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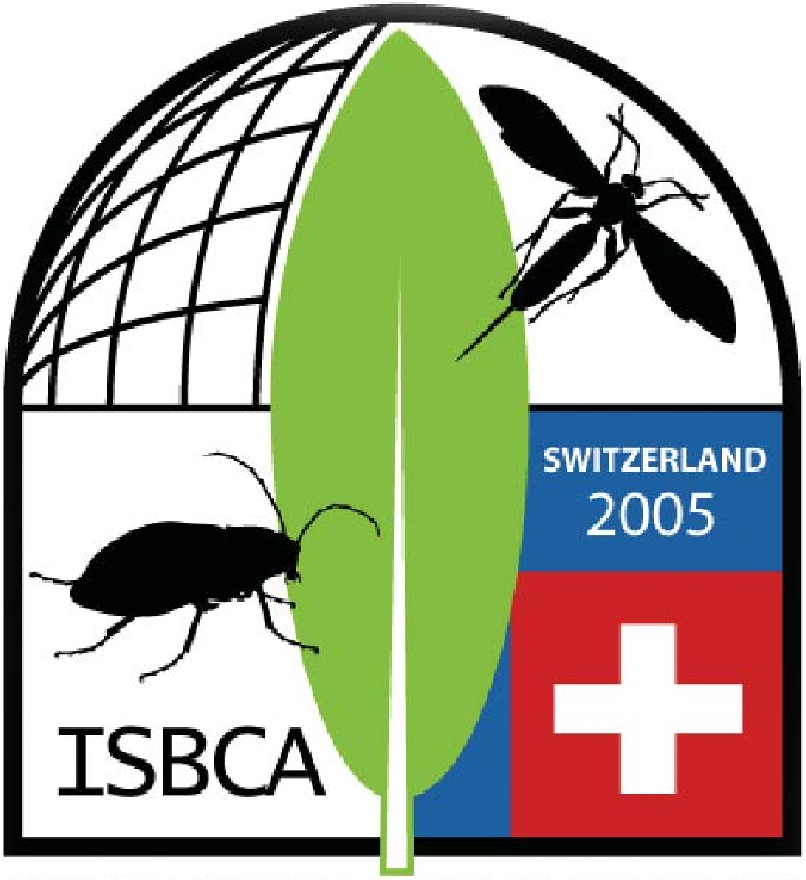
Biological Control



INTERNATIONAL SYMPOSIUM ON BIOLOGICAL CONTROL OF ARTHROPODS

September 12-16, 2005

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United States
Department of
Agriculture

Forest
Service

FHTET-2005-08
September 2005
Volume I

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SECOND INTERNATIONAL SYMPOSIUM ON BIOLOGICAL CONTROL OF ARTHROPODS

DAVOS, SWITZERLAND
SEPTEMBER 12-16, 2005

USDA Forest Service
Publication FHTET-2005-08

SECOND INTERNATIONAL SYMPOSIUM ON THE BIOLOGICAL CONTROL OF ARTHROPODS

The Second International Symposium on the Biological Control of Arthropods held in Davos Switzerland builds upon the foundation laid at the first meeting in Hawaii in January 2002. The intent of the ISBCA meetings is to create a meeting for practitioners, a forum for information exchange, an event to build cohesion among the research community, and to foster discussions of issues effecting biological control work, particularly pertaining to the use of parasitoids and predators as biological control agents.

To this end, a 14 session conference with invited has been designed to address the most interesting and relevant research topics that have broad international application. The oral sessions have been complimented with unsolicited poster presentations prepared by over 100 different scientists from around the world. Topics covered at ISBCA II are diverse and include invasion biology and application to biological control, biological control of arthropod pests of conservation importance, the role of biological control for pest management in developing nations, and emerging experimental protocols and legislation for assessing natural enemy specificity and safety.

The printed ISBCA II conference proceedings are large, indicating the great interest in the content of this meeting. The two volume proceedings only include the articles prepared by invited speakers. The accompanying CD has an electronic version of the conference proceedings and the abstracts of approximately 115 posters that were presented at the meeting and perused by over 200 meeting attendees representing the international biological control community.

ACKNOWLEDGEMENTS

Credit is due to the following participants in helping to organize ISBCA II:

SCIENTIFIC SESSION ORGANIZING COMMITTEE MEMBERS

Session 1: Sandy Smith, University of Toronto, Ontario Canada, and Heikki Hokkanen, University of Helsinki, Helsinki Finland. Session 2: Mark Hoddle, University of California Riverside, California U.S.A., and Matthew Cock, CABI Bioscience, Delémont Switzerland. Session 3: Nick Mills, University of California Berkeley, California U.S.A., and Jacques Brodeur, Université de Montréal, Quebec, Canada. Session 4: Steve Wratten, Lincoln University, Canterbury New Zealand, and Geoff Gurr, University of Sydney, Orange NSW, Australia. Session 5: Juan Manuel Alvarez, University of Idaho, Aberdeen Idaho, U.S.A., and Shu-sheng Liu, Zhejiang University, Hangzhou, P.R. China, China. Session 6: Janny Vos, CABI Bioscience Nederland, Leusden The Netherlands, and Peter Ooi, FAO, Bangkok Thailand. Session 7: Joerg Romeis, Agroscope-FAL, Zurich-Reckenholz Switzerland, and Tony Shelton, Cornell University Geneva, New York U.S.A. Session 8: Felix Wäckers, NL Royal Academy of Sciences, Harlem The Netherlands, and Henry Fadamiro, Auburn University, Alabama U.S.A. Session 9: Bob Pfannenstiel, USDA-ARS, Weslaco Texas, U.S.A., and Matthew Greenstone, USDA-ARS Beltsville, Maryland U.S.A. Session 10: Steve Naranjo, USDA-ARS Phoenix, Arizona U.S.A. and Patrick de Clercq, Ghent University, Ghent Belgium.

Session 11: Dave Gillespie, Agriculture and Agri-Food Agassiz, British Columbia Canada, and Karel Bolckmans, Koppert, Amsterdam The Netherlands. Session 12: Franz Bigler, Agroscope-FAL, Zurich-Reckenholz Switzerland, and Joop van Lenteren, University of Wageningen Wageningen, The Netherlands. Session 13: Roy Van Driesche, University of Massachusetts Amherst, Massachusetts U.S.A., and Peter Mason, Agriculture and Agri-Food Ottawa, Ontario Canada. Session 14: Barbara Barratt, AgResearch, Mosgiel New Zealand, and Ulli Kuhlmann, CABI Bioscience, Delémont Switzerland.

SCIENTIFIC COMMITTEE

Members of the Scientific Committee are thanked for providing input into session themes, and development and review of presentation content and relevance to ISBCA. The Scientific Committee included: Mark S. Hoddle, Barbara Barratt, Matthew Cock, Cliff Gold, IITA/ESARC, Kampala Uganda, Geoff Gurr, Heikki Hokkanen, Molly Hunter, University of Arizona, Tucson Arizona, U.S.A., Moses Kairo, Florida A&M University, Tallahassee, Florida U.S.A., Nick Mills, Banpot Napompeth, National Biological Control Research Center (NBCRC), Kasetsart University, Chatuchak Bangkok, Thailand.

REGIONAL ORGANIZERS

We thank Regional Organizers for coordinating ISBCA II advertising, soliciting interest in the conference, and for helping with local inquiries about ISBCA II. Regional Coordinators included: Mark S. Hoddle, Dave Gillespie, Juan Manuel Alvarez, Moses Kairo, Tania Zaviezo, Departamento de Fruticultura y Enología, Casilla , Santiago, Chile, Willie Cabrera Walsh, USDA-ARS South American Biological Control Laboratory, Argentina, Ulli Kuhlmann, Stefan Toepfer, Plant Health Service, Hodmezovasarhely Hungary, Victor Fursov, Institute of Zoology of National Ukrainian Academy of Sciences, Kiev Ukraine, Malika Bounfour, Ministry of Agriculture, Rabat Morocco, Cliff Gold, Manuele Tamo, Biological Control Center for Africa, Republic of Benin, West Africa, Rami Kfir, Plant Protection Research Institute, Pretoria South Africa, Ashraf Poswal, CABI Bioscience Rawalpindi , Pakistan, Banpot Napompeth, Feng Zhang, CABI Office, Beijing P.R. China, , Masami Takagi, Kyushu University, Fukuoka Japan, Barbarra Barratt, Don Sands, CSIRO Entomology, Indooroopilly Queensland, Australia.

LOCAL ORGANIZATION

Ulli Kuhlmann in collaboration with *KCS* Convention Service, Delémont, Switzerland made all the pertinent venue, accommodation, field trip, and culinary decisions for Davos.

FINANCIAL ASSISTANCE

ISBCA would not have been possible with generous contributions from Richard Reardon USDA-Forest Service for supporting the publication and distribution of the ISBCA II Conference Proceedings. Eric Rosenquist, USDA-ARS, National Program Leader Tropical Agriculture and Kevin Hackett, USDA-ARS, National Program Leader for Beneficial Insects, Beltsville, U.S.A. for supporting the travel and accommodation of invited speakers as well as those attending from developing countries. We also appreciate very much the support from Katharina Jenny and Ueli Stürzinger, Swiss Agency for Development and Cooperation (SDC), Natural Resources and Environment Division and Asia II Division, for providing financial resources to support participants from developing countries. We like to thank Rudolf Strasser, SDC Swiss Cooperation Office of DPR Korea, Pyongyang, for supporting the participation of three scientists from DPR Korea. In addition we would like to thank the Swiss National Foundation (SNF), International Relations Division for supporting scientists from Ukraine, Kyrgyzstan, Romania and Bulgaria to attend this symposium in Switzerland.

CONFERENCE PROCEEDINGS

Mark Riffe (USDA Forest Service - FHTET) is thanked for many hours patiently and diligently spent on page layout and design of the ISBCA II conference proceedings.

CONFERENCE LOGO

Vincent D'Amico III, USDA Forest Service Hamden Connecticut U.S.A., is thanked for designing the ISBCA II logo.

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Volume I

RECONSTRUCTING AN ECOSYSTEM: A THEORY UNIFYING INVASION BIOLOGY AND BIOLOGICAL CONTROL

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ABSTRACT

Invasion biology is centered around two focusing themes – (i) will an invasion result in an establishment, and (ii) does the establishment of a new species result in a threat to existing species or critical community or ecosystem characteristics. In biological control, we are continually faced with two different types of invasions. The first of these is the unintentional (or sometimes intentional!) invasion of a plant or animal species into a new area. The second type is the subsequent, and intentional, invasion of natural enemies introduced for the purpose of ameliorating the negative effects of some previously invasive species that has become a threat to other species or to some characteristic of the invaded ecosystem. This paper presents a theory of interactions, based on both guild structures in a community and on population dynamics, that brings together these two types of invasions and connects invasion biology and biological control. In essence, the connections between these two different types of invasion share common population dynamic structure, and a common guild structure. However, the impact on the ecosystem in which these invasions takes place is vastly different between the unintentional introduction of a damaging species compared with the introduction of a species in a higher trophic level. The theory is presented in both ecosystem structure form and in the form of a model of the population dynamic processes involved. The model outcomes are related to current issues and recent projects in biological control.

INTRODUCTION

First, I would like to express my thanks to the symposium organizers for all their hard work putting together such a wonderful meeting, and secondly I would like to thank them for the opportunity of addressing this august body about some ideas I have relating ecosystem or community disturbance and biological control.

I am strongly of the opinion that biological control is the most powerful ally that a disturbed ecosystem has in the efforts to return it to ecological health. In this paper I will try to demonstrate why I think that this is so by following a path from simple ideas to more complex ones in order to demonstrate the power that biological control may have in helping manage ecosystems at risk from invading species. The path I will take is this: I will look at what happens in nature in biological control programs. I will then show that we can capture what happens in nature in a simple model of population dynamics. Then I will expand the model to include migration in four different types of ecosystems. In these ecosystems, I will simulate the invasion of a species and its remedy through the introduction of a natural enemy. And in these cases, we find the same power and results in a complex ecosystem as we do in the simpler, two-species cases.

MATERIALS AND METHODS

To develop these themes about biological control and ecosystem-wide biology, I am going to draw upon real-world data to first give us an impression of what biological control of arthropods can do in terms of population suppression (Van Driesche & Bellows 1996). Of the some 1200 biological control programs developed against arthropods and other pests, there is relatively little data on what we would consider modern quantitative population dynamics. Early qualitative reports of biological control success were exuberant about their impact. More recently, we can find in the literature (and in our own work) quantitative studies on the impact of natural enemies on harmful invasive species.

Fig.1 shows a few examples of the impact of introducing a natural enemy into an already established population of a harmful or pest species. In every case we find important and repeated characteristics. Although the figure only indicates three examples, we find throughout the quantitative literature four features common to such programs. One, that whenever an arthropod species invades a new territory in which it can survive and reproduce well, its population grows to a size that occupies all the available resource that is critical for that species (such as leaf area, for example). Second, we find that when an effective natural enemy is introduced, it requires some 10-15 generations for the population of the natural enemy to catch up to the pest species density and exert a sufficient force on it that the pest population collapse. Third, we see that in a biological control program, once the natural enemy’s population density has caught up with the pest, the pest population crashes to phenomenally low densities, typically 4-8 orders of magnitude lower than prior to the natural enemy’s introduction, a control level unsurpassed by any other mechanism or pest control known. The final feature of this control is that it appears permanent. The pest and natural enemies continue to exist at very, very low densities without disruptions or outbreaks.

These four characteristics of biological control programs and their impact on pest species appear pervasive, and many examples can be found from the biological control of pests in many different environments. This pervasiveness implies that there are intrinsic features of pest-natural enemy systems that are found across many taxa, in many different environments. So a task is set before us to uncover, or at least mimic, what some of these features are, so that we might come closer to an understanding of what makes biological control so powerful in so many different settings. As a beginning, I will present a graphical view of what happens to an ecosystem when it suffers an invasion of a harmful species.

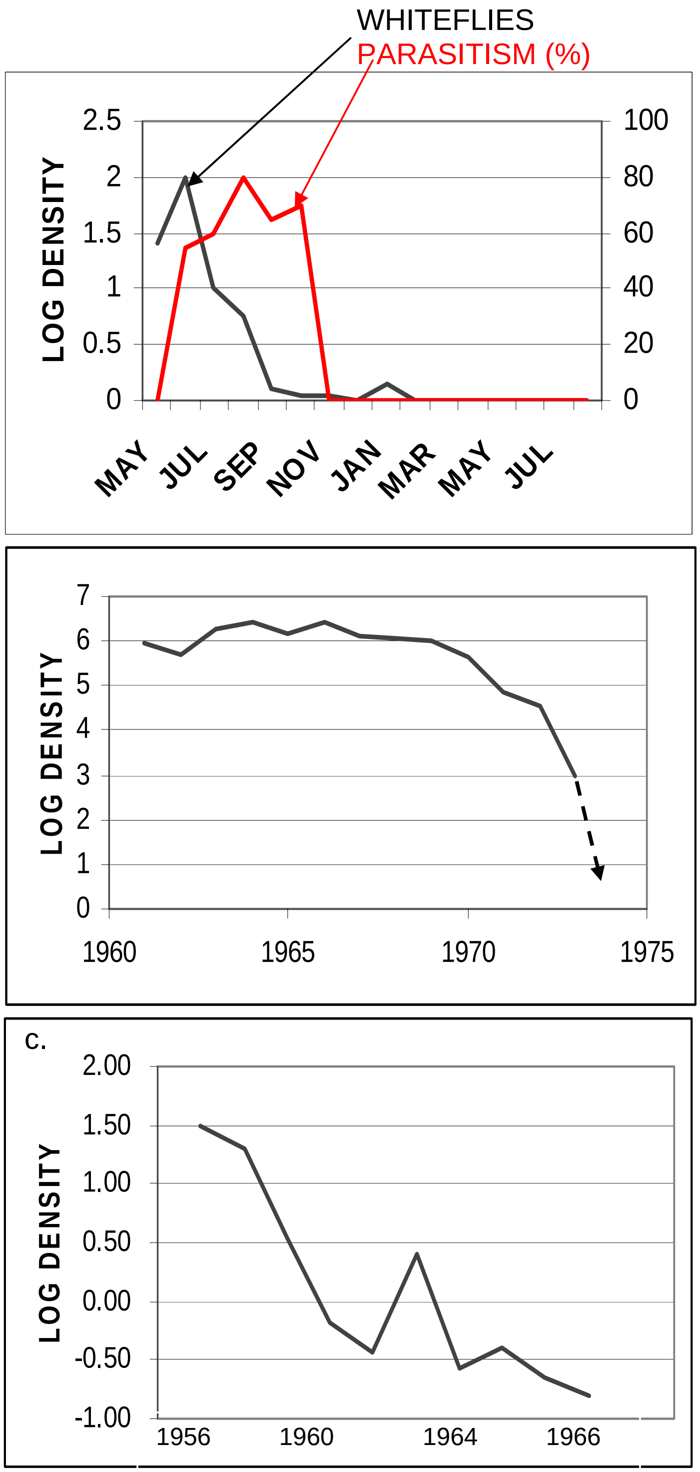

Figure 1. Three examples of biological control outcomes that emphasize the four important points of biological control: first, that an invasive species can reach phenomenal densities. Second, biological control can bring about major suppression of target pest. Third, an expectation that it will take 10 to 15 generations to achieve such suppression, and fourth, that such suppression is permanent. (a), giant whitefly, Bellows unpublished; (b) larch sawfly after Ives 1976; (c), olive scale, after Huffaker 1971.

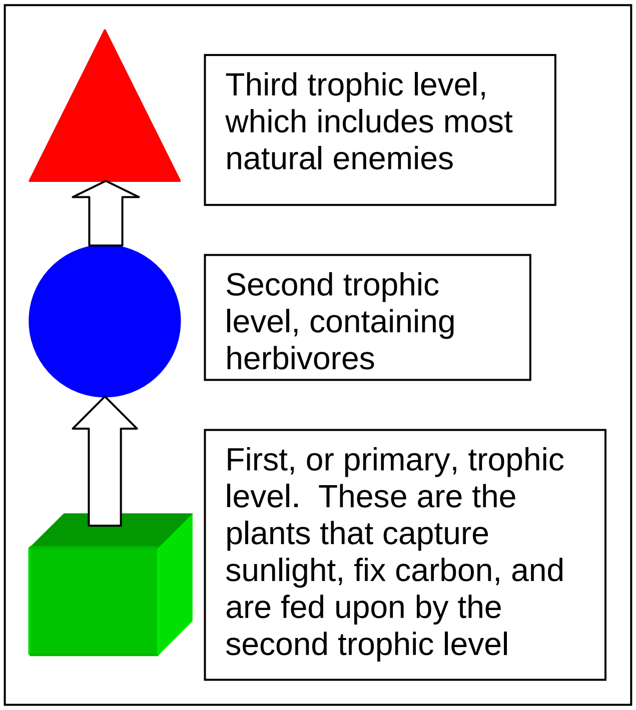

Figure 2. A fundamental community consists of plant species, herbivore species, and predator or parasitic species.

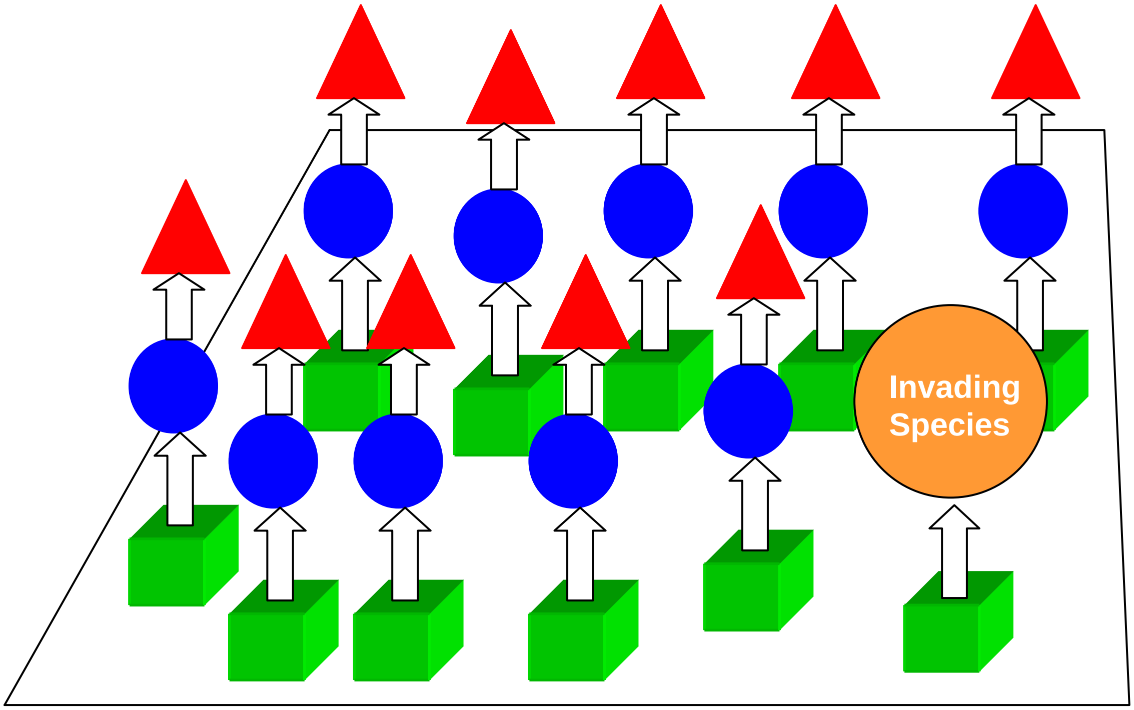

Figure 3. An ecosystem is composed of many communities operating together in the same space and time. One community may be present in many numbers. There also may be many different communities that interact with one another. An invasion by a species not a member of the ecosystem may reek havoc on ecosystem function.

In Fig. 2 I have characterized the structure and function of a simple, three-species community. Having many such communities, and mixing them with similar communities made up of different species (Fig. 3), provides the basis for an ecosystem in dynamic homeostasis. A major problem arises when a species new to the ecosystem, an invading species, arrives and begins to grow out of control, using up nutrients, or competing for foliage, and changing the homeostasis to the point that the ecosystem can no longer function as is had.

We now need a tool, or a model, that captures the four essential features of a successful biological control campaign. The structure of such models has been the object of much study, from the initial work of Thompson (1924) and Nicholson and Bailey (1933), through to the present day. The simplest and most appropriate form is a pair of difference equations, as shown here:

$$h(t+1) = F \bullet h(t) \bullet g(h(t) \bullet f[h(t),p(t)]$$

$$p(t+1) = h(t) \bullet g(h(t) \bullet \{1 - f[h(t),p(t)]\}$$

A bit of explanation makes these equations very simple to follow. The herbivore population that is the target of a biological control program is represented by ‘h’. The value of *t* is the generation in which the equations are operating, and is iterated or added to each time we use the equations. The symbol *F* represents the fecundity of the herbivore. Now we have only the functions g and f to deal with. At extremely high densities, the herbivore may run out of resources necessary for optimal reproduction. So the function g(h) is simply the proportion of offspring that survive in that generation due to any limit placed on the population by lack of resources. The function f(g,p) tells us the proportion of herbivores that escape parasitism, and hence go on to survive to the adult stage and produce the generation h(t+1). The proportion that does not escape parasitism (in the second equation) eventually become parasitoid adults, p(t+1), in the next generation. This pattern repeats itself over and over, generation after generation.

I will not bore you with nearly a hundred years of research trying to find functions for g and f that actually describe what we see in biology and in biological control situations. Suffice to say that, after many trials, two have come to be recognized as the most suitable functions. For density dependence in the herbivore population, a model with the necessary properties was provided by Maynard Smith and Slatkin in 1973 (Bellows 1981), where

$$g(h) = 1 / (1 + (ah)^{b})$$

In this model the parameter *a* is simply a scaling parameter, helping to fix the equilibrium density of the herbivore population in the absence of any predation or parasitism. The parameter *b* has bearing on the degree of severity of the density dependence in the herbivore populations. In most insect populations, density dependence found in nature is mild (Bellows 1981), and in all the simulations reported here *b* has the value 1, which represents simple contest competition. The parameter a was given the value 0.001 for every location, and the fecundity (F), that is, the number of offspring that do not perish due to density independent causes, was assigned the value 4. This provides an equilibrium herbivore density, in each location (and in the absence of parasitism), of 3000 individuals.

Of the many models employed by many authors for predation or parasitism (function f(h,p)), only one has emerged that can demonstrate the properties we see in nature: major suppression of an invading species, with stable, continuing populations (May and Hassell 1988). This model employs the negative binomial distribution of attacks by a natural enemy, that is, the attacks tend to be clumped or aggregated (as are also many herbivorous insects in nature). The function takes the form:

$$f(h,p) = (1 + (a'P)/k)^{-k}$$

The parameter *a'* represents the search efficiency of a single female parasitoid, and the parameter *k* indicates the degree of aggregation of her attacks. In the simulations reported here, these parameters were given values of 0.1 and 0.5, respectively, indicating a moderately efficient natural enemy with a mild degree of aggregation in her attacks.

Before continuing to explore the simulations of ecosystem-level invasions, we must first determine if the tool, that is, the model, is an effective mimic of what we see in natural invasions followed by a biological control program. In order to do so, I ran simulations of just an isolated pair of populations, one herbivore and one parasitoid. Each simulation ran for 20 generations with only the herbivore. At that time, a single natural enemy was added to the population, which was then run for an additional 15 generations. The outcomes are discussed in the Results section, but suffice to say here that they were in keeping with expectations, and validated the use of the model as a tool to explore more complicated ecosystem-level invasions.

In exploring the potential ecosystem-level impact of a natural enemy working against an invasive species, there are several significant questions to explore. One is to what degree is the invaded species suppressed when it is capable of movement from one part of an ecosystem to another. Another important issue is whether any suppression achieved is stable, or is subject to erratic fluctuations.

In order to explore these issues, I developed a model of an ecosystem which consisted of 400 locations, each adjacent to two or more locations, placed on a square grid of 20 rows and 20 columns (Fig. 5). Each location was characterized by a parameter $\tilde{a}$, which was varied in different ways in the four ecosystems. The parameter $\tilde{a}$ varied from 0 to 1, and was used to characterize the suitability of each location for herbivore reproduction. The parameter $\tilde{a}$ was multiplied into the herbivore reproduction equation:

$$h(t+1) = \tilde{a} \bullet F \bullet h(t) \bullet g(h(t) \bullet f[h(t),p(t)]$$

A location with a value of 1 is highly suitable, and imposes no limit on the herbivore. A location with a lower value of $\tilde{a}$ would limit the potential reproduction of the herbivore.

Using this model ecosystem I investigated four different patterns for the value of the parameter $\tilde{a}$, to represent four different types of ecosystems. In one simulation, all locations in the environment had a value of 1. This, in a way, was the most basic evaluation of the behavior of biological control over a wide, uniform area. In the second simulation type, I placed values of 1 in a regular pattern over the ecosystem, separated by values of 0 (Fig. 5). This might be viewed as representing an orchard, or an urban ecosystem, with each house and yard separated by streets or avenues. In the third simulation, I assigned each location in the ecosystem a random number between 0 and 1. This is the most diverse and complex system (Fig. 6). In the fourth case, I placed specific areas on the ecosystem map in which the locations had high values of ã, separated by locations with values of 0. This represents an ecosystem with certain vulnerable habitats, in which conditions allow the invading species to flourish, surrounded by areas where the invading species can not reproduce.

Migration in the ecosystem was handled simply, and in keeping with most of what we know about movement in arthropod populations. The premise I followed was as follows: most offspring tend to stay close to their area of birth and rearing (close at least in the sense of the perceived size of the grid). Hence after herbivore reproduction and parasitism, there was a migration phase in which only half of the surviving individuals in a location remained there. Of the other half, 80% were distributed equally in the locations surrounding the one of their birth, indicating moderate amounts of migration. The remaining 10% were considered "far migrators", and were distributed evenly over the entire ecosystem, into locations from the farthest to the closest to where they were born.

RESULTS

TWO SPECIES, SINGLE LOCATION MODEL

Validating a tool such as a model for use in exploring the potential impact of an invading species requires that we examine the behavior of the model in its simplest possible form, and compare the model outcomes with what we know occurs in nature. If the model passes such a test, then it may be useful to generate hypotheses about what may happen in a larger context, that is, in our study what may happen in an ecosystem. Of course, if the model cannot capture the essentials of natural populations, it is an untrustworthy tool and another one must be sought for use in its place.

Of the four essential and common features of an invading species, a biological control program, and the impact on the two populations involved, we can examine each one in turn after conducting simulation studies with the proposed model. I conducted several such studies, with varying values for the parasitoid search parameters and the herbivores growth parameters. One such simulation study, the results of which were typical of all the trials I ran, is shown in Fig. 4.

In this study, we see the rapid rise in density of the invading species, reaching its carrying capacity in just 5 generations. This is equivalent to the invading species occupying all the available resources, as is common when a species is adventive to an area and has no effective natural enemies. In the absence of natural enemies, it rapidly reaches the environmental limit of its growth, and the population is limited by its own density dependence. Here, the model mimics the first of the four common features of a biological control program.

In generation 18, when the invaded species had reached its equilibrium density of 3000 individuals, 10 parasitoids were added to the system. These parasitoids amounted to 0.3% of the host population. But such is often the case in biological control – by the time a natural enemy has been located, screened for safety, and rearing has begun, the invaded species which is its target has been reproducing without limit for many generations. So the entry of 10 adult

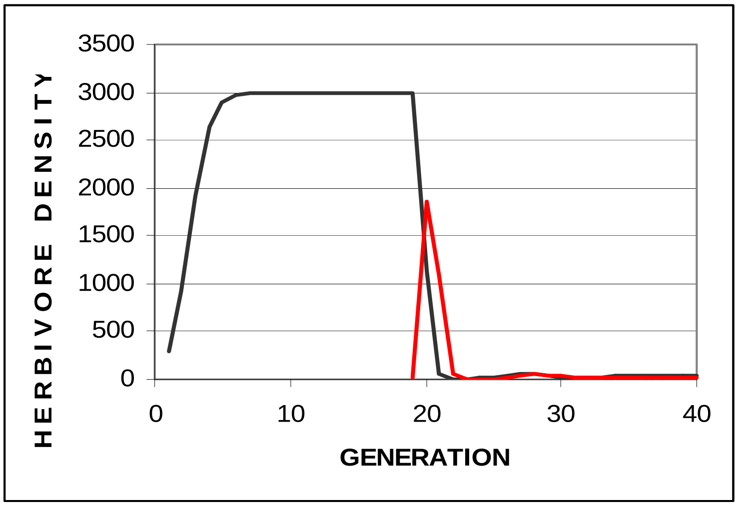

Figure 4. A typical simulation run of the model for an invading herbivorous species (black) followed by the introduction of a natural enemy (red) at generation 18.

parasitoids into the system is typical of such programs. As the impact of 10 natural enemies on a population of 3000 herbivores is nearly undetectable, these releases are often referred to as “inoculation” releases, with the expectation that their offspring will start a population growth that will eventually catch up to, and then control, the invading species.

In natural systems where such data are available, approximately 10-15 generations pass before the density of the invader begins to decline, and when it does so, it does so very rapidly. If a sample is taken at the right time, one can often find a leaf covered with the invading herbivore, together with very large numbers of the natural enemy. Here the model appears to be slightly more efficient than what data we have on such matters, bringing the invaded species density down dramatically within five generations.

The last two matters, the degree of suppression and the stability of that suppression, we also see in the modeled populations. The average density of the invaded species after the suppression (generations 36-40) is 31.2, a drop of two orders of magnitude below its previous high level. The standard deviation of this mean population level is 0.95, which is 3% of the mean, and indicates a very stable population. Clearly the impact of the natural enemy in the model has given us an appropriate decline in density, and maintains it with considerable stability.

Note the major drop in density of the invaded species, followed by a moderately stable and consistently low density of both the herbivore and its natural enemy.

ECOSYSTEM SIMULATIONS

Having concluded an evaluation of the model in its simplest setting, we have found that it does have the characteristics and behavior that we see in nature. This does not make the model “correct” in any context – a model is simply an abstract representation a dynamic process. But the fact that the model does capture the dynamical behavior we see among natural populations does give some confidence that we can now apply the model to a wider scope, and use it to ask questions about the behavior of similar systems, such as an ecosystem which we envisage as a collection of communities each behaving in the manners that the model has been able to replicate. So here we are using the model to explore possibilities, create hypotheses; but we are not attempting to prove something. Hypotheses only get tested by using biological data, and the most useful and related thing a model does is help us create sensible hypotheses to test.

Such testing is beyond the scope of this paper, but we can examine what the model behavior is in the ecosystems that I have proposed testing. Two of these are somewhat trivial, and I will present them *de novo* at the symposium. Two others will suffice to demonstrate what new insight might be awaiting us in the continued study of ecosystems affected by invasive organisms.

The first of these (Fig. 5) represents an ecosystem that has areas suitable for the reproduction of the invading species, separated by regions that are not. Some have termed such a layout an “orchard” model of an agricultural ecosystem (to distinguish it from monocultural agricultural regions). One of the first things we would expect is that the equilibrium density of the invading species should be lower here than in an ecosystem that was uniformly suitable for reproduction, as there is less resource in this ecosystem. Such turns out to be the case, and the equilibrium density for an invading species in this ecosystem is only 36% that of when it is growing in a uniform ecosystem.

Turning to our four expectations from biological control systems, we see that in the absence of natural enemies, the invading species does reach the carrying capacity of this environment quickly. We also note that the population density of the pest falls rapidly following the introduction of the natural enemy, in this case in approximately 7 generations (but this was also the case for this model when evaluated in the simplest trial). The pest population is reduced, and appears to be low and stable for many generations. However, a closer quantitative look shows that the average population density of the invaded species in only 1.5 orders of magnitude lower that its carrying capacity. The standard error of the mean is very low (3% of the mean density), so the new stasis appears very stable. But it does appear that the model does not predict as great a population density reduction as we have seen in many field studies.

Lastly, we turn to an ecosystem that is rather like an abandoned field, or a heterogenous forest, or any other kind of ecosystem that has great variation in its resources over the space it occupies. The average value of the parameter ã in this ecosystem is 0.500, thus we might expect that the carrying capacity of the ecosystem would only be half of what we would find in an ecosystem that was uniformly suitable for an invading species. This is indeed the case. But let us turn to our four characteristics and evaluate what the model says about biological control as a tactic in such a diverse system.

We find that an invaded species does indeed climb to the overall carrying capacity very quickly. When the natural enemy is introduced, a new stasis is reached in approximately 10 generations, fitting well with what we see in nature. However, the new equilibrium of the invaded pest is again approximately 1.5 orders of magnitude lower than the environment’s carrying capacity.

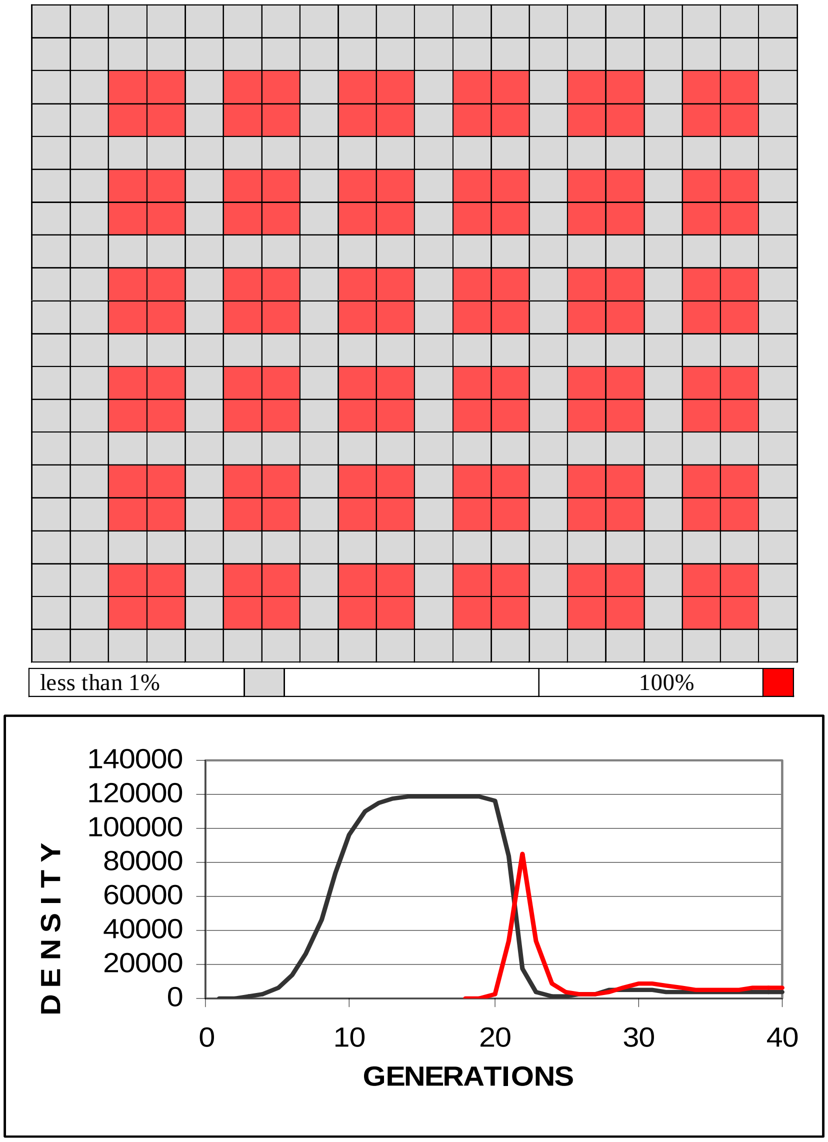

Figure 5. An environment with regularly distributed suitability for pest survival, separated by regions where the pest cannot survive. The density of the pest (black) increased until the introduction of the natural enemy (red).

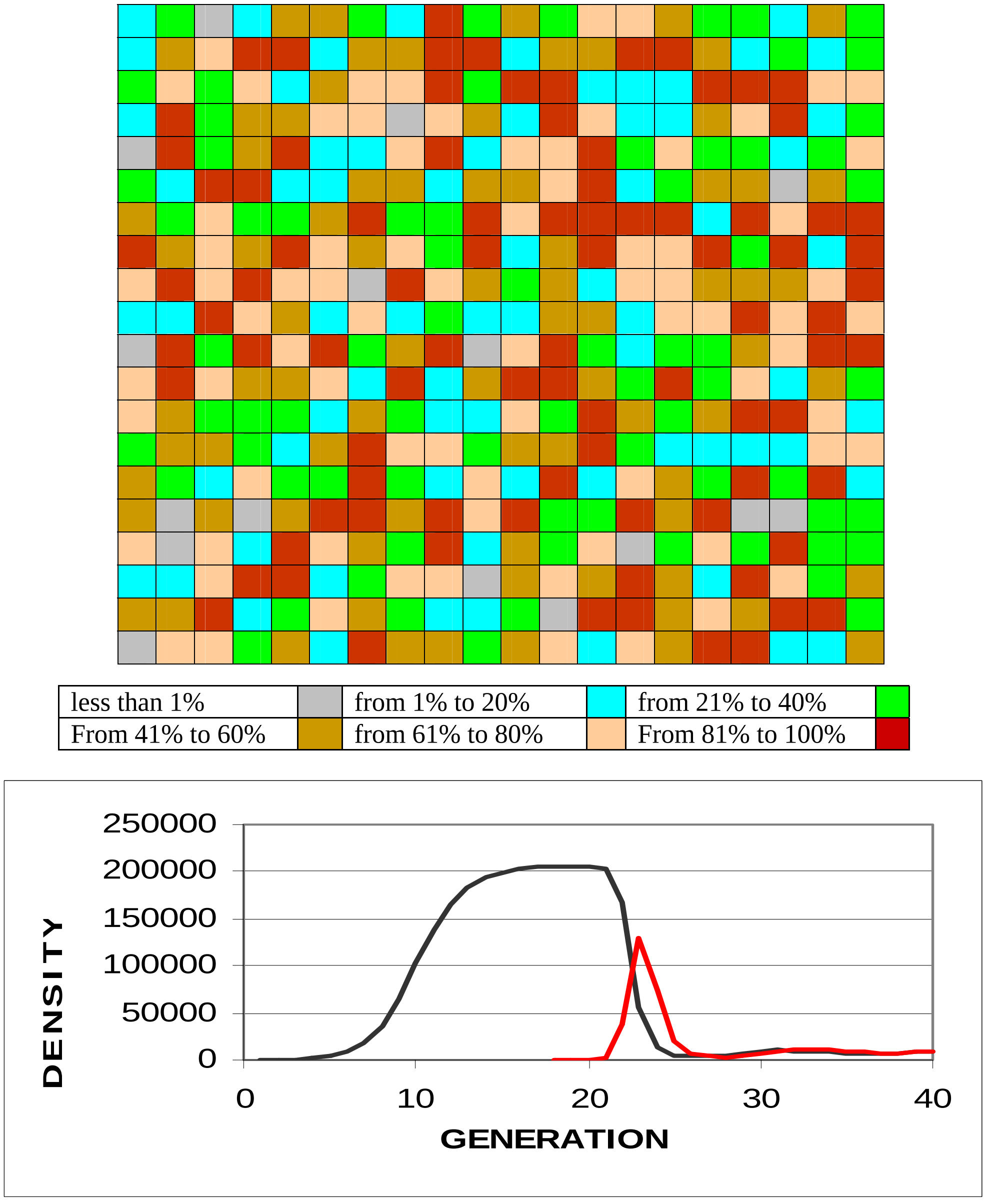

Figure 6. An environment with randomly distributed suitability for survival of an invaded species, and the dynamics of the pest (black) increasing in number until the introduction of the natural enemy (red).

DISCUSSION

When discussing models and their uses, particularly I think in the field of population dynamics, one can be tempted to wax poetic on the charming attributes of this equation or that formulation, or on the elegance of a particular mathematical framework. But jesting aside, models rally only serve two sensible purposes. First, models are of great value when they shed light on some piece of truth that was previously hidden from us. How we can tell whether what the model tells us is true, since we had not discovered it ourselves, is a bit of a philosophical conundrum. But we must pass on and leave the philosophers such puzzles.

Models are also useful to us when we have confidence that they actually capture the essence of an interaction, such as in a host-parasitoid interaction. We can gain such confidence by comparing certain features of a model with similar features of a living system. If the two systems, one living on its own, and the other living in our heads, provide similar answers to questions put to both, then our confidence in the model's ability grows. It becomes a tool that we can apply to other systems. We can use it to ask questions about bigger, or broader, systems. That is what we did here.

The model that I worked with gave confidence in the beginning because it could readily mimic the behavior of simple real-world systems. This prompts the questions: Can it tell us something we do not know about more complex systems? So I applied it to imaginary ecosystems, with imaginary properties (such as migration), and gathered some new information. The model showed that biological control may well be a phenomenal tool to solving invaded species problems. It indicated that several different kinds of ecosystems that biological control could reduce the density of an invading species from 10-100-fold (although we see values far higher than that in studies of natural systems). The model also indicated that the new lower population density would be extremely stable, and would remain so for a long time. Both of these points are reasons for biological control to stand ready to aid whenever an invasive species problems crops up – not only in our traditional agricultural arenas, but also in urban areas, in forests, in the invasions of wetlands.

The question of whether or not biological control can contribute to restoring ecosystems disturbed by species invasions still needs to be answered in the field. But everything I have found, using tools as disparate as simple populations models to holistic systems analysis, tells me that the answer may well be a resounding yes.

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CLASSICAL BIOLOGICAL CONTROL OF THE ALFALFA WEEVIL IN JAPAN

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ABSTRACT

The alfalfa weevil was accidentally introduced into Japan in early 1980's and has been heavily infesting the Chinese milk vetch in paddy fields ever since. Chinese milk vetch is main source of honey products for apiarists in spring. Thus, the alfalfa weevil is not a pasture pest but a pest of apiculture. Four species of parasitoids were introduced into Japan as biological control agents for this pest from U.S.A. in 1988 and 1989. One of the parasitoids, *Bathyplectes anurus* was recovered in 1997. The percentage parasitism by *B. anurus* is higher on Narrow-leafed vetch in surrounding grasslands than on the Chinese milk vetch in paddy fields, suggesting that the conservation of these grasslands is important for the biological control of the alfalfa weevil.

INTRODUCTION

The alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) is the most serious pest of alfalfa in the United States (USDA 1991). This pest is of Eurasian origin and was accidentally introduced into North America. Classical biological control of this pasture pest has a had almost 100 years of history in the U.S.A. and this has been summarized by Radcaliffe *et al*. (1998). In all, 12 species of natural enemies were involved in the combined USDA biological control program against the alfalfa weevil (Bryan *et al*. 1993). In most major American alfalfa production areas, several natural enemies of the alfalfa weevil are now well established and alfalfa growers benefit from their effects (Radcaliffe *et al*. 1998).

The alfalfa weevil was also accidentally introduced into Japan during the early 1980's (Okumura 2002), however, its impact on alfalfa has thus far been limited because it has yet to reach the commercial alfalfa production areas located near Hokkaido. The crop heavily infested by the weevil is Chinese milk vetch (*Astragalus sinicus* L.), grown in paddy fields during the early spring. Japanese rice farmers keep Chinese milk vetch as green manure in winter and the flowers of the plant provide the main source of honey products for apiarists during spring. Thus, the alfalfa weevil is not a pasture pest but more a pest of the paddy field agro-ecosystem during the non-crop season in Japan.

Moji Plant Protection Station introduced four species of parasitoids from U.S.A. into Japan for the biological control of this pest, (Kimura and Kaku 1991). One of the parasitoids, *Bathyplectes anurus* (Thomson) (Hymenoptera: Ichneumonidae) was recently recovered and is expanding its distribution. In this paper, we summarize the invasion biology of the alfalfa weevil in Japan and efforts towards its classical biological control. We also discuss the importance of natural enemy conservation and the future prospects of biological control of the alfalfa weevil.

INVASION BIOLOGY OF THE ALFALFA WEEVIL IN JAPAN

The alfalfa weevil was first discovered near Fukuoka airport in 1982 (Kimura *et al*. 1988). Subsequent surveys in the same year revealed that it was present in an area close to Fukuoka airport and widely distributed in Okinawa Island (Fig. 1). After that, the alfalfa weevil expanded its distribution towards eastern and northern Japan and has now reached Tokyo. The alfalfa weevil infests leguminous plants such as *Astragalus sinicus* (Chinese milk vetch), *Medicago lupulina* (black medick), *M.hispida* (burr medic), *M. sativa* (alfalfa), *Melitotus officinalis* (yellow sweet clover), *Trifolium pratense* (red clover), *T. repens* (white clover), and *Vicia sativa* (narrow-leaved vetch) in Japan (Yoshida *et al*. 1987). All of these leguminous plants in Japan are wild except alfalfa in Hokkaido, a northern island of Japan, and Chinese milk vetch in paddy fields of south and western Japan.

The larvae of the alfalfa weevil infest and seriously damage Chinese milk vetch flower buds and flowers in early spring. The production of honey from this source is greatly decreased as a consequence. When the farmers begin to flood the fields in May, the new adult weevils are forced to emigrate from paddy fields to aestivate following their emergence. These flooding events also displace natural enemies. After harvesting rice in the autumn, farmers seed with Chinese milk vetch to fertilize the fallow paddy fields and adult weevils are able to return to the fields. Adult female weevils oviposit on alfalfa during the winter and early spring, and larvae hatch and start damaging alfalfa from February to May.

INTRODUCTION EFFORTS OF NATURAL ENEMIES

Some indigenous parasitoids attack the alfalfa weevil but the percentage parasitism is very low and ineffective in suppressing populations of the alfalfa weevil (Okumura 1987). In order to conserve the paddy field agro-ecosystem during winter and spring, four species of parasitoids, *Bathyplectes anurus*, *B. curculionis* (Thomson), *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) and *M. colesi* Drea, were introduced as biological control agents of the alfalfa weevil from U.S.A. into Japan during 1988 and 1989 (Kimura and Kaku 1991).

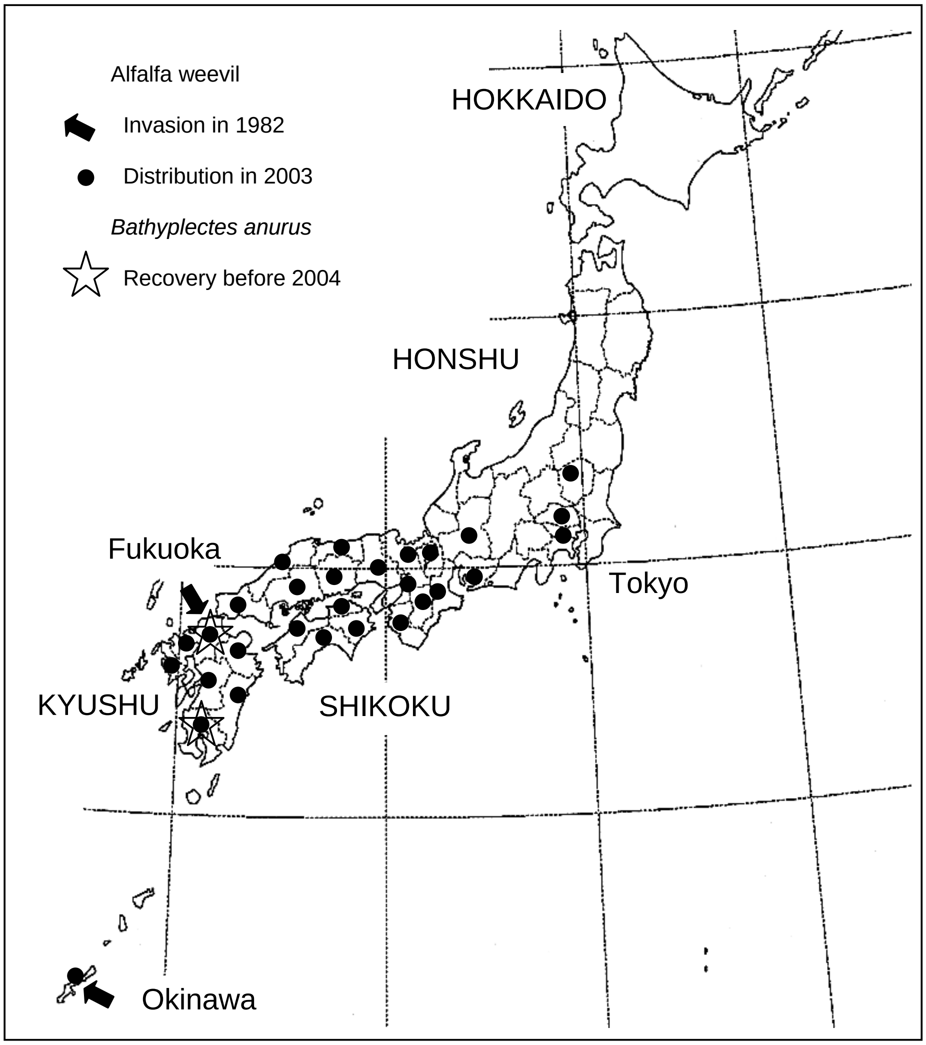

Figure 1. Invasion and geographic spread of the alfalfa weevil, *Hypera postica,* and recovery of its parasitoid, *Bathyplectes anurus* in Japan.

These parasitoids, except for *M. colesi,* were repeatedly released mainly in Kyushu after mass production in the laboratory. Despite these efforts, no parasitoids were considered established until 1996 (Okumura *et al.* 2002). One of the reasons for failure is that rice fields are tilled and irrigated before they are planted in the growing season. Because of these agricultural practices, both the alfalfa weevil and natural enemies disappear from the paddy fields in summer and this limits establishment. One parasitoid, *B. anurus,* however, was recovered in Fukuoka during 1997. Initially during establishment, the percentage parasitism was low but it increased gradually, reaching about 40% by 2003 (Shoubu *et al.* unpubl.). *B. anurus* is a univoltine, solitary, endoparasitoid of the alfalfa weevil larvae and its life cycle is well synchronized with its host in Kyushu (Okumura *et al.* 2002). Our survey showed that weevil damage on Chinese milk vetch was reduced from 2001 to 2003. Another survey reported higher parasitism by *B. anurus* on weevils in wild leguminoseae (e.g., *M. hispida and V. sativa*) in surrounding grasslands than on weevils in Chinese milk vetch from paddy fields (Okumura 2002).

NOTE: no in-text reference for Figure 2.

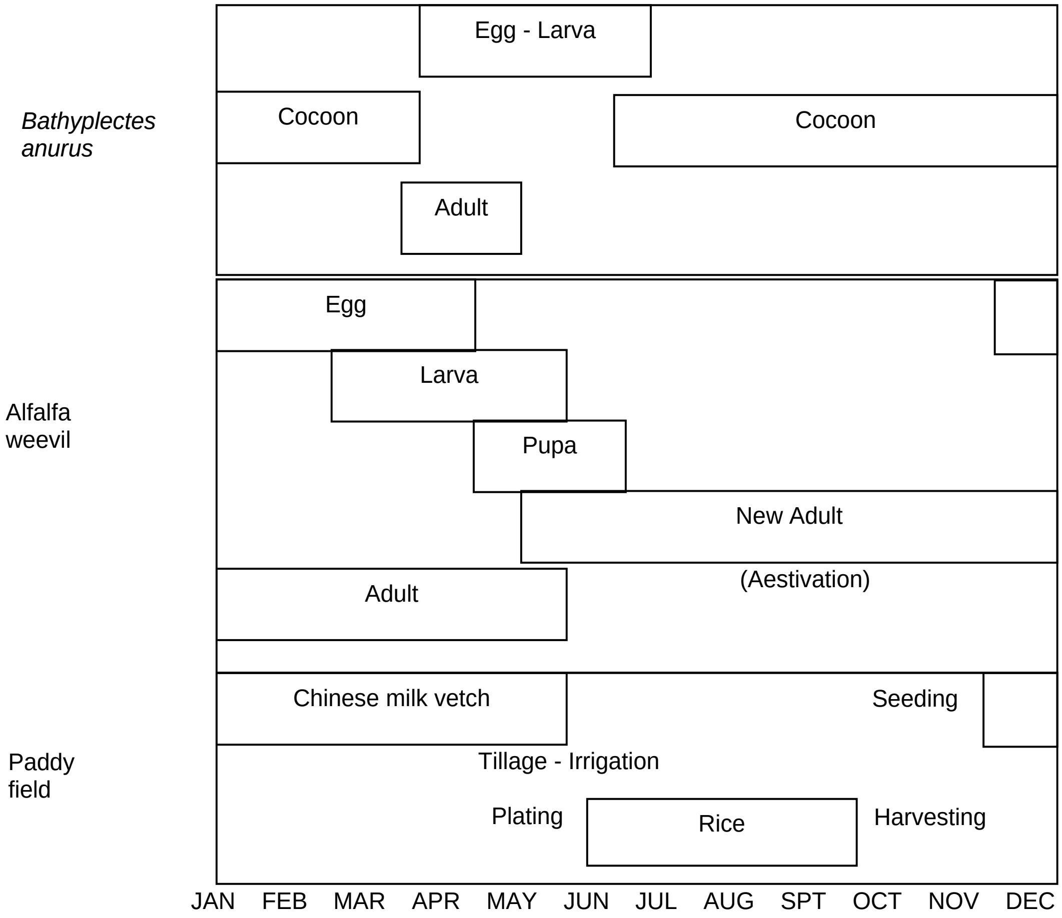

Figure 2. Life cycle of the alfalfa weevil, *Hypera postica* and its parasitoid, *Bathyplectes anurus* in a Japanese paddy field system.

DISCUSSION

Biological control of the alfalfa weevil has been of great economic benefit to U.S.A. alfalfa producers (Radcliffe *et al.* 1998). Shoubu *et al.* (2004) suggested that *B. anurus* was a promising agent for classical biological control of alfalfa weevil in Japan, Thus, a redistribution program for *B. anurus* is highly recommended for management of the alfalfa weevil in Japan. However, Japanese paddy agro-ecosystems are not very favourable for classical biological control because they are completely disrupted once a year during the late spring. Populations of *B. anurus* seem to be better maintained on wild leguminous plants in surrounding grasslands than on Chinese milk vetch in paddy fields. This suggests that conservation of grasslands surrounding paddy fields is very important if the effectiveness of *B. anurus* as a control agent for the alfalfa weevil is to be increased.

ACKNOWLEDGEMENTS

I thank Richard Shaw for help with the manuscript.

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THE INVASION OF THE WESTERN CORN ROOTWORM, *DIABROTICA VERGIFERA VIRGIFERA*, IN EUROPE AND POTENTIAL FOR CLASSICAL BIOLOGICAL CONTROL

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ABSTRACT

The maize-destroying western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) originates from Mexico and is an invasive species in the United States and in Europe. *Diabrotica v. virgifera* was accidentally introduced into Europe near Belgrade in Serbia in 1992. Within 10 years, this invasive alien species spread throughout Central Europe. Recently, several new spots of isolated invasions were reported in Europe showing that *D. v. virgifera* is a very successful invader. Its major success is suggested to result from: (a) suitable pathways for multiple introduction events; (b) the high flight ability of the beetle, which allows a successful initial colonization movement towards maize fields; (c) a high rate of success in pheromone-mediated mate location even at small initial population sizes; and (d) a high potential fecundity. Furthermore, this success of invasion does not seem to be reduced by the high generational mortality of more than 99% or the low realized fecundity, both of which should reduce the probability of establishment and population growth. Conclusively, European maize production is threatened. Classical biological control could be one element of a sustainable management strategy against *D. v. virgifera.* After conducting reviews, surveys and experiments on potential classical biological control agents, the following conclusions were compiled from a detailed step-by step approach: (1) effective indigenous natural enemies are not attacking any life stage of *D. v. virgifera* in Central Europe; (2) in the area of origin surveyed, *Celatoria compressa* Wulp (Diptera: Tachinidae) was the only parasitoid found on the target species, *D. v. virgifera*, and its host range is considered to be restricted to Diabroticite beetles; (3) prior to its potential importation, the parasitoid's basic and reproductive biology has been clarified; and (4) according to the results of host specificity testing, *C. compressa* would be safe for introduction as direct and indirect impacts on other organisms would be extremely low. Therefore a sustainable integrated management approach is likely to incorporate classical biological control with other control measures such as tolerant maize varieties and crop rotation.

INTRODUCTION

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) as well as its host plant *Zea mays* L. (Poaceae) evolved together in the subtropics of Mexico and Central America, and are non-native species in Europe. *Diabrotica v. virgifera* was accidentally introduced into Europe and was first observed near Belgrade in Serbia in 1992 (Kiss *et al.* 2005). Within 10 years, this invasive alien species spread over 310,000 km^2 throughout Central Europe and its eradication became impossible (Kiss *et al.* 2005). Recently, several new spots of isolated invasions were reported, such as in Lombardy (Italy), near Paris (France), near Basel (France and Switzerland), Amsterdam (The Netherlands), and London (UK) showing that *D. v. virgifera* is a very successful invader (Kiss *et al.* 2005), and will significantly change European maize production systems. Pathways of introductions of *D. v. virgifera* have never been formally investigated. The theory of introduction into Europe from North America via airplanes is generally accepted (Kiss *et al.* 2005). Possible causes of the successful invasiveness of *D. v. virgifera* theoretically include: (a) suitable pathways for multiple introduction events; (b) a remarkable flight ability of the beetle, which allows successful initial colonization movements towards maize fields; (c) a low viable population size required to build up a population; (d) a high capability of finding the counter sex for copulation at small initial population sizes by using sex pheromones; (e) a low mortality of developmental stages of *D. v. virgifera* in the invaded areas; and (f) a high fecundity.

Due to the successful invasion of *D. v. virgifera* in Europe, a sustainable management strategy against this invasive alien pest is needed. Classical biological control may have an important application in such a management policy as it provides an opportunity to partially reconstruct the natural enemy complex of an invading alien pest. *Diabrotica v. virgifera* is considered a prime target for a classical biological control approach. In order to investigate the initial colonization process of this invasive alien pest towards maize fields, mark-release-recapture studies were conducted in southern Hungary in 2003 and 2004. To study the potential use of classical biological control, life table studies were used to find host niches of *D. v. virgifera* that are not presently occupied by indigenous natural enemies in the area of invasion. Based on this information, specific and effective natural enemies from the area of origin were selected and investigated for potential introduction into Europe. Over the last five years we evaluated the potential of classical biological control applying a step-by-step approach: (1) investigating the initial colonization process of introduced *D. v. virgifera*; (2) conducting life table studies for *D. v. virgifera* in the area of invasion and studying the minimum viable population size of *D. v. virgifera*; (3) surveying for natural enemies in the area of invasion and origin; and (4) assessing the suitability and host specificity of candidate biological control agents.

MATERIALS AND METHODS

MARK-RELEASE-RECAPTURES DURING THE INITIAL COLONIZATION PROCESS OF INTRODUCED *D. V. VIRGIFERA*

Mark - release - recapture studies were conducted in southern Hungary in order to investigate the movements of *D. v. virgifera* towards suitable habitats, such as to its host plant maize (for details refer to Toepfer *et al.* 2004). In 2003 and 2004, nine mark-release-recapture experiments were carried out in a grass steppe-area and alfalfa field, in which two small maize fields had been planted 300 m distant from the release points. After each release of 5,500 to 6,000 *D. v. virgifera*, adult beetles were recorded three times every second day by non-baited yellow sticky traps placed at regular intervals around the release point. The probability of arrival of female beetles in maize field was estimated to assess the risk of establishment of starter populations.

LIFE-TABLE STUDY TO MEASURE MORTALITY FACTORS AND MINIMUM VIABLE POPULATION SIZE

Life-table studies of this univoltine species were conducted in two maize fields in southern Hungary from 2000 to 2003 in order to provide an ecological understanding of mortality factors regulating population dynamics of this invasive pest (for details refer to Toepfer and Kuhlmann 2005). The mortality affecting the egg stage was assessed by exposing several thousand eggs to pre-overwintering, overwintering and post-overwintering conditions and by recovering the surviving eggs. The mortality levels of larval to adult stages were measured by artificially infesting 286 maize plants and subsequently recovering (1) the three larval instars and pupae at six time intervals using soil-root sampling and (2) the adults using emergence cages. Three age-specific life-tables were constructed. Finally, the reproductive rates of small founding populations were calculated to discern the minimum viable population size.

NATURAL ENEMY SURVEY IN THE AREA OF INVASION AND ORIGIN

A three-year field survey was conducted in Hungary, Yugoslavia, and Croatia, which are currently the focal points of invasion, to determine the occurrence of indigenous natural enemies of *D. virgifera* in Europe. A total of 9,900 eggs, 550 larvae, 70 pupae and 33,000 adults were examined for the occurrence of parasitoids, nematodes, and fungal pathogens. Moreover, the above-described life-table study was used to determine host niches of *D. v. virgifera* that are not presently occupied by indigenous natural enemies. In a next step, the structure and function of natural enemies in the area of origin of *D. v. virgifera* was assessed, with a special emphasis placed on parasitoids of *Diabrotica* adults. Surveys were conducted in collaboration with Dr. Astrid Eben (Instituto de Ecologia, Xalapa, Mexico) and Dr. Rebeca Alvarez Zagoya (Instituto Politecnico Nacional, CIIDR-IPN, Durango, Mexico). Adults of *Diabrotica* spp. were collected in agricultural and natural habitats containing a high species diversity including the target species *D. v. virgifera* in northern Mexico. In collaboration with G. Cabrera Walsh, (USDA-ARS South American Biological Control Laboratory, Buenos Aires, Argentina) *Diabrotica* adult natural enemy surveys were carried out in central and northern Argentina as well as southeastern Brazil. *Diabrotica* adults were collected directly from leaves and flowers of maize, beans, squash or wild plants within the fields. In all surveys, every available species of the sub-tribe Diabroticina, e.g. *Diabrotica spp., Acalymma spp.*, and *Ceratoma spp.*, were collected. *Diabrotica* adults were separated based on species, collection site and collection date and colonies were maintained in cages until emergence of parasitoid larvae. Based on the original host ranges of the parasitoids discovered, as well as their respective parasitism rates and overwintering strategies, the most promising parasitoid species was selected for further studies.

SUITABILITY AND HOST SPECIFICITY OF THE CANDIDATE BIOLOGICAL CONTROL AGENT

In order to study in detail the suitability of the selected candidate biological control agent, Celatoria compressa Wulp (Diptera: Tachinidae), the following attributes were investigated: (a) behavior of host attacks by C. compressa females, (b) the larviposition period, (c) the number of daily larviposition attempts per female, (d) the number of puparia produced daily per female and (e) the cumulative puparia production per female over the entire larviposition period (for details refer to Zhang et al. 2003). The functional response of C. compressa was also studied. A randomly chosen density between one and 50 adults of D. v. virgifera was offered to an individual 8 to 10 day-old mated female for 24 hours. After exposure, the hosts were kept for 20 days and emerged C. compressa larvae were recorded for each host density.

With respect to the safety of biological control, standards and frameworks recently developed for the release of exotic biological control agents were followed (van Lenteren et al. 2003). This study focused first on the selection of potential non-target species at risk in *D. v. virgifera* invaded areas in Europe, and secondly on host specificity testing of *C. compressa*. According to the phylogenetic centrifugal method proposed for weed biological control agents and a practical approach suggested by Kuhlmann and Mason (2003), a simplifying procedure was applied and indigenous Coleopteran species were selected for testing under quarantine laboratory conditions (for details refer to Kuhlmann et al. 2005). Thereafter, the host specificity of the candidate classical biological control agent C. compressa was assessed in (1) no choice tests, (2) sequential no choice tests, (3) choice tests, and (4) sequential choice tests.

RESULTS

INITIAL COLONIZATION PROCESS OF INTRODUCED *D. V. VIRGIFERA*

In five out of 15 recapture periods, released *D. v. virgifera* populations performed a unidirectional movement (38%), in three cases beetles performed a bi-directional movement (20%), and in seven cases no directional movement was found (46%). In ten out of 15 recapture periods, the released populations were moving in a direction that was comparable with the mean wind direction. Averaging over sites and years, *Diabrotica* beetles did not move in the directions of the two small maize fields more frequently than expected if assuming random movement. However, beetles did travel significantly more frequently in the direction of naturally occurring maize fields (within a radius of 1,500 m) than towards other habitats. On average, 2.8% ± 3.2 SD of all recaptured *Diabrotica* beetles arrived in a 300 m distant small maize plot.

MORTALITY FACTORS AND MINIMUM VIABLE POPULATION SIZE

A total mortality of 99.6% ± 0.16 SD was determined during the time period between oviposition and the emergence of adults. Highest losses were generated by mortality during the first instar larval stage (94.2% marginal death rate) and by the inability of adults to realize their potential fecundity (80.4%). The most successful age intervals were pre- and post-diapausing eggs, with marginal death rates of only 17.3% and 18.4%, respectively. The third instar larvae and pupae also had a moderately low marginal death rate of 36%. Factors that varied greatly between years and sites, such as realized fecundity and mortality of second and third instar larvae and overwintering eggs, had the highest potential to change population growth. *Diabrotica v. virgifera* net reproductive rates were generally below 1 (mean R_0 = 0.62) indicating declining pest populations. Therefore, the risk is low that newly introduced females of this alien beetle would produce enough eggs, larvae and emerging adults to establish a new generation. However, when considering the maximum potential progeny of females, the capacity of increase could reach growth factors between 2 and 4.5. Luckily, only 19.6% of this potential fecundity was usually realized under field conditions and the mean realized fecundity of starter populations varied substantially between years and between individual females.

NATURAL ENEMY SURVEY IN THE AREA OF INVASION AND ORIGIN

The natural enemy survey as well as the life-table study revealed that effective indigenous natural enemies were generally not attacking any of the life stages of *D. v. virgifera* in Europe. Two exceptions were the fungi *Beauveria bassiana* (Bals.) Vuill. (Mitosporic fungi; formerly Deuteromyces) and *Metarhizium anisopliae* (Metsch.) Sorok (Mitosporic fungi) attacking adults of *D. v. virgifera* on an extremely low level (< 1%). However no other entomopathogenic fungi, entomopathogenic nematodes, or parasitoids were found on eggs, larvae, pupae or adults. Therefore, each life stage of *D. v. virgifera* would be a suitable target for a classical biological control agent, and the natural enemy complex of the invading alien *D. v. virgifera* may be reconstructed in Europe.

Based on survey results and literature records in the area of origin of *Diabrotica spp.* (Cabrera Walsh *et al.* 2003; Eben and Barbercheck 1996; Guimaraes 1977; Heineck-Leonel and Salles 1997), adult parasitoids are probably the most common natural enemies of species in the *virgifera* group of the genus *Diabrotica*, followed by mermithid nematodes of the genus *Hexamermis* (Eben and Barbercheck 1996; Kuhlmann and Burgt 1998). As the focus of this survey was to obtain parasitoids of adult *Diabrotica*, knowledge of natural enemies attacking the soil dwelling larval stages of *Diabrotica* beetles is still incomplete. In the classical biological control agent selection process, five of the six known parasitoid species of adult *Diabrotica* in the area of origin were excluded from consideration based on information gleaned from the literature. The North and Central American tachinid fly, *Celatoria setosa* was not selected as Fisher (1983) indicated that this fly is almost exclusively a parasitoid of *Acalymma* species. Similarly, the North American tachinid fly, *Celatoria diabroticae* Gahan was also not chosen because it appears to be strongly associated with only *Diabrotica undecimpunctata howardi* Barber (Summers and Stafford, 1953), and *D. undecimpunctata undecimpunctata* Mannerheim (Fischer, 1981). The North American braconid *Centistes diabroticae* was not considered as it has been only reared from *Acalymma vittata* (F.) (Gahan, 1922; Fischer, 1981). Experimental data indicated that the South American *Celatoria bosqi* will not accept *D. v. virgifera* adults as hosts, which suggested that this tachinid appears to be specific to the *fucata* group within the genus *Diabrotica*. The South American braconid *Centistes gasseni* Shaw was rejected as a potential biological control agent due to the parasitoid's incompatible overwintering strategy and rearing difficulties. The only parasitoid actually found on the target species, *D. v. virgifera,* was the tachinid *Celatoria compressa* from northern Mexico. It attacked a range of species in four different genera of Diabroticite beetles suggesting a large number of host species, but nonetheless restricted to Diabroticite beetles. *Celatoria compressa* was ultimately the only parasitoid that was selected as a candidate biological control agent for *D. v. virgifera* in Europe based on its availability in northern Mexico, its known host range including the target host record, and its suitability for rearing under laboratory conditions. Generally, it should be noted that distribution and the efficacy known for *C. compressa* should be considered as provisional due to the fact that these tachinid species have been little studied.

SUITABILITY AND HOST SPECIFICITY OF THE CANDIDATE BIOLOGICAL CONTROL AGENT

The age of *C. compressa* adults was found to be the most crucial factor in achieving mating. Only newly emerged, one hour-old females mated successfully with 2 to 5 day-old males. During the pre-larviposition period, the egg load of females increased steadily from day one (mean = 16.6 ± 1.1 SE) to a maximum egg load on day four (mean = 69.3 ± 0.8 SE). During a female's larviposition period (mean = 22.5 ± 0.6 SE days; n = 19), a total of 33.2 ± 0.9 SE first instars were larviposited into hosts. This represents only half of the female's egg load. The cumulative number of larviposition attempts per female reached a mean of 120 ± 2.2 SE, whereas the mean cumulative puparia production per female was only 29.7 ± 5 SE. The number of hosts parasitised by *C. compressa* increased with increasing host density until an upper limit was reached. This functional response of *C. compressa* fit the Holling type II response ($R^2 = 0.239$; $F = 96.40$; df = 2, 80; $P < 0.001$).

Nine European non-target Coleopteran species potentially at risk of being attacked by *C. compressa* were selected as representative species for testing in the quarantine laboratory: two-spotted lady beetle, *Adalia bipunctata* L. (Coleoptera: Coccinellidae); red pumpkin beetle, *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae: Galerucinae: Luperini); thistle tortoise beetle, *Cassida rubiginosa* Müller (Coleoptera: Chrysomelidae: Cassidinae); golden loosestrife beetle, *Galerucella pusilla* Duft (Coleoptera: Chrysomelidae: Galerucinae: Galerucini); green dock beetle, *Gastrophysa viridula* Deg. (Coleoptera: Chrysomelidae: Chrysomelinae); *Gonioctena fornicata* Brüggemann (Coleoptera: Chrysomelidae: Chrysomelinae); cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae: Criocerinae); elm leaf beetle, *Pyrrhalta luteola* (Müller) (Coleoptera: Chrysomelidae: Galerucinae: Galerucini); and pea and bean weevil, *Sitona lineatus* Linnaeus (Coleoptera: Curculionidae).

In no-choice or choice tests, naïve females of C. *compressa* never parasitised eight of nine non-target species tested. In the absence of *D. v. virgifera* adults, *A. foveicollis* was occasionally accepted (6 larvae in 260 hosts), but complete development by *C. compressa* was not achieved. The acceptance of *A. foveicollis* by *C. compressa* was significantly lower than that of the target host, *D. v. virgifera*, 2.3% versus 28.7%. In the sequence of no-choice tests, *A. foveicollis* was accepted (4 larvae in 260 hosts) but it was again significantly lower than that of the target species compared within the same day of three successive days. From the four *A. foveicollis* adults parasitised, a single *C. compressa* larva completed its development and formed a puparia. In the presence of *D. v. virgifera* in the choice test, *A. foveicollis* was never accepted by *C. compressa* but during the sequence of choice tests *A. foveicollis* was again accepted by

C. compressa in a few cases. However, host acceptance was significantly lower than that observed for *D. v. virgifera* on the first, second and third day. In contrast to the results of the sequence of no-choice tests, host suitability for *A. foveicollis* by *C. compressa* was not found.

It can be predicted that the candidate biological control agent *C. compressa* will have a narrow host range in Europe, being restricted to a few genera on the tribe level of Luperini among the subfamily Galerucinae. These results of the physiological host range of *C. compressa* obtained under quarantine conditions are in agreement with the known field host range from the area of origin in Mexico.

DISCUSSION AND CONCLUSIONS

Accidentally introduced specimens of the invasive alien maize pest, *D. v. virgifera*, must initially migrate to their target habitat, maize, in order to feed, reproduce, establish and then to invade other areas. Mark-release-recapture experiments revealed that small introduced *Diabrotica* beetle populations mainly show non-directional dispersal. However, beetles were moving significantly more frequently towards commercial scale maize fields within a radius of up to 1,500 m than in the direction of other habitats. Adult populations of *D. v. virgifera* were spreading over more than 100 hectares of non-maize areas, and 2.8% of all recaptured beetles arrived in maize plots at a distance of 300 m from the release point. This probability of arrival would increase linearly with the number of maize fields. Next to this number of maize fields, the number of introduced specimens is essential to estimate risk of arrival of *D. v. virgifera* in their target habitat. Literature references suggest that in most cases hardly more than 10 specimens are introduced in a single event of introduction (Wittenberg and Cock 2001). However, in a hypothetical case where 10 maize fields are in close proximity to a point of alien introduction, already one to two females out of the ten *Diabrotica* beetles would reach a maize field, leading to a risk of establishment of a starter population of the introduced species. Fortunately, there would be a less than 1 % chance that those newly arriving female beetles would produce enough progeny to ensure the successful emergence of a new adult generation. The rate of increase from a starter population to the following generation was often found to be less than factor one, which indicates declining populations and would result in the extinction of introduced founder populations. However, when considering the maximum potential progeny of females, the capacity of increase could reach growth factors between 2 and 4.5. Luckily, only 19.6 % of this potential fecundity was usually realized under field conditions and the mean realized fecundity of starter populations varied considerably between years and between individual females. Due to this variability it remains difficult to predict the probability and quarantine risk for establishment of newly introduced starter populations. Still, the high percentage of non-directional flight by adults, the moderate probability of accidentally introduced specimens to arrive in maize, the low realized fecundity of females as well as the extremely low survival probability until the subsequent generation very much decrease the probability of a successful invasion of this alien *D. v. virgifera*. Reasons for the ongoing invasions in Europe must be explained by either optimal oviposition conditions or by an increased frequency of introduction events.

With regard to the use of classical biological control as a sustainable management strategy against *D. v. virgifera* the following conclusions are compiled:

1. Effective indigenous natural enemies are not attacking any life stage of *D. v. virgifera* in Central Europe;
2. In the area of origin surveyed, *Celatoria compressa* was the only parasitoid found on the target species;
3. Prior to its potential importation, the parasitoid's basic and reproductive biology was clarified;
4. According to host specificity testing, *Celatoria compressa* would be safe for introduction as direct and indirect impacts on other organisms would be extremely low (host range is considered to be restricted to Diabroticite beetles).

Regarding the tachinid *C. compressa*, we have studied a promising candidate classical biological control agent that would be safe for introduction against *D. v. virgifera* in Europe. Nonetheless, there are a number of questions that remain to be answered before its potential importation, such as the hibernation strategy of *C. compressa*, its cold tolerance and its impact under more natural conditions than in bioassays in quarantine laboratories. In conclusion, a sustainable integrated approach against *D. v. virgifera* in Europe is likely to incorporate classical biological control with other pest management options, such as tolerant maize varieties, crop rotation, and cultural techniques which have the potential to enhance the conservation of natural control.

ACKNOWLEDGEMENTS

This work was possible due to the hospitality offered by the Plant Health Service in Hodmezovasarhely in Hungary. We would like to thank for the kind collaboration Ibolya Hatala Zseller and team, Plant Health Service, Hodmezovasarhely, Hungary; as well as Michael Hatala of the Hodmezogazda RT Agricultural Company. We are grateful to Dr Astrid Eben (Instituto de Ecologia, Xalapa, Mexico), Rebeca Alvarez Zagoya (Instituto Politecnico Nacional, CIIDR-IPN, Durango, Mexico) and Guillermo Cabrera Walsh (USDA South American Laboratory, Buenos Aires, Argentina) for the collection of parasitoids. We gratefully acknowledge the continuous support with eggs of *D. v. virgifera* by Chad Nielson and Michael Ellsbury (USDA-ARS, Northern Grain Insect Research Laboratory at Brookings, South Dakota, U.S.A.). We appreciated very much the technical assistance of Emma Hunt, Christine Gueldenzoph, Tara Gariepy, Rike Stelkens, Kim Riley and Leyla Valdivia Buitriago during the experiments and the parasitoid rearing. In Hungary, technical support by Lars Reimer, Edit Kiss, Szabolcs Meszaros, Erzsebet Kovacs, Nora Levay and Marianna Szucs was greatly appreciated. We also thank Wade Jenner, Ottawa, Canada, for reviewing the English text. This study was funded by the Bundesamt für Bildung und Wissenschaft, Bern, Switzerland, within the EU project (QLK-5CT-1999-01110) as well as by the EU M. Curie Fellowship program (QLK5-CT-2002-51515).

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IDENTIFYING THE DONOR REGION WITHIN THE HOME RANGE OF AN INVASIVE SPECIES: IMPLICATIONS FOR CLASSICAL BIOLOGICAL CONTROL OF ARTHROPOD PESTS

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ABSTRACT

Foreign exploration is a major component of a classical biological control program. Exploration within the home range of the target can be a daunting proposition as the target is most likely rare with an irregular distribution. As costs and greater regulatory oversight increase, classical biological control programs targeting arthropods need to complete preliminary steps prior to embarking on a collecting trip that will return live natural enemies to quarantine for rearing and evaluation. These preliminary steps should be designed to maximize the likelihood of identifying the donor region within the pest's home range from which the invading population originated. Two techniques can be used to help identify the target's donor region(s): climate matching and haplotype identification. These techniques increase the likelihood of collecting natural enemies well suited to the climate of the receiving area and will result in natural enemies best adapted to the genetic structure of the invasive target population. Further, haplotyping studies may help clarify taxonomic uncertainty regarding the "true" identity of the target pest and may also help uncover conduits by which these unwanted organisms are moving from area to area. This paper details work completed on determining the home range of the avocado thrips, *Scirtothrips perseae*, and delineating the donor region within the prescribed home range. The implications of this approach for increasing the success rates of classical biological control of arthropods are discussed.

INTRODUCTION

Globally, exotic species pose a perennial invasion threat, and successful invaders can be extremely damaging to agricultural, urban, and natural areas should they establish outside of their home range. Exotic pioneers are infiltrating previously unexploited ecosystems at unprecedented rates as the human population grows, as goods are transported ever more rapidly over an increasingly larger global scale, and then passed through border inspection stations with cursory examination before release tens of thousands of kilometers from the point of origin. Economic losses and associated management costs for exotic arthropod pests in the U.S.A. have been estimated to cost in excess of $20 billion annually (Pimentel *et al*. 2002), and over $2 billion for New Zealand (Barlow and Goldson 2002).

Clearly, invasive arthropod pests have not only major economic impacts but they can cause devastating effects on ecosystem functioning once they become an established unit within the accommodating system. Classical biological control, that is, the deliberate importation and release of exotic natural enemies for control of an exotic pest in a newly invaded range, can be an extremely powerful tool for suppressing pests in agricultural, urban, and natural environments (Hoddle and Syrett 2002). Many problematic arthropods of exotic origin may be good potential targets for classical biological control. In some instances, exotic natural enemies may be the only rational control option once eradication, containment, and long-term chemical suppression are deemed ineffective or unsustainable by regulatory agencies (Hoddle 2004a). Despite over 100 yrs research and application of classical biological control by arthropods against arthropods, the success rate per decade has been consistent at around 10-12% (Gurr *et al.* 2000). The success rate for classical biological control of arthropods is substantially lower than that observed for terrestrial weeds where control has been estimated at around 30% (Syrett *et al.* 2000).

Classical biological control has come under increasing scrutiny by ecologists and conservationists because natural enemies used for exotic pest suppression may pose serious and irreversible threats to non-target organisms in ecosystems in which they are released or infiltrate post-release (Louda and Stiling 2004). Despite the widely recognized economic, environmental, and social benefits that accrue from successful biological control programs it is recognized that more needs to be done to ensure greater efficacy and safety to mitigate adverse non-target effects (Hoddle 2004b). Concerns over the specificity of natural enemies used in classical biological control programs against arthropods is leading to the development and implementation of legislative guidelines that are designed to promote assessment of the safety of potential candidate biological control agents prior to release (see papers in session 13). This had the effect of promoting greater research activity on techniques that can be used to predict (see papers in session 13) and assess environmental risk (see papers in session 12) arising from the introduction of novel upper trophic level organisms into areas that have historically lacked such organisms.

In an environment of increasing regulation and given the well justified need to address concerns over natural enemy safety, it is likely that project costs will significantly increase and the numbers and types of classical biological control projects that are launched may decrease and be removed from the research agenda of public Universities to become a government enterprise (Van Driesche and Hoddle 1997). Consequently, it is becoming increasingly necessary to take as many preliminary steps as possible to gather pertinent data to maximize the likelihood of finding suitable biological control agents when initiating a classical biological control project prior to launching an expensive and time consuming foreign exploration program that will locate natural enemies that must be later subjected to safety testing. Preliminary steps must aim to increase the chances of locating host specific natural enemies in the pest's home range that are likely to have maximal impact on target population growth after being imported, cleared through quarantine, and established in the invaded range. One preliminary approach that may increase the likelihood of locating the most efficacious natural enemies is to search within specific areas of the pest's home range for agents that are most closely associated with pest populations that have the most similar biological, ecological, and genetic profile as the invading population that needs to be controlled. This article focuses on using a climatic modeling and haplotyping approach to locating natural enemies for the biological control of avocado thrips, *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae).

DESIGNING A FOREIGN EXPLORATION PROGRAM FOR AVOCADO THRIPS

BIOLOGY AND IMPACT OF THE INVADER

Avocado thrips (Fig. 1) was first discovered on avocados in June 1996 near Port Hueneme (Ventura County) and in Irvine (Orange County) California U.S.A. This pest currently infests 95% of California's avocado acreage and approximately 80% of commercial orchards require pesticide applications to control this pest (Hoddle *et al.* 2002). Economic losses are incurred when avocado fruit is scarred (Fig. 2) by feeding thrips adults and larvae and this pest has been estimated to cost the California avocado growers $4-5 million each year now reliable management practices have been implemented (Hoddle *et al.* 2003). At time of discovery in California, avocado thrips was a species new to science (Nakahara 1997), and nothing was known about its area of origin, biology, or ecology (Hoddle *et al.* 2002). This thrips is unusual in the genus *Scirtothrips*. It is the only species known to outbreak and cause economic damage in cool weather, all other pest species of *Scirtothrips* outbreak and cause damage when temperatures are high. Cool weather in late winter and spring in California synchronizes pest population growth with leaf flush and immature fruit growth which are initiated during spring each year (Hoddle 2002a). These two substrates are most favored by *S. perseae* for feeding and oviposition (Hoddle 2002b).

In California, extensive host plant surveys indicate that *S. perseae* may be monophagous as it has only been recorded breeding on avocados (Hoddle *et al*. 2002c). When taken together, pest ecology and host plant preferences, strongly suggest that *S. perseae* has a close evolutionary history with avocados and may have originated somewhere in the home range of this plant. Three distinguishable ecological races or subspecies of avocado (*Persea americana*) are recognized; these being (1) Mexican (*P. americana* var. *drymifolia*), (2) Guatemalan (*P. americana* var. *guatemalensis*) and (3) West Indian or Caribbean (*P. americana* var. *americana*)

Figure 1. Avocado thrips, *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae). Photo: UC Regents. UGA1390031

Figure 2. Avocado fruit showing scarring that has resulted from avocado thrips feeding damage when these fruit were < 2 cm in length. Photo M. S. Hoddle. UGA1390032

types. The areas of origin for these avocado races indicated where foreign exploration for avocado thrips should commence (Hoddle *et al.*, 2002c).

FOREIGN EXPLORATION STEP I: DELINEATING THE HOME RANGE OF THE PEST

Over the period 1997-2000 avocados (wild and cultivated) in Mexico, Central America, Brazil, and the Caribbean were intensively searched for avocado thrips and associated natural enemies. Collecting was repeated in Mexico and Central America in 2003 and 2004. Collection records enabled the preparation of a distribution map for *S. perseae* in Mexico and Central America (Fig. 3).

Scirtothrips perseae has a highly restricted range (Fig. 3) and is only found at elevations exceeding 1500 m. This high altitude range explains the predilection of this pest for cool growing conditions and suggests that it was pre-adapted to cool growing conditions in coastal avocado orchards in California (Hoddle *et al*. 2002c). Survey work in California indicates that this pest is most severe within 20 kms of the coast because of cooler conditions resulting from the marine influence, and hotter conditions typical of more interior and arid areas do not experience problems with this insect (Hoddle 2003).

FOREIGN EXPLORATION STEP II: DELINEATING THE DONOR REGION WITHIN THE HOME RANGE

Avocado thrips occupies a vast range within Mexico that extends into the mountainous areas of central Guatemala. Somewhere within this range it is likely that an invading population of *S. perseae* originated and entered California. More precisely defining this donor region within the home range may provide natural enemies that are closely adapted to the ecological and climatic requirements of the pest, and they may also be better suited to exploiting the invasive population if it is comprised of individuals with a genetic constitution that is most similar to those of the donor region. A CLIMEX model (Baker 2002) was parameterized with laboratory derived biological data for S. perseae (Hoddle 2002a) and used to model the distribution of *S. perseae* in the home and invaded range. CLIMEX returns an Ecoclimatic Index (EI) value that indicates the climatic suitability of particular area for a species. The larger the EI (as represented by a blue dot on the maps in Fig. 4) the better the climatic conditions for the organism of interest (Baker 2002).

Thrips specimens and associated natural enemies collected during foreign exploration were preserved in 95% ethanol and a subset of collected material was subjected to DNA analysis. The population genetics of *S. perseae* were examined using mitochondrial DNA (mtDNA) and microsatellite markers. The mtDNA sequences revealed three geographically distinct and divergent lineages, of which the mtDNA haplotypes of Californian individuals were most closely related to populations in the centre of the pest's native range. Analysis of allele frequencies at four microsatellite loci indicated Coatepec-Harinas, Mexico, as the most likely source of the Californian population. Statistically, we did not detect any bottleneck in population size associated with the invasion of California. However, estimates of the effective population size of the invading population suggest that a severe bottleneck occurred indicating that the quantity of host plant material entering California was small. Our findings implicate Coatepec-Harinas, a large avocado germplasm and breeding centre, as the most

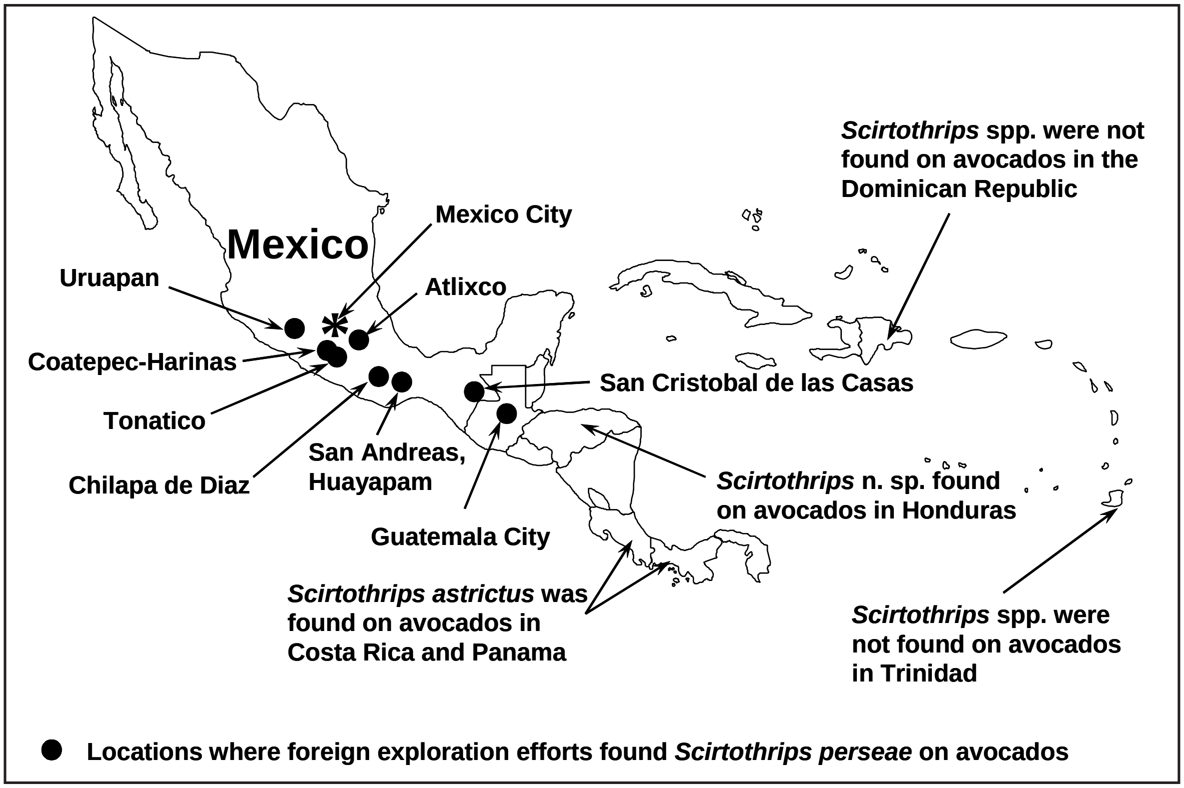

Figure 3. The distribution of *Scirtothrips perseae* in Mexico and Central America.

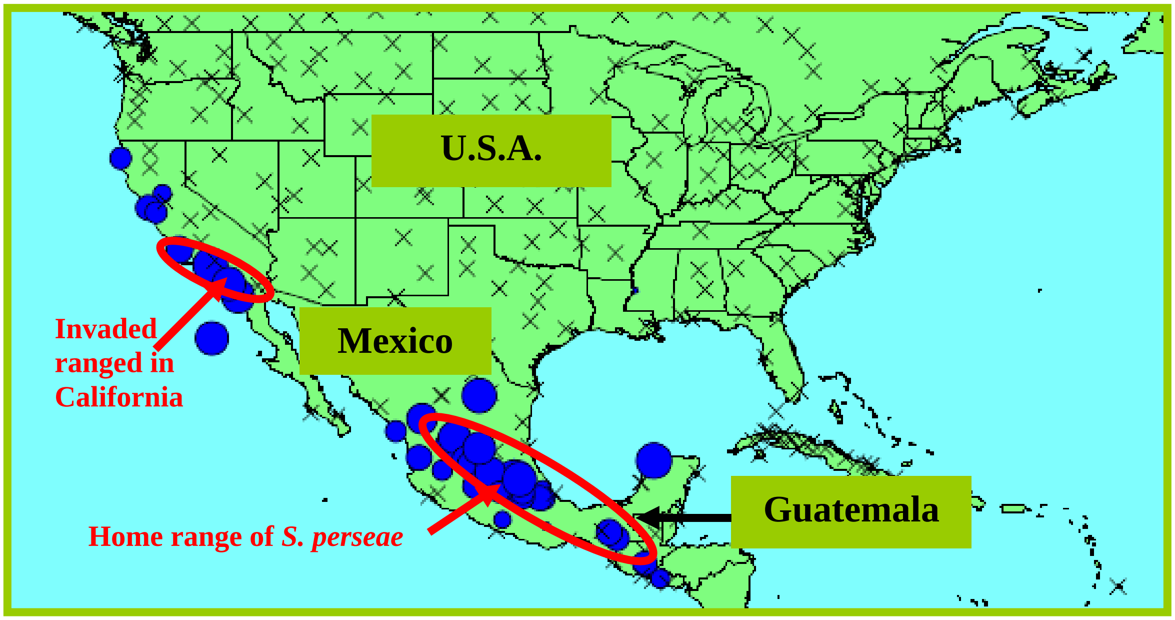

Figure 4. CLIMEX predictions for the distribution for the distribution of *Scirtothrips perseae* in Mexico and Guatemala, and the invaded range in California. Crosses indicate areas that are climatically unsuitable for *S. perseae*. California is the only state in the continental U.S. that has a climate suitable for *S. perseae* populations.

likely source of the introduced Californian population of *S. perseae*, and that just one invasion event into California occurred (Rugman-Jones *et al.* 2005).

FOREIGN EXPLORATION STEP III: COLLECTING NATURAL ENEMIES FOR IMPORTATION AND EVALUATION

Our genetic analyses strongly suggest that the *S. perseae* population that colonized California originated from an area around Coatepec-Harinas in Mexico implying that natural enemies well adapted to the California haplotype of *S. perseae* should be found in this region. Further, CLIMEX indicates a very good climatic match for this area of Mexico with infested avocado growing regions in California. Future foreign exploration for host-specific natural enemies of *S. perseae* for importation into quarantine for evaluation for release in California should be focused around Coatepec-Harinas as it will likely yield natural enemies adapted to both the climate in California and the pest haplotype.

DISCUSSION

Foreign exploration is a critical element of classical biological control and it is a well accepted fact that projects have either failed or been severely impeded by importing and releasing natural enemies that are either poorly adapted to the climate in the receiving area (Van Driesche and Hoddle 2000) or fail to perform adequately on the pest biotype against which they are released (Hufbauer 2002). Given the high level of cost associated with prospecting for natural enemies overseas, the difficulty in extracting and translocating material from one country to another, and the time consuming and anxiety ridden chore of establishing viable colonies in quarantine, as much preliminary work as possible should be conducted to ensure that the best adapted natural enemies for use against the target are found and imported. Further, as momentum steadily gains towards mandatory host-specificity testing, resources can not afford to be wasted on evaluating natural enemies that may not be the most efficacious when released in the field. Setbacks of this nature should they occur may delay programs by several years as momentum is again built to commence overseas prospecting, or the program may be shut down either due to a lack of funds or based on the possibly incorrect assumption that no effective natural enemies exist for use against the target.

When compared to the "success rate" for biological control of terrestrial weeds, successful suppression of arthropod pests with natural enemies appears to be approximately 3x lower (~10% vs. ~30%) (Gurr *et al.* 2000; Syrett *et al.* 2000). One possible reason for this difference in measured success could come from the amount of preliminary prospecting in the targets home range and follow up screening and testing in quarantine before release. Typically, natural enemies used for weed control are subjected to much higher levels of scrutiny than arthropod natural enemies before release. The time from inception to release of weed natural enemies is around three scientist years at an average cost of approximately $0.5 million per agent (McFadyen 1998). In some instances, it may take up to 20 scientist years for a successful weed biological control program to be realized (Pemberton 2002). This level of effort in selecting natural enemies for release may in part explain why weed biological control programs have more successful than those for arthropods – weed programs by legislative necessity has resulted in careful selection of targets and thorough preliminary work on identifying the important key players within the weed's home range. In fact, climate matching and genetic analyses of target populations in the invaded range are becoming routine for weed programs to facilitate the collection of agents from the same strain within the historic home range (McFadyen 1998).

Similar economic data for costs of arthropod biological control programs for comparison to weed programs are difficult to locate. However, I think it is accurate to state that historically less attention and money has been devoted to screening and evaluating arthropod natural enemies when compared to weed natural enemies. As a consequence of increased testing of natural enemies in Australia, the cost per agent has increased by 80% and the number of projects has decreased by around 30% (Van Driesche and Hoddle 1997). Greater scrutiny of arthropod natural enemies under an evolving framework of host specificity testing theory and protocols may ultimately increase not only the safety of programs, but also the efficacy because targets will need to be selected more carefully to ensure likelihood of success, and greater attention will be given to identifying promising candidates because of an economic need to eliminate at an early stage those agents with little control potential.

As illustrated here with the *S. perseae* biological control project, preliminary steps in this foreign exploration project clearly delineated the target's home range and identified the donor region within the home range of the pest. It is suggested that this type of groundwork is required to maximize success of an arthropod biological control program before expensive foreign exploration is undertaken with the intention of importing life material for evaluation in quarantine. Matching the climate of the invaded range to areas within the pest's home range is standard practice. Even greater resolution of the donor region within the home range can be achieved with genetic analyses. Haplotyping of target arthropod populations in the invaded and home range for a classical biological control program may become a standardized first step in a new project, akin to another basic first step such as climate matching. This molecular assessment as requisite preliminary step in a biological control program will not only aid in selection of natural enemies most adapted to the genetic strain(s) in the invaded range but would also identify taxonomic problems, possible cryptic species complexes, as well as indicating possible conduits by which invaders are moving. Molecular tools are promising to provide quickly and cheaply resolution to questions concerning species identity and strains or biotypes of invasive species targeted for classical biological control.

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IMPACT OF INTRASPECIFIC AND INTRAGUILD PREDATION ON PREDATOR INVASION AND COEXISTENCE: CAN EXOTIC LADYBEETLES DISPLACE NATIVE SPECIES?

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ABSTRACT

Exotic predators are more likely to replace related native species when these species not only compete for similar prey species, but also predate on the offspring of the native predators. In several groups of arthropods, however, this intraguild predation (IGP) is not only mutual, but also co-occurs with intraspecific predation (ISP or cannibalism). These different processes may have counteracting effects on species invasion and coexistence. In this study, we derived simple rules that describe under which combinations of IGP and ISP a predator species is able to invade into a stable predator-prey system, and under which conditions an invasion will results in displacement or in coexistence. This theory is then applied to species pairs of exotic and native lady beetles, to test if differences in IGP and ISP may play a role in the establishment of introduced exotic ladybeetles species (Coleoptera: Coccinellidae) such as *Harmonia axyridis* in Europe and *Coccinella septempunctata* in North America. For an accurate estimation of the key processes we cannot rely on specific experimental data only, but take allometric relationships into account as well. For ladybeetles, IGP and ISP seem to be determined largely by size differences of the interacting larvae, thereby giving an overall advantage to the larger species. On the other hand, larger species generally have higher food requirements, which may give them a disadvantage in resource competition. The estimated levels of IGP, ISP and competitive ability of the interacting species can not fully explain the invasion by the two exotic ladybeetles species.

INTRODUCTION

In recent years the invasive nature of two ladybeetles (Coleoptera: Coccinellidae) has drawn considerable attention in the scientific literature. The originally Eurasian *Coccinella septempunctata* L. established and spread through the whole of North-America in the 70s and 80s (Alyokhin and Sewell 2004; Elliott *et al.* 1996). Later, in the mid 90s, the originally Asian *Harmonia axyridis* (Pallas) became established in various parts of North-America and more recently in some parts of Western Europe as well (Adriaens *et al.* 2003).

Simultaneous with their establishment in new habitats a population decline of native species was observed. The establishment of *C. septempunctata* in arable fields in North America was followed by a dramatic decline of several native ladybeetles (including *Adalia bipunctata* L.) in these fields (Alyokhin and Sewell 2004; Elliott *et al.* 1996; Evans 2004; Wheeler and Hoebeke 1995). *C. septempunctata* also became the dominant ladybeetle species in apple orchards, pushing *A. bipunctata* to a second position (Brown 2003; Brown and Miller 1998). The later establishment of *H. axyridis* in orchards resulted in a local decline of especially this other exotic *C. septempunctata* (Brown 2003), but this pattern is not yet apparent in arable fields (Nault and Kennedy 2003). In none of the cases the exotic species has resulted in the exclusion of native species.

Resource competition for aphid prey is a possible explanation for the decline in native species following the establishment of the exotic one (Evans 2004). However, no evidence is yet provided that the exotic species are better resource competitors than the native ones.

Intraguild predation (IGP) between the exotic and native ladybeetles is regarded as the most likely reason for the spread of the exotic species and the subsequent reduction of native or earlier-established species (Yasuda and Ohnuma 1999). Lab studies indeed show that the IGP by the exotic species on native species is generally bigger than the reverse predation (Snyder *et al.* 2004; Yasuda *et al.* 2004), and that IGP between the two exotic species is in favour of *H. axyridis* (Yasuda and Ohnuma 1999).

A complicating factor is that these predators not only feed on the juveniles of other predator species, but also on those from their own species. This cannibalism or Intraspecific Predation (ISP) may partly reduce the effect of IGP on population dominance.

In this study we therefore start with reviewing the theory on the combined impact of IGP and ISP on population dynamics. Then we show how the strength of the different inter- and intraspecific interaction may be calculated, and use these values to derive predictions on invasibility and species coexistence. In the second part we include resource competition in our theory. How will the various coccinelids differ in competitive ability, and how will this alter our conclusions. Finally, we will discuss the realism of our simplifying assumptions, indicate how spatial and temporal avoidance, resource partitioning and metapopulation dynamics may affect our conclusions.

IMPACT OF IGP AND ISP ON INVASION AND COEXISTENCE

THE MODEL

In order to derive simple rules on how IGP and ISP affect the chance that an exotic predator can invade a habitat and eventually replace native guild members, we consider pairs of predators that feed on the same prey and that are equal in their ability for resource competition, i.e. have equal functional and numerical responses. The occurrence of IGP and ISP is very stage-dependent, with the smaller juveniles falling victim by older and bigger stages, which requires a stage-structured predator-prey model. The simplest version has two stages only, with only the juvenile stage vulnerable for predation, with both stages feeding, and the adult stage reproducing. The strength of IGP and ISP (c_{xx}) is expressed as the attack rate on intraguild and intraspecific juveniles relative to that on the basic (aphid) prey (see Fig. 1).

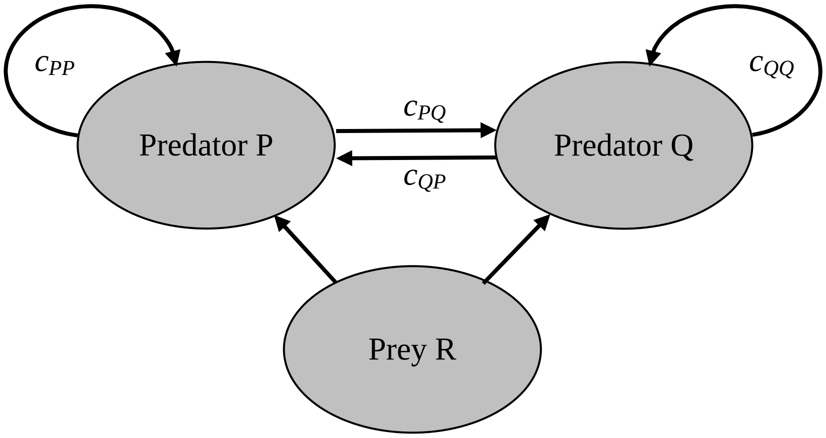

Figure 1. Food web diagram of system studied in this paper. Arrows indicate flow of biomass. Symbols show the parameters used to indicate the strength of intraspecific and intraguild predation (attack rate) relative to predation on the basic prey (resource) *R*.

In the presence of basic prey the feeding on other predators is reduced, and the reverse, according a two-prey version of Holling's disk equation (Hassell 1978). The conversion of intraguild and cannibalistic prey into predator offspring is important as well, and expressed relative to that of the basic (aphid) prey (ϕ_x).

Invasibility is studied under the assumption that a system of one predator and its prey is in equilibrium, and the question is answered: can the other predator, when still at low numbers, invade this system? When predator Q can invade a system with predator P, and predator P can invade a system with predator Q, the two predators can coexist on the same prey. By this approach the parameter conditions that allow for invasion or coexistence can be derived analytically (see van Rijn and Thomas 2005), and are shown in Fig. 2. Figure 2 shows that in absence of cannibalism no coexistence is possible. At increasing levels of cannibalism, especially when performed by both species, a wider range of IGP values leads to coexistence.

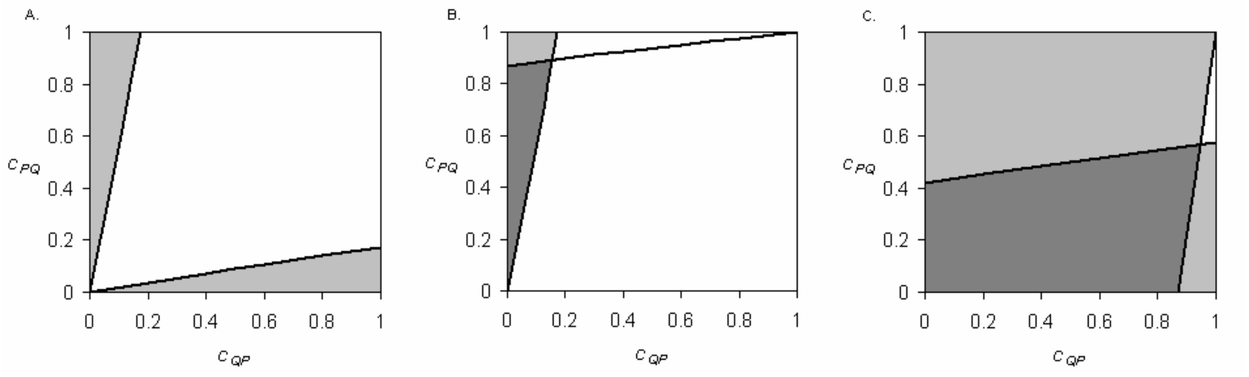

Figure 2. Mutual invasibility plots in IGP space (c_{PQ} indicates strength of IGP by species P on species Q) for different levels of ISP. A. No ISP (c_{PP}=0, c_{QQ}=0). B. ISP in P only (c_{PP}=1, c_{QQ}=0). C. ISP in both P and Q (c_{PP}=0.5, c_{QQ}=1). Light grey areas indicate IGP parameter combinations at which one species can invade the other, and dark grey areas indicate parameter combinations at which both species can mutually invade the other (allowing coexistence of P and Q).

ESTIMATING ISP AND IGP IN COCCINELLIDS

In this study we choose to focus on three species: *H. axyridis* (exotic), *C. septempunctata* (exotic in America, native in Europe), and *A. bipunctata* (native, common in America and Europe).

Experimental studies on IGP and ISP have focussed, for practical reasons, on interactions between one or two stage combinations, such as L3 - L4 (Snyder *et al.* 2004) or L2 - L2 and L2 - L4 (Yasuda *et al.* 2004). For a proper estimation of the interaction strength between the two populations all possible stage combinations should be considered. To fill this omission we utilize the commonly observed pattern that, apart for species specific effects, size difference between the two combatants strongly determine the outcome. A logistic function is fitted to the observations and used to extrapolate to unstudied interactions on the basis of the size ratio of the interaction larvae. Feeding on eggs and pupae is not considered size dependent. Conspecific eggs are strongly preferred (by all stages) over heterospecific eggs, party as a result of toxins (Agarwala and Dixon 1992). Feeding on pupae is assumed to be negligible (Schellhorn and Andow 1999). The overall intraguild or intraspecific predation rate is calculated as the mean of all the stage combinations weighed by their relative contributions to a stable population, i.e. weighed by the stage durations (including the non feeding stages, such eggs and pupae). The results of these estimations are, relative to the predation rate on aphid prey, presented in Table 1. *C. septempunctata* clearly show lower levels of cannibalism than the other species, but take intermediate positions regarding IGP (Kajita *et al.* 2000; Yasuda *et al.* 2001).

The overall conversion efficiency of cannibalistic and intraguild prey relative to aphid prey (f_x) is expected to be somewhat higher for *H. axyridis* than for the other species (Michaud and Grant 2003; Yasuda and Ohnuma 1999), but is not varied between species, and is assumed to equal 1. Other parameters (assumed to be equal for all predators) are based on the life history and predation data of *A. bipunctata* and *Myzus persicae* (Burgio *et al.* 2002; Lanzoni *et al.* 2004) and have only minor impacts on the invasion boundaries.

Table 1. Parameter (*c*) estimations for ISP and IGP for three coccinellid species and their combinations. *R** (critical resource density) estimations based on size; lower value indicates higher competitive ability.

Adult size (mm)	Predator	c-value, Victim			R* (relative to *h*)
		Ab	*Cs*	*Ha*	
4.5	*A. bipunctata*	0.32	0.25	0.19	0.26
7	*C. septempunctata*	0.35	0.22	0.25	0.39
8	*H. axyridis*	0.39	0.35	0.51	0.45

PREDICTIONS

The preceding theory and the given parameter estimations predict different outcomes for each of the three combinations of coccinellid predators, as shown in Fig. 3. *C. septempunctata* is able to invade a system with the native *A. bipunctata*. But since *A. bipunctata* is not able to invade a system with *C. septempunctata*, *C. septempunctata* will gradually displace *A. bipunctata*. *H. axyridis* is also able to invade a system with *A. bipunctata*, but reversely as well, so that the two species can coexist. Finally, the exotic *H. axyridis* may be able to invade a system with *C. septempunctata*, but this is uncertain, as the point is close to the invasion boundery. Since *C. septempunctata* is able to invade *H. axyridis*, the interaction will not result in its full displacement.

That, according to these calculations, *H. axyridis* will not displace *A. bipunctata* whereas the less aggressive predators *C. septempunctata* will, results from the relatively high level of cannibalism in *H. axyridis*, which, as shown before, creates room for coexistence.

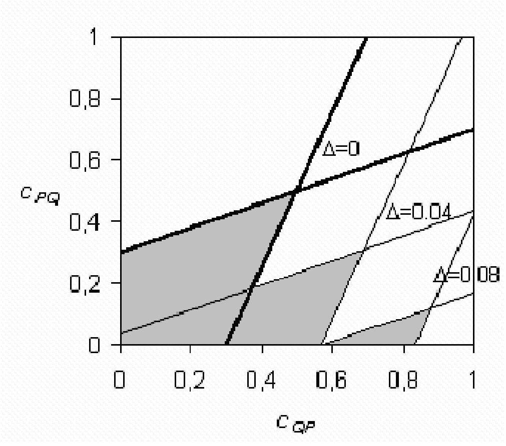

Figure 3. Mutual invasibility plot for unequal predators, based on linear functional responses. Difference in resource utilisation (*R**) between predator species is indicated by Dð, which is positive when in the advantage of *P*. IGP parameter combinations that allow for species coexistence are represented by the shaded area below and left of the two invasion bounderies. ISP levels are assumed to be moderate ($c_{PP} = c_{QQ} = 0.5$).

IMPACT OF RESOURCE COMPETITION

MODEL EXTENSIONS

Under well-mixed conditions and in absence IGP or other types of interference, the outcome of competition is predicted by Tilman's R^* rule (Tilman 1982). This rule says that the species that can maintain an equilibrium population at a lower resource (or prey) density (R^*) than other species, will outcompete these other species. Since at equilibrium reproduction equals mortality, these R^* levels result from the way reproduction and mortality is affected by prey density, i.e. the numerical response. When differences in competitive ability (differences in R^*) are incorporated in the preceding theory, the results become much more complex (see van Rijn and Thomas 2005). The basic impact, however, can be shown graphically, see Fig. 3. Increasing the competitive differences between the two predators will shift the different invasion boundaries on one direction. The positive parameter domain that allow for coexistence will consequently shrink and will ultimately become nonexistent.

ESTIMATING COMPETITIVE ABILITY IN COCCINELLIDS

Studies on the functional and numerical response of coccinellids are generally too sparse and variable to quantify differences between species. To overcome this gap in experimental data, we draw on the theory of allometric relations. The relative uniformity within the group of aphidophagous ladybeetles makes us expect that differences in prey consumption and utilisation will to a large extent result from the differences in their sizes (Dixon 2000).

Critical for the position of R^* is the shape of the functional response, and especially its half-saturation density (h). This parameter can be seen as prey handling rate divided by effective search rate. Since both these parameters should similarly scale with size, h is therefore expected to be largely independent of predator size (Gurney and Nisbet 1998). The scaling parameters of other critical parameters were estimated by analysing published data on Coccinellidae (see also Dixon 2000; Sabelis and van Rijn 1997). We estimated that maximum consumption rate and basal maintenance rate scales with length to the power 1.5 and 2.3 respectively, resulting in a scaling parameter for reproduction of 1.3.

Given the dependency of the prey equilibrium these parameters (van Rijn *et al.* 2002), R^* will increase with predator size, indicating that smaller species are better competitors. Another conclusion is that bigger species require a higher level of food supply than smaller ones to survive, which is confirmed by experiment. The study by Giles *et al.* (2002) shows that to obtain similar levels of survival *C. septempunctata* requires a higher level of food supply than the smaller *Hippodamia convergens*. The comparative studies by Obrycki *et al.* (1998) and Yasuda and Kimura (2001) show that at low prey supply the starvation related mortality of *C. septempunctata* and H. *axyridis* is much higher than of the smaller *Coleomegilla maculata* or *Propylea japonica*.

PREDICTIONS FOR EXOTIC AND NATIVE COCCINELLIDS

Based on the scaling rules we calculated species specific values for the critical parameters in the model (consumption rate and basal maintenance rate, together defining reproduction rate). The resulting critical (or equilibrium) prey density, R*, is indicated in Table 1.

These size-related differences in resource utilisation between the various coccinellid species do affect the predictions for invasion and coexistence, as shown in Fig. 4. The higher competitive ability of *A. bipunctata* now shifts the invasion boundary downward, so that *C. septempunctata* is no longer able to invade the system. The same is true for *H. axyridis*. In the latter case *A. bipunctata* is even able to invade a system with *H. axyridis*. In the interaction between *H. axyridis* and *C. septempunctata* it is more clear that *C. septempunctata* should win the competition.

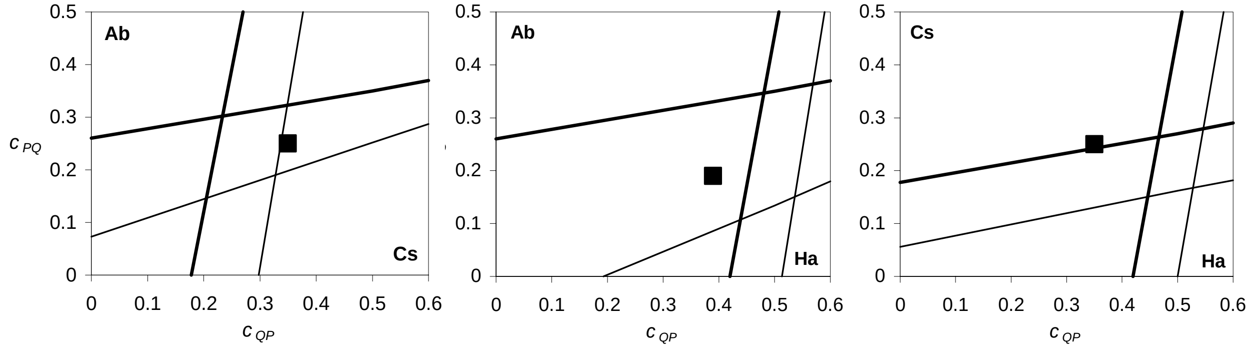

Figure 4. Mutual invasibility plots for specific coccinellid interactions, based on type II functional responses. Thick line indicates the invasion boundaries based on equal competitive abilities; thin line based on the assumption that smaller species are better competitors. Horizontal lines when native species is resident; vertical lines when exotic species is resident. See Table 1 for parameter values.

DISCUSSION

The model approach outlined above should be seen as a first step in analysing the contribution of IGP and ISP to invasion and species coexistence, taking only a few aspects of coccinellid biology into account. More general, however, the analysis shows that knowledge on the asymmetry in IGP is not sufficient to draw conclusions on the ability of a species to invade an existing predator-prey system and to displace the original predator. The level if IGP should, first of all be compared with the level of ISP. When the latter is higher than the former, the level of IGP may be sufficient to invade, but not to replace the other predator. Secondly, competition for common resources may be equally important for the outcome of the interaction. Whereas size generally is an advantage in direct interactions such as IGP, it may be a disadvantage in resource competition. Resource competition, however, can be affected by much more aspects than size, including prey specialisation, habitat dependent foraging efficiency, etc.

ADDITIONAL MECHANISMS FOR COEXISTENCE

Without the impact of resource competition the analysis can be seen as a worst case scenario regarding species replacement, since adding more realism and complexity into that system is likely to increase the likelihood of species coexistence.

SPATIAL AVOIDANCE OF COMPETITION

Intraguild predators may perform behavioural responses that reduces the intraguild interactions. On the other hand, avoidance of intraspecific predation and competition is likely to occur as well. Larval tracks of conspecific ladybirds are known to deter ovipositing females (Yasuda *et al*. 2000). Sibling and kin discrimination by ladybird larvae and females (Joseph *et al*. 1999; Osawa 1992) may reduce ISP as well.

RESOURCE PARTITIONING

All the coccinellid predators and especially the invasive exotics, can feed on a wide range of aphid species, as well as lepidopterans, psyllids, and even pollen (Dixon 2000). Those food ranges, as well as the host plant ranges, may not fully overlap between the different species, which clearly reduces the level of competition, as well as IGP.

TEMPORAL VARIATION

Differences in time of emergence from the overwintering stage may create temporal segregation of resource utilisation. It may, however, also enhance or reverse the asymmetry in competition and IGP, as early arrivals at a resource patch have a headstart in prey depletion, and may benefit from a size advantage in direct intraguild interactions as well (Yasuda *et al*. 2004).

METAPOPULATION DYNAMICS

Finally, asynchrony in prey patch depletion, recreation and recolonisation by predators (as part of a metapopulation process) may provide additional room for coexistence when the best competitor is not the fastest coloniser as well (Osawa 2000).

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BIOLOGICAL CONTROL OF ARTHROPODS OF CONSERVATION IMPORTANCE

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SESSION 2 INTRODUCTION

Invasive species, and the associated development of a relatively new biological discipline broadly known as invasion biology, are a mainstream and widely recognized scientific endeavor. Parties interested in invasive species and their management, modes of infiltration, and impact represent widely disparate groups including: political officials, conservationists, ecologists, agricultural, silvicultural, horticultural, and aquacultural producers, the lay public, and entomologists, including biological control scientists (Wittenberg and Cock 2001). Invasive species and their management currently enjoy a high public profile. Books, T.V. shows, and magazine and newspaper articles regularly appear on this subject, particularly in North America, often including discussion of biological control as a means of environmentally-friendly suppression.

Biological control practitioners are interested in invasion events from a variety of angles, but manipulating two dynamic processes form the pragmatic basis for applied biological control: (1) reducing biotic drivers that promote invasion by pest organisms (i.e., reducing population growth rates, spread, and resulting economic and ecological damage), and (2) promoting biotic drivers of invasive upper trophic level organisms deliberately released for the suppression of unwanted invaders (i.e., carefully increasing the likelihood of population growth, spread, and impact of natural enemies by selecting species with close biological and ecological links to the target pest).

Biological control has been applied widely and often with some level of success against a variety of insect, weed, and vertebrate pests. Most biological control applications have targeted pests of agricultural and forestry importance. This is particularly true for arthropod pests (i.e., insects and mites), and until relatively recently this was mostly the situation for weeds too. However, most weeds are multi-sectoral in their impact, and earlier weed biological control programs yielded environmental benefits as well as the traditional economic benefits. There is now an increasing trend for the environmental impact of alien invasive plants to recognized, so that there is demand and resources for the biological control of weeds of conservation importance (terrestrial and aquatic). A similar trend is emerging for arthropod pests of conservation importance as the ecological impact of these incursive species in infiltrated ecosystems is widely appreciated and the need for remedial action recognized. Indeed, biological control of arthropods of conservation importance has gone a step further. New projects are assessing the feasibility of controlling arthropod pests in aquatic situations, most remarkably the marine environment. Aquatic arthropod pests, most notably an invasive crab, the European green crab, *Carcinus maenas* (L.) (Brachyura: Cancridae) are being assessed as targets for classical biological control and this novel area is providing unique challenges in determining and assessing host specificity, threats to non-target organisms, and the complicated theoretical issues arising from open vs. closed systems for pest and natural enemy recruitment which are being investigated with mathematical models.

One of the first steps in a biological control program is to assess the area of origin of the target pest. When the target is an economic pest, there is usually information available on the status of the pest in the economic literature from around the world, which provides a starting point for projects. The exact origin may still be obscured by subsequent spread, and taxonomic problems related to correct identification (e.g., coffee mealy bug, *Planococcus kenyae* Le Pelley [Greathead 2003]), and sometimes the pest has not been recognized as such in its area of origin (e.g., cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero [Neuenschwander 2003] and Levuana moth, *Levuana iridescens* Bethune-Baker [Tothill *et al*. 1930; Kuris 2003]). When dealing with pests that affect indigenous plants of conservation importance, it may well be that the origin of a newly introduced species is unknown, since the species attacks no plants of economic importance in its area of origin. *A priori*, it seems likely that this may happen more frequently in the case of environmental pests, and we include one such example here: the horse chestnut leaf miner (Kenis *et al*.). Another example from the field of insects of conservation importance is the web spinning pamphilid sawfly, *Cephalcia tannourinensis* Chevan, of unknown origin, which appeared in Lebanon more than 15 years ago and started to cause substantial damage to the few remaining stands of cedar of Lebanon (*Cedrus libani*). At present, it is being managed with an IPM program (Nemer and Naser 2004), as no classical biological control options have been identified.

Many problems associated with determining true species identity and exact area of origin may be overcome through the use of molecular tools. "DNA fingerprints" such as microsatellites can theoretically be used to identify haplotypes within populations that are naturally spread over vast areas within the home range. It should be possible to use a molecular approach to circumscribe within an area of origin a more exact range for the invading population and focus foreign exploration efforts here. One potential benefit arising from such a highly focused search would be the selection of natural enemies from within the pest's home range that are most adapted to the particular haplotype that is the target of the biological control project. A highly focused search within the pest's home range using a molecular-based rationale is feasible when an invasion has resulted from a limited number of founders sourced from a highly localized area and a low number of successful invasion events resulting in establishment occurred. Focused searches based on haplotype matching may increase the success of classical biological control of arthropod pests beyond the somewhat static rate of 10-12% (Gurr *et al.* 2000). Conversely, there is the possibility of rapid evolution through a bottleneck on arrival in a new habitat, so that there is no exact match between the introduced population and any source populations. Similarly this approach can be used to demonstrate multiple introductions, and the introduction of a new biotype with different, more damaging, characteristics.

Despite the recognized potential for natural enemies to suppress pests of economic and conservation importance in a variety of different environments, there has been a very vocal outcry from concerned ecologists and conservationists that biological control is not always a safe and risk free method of pest suppression (e.g., Louda and Stiling 2004). These criticisms have been challenged and arguments have been forwarded that biological control has the potential to be highly effective and safe when conducted according the recognized scientific protocols regardless of whether the target is of economic or conservation concern (Hoddle 2004a,b,c). We reaffirm that, in many instances, once prevention, containment, and eradication options have been exhausted or deemed infeasible, carefully orchestrated biological control programs against well selected targets may be the only feasible option for bringing invasive species of conservation importance under permanent control. The goals of these conservation-oriented biological control projects are to restore population balance and ecosystem health to similar levels seen prior to the invasion without radically disrupting pre-existing food webs (it should be noted that food webs are almost always disrupted in native systems by invasive alien species that reach extremely high densities) or perturbing natural ecosystems in unforeseen ways. Conservation land managers are increasingly recognizing that the options for sustainable management of alien invasive plants are frequently very limited, and that biological control is often the best option. It is becoming ever more apparent that the same also applies to arthropod pests having environmental impact.

The purpose of session 2 in the Second International Symposium on the Biological Control of Arthropods is simple – to showcase examples of the application of classical biological control for suppressing invasive pests of conservation importance. The five selected speakers will present projects that have either been completed (projects by Causton and Fowler) or are underway (projects by Kenis *et al*. and Frank and Cave) against insects causing the demise of native plant plants in either island (cottony cushion scale on the Galapagos Islands [Causton] and orthezia scale on St. Helena [Fowler]) or mainland habitats (bromeliad weevils in Florida, U.S.A. [Frank and Cave] and horse chestnut leaf miner in Europe [Kenis *et al*.]). Kuris *et al*., review green crab biological control and the potential use of castrating barnacles for the suppression of this globally aggressive marine invader. Additionally, the five presentations in session 2 will be complimented by other presentations on biological control of arthropod pests of conservation importance that are being discussed in different contexts in other sessions, fire ants [Porter and Gilbert session 13], and wooly hemlock adelgid [Zilahi-Balogh session 13]).

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THE SUCCESSFUL CONTROL OF *ORTHEZIA INSIGNIS* ON ST. HELENA ISLAND SAVES NATURAL POPULATIONS OF ENDEMIC GUMWOOD TREES, *COMMIDENDRUM ROBUSTUM*

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ABSTRACT

The small South Atlantic island of St. Helena has a highly degraded but internationally significant terrestrial flora, now covering only 1% of its land area. The 2500 gumwood trees, *Commidendrum robustum*, in the last two natural stands, are an important part of this remnant flora. In 1991, a scale insect infesting the gumwoods was identified as *Orthezia insignis*. This South American pest is widespread in the tropics, but this was the first record from St. Helena. By 1993, there were severe patches of infestation of the scale, and over 100 gumwood trees were dead. If the exponential increase in the number of dead trees had continued, all 2500 trees would have been killed by 1995. This was a likely outcome given the lack of natural enemies, and abundance of alternative host plant species for the scale. Fortunately, *O. insignis* had a history of successful biological control in Hawaii, and several African countries, through the introduction between 1908 and 1959 of the predatory South American coccinellid beetle, *Hyperaspis pantherina*. The life history and environmental safety of the predator were studied in quarantine in the U.K., and in 1993 the St. Helena government gave permission for its introduction onto the island. In May 1993, 80 *H. pantherina* survived the 6-day journey to St. Helena, and were used to establish a laboratory colony, from which over 5000 beetles were released from June 1993 to February 1994. Monitoring was undertaken using visual counts of *O. insignis* and *H. pantherina* on 300 labelled branchlets on the gumwood trees. Although the cause of tree death was visually obvious, monitoring demonstrated significant correlations between the levels of attack by the scale and tree mortality. *H. pantherina* was detected on the labelled shoots in February 1994, and numbers then increased, coinciding with a 30× decrease in mean scale numbers. This measured reduction is conservative, because the number of live scales tended to be underestimated when debris from recent feeding by the coccinellid was present. There have been no further problems reported with the scale on St. Helena since 1995. Laboratory rearing of *H. pantherina* was discontinued in July 1995 because insufficient *O. insignis* could be found anywhere on the island. Biological control of *O. insignis* was successful, but the extensive blackening from sooty moulds on all surviving gumwood trees in February 1995, suggested that the predator was effective only just in time to prevent most of

the trees being killed. Experimental transfers of *O. insignis* showed that the other three members of the endemic genus *Commidendrum* could also be at risk from the scale. The deliberate introduction of *H. pantherina* into St. Helena is an early example of biological control being initiated solely for conservation of indigenous biodiversity. It appears that this successful programme has saved the field population of a rare endemic plant from extinction.

INTRODUCTION

The 122-km^2 island of St. Helena is situated in the South Atlantic Ocean (15º 56´ S, 5º 42´ W). Despite widespread environmental degradation since the 16th century, the extant biota of the island is of international significance (Pearce-Kelly and Cronk 1990). St. Helena's flowering plants, for example, include 30 endemic species in 23 genera, and 10 of these genera are also endemic (Pearce-Kelly and Cronk 1990). Native vegetation covers less than 1% of the land area, and many of the indigenous plant species exist in only very small numbers (Cronk 1989). On the positive side, plant species thought to be extinct for over 100 years have been rediscovered, and ambitious restoration programs have been started (Cronk 1989). The endemic genus *Commidendrum* contains four species, including *C. robustum* (Roxb.) DC. (St. Helena gumwood) (Asteraceae), the island's national tree. The once extensive forests of gumwoods are now represented by 2500 trees in two small stands.

In 1991, an insect was noticed attacking gumwood trees at Peak Dale (G. Benjamin, pers. comm.) (Fig. 1). This was identified as the South American scale, *Orthezia insignis* Browne (Homoptera: Ortheziidae), a polyphagous pest that has been accidentally introduced into many tropical countries on imported plants. The first gumwood deaths attributed to *O. insignis* occurred in 1992. Control of *O. insignis* using insecticides was not an option because of the steep terrain, strong winds and risk to indigenous insects. CAB International suggested biological control as an option.

Figure 1. Gumwood branch heavily infested with *Orthezia insignis*, Peak Dale, St. Helena, May 1993. Note the blackening from sooty molds. UGA1390005

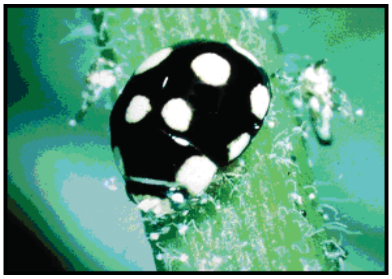

Figure 2. Adult *Hyperaspis pantherina*. UGA1390006

A predatory beetle, now known as *Hyperaspis pantherina* Fürsch (Coleoptera: Coccinellidae) (Fig. 2), was a recognised biological control agent for *O. insignis*, but because of poor past monitoring it's success record was uncertain, and little was known about it's biology. Consequently, the first steps were to review the past record of the agent, and study its life history with an emphasis on assessing its host specificity and optimising methods for rearing and transportation.

PAST USE OF *HYPERASPIS PANTHERINA* FOR BIOLOGICAL CONTROL OF *ORTHEZIA INSIGNIS*

Attempts to control *O. insignis* biologically using *H. pantherina* began with the first introduction of just five individuals from its native Mexico to Hawaii in 1908 (Clausen 1978). Since then *O. insignis* has reportedly been under effective control by *H. pantherina* (Zimmerman 1948). In 1948, *H. pantherina* from Hawaii were introduced into Kenya: *O. insignis* is no longer considered a major pest in Kenya, which again was attributed to *H. pantherina* (Greathead 1971). From Kenya, *H. pantherina* was distributed to Tanzania, Uganda and Malawi, where reports indicated that control of *O. insignis* was generally successful (Greathead 1971), although the outcome was disputed in Malawi. *H. pantherina* is the only biological control agent for *O. insignis* that has definitely established and achieved substantial control of the pest, although the evidence for this is non-quantitative and often anecdotal (Booth *et al*. 1995).

BIOLOGY AND CULTURING OF *ORTHEZIA INSIGNIS* AND *HYPERASPIS PANTHERINA*

Orthezia insignis is a mobile scale insect, which as an adult female has a large wax ovisac (Fig. 3). The species is parthenogenetic. The eggs hatch inside the ovisac and the 1st instar nymphs then move out to feed. *Orthezia insignis* was reared in large cages on various plant species as described by Booth *et al*. (1995).

Hyperaspis pantherina is difficult to rear successfully in large cages because the supply of prey can easily become exhausted, resulting in cannibalism by the predator, and collapse of the culture. Rearing methods were developed that used large numbers of small containers, with fresh *H. pantherina* eggs (normally attached to an adult scale) transferred into fresh containers every 2-3 days (Booth *et al*. 1995). Regular transfers of eggs, and provision of prey, reduced cannibalism because there were only small numbers of *H. pantherina* at similar growth stages in each container. After hatching, the first instar larvae of *H. pantherina* usually enter the ovisac of the female scale, where they consume scale eggs and hatching nymphs. *Hyperaspis pantherina* larvae normally became visible in the containers in the third instar when they leave the ovisac, in the process they normally kill and consume the adult scale.

When four female *H. pantherina* were closely observed for their adult life, over 90% of the total of 657 eggs produced were laid on adult female *O. insignis*. Almost all of the eggs laid on female *O. insignis* were either on the dorsal surface of the abdomen (Fig. 3) or on the dorsal surface of the ovisac. The few eggs laid on the substrate include those laid on the exuviae of *O. insignis*, on other fragments of the prey, nearby on the host plant, and on other suitable surfaces such as filter paper. In the complete absence of *O. insignis* as live individuals, exuviae or other remains, only one egg was laid in nearly 2 years of culturing. That *H. pantherina* almost never laid eggs in the absence of *O. insignis* (insects, exuviae or debris after predation), and that over 90% of eggs were laid on the adult female scales, suggest a very close predator-prey relationship. However, when deprived of *O. insignis*, caged adult beetles did attack *Planococcus* and *Pseudococcus* species (Homoptera: Pseudococcidae) (Booth *et al*. 1995). A risk of attack on other mealybug or scale species (Homoptera: Coccoidea) on St. Helena was not considered important, as all such insects recorded on the island are accidental introductions, and most are pests (Booth *et al*. 1995; Fowler 1993).

Figure 3. Adult *Orthezia insignis* with the 0.7mm oval, grey egg of *Hyperaspis pantherina on its abdomen*. UGA1390007

SHIPMENT TO ST. HELENA, REARING, AND RELEASE

St. Helena is remote, with the fastest access route being a military flight from the U.K. to Ascension Island, followed by a sea voyage to the island itself. No *O. insignis* were allowed in the shipment of *H. pantherina*, so the predator had to survive on water and honey agar (details in Booth *et al*. 1995). In May 1993, 80 larvae, pupae and adults of *H. pantherina* survived the 6-day journey to St. Helena, and were used to initiate a rearing programme using the methods previously developed. The first releases of *H. pantherina*, from June to November 1993, used a total of approximately 50 surplus ovipositing females from the laboratory culture. Each female was placed into a small 1mm mesh sleeve enclosing a scale-infested gumwood branchlet at Peak Dale. At weekly intervals, each sleeve was moved to a new branchlet, until the female died. The first three individual *H. pantherina* released into sleeves were checked after 4 days: all three females had survived, and eggs could be seen on the dorsal surface of several adult scales. No further assessment of this release method was made, because detecting *H. pantherina* eggs or young larvae in the field was difficult. In February 1994, 5000 beetles were released onto the gumwoods without sleeving. The rearing facility operated for 2 years until July 1995, when insufficient numbers of *O. insignis* could be found on St. Helena to maintain the culture of the predator. After the mass release onto the gumwoods, various other releases were made onto exotic ornamental plants at the request of private landowners. No parasitoids or other natural enemies of *O. insignis* were noticed in any of the field collections of the scale used in the rearing facility.

DISTRIBUTION OF *ORTHEZIA INSIGNIS* ON ST. HELENA

Monitoring was undertaken in stands of *L. camara* at 27 additional sites across the island in 1993 (Fowler 2003). At all these sites, *O. insignis* infestations on *Lantana camara* L. (Verbenaceae) were visually categorised as abundant, present (but not abundant), or absent. *Lantana camara* was selected because it is a common host plant for *O. insignis* found all over the island at all altitudes. In contrast, gumwood trees are only common at Peak Dale and at the new restoration plantings at Horse Point (Fig. 4).

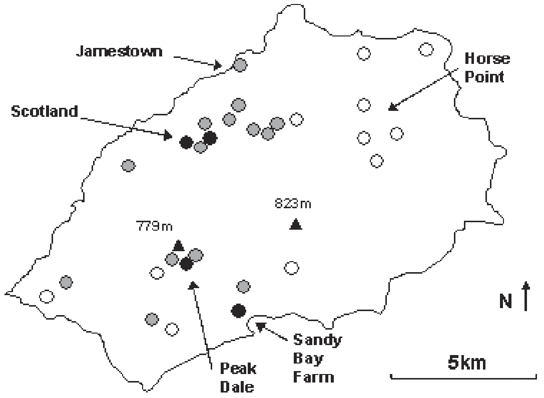

Figure 4. The island of St Helena. Circles indicate areas where the weed, *L. camara*, was checked for *O. insignis* infestations in 1993 (black = abundant scale; gray = scale present, but not abundant; open = scale not detected).

During May/June 1993, *O. insignis* was only abundant at Scotland (near to the main urban centre and only port at Jamestown), at Sandy Bay Farm and Peak Dale. This pattern of occurrence was consistent with *O.* insignis being a recent introduction onto the island, followed by accidental translocation to Sandy Bay Farm on cultivated plants, and then dispersal in the prevailing SE trade winds up to Peak Dale. *Orthezia insignis* was absent in the north-east part of the island and from many of the steep coastal cliffs in the south (Fig. 4). By February 1995, *O. insignis* had become abundant on *L. camara* at Horse Point, suggesting continued dispersal. Eggs and larvae of *H. pantherina* were seen in February 1995 on lantana about 1 km from a release site, demonstrating that the predator was also dispersing. The limited distribution of *O. insignis* in 1993 suggested that other *Commidendrum* spp., particularly the large recovering areas of scrubwoods (*C. rugosum* [Ait.] DC.) in the steep, dry coastal zone, might not have been exposed to the pest. Consequently, some simple investigations of the potential acceptability of several plant species endemic to St. Helena, including scrubwood, were undertaken in 1993 (see next section).

THREATS TO OTHER ENDEMIC PLANT SPECIES

Single cut shoots (10–15cm long) were taken from 2–9 plants each of *Lantana camara* and of 8 species of endemic trees and shrubs; gumwood, *C. robustum*; scrubwood, *C. rugosum*; bastard gumwood, *C. rotundifolium* (Roxb.) DC.; false gumwood, *C. spurium* (Forst.f.) DC.; he-cabbage tree, *Pladaroxylon leucadendron* (Forst.f.) Hook.f.; she-cabbage tree, *Lachanodes arborea* (Roxb.) B. Nord (all Asteraceae); St. Helena ebony, *Trochetiopsis melanoxylon* (Ait.f.) Marais; ebony/redwood hybrid, *T. erythroxylon* (Forst.f.) Marais x *T. melanoxylon* (Sterculiaceae). Fifty *O. insignis* nymphs were allowed to transfer onto each cut shoot from small pieces of infested lantana that were placed onto the cut shoots. The shoots, in vials of water, were positioned so that the foliage of each shoot was not touching anything. Remaining nymphs were counted after 3–4 days. The results are shown in Table 1. Mean survival of

O. insignis nymphs on the genus *Commidendrum* (gumwoods and scrubwoods) (Asteraceae) ranged from 34% for *C. robustum* to 70% for *C. spurium*. This was markedly higher than the 2.5% nymphal survival on the *Trochetiopsis* species (ebony and ebony/redwood hybrids).

Only the comparison of *Trochetiopsis* species/hybrid with *C. rugosum* and *C. spurium* was statistically significant. Given the suitability of *C. robustum* to *O. insignis* in the field, this result suggests that all 4 *Commidendrum* species were likely to be suitable hosts, but that the highly endangered *Trochetiopsis* species/hybrid in the family Sterculiaceae might be relatively much less suitable. The cut shoots probably deteriorated in the time required for scale nymphs to transfer from the drying pieces of *L. camara*, so even transfers to cut shoots of *L. camara* only had a 40% survival rate. Given the low number of replicates and high variability, little can be concluded about the suitability of the two species of cabbage trees (Asteraceae), although mean nymphal survival was 40% on the two replicates of he-cabbage trees, *P. leucadendron* (Table 1).

Table 1. Summarized results of the laboratory host range test, exposing cut shoots of selected endemic plants species in St. Helena to 50 nymphs of *O. insignis* for 3–4 days. The introduced weed *L. camara* was used as a control. Means followed by the same lower case letters are not significantly different. (Overall ANOVA on arcsin transformed data, $F_{8,29}=4.66$, $P<0.01$, comparison of means used Tukey HSD, $P<0.05$, SYSTAT [SPSS 1997]). Data from Fowler (2003).

Plant species	Replicates	Mean % survival (± SE)
Lantana camara	4	40 (±7.4)^{ab}
Gumwood, C. robustum	9	34 (±3.4)^{ab}
Scrubwood, C. rugosum	9	50 (±4.3)^{a}
Bastard gumwood, C. rotundifolium	2	50 (±15)^{ab}
False gumwood, C. spurium	2	70 (±5.0)^{a}
He-cabbage tree, L. leucadendron	2	40 (±10.0)^{ab}
She-cabbage tree, L. arborea	2	10 (±5.0)^{ab}
St Helena ebony, T. melanoxylon	4	2.5 (±0.8)^{b}
Ebony/redwood hybrid, *T. erythroxylon* x *T. melanoxylon*	4	2.5 (±1.3)^{b}

MONITORING PREDATOR/PREY ABUNDANCE AND IMPACT

Prior to 1993, the numbers of trees dying after infestation by *O. insignis* were counted, but infestation levels of the scale were not quantified. Nevertheless, the infestation levels were clearly very high as shown in Fig 1. In 1993 a monitoring program was started to relate scale infestation levels to damage or death of trees, and determine whether these measurements changed in response to increasing levels of the introduced predator. The two relict stands of gumwood trees at Peak Dale were designated sites A and B: site A being where infestations of *O. insignis* were first noticed in 1991, and site B being the stand approximately 0.5 km further to the south-west. At both sites, 15 trees were selected and labelled, with 5 trees in each of 3 visually assessed damage categories: severely infested (>50% of canopy affected); moderately infested (<50% of canopy affected); and uninfested. The selection of these 30 trees was as random as possible, although heavily shaded trees, and those with most of their canopy out of easy reach, were avoided. Gumwood trees have a simple, sparse canopy that was divided conveniently into approximately 20 cm long branchlets, comprising a group of 1–3 growing points, each with 10–20 leaves. Ten branchlets per tree were selected randomly and labelled for the non-destructive sampling program. At 1–3 month intervals, the numbers of adult and nymphs of *O. insignis* on each branchlet were visually estimated, and numbers of *H. pantherina* larvae and adults counted. The presence, or approximate percentage damage, due to other herbivores or predators was also assessed by eye. A visual estimate was made of the percentage of canopy of each tree that was heavily infested with scale.

The total numbers of dead gumwood trees at Peak Dale increased exponentially from 1991 to 1993–4 (Fig. 5). By 1995 mortality of gumwoods had reached an asymptote, with only 12% dead from a total of 2500 trees. If the exponential rate of loss of gumwoods from 1991 to 1994 had continued, all trees in the two relict stands at Peak Dale would have been killed by 1995.

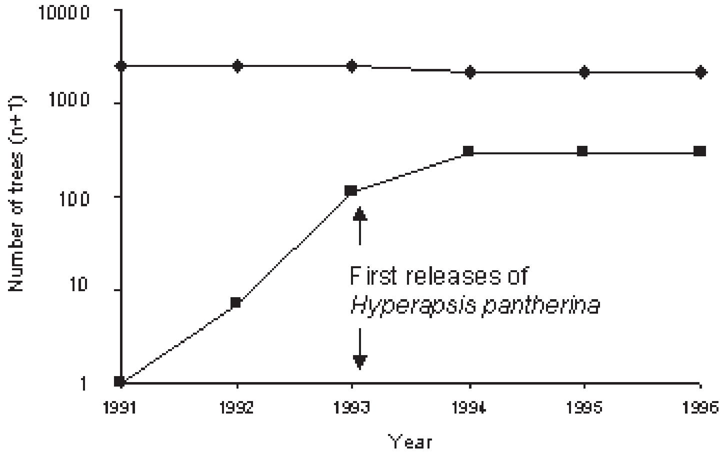

Figure 5. The numbers of live (♦) and dead (■) gumwood trees at Peak Dale, during the outbreak and subsequent biological control of *Orthezia insignis*. The exponential increase of the scale numbers from 1991 to 1993–4 was significant ($F_{(1,2)}$=66.3, P<0.05, r^2=0.97, log(y+1)=0.77x-1532, SYSTAT [SPSS 1997]). Data from Fowler (2003).

Mortality among labelled trees was highest at site A, and higher among trees that were severely damaged at the start of the release program (Table 2). However, all labelled trees became infested during the monitoring period, and mortality increased rapidly between 1993 and 1994 (Table 2) showing a similar pattern of tree death as the overall stand (Fig. 6). Mean numbers of *O. insignis* per 20 cm branchlet on severely infested trees peaked at over 3000.

The causative link between infestation by *O. insignis* and death of gumwood trees was visually obvious at the site, and is supported by data: the mean percentage of canopy infested with *O. insignis* on trees that were killed was significantly higher than on surviving trees (Fig. 7).

Data from trees in plots A and B, that were initially severely or moderately infested, were combined in Fig. 7. Data from the initially uninfested trees were excluded because *O. insignis* numbers mostly remained low. However, the numbers of *O. insignis* on the initially uninfested trees rose during the monitoring period, and then fell towards the end (as in initially severely and moderately infested trees).

Table 2. Cumulative mortality of gumwood trees at sites A and B in Peak Dale. There were 30 labelled trees, with five in each initial infestation category at each site. Dates were simplified to show numbers of trees dead at the end of 6-month periods, plus the final sampling date in February 1995. Data from Fowler (2003).

Initial Infestation Category	Site	June 1993	December 1993	June 1994	December 1994	February 1995	Total % Mortality
Severe	A	0	4	4	4	4	80%
	B	0	0	2	2	3	60%
Moderate	A	0	0	3	3	3	60%
	B	0	0	0	0	0	0%
Uninfested	A	0	0	0	1	1	20%
	B	0	0	0	0	0	0%
Total dead trees		0	4	9	10	11	37%

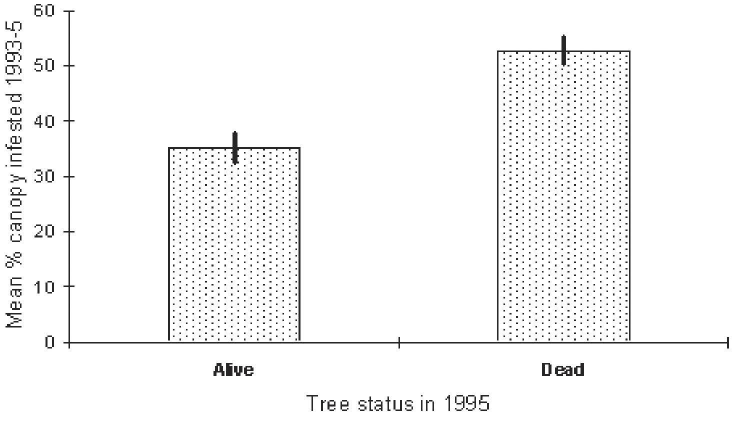

Figure 6. Mean (+/- SE) percentage of the tree canopy severely infested by *O. insignis* from 1993 to 1995. Trees that died had higher mean % canopy infestations by *O. insignis* than trees that survived (t = 4.62, df = 18, $P<0.001$, SYSTAT [SPSS 1997]). Initially uninfested trees were excluded as only one died. Data from Fowler (2003).

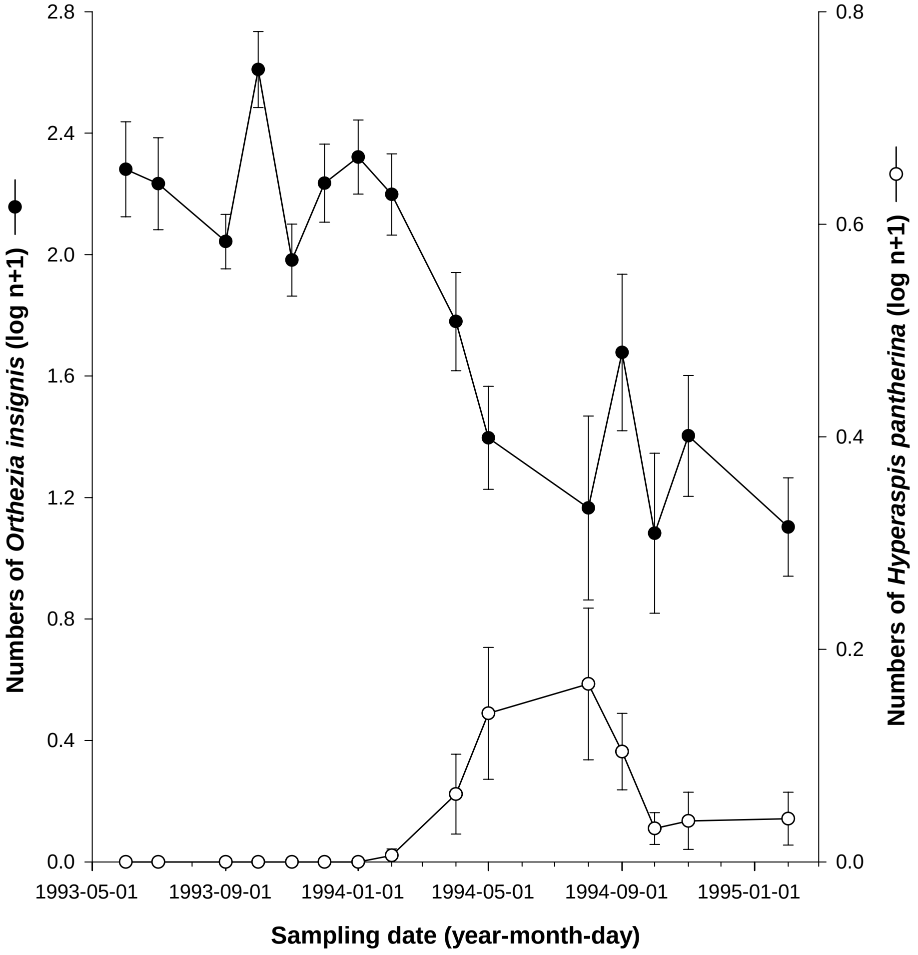

Figure 7. The mean numbers of *O. insignis* and *H. pantherina* on the labelled shoots of initially severely and moderately infested gumwood trees at Peak Dale. Error bars show the standard error for each mean, calculated on log-transformed data.

Data in Fig. 7 show an approximately 30× reduction in mean scale numbers per 20 cm branchlet, from >400 adults and nymphs (in September 1993) to <15 (in February 1995) when sampling ceased. The reduction in *O. insignis* populations was probably greater than this because monitoring staff overestimated scale numbers as the predator became common; in the field it was difficult to distinguish live scales from dead and partially consumed scales.

Numbers of *H. pantherina* counted on the labelled shoots were highly variable and usually low. High mean *H. pantherina* numbers were recorded occasionally on individual trees, in one case peaking at 1.3 adults and 3.4 larvae per 20 cm branchlet. The mean numbers of *H. pantherina* increased from January to August 1994, coinciding with the only consistent decrease in the mean numbers of *O. insignis* (Fig. 7). Earlier decreases in scale numbers might also have coincided with increases in predator numbers, but at this stage predator numbers were too low to be detected in the monitoring program.

Other herbivores detected in the monitoring program were limited to occasional lepidopteran larvae and mealybugs (*Pseudococcus* spp.). Few predatory arthropods, other than *H. pantherina,* were recorded: eggs of *Chrysoperla* species (Neuroptera: Chrysopidae) were found several times on gumwood shoots infested with *O. insignis*, but there was no evidence that the larvae were attacking the scale; one adult *Cheilomenes lunata* (F.) (Coleoptera: Coccinellidae) was collected from gumwoods infested with *O. insignis*, but did not attack *O. insignis* in confinement.

The relict natural population of St. Helena gumwoods, and probably related endemic species, were under severe threat from the alien scale insect, *O. insignis*. The number of dead gumwood trees increased exponentially from 1991 to 1993–4, and if this trend had continued, all the gumwoods in the two main stands would have been killed by 1995 (Fig. 5). Though speculative, this prediction is supported by the appearance of the gumwoods in February 1995: the foliage of most surviving trees in both sites showed very substantial blackening from sooty molds indicating that scale populations had been high and extensive (Fig. 8). There was also direct evidence from the abundance of exuviae and partly-predated scales that the densities of *O. insignis* had been high. It appeared that biological control had been successful only just in time to save most of the trees. The gumwoods were showing signs of recovery from the outbreak of *O. insignis*, with new growth appearing that was uninfested by the scale.

A number of factors contributed to the severity of the threat to the gumwoods from *O. insignis*. There were large numbers of alternative host plants for *O. insignis*, such as *L. camara*, around both sites. Hence, a decline in the numbers and/or health of the gumwoods was unlikely to have limited scale numbers sufficiently to save any of the gumwoods at Peak Dale. It was also unlikely that existing predators or parasitoids on St. Helena would have suppressed *O. insignis*, as there was no sign of any mortality from natural enemies other than *H. pantherina* during the 2-year study. Finally, the gradual spread of *O. insignis* onto all of the initially uninfested gumwood trees, and the blackened appearance of most trees in 1995, gave little indication that any trees were less susceptible to the pest, and hence might have survived the outbreak.

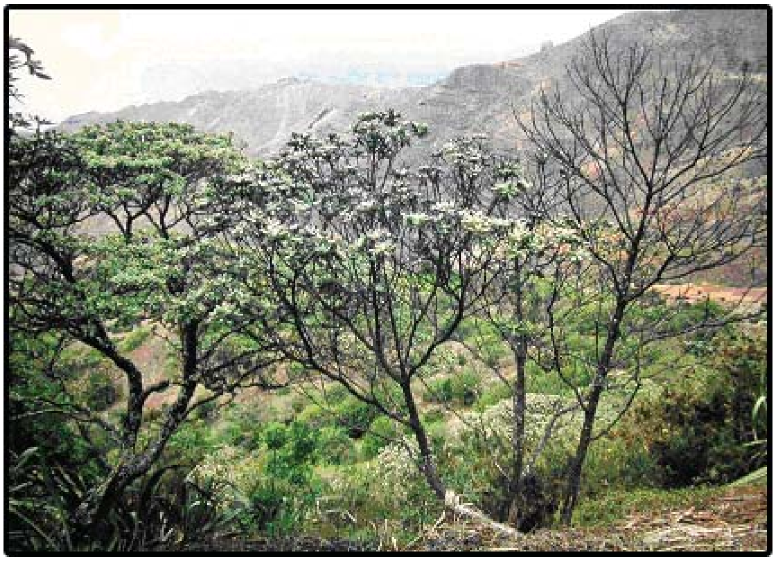

Figure 8. Surviving and dead gumwoods at Peak Dale in 1995. The central tree has uninfested regrowth emerging from leaves that are blackened by sooty molds. UGA1390011

Although the data in this study are only correlative, the success of *H. pantherina* as a biological control agent for *O. insignis* on St. Helena is consistent with its past record in Hawaii and Africa (Booth *et al*. 1995). Since 1995 there have been no further problems with *O. insignis* reported from St. Helena. Restoration projects, to encourage natural gumwood regeneration by controlling weeds in and around the two relict stands, can now proceed. These projects were considered pointless unless biological control of *O. insignis* was achieved (T. Upson, pers. comm.). A program to establish a millennium forest of gumwoods on a previously wooded site on the island began in 2000. Molluscs and lepidopteran larvae cause minor pest problems on these young trees, but *O. insignis* has not been noticed (I. Peters, pers. comm.), providing further evidence that the scale is under satisfactory biological control. The introduction of *H. pantherina* to St. Helena provides a particularly clear, quantitative study where the field population of a rare endemic plant was likely saved from extinction by biological control of an alien insect pest. Conservation benefits from biological control need to be considered in the current debate on the harm that introduced biological control agents can do to indigenous species (Howarth 1983; Louda *et al*. 1997; Simberloff and Stiling 1996)

ACKNOWLEDGEMENTS

G. Forrester provided statistical advice, and K. Alders helped sort the raw data. The Agriculture and Forestry Department (A & F), St. Helena, helped with many aspects of the project. A. Hill (A & F) managed the monitoring program. A. Cross, R. Mitchell and R. Shaw (CAB International) developed rearing methods in the UK, under license from the Ministry of Agriculture, Fisheries and Food (licence PHF 873A/667/73). A. Cross took the photographs for Figs. 3 and 4. P. Markham and I. Bedford (John Innes Institute) provided the culture of *O. insignis*. Funding was provided by the UK Government (ODA project OMC 92/94 349/053/001A).

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EVALUATING RISKS OF INTRODUCING A PREDATOR TO AN AREA OF CONSERVATION VALUE: *RODOLIA CARDINALIS* IN GALÁPAGOS

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ABSTRACT

Conservation areas can be highly susceptible to alien species introductions, and therefore extra care should be taken to assess potential interactions between a biological control agent and its new environment. In the Galápagos Islands, biological control was first considered to mitigate the threats posed to flora and specialist phytophagous Lepidoptera by the invasive scale *Icerya purchasi* Maskell (Hemiptera: Margarodidae). *Rodolia cardinalis* Mulsant (Coleoptera: Coccinellidae) was selected as a candidate agent, but a risk assessment that included prey range studies was considered necessary to evaluate the threats to Galápagos biota. In this paper important considerations for assessing the potential impacts of introducing an insect predator into a vulnerable ecosystem and for defining test procedures are discussed. These include the assessment of potential ecological impacts at different trophic levels, criteria for identifying and ranking non-target species, and the use of the precautionary principle for assessing acceptable levels of risk. Limitations encountered in conducting tests are also discussed, such as test species availability and the influence of host volatiles.

INTRODUCTION

The implementation of a biological control program of a pest of conservation importance will often involve liberating agents into areas of high conservation value. These may be habitats of threatened populations that are highly susceptible to the introduction of alien species. Island ecosystems, because of their isolation and late colonization, are especially sensitive to invasion because of an underrepresented, disharmonic and genetically impoverished biota that has developed few strategies to deal with alien species (Elton 1958; Loope *et al.*1988). Biological control in areas of conservation importance therefore requires careful deliberation to evaluate potential ecological impacts of introducing a new exotic species. Release of a biological control agent should proceed only if non-target effects are shown to be minimal or highly improbable in comparison to the damage inflicted on native species by the target pest.

The Galápagos archipelago, a UNESCO world heritage site and biosphere reserve, is under threat from introduced species. A recent inventory reported 463 introduced insects (Causton *et al.*, unpubl. data). One of the most serious of these species is the invasive scale *Icerya purchasi* Maskell (Hemiptera: Margarodidae) which threatens both the endemic flora and specialist phytophagous Lepidoptera that feed on them (Causton 2001, 2003a; Roque-Albelo 2003). To mitigate its impacts, biological control was considered for the first time in Galápagos. The much-used coccinellid predator, *Rodolia cardinalis* Mulsant (Coleoptera: Coccinellidae), was selected as a potential control agent. Although renowned for its narrow prey range elsewhere, this was unconfirmed and a risk assessment that included prey range studies was carried out to determine if its introduction would harm the Archipelago's indigenous biota (Causton 2004; Causton *et al.* 2004).

Using *R. cardinalis* as an example, this paper discusses the procedures used to evaluate the risk of introducing a predator into a vulnerable ecosystem and includes important considerations for: 1) assessing potential ecological impacts; 2) identifying criteria for selecting non-target species; 3) conducting tests; and 4) assessing acceptable levels of risk for introducing a biological control agent.

POTENTIAL ECOLOGICAL INTERACTIONS

Areas of conservation value are often ecologically complex compared with human-modified environments and the introduction of a biological control agent and removal of the prey species in such a system is likely to have consequences (Louda *et al.* 2003; Strong and Pemberton 2001). Predators such as *R. cardinalis* are high in the food chain and the outcome of their feeding and related population explosion and declines could impact the food web widely. Furthermore, both larvae and adults may be voracious feeders and may have high dispersal and searching abilities increasing potential encounters with non-target species. Because of this, risk assessments should consider not only the direct impacts of feeding on non-target species, but also other interactions that might occur, and, in response to these, appropriate research methods should be designed to quantify risk. Interactions that might take place with the introduction of a predator such as *R. cardinalis* are shown in Fig. 1 and are discussed below.

INTERACTIONS WITH LOWER TROPHIC LEVELS

A predator may feed on and cause the decline of species other than the target prey in the proposed region of introduction, particularly when the target prey is scarce or when the predator disperses to areas outside the distribution of the target prey. Species at highest risk will be those closely related to the preferred prey of the control agent or species that overlap ecologically. Non-target species may be at greater risk of exposure if the agent is not efficient at controlling the target prey but is maintained in high numbers (Holt and Hochberg 2001). On the other hand, some interactions might be beneficial to both the biological control agent and a non-target species; such as an insect defended from natural enemies and/or transported to new locations, or a plant pollinated by the control agent. The consequences of such synergistic interactions for indigenous biota could be negative if they increase the fitness of another introduced species (Simberloff and Von Holle 1999).

For example, predators that feed on pollen or nectar may compete with insect pollinators. Although not considered a risk group in Galápagos (Causton 2003a), pollinating species in other conservation areas may be impacted if they specialize on threatened plant species

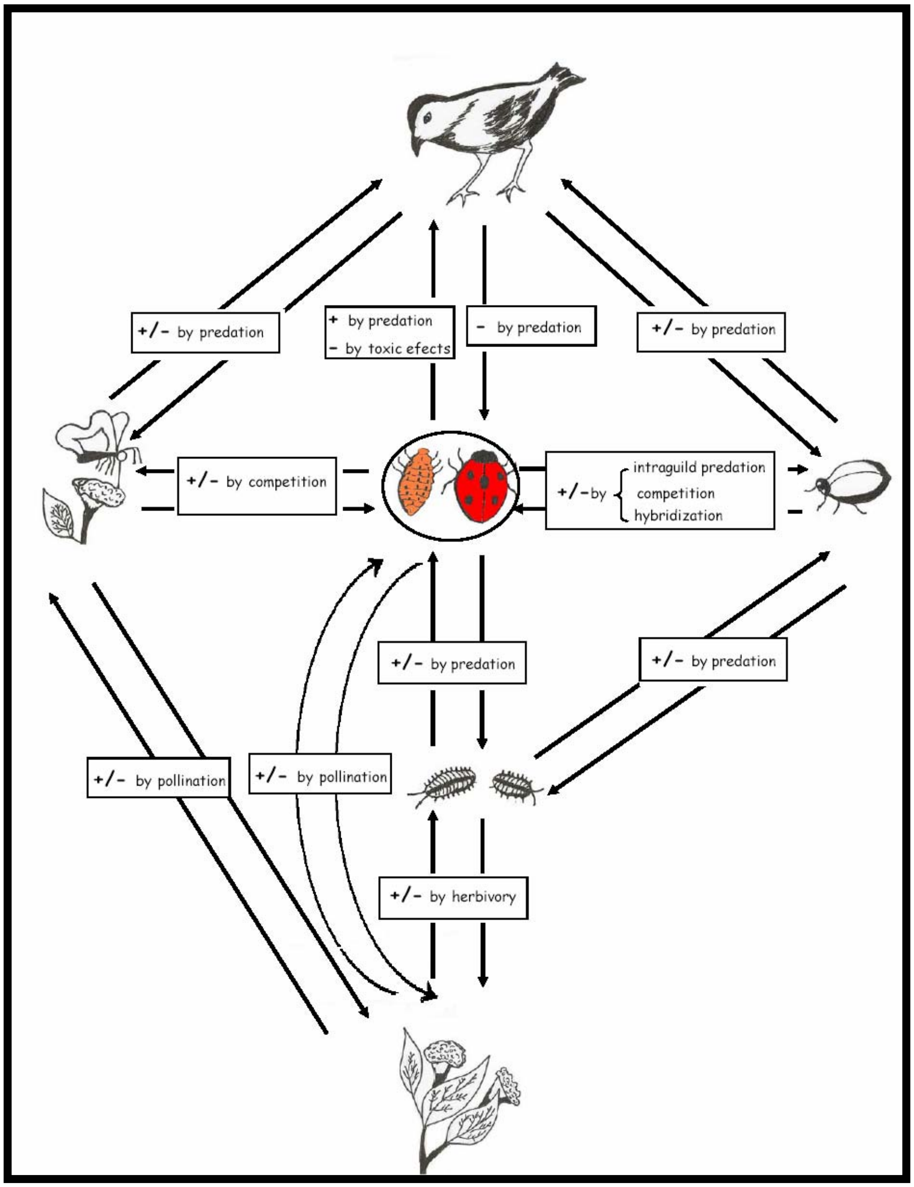

Figure 1. Overview of potential interactions between an introduced predator and its new environment.Introduced predators may remove or reduce resources such as food and nesting sites from other invertebrate species (e.g., Dixon 2000; Obrycki *et al.* 2000).

with small populations. Some coccinellids have been shown to deter parasitoids from ovipositing purely by their presence near the prey (Dixon 2000). On the other hand, the presence of a predator may benefit other predators by making a prey species more accessible (Charnov *et al.* 1976). Another potential negative result of a species introduction is hybridization with a species of conservation value or another biological control agent (causing extinction or a decline in fitness through the production of sterile hybrids), or with an introduced species (causing increased fitness which in turn may affect indigenous species).

INTERACTIONS WITHIN THE SAME TROPHIC LEVEL

Species at the same trophic level can also be directly impacted by feeding (intraguild predation). For example, coccinellids can feed on conspecific and heterospecific ladybirds, other predators and parasitoids (see Dixon 2000). In the laboratory, larvae of *R. cardinalis* have been observed to kill and or displace larvae of *Rodolia iceryae* Janson (Coleoptera: Coccinellidae), even when target prey were available (Mendel and Blumberg 1991). Ultimately, the outcome of the interaction between predators will depend on their size, aggressiveness, defense mechanisms, and whether the species is a top or primary predator (Dixon 2000). In Galápagos, the only native predator of *I. purchasi* is the lacewing, *Ceraeochrysa cincta* Schneider, (Neuroptera: Chrysopidae), which was not considered at risk because it was found to attack both larvae and adults of *R. cardinalis* in captivity (Causton *et al.* 2004). On the contrary, the foraging behavior of *R. cardinalis* in Galápagos could be affected by the presence of this lacewing as well as by introduced species of ant that defend *I. purchasi* in return for honeydew.

The consequences to biota of removing the target prey should also be considered. In Galápagos, negative effects on indigenous species were improbable because there were few species (one lacewing and some bird species) that fed on *I. purchasi*, and all of these were generalist predators that did not rely exclusively on the target prey. In contrast, a positive response was expected because the removal of the target prey would eliminate an important food source for two species of invasive wasp and the invasive fire ant, *Wasmannia auropunctata* Roger (Hymenoptera: Formicidae).

INTERACTIONS WITH HIGHER TROPHIC LEVELS

Insectivores at higher trophic levels may benefit directly from the abundance of the predator (as a food source in its own right) producing either positive or negative spillover effects on other species down the food chain (e.g., Holt and Hochberg 2001; Pearson and Callaway 2003). Hoddle (2004) refers to this as natural enemy subsidization of food webs. Alternatively, a decline or a change in behavior in species at higher trophic levels could occur as a direct result of feeding. Because some coccinellids produce toxins as a means of defense (Dixon 2000; Marples *et al.* 1989), insectivorous vertebrates were considered at risk from the introduction of *R. cardinalis*. Vertebrates may also be affected if the introduced biological control agent competes for the same food.

In conclusion, detrimental impacts are normally perceived as effects that negatively change the status of indigenous biota. However, interactions that increase the fitness of an introduced species should also be of concern because they may also have an impact on the ecosystem. In the long-term, any of these interactions could lead to species displacement, alteration of community structure and dynamics, and disruption of natural evolutionary patterns. Although it will not be possible to test all the potential interactions, prioritization of potential impacts according to their likelihood should be carried out to identify non-target species at risk and assess the safety of the biological control agent.

CRITERIA FOR IDENTIFYING NON-TARGET SPECIES POTENTIALLY AT RISK

To best evaluate non-target species at risk, a thorough understanding of the biology of the predator and target prey is essential and may involve studies in their native range (budget permitting) or introduced range, as well as in the laboratory. Research topics should include: 1) phylogeny, 2) feeding range, 3) stimuli that prompt foraging and host location (such as specific plant and insect chemicals and morphological features), 4) habitats occupied, 5) geographical range and climatic tolerance, 6) phenology, and 7) place in the food web.

It should not be assumed that the potential non-target impacts of a predator are only restricted to the geographical extent of the target prey. Strong flying species and/or species that are wind or water dispersed may reach areas outside that range. Furthermore, humans may assist spread. For example, *R. cardinalis* is adapted to a wide range of climatic regimes and adults are strong fliers suggesting that it would be able to reach and adapt to most parts of Galápagos if food were available.

Criteria that we used for *R. cardinalis* (Causton 2004; Causton *et al.* 2004) and that might apply for other predators are listed below:

- Species closely related to *I. purchasi* or the Margarodidae. Centrifugal testing (Wapshere 1974), used for weed and arthropod biological control agents, assumes that the closer the species is taxonomically to the target pest, the more likely it is to be attacked.

- Species previously reported as prey for any *Rodolia* species. Because coccinellids that prey on scales are known to exhibit restricted feeding ranges (Dixon, 2000), the feeding habits of congenerics were also considered to be a useful indicator of the potential feeding range of *R. cardinalis*.

- Species morphologically or physiologically similar to *I. purchasi*. Olfactory and visual cues such as wax filaments produced by scale insects are often necessary to prompt coccinellid foraging and oviposition (Dixon, 2000; Merlin *et al.* 1996). We assumed that such prey characteristics would influence prey selection by *R. cardinalis* in Galápagos.

- Species that live in close proximity to the prey of *R. cardinalis*. The greater the host plant range of the target prey the greater the number of species interactions. Species of insects, in particular, Homoptera or endangered insects, were considered to be at risk if they occupied niches close to *I. purchasi*. Furthermore, natural enemies that fed either on the pest *I. purchasi*, or on other taxa identified as potential prey of *R. cardinalis* were also considered to be at risk due to competition or intraguild predation.

- Species of conservation value that might interact with *R. cardinalis* in other ways. For example, insectivorous vertebrates that feed on coccinellidae.

RANKING NON-TARGET SPECIES

Prioritization is an important tool when a large number of non-target species have been identified, or as in our case, when information about the non-targets was sparse and field collections were limited by budget constraints. Species had to be ranked according to conservation priority and/or importance as an indicator of the prey range of *R. cardinalis*. For our purposes, host plant distribution was often used as an indicator of the distribution and abundance of phytophagous non-target species because more information is available on the Galápagos flora. Species of highest priority were the species endemic to a single island and specialized feeders with a small host range, especially those that are closely related to *I. purchasi* or in genera reported as prey, or those that feed on rare plant species that are attacked by *I. purchasi*. An endemic, but subterranean margarodid, *Margarodes similis* Morrison (Hemiptera: Margarodidae) was also considered a priority for testing because it is the closest species phylogenetically to *I. purchasi*.

IMPORTANT CONSIDERATIONS FOR CONDUCTING TESTS

Prey range tests form the bulk of a risk assessment allowing one to assess some of the direct and indirect impacts that could occur with the introduction of a biological control agent. This is the traditional focus of testing. However, other interactions such as competition may also require testing experimentally, which will depend on the potential ecological interactions identified. The efficacy of the agent in controlling the target pest should also be studied to reduce the risks of spillover and other indirect effects (see Hoddle 2004; Holt and Hochberg 2001).

The order in which studies are conducted and species tested will also influence the type of tests that need to be carried out. By defining the prey range of the predator first, one can better identify the species that might be affected (by niche overlap, intraguild predation, or competition) and thus reduce the number of species that need to be tested.

Important considerations for designing tests for *R. cardinalis* and other predators are listed by Causton (2004). Some of the salient points for ensuring the success and accuracy of the tests are listed below.

TESTING CORRECT BIOTYPE

The prey range and behavior of geographically distinct predator populations may vary (Phillips *et al.* 2002; Sands and Van Driesche 2004), and as a precaution, tests should be conducted on the biotype that will be introduced and future introductions restricted to the same biotype.

SELECTION OF PREDATOR STAGES FOR TESTING

Predator stages that need to be tested will depend on the biology of the predator. For example, both *R. cardinalis* adults and larvae are vagile entomophages and required separate tests to determine feeding on non-targets. On the other hand, neonate larvae were tested to determine life cycle development because adult oviposition was shown to be an unreliable parameter (Causton 2004).

CONDITION OF PREDATOR STAGES USED IN TESTS

Several factors may influence the test outcome and should be considered:

1. *Prior feeding experience.* Naïve, unfed individuals may feed on a test species that is rejected by individuals that have fed previously on the target prey. Our studies did not show any behavioral differences but other studies suggest that some predators may be conditioned to feeding on a preferred prey (e.g., Rayor and Munson 2002).
2. *Hunger levels.* Satiated individuals often do not respond quickly to prey, while naïve (unfed) individuals may become weak and therefore uninterested in feeding if not tested immediately. Mature individuals are less likely to feed.
3. *Reproductive status.* Mated individuals may behave differently to non-mated individuals.
4. *Fitness of test individual.* Crowded rearing conditions produce smaller and less fecund individuals that are less likely to eat and develop normally (e.g., Booth *et al.* 1995).

CHOICE OF PREY LIFE STAGES AND HOST PLANT

The prey life stage that is tested should reflect the life stage of the predator. For example, neonate predator larvae may be unable to feed on tougher later instars.

The host plants of the non-target test species should also be considered carefully because they may affect the predator‘s choice of prey. Phytophagous insects can sequester alkaloids from some host plants that deter predation or predator development (e.g., Mendel and Blumberg 1991; Mendel *et al.* 1992). Host plant volatiles and plant defenses, and the condition of the plant may also influence behavior by attracting or deterring predators (e.g., de Boer and Dicke, 2003; Eisner *et al.* 1998; Palmer 1999).

To reduce these effects we recommend:

1. preliminary tests be run to evaluate predator feeding on target prey reared from as wide a range of plants as possible,
2. where possible, more than one food plant be used for testing a non-target species, and
3. plants with alkaloids, trichomes or pronounced pubescence be avoided in tests.

TEST ENVIRONMENT AND TEST TYPE

The test environment should not be too small to disrupt host location cues or too big making host location impossible.

Test designs should accurately reflect the physiology and behavior of both the control agent and the test species, be standardized and well replicated. Experimental controls should be used in all trials. Both positive controls (target prey) and negative controls (no food) are recommended to understand better the response of the agent to a test species. No-choice tests were considered to be the most appropriate for testing *R. cardinalis* because they allowed us to quickly determine whether a test species was an acceptable prey, and avoided the risk of contamination with target prey semiochemicals. Other options for testing predators are summarized by Van Driesche and Murray (2004).

TEST SPECIES AVAILABILITY

1. Substituting near relatives to confirm general patterns. Because it was hard to locate some priority species for testing, our field collections were sometimes opportunistic using a find and test approach. Testing alternative species (including introduced species) in the same genus or family as those non-target species that could not be located allowed us to test a greater number of species. The rationale used here was that as long as we could define the prey range of *R. cardinalis*, it did not matter if we could not find all the non-target species desired for testing. This approach has been used for testing the host range of other candidate agents and is considered a solution for analyzing the risks of the agent to rare or endangered species (Barratt 2004; Coombs 2004).

2. Acceptability of using field-collected versus laboratory-reared test species. Rearing non-target species in the laboratory was not feasible in Galápagos because: 1) little information was available about the biology/phenology of the non-target species; 2) techniques for growing host plants of phytophagous insects were few, and 3) there was insufficient infrastructure to rear species from other islands under quarantine conditions. By using field-collected specimens we were able to test a wide range of species. Testing field-collected material was deemed acceptable because few Galápagos Homoptera seemed to have parasitoids or pathogens (except for aphids), which could alter the feeding behavior of the biological control agent. Rearing non-target species in organdy sleeves in the field has been suggested as a possible alternative but would only be feasible in areas within easy reach (Van Driesche, 2004). We also found that this ad hoc method of prey collection made coordinating test species collection with predator rearing difficult. In addition to this, the repetition of experiments was not guaranteed unless the test species was available year round. In retrospect, it would have been better to attempt to rear at least the high priority test species in the laboratory to simulate field conditions better.

WHAT IS AN ACCEPTABLE LEVEL OF RISK?

In any risk assessment for biological control two questions will arise: 1) what types of interaction between a biological control agent and the ecosystem are likely and what is acceptable? 2) How much research is necessary before a conclusion can be reached?

There is a general consensus that a biological control agent should not be released if it can complete its life cycle on non-target species of ecological or economic significance, but levels of risk of short-term feeding are not well defined. Moreover, the consequences of interactions other than feeding are rarely considered. Temporary foraging on non-target species is in some cases considered acceptable and necessary for sustaining population numbers of a biological control agent when its target prey population is low (e.g., Sands 1997; Sands and Van Driesche 2000). However, if species of conservation importance are implicated it is preferable to apply the precautionary principle (Cooney 2003). In ecosystems such as Galápagos, short-term feeding and some of the other interactions listed earlier may have considerable impact on non-target species, especially on already threatened endemic species. Even common or non-endemic indigenous species all have some role in ecosystem dynamics and should be protected. Moreover, interactions that cause an increase in an introduced species that is already or could be damaging should be avoided at all cost. In a conservation context, short-term feeding should therefore only be acceptable if it involves a species that is of no value (i.e., introduced species), and this, only if the consequences of this interaction would have no indirect impact on the ecosystem.

Accurate prediction of all potential interactions between a biological control agent and species in the proposed area of introduction is difficult because of a lack of understanding of ecosystem structure and dynamics. Furthermore, even when risk species are identified it may be hard to find or rear them. Nevertheless, the goal of a biological control program should be to understand these interactions as much as possible and to introduce an agent that will interact least with the environment. It is clear that the more restricted the feeding and the ecological habitats of the biological control agent, the fewer the interactions that are likely to occur (Hoddle, 2004).

Determining an acceptable level of risk for introducing a biological control agent will ultimately depend on the immediate and long-term impacts of the target pest and the urgency of mitigating that threat. In Galápagos, evidence about the lack of risk of immature *R. cardinalis* to Galápagos fauna was conclusive (Causton 2003; Causton *et al.* 2004), but the potential impacts of adults could not be tested to the desired level because trials were limited by test species availability. The decision of whether research should continue in order to better understand the potential interactions of adult *R. cardinalis* with indigenous biota was guided by the precautionary principle and the perceived cost-benefit of releasing the biological control agent. Because key habitats and endangered plant species were being seriously affected by *I. purchasi*, the Galápagos National Park concluded that compared to the high costs of not controlling *I. purchasi* immediately, the potential negative effects of releasing *R. cardinalis* into the Galápagos environment would be minimal. The results of the risk assessment indicated that:

- *R. cardinalis* would be effective in controlling the target pest.
- Immature *R. cardinalis* were highly prey specific (demonstrated through tests on a wide range of species).
- A narrow prey range of adult *R. cardinalis* was suggested by feeding trials.
- *R. cardinalis* was distasteful to some vertebrates and should not affect insectivorous predators.
- Damaging interactions with other indigenous biota would be minimal. because of a restricted feeding range resulting in little niche overlap with other species.

Rodolia cardinalis was consequently released in 2002 and has since been liberated on eight islands (Causton 2003b). Monitoring results to date show a decline in *I. purchasi* numbers, recuperation in some plant species, and no interactions with non-targets. Longer term studies will reveal whether this species has had any negative impacts on this fragile ecosystem.

ACKNOWLEDGEMENTS

My thanks to Chris Buddenhagen, Heinke Jäger and Alan Tye for constructive comments on the manuscript. I would particularly like to thank Piedad Lincango for help with the figure. This project would not have been possible if not for the help of a long list of people the names of whom are available in Causton (2004).

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THE HORSE-CHESTNUT LEAF MINER IN EUROPE – PROSPECTS AND CONSTRAINTS FOR BIOLOGICAL CONTROL

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ABSTRACT

The horse-chestnut leaf miner, *Cameraria ohridella* Deschka and Dimic (Lepidoptera: Gracillariidae), is a moth of unknown origin that was first observed in Macedonia in the late 1970s. Since then, it has spread over most of Europe, causing permanent outbreaks on the European horse-chestnut, *Aesculus hippocastanum* L. (Sapindales: Sapindaceae), a major urban tree in Europe. The horse-chestnut is endemic to the Balkans where the few remaining natural stands are also severely attacked, causing concern for the survival of this rare tree species. Classical biological control is considered as the only long-term control option, but shows two major constraints i.e. the fact that the origin of the moth is unknown, and the low number of specific species among the natural enemy complex of Gracillariidae. A collaborative programme has been set up to locate the area of origin of the moth, including the following methods: (1) studies on the parasitoid communities of *C. ohridella* in the Balkans; (2) host tree screening tests; (3) surveys in the potential areas of origin, i.e. the Balkans, Asia and North America, with the help of pheromone traps; (4) molecular studies on *C. ohridella* and congeneric species. If the area of origin is not found, the introduction of parasitoids of congeneric species from Asia or North America could be considered, provided parasitoids specific at genus level are found.

INTRODUCTION

The horse-chestnut leaf miner, *Cameraria ohridella* is a moth of unknown origin that was first observed attacking the European horse-chestnut, *Aesculus hippocastanum* L. in Macedonia in the 1970's, and described as a new species in 1986 (Deschka and Dimic 1986; Simova-Tosic and Filev 1985). In 1989, it was found in Austria, from where it spread to most of Europe. Thanks to two to four generations per year, a high fecundity and the low impact of indigenous natural enemies (Freise *et al.* 2004; Girardoz and Kenis unpublished data; Grabenweger 2003; Grabenweger *et al.* 2005), *C. ohridella* reaches outbreak densities a few years only after its arrival (Gilbert *et al.* 2004). Despite yearly fluctuations in population levels, no outbreak has ever collapsed, even in Macedonia after 30 years of permanent defoliation. Horse-chestnut is one of the most commonly planted shaded trees in European towns and cities. In regions where the pest occurs, the trees are defoliated before the end of the summer, year after years. Studies in Italy have shown that the pest does not affect the survival of the tree (Salleo *et al.* 2003). However, in more northern climates, *C. ohridella* is suspected to cause the decline of horse-chestnut because defoliation induces a second flowering, decreasing frost hardness (Balder *et al.* 2004). Despite a low risk for the survival of the trees in urban areas, the aesthetic damage is so severe that some municipalities are already replacing this highly valuable tree by other species. It has been estimated that the replacement of 80% of the horse-chestnut trees in Berlin alone would cost about 300 million Euros (Balder and Jäckel 2003). The environmental impact of the pest may be even more serious. The horse-chestnut is endemic to the Balkans. The few natural stands remaining in Greece, Macedonia, Albania and Bulgaria are also severely attacked (Avtzis and Avtzis 2003; Grabenweger *et al.* 2005, Tomov and Kenis unpublished data). Studies have shown that, in these areas, the permanent outbreaks may hamper the regeneration process, causing concern for the survival of this rare tree species (Thalmann 2003). In addition, *C. ohridella* is occasionally found attacking and developing on maple trees (*Acer pseudoplatanus* and *A. platanoides*), in which case damage levels may be as high as on horse-chestnut (Freise *et al.* 2003a; Hellrigl 2001). It cannot be ruled out that the damage on maple will increase with time, considering the constant pressure on the moth to find new suitable host trees when horse-chestnut trees are totally defoliated. The reasons for seeking control measures for this pest therefore relate to minimising the impact on planted ornamentals, to the conservation of the remaining natural stands and to minimising the chance of developing strains that may become serious pests of maples in Europe.

Control measures include the aerial spraying of diflubenzuron, the injection of systemic insecticides and the removal of dead leaves, in which pupae overwinter (Heitland *et al.* 2003; Kehrli and Bacher 2003). However, these methods are not sustainable. They are expensive, have to be repeated every year, and chemical treatments are not well perceived by the public. Therefore, biological control methods have been considered. Invasive leaf miners are notorious for being recruited by indigenous parasitoids in the region of introduction, which may results in the control of the pest (e.g., Digweed *et al.* 2003; Urbaneja *et al.* 2000). As expected, *C. ohridella* has been adopted by a whole complex of polyphagous parasitoid species (e.g., Freise *et al.* 2002; Grabenweger 2003; Grabenweger *et al.* 2005; Hellrigl 2001). Nevertheless, parasitism remains unusually low for a leaf miner, even at the type location 30 years after its arrival, and apparently of low incidence on moth populations (Grabenweger *et al.* 2005). Kehrli *et al.* (2005) have developed an ingenious system to augment parasitism at local scale. They stored dead leaves with overwintering moths in containers that, at emergence, allow the parasitoids to escape without their host. They observed increased parasitism rates at the experimental plots, but no effect on moth populations. This system is presently being improved and may, ultimately, provide an interesting method to be used in specific urban environments. Nevertheless, it will never provide a solution for natural horse-chestnut stands. In the long run, unless a native European natural enemy suddenly improves its capability of controlling the moth, the only sustainable solution to the *C. ohridella* problem will be the introduction of an exotic natural enemy, most likely a parasitoid. Classical biological control against *C. ohridella* shows potential, but also major constraints, the two main ones being the fact that the origin of the moth remains unknown, and the low number of specific species among the natural enemy complex of Gracillariidae. This paper reviews these two major constraints and describes collaborative efforts to resolve them.

REGION OF ORIGIN OF *C. OHRIDELLA*

Since the discovery of *C. ohridella* in Macedonia in 1984, the origin of the moth has been a matter of debate. It was first suggested to be a relict species that has survived the Ice Age with its host in southeastern Europe (Deschka and Dimic 1986; Grabenweger and Grill 2000). However, there are several arguments against this theory (Holzschuh 1997; Kenis 1997). Firstly, *C. ohridella* has shown high dispersal capacities in recent years (e.g., Gilbert *et al.* 2004). Thus, if the moth was native to the Balkans, why would it spread only now? Secondly, *Cameraria* is not a European genus. The 74 described, and many undescribed species all occur in North and South America and East and Central Asia (Grabenweger and Grill 2000). Thirdly, parasitism is unusually low for a leaf miner, and composed only of polyphagous species. Finally, since its first observation in Macedonia about thirty years ago, outbreaks in the region have continued unabated and recent surveys in natural stands of *A. hippocastanum* in Greece, Macedonia and Bulgaria have shown that these natural stands are as heavily attacked as planted trees in urban areas (Avtzis and Avtzis 2003; Grabenweger *et al.* 2005, Tomov and Kenis unpublished data). This situation characterizes an exotic rather than a native herbivorous insect. Hellrigl (2001) proposes another hypothesis that needs consideration and may explain some of the anomalies mentioned above. He suggests that the moth may have shifted from another host tree (e.g., an *Acer* species) in the Balkans or the Near East but does not provide conclusive evidence for this hypothesis.

In recent years, various activities have been carried out to help locating the area of origin of the moth. These include (1) studies on the parasitoid complex of the moth in the Balkans; (2) screening tests to assess the host range of *C. ohridella*; (3) surveys in potential regions of origin of the moth; (4) molecular studies on *C. ohridella* and other *Cameraria* spp. The present state-of-the-art of these studies is briefly described herein.

STUDY OF THE PARASITOID COMPLEX OF THE MOTH IN THE BALKANS

Parasitism of *C. ohridella* in Europe has been abundantly studied (e.g., Freise *et al.* 2002; Grabenweger 2003; Hellrigl 2001) and the low parasitism rates and lack of specific parasitoids have since long been used as an argument in favor of a non-European origin of the moth (e.g., Holzschuh 1997; Kenis 1997; Pschorn-Walcher 1994). However, until recently, all these studies on parasitism had been made in invaded areas whereas no data existed on the parasitoid complex in natural stands in the Balkans, where the moth is supposed to have originated. Thus, we conducted surveys for parasitism in these habitats and compared them with data from plantations in the Balkans and Central Europe (Grabenweger *et al.* 2005). The parasitoid complexes are summarized in Table 1. We found marked differences neither in the parasitoid complexes nor in the parasitism rates between natural and artificial stands. Furthermore, all species found during this study were polyphagous parasitoids of various leaf miners. The major difference between the Balkans and the rest of Europe is the prevalence of the eulophid pupal parasitoid *Pediobius saulius*, the dominant species in artificial and natural stand in the Balkans. In Central and Western Europe, it is a common parasitoid of other leaf miners but rarely attacks *C. ohridella.*

HOST RANGE SCREENING TESTS

Screening tests were carried out to assess the present or potential host range of *C. ohridella*. No-choice tests in field and laboratory cages and open-field tests were carried on *Aesculus* spp. and *Acer* spp. Oviposition rates and development successes were observed (see Freise *et al.* 2003a; 2003b for methods). Eleven out of the 13 world *Aesculus* species and many hybrids and *A. hippocastanum* cultivars were screened by Freise *et al.* (2003a,b) (Table 2). The two most suitable hosts were *A. hippocastanum* and the Japanese horse-chestnut *A. turbinata*, whereas successful development also occurred on the American species *A. glabra*, *A. sylvatica* and *A. flava* (= *A. octandra*). In contrast, it did not develop successfully on the Asian *A. chinensis*, *A. assamica* and *A. indica* and on the American *A. pavia*, *A. californica* and *A. parviflora*. *A.* X *carnea*, a hybrid of *A. hippocastanum* and *A. pavia*, often planted in European cities, is also unsuitable for development (Freise *et al.* 2003a,b).

Screening tests on *Acer* spp. are still on-going. Twenty-six European, Asian and North American species have been preliminary screened in non-choice tests in field and laboratory cages. *C. ohridella* oviposited on most of the species but, in general, larvae died in the early stages. Larvae developed successfully in the North American *A. circinatum* and, occasionally, in the European *A. pseudoplatanus*, *A. tataricum* and *A. heldreichii*, and the Asian *A. japonicum* (Heitland and Schlinsog, unpublished data). For unknown reasons, larvae usually failed to pupate. Strong intra-specific variations were observed with several *Acer* species, i.e. larvae developing well in some individual trees and not at all in others. Similar observations

Table 1. Parasitoids of *C. ohridella* found in the Balkans in natural horse-chestnut stands and plantations (Grabenweger *et al.* 2005) and in Austria and Switzerland by the same investigators (Girardoz, Kenis and Quicke, submitted; Grabenweger 2003; Grabenweger and Lethmayer 1999). X = rare; XX = common; XXX = dominant.

	Balkans-Natural stands	Balkans-Plantations	Austria-Switzerland
Eulophidae			
Baryscapus nigroviolaceus (Nees)	XX	XX	X
Chrysocharis nephereus (Walker)	XX	XX	XX
C. pentheus (Walker)		X	X
C. phryne (Walker)	XX		
Cirrospilus elegantissimus Westwood		X	
C. pictus (Nees)			X
C. variegates (Masi)		X	
C. viticola (Rondani)	XX	XX	X
C. vittatus (Walker)		X	X
C. talitzkii (Boucek)	X	X	
Closterocerus lyonetiae (Ferriere)	X	X	
C. trifasciatus Westwood	XX	XX	XX
Elachertus inunctus Nees	X	X	
Minotetrastichus frontalis (Nees)	XX	XX	XXX
Neochrysocharis chlorogaster (Erdos)	X	X	
Pediobius saulius (Walker)	XXX	XXX	X
Pnigalio agraules (Walker)	X	XX	XX
P.pectinicornis (L.)	X	X	X
Sympiesis sericeicornis (Nees)		X	X
Eupelmidae			
Eupelmus urozonus (Dalman)	X	X	X
Pteromalidae			
Pteromalus semotus (Walker)	X	X	X
Braconidae			
Colastes braconius (Haliday)	X		X
Ichneumonidae			
Itoplectis alternans (Gravenhorst)			X
Scambus annulatus (Kiss)	X	X	X

are made in the field in Europe. Most maple trees are apparently resistant to *C. ohridella*, but fully-grown mines are occasionally found on *A. pseudoplatanus* and, rarely, on *A. platanoides*, in which case the tree is usually heavily attacked. Further screening tests are presently being continued with the same and other *Acer* species.

SURVEYS

Surveys have been carried out in potential regions of origin. Two methods have been used: visual inspections of potential host trees and set up of pheromone traps. Surveys have been primarily focused on *Aesculus* spp., but other trees have been inspected to collect *Cameraria* spp. for molecular studies (see below). The regions and *Aesculus* spp. that have been investigated (as in April 2005) are listed in Table 2. At least 13 *Aesculus* spp. are clearly identified in the world, five in Asia, seven in North America and one in Europe. Six additional species have been described from China, but they are nearly indistinguishable from previously described species and their recognition remains tentative (Xiang *et al.* 1998). Most of the 13 *Aesculus* spp. have already been inspected, albeit not all extensively. *C. ohridella* was not found, but other *Cameraria* spp. were collected on *A. turbinata* in Japan, *A. flava* and *A. parviflora* in U.S.A. (M. Kenis, G. Grabenweger and C. Lopez Vaamonde, unpublished data).

Surveys were also made in Greece and Bulgaria, in regions where *A. hippocastaneum* is endemic, to check for the presence of *C. ohridella* on *Acer* spp. The following *Acer* species were inspected: *A. heldreichii*, *A. hyrcanum*, *A. monspessulanum*, *A. obtusatum*, A, *platanoides*, *A. pseudoplatanus* and *A. tataricum*. No mines were found on any *Acer* species. A network of pheromone traps placed in Greece on *Acer* spp., far from infested horse-chestnut trees, did not catch any *C. ohridella* (N. Avtzis, unpublished data).

MOLECULAR STUDIES

Molecular analyses may help locating the area of origin of *C. ohridella* in various ways. Firstly, the genetic variability of European populations can be assessed to describe the phylogeography of the moth in Europe and, especially, to evaluate if European populations come from a single introduction. In other words, a genetic homogeneity in European populations would suggest a non-European origin. Preliminary analyses using isozymes (Perny, 1997) and RAPD-PCR (Kovács *et al.* 2000) showed very little variability among central European populations. However, investigations using additional genetic methods (i.e., AFLPs) and including populations from natural horse-chestnut populations in the Balkans are needed before drawing firm conclusions regarding the genetic variability of European populations.

Molecular techniques can also be used to reconstruct the phylogeny of the genus *Cameraria*. Knowing where, and on which host tree the sister species of *C. ohridella* occurs would be of tremendous help in the search for the area, and tree of origin of the pest. Lopez Vaamonde *et al.* (2003) studied the evolution of host plant use of *Phyllonorycter*, the genus most closely related to *Cameraria*. They used the molecular phylogeny of the moths to reconstruct the ancestral host use and different host switches. The main result was that closely related *Phyllonorycter* species often feed on closely related host plant species, regardless of their geographic distribution. This is interpreted as due to host switching (colonization followed by speciation) being more likely to happen amongst phylogenetically related plants. A

Table 2. Visual surveys and pheromone trapping for *Cameraria* spp. on *Aesculus* spp. (M. Kenis, G. Grabenweger and C. Lopez Vaamonde, unpublished data) and host suitability for *C. ohridella* in screening tests (Freise *et al*. 2003a,b). Only the 13 traditional *Aesculus* spp. of Hardin (in Xiang, 1998) are mentioned here.

***Aesculus* sp.**	**Region**	**Level of Surveys**	**Pheromone Trapping**	**Host Suitabilty**[3]	***Cameraria* sp. Found**[1]
A. turbinata	Japan	high	yes	yes	yes
A. wilsonii	Central China	medium	yes	not tested	no
A. chinensis	China	medium	yes	no	no
A. indica	Himalaya	high	yes	no	no
A. assamica	South-East Asia	none	no	no	-
A. flava	Eastern USA	medium	yes	(yes)	yes[2]
A. glabra	Eastern & Central USA	low	no	yes	no
A. parviflora	Eastern USA	high	no	no	yes[2]
A. sylvatica	Eastern USA	none	no	(yes)	-
A. pavia	Eastern USA	medium	no	no	no
A. parryi	Baja California	medium	no	not tested	no
A. californica	California	high	no	no	no
A. hippocastanum	South-East Europe	high	yes	yes	yes

[1] *Cameraria* sp. found during visual surveys. No *Cameraria* sp. has been caught with pheromone traps.
[2] It is not yet clear whether the same *Cameraria* sp. was found on *A. flava* and *A. parviflora*. *Cameraria aesculisella* is known from *A. flava* and *A. glabra*.
[3] Host suitability: yes = at least 20% of *C. ohridella* reaching the adult stage; (yes) 5-20% reaching the adults stage; no = less than 1% of the mines reaching the adult stage.

similar phylogenetic study is presently being carried out with the genus *Cameraria*. The nuclear gene 28S rDNA (D1-D3 expansion regions) is used to reconstruct a combined molecular phylogeny at species level. Over 30 species have already been collected both in North America (U.S.A. and Mexico) and Asia (Japan and China) on several tree and shrub species, including *Aesculus* spp. and Acer spp. If *Cameraria*'s host range shows a similar phylogenetic conservatism as *Phyllonorycter*, we can expect a molecular phylogeny where *Cameraria* species cluster according to their host plant group. Thus, if *A. hippocastanum* is the original host plant of the latter moth species and not the result of a recent colonization event, we would expect to obtain a clade of *Aesculus* -feeding *Cameraria* spp. including *Cameraria ohridella*. The analyses are presently on-going, but preliminary results show that *C. ohridella* is genetically very distinct from two Asian and North American *Cameraria* spp. collected on *Aesculus* spp. In contrast, it clusters with another species, probably *Cameraria niphonica* Kumata, feeding on *Acer palmatum* and *A. japonicum* in Japan and *A. pseudosieboldianum* in China.

DISCUSSION

LOCATING AREA AND HOST OF ORIGIN

Finding the area and host of origin of *C. ohridella* remains a challenge and no option can yet be discarded. The moth could originate (1) from *Aesculus hippocastanum* in the Balkans; (2) from another host genus in the Balkans; (3) from another *Aesculus* sp. in Asia or North America; (4) from another host genus in a non-European region. However, our investigations described above allow us to better evaluate these possibilities and to narrow our future surveys.

FROM *A. HIPPOCASTANUM* IN THE BALKANS

Although this solution cannot be totally ruled out, it is probably the least likely. The dramatic increase in population densities of a previously undetected insect still needs to be explained. Furthermore, our surveys confirmed that natural, endemic horse-chestnut stands in the Balkans are also seriously affected and that parasitism in these stands is as low as elsewhere in Europe. This situation strongly suggests an "unnatural host-herbivore association". The best way to verify whether *A. hippocastanum* is the original host tree of *C. ohridella* would be to perform molecular analyses to measure the genetic heterogeneity of moth populations in natural horse-chestnut stands in the Balkans.

FROM ANOTHER HOST PLANT IN THE BALKANS

A host-shift from another host plant could partly explain the persistent outbreak populations, limited parasitism and sudden spread in Europe. However, the original host remains to be found. The lepidopteran fauna of the Balkans is relatively well known, and the moth has never been observed on any plant species before its first record on horse-chestnut in the 1980s. The only other plant genus which is suitable for the development of *C. ohridella* is *Acer*, which is also the closest relative to *Aesculus* in Europe. All *Acer* species occurring in the Balkans have been checked for *C. ohridella*, without success. Dramatic host-shifts have been reported from many insects, including gracillariid leaf miners. For example, *Phyllonorycter messaniella* (Zeller), a polyphagous European leaf miner largely increased its host range to other families when introduced in New Zealand (Wise 1953). Similarly, the Californian species *Marmara gulosa* Guillén and Davis apparently expanded its host range from native willows to various introduced plants such as citrus, avocado, cotton and oleander (Guillén *et al.* 2001). However, host-shift in insects usually occurs when an insect encounters a new plant, i.e. when either the plant or the insect is exotic. Since *A. hippocastanum* is endemic to the Balkans, a host-shift would be more conceivable if *C. ohridella* was exotic. Nevertheless, to verify the hypothesis of another original host plant in the Balkans, networks of pheromone traps could easily be set up in various environments.

FROM ANOTHER *AESCULUS* SP. IN ASIA OR NORTH AMERICA

This option remains plausible although host screening tests and surveys have seriously limited the possible host trees. *Aesculus turbinata*, the sister-species of *A. hippocastanum* (Xiang 1998) was also the most suitable alternative host for *C. ohridella* in our screening tests. However, despite extensive surveys in Japan, *C. ohridella* was not found. Other Asian *Aesculus* spp. are apparently unsuitable for the development of the moth, and surveys in China and Pakistan also remained unsuccessful. Among the North American species, three species are suitable hosts for *C. ohridella*: *A. sylvatica*, *A. glabra* and *A. flava*. *A. sylvatica* has a very limited distribution in south-eastern U.S.A. and has never been checked for *C. ohridella*. *A. glabra* has not yet been surveyed extensively and has a large geographic range. *Aesculus* spp. are rather insignificant tree and shrub species in North America and their fauna has rarely been studied. Therefore, a rare, specific leaf miner may have remained undetected. Additional surveys should focus on these three species.

FROM ANOTHER HOST GENUS IN A NON-EUROPEAN REGION

The low probability that *C. ohridella* originates from the Balkans and the limited number of *Aesculus* spp. that remain plausible hosts of origin have made this hypothesis more likely. *Acer* has been sometimes cited as potential host genus of origin (e.g., Hellrigl, 2001) and it is indeed the most likely. *Acer pseudoplatanus* and *A. platanoides* are, until now, the only two non-*Aesculus* species that are occasionally attacked in Europe. Screening tests showed that *C. ohridella* oviposits on most tested *Acer* spp. and develops at least to mature larvae on a few of them. Furthermore, preliminary results of the phylogenetic study of the genus *Cameraria* suggest that the sister species of *C. ohridella* feeds on *Acer* spp. in China and Japan. Based on recent phylogenetic studies, *Acer* and *Aesculus* are now both placed in the family Sapindaceae (APG 1998) a family which comprises mainly tropical and sub-tropical genera. Considering that overwintering *C. ohridella* pupae are easily able to survive low winter temperatures in eastern and northern Europe, it can reasonably be expected that *C. ohridella* originates from a temperate region. Thus, most other members of the Sapindaceae family can be discarded. Interestingly, during our surveys in Japan, another *Cameraria* sp. has been found mining both *Aesculus turbinata* and *Acer mono*, suggesting that the switch from *Aesculus* to *Acer* and vice-versa is possible. Nevertheless, host-shifts such as those of *Phyllonorycter messaniella* and *Marmara gulosa* show that phyllogenetically unrelated hosts can sometimes be adopted. Thus, other host genera in other families cannot be excluded for *C. ohridella*. Surveys should be carried out in temperate regions in the distribution range of *Cameraria* spp., i.e. North America and East or Central Asia. Considering that the Lepidopteran fauna, particularly that of *Acer* spp., is quite well known in North America and Japan, surveys should focus primarily on China and other rather unexplored countries.

PROSPECTS FOR FINDING SUITABLE BIOLOGICAL CONTROL AGENTS

If the area of origin of *C. ohridella* is found outside Europe, studies on its natural enemies should be carried out in this region to select biological control agents, probably parasitoids, to be introduced into Europe. If it appears that the moth originates from the Balkans, or if the area of origin remains undiscovered, an alternative would be to look for parasitoids of other *Cameraria* spp. (Kenis 1997). Studies should focus either on other *Cameraria* spp. on *Aesculus* spp. or on the sister species, *C.* ? *niphonica* feeding on *Acer* spp. in China and Japan. However, whatever the source of parasitoids, it is not certain that suitable species will be found for introduction into Europe. Nowadays, natural enemies used in classical biological control have to show a high degree of host specificity, to avoid non-target effects in the region of introduction (Kuhlmann and Mason 2003). Gracillariid leaf miners are known to be attacked mainly by polyphagous parasitoids (Askew 1994). No other *Cameraria* species occurs in Europe and, thus, a parasitoid specific at genus level would be acceptable. Similarly, since no native leaf miner occurs on horse-chestnut in Europe, a parasitoid that would be specific to leaf miners on *Aesculus* spp. would be suitable as well. Parasitoid complexes of *Cameraria* spp. are poorly known. Literature data are lacking for Asian species and scarce for North American species, usually with little or no information on parasitism rates or host specificity. Host-parasitoid data bases such as those of Krombein *et al.* (1979), Yu (1999) or Noyes (2002) provide useful reviews, but are poor information sources for parasitoid host range because they include unchecked literature records which are full of errors in parasitoid or host identification and wrong host-parasitoid associations. Among the 46 parasitoid species recorded from *Cameraria* spp. in these reviews, only seven are not recorded from another host genus (Table 3). Many genera, however, are typical koinobiont endoparasitoids: *Mirax*, *Phanomeris*, *Orgilus* (Braconidae), *Ageniaspis*, *Copidosoma* (Encyrtidae), *Achrysocharoides* (Eulophidae). Most of these are surely more host specific than the usual idiobiont leaf miner parasitoids. These genera are not represented in the parasitoid complex of *C. ohridella* in Europe.

Besides *C. ohridella*, the only other *Cameraria* species that has been the target of a specific study on its parasitoid complex is *C. caryaefoliella* (Clemens), a minor pest of pecan in North America. Heyerdahl and Dutcher (1985) listed 22 parasitoid species attacking *C. caryaefoliella*. More interestingly, they collected, with *C. caryaefoliella,* three other pecan leaf mining moths, the nepticulid *Stigmella juglandifoliella* (Clemens), the heliozelid *Coptodisca lucifluella* Clemens and the gracillariid *Phyllonorycter caryaealbella* (Chambers). Seven of the 22 parasitoids found on *C. caryaefoliella* were found only on this host, among which three were qualified as “common”: the encyrtid *Copidosoma* sp., and the eulophids *Achrysocharoides bipunctatus* (Girault) and *Pnigalio* sp. nr. *pallipes* (Heyerdahl and Dutcher 1985). This suggests that parasitoids of *Cameraria* spp. may be more specific than indicated in the parasitoid lists as those presented in Table 3.

Few publications mention parasitism rates data on the impact of natural enemies on the control of *Cameraria* spp. Heyerdahl and Dutcher (1990) measured mean parasitism rates of 24-34% and 14-42% in larvae and pupae of C. *caryaefoliella,* respectively. Faeth (1980) found a parasitism rate of 48% in an undescribed *Cameraria* sp. on oak and Connor (1991) measured 22 to 55% apparent parasitism in *C. hamadryadella* (Clemens) on four different oak species. These parasitism rates are higher than those observed on *C. ohridella.* Furthermore, they probably underestimated the impact of parasitism in the *Cameraria* populations. Faeth (1980) and Connor (1991) noted that the total larval and pupal mortality was 94-100%, predation and “unknown” mortality being mentioned as other main mortality factors. As noted by Connor (1991), high predation rates mask or reduce the estimated rate of parasitism because predators prey as well on parasitized mines. Furthermore, unknown mortality probably includes host feeding and pseudoparasitism, as observed by Heyerdahl and Dutcher (1990).

Hardly anything is known of parasitism of *Cameraria* spp. in Asia. In our recent surveys, we made opportunistic observations on parasitism in *C.* ? *niphonica* on *Acer* spp. in China and Japan and a *Cameraria* sp. on *Aesculus turbinata* and *Acer mono* in Japan. Based on emergence data only, parasitism of *C.* ? *niphonica* was 92% (n=26) in China and 62 %

(n=52) in Japan, whereas parasitism of *Cameraria* sp. in Japan was 76% (n=21) (Kenis, unpublished data). Parasitism was equally shared between braconids and eulophids. These numbers have to be taken with great caution because mortality in rearing was high and probably affected more the hosts than the parasitoids. Nevertheless, these data and the previously cited American publications clearly show that parasitism is more important on native *Cameraria* spp. than on *C. ohridella* in Europe. The exact role of parasitism on the population dynamics of *Cameraria* spp. in their native range is not known but we believe that parasitoids are at least partly responsible for the lower population levels compared to *C. ohridella*. Life table studies have been carried out for *C. ohridella* in Switzerland and Bulgaria, which include assessments of the respective mortality factors on the different developmental stages (Girardoz, Tomov and Kenis, in preparation). Similar studies should be made on native *Cameraria* spp. to better understand the factors causing the long-lasting outbreaks observed in Europe.

ACKNOWLEDGEMENTS

This project is a collaborative work between several teams. In addition to the authors of the paper, many more colleagues and students participated surveys, collections, laboratory work, placement of pheromone traps in various regions of the world, and parasitoid identification. They are too numerous to be cited here but are all warmly thanked. We also thank Mathew Cock for his comments on the manuscript. This study was funded by the European Commission and the Swiss Federal Office for Education and Science, as part of the FP5 project CONTROCAM, QLK5-CT-2000-01684.

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METAMASIUS CALLIZONA IS DESTROYING FLORIDA'S NATIVE BROMELIADS

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ABSTRACT

Bromeliads (Bromeliaceae) are a family of about 2,500 species native to the Neotropics, with 16 native to Florida. For decades, enthusiasts have imported into Florida numerous species from Neotropical countries for their attractive foliage and colorful flowers. The impression of enthusiasts, still fostered by growers' manuals, was that bromeliads have no serious pest insects – only a few easily controllable scale insects and mealybugs (Coccoidea). In 1989, an unidentified weevil was detected on ornamental bromeliads at a nursery in Ft. Lauderdale. The nursery was treated with chemicals to eradicate the population, but too late – surveys showed that this weevil was established on native Florida bromeliads in nearby county parks. It was *Metamasius callizona* (Chevrolat) (Coleoptera: Dryophthoridae, formerly Curculionidae). It had been shipped from infested shadehouses in the state of Veracruz, Mexico. USDA-APHIS records showed interceptions year after year of this and other *Metamasius* weevils on bromeliads imported to Florida. There was little knowledge of it in Mexico except as an occasional pest of cultivated pineapples, which was unpublished information at the time. A chemical eradication attempt was not supportable because (1) the weevil was already in county parks that may not be treated with chemicals, and (2) there were no funds except for eradication of major agricultural pests. Bromeliad enthusiasts called for research but could raise little money. Their immediate needs were met by the discovery that carbaryl (Sevin®) would control bromeliad weevil in plant collections. As the multivoltine weevil population spread from county to county destroying native bromeliad populations and invading state parks, botanists of Florida's Endangered Plant Advisory Council became alarmed and declared two more of Florida's native bromeliad species to be endangered (Florida Administrative Code). Brief searches for potential biological control agents were carried out in Mexico, Panama, and Honduras. In Honduras, an undescribed fly (Diptera: Tachinidae, cf. *Lixophaga* sp.) was found as a parasitoid of the closely related *M. quadrilineatus* Champion, a species that inhabits remnant cloud forests on hilltops. Climatic conditions could not be duplicated in Florida's unsuitable main quarantine facility, and attempts to rear this fly failed. Recently, Florida Park Service personnel saw the devastation to native bromeliad populations and became alarmed. Increased funding led to further exploration in Guatemala, Belize, Mexico

(again) and even Paraguay for candidate biological control agents. None, other than the Honduran tachinid fly (also found in Guatemala), was detected. Research efforts were then concentrated in Honduras where adequate stock of the fly could be obtained and maintained at temperatures cooler than obtainable in Florida's Gainesville quarantine facility. Stock of *M. callizona* and *M. mosieri* Barber (a native Florida non-pest species) has been supplied to Honduras for tests. A new Biological Control Research and Containment Laboratory at Ft. Pierce has just become operational. At time of writing, the weevil's population was outside the northern edge of Everglades National Park.

INTRODUCTION

Bromeliads (Bromeliaceae) are a family of about 2,500 species native to the Neotropics, with 16 native to Florida. For decades, enthusiasts have imported to Florida numerous species from Neotropical countries for their attractive foliage and colorful flowers. From these have been created numerous hybrids. The impression of enthusiasts was that bromeliads have no serious pest insects – only a few controllable scale insects and mealybugs – a false idea that is perpetuated in horticultural advice to growers (e.g., Black and Dehgan 1993; Wall 1988). *Ananas comosus* (L.), pineapple, is the only major agricultural bromeliad crop, but its extent is trivial in Florida.

In 1989, an unfamiliar weevil was detected on ornamental bromeliads at a nursery in Ft. Lauderdale. The nursery was treated with chemicals to eradicate the population, but too late – surveys showed that this weevil was established on native epiphytic Florida bromeliads in nearby county parks. It was found to be *Metamasius callizona* (Chevrolat), native to Mexico and Guatemala, and believed at the time also to occur in other Central American countries (O'Brien and Thomas 1990). It was the realization that the larvae do not merely feed on native bromeliads, but kill them (Frank and Thomas 1991a), that made this pest serious. The probable origin was traced to infested shadehouses in the state of Veracruz, Mexico (Frank and Thomas 1994). USDA-APHIS records showed interception year after year of this and other *Metamasius* weevils on bromeliads imported to Florida. Exporters (in Latin America) and importers (in Florida and other parts of the U.S.A.) had been careless. USDA-APHIS inspection of imported plants at U.S. ports and airports examines fewer than 2% of shipments (Frank and Thomas 1994). This had been a potential disaster waiting for years to manifest itself. If the infested plants had been shipped to a nursery in most states of the U.S.A., the weevils might have harmed only the stock of that nursery. But in southern Florida, susceptible native bromeliads grow in trees in close proximity to nurseries, easing establishment barriers for weevils outside of cultivation.

A chemical eradication attempt in Florida was not supportable because (1) the weevil was already in county parks which may not be treated with chemicals, (2) the situation was unprecedented and could not be evaluated confidently, (3) there were no funds for eradication except of major new pests of major agricultural crops (e.g., citrus), and (4) there are no low density monitoring tools to accurately measure the efficacy of eradication attempts.

There was little knowledge of *M. callizona* in Mexico except as an occasional pest of cultivated pineapples, unpublished until Rebolledo *et al.* (1998). *M. callizona* invades pineapple fields in newly-cleared land close to forest edges and has been controlled by use of broad-spectrum chemicals. Bromeliad enthusiasts in Florida called for research for control but could raise little money. Their immediate needs were met by the discovery that carbaryl (Sevin®) would control *M. callizona* in their collections. However, all of Florida's native bromeliads are epiphytic, and chemical control of weevils in bromeliads growing high above ground over large areas of land is an infinitely more difficult proposition technically, environmentally, legally, and financially.

Lack of feasibility of chemical eradication led to initiation of a biological control project. The project is far from complete. This paper reviews its progress to date.

M. CALLIZONA DEVASTATES NATIVE FLORIDA BROMELIAD POPULATIONS

As the *M. callizona* population spread from county to county in native bromeliad populations, botanists of Florida's Endangered Plant Advisory Council became concerned. They declared two more of Florida's native bromeliad species to be endangered. This brought to 10 the number of native species listed as threatened or endangered under the Florida Administrative Code (1998) (Table 1). Florida law is independent of federal U.S. law in this respect, for none of these species is so listed under the federal Endangered Species Act. Suffice it to say that all the native Florida bromeliads but one are known at the species level to occur also in the West Indies. So of course is the West Indian manatee, a 'charismatic' species whose Florida populations have been declared to be a distinct subspecies, and it is the Florida subspecies which is 'an endangered species' under federal law. One Florida bromeliad species occurs only in Florida (is precinctive) and has now been found to be attacked by *M. callizona*. Twenty years ago this might have resulted in its federal listing as an endangered species, but now the Environmental Protection Agency is under pressure to prevent expansion of the list of endangered species. That species, *Tillandsia simulata*, was only belatedly found to be attacked because its range is in central Florida, north of the counties where weevil-caused damage was at first concentrated.

Monitoring of the spread of *M. callizona* in Florida has been intermittent since 1989 (Frank 2005). The spread of the weevil now seems likely to continue until it has overrun all parts of Florida having those 12 native bromeliad species with individuals capable of growing to a considerable size (Table 1, Fig. 1). Spread is not only by flight of adults but also caused by people carelessly moving infested ornamental bromeliads from place to place. There is one curious anomaly: by 1991 the weevil was found in a county park in southern Miami-Dade County (Frank and Thomas 1991b), but was not found in that park after hurricane Andrew (August 1992), and has not been detected elsewhere in that county despite relatively frequent surveys.

Invasion of Broward County parks in 1989-1991 left drifts of fallen large bromeliads (mostly *T. utriculata*, Fig. 2). Visits to those same parks in 1999 showed very sparse *T. utriculata* populations, with *M. callizona* infesting some of the few remaining larger plants. The slow growth of the plants to flowering size (upward of 10 years), and apparent preference of *M. callizona* for attacking large plants strongly suggested a powerful negative effect on *T. utriculata*

Table 1. Florida bromeliad species, their abundance and status under Florida law, and susceptibility to attack by *M. callizona* larvae.

Bromeliad Species	**Florida Status**	**Attacked by *M. callizona* Larvae?**
Catopsis berteroniana Schult. (f.) Mez	Rare, endangered	probably[1]
Catopsis floribunda L.B. Sm.	Rare, endangered	probably[1]
Catopsis nutans (Sw.) Griseb.	Very rare, endangered	probably[1]
Guzmania monostachia (L.) Rusby *ex* Mez	Rare, endangered	yes[2]
Tillandsia balbisiana Schult. and Schult. f.	Occasional, threatened	yes
*Tillandsia fasciculata S*w.	Frequent, endangered[3]	yes
Tillandsia flexuosa Sw.	Infrequent, threatened	yes
Tillandsia paucifolia Baker	Occasional	yes
Tillandsia pruinosa Sw.	Rare, endangered	probably[1]
Tillandsia simulata Small	Frequent[4]	yes
Tillandsia utriculata L.	Frequent, endangered	yes
Tillandsia variabilis Schltdl.[5]	Occasional, threatened	yes
Tillandsia bartramii Elliott	Frequent	no, too small
Tillandsia recurvata (L.) L.	Common	no, too small
Tillandsia setacea Sw.	Common	no, too small
Tillandsia usneoides (L.) L.	Common	no, too small

[1] The three *Catopsis* spp., *G. monostachia*, and *T. pruinosa* have not yet been observed to be attacked in nature, probably because of their rarity.
[2] Cultivated specimens of *G. monostachia* and *Catopsis* spp. have been attacked.
[3] *T. fasciculata* is much less susceptible than T. *utriculata* perhaps because of its high fibrosity. Perhaps it was listed as endangered because of its high phenotypic variability. It is still illogical that it should have been listed as endangered, but *T. paucifolia*, which is readily attacked and much less common, is not.
[4] Precinctive; native to and occurring only in central Florida.
[5] Listed as *T. valenzuelana* Richard in Florida Administrative Code (1998).

population sustainability. The research project of graduate student Teresa Cooper (Cooper 2005) is an evaluation of the dynamics of the bromeliad populations attacked by *M. callizona*, especially in the Myakka River State Park.

Most of the larger native bromeliads (Table 1), led by *T. utriculata* and *T. fasciculata*, the species of *Catopsis* and *Guzmania*, down to *T. flexuosa*, *T. simulata* and *T. variabilis*, impound water in their leaf axils, forming phytotelmata. The phytotelmata house an aquatic invertebrate fauna causing no harm to the plants (Frank 1983). An estimated 15 of these invertebrate species, several of which have not yet been described by taxonomists, seem to be specialists, living only in bromeliad phytotelmata (Frank 1983 and unpublished). Destruction of their host plants by *M. callizona* necessarily destroys populations of these invertebrates. A

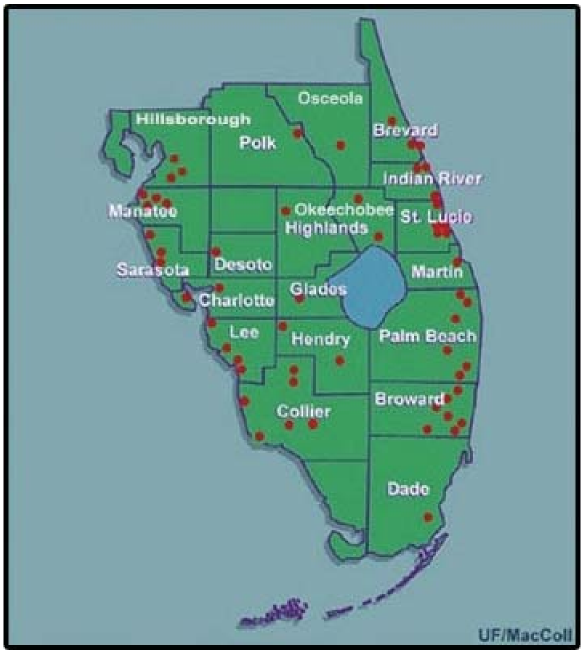

Figure 1. Recorded distribution of *Metamasius callizona* in central and southern Florida and showing county boundaries and their names, as of early 2005.

Figure 2. A fallen *Tillandsia utriculata* showing *Metamasius callizona* cocoons in mined stem. Photo: J. L. Castner. UGA1390013

Figure 3. An adult *Metamasius callizona*. Length 11-16 mm. Photo: J. L. Castner. UGA1390014

few of these invertebrate species, especially the mosquitoes among them, were found to have adapted their existence in phytotelmata of nonnative ornamental bromeliads cultivated in urban habitats (Frank *et al.* 1988), and some of these ornamental bromeliads seem not to be attacked by *M. callizona.* Nevertheless, dependence upon cultivated bromeliads in urban habitats in southern Florida makes for a tenuous existence. The two most abundant mosquito species in native bromeliads are *Wyeomyia mitchellii* (Theobald) and *W. vanduzeei* Dyar and Knab. Females of both species take blood from humans and are pests. However, they are specialists to the bromeliad habitat and, in bromeliads in Florida, their larvae outcompete and exclude invasive mosquitoes whose females are known to be able to transmit viral diseases to humans (Lounibos *et al.* 2003).

If *M. callizona* (Fig. 3) eradicates 12 native bromeliad species in Florida, it also is likely to eradicate up to 15 invertebrate species. A loss of 27 species from a single cause may be the worst ecological disaster to befall Florida by an invasive terrestrial arthropod.

BEHAVIOR OF SOME *METAMASIUS* WEEVILS

Thirty-two bromeliad-eating weevil species are known in the Neotropics (Frank 1999). Three of them, and one other *Metamasius* species, are important to this project.

METAMASIUS MOSIERI BARBER

This weevil was described from Florida and Cuba in 1920, and later was reported from the Dominican Republic. For lack of other evidence, it has been assumed to be native to all three land masses. Little was known about *M. mosieri* (Fig. 4) until it was encountered sometimes in searches for *M. callizona*. Adults and larvae are considerably smaller than those of *M. callizona*. Larvae develop in small *Tillandsia* bromeliads, including *T. balbisiana*, *T. paucifolia*, *T. setacea*, *T. simulata*, *T. utriculata*, *T. variabilis* (Larson *et al.* 2001) and occasionally small nonnative *Tillandsia* in growers' collections (Frank 1999). When *T. utriculata* is attacked, it is only small specimens that are attacked. When *T. balbisiana* is attacked, it is often the flower spike that is mined. Why larger plants are not attacked is unknown. Adults are distinctively colored. Larvae are very difficult to distinguish from small *M. callizona* larvae. The species has been detected only in a few counties in the southwest and the southeast. Why it is not more widely distributed, and why its populations are not abundant are unknown. A few score larvae have been collected in the field and reared, but none has produced adult parasitoids. Larvae are very sensitive to ambient conditions and are harder than those of *M. callizona* to rear. In our biological control attempt against *M. callizona* in Florida, *M. mosieri* will be treated as a nontarget native species.

METAMASIUS QUADRILINEATUS CHAMPION

This weevil attacks bromeliads in Mexico, Guatemala, El Salvador, and Honduras. At least in Honduras and Guatemala, it is host to the only parasitoid of bromeliad-eating weevils that we have yet discovered (see below). Females are believed to oviposit only in fallen epiphytic bromeliads that have tipped, draining the water impounded in their leaf axils (Alvarez del

Figure 4. An adult *Metamasius mosieri***.** Length 6-9 mm.
Photo P. M. Choate. UGA139000515

Hierro and Cave 1999). Thus, the weevil is of little or no threat to bromeliad populations. Indeed at the high altitudes in Honduras where this weevil has been found, native bromeliad populations seem to thrive in the presence of a thriving weevil population and seasonally abundant parasitoids. The bromeliads, the weevil and its parasitoid may exist at high elevations (in remnant cloud forest above 1600 m) by climatic restriction, or they may have existed at lower elevations before clearing of vast tracts of forest for agriculture; we do not know which. In the former case, none of them (including the parasitoid) may be able to exist at Florida's low elevations because of higher summer temperatures.

METAMASIUS HEMIPTERUS (L.)

This weevil was detected in Florida in 1984 as an invasive species from the Neotropics (O'Brien and Thomas 1990). It attacks banana, some ornamental palm trees and, rarely, ripe pineapple fruits. Its larvae have not been found to attack native Florida bromeliads. It is to be treated as a nontarget species in our project. Attack on it by any biological control agent that we establish against *M. callizona* could only be beneficial, but this would indicate a wider host range and might require testing of the susceptibility of other weevil genera.

METAMASIUS CALLIZONA

The objective of our project is a substantial reduction in population densities of this weevil. Adult weevils nibble on leaves of a wide range of bromeliads. Females oviposit in a narrower taxonomic range of bromeliads. Eggs are laid in slits that are cut in leaf bases and egg-laying is restricted to bromeliads of a size that will allow development of at least one larva mining in the meristematic tissue. The minimal size of *T. utriculata* plants in which *M. callizona* will oviposit is larger than 11.9 cm diam. (length of longest leaf 9.8 cm) (Sidoti and Frank 2002). The egg takes 7-10 d at 26°C to incubate (Salas and Frank 2001). Hatchling larvae tunnel into the meristematic tissue and begin to mine. When larvae were reared on pineapple stems at

26°C, five instars were detected before larvae pupated, the pupal stage lasted 9-15 days, and the total developmental time from egg to adult was about 8 wk (Salas and Frank 2001). Prepupae construct cocoons of plant fiber. Longevity of adults, preovipositional period of females, and total fecundity are under investigation. All life stages may be found throughout the year in natural areas of Florida. Generations are not discrete and the mean generational time of eggs to adults is speculated to be 13-17 wk (Salas and Frank 2001).

COMPONENTS OF A PROJECT AGAINST *M. CALLIZONA*

The initial components included (a) a literature search, (b) monitoring spread of *M. callizona* in Florida, (c) recording native host plants attacked, (d) collecting living specimens from nature in Florida to determine whether any contained parasitoids, (e) studying the life cycle of *M. callizona*, (f) collecting and studying the nontarget species *M. mosieri*, (g) testing and recording effects of *M. callizona* on ornamental (non-native) species, (h) answering grower questions about control methods, including feasibility of chemical control, and (i) preparing and delivering information to people concerned with the native bromeliads in various parts of Florida, including construction of websites (http://BromeliadBiota.ifas.ufl.edu/wvbrom.htm and http://SaveBromeliads.ifas.ufl.edu).

All of this led to the need to obtain funds to hire personnel and for foreign exploration to study *M. callizona* in its native habitats, and to detect potential biological control agents. The first funding agency strongly suggested it might contemplate funding the project if a seed-collecting project were begun. The idea was widespread collection of viable seed of the species at risk, their germination and growing out to replace the seedlings in nature once the weevil population had begun to decline. In desperation for funds, we undertook this. We were greatly helped by two professional growers who handled the seeds and their germination once these were supplied, but we needed state permits for these growers to grow the seed. Volunteers helped to collect the seed, but we needed state and county permits for them to do so as well. Further, a computerized database had to be constructed to document and catalogue the seed collections.

THE SEARCH FOR A BIOLOGICAL CONTROL SOLUTION

Lack of feasibility of chemical eradication of *M. callizona* stimulated interest in biological control. Nothing was known about parasitoids of any of the bromeliad-attacking *Metamasius* spp. Brief searches for potential biological control agents were carried out in Mexico and Panama. In Veracruz and Oaxaca, Mexico (1992), *M. callizona* was found abundantly only in the infested shadehouses of the grower whose carelessness caused its invasion of Florida. Over 100 larvae brought to a containment facility in Florida produced healthy adults, with no evidence of parasitoids. In Chiriquí, Panama (1994) *M. callizona* was not found, but a close relative, *M. cincinnatus* Champion with the same behavior was found abundantly only at one locality in nature. Again, over 100 larvae brought to Florida produced only healthy adults. In Honduras, an undescribed fly (Diptera: Tachinidae, cf. *Lixophaga*, Fig. 5) was found as a parasitoid of the closely related *M. quadrilineatus* Champion, a species that inhabits remnant cloud forests on hilltops. The fly and its host became the subject of a Honduran student project, and were reared successfully at 20°C (Alvarez del Hierro and Cave 1999). Climatic conditions for rearing could not be duplicated in Florida's unsuitable main quarantine facility. A few fly larvae were transferred successfully to *M. callizona* larvae in 1998-1999 and developed, but attempts to establish a colony failed.

Figure 5. An adult male tachinid cf. *Lixophaga*. Photo: B. C. Larson. UGA1390016

More recently, Florida Park Service personnel saw the devastation to native bromeliad populations and became alarmed. Increased funding led to further exploration in Guatemala, Belize and Mexico without discovery of additional parasitoids (Cave *et al.* 2004). Even Paraguay in 2004, and Peru in 2005 were explored for candidate biological control agents of any bromeliad-attacking *Metamasius* sp. None, other than the Honduran tachinid fly (also found in Guatemala in *M. quadrilineatus*), was detected. Research efforts were concentrated in Honduras where adequate stock of the fly could be obtained and maintained at temperatures cooler than obtainable in Florida's Gainesville quarantine facility. Larvae of *M. callizona* and *M. mosieri*, grown in Florida, have been supplied month after month to Honduras for tests. Studies there have confirmed that the tachinid cf. *Lixophaga* will attack *M. callizona*, and does so at least as readily as it will attack *M. quadrilineatus* under experimental comparison. *Metamasius mosieri* has been demonstrated to be an appropriate host for the tachinid cf. *Lixophaga*, but *M. callizona* appears to be a preferred host. Many other questions are being investigated by postdoctoral researcher Alonso Suazo. A new Biological Control Research and Containment Laboratory became operational in February 2005 in Ft. Pierce, Florida, and it is to that facility that a stock population of the tachinid will be shipped as soon as possible. The weevil's population is now at the edge of Everglades National Park (Fig. 6), and the control situation is critical.

Figure 6. A scene from the Everglades showing the color form (yellow bracts) of *Tillandsia fasciculata* that exists there and, above and to the left, *Tillandsia paucifolia*. Both species are under attack by *Metamasius callizona*. Photo: J. H. Frank. UGA1390017

CONCLUSIONS

This is an unusual biological control project in that it attempts to control an invasive species that endangers native flora. As such, it has little easily-documented economic effect. Adequate funding for the necessary components of the project has been especially difficult to obtain and there is no certainty of its continuation. Progress has been made, but we do not yet know whether the only detected potential biological control agent will survive in Florida's climate, what effect it will have on non-target species, nor whether a release permit will be issued. We do not know whether it will be able to reduce populations of *M. callizona*. We do not know to what extent *M. callizona* will eradicate bromeliad species from Florida if left uncontrolled. It would take little phytophagy to completely eradicate the rarest species. The current measured rate of disappearance of *T. utriculata*, still widespread but becoming rarer, if it were continued, suggests that total eradication would be possible for that bromeliad. Most of the other bromeliads now under attack or likely to be attacked in nature may meet the same fate. Expect publications by Teresa Cooper on the dynamics of weevil-attacked bromeliads in Florida, and by Alonso Suazo on rearing conditions and some host-range testing of the tachinid cf. *Lixophaga* in Honduras. Also, we expect to publish a taxonomic description of the tachinid fly by Monty Wood (Ottawa, Canada). Wish us luck in Florida in successful importation of the fly, laboratory colonization, nontarget testing, release permitting, establishment in the field, and control of *M. callizona*.

ACKNOWLEDGEMENTS

The inspiration and early support for a biological control project against *M. callizona* came from the Florida Council of Bromeliad Societies. The Florida Department of Agriculture and Consumer Services supported the project for two years. Dr. Barbra Larson, as postdoctoral associate with the project, developed the website http://SaveBromeliads.ifas.ufl.edu, other educational materials, and grant applications.The Park Service of the Florida Department of Environmental Protection supported it for three years. The U.S. Environmental Protection Agency provided a grant to allow production of materials for public education. Drs. Frank Slansky Jr. and Oscar Liburd kindly reviewed a manuscript draft and made critical comments. Useful editorial suggestions were made by Dr. Mark Hoddle.

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BIOLOGICAL CONTROL OF THE EUROPEAN GREEN CRAB, *CARCINUS MAENAS*: NATURAL ENEMY EVALUATION AND ANALYSIS OF HOST SPECIFICITY

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ABSTRACT

Many introduced marine organisms are ecological and economic pests. Nevertheless, no management approach is available to mitigate their impacts. Now, a theoretical perspective borrows principles from classical biological control, as widely applied to terrestrial and fresh water systems, to control the abundance of introduced marine pests.

A banner example of a marine pest is the European green crab, *Carcinus maenas* (L.) (Brachyura: Cancridae). This crab invaded San Francisco Bay in the late 1980s and has expanded its geographic range at a record rate. Studies on the Pacific coast of the U.S.A. and elsewhere strongly indicate that it is a worst-case introduction, affecting native organisms and potentially harming fisheries and aquaculture. Extensive studies show that a significant element of its success, where introduced, has been release from its natural enemies, notably parasites.

Natural enemies are infectious agents with potential to contribute to green crab biological control include two parasitic castrators: *Sacculina carcini* Thompson (Rhizocephala: Sacculinidae) and, *Portunion maenadis* Giard (Isopoda: Entoniscidae), the parasitoid flatworm, *Fecampia erythrocephala* Giard (Fecampiida: Fecampiidae), and the symbiotic nemertean egg predator, *Carcinonemertes carcinophila* (Kolliker) (Hoplonemertea: Carcinonemertidae). Evidence for their potential efficacy derives from quantitative natural history studies and ecological modeling. Their safety with respect to non-target organisms is of great concern. Relevant information is available from qualitative field studies, host specificity experimentation, evolutionary theory, and the ability to detect and estimate the frequency of unsuccessful parasite attack rates in natural populations.

We interpret host specificity in the context of the double filter paradigm of Combes (2001). For a host to be suitable, two filters must be partially open. When the encounter filter is open, the infective stage can locate and enter the host because it shares the appropriate habitat and has suitable host location and attack behaviors. For the parasite to be successful, the compatibility filter must also be partially open. This occurs if the host provides appropriate nutrition and its defensive mechanisms cannot kill nor block reproduction of the infectious agent. For both rhizocephalan barnacles and entoniscid isopods in native (coevolved) regions, potential alternative hosts appear to be protected via inability to encounter the host, or due to incompatibility, or both filters are closed. Naïve hosts from an introduced area are located under permissive encounter experimental conditions, but the compatibility filter is closed.

INTRODUCTION

We now recognize the ubiquitous importance of introduced marine organisms as pests (Carlton 1989). Several international conferences have been organized in the past eight years and research funding has been substantially expanded. Studies of impacts of some of the most successful invaders such as the *Caulerpa taxifolia* alga in the Mediterranean (Meinesz 2004), *Musculista senhousia* mussels in southern California (Crooks and Khim 1999), and *Carcinus maenas* green crabs in Tasmania and on both coasts of North America (Grosholz *et al.* 2000) demonstrate that marine invaders can deplete native species through competition, predation, and alteration of habitat. Secondary facilitation of other exotics and other tri-trophic consequences also significantly and substantially alter natural communities in ways deemed undesirable. Most invasion problems are tackled at two levels: prevention and mitigation. For agricultural insect and weed pests both elements are actively developed. For marine pests, a major international effort has been mounted to prevent further introductions with increased regulation of major vectors such as ballast water, hull fouling and oyster mariculture (Cangelosi 2002). Some of these options are very costly (mid-ocean ballast exchange is estimated to add $112-362 million per annum to the cost of shipping just to the U.S.A. (Hayes 2001). Amelioration of the impacts of the marine invaders that are already here receives little attention. The cause for this defeatist attitude is unclear. The vastness of the marine habitat and the pelagic larval dispersal strategies of many marine organisms do make it seem that an invader is unstoppable once it arrives (Kuris and Lafferty 2001). It could also relate to the training of most marine ecologists, which in accordance with the general ecological literature, extrapolates nonscientific biological control horror stories to an *a priori* suspicion of scientific classical biological control.

For marine exotics, Thresher and Kuris (2004) showed that for control options there is an inverse relationship between likelihood of success and the perceived willingness of the approach to be supported by investigators and regulators. One might conclude that, at present, the problems caused by marine exotics are not sufficiently severe to risk costs associated with mitigation. Of course, that begs the question: why then impose the substantial costs to prevent this problem?

Our perspective is that if costs imposed by introduced marine species are great, control strategies are worth developing. This has paid off with the first successful eradications of established marine pests (Culver and Kuris 2000; Kuris 2003a; Myers *et al*. 2000). For use of natural enemies, we showed that a theoretical window for biological control of marine pests was available (Kuris and Lafferty 1992). By extending models of the impact of parasitic castrators and symbiotic egg predators on crustacean fisheries, we proposed that at least these types of natural enemies could act as classical biological control agents against marine exotics and devised a general protocol for development of this approach. This was also extended for the use of marine parasitoids (Kuris *et al*. 2002). Efficacy models were generally related to those developed for insect pest control by parasitoid natural enemies (Lafferty and Kuris 1996) and modified by experiences controlling infectious diseases impacting crustacean fisheries (Kuris and Lafferty 1992). Safety concerns could be evaluated experimentally following protocols for weed pest biological control (Kuris and Lafferty 2001; Lafferty and Kuris 1996).

With the discovery of a population of the European green crab, *Carcinus maenas*, (L.) (Decapoda, Portunidae) in South San Francisco Bay in 1991 (Cohen *et al*. 1995), its rapid spread via larval dispersal to bays and estuaries as far north as Nootka Sound, British Columbia in less than 10 years (Behrens-Yamada 2001) was a record linear range expansion rate for a marine animal. Its eurytopic, euryhaline, and generalist feeding habits suggest European green crab is a worst case pestiferous invader. Experimental and empirical studies demonstrate its strong negative impact on other species of crabs, other invertebrates, and perhaps as a competitor with shorebirds for food (Cohen *et al*. 1995; Grosholz and Ruiz 1996; Grosholz *et al*. 2000). It has now also been shown to facilitate the adverse impact of an earlier introduction, the small clam, *Gemma gemma* (Totten) (Grosholz 2005). Hence, a control campaign against the introduced green crab appears warranted. These studies in California are supported by similar studies of other introduced green crab populations from the east coast of North America, South Africa, and Victoria and Tasmania in Australia (Glude 1955; Le Roux *et al*. 1990; MacPhail *et al*. 1955). Its sibling species, the Mediterranean *C. estuarii* Nardo has also been introduced in Japan.

Here, we will summarize the biology of the natural enemies of European green crab, evaluate their potential with respect to efficacy and safety, note technological difficulties impeding further work, and expand on the evaluation of the safety of parasitic castrators using experiments and field observations in the context of the host specificity encounter-compatibility paradigm of Combes (2001). This will focus on the potential safety of the most promising agent (in terms of efficacy), *Sacculina carcini* Thompson (Rhizocephala: Sacculinidae).

EVALUATION OF GREEN CRAB NATURAL ENEMIES

The green crab, *Carcinus maenas*, is the most common crab along the shores of Europe, and as an introduced species in New England and the Canadian Maritimes. Thus, it is the most studied crab with respect to its growth, reproduction, physiology, role in community ecology, and its parasitofauna. Based on the considerable available literature, and our extensive search for natural enemies in Europe, we provide an evaluation of natural enemies consistent in the context of scientific classical biological control. We briefly considered and rejected the use as predators as control agents. Although many species of birds and fishes eat green crabs, none are specialist green crab predators. The use of a generalist predator is unlikely to be an effective control agent and it is likely to have considerable unwanted consequences for non-target species.

MATERIALS AND METHODS

In Table 1, we summarize the published information of the infective agents of green crab. Although extensive and offering considerable detail for a few species of parasites, it does not include even a single study across a variety of taxa. Hence, our first task was to survey native populations of green crab over its entire range in Europe. We conducted several surveys of European green crab populations (20 sites, ~3000 crabs dissected) that extended from Tromso, Norway to Gibraltar. These data were compared with our surveys of introduced green crab populations along the coasts of North America, in South Africa, Victoria and Tasmania, Australia, and of the closely related *C. estuarii* population in Japan (N=~2000) (Torchin *et al.* 2003). For metazoan parasites, we recovered all but one of the agents reported in the literature and discovered two that had not been previously reported. Table 1 summarizes our findings and provides a few details about their abundance and pathogenicity. Because certain types of host-parasite interactions have general implications for their use as natural enemies we have grouped these by trophic adaptive syndromes according to Lafferty and Kuris (2002).

RESULTS

Crabs in introduced populations are infrequently parasitized and parasite species richness in all invaded regions falls well below the European total (California: one species, east coast of North America: 3 species, Victoria: four species, Tasmania: 2 species, South Africa and Japan: no parasites) (Kuris and Gurney 1997; Kuris *et al.* 2002; Torchin *et al.* 1996). Further, no parasitic castrators, nor parasitoids, have been recovered from any introduced green crab population.

EVALUATION OF NATURAL ENEMIES

The parasites listed in Table 1 vary with respect to their likely efficacy and safety as biological control agents for *C. maenas.*

Pathogens. Pathogens are intensity-independent infectious agents whose abundance increases in the host through reproduction. Their populations are generally limited by host defensive responses. They are suitable for modeling as microparasites (Anderson and May 1979). The microbial pathogens reported from the green crab are rarely seen in natural populations. These diseases, particularly the ciliates, appear to be associated with stressed, high density, captive host populations. We conclude that they are inefficiently transmitted, requiring high host densities and cofactors. Stressors including pollution, high temperature, low oxygen, or confinement are some of the needed cofactors to produce disease. Their host specificity has not been experimentally examined, but evidence from related agents in other crustaceans suggests that they may not be host specific (except perhaps Microspora) for non-target crabs.

Table 1. The abundance of infective agents of the green crab in Europe from the literature (Lit.) and our surveys (Surv.), + is reported, 0 is not found, – is not investigated. For Type of agent, Path is microbial pathogens (microparasite), TTPF is trophically transmitted parasite in its final (predator) host (macroparasite), TTPI is trophically transmitted parasite in its intermediate (prey) host, PC is parasitic castrator, Ptoid is parasitoid, SEP is symbiotic egg predator (terminology from Lafferty 2002); for references see (Provenzano 1983; Behrens-Yamada 2001; Torchin *et al.* 2001, Stentiford and Feist 2005).

Infective Agent	Type	Lit.	Surv.	Abundance	Remarks
Viruses (6 spp.)	Path	+	-	?	Sporadic, often in captive populations. Several species sometimes lethal.
Bacteria (3 spp.)	Path	+	+	?	Sporadic, sometimes lethal.
Anophrys (histophagic ciliates)	Path	+	0	rare	Only captive populations?
Haematodinium perezi (dinoflagellate)	Path	+	-	?	Can cause mortality in natural populations.
Thelohania maenadis, Abelspora portulacensis (microsporans)	Path	+	0	?	Sporadic, several species, sometimes lethal.
Nematopsis sp. (gregarine)	TTPF	+	+	common	Avirulent
Fecampia erythrocephala (Fecampiida)	Ptoid	+	+	to 20%	Lethal, geographically localized, habitat specialist.
Microphallus lasmob, M. primas, Spelotrema excellens (microphallid trematodes)	TTPI	+	+	often abundant (0-100%)	Metacercaria, birds are final hosts.
Trypanorhynch and *tetraphyllid cestodes*	TTPI	0	+	rare	Plerocercoid larvae, lasmobranches are final hosts.
Profillicolis botulus (Acanthocephala)	TTPI	+	+	common	Acanthella and cystacanth larvae, geographically localized, birds are final hosts.
Pararcuaria tridentata, Cosmocephalus obvelatus (larval nematodes)	TTPI	+	0	rare	Found once, birds are final hosts.
Carcinonemertes carcinophila (Nemertean)	SEP	+	+	common (0-100%)	High intensities at some locations.
Lecithomyzon maenadis (nicothoid copepod)	SEP	+	-	common at one location	Sporadic and localized? eats eggs.
Sacculina carcini (rhizocephalan barnacle)	PC	+	+	common (0- 70%)	Stunts crab growth, feminizes males, blocks reproduction, varies with habitat.
Portunion maenadis (entoniscid isopod)	PC	+	+	Can be common (0- 15%)	Blocks reproduction.

Typical parasites and trophically transmitted parasites in final (predator) hosts. These infectious agents induce pathology in an intensity-dependent manner and are effectively modeled using the macroparasite models of May and Anderson (1979). The only such parasites in the green crab are gregarines. These appear to be avirulent and as such have no potential as biological control agents.

Parasitoids. Parasitoids are so closely associated with the study of insect natural enemies that some consider the term taxonomic (applying only to insects). "Parasitoid" is now applied to all intensity-independent infectious agents that require the death of their hosts to complete their life cycles. Examples include viruses (bacteriophage) mermithids, monstrilloid copepods and hyperiid amphipods (Kuris 1974; Kuris and Lafferty 2000; Lafferty and Kuris 2002). One of the most unusual parasitoids is the fecampiid flatworm, *Fecampia erythrocephala* Giard (Kuris *et al.* 2002). As with most parasitoids, it infects very early instars (crabs less than 12 mm carapace width [CW]). It is only known from the coasts of England, Ireland and Atlantic France (a related undescribed species may be present in the Mediterranean Sea [Brun 1967]). It is also a habitat specialist, occurring only in rocky or cobble habitats in the middle intertidal zone of semi-protected shores (Kuris *et al.* 2002). Estimates of its growth rate and its prevalence suggest that it is a major mortality factor of young green crabs in these habitats. It can infect other species of crabs (especially *Cancer pagurus* Linnaeus [Decapoda, Cancridae]), although, in its preferred habitat, only *C. maenas* is commonly encountered. As a habitat specialist, *F. erythrocephala* may be a useful biological control agent if those habitats are deemed worthy of protection, or serve as sources for green crab populations on a regional scale. Its host specificity remains to be experimentally investigated. In certain regions, presumably appropriate habitats are common and few other native crab species are found in the habitats of introduced green crabs (e.g., east coast of North America). It is likely that its life cycle can be maintained in the laboratory as the adults are free-living and its larvae are non-feeding.

Trophically transmitted parasites in their intermediate (prey) hosts (TTPIs). Several trophically transmitted parasites (TTPs) use *C. maenas* as their prey (intermediate) host. Predatory final hosts include birds for the nematodes, theacanthocephalan, *Profilicollis botulus*, in the northern range of the green crab in Europe, the trematodes throughout its range, and elasmobranches for trypanorhynch and tetraphyllid larval tapeworms in the southern part of its range. Accumulating evidence suggests that these TTPIs may reduce host abundance through behavioral modifications leading to increased rates of predation by final hosts on infected intermediate hosts (Kuris 1997). There is some evidence that these parasites may play a role in green crab population control in Victoria, the only region where introduced green crabs are not considered pests. At sites sampled in Victoria, green crabs are frequently parasitized by large trypanorhynch plerocercoids (*Trimacanthus aetobatidis* [Robinson]: Trypanorhyncha: Eutetrarhynchidae). The site of infection of these large encysted parasites overlies the large nerves leading anteriorly from the thoracic ganglion. This site is likely to promote behavioral modifications contributing to higher rates of predation on infected hosts by the abundant final host, the fiddler ray, *Trigonorhinus fasciata* Muller and Henle (Rhinobatidae) (Kuris and Gurney 1997). While TTPIs may markedly reduce the fitness of their prey hosts, they generally have low pathogenicity in their vertebrate predato hosts (Kuris

2003b; Lafferty 1992), some (notably acanthocephalans) are certainly pathogenic in their final hosts. Since there are evident safety issues with respect to vertebrates and since the ability of these TTPIs to regulate prey populations may be modest and ephemeral (only when infected prey hosts are abundant – a condition only met for the microphallids), the available TTPIs do not seem to merit continued investigation. Native TTPIs that use exotic *C. maenas* may be suitable as augmentative natural enemies.

Symbiotic egg predators (SEPs). This distinctive type of natural enemy offers the infestation dynamics of a parasite, but its trophic impact is solely on the developing embryos brooded by the ovigerous crabs. These can have very strong effects on host populations. They have been associated with the collapse of a major fishery (red king crab in Alaska) and the non-recovery of others (Dungeness crab in Central California (Hobbs and Botsford 1989; Kuris and Lafferty 1992; Kuris *et al.* 1991). *Carcinonemertes carcinophila* (Kollicker) (Hoplonemertea, Carcinonemertidae) can occur at high infestation rates, causing catastrophic brood mortality (Plymouth, England, Mira River estuary, Portugal, our personal observations). There is strong circumstantial evidence for host specificity of nemertean SEPs. The introduced populations of the green crab in the Atlantic coast of North America have never acquired *C. carcinophila* (= *C. c. immunita*, a closely related undescribed species, Kuris and Sadeghian, unpublished information) found at high prevalences on two portunid crabs, *Callinectes sapidus* Rathbun and *Ovalipes ocellatus* (Herbst) despite their considerable habitat overlap with the confamilial green crab (Coe 1902; Torchin *et al.* 1996). However, there is incontrovertible evidence that other SEP nemerteans have transferred to the green crab (Torchin *et al.* 1996). Hence, nemertean SEP natural enemies merit further investigation because they may be efficacious control agents. No safety tests have been conducted but will obviously be required. Experimental studies of host specificity remain elusive for these nemerteans, as larval transfer to new hosts has not been achieved. Brood mortality remains to be quantified.

Nicothoid copepod SEPs have been reported from both native and introduced populations of green crabs. However, these have only twice been observed (Gallien and Bloch 1936; Johnson 1957). These natural enemies appear to be geographically patchy and sporadically abundant. The relatively brief brooding period for the green crab and trap aversion by ovigerous females impedes detection and evaluation of SEPs as potential control agents.

Parasitic castrators. There is considerable theoretical and empirical evidence that parasitic castrators can control host populations (Blower and Roughgarden 1987; Kuris and Lafferty 1992; Lafferty 1993). *Sacculina carcini* appears to have the most dramatic effects on green crab growth and abundance (Lafferty and Kuris 1996; Torchin *et al.* 2001; 2002), and thus on the ecological impact of *C. maenas* on native organisms. Our analysis of crab size and crab population biomass indicated that prevalence of the two parasitic castrators (*S. carcini*, and *P. maenadis*) are inversely correlated with mean and maximum crab size and crab biomass (trapped catch per unit effort). Parasitic castrator prevalence accounts for 60-65% of the variance in these indicators of crab performance. Green crabs in Europe rarely exceed 70 mm carapace width (CW), while in California and Tasmania crabs exceeding 90 mm CW are common. *Sacculina carcini* is the most studied parasite of the green crab. Its life cycle can be completed in the laboratory. Its effects on the host, and its host specificity have been investigated.

Parasitic castrator entoniscid isopods have been less studied. *Portunion maenadis* is not common on the Atlantic coast of Europe. However, it is often highly prevalent in Mediterranean populations of the closely related *C. estuarii* (Veillet 1945). This contrast may reflect differences in abundance or infection rates of the intermediate planktonic copepod host in the Mediterranean versus in the Atlantic.

HOST SPECIFICITY OF CRUSTACEAN PARASITIC CASTRATORS AND THE SAFETY OF *SACCULINA CARCINI*

Evidence from host use patterns of *Sacculina carcini* and other rhizocephalans indicates that most species have narrow host specificity (one host or a few closely related hosts). We have been able to develop the life cycle of *S. carcini* as a reliable laboratory system, and have conducted host specificity experiments on green crabs and four native California crab species that were ecological analogs or economically important (*Cancer magister* Dana).

We interpret host specificity in the context of the double filter paradigm of Combes (2001). For a host to be suitable, two filters must be partially open. When the encounter filter is open, the infective stage can locate and enter the host because it shares the appropriate habitat and has suitable host location and attack behaviors. For the parasite to be successful, the compatibility filter must also be partially open. The host can provide appropriate nutrition and its defensive mechanisms cannot kill nor block reproduction of the infectious agent. Our experiments were designed to bypass the encounter filter (host location, behavior) and examine the compatibility filter (nutritional suitability, host defenses) (Goddard *et al.* in press). We readily infected all four native California species. However, in the naïve hosts, it never completed its development. Rather, pathology was markedly neurotropic and infected crabs became paralyzed and died. Hence, *S. carcini* can only pose a threat to these non-target species if it is well established in its natural host. In other words, its safety as a biological control agent is directly related to its efficacy. It would have to attain a high prevalence in green crab populations to provide sufficient excess larvae to be available to attack native crabs.

Additional experimental studies showed that infective *S. carcini* larvae could also attack a European crab, *Pachygrapsus marmoratus* (Kuris *et al*., submitted). However, this crab mounted a fully successful defense by melanizing early internal stages in the thoracic ganglion. We were able to use the presence of these characteristic melanized lesions to estimate the effectiveness of the encounter filter for *S. carcini*. At the Mira River estuary, Portugal, green crabs are heavily infected with *S. carcini* (~ 50% prevalence), and *C. maenas* and *P. marmoratus* exhibit considerable habitat overlap. Here, we examined *P. marmoratus* for the melanized lesions in the thoracic ganglion and compared their presence with crabs from a site on the outer coast, remote from *C. maenas*. No lesions on *P. marmoratus* were associated with *S. carcini*. Consequently, for this species, both the encounter and the compatibility filters are closed. Processes governing the encounter filter have been difficult to investigate. Our use of melanized parasite early infective stages provides a demonstration of the importance of the encounter filter for the maintenance of host specificity in Rhizocephala and entoniscid isopods (Table 2).

Most recently, we were able to use the lesion-detection squash technique to evaluate encounter and compatibility for another parasitic castrator, the entoniscid isopod, *Portunion conformis* (Kuris *et al*. in prep.). This internal parasite is widespread and often highly prevalent (>90%) in *Hemigrapsus oregonenesis* and *H. nudus* (where the latter co-occurs with *H. oregonensis*) (Kuris *et al*. 1980). At Elkhorn Slough in Central California, both *H. oregonensis* and *Pachygrapsus crassipes* are common and syntopic. The infective stage of the parasite is the cryptoniscus larva. In squashes of crab internal organs (gonads, digestive glands, thoracic ganglia), this stage is readily apparent because it retains its isopodan features as an exuvia even after it molts to the apodous juvenile. The latter becomes encased in a sheath of host blood cells and continues to grow and develop to the adult in a natural host (Kuris *et al*. 1980). By examining its host, *H. oregonensis*, and its potential host, *P. crassipes*, we were able to show that the encounter filter is as open for *P. crassipes* as it is for *H. oregonensis* (both crabs had similar prevalences and intensities of *Portunion conformis* larvae and juveniles, Kuris *et al*. in prep.). However, for *P. crassipes*, the compatibility filter was completely closed. All *P. conformis* larvae and juveniles were dead and had elicited a melanization defensive response. Most were still in the cryptoniscius stage; some had successfully molted. These were ensheathed by host blood cells, but had died and were thus melanized (Kuris *et al*. 1980). Thus, for this native parasite against a native non-host crab, the encounter filter was fully open while the compatibility filter was closed (Table 2); it could not evade the host's defensive response.

Table 2. Experiments and observations evaluating the encounter and compatibility filters for some parasitic castrators of crabs. Crabs categorized by their evolved relationship (E.R.) with the parasite and to whether encounter is possible (E.P.) and compatibility is possible (C.P.) *not evaluated, experimentally wedged open.

Parasite	Natural Host	Non-host	E.R.	E.P	C.P	Reason
Sacculina carcini	*Carcinus maenas*	*Cancer magister*	No	*	No	Parasite could not regulate its growth. A variable, partially successful cellular defensive response elicited
		Hemigrapsus oregonensis	No	*	No	same
		H. nudus	No	*	No	same
		Pachygrapsus crassipes	No	*	No	same
		P. marmoratus	Yes	No	No	Elicits a powerful and fully effective cellular defensive response
Portunion conformis	*H. oregonensis*	*H. nudus*	Yes	Yes	Yes	Lower prevalence suggests encounter filter may be less open (Kuris et al. 1980). Both hosts mount successful defensive responses against dead parasites.
		P. crassipes	Yes	Yes	No	Elicits powerful cellular defensive response.

FUTURE WORK

Further studies of host specificity of *Sacculina carcini* are needed to evaluate the encounter filter as a potential barrier to infection of non-target hosts. Models investigating the joint effect of *C. maenas* as a competitor/intraguild predator on native crabs, and the lethal parasitization of native crabs from larvae produced by infected green crabs, are needed to bound the costs and benefits of *S. carcini* as a control agent.

The technical ability to conduct experimental infections of both *Fecampia erythrocephala* and *Carcinonemertes carcinophila* should be developed. This will permit experimental investigations of the safety of these agents. A model for a parasitoid of a marine host with open recruitment should be developed to examine the potential of *F. erythrocephala* as a control agent in its specific habitat. Studies of dispersal and recruitment of *C. maenas* larvae to adult habitats will aid the recognition of the importance of different habitats as sources or sinks for larval production. Further field studies to detect Microspora and viruses will improve our understanding of the epidemiology of these potential natural enemies. For symbiotic egg predators (nemerteans and nicothoid copepods), we need to estimate their impact on crab natality. The habitat use of *Portunion maenadis* Giard and Bonnier (Peracarida, Entoniscidae) should be evaluated to see if this parasite might be more common under environmental conditions available in regions where green crabs have been introduced. In addition, the regulatory potential of a parasitic castrator with a two-host life cycle (such as *P. maenadis*) should be modeled. Finally, continued investigations of the impact of green crabs on native species are needed to aid management agencies' decision-making processes. They must have a reliable estimation of costs of this pest, the predicted benefit of a control approach and the risk associated with control approaches. The public can then make a more rational decision to institute policies for mitigation of introduced marine pests such as the green crab.

ACKNOWLEDGEMENTS

We thank Jeffrey Goddard, Patricia Sadeghian, Nadia Talhouk, Rob Gurney, Nicole Murphy, Nic Bax, Ron Thresher and James Carlton for their collaborative efforts with aspects of this study, and for their thoughtful contributions towards the development of these ideas. This research was funded by a grant from the National Sea Grant College Program, National Oceanographic and Atmospheric Administration (NOAA), US Department of Commerce under grant number NA06RG0142, project number R/CZ-162 through the California Sea Grant college system, and in part, by the California State Resources Agency as well as by NSF through the NIH/NSF Ecology of Infectious Diseases Program (DEB-024565). The views expressed herein are those of the author and do not necessarily represent the views of NOAA or any of its sub-agencies. The US government is authorized to reproduce and distribute this paper for governmental purposes.

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ESTABLISHMENT OF *PERISTENUS* SPP. IN NORTHERN CALIFORNIA FOR THE CONTROL OF *LYGUS* SPP.

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ABSTRACT

Lygus hesperus is native to western United States and is a pest to numerous field and seed crops. In California, it is a key pest of cotton and strawberries, both highly valued crops. Extensive surveys for natural enemies in western United States have found one egg and two nymphal parasitoids attacking *Lygus* species, primarily *L. hesperus*. However in central California surveys in alfalfa by ourselves and others have failed to find any nymphal parasitoids. Beginning in the early 1970's the USDA ARS initiated importation of parasitoids associated with *Lygus rugulipennis* infesting alfalfa in central Europe. Van Steenwyk and Stern attempted but failed to establish *Peristenus stygicus* during the mid 70's in the southern region of the San Joaquin Valley in central California. Importation of nymphal parasitoids into eastern United States during the 1980's, however, successfully reduced *Lygus lineolaris* infesting alfalfa, a close relative of *L. hesperus*.

Several populations of *Peristenus stygicus* and *Peristenus digoneutis* were released in Sacramento, California in alfalfa managed by CDFA. Parasitoids were collected from southern France, central Italy and Spain by CABI Bioscience and the European Biological Control Laboratory, USDA ARS. Beginning in 1999, parasitoids have been released at several sites in central California, both inland and on the coast. Parasitism has increased each year at our original release site of alfalfa in Sacramento. Three years following our last releases there, we continue to find abundant numbers of both *P. stygicus* and *P. digoneutis*. Maximum summer parasitism has increased each year since releases were made, reaching 90% summer 2004.

Parasitized nymphs of *L. hesperus* and *Closterotomus norvegicus* have been collected from nearby vacant lots infested with black mustard and wild radish. Identification of adults is pending. Results indicate that these parasitoids are permanently established in the Sacramento region. Over the same period of time, maximum *Lygus* counts has varied from 3 to 14 per sweep, and appears to be declining.

In contrast to results at the first release site in Sacramento, parasitism at our other central California release sites, including one at UC Davis has yet to increase, despite additional releases in 2002 and 2003. However at one of our new central coast sites we recovered parasitoids, as larvae, at a control site 300 m from where they were first released 6 weeks earlier. Only the introduced parasitoids *Peristenus stygicus* and *P. digoneutis* were recovered, i.e. no native braconids. Native parasitoids, *Peristenus* nr. *howardi*, have been recovered from *Closterotomus norvegicus* at the same locations.

INTRODUCTION

Lygus hesperus Knight (Heteroptera: Miridae) is native to western United States and a pest to several field and seed crops in California (University of California Cooperative Extension 2000; Zalom *et al*. 1990) and across North America (Broadbent *et al*. 2002; Coulson 1987; Strong 1970). Currently *Lygus* spp. in North America are managed on most crops through applications of broad spectrum insecticides. Cultural and biological alternatives are not considered useful. Importation of nymphal parasitoids in eastern United States during the 1980's, however, successfully reduced *Lygus lineolaris* Palisot de Beauvois infesting alfalfa, *Medicago sativa* L., a close relative of *L. hesperus* (Day 1996; Day *et al*. 1990).

Extensive surveys for natural enemies in western United States have found one egg and two nymphal parasitoids commonly attacking *Lygus* spp. (primarily *L. hesperus* and some *L. elisus* Van Duzee; [Clancy and Pierce 1966; Clancy 1968; Craig and Loan 1987; Graham *et al*. 1986]). In California, *Lygus* eggs are commonly attacked by *Anaphes iole* Girault (=*ovijentatus*) (Hymenoptera: Mymaridae) (Graham *et al*. 1986), and in Idaho *Peristenus howardi* Shaw (Hymenoptera: Braconidae) has been reported attacking nymphs on alfalfa (Day *et al*. 1999; Mayer *et al*. 1998). Although *Euphoriana uniformis* (Gahan) (Braconidae) has been reported in southern California, only rarely has it been found attacking *Lygus* in alfalfa. In Europe, nymphal parasitoids were reported attacking *Lygus rugulipennis* (F.) to a higher degree (20-32%) than *Lygus lineolaris* found in eastern United States (8-13%) prompting their importation (Day *et al*. 1990).

Alfalfa is a major crop in central California (over 623,000 ha of hay alfalfa in 2004, California Agricultural Statistics Service, www.nass.usda.gov) and considered a major source for *Lygus* infesting other crops (Goodell *et al*. 2000; Stern *et al*. 1969). Surveys by Clancy and Pierce (1966) and others (S. Rao and S. Mueller pers. comm.) have found *Lygus* nymphs infesting alfalfa in central California free of any parasitoids. An attempt at classical biological control of *Lygus spp.* in Canada and parts of western U.S.A. over the last 30 years using *Peristenus* spp. imported from Europe has met with failure (Broadbent *et al*. 2002; Coulson 1987). Attempts to colonize *P. stygicus* in central California in the 1970's resulted in limited recoveries, but no permanent establishment (Van Steenwyk and Stern 1977). A similar attempt at classical biological control on the east coast of the United States against *L. lineolarus* in the 1980's has met with much better success (Day 1996; Day *et al.* 1990). *Peristenus digoneutis* Loan collected off *Lygus rugulipennis* was imported from central Europe, where alfalfa is native. A recent survey showed that *P. digoneutis* is established over a wide area and has reduced *L. lineolaris* to much lower levels in alfalfa than prior to importation of this natural enemy. Parasitism of nymphs increased from 15% by native parasitoids to 50% two years later following establishment of *P. digoneutis*. *Lygus* numbers in alfalfa decreased by 75%. Correlative data suggests *P. digoneutis* is responsible for a reduction of damage to apples by *L. lineolaris* on the east coast (Day *et al.* 2003).

Since attempts to colonize parasitoids collected on closely related *Lygus* in Europe were successful in reducing populations of *Lygus lineolaris* in alfalfa on the east coast of the United States (Day 1996), we felt another, more enduring effort was warranted in California. Furthermore, *L. hesperus* attacks a broad range of crops, including strawberries and cotton in California, both of high economic value (Schuster 1987; Zalom *et al.* 1990). Laboratory and field studies show that these parasitoids have a high degree of host specificity (Condit and Cate 1982; Day 1999; Haye 2004; Kuhlmann *et al.* 1999; Lachance *et al.* 2001), supporting the notion that imported parasitoids would have a minimal, if any negative side affect on the environment. We report on an on-going effort to permanently establish these parasitoids in several regions of California where *Lygus* is a serious pest to high value crops. Central California was again surveyed for the presence of nymphal parasitoids and two species of *Peristenus* were imported and released at several locations.

MATERIALS AND METHODS

In 1997 and 1998 we surveyed alfalfa in Kern, Fresno, Sacramento and Yolo counties for the presence of nymphal parasitoids in Lygus spp. Three alfalfa fields in each county were sampled each year during July and August using a standard 37 cm diameter sweep net. Nymphs were dissected by teasing apart the abdomen and examining their contents for the presence of immature parasitoids with the aid of a dissecting microscope.

Foreign exploration for *Lygus* spp. was conducted by CABI Bioscience and the USDA ARS European Biological Control Laboratory beginning summer 1998. The first release of *P. digoneutis* and *P. stygicus* was in September 1998. Parasitoids were collected in regions of southern France (Herault, Lattes), northern (San Dona'de Piave) and central (Umbria) Italy, and northeastern Spain (Catalongnia, Navata), south to the province of Granada. Parasitoids were collected from native *Lygus* (mainly r*ugulipennis*) infesting alfalfa, shipped as cocoons, and sent to either the USDA ARS quarantine facility in Newark, Delaware, or the Agriculture Agri-Food Canada quarantine in London, Ontario. Both agencies stored cocoons through the winter, then shipped adult parasitoids to CDFA in Sacramento, California. Each year collections were made in increasingly more southern sites starting with southern France then moving to southern Spain, which closely matches the climate of central California (Climex© climate matching software).

A quarter ha plot of alfalfa was planted at CDFA's field insectary in Sacramento fall 1997 for the sole purpose of colonizing imported parasitoids of *Lygus*. In 1999 and 2000, three other plots of alfalfa were planted in central California also for establishing *Lygus* parasitoids: the University of California, Davis (Student Experiment Farm), ca. 0.5 ha, University of California Kearney Agricultural Center near Fresno (ca. 0.5 ha), and the Shafter Research and Extension Center, near Shafter (ca.1 ha). In 2002 (Santa Cruz County), and in 2003 (Monterey County), parasitoids were released within 10 km of the coast into non-crop vegetation near strawberry (*Fragaria* L.) farms.

Parasitoids received from these two quarantine facilities were either released directly into study plots of alfalfa or reared for future release. About 100 to 500 parasitoids were shipped to us each summer. Additional parasitoids released into fields were either produced ourselves or collected from our field insectary, the initial release site in Sacramento. Each year from 1998 to 2003 1,100 to 20,000 were released among these 6 locations. Beginning in 2001, we reduced our cultures of *Peristenus* for release to two populations of *P. stygicus* (Umbria, Italy and Granada, Spain) and one culture of *P. digoneutis* (Catalonia, Spain). All populations and species were released at all locations. After four years (summer 2001), releases of *Peristenus* spp. ceased at the first release site (North B St., Sacramento). Releases were discontinued at the Shafter site in 2003 due to poor recoveries.

Lygus were reared on a mix of green beans and artificial diet following methods developed by the USDA ARS and others (Cohen 2000a,b; Patana and Debolt 1985). Parasitoids were reared on *L. hesperus* nymphs, both placed in 1 liter clear plastic containers, fitted with a screened false bottom. The bottom of the container was layered with autoclaved vermiculate for diapausing parasitoids.

Parasitoids and *Lygus* were monitored at release sites beginning one to two years following initial releases. The proportion of nymphs parasitized by *Peristenus* spp. was measured by subsampling from nymphs swept while monitoring Lygus densities. Four sets of 10 to 50 , 180° sweeps were made across the tops of alfalfa plants. Numbers of *Lygus* were recorded when aspirating nymphs dumped onto a beat sheet. A subset of the same nymphs of all instars were returned to the laboratory and used for dissections or identification of parasitoids. The abdomens of 15 to 60 nymphs were teased apart and examined using a dissecting scope. Nymphs in which we found eggs or larvae of *Peristenus* were scored as parasitized. Samples of 100 or more nymphs from the same sampling event were placed in rearing cages (above) allowing for adult development and identification. Sampling was initiated each spring just prior to making the first releases of additional parasitoids.

Beginning in 2004, three vacant fields within 5 km of our original release site in Sacramento were surveyed for the presence of *Peristenus* spp. Herbacious annuals known to harbor *Lygus* were swept in mid spring, March – June. Nymphs were returned to the laboratory and dissected as above for the presence of parasitoids. If enough nymphs could be collected (>50), some were reared to adults.

RESULTS

Roughly equal numbers of nymphs were collected from each of four counties while surveying for alfalfa. No nymphal parasitoids were dissected from the1,980 Lygus nymphs collected in 1997 and 1998 . Of 400 adult *Lygus* collected at the same time and then later identified, 98%

were *Lygus hesperus* and 2% *L. elisus* Van Duzee (det. M. Schwartz, Agriculture and Agri-Food Canada, Ottawa)

Peristenus spp. were first recovered May 2000 at our first release site located in Sacramento, two years after the first releases of parasitoids and when we first began to dissect nymphs. Each subsequent year annual maximum parasitism levels have climbed at this location reaching a high of 90% in 2004 (Fig. 1). However, recoveries and levels of parasitism from our other locations in the central valley have remained low and highly variable (Table 1). Releases at the Shafter Research and Extension Center were discontinued due to poor recoveries. Recoveries at the two more recent coastal sites while still low, have generally increased and have had far less time for increase.

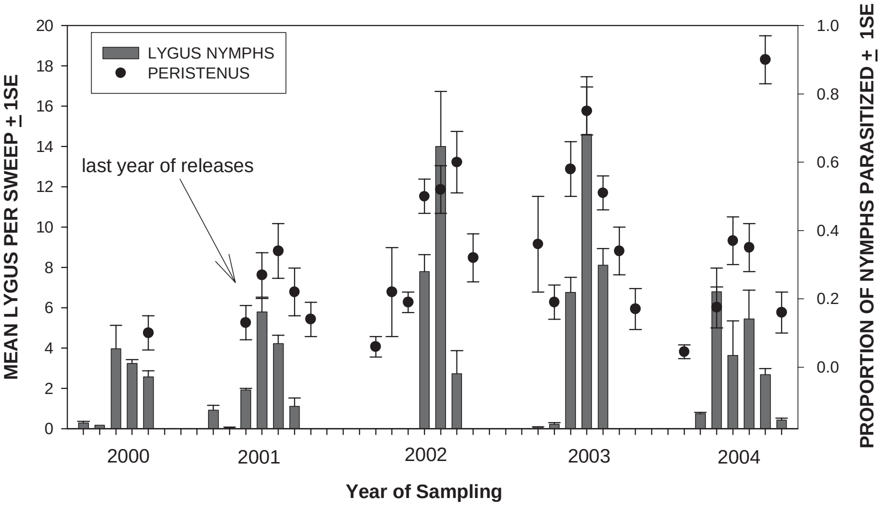

Figure 1. Density of Lygus and proportion in parasitized monthly averages, April-October, North B St., Sacramento.

Table 1. Maximum parasitism levels of releases *Peristenus* spp. at release sites.

Location	Maximum Parasitism (year)				
	2000	**2001**	**2002**	**2003**	**2004**
Sacramento, N B St.	10.0	34.0	60.0	75.0	90.0
UC Davis	0.0	4.0	2.0	3.5	—
Merced Ranch	—	4.0	2.0	3.5	—
UC Kearney Ag Ctr.	24.0	12.0	10.0	3.3	7.0
Shafter Res. and Ext. Ctr.	—	5.0	0.0	0.0	—
Castroville1	—	—	24.0	7.14	23.0
Castroville2	—	—	—	—	15.0
Watsonville1	—	—	—	7.0	25.0

Parasitism at the Sacramento site steadily increased the three years following last releases of parasitoids. Increases in parasitism have paralleled increases in the *Lygus* population in alfalfa and were positively correlated (Fig. 1; r = 0.55, p = 0.01, n = 20 sample months), suggesting a density dependent relationship between these two insect populations. In 2004 parasitism reached a maximum of 90% in August. Lygus densities dropped dramatically from a seasonal average of 7.4 nymphs per sweep in 2003 to 3.42 in 2004. Densities of *Lygus* increased during the first 6 years of the alfalfa plot most likely because it was the first field of its kind in the area in many years, an industrial region of the city.

Both species of *Peristenus* have persisted at the Sacramento release site. *Peristenus stygicus* has in general been the dominant species, varying each year from 29 to 95% of the species identified (Table 2). However, *P. digoneutis* towards the end of summer's 2002 and 2004, increased in relative proportion and was equal or dominant in numbers by the end of the summer.

Parasitized *L. hesperus* were found near the original release site in Sacramento. Weedy annuals swept at these lots included wild radish, (*Raphanus sativus* L.) black mustard, (*Brassica nigra* (L.) and vetch (*Vicia* sp.). In 2003 and 2004 parasitized Lygus were found in vacant lots 0.16, 0.50, and 2.0 km from the original release site. On one occasion, a single adult *P. digoneutis* was reared from a collection of *Lygus* made at the vacant lot 0.16 km from the release site.

Table 2. Species composition of *Peristenus* sp. at Sacramento release site.

Date Sampled	***P. stygicus* Recovered**		***P. digoneutis* Recovered**	
	#	%	#	%
July 2002	76	95.0	4	5.0
August 2002	86	82.7	18	16.3
October 2002	5	50.0	5	50.0
January 2003A	8	66.6	4	33.4
June 2003	12	85.7	2	14.3
July 2003B	16	84.2	3	15.8
March 2004 A	3	30.0	7	70.0
June 2004	37	80.0	9	20.0
July 2004	3	75.0	1	25.0
September 2004	2	29.0	5	71.0

[A] All recoveries made from soil samples

[B] 2 *P. stygicus* and 3 *P. digoneutis* recovered from soil samples

DISCUSSION

No *Peristenus* spp. were recovered from *Lygus* nymphs collected from alfalfa in central California in 1997 and 1998, prior to releases of exotic parasitoids reported herein. The same was found more recently by S. Mueller (unpubl. data). These results show that *Lygus* spp. infesting alfalfa in central California have remained free of nymphal parasitoids since earlier surveys by Clancy and Pierce (1966) and that releases of *P. stygicus* by Van Steenwyk and Stern (1977) in the southern part of the valley have never established. The vast majority of the Lygus were *L. hesperus* (98%), the remainder being *L. elisus*.

Populations of *P. stygicus* and *P. digoneutis* have persisted and increased in numbers at our original release site since last releases in 2001. Correlative data suggests together they have caused the local population of Lygus in alfalfa at this site to drop from a high of 7.4 to 3. 4 per sweep. Parasitized Lygus nymphs have been collected up to 2 km from this release site suggesting that their populations are spreading. Both species of *Peristenus* have coexisted since the last releases in 2001, with *P. stygicus* dominating in numbers recovered. Recent work on its biology shows that *P. stygicus* has twice the lifetime fecundity as *P. digoneutis* (Haye *et al*. 2005). However, seasonal trends at this same site also suggest that the proportion of each species approaches 50:50 towards the end of the summer, similar to findings by Haye (2004) who surveyed Europe in the native range of these parasitoids. Although *P. stygicus* has a higher reproductive output, *P. digoneutis* may outcompete this species over the summer. Laboratory studies show that *P. digoneutis* is a superior intrinsic competitor (LaChance *et al*. 2001). Furthermore, host range studies show that *P. digoneutis* has a higher degree of host specificity (Haye 2004), an attribute often associated with greater searching ability and survivorship at low host densities (Varley *et al*. 1973).

There may be several reasons for the lack of parasitoid establishment at sites other than Sacramento. The two most likely reasons are poor climatic match and lack of *Lygus* nymphs at key times of the year. The Shafter Research and Extension Center is about 580 km south of Sacramento (38.5° N). The University of California Kearney Agricultural Center is in between. Many of the released *Peristenus stygicus* have come from the Granada region of southern Spain which has a climatic match of 77 (out of 100) with Sacramento, and climatic match of 55 with Bakersfield about 20 km southeast of Shafter (Climex software, Sutherst *et al*. 1999). Therefore, Sacramento may be at the southern range, in terms of climatic limits, for these parasitoids. Day *et al.* (2000) found that *P. digoneutis* collected from central Europe has been limited in its dispersal southward on the eastern seaboard of the United States. He found a good agreement between summer high temperatures of 30° C for 14 to 30 days and this parasitoids southern-most establishment. However, the UC Davis release site is in the exact same climatic region as the Sacramento site, being only 20 km away in a flat valley. The most likely cause for poor establishment there is the low numbers of *Lygus*. While densities of nymphs at the Sacramento site have varied from an annual average of 1.32 to 7.4 per sweep each year, densities at UC Davis have varied from 0.4 to 1.2 per sweep.

Another key factor in establishment of *Peristenus* spp. at Sacramento is how the alfalfa has been grown. At Sacramento, unlike the other sites, the cuttings were never baled, allowing for buildup of thatch on the ground. This may have provided additional protection from desiccation for the parasitoids pupating in the soil. The ground at other locations was far more barren. We also used overhead sprinkler systems rather than surface irrigation. Although these latter two practices were adopted at the Shafter research center in 2002 and 2003, parasitoids still failed to colonize.

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CLASSICAL BIOLOGICAL CONTROL OF CODLING MOTH: THE CALIFORNIA EXPERIENCE

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ABSTRACT

Codling moth is a notorious fruit-boring pest that has extended its original distribution from the natural apple forests of Central Asia to cover all apple growing regions of the world. Having been discovered in California as early as 1872, codling moth has continued to be the dominant pest of apple, pear and walnut production causing extensive damage in the absence of insecticide treatment. In an effort to reduce reliance on insecticides, a classical biological control program was initiated in 1992. Following an initial survey for parasitoids of codling in Central Asia, three species were selected for importation and release in California; one larval parasitoid, *Bassus rufipes* (Braconidae) and two cocoon parasitoids *Liotryphon caudatus* and *Mastrus ridibundus* (Ichneumonidae). The outcome of releases made from 1993 to 2000 was that insufficient *B. rufipes* were released to gain establishment, *L. caudatus* established at least temporarily, and *M. ridibundus* became well established and continues to impact codling moth populations in the region. Although not a dramatic success in terms of the level of reduction of codling moth population densities, parasitism has played an important role in reducing the frequency of fruit and nut damage in orchards. This project provides an interesting example of what can be expected from parasitoid introductions against a notorious direct pest that belongs to a taxonomic family with a very poor history of success in the biological control record.

INTRODUCTION

California has a long history of classical biological control (henceforth referred to simply as biological control) originating with the successful control of the cottony cushion scale, *Icerya purchasi* Maskell (Homoptera: Margarodidae), as a pest of citrus in southern California in 1889 (Caltagirone and Doutt 1989). Since this first historical success, more than 100 years ago, California has been one of the most active regions of the world with regard to the pursuit of biological control solutions for invasive pests. There have also been numerous subsequent successes in California, including the suppression of invasive armored scales, whiteflies and mealybugs on citrus, as well as other notable cases such as olive scale and walnut aphid (Mills and Daane 2005).

The biological control record provides consistent evidence that homopteran pests have been the most successful targets for biological control and that lepidopteran pests have been more difficult to control through natural enemy importation (Greathead 1995; Mills 2000; 2005a). In addition, both Lloyd (1960a) and Gross (1991) have shown that projects against borers have been less successful than those against pests that have less of a physical refuge from parasitism. Thus, the codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), a notorious fruit boring pest of pome fruit, walnuts, and some stone fruits, would appear to rank very low in terms of the chances for success as a target for biological control. However, as argued by Mills (2005b), it should not be neglected as a target, due to its economic importance in California and the fact that it is an invasive species in the western U.S., on an exotic crop plant, in a relatively undisturbed environment, and has a lower level of abundance in its region of origin in Central Asia.

Codling moth first appeared in California in 1872 (Simpson 1903), and has since become a devastating pest of apples causing almost complete crop loss in the absence of effective management, and up to 40% loss of early-harvest pear cultivars and early-harvest walnut cultivars (Barnes 1991; Mills unpublished observations). As an invasive species it has extended its original distribution from the natural apple forests of Central Asia to cover all apple growing regions of the world, with the exception of eastern China and Japan (Mills 2005b). Here, I review the biological control project against codling moth in California that ran from 1992-2000, with an emphasis on the selection of parasitoids for introduction and the outcome of the parasitoid releases.

SELECTING EFFECTIVE PARASITOIDS FOR INTRODUCTION

Codling moth in California supports a small assemblage of indigenous parasitoids (Mills 2005b), including an egg parasitoid *Trichogramma platneri* Nagarkatti (Hymenoptera: Trichogrammatidae), a larval-prepupal parasitoid *Macrocentrus ancylivorus* Rowher (Hymenoptera: Braconidae), a cocoon parasitoid *Mastrus carpocapsae* (Cushman) (Hymenoptera: Ichneumonidae), and a pupal parasitoid *Coccygomimus hesperus* Townes (Hymenoptera: Ichneumonidae). It is also attacked by an egg-larval parasitoid *Ascogaster quadridentata* Wesmael (Hymenoptera: Braconidae) that was introduced into Washington State in the 1920s. In general, using corrugated cardboard bands to intercept codling moth larvae seeking cocooning sites on the trunk of orchard trees, parasitism of codling moth in California at the start of this project was low and typically less than 5% both in coastal and inland regions (Mills unpublished observations). However, egg parasitism by *T. platneri* frequently rose to 30-60% later in the season in unsprayed orchards, and parasitism of overwintering cocoons by *M. carpocapsae* was recorded to be as high at 23% in 1995 in one apple orchard on the Central Coast.

In contrast, in Central Asia, codling moth supports a more diverse parasitoid assemblage (Fig. 1), including two hyperparasitoids *Perilampus tristis* Mayr (Hymenoptera: Perilampidae) and *Dibrachys cavus* (Walker) (Hymenoptera: Pteromalidae). Levels of parasitism were greater in this region (Mills 2005b), with a maximum of 33.3% recorded for *Bassus rufipes* (Nees) (Hymenoptera: Braconidae) and 43.9% for *Mastrus ridibundus* (Gravenhorst)

(Hymenoptera: Ichneumonidae). A stage-structured model of codling moth population growth also identified that, of the life stages in the codling moth life cycle that are vulnerable to parasitism, the 2nd instar and cocoons stages would be most vulnerable to additional parasitism (Mills 2005b). In selecting parasitoids for introduction, the criteria used were the absence of antagonistic interactions between parasitoid species (Mills 2003), greater than

30% parasitism observed in the region or origin (Hawkins and Cornell 1994), and parasitoids targeting the 2nd instar and cocoon stages (Mills 2005b). Using these combined criteria, the larval endoparasitoid *B. rufipes*, and the two prepupal ectoparasitoids *Liotryphon* spp. and *M. ridibundus* were selected for introduction to California (Fig. 2).

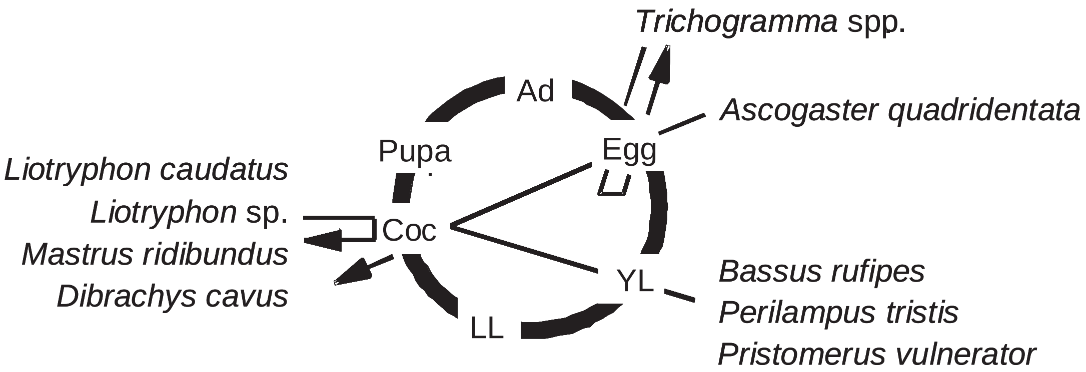

Figure 1. The parasitoid assemblage associated with codling moth in Central Asia. The circle represents the life cycle of the codling moth with YL = young instar larva, LL = late instar larva, and Coc = cocoon. The arrows represent the life stages attacked and killed by the associated parasitoids, those remaining outside of the circle being ectoparasitoids, and those passing through the circle being endoparasitoids.

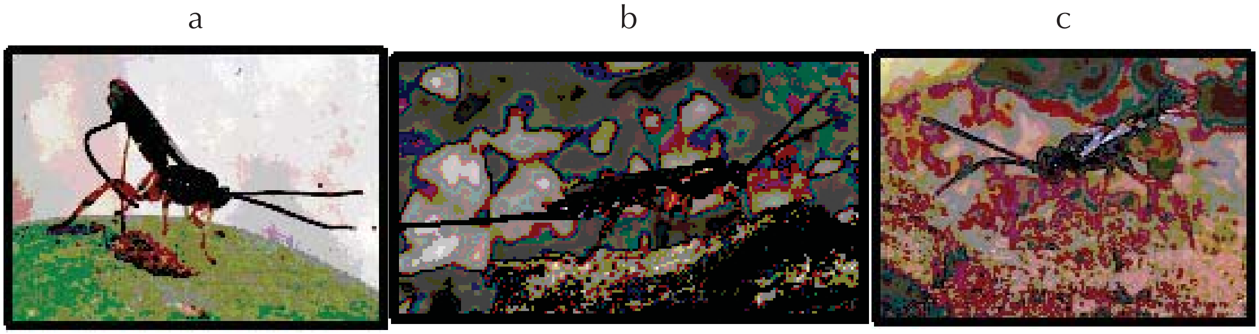

Figure 2. Parasitoids selected for introduction into California, (a) *Bassus rufipes*, (b) *Liotryphon caudatus*, and (c) *Mastrus ridibundus*. UGA1390053, UGA1390054, UGA1390055

CURRENT STATUS OF THE OUTCOME OF THE PROJECT

The introduced parasitoids were released in a total of 130 orchards in California, comprising 37 apple orchards, 21 pear orchards, and 72 walnut orchards. *L. caudatus* was the first parasitoid to be released, from 1992-1997, with a total of 45,981 individuals (males and females) released (Table 1). In the case of *M. ridibundus*, a total of 316,986 individuals were released from 1995-2000. A much larger number of individuals were released for two reasons. Firstly, *M. ridibundus* is a gregarious parasitoid with an average of 4 individuals per host cocoon, and thus greater numbers could be produced in rearing, and secondly, during the course of the release phase of the project it became clear that it was more effective than *L. caudatus* and thus greater effort was devoted to the release of this species. Releases of *B. rufipes* were constrained to 196 individuals from 1995-1997 due to the lack of success in rearing this species effectively in captivity.

Recoveries were monitored in several, but not all, orchards each year by installing corrugated cardboard bands on the trunks of at least 50 trees in the orchard early enough in the season to trap the naturally descending codling moth larvae. Both *L. caudatus* and *M. ridibundus* were recovered from codling moth cocoons in the year of release (Table 2), but there have been no recoveries of *B. rufipes*. Average rates of parasitism varied between parasitoid species ($F = 5.84$, df = 1, 85, $P < 0.02$, arcsine transformed data), but not between commodities ($F = 1.15$, df = 2, 85, $P = 0.32$), although there was a significant interaction ($F = 3.19$, df = 2, 85, $P = 0.05$) as in contrast to the situation in apple and walnut, *L. caudatus* appeared more successful in pears than *M. ridibundus*.

Table 1. The numbers of individuals of each of the three parasitoids that were released against codling moth in California orchards during the course of the project.

	1992	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**
Liotryphon caudatus									
Current year	1,464	7,053	6,452	10,467	11,382	9,000			
Cumulative	1,627	8,680	15,132	25,599	36,981	45,981			
Mastrus ridibundus									
Current year				10,850	29,186	39,150	82,800	115,000	40,000
Cumulative				10,850	40,036	79,186	161,986	276,986	316,986
Bassus rufipes									
Current year				38	127	31	0	0	0
Cumulative				38	165	196	196	196	196

Table 2. The percent parasitism of codling moth cocoons, pooled across orchards and years, for each of the parasitoids and commodities from which they were recovered in the year of release.

	Apple		**Pear**		**Walnut**	
	L. caudatus	*M. ridibundus*	*L. caudatus*	*M. ridibundus*	*L. caudatus*	*M. ridibundus*
Mean ± SD	1.58 ± 0.03	16.39 ± 0.19	7.51 ± 0.17	4.58 ± 0.08	1.59 ± 0.05	8.64 ± 0.11
Maximum	14.29	56.29	50.00	21.74	24.57	36.86
N	17	13	8	6	25	22

Although *L. caudatus* has been recovered occasionally from orchards in years after the release it seems likely that this species has not become established in California. In contrast, *M. ridibundus* has continued to be recovered from orchards and is almost certainly established in the region.

CONCLUSIONS

Although considered a target for classical biological control both in the 1920s and again in the early 1960s (Lloyd 1960b), codling moth has been ignored more recently by biological control practitioners due to the fact that it is a direct pest. However, a need to reduce insecticide residues in fruit crops (e.g., Melnico 1999) together with a consideration (Mills 2005b) of orchards as a suitable environment for biological control success, the lower level of abundance of codling moth in its region of origin, and the vulnerability of 2nd instar and cocoon stages of the life cycle to parasitism, raised the profile of codling moth as a target for biological control in California.

Codling moth damage in walnuts in California has declined since the release of *M. ridibundus* in 1995, with parasitism of overwintering cocoons reaching 56% in some unsprayed orchards (Mills unpublished observations). The outcome of the project cannot be considered a dramatic success, as should be expected in the case of a direct pest (Gross 1991; Lloyd 1960a), but as noted by Goldson *et al.* (1994), the value of parasitism and the contribution of partial biological control to the overall management of such notorious and intractable pests as the codling moth should not be underestimated.

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THE IMPACT OF PARASITOIDS ON *PLUTELLA XYLOSTELLA* POPULATIONS IN SOUTH AFRICA AND THE SUCCESSFUL BIOLOGICAL CONTROL OF THE PEST ON THE ISLAND OF ST. HELENA

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ABSTRACT

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the most injurious insect pest of brassica crops throughout the world. In many countries it has developed resistance to almost every synthetic insecticide used against it including *Bt* formulations. In addition to resistance, the destruction of its natural enemies through indiscriminate use of broad-spectrum insecticides is considered responsible for its high pest status. Population studies of *P. xylostella* and its parasitoids in the Eastern Cape, Gauteng and North-West Provinces of South Africa revealed that the pest is naturally controlled if insecticides are not used. A total of 3 egg-larval parasitoids, 8 larval parasitoids, 4 larval-pupal parasitoids, 6 pupal parasitoids and 12 hyperparasitoids have been identified as being associated with *P. xylostella* in South Africa.

An insecticide check method was used to assess the impact of parasitoids on levels of infestations by *P. xylostella*. In the sprayed plots parasitism of *P. xylostella* larvae and pupae fluctuated between 5-10% whereas in the untreated plots parasitism peaked above 90%. As a result population levels of *P. xylostella* on the sprayed plants were about five times higher than on the control plants, which is an indication that parasitoids played an important role in controlling the pest populations.

Plutella xylostella was a severe pest on the Island of St Helena, South Atlantic Ocean. Farmers were heavily depended on chemical control, often spraying cocktails of several insecticides when the required control failed. A survey in brassica crops on St Helena revealed that natural enemies were not an important factor in controlling *P. xylostella* and that the only parasitoid on the Island was the larval-pupal parasitoid *Diadegma mollipla* (Holmgren) (Hymenoptera: Ichneumonidae). Following an agreement between NRInternational and the Plant Protection Research institute (PPRI) of South Africa two consignments of the larval parasitoid, *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae), and the pupal parasitoid, *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae), were shipped in 1999 from South Africa to St Helena. The parasitoids were mass reared on the Island and released on 10 different farms. An early survey of 19 farms (release and non-release sites) in 2000 indicated that both introduced parasitoids became established. *Cotesia plutellae* was found in 15 farms

with up to 80% parasitism and *D. collaris* on 5 farms with up to 55% parasitism. Further surveys during 2002 - 2004 indicated very low levels of *P. xylostella* populations. However, *C. plutellae* cocoons were present throughout the Island which is an indication that parasitoids had been the cause for the decline in the pest populations. Farmers in St Helena reported that *P. xylostella* infestations remain low and that no chemical control has been necessary since 2001. This is a strong indication for the success of the biological control of *P. xylostella* on St Helena.

INTRODUCTION

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is cosmopolitan in its geographical distribution, occurring in all major zoogeographical regions of the world wherever crucifer crops are cultivated (Talekar and Shelton 1993). It is the most universally distributed of all Lepidoptera and has the ability to migrate and disperse over very long distances.

The host range of *P. xylostella* is limited to plants of the family Brassicaceae that contain mustard oils and their glucosides. Cultivated crops on which the diamondback moth feeds include cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*), broccoli (*B. oleracea* var. *italica*), radish (*Raphanus sativus*), turnip (*B. rapa pekinesis*), brussels sprouts (*B. oleracea* var. *gemmifera*), kohlrabi (*B. oleracea* var. *gongylodes*) and more (Fig. 1). In addition, *P. xylostella* feeds on numerous crucifer weeds (Talekar and Shelton 1993). Lohr (2001) observed for the first time the ability of *P. xylostella* to switch hosts; severe outbreaks of *P. xylostella* on commercial peas (*Pisum sativum*) in the Rift Valley of Kenya.

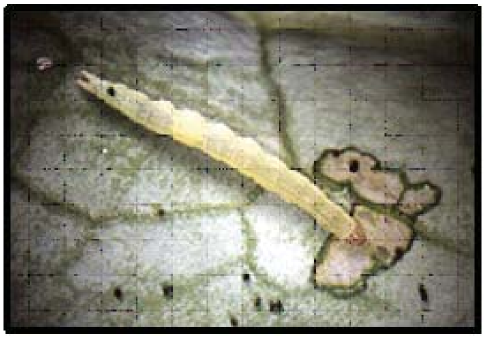

Figure 1. A young *Plutella xylostella* larva feeds on a cabbage leaf.
UGA1390043

In many countries, *P. xylostella* has developed resistance to almost every synthetic insecticide used against it in the field including *Bacillus thuringiensis* Berliner (*Bt*) formulations (Liu *et al.* 1995; Tabashnik *et al.* 1990). *Plutella xylostella* was the first crop pest to develop resistance to DDT and the first insect to develop resistance to *Bt* in the field. The increasing usage of *Bt* products resulted in an increasing number of reports of field resistance by *P. xylostella* populations (Tabashnik 1994). New insecticides are continuously being developed as existing insecticides become useless, but *P. xylostella* has developed resistance very quickly to many of these (Nisin *et al.* 2000; Shelton *et al.* 2000). The pest has also developed cross-resistance and multiple-resistance to different chemical pesticides (Shelton *et al.* 2000).

Plutella xylostella is the most injurious insect pest of cabbage and other crucifer crops throughout the world (Fig. 2). Lack of effective natural enemies is considered to be the major reason for its high pest status in most parts of the world (Lim 1986). In many countries, in addition to the development of resistance, the destruction of natural enemies by the widespread use of broad-spectrum insecticides is also considered responsible for this imbalance (Talekar and Shelton 1993). The annual cost of managing *P. xylostella* worldwide is estimated to be about US$1 billion (Talekar 1992).

Figure 2. Severe Damage to cabbage caused by *Plutella xylostella* in St Helena Island before the introduction of parasitoids. UGA1390044

Mediterranean area of origin has been repeatedly suggested for *P. xylostella* (Harcourt 1954; Hardi 1938) but no evidence for such assumption has ever been provided. This was based on the idea that the pest evolved there on crucifer plants and has been accidentally distributed from Europe around the world with the cultivated brassicas. More recently, Kfir (1998) challenged this hypothesis by suggesting a South African origin for *P. xylostella*. This was based on the diversity of wild crucifer plants and the numerous *P. xylostella* parasitoids recorded in South Africa. However, there is not yet conclusive evidence for the exact origin of the pest.

Numerous parasitoids and predators attack all developmental stages of *P. xylostella*. In addition, general predators such as birds and spiders often consume adult moths. Over 90 species of parasitoids have been recorded worldwide (Goodwin 1979) attacking all developmental stages of *P. xylostella*. Of these, the most predominant and effective larval parasitoids belong to three major genera, *Apanteles*, *Cotesia* and *Diadegma* and pupal parasitoids belonging to the genus *Diadromus*. For biological control of *P. xylostella* some parasitoid species have been introduced to Southeast Asia, the Pacific Islands, North and Central America, Africa, the Caribbean, Australia and New Zealand with various degrees of success (Lim 1986).

In South Africa Ullyett (1947) studied *P. xylostella* and its natural enemies and recorded parasitoids, predators, bacteria and an entomopathogenic fungus associated with it. He concluded at the time that in South Africa *P. xylostella* was well controlled by its natural enemies. Later Dennill and Pretorius (1995) demonstrated that high infestation levels by *P. xylostella* are a result of excessive insecticide applications. At one study site where insecticides were applied only once every three weeks, parasitism of *P. xylostella* reached 90% and the pest did not cause economic losses. In contrast, at a second study site with regular and excessive chemical applications, parasitism levels were negligible and serious outbreaks of *P. xylostella* caused total crop loss. Other studies in the Eastern Cape, Gauteng and North-West Provinces of South Africa revealed very high parasitism levels of *P. xylostella* in unsprayed cabbage crops (Kfir 1997a,b; Smith and Villet 2002; Waladde *et al.* 2001) whereas at the same regions economic damages were recorded by farmers who regularly sprayed their cabbage fields. This indicated that insecticides interfered with the natural control of *P. xylostella* in South Africa. During these studies a total of 3 egg-larval parasitoids, 8 larval parasitoids, 4 larval-pupal parasitoids, 6 pupal parasitoids and 12 hyperparasitoids have been identified as being associated with *P. xylostella* in South Africa (Kfir 2003; Lohr and Kfir 2004).

This paper reviews the impact of parasitism by indigenous parasitoids on populations of *P. xylostella* in South Africa and a successful biological control of *P. xylostella* on the Island of St Helena with parasitoids introduced from South Africa.

IMPACT OF PARASITOIDS ON *P. XYLOSTELLA* POPULATIONS

An insecticide check method was used to assess the effect of parasitoids on levels of infestation by *P. xylostella* in cabbage (Kfir 2004). The field trials were conducted at Gauteng and North-West Provinces in South Africa. Previous studies at these regions indicated that the number of *P. xylostella* moth caught in pheromone traps, and *P. xylostella* larval infestations on the crops normally peaked during the spring months of September-October (Kfir 1997b). The planting dates in this study were chosen to coincide with high populations of DBM in the field to ensure maximum natural infestations.

To suppress natural enemies a selective insecticide, dimethoate, an organophosphate compound with both systemic and contact action was applied twice weekly to cabbage plots. Similar untreated plots were used as control. Dimethoate was shown to suppress natural enemies in California cotton fields, which in turn caused an increase in abundance of *Spodoptera exigua* Hübner (Eveleens *et al.* 1973), and *Trichoplusia ni* Hübner (Ehler *et al.* 1973). This indicates that dimethoate can be detrimental to natural enemies of Lepidoptera but causes no harm to the pests.

At weekly intervals ten plants were randomly selected from each plot and thoroughly scouted for *P. xylostella* larvae, pupae and parasitoid cocoons. To determine parasitism all collected material was kept individually in glass vials in the laboratory until either parasitoids or moths emerged. All emergent parasitoids were identified and their incidence calculated.

At North-West Province, populations increased very rapidly from the second half of September and peaked during the second half of October at 47.0 larvae/plant in the sprayed plots and 12.4 larvae/plant in the control plots (Fig. 3a). At Gauteng Province populations peaked at 27.7 larvae/plant in the sprayed plots and at 4.7 larvae/plant in the control plots (Fig. 3b). At the two sites population levels of *P. xylostella* on the sprayed plants were significantly higher than on the control plants (t-test between two independent samples (Snedecor and Cochran 1967). At North-West Province, a total of 8205 DBM larvae and pupae were collected from the sprayed plants and 1607 from the control plants ($t = -16.59$, 4 df, $P < 0.001$). At Gauteng Province 3648 DBM were collected from the sprayed plants as compared with 734 DBM from the control plants ($t = -16.28$, 4 df, $P < 0.001$) (Kfir 2004).

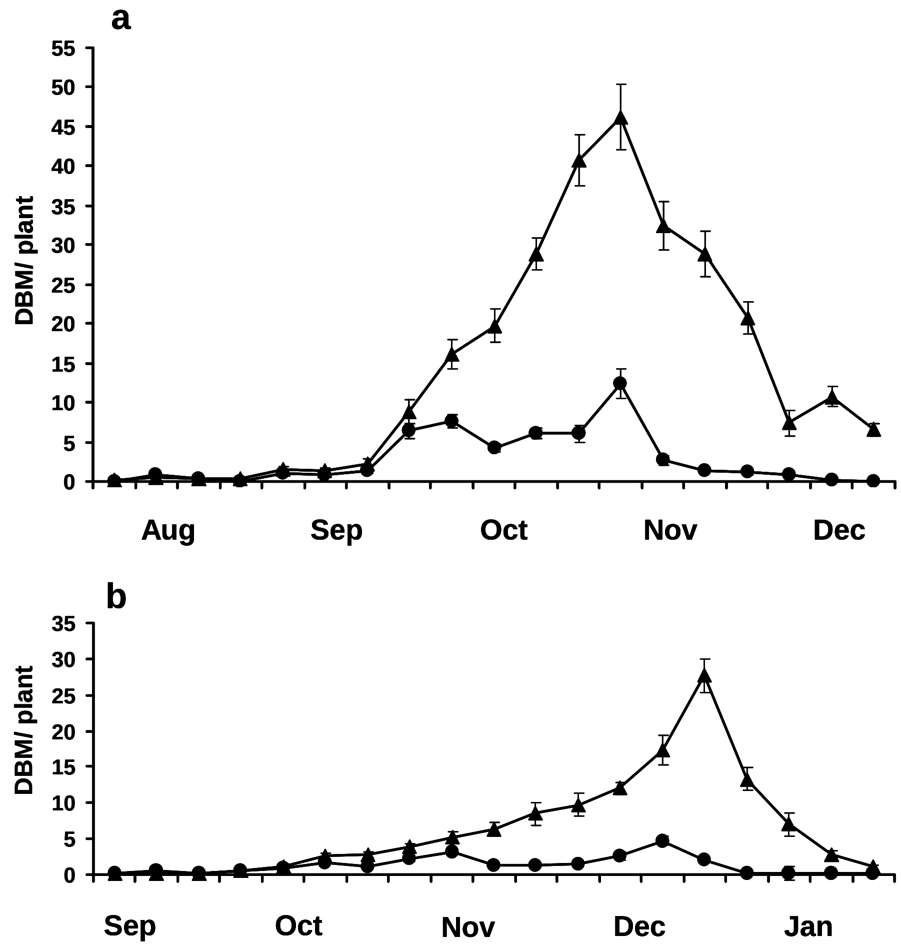

Figure 3. Abundance of diamondback moth, *Plutella xylostella*, larvae and pupae on sprayed (triangles) and control (circles) cabbage. Bars represent standard errors (SE) when larger than symbol size. (a) North-West Province, (b) Gauteng Province, South Africa (from Kfir 2004).

Percent parasitism of *P. xylostella* at both sites throughout the season was higher on the unsprayed plots (Fig. 4). At North-West Province, in the sprayed plots percent parasitism fluctuated around 5% (seasonal mean of 4.9%) whereas in the control plots parasitism increased rapidly to above 90% towards the end of the season (seasonal mean of 65.9%) (Fig. 4a). At Gauteng Province, parasitism in the sprayed plots fluctuated around 10% with a peak of 17.9% in middle of December (seasonal mean of 12.8%) and in the control plots parasitism was high (70-95%) from the middle of November to the middle of January (seasonal mean of 64.9%) (Fig. 4b).

The most abundant parasitoids were the larval parasitoids *Cotesia plutellae* (Kurdjumov) and *Apanteles halordi* Ullyett (Hymenoptera: Braconidae) (Fig. 5), the larval-pupal parasitoid *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae), which is the only known gregarious primary parasitoid *of P. xylostella*, the pupal parasitoid *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae) and the hyperparasitoids *Mesochorus* sp. (Hymenoptera: Ichneumonidae) and *Pteromalus* sp. (Hymenoptera: Pteromalidae). Both emerged from cocoons of their primary parasitoid hosts (Kfir 2004).

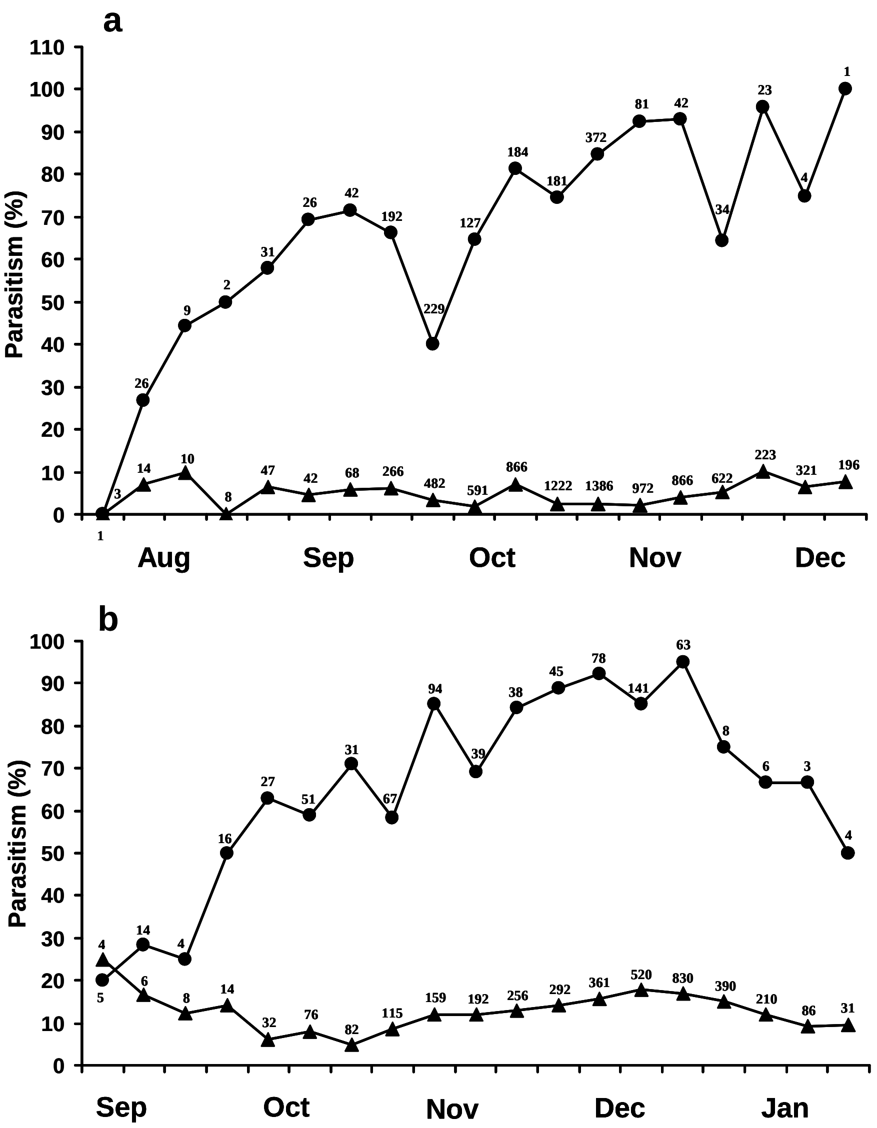

Figure 4. Percentage parasitism of diamondback moth, *Plutella xylostella,* larvae and pupae on sprayed (triangles) and control (circles) cabbage. Numbers represent sample size. (a) North-West Province, (b) Gauteng Province, South Africa (from Kfir 2004).

Figure 5. Adults of *Apanteles halfordi* (right) and *Cotesia plutellae* feeding on honey.
UGA1390045

The findings from this study demonstrated that the higher infestation level of cabbage by *P. xylostella* in the insecticide-treated plots was caused by partial elimination of parasitoids and that parasitoids play an important role in the natural control of *P. xylostella* populations in South Africa.

BIOLOGICAL CONTROL OF *P. XYLOSTELLA* ON ST. HELENA

Until recently *Plutella xylostella* was a serious pest of crucifer crops on the island of St Helena, a small British volcanic Island (122 sq km) in the South Atlantic Ocean (15 57'S, 5 42'W), 1,850 km from the west coast of Africa. Farmers on St. Helena were heavily dependent on insecticides to control *P. xylostella*, often overdosing and mixing cocktails, containing several pesticides, when the recommended dose failed to control the pest. Surveys in crucifer fields revealed that the only parasitoid of *P. xylostella* present on St Helena was *Diadegma mollipla* (Holmgren) (Hymenoptera: Ichneumonidae), which also occurs on the African mainland and some Indian Ocean Islands (Azidah *et al*. 2000).

Since most supplies, including fresh produce, are shipped from Cape Town in South Africa to St Helena, it was assumed that *P. xylostella* together with this single parasitoid had been introduced into the Island on imported cabbages from South Africa. However, because *D. mollipla* on its own was unable to reduce *P. xylostella* to below economic damage levels a biological control project, funded by DFID, UK, was hence initiated. The Plant Protection Research Institute (PPRI) of South Africa was contracted by the IPM Project on St Helena and NRInternational to supply additional parasitoids of *P. xylostella* to St Helena and to train the IPM Project personnel in mass rearing and handling procedures for *P. xylostella* and its parasitoids, release techniques for parasitoids and methods to follow-up parasitoid dispersal and establishment in the field (Kfir and Thomas 2001).

In order to reduce likelihood of competition between the introduced parasitoids and the resident larval-pupal *D. mollipla* parasitoid, it was decided to introduce into St Helena the larval parasitoid, *C. plutellae*, and the pupal parasitoid, *D. collaris.*

During 1999 two consignments of *C. plutellae* and, *D. collaris* were sent to St Helena by ship since there is no airport on the Island. The consignments contained all developmental stages of these two parasitoids, i.e. adult wasps, parasitoid cocoons and parasitised *P. xylostella* larvae and pupae (Fig. 6). During the 6-day voyage adult wasps were fed daily with honey and water, while the parasitised, but active, *P. xylostella* larvae were provided with fresh cabbage leaves, until parasitoid cocoons formed or until the hosts pupated.

Figure 6. *Diadromus collaris* parasitising a pupa of *Plutella xylostella*. UGA1390046

The parasitoids were mass reared in a rearing facility established for the project by the IPM Project of the Department of Agriculture and Natural Resources on the Island. Before releases were undertaken, extension officers visited the intended release sites, and spoke to all farmers using the local radio station advising them to stop using insecticides and to switch to more selective Bt sprays, so as to give the introduced parasitoids the best possible chance of survival. A total of 17,500 *C. plutellae* and 23,500 *D. collaris* were then released on ten different farms across the Island, continuously from May 1999 to September 2000 (Kfir and Thomas 2001).

A follow-up survey of 19 farms, conducted during 2000, at the release sites and on another 9 non-release farms, found that both parasitoids were well established: *C. plutellae* was present on 15 out of 16 farms sampled, 8 of which were farms where no parasitoids had been released. The percentage parasitism of *P. xylostella* larvae by *C. plutellae* was relatively high. For example, on Briars farm it was 32.7% (n = 104 larvae), on Mulberry Gut farm it was 27.7% (n = 70) and on Pouncey's farm (a non-release site) it was 80% parasitism (n = 30). *Plutella xylostella* pupae were likewise collected on 14 farms and *D*. collaris parasitoids were found on 5 of these, one of which was a non-release site. Percentage parasitism of pupae by *D. collaris* ranged from zero up to 55% on Nr Half Way farm (n = 20). This was an indication that both parasitoids had survived their initial release and had found and successfully parasitized the respective host stages. They were also actively dispersing into adjacent farms. However, at this stage of the project, *D. mollipla*, the resident species, still proved the most abundant and widely distributed parasitoid present. It emerged in *P. xylostella* samples from 17 out of 19 farms surveyed (Kfir and Thomas 2001).

Further surveys during 2001 – 2004, even in spring (September-October), which is normally a time when *P. xylostella* outbreaks occurred on St Helena, indicated low levels of *P. xylostella* populations. Moreover, cocoons of *C. plutellae* were reportedly found to be present throughout the Island, which is an indication that parasitoids had been the cause for the decline in the pest populations. Farmers in St Helena reported that *P. xylostella* infestations remain low and that no insecticides or *Bt* applications have been necessary since 2001. This is a strong indication for the success of the biological control of *P. xylostella* on St Helena.

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ESTABLISHMENT IN NORTH AMERICA OF *TETRASTICHUS SETIFER* THOMSON (HYMENOPTERA: EULOPHIDAE), A PARASITOID OF *LILIOCERIS LILII* (COLEOPETERA: CHRYSOMELIDAE)

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The lily leaf beetle, *Lilioceris lilii* (Coleoptera: Chrysomelidae), first reported in North America in 1945, was found in Boston in 1992 and has since spread throughout the New England States, New York, and five Canadian provinces. This pest of ornamental and native lilies in North America is generally under good biological control in Europe from a complex of larval parasitoids, including three ichneumonid species and *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae). Based upon surveys in Europe and host range testing in the U.S.A. and Europe, we determined that *T. setifer* was the best candidate to control *L. lilii* throughout its current range and it was the first species released against this pest. *Tetrastichus setifer* is a univoltine, gregarious parasitoid which overwinters in a host cocoon in the soil. Adults emerge in the spring and females oviposit in all four larval instars, spending in excess of 15 minutes laying an average of nine eggs per host. They are host specific to the genus *Lilioceris*, with a preference for *L. lilii*.

Larvae of *L. lilii* were collected throughout Europe and fed lily leaves until forming a cocoon in vermiculite. Host cocoons containing overwintering parasitoid larvae were held at 2°C for a minimum of five months before shipment to Rhode Island. In Rhode Island they were stored at 4°C before warming to 25°C for adult emergence and field release into 6m x 6m plots of approximately 800 lilies. We released *T. setifer* in Wellesley, Massachusetts in 1999 and 2000. Following release, we recorded in-season parasitism, but no winter survival. In 2001 we removed the shredded bark mulch from our plot and released 810 parasitoids. Again, we recorded high levels of in-season parasitism, but parasitoids also successfully overwintered in the plot. With no further parasitoid releases, we recorded 37% parasitism of fourth instars at peak density in 2002, followed by 100% and 57% parasitism of fourth instars in 2003 and 2004, respectively. Peak *L. lilii* larval density in the plot declined from seven per stem in 2000 to one per stem in 2004. We had similar results with a release plot in Cumberland, Rhode Island, where a release of 584 parasitoids in 2001 resulted in high in-season parasitism, but no winter survival in mulched plots. Following mulch removal, 984 parasitoids were released in 2002 and *T. setifer* successfully overwintered, causing 95% parasitism of peak fourth instar larvae in 2003 and 75% in 2004. Peak *L. lilii* larval density in the plot declined from six larvae per stem in 2001 to two per stem in 2004.

In 2003 we began distributing *T. setifer to* cooperators in other New England States who established lily plots, monitored beetles, and released parasitoids at four sites. We recovered successfully overwintered *T. setifer* in Bridgton, Maine in 2004 with 6% parasitism of fourth instar larvae. Based upon high parasitism rates following releases in 2004, we expect to find overwintered *T. setifer* in New Hampshire in 2005.

Tetrastichus setifer is established in at least three New England States and is substantially impacting populations of *L. lilii* in release plots in Massachusetts and Rhode Island. We are presently evaluating the spatial distribution of this parasitoid around release sites and evaluating release protocols.

RETROSPECTIVE EVALUATION OF THE BIOLOGICAL CONTROL PROGRAM FOR *BEMISIA TABACI* BIOTYPE "B" IN THE U.S.A.

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ABSTRACT

A retrospective evaluation of the biological control program for *Bemisia tabaci* biotype B in the U.S.A. was conducted. The use of climate matching to direct foreign exploration led to discovery of *B. tabaci* parasitoids from diverse climates, which proved useful in selecting species which would establish in the varied climates of the impacted agricultural areas of the U.S.A. The parasitoids which established on the B biotype in the U.S.A. came from several Old World biotypes. Field and laboratory evaluation demonstrated significant differences in their attack rates when searching for *B. tabaci* on cotton, broccoli, or melons. These tritrophic interactions could also have influenced their competitiveness and is also evidence of how plant hosts influence host range of parasitoids. It is also suspected that hybridization of the *Eretmocerus* spp. may have occurred, and molecular methods for testing this hypothesis are discussed. This retrospective evaluation of the program in the U.S.A. was used to develop predictive tools for selection of agents for biological control of *B. tabaci* in Australia and China.

INTRODUCTION

Bemisia tabaci biotype 'B' Gennadius (Homoptera: Aleyrodidae) (= *Bemisia argentifolia* Bellows and Perring) became a major pest of cotton, cucurbits, winter vegetables and ornamental plants in the southern U.S.A. during the 1990's. Foreign exploration for natural enemies of *B. tabaci* was implemented, and as a result, over 56 populations of parasitoids were established in quarantine culture from collections made between 1992 and 1998 (Kirk and Lacey 1995 Kirk *et al*. 1993; Kirk *et al*. 2000; Legaspi *et al*. 1996). Imported natural enemies were evaluated in laboratory and field cage tests and then released in AZ, CA, and TX (Goolsby *et al*.

1996; Goolsby *et al*. 1998; Goolsby *et al.* 2000; Gould *et al*. 1998; Hoelmer *et al*. 1998; Simmons *et al*. 1998; Pickett *et al*. 1999). Several species of parasitic Hymenoptera are now established in Texas, Arizona and California (Goolsby *et al.* 2005). A retrospective analysis of the program was conducted, and a set of predictive tools was developed to assess the parasitoids of *B. tabaci* being considered for importation by other countries (Goolsby *et al.* 2004). These tools were put into practice to predict which parasitoid species would be the best candidate for introduction into areas of Queensland, Australia, and more recently southern China, which are currently affected by *B. tabaci.*

DISCUSSION

FOREIGN EXPLORATION

Due to the wide distribution of *B. tabaci* and the unknown origin of the B biotype population, a worldwide search for natural enemies was conducted. By the early 1990's, *B. tabaci* biotype B was distributed across the southern tier of North America, including Florida, Texas, Arizona, and California. The climates and agroecosystems of these areas were climatically similar in that they supported a mix of year-round crops, including winter vegetables and summer row crops. However, their climates differed in terms of their seasonal minimum and maximum temperatures, relative humidities, and rainfall patterns and amounts. Climate-matching using CLIMEX (Sutherst *et al.* 1999) showed strong affinities between: Southeast Asia and south Florida; Mediterranean Europe with the San Joaquin Valley of California; the Arabian Peninsula with the Imperial Valley of California and South Asia and the Lower Rio Grande Valley of Texas as shown in Fig. 1.

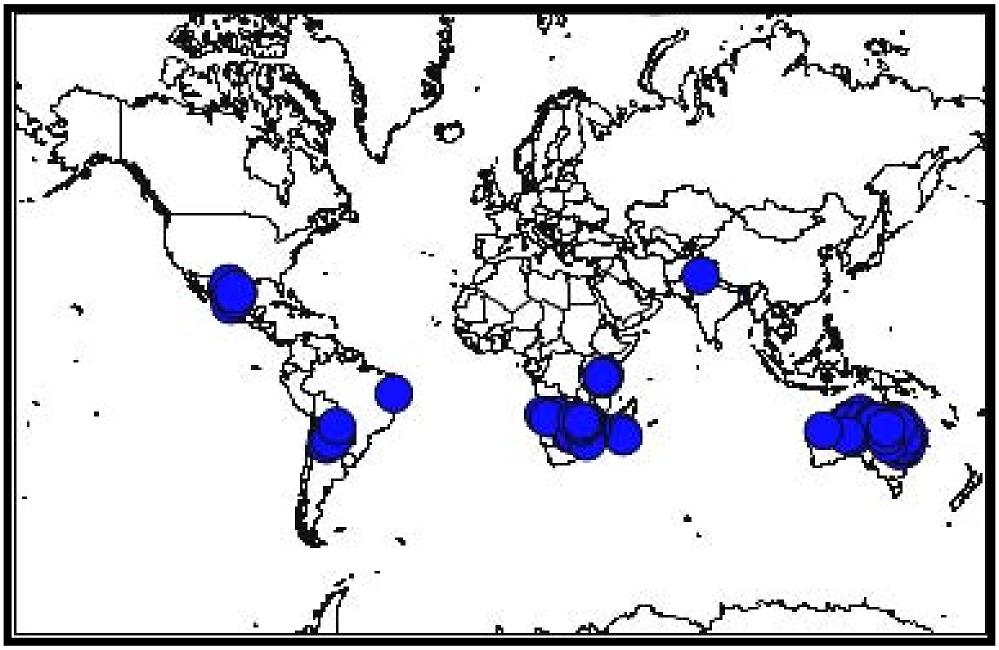

Figure 1. Worldwide locations with climatic similarity to the Lower Rio Grande Valley of Texas, U.S.A. Blue dots indicate a CLIMEX match of 75% or better.

Foreign exploration focused entirely on natural enemies of the *B. tabaci* complex, which included several known biotypes (Brown *et al.* 1995; Frohlich *et al.* 1999). It was not known at the time if the parasitoids from non-B biotypes would find the B biotype suitable, but now in retrospect, we know that the Old World *Eretmocerus* parasitoids readily accepted it. With the introduction of the Q Biotype into North America it is likely that the established parasitoids will find this biotype equally suitable. This is the case in southeast Spain, where the Q biotype is established and is readily accepted as a host by *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae). The other important outcome of the foreign exploration involved the *a priori* decision to collect natural enemies only from the *B. tabaci* complex. This decision was manifested in the host range and host specificity of the biological control agents. A biological risk assessment was conducted in 1994 prior to release, and it was determined that only natural enemies reared from *B. tabaci* would be permitted for release, and further only primary and autoparasitoids in the genera *Eretmocerus* and *Encarsia* (USDA 1995a,b). The requirement set forth in this assessment precluded the 'new association' strategy, but appears to have resulted in natural enemies with narrow host specificity. A decade later, this decision seems appropriate, given the trend towards natural enemies with narrow host ranges and the call for host-range testing of arthropod biological control agents in the U.S.A. (Van Driesche and Reardon 2004).

QUARANTINE

Several *Eretmocerus* and *Encarsia* species were established in culture between 1993-1998 (Goolsby *et al.* 1998). These candidate biological control agents came from varied climates and from *B. tabaci* infesting a wide variety of host plants. Early experiences with the indigenous North American natural enemies showed that parasitism by a key native species, *Eretmocerus eremicus* Rose and Zolnerowich, was low on *B. tabaci* infesting fall/winter cole crops. This resulted in very low numbers of overwintering *E. eremicus* in the spring and outbreaks of *B. tabaci* on melons (Hoelmer 1995). The same drop in parasitism by the native *Encarsia* was not noted on winter cole crops. This was evidence of a tritrophic interaction between *E. eremicus, B. tabaci* and its cole crop host. To evaluate potential host plant effects (tritrophic interactions) of the imported parasitoids their attack rate was evaluated on several key crop plants, including cotton, broccoli, and cantaloupe melons. Significant differences were noted between plant types (Goolsby *et al.* 1998). For example, *Eretmocerus hayati* Zolnerowich and Rose, performed best on cotton, but was lower ranked on melons and broccoli. In general, the Old World *Eretmocerus* spp. performed the best and were prioritized in the biological control program.

As the science of biological control moves toward more extensive host range testing for arthropod agents, we should use this evidence of the tritrophic effects on the *B. tabaci* parasitoids when we predict the realized host range of agents. Realized host range is a term that is gaining acceptance in the biological control of weeds community. It is defined as the innate host specificity of an organism, including its fundamental or physiological host range, relative acceptability and suitability of hosts, ability to learn, and influence of time-dependent effects in the post-release environment (van Klinken 2000). In the case of the Old World *Eretmocerus* spp., their realized host ranges were influenced by the host plants where they were released. Preference for *B. tabaci* on selected host plant showed evidence of habitat specialization. For these parasitoid species, plant cues, rather than the host insect, may be more important in their host-finding mechanisms. This is similar to the results of Kuhlmann *et al.* (2000) who found distinctive habitat preferences and host plant associations for mirid plant bug parasitoids in Europe. If non-target whitefly species were at risk in future biological control programs involving *Eretmocerus* spp., then the influence of the plant host should be considered in host-range testing. Non-target attack may be ameliorated by the effect of the plant host on their searching behavior. It appears from the research on *Eretmocerus* spp. in the *B. tabaci* program that even studies done in the confinements of quarantine laboratories can produce meaningful results and add to predictions of the realized host range of a biological control agent.

ESTABLISHMENT OF PARASITOIDS IN THE U.S.A.

Five species of Aphelinidae became established in the U.S.A.: *E. mundus*, *E. hayati*, *Eretmocerus emiratus* Zolnerowich and Rose, *Eretmocerus* sp. (ex. Ethiopia), and *Encarsia sophia* Viggiani. A sixth species *Eretmocerus melanoscutus* Zolnerowich and Rose, may be locally established in greenhouses in South Texas (T. X. Liu, pers. comm.). Climatic effects and the influence of tritrophic interactions appears to have had a strong influence on their establishment (Goolsby *et al.* 1998; Goolsby *et al.* 2005). However, several questions remain regarding establishment patterns. *Eretmocerus hayati* only established in Texas despite what appears to be ample heat tolerance for the irrigated desert agriculture of Yuma and the Imperial Valley. Did the crop mix of the Imperial Valley, i.e. large plantings of alfalfa, put it at a competitive disadvantage versus *E. emiratus* and *E.* nr. *emiratus* (ex. Ethiopia)? One of the obvious differences in the crop mix between the two areas is the large plantings of alfalfa in the Imperial Valley. Although year-round densities of *B. tabaci* in alfalfa are low, it is an important bridging host in the fall between cotton and winter vegetables. Field cage evaluations of the parasitoids conducted in the Imperial Valley showed that levels of parasitism on alfalfa were much lower than with cantaloupe melons, cotton or broccoli (Hoelmer 1998; Hoelmer and Roltsch in press). These tests did not include *E.* nr. *emiratus* (ex. Ethiopia), which became available later in the program. This parasitoid species may have been more effective at searching for *B. tabaci* in alfalfa, a trait that would have favored its establishment. In the Lower Rio Grande Valley the situation is reversed, where *E. hayati* has become established, and to date, no recoveries of *E.* nr. *emiratus* (ex. Ethiopia) have been made. Could the lack of alfalfa have influenced the establishment patterns in this agroecoystem? Field studies are planned for Arizona, California and Texas to evaluate the impact of the introduced parasitoids on *B. tabaci* using the methods developed by Naranjo and Ellsworth (In press). The proposed studies will be conducted on series of crops which may also explain the tritrophic interactions of the plant hosts with the introduced parasitoids.

In Yuma and the Imperial Valley, two species, *E. emiratus* and *E.* nr. *emiratus,* have become established. The population of *E.* nr. *emiratus* in Yuma is morphologically identical to the original voucher specimens (Mike Rose, pers. comm.). However, in the Imperial Valley the species appear to grade together, which suggests that the two populations are reproductively compatible and may be hybridizing. Could this be evidence that a more fit hybrid form of the two species exists in the field? Molecular tools may be able to validate this hybridization event. More importantly though, could molecular markers be used in the laboratory to identify hybrids and follow their success or failure in the field? If so, these techniques may open the possibility for selective breeding in quarantine of reproductively compatible species and/or populations followed by field studies to evaluate their efficacy. Biological control programs often seek populations of a natural enemy species from different climates with the belief that they will be more suited to the area of introduction. However, climatically adapted species may lack important biological attributes found in other populations. Multiple populations may have the opportunity to mate in the introduced range which leads to selection of the most fit individuals. However, the opportunity for this to occur may be lacking due to the stochastic effects of the environment and small initial release populations of the biological control agents. The opportunity for hybridization of species may be best done in the laboratory after which molecular markers can be employed to track the failure or success of these hybrids in the field.

The apparent intergradation of the two *Eretmocerus* species in the Imperial Valley could provide an opportunity to test these hypotheses and propose new research that would integrate the use of molecular techniques and crossing studies at the outset of biological control program. To test this hypothesis, material from the sources populations in Ethiopia and the United Arab Emirates would need to be recollected and reared in quarantine for the genetic studies. Other keys species in the program, including *E. hayati* from Pakistan, *E. mundus* from Spain, and *E. melanoscutus* from Thailand, should also be recollected and analyzed in the study. By comparing the genetics of the source populations with the established field populations, we may discover that hybridization occurred between these closely related species. Understanding which parental populations contributed to the hybrid forms may help us determine which biological traits (i.e., climatic tolerance, host range) were contributed from each source population. In the future, we may be able to determine which genes are responsible for the desired traits and select for individuals with the highest potential for success.

USING THE PREDICTIVE TOOLS TO SELECT AGENTS FOR AUSTRALIA AND CHINA

Predictive tools for prioritizing agents were used throughout the biological control program to prioritize agents for mass rearing and release (Goolsby *et al.* 1996; Goolsby *et al.* 1999; Hoelmer and Goolsby 2003). A retrospective study of the establishment of parasitoids in the U.S.A. showed the predictive value of the climate matching and quarantine attack rate studies (Goolsby *et al.* 2005). Based on this retrospective evaluation, a set of guidelines or tools were developed to help select the first agent for evaluation and release in Australia. Regulations in Australia require host range testing of arthropods, which is a considerable commitment in terms of time and resources. Therefore, selecting the best first candidate for testing was imperative. *Eretmocerus* spp. were prioritized because they had generally done well in quarantine attack rate studies. Climate matching showed that McAllen, TX in the Lower Rio Grande Valley was the most similar part of N. America to Queensland, Australia where silverleaf whitefly has become a pest (Fig. 2). In addition, the crop mix in this region of Australia is similar to the Lower Rio Grande Valley. Since *E. hayati* has established in Texas and is the dominant parasitoid in field collections, this species was shipped to Australia for host range testing and evaluation as a biological control agent. In the quarantine studies, *E. hayati* was shown to only attack *B. tabaci* and one other closely related whitefly. Australian regulatory authorities granted a release permit for *E. hayati* and it was released in late 2004. Early indications from the program in Australia are that *E. hayati* is successfully reproducing in the field and dispersing. While it is too early to tell if this species will become permanently established, it appears that the predictive tools worked well in selecting a candidate. It is possible that other parasitoid species might have shown similar results, but given the regulatory framework in Australia, this hypothesis cannot be fully tested unless *E. hayati* is shown to be ineffective and release of a second species is warranted.

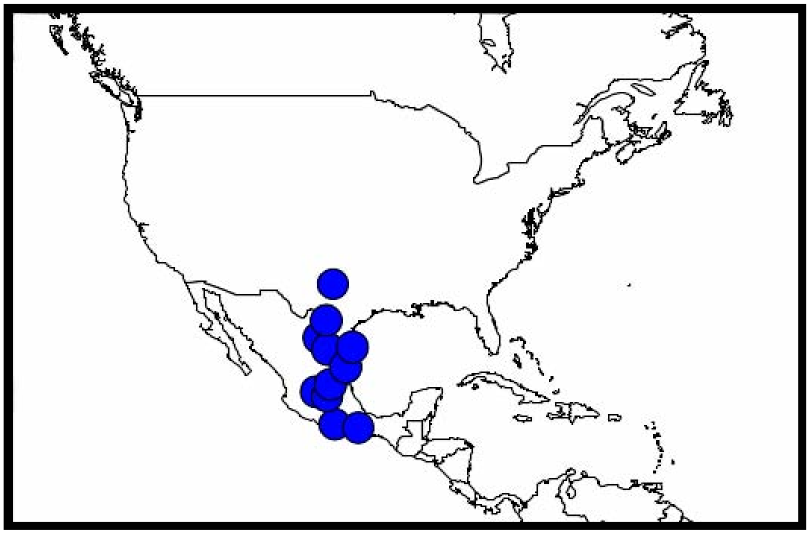

Figure 2. North American locations with climatic similarity to Emerald, Australia. Blue dots indicate a CLIMEX match of 75% or better.

Bemisia tabaci biotype B has also recently become a pest in China (Luo *et al.* 2002). Like Australia, other biotypes of *B. tabaci* are endemic to China along with a suite of indigenous parasitoids (Huang and Polaszek 1998). It is not known if the introduced species will provide additional biological control in the midst of the endemic parasitoids, but the experience in Australia has been that the endemic parasitoids were ineffective (DeBarro 2000). Using our experience in Australia, the predictive tools were used again to select candidates for release in China. The areas of China that are impacted by *B. tabaci* include the subtropical areas of southern China north to the warm temperate areas of Shanghai. In the Shanghai area, *B. tabaci* overwinters in greenhouses and infests field plantings each spring. Climate-matching using Guangzhou, Guandong as the home location showed that the best matches for N. America occurred from Florida westward to Texas (Fig. 3). Both *E. hayati* and *E. melanoscutus* have been recommended for release in China. This provides an opportunity to test the release of two species simultaneously and evaluate their tritrophic interactions in the agroecosystem. In China, banker plant, first developed for augmentation of *Eretmocerus* spp. in melon crops in TX and CA, may be a useful method for passive dispersal of the parasitoids from the greenhouses to field crops (Goolsby and Ciomperlik 1999; Pickett *et al.* 2004).

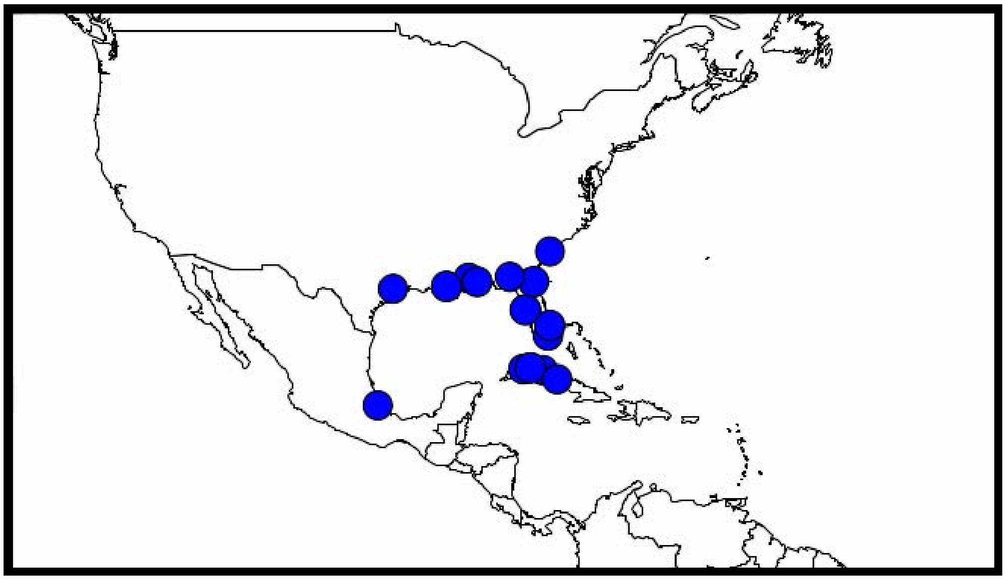

Figure 3. North American locations with climatic similarity to Guangzhou, China. Blue dots indicate a CLIMEX match of 65% or better.

The biological control program for *B. tabaci* provided novel opportunities to use predictive tools to direct foreign exploration and evaluate a suite of natural enemies in quarantine prior to release. These experiences have been used to develop a set of predictive tools for biological control of *B. tabaci*, which have used in the selection of agents for release in Australia and now China. The influences of climate and tritrophic effects appear to have been important factors in the establishment of the *Eretmocerus* spp. for *B. tabaci*. Further studies on the introduced parasitoids, including their impact on *B. tabaci*, the influence of the host plants in the agroecosystem, and their genetics, are warranted, and may provide useful insights and new scientific directions for biological control of arthropod pests.

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CULTURAL MANIPULATIONS TO ENHANCE BIOLOGICAL CONTROL IN AUSTRALIA AND NEW ZEALAND: PROGRESS AND PROSPECTS

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ABSTRACT

Increasing social and government awareness of ecosystem services has facilitated a significant increase in conservation biological control (CBC) research in Australasia. Coupled with the recent development of new methods and theoretical insights, this is an exciting period for the discipline of CBC. Increasingly, this branch of arthropod biocontrol is taking a rigorous, directed approach, 'ecological engineering', rather than relying on a crude approximation that diversifying vegetation in a 'shotgun' manner will tend to reduce pest numbers. Funding agencies are supporting such work in several agricultural systems. Industry too has become an important supporter of such work, especially in New Zealand. This paper will review conservation biological control work in Australasia to illustrate the ecological engineering approach and prospects for wider adoption of conservation biological control.

INTRODUCTION

Ecosystem services (ES) such as nutrient cycling, pollination and biological control support agricultural production. Costanza *et al.* (1997) calculated the total ES value for the world at US$ 55 trillion per year, the annual gross global production is in contrast only US$18 trillion.

Many current pest and disease problems are the result of a disruption of the restraints formerly imposed by ES. More than 40% of all world food production is being lost to insect pests (15%), plant pathogens (13%) and weeds (12%). 3 billion kilograms of pesticides are currently used each year to ensure food production, but there is no evidence that this use has led to sustainable pest population reductions (Pimentel 2004). Continued reliance on frequent, high-rate use of artificial inputs is ineffective and unsustainable. Problems associated with that approach include pesticide resistance and suppression of natural enemies like parasitoids and predators (Theiling and Croft 1988). Further, because only an estimated 0.1% of the 3 billion kilograms of the formulated product applied each year actually reaches the target organisms, a large proportion is available in the environment to affect non-target species (Metcalf 1994). Demographic studies have shown repeatedly that natural enemies like predators and parasitoids inflict the largest proportion of insect herbivores' mortality, when compared with other factors such as competition, weather and plant effects. Reducing reliance on pesticides by widespread implementation of integrated pest management is therefore a critical objective for 21st century agriculture. Biological control is an extremely important alternative to widespread pesticide use and one of its most promising components is the conservation of natural enemies.

Conservation biological control (CBC) involves the provision of resource subsidies (Polis and Strong 1996) such as pollen, nectar, shelter and/or alterative prey or hosts for predators and parasitoids. However, the complexities inherent in any plant-pest-beneficial system require detailed understanding through theoretical and empirical analyses before CBC measures can be implemented in a rational manner. It is not enough merely to observe direct, ''beneficial'' predator–prey or parasitoid–host interactions and to attempt to recreate them in agricultural settings. The mechanisms driving these interactions, and their indirect effects on other organisms, should be elucidated to confirm that a CBC program is both effective and environmentally responsible (e.g., Berryman 1999; Ehler 1994; Gurr and Wratten 1999; Kareiva 1996; Landis *et al.* 2000; Simberloff and Stiling 1996; Strong and Pemberton 2001; Waage 1990).

ECOLOGICAL PRINCIPLES

Two main ecological principles are at the heart of natural enemy enhancement by floral foods. The first is the concept of life-history omnivory, whereby a species feeds at different trophic levels during different life-history stages (Polis and Strong 1996). Many natural enemies, for example certain parasitoids, lacewings, and hoverflies, are carnivorous during their larval stage and become herbivorous as adults. This ecological phenomenon undermines the concept of discrete trophic-level paradigm and replaced it with complex multispecies food webs and interaction webs (e.g., Hawkins 1992; Janssen *et al.* 1998; Polis and Strong 1996). It is the seemingly minor interactions with non-host or non-prey species that have been largely overlooked. Understanding these interactions not only reduces the probability of unforeseen environmental harm (e.g., Strong and Pemberton 2001), but also provides the theoretical tools necessary for successful biological control (Berryman 1999; Gurr and Wratten 1999; Janssen *et al.* 1998; Landis *et al.* 2000; Lewis *et al.* 1998).

The second component of ecological theory that is integral to CBC is that of resource subsidies. Concomitant with the breakdown of the trophic-level paradigm was the understanding that many species obtain resources from outside their target habitat. These ''spatial subsidies'' allow an increase in consumer abundance, beyond that which can be sustained by the resources present within the local habitat alone (Polis and Strong 1996). Analogous processes occur when natural enemies feed on non-crop plants within the crop habitat. During an outbreak of pests, their natural enemies will be constrained by the availability of other resources that may, for example be required by the adult rather than by predacious/parasitic larvae. The provision of any non-crop plant or resource, from which a natural enemy may derive benefits, is particularly important in agricultural systems, where expansive monocultures are typical. Aggregation of natural enemies around resource-providing plants has frequently been recorded (e.g., Berndt *et al.* 2002; Hickman and Wratten 1996; Hooks *et al.* 1998; Root 1973; van Emden 1963) and several studies have shown that floral resources allow parasitoids to maximize their reproductive success via increases in longevity and egg load (e.g., Arthur 1944; Dyer and Landis 1996; Jacob and Evans 2000; Heimpel *et al.* 1997; Jervis *et al.* 1993; Wheeler 1996), and that this may lead to reduced populations of arthropod pests in the field (Irvin *et al.* 2000; Patt *et al.* 1997). Even where natural enemies do not exhibit life-history omnivory, they may be sufficiently polyphagous to use alternative diets doing periods of low pest density. Habitat manipulation may provide foods such as pollen and non-pest herbivores so that communities of natural enemies may be maintained on farms until pest numbers begin to build up.

RISKS

CBC most commonly involves the enhancement of natural enemy species that are already present in the system, so assessment of the potential for host-range expansion is not imperative. Other non-target effects of conservation biological control must nonetheless be considered before floral resource subsidies can be applied responsibly to an agricultural setting. Possibly the greatest environmental threat posed by non-crop resource subsidies is the potential for exotic plant species that are introduced for CBC to become invasive (e.g., Cheesman 1998). There are also several potential indirect effects that may reduce the effectiveness of a conservation biological control program, or contribute to environmental harm. First, intraguild competition and predation may influence the success of natural enemy enhancement using floral resources. Another potential risk is the enhancement of species other than the targeted beneficials. It is reasonable to assume that while beneficials are attracted to flowers and benefit from the provided nectar and pollen, so too may predators and hyperparasitoids of the natural enemies, as well as the pests themselves. Such risks highlight the desirability of avoiding a ‘shotgun approach’ (sensu Gurr *et al.* 2005) to providing food plants but also illustrates that achieving adequate suppression of multiple pest species within a given crop system may not always be tractable. Thus, theoretical and mechanism-based approaches as well as practical guidelines are imperative if success rates of biological control are to improve.

For example, CBC approaches have to consider the complexity of ways in which flowers may affect the population dynamics of pest/beneficial systems. There is a hierarchy of levels at which floral resources may selectively favour the beneficials more than the pests and their own antagonists. Availability of resources only to a beneficial may be achieved via: the morphology of the flowers, the quality of nectar and pollen, floral attractiveness and the morphology of insects targeted. A relative advantage to the beneficial may further be provided by: a greater fitness improvement of the beneficial compared with that of the pest, a spatial or temporal difference in the foraging of beneficials and pests and the beneficial benefits from a prey/host of improved quality more than does the prey/host itself. The effectiveness of a beneficial may also be enhanced by changes in its sex ratio and a relatively greater enhancement of its fitness than that of its fourth-trophic-level antagonists.

Partial information, based on anecdote, may lead to the accidental introduction of noxious weeds, and the enhancement of pest populations (Baggen and Gurr 1998) or higher-order predators/hyperparasitoids (Stephens *et al.* 1998). Practical guidelines for employing plant foods in farming systems are therefore required. These guidelines must be based on sound theoretical and empirical foundations and incorporate the following steps (Gurr *et al.* 2005): (1) Field surveys to determine which natural enemies of the key pest are present, (2) literature review for available information on ecology of natural enemies and pests, (3) modelling to predict benefits and avoid risks, (4) consultation with farmers to determine agronomic acceptability of possible resource plants and avoid risks (e.g., weed potential, product contamination, and toxicity to livestock), (5) laboratory assays to measure the effect of candidate plant species on important natural enemy species (e.g., longevity, fecundity, and flight propensity), (6) laboratory assays to measure the effect of candidate plant resources on target pest (e.g., to avoid nectar feeding by adult Lepidoptera or foliar feeding by larvae), (7) field experiments to check for attraction of beneficials and unpredicted effects including enhancement of secondary pests or agonists of important natural enemy species. CBC approaches that incorporate theoretical and mechanism-based approaches and follow practical guidelines are likely to be more successfully and become more widely practised. Collectively, these approaches constitute the foundation for ecological engineering.

ECOLOGICAL ENGINEERING EXAMPLES

The ecological engineering approach to CBC (Gurr *et al.* 2004), is characterised by being based on ecological theory (as explored in preceding sections) and by being developed via rigorous experimentation. The process of development typically aims to identify and provide the most functional components of biodiversity, rather that simply increasing diversity in a ‘shotgun’ fashion. By doing so, it minimizes the risks discussed above. This directed approach to understanding and using agricultural biodiversity is important because there are a number of pitfalls in the simplistic assumption that enhanced biodiversity will suppress pests in a risk-free fashion. Essentially, ecological engineering uses a range of modern techniques to identify the ‘right kind’ of diversity.

An early example of CBC in Australia was the work of Baggen and Gurr (1998) that used laboratory bioassays and small scale field experiments to identify plant species suitable for enhancing the potato moth parasitoid *Copidosoma koehleri* (Fig. 1).

Figure 1. Small-scale field experiments (pictured) coupled with laboratory bioassays are an important preliminary phase before open field studies in commercial crops. (Photo: K. Waite).

Several plant species enhanced this hymenopteran parasitoid's fecundity and adult longevity but were fed upon also by the target pest, potato moth (*Phthorimaea operculella*). Use of an ecological engineering approach identified the 'selective food plant' phenomenon, whereby plant species that benefit the target natural enemy whist denying feeding by pests. In the *Copidosoma/Phthorimaea* system, one such selective food plant was *Phacelia tanacetifolia*. Observations of floral morphology revealed that nectaries were at the base of the corolla and access to these was restricted by the presence of stamen appendages (Fig. 2a). The presence of outward pointing hairs arising from the style may also have been important in preventing the moth inserting its proboscis (Fig. 2b).

Figure 2. (a) Inflorescence of *Phacelia tanacetifolia* showing the presence of stamen appendages that block access to the nectaries and are thought to be responsible for its 'selective food plant' status (see text for explanation) (b) outward pointing hairs on style though to interfere with proboscis insertion by moths (Photos L. R. Baggen). UGA1390025, UGA1390026

Subsequent farm-scale field (Baggen *et al.* 1999) work validated the observations from smaller scale experiments and showed the value of laboratory studies in plant species selection.

Such work has led to the ecological engineering approach being used in more recent projects. The vineyard system offers especially good scope for CBC because its perennial nature affords a higher degree of stability than is common to annual crops. The vineyard 'floor' is also an important potential location for non-crop plants. Progress in this system is summarised in the following case study.

CASE STUDY: CONSERVATION BIOLOGICAL CONTROL OF PESTS IN VINEYARDS IN NEW ZEALAND AND AUSTRALIA

Biological control of insect pests in New Zealand and Australian vineyards is focused on the management of leafrollers, specifically the lightbrown apple moth (LBAM), *Epiphyas postvittana* Walker (Lepidoptera: Tortricidae) which is considered to be the most damaging to grapevines (Nicholas *et al.* 1994; Bailey 1997). The larvae of *E. postvittana* damage grapevines by feeding on new shoots, flowers, berries, stalks and leaves (Lo and Murrell 2000). Damage is also caused by the transmission of *Botrytis cinerea* amongst grape bunches by the larvae (Bailey 1997; Nair *et al.* 1988) or by providing infection sites for the Botrytis fungus by larval feeding on the bunches (Nicholas *et al.* 1994). Such damage may cause significant losses in grape production; for example in New Zealand, mid-season losses, as a result of Botrytis infections, may exceed 20% under favourable conditions, and complete losses of crops can occur before harvest in very wet seasons (Nicholas *et al.* 1994). Currently, the most common method of control of insect pests in vineyards is via insecticides.

Though there are benefits from providing natural enemies with flowering plants, the ultimate aim of CBC is to determine whether, by adding flowering plants, the five steps in an established hierarchy of research outcomes (Gurr *et al.* 2003) are met. The hierarchy of research outcomes include:

1. Aggregation of parasitoids at or near the flowers
2. An enhancement of the parasitoids' 'fitness' (longevity, fecundity and searching efficiency)
3. An increase in parasitism rate
4. A decrease in pest populations
5. Pest populations are brought below an economic threshold

In this case study we consider the levels of success that have been achieved in this hierarchy through understorey management in vineyards in relation to managing populations of leafrollers, such as *E. postvittana* in New Zealand and Australia.

E. postvittana is attacked by a wide range of parasitoids and predators in New Zealand during most of its developmental stages (Thomas 1989). However, it is *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) which is the most common parasitoid attacking leafroller larvae in New Zealand (Berndt 2002; Charles *et al.* 1996). In Australia, however, it is the egg parasitoid, *Trichogramma carverae* (Oatman and Pinto) (Hymenoptera: Trichogrammatidae) which is commonly used as a commercial biological control agent of *E. postvittana* (see below).

Berndt *et al.* (2002) worked on CBC to enhance natural enemy populations of *E. postvittana* in vineyards in New Zealand. In that study, the first level of the hierarchy of research outcomes (see above) was met when significantly more male *D. tasmanica* were collected on yellow sticky traps in buckwheat plots compared with control plots where no flowering plants were present (Berndt *et al.* 2002). Buckwheat did not appear to increase local adult parasitoid populations and the low numbers of parasitoids captured overall may explain this. In an earlier study, Irvin *et al.* (in press) examined the effects of buckwheat plants on *D. tasmanica* 'fitness' (longevity and fecundity) in laboratory experiments. The results showed that longevity of female *D. tasmanica* was increased from 12 days (water only) to 35 days when they were exposed to buckwheat, and that buckwheat enhanced potential fecundity by 62 % (Irvin *et al.* in press); thus the second level of the hierarchy was reached. The third level was achieved when parasitism rates of leafroller larvae were increased by more than 50 % in one vineyard of three when buckwheat flowers were present. At the other two vineyards, buckwheat had no effect on parasitism rates, but at these locations, leafroller populations were low, because insecticides had been used in that growing season.

Although the research described here has shown that the first three levels in the hierarchy of research outcomes can be achieved in the vineyard system, the fourth and fifth levels have not yet been demonstrated in New Zealand. Current research is addressing whether the fourth level can be achieved; however, preliminary results (Scarratt, unpublished) indicate that there is no reduction in the numbers of leafroller larvae in areas of the vineyard where buckwheat was planted. Possible reasons for this include the possibility that leafroller adults also benefit from flowering buckwheat. However, Irvin (1999) showed that *E. postvittana* larvae fed on buckwheat leaves in the laboratory but could not successfully develop to adults and that the longevity and fecundity of adult *E. postvittana* were not increased when they were provided with buckwheat. Another reason why reductions in the pest population may not have occurred when buckwheat was present may be that *E. postvittana* has a high fecundity (Danthanarayana 1975) and that *D. tasmanica* induced mortality, even via the provision of resource subsidies cannot overcome the effects of high pest fecundity. This is also thought to be the reason why predation by ladybugs (Coleoptera: Coccinellidae) may not reduce the numbers of mealybugs (Hemiptera: Pseudococcidae) (Dixon 2000). These questions associated with adding floral resources to vineyards may be usefully explored further with the support of ecological modelling (Kean *et al.* 2003).

Recent studies in the Australian vineyard system have revealed still greater levels of complexity in plant species choice for cultural manipulations to enhance biological control. The egg parasitoid, *Trichogramma carverae*, is an important Australian endemic biocontrol agent for *E. postvittana*. Studies adult feeding by *T. carverae* showed that its longevity and realised fecundity were markedly improved by several flower species (including buckwheat as used in New Zealand to enhance other parasitoids) but there were remarkably strong within-species differences conferred by different varieties of alyssum, *Lobularia maritima* (Begum *et al.* 2004). Exposure to white flowers gave realised fecundity levels significantly in excess of those for other flower colours for three-day old adults (Fig. 3). Differences were still greater after 6 days to the extent that realised fecundity for non-white flower treatments was no greater than in the control treatment in which flowers had been removed from shoots.

A further dimension to the selective food plant syndrome explored by Begum (2004) was the ability of pest larvae to feed on the foliage of plants used in CBC. In this work on *T. carverae*, not only was *L. maritima* (white flowering variety) the optimal species for adults, larvae of the pest (*E. postvittana*) were unable to develop on its foliage. Such experimental work in the laboratory and glasshouse was important in identifying plant species to be included in larger scale field evaluations.

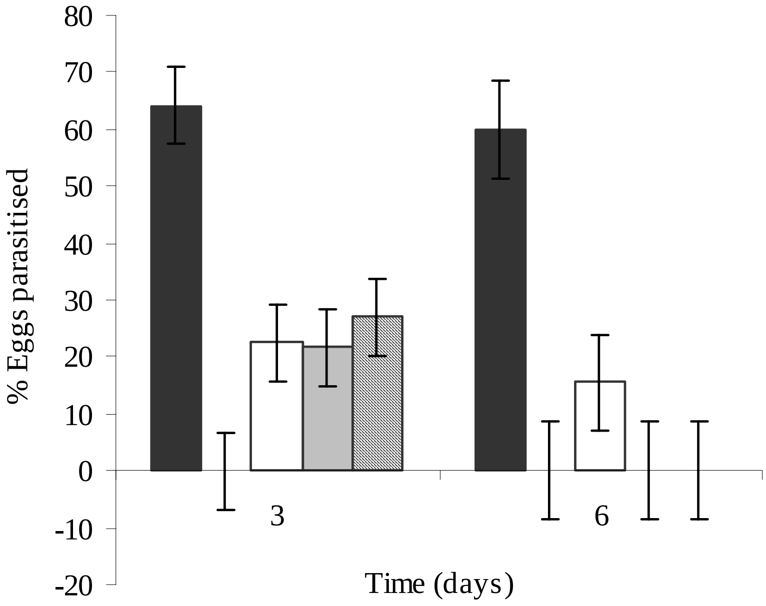

Figure 3. Parasitism of *E. postvittana* eggs by *T. carverae* is markedly affected by within-species differences in flower colour for alyssum: shaded = white flowers present, unshaded = control, white flowers removed (zero for both dates), unshaded = light pink flowers present, grey = dark pink flowers present, crosshatched = purple flowers present. (from Begum *et al.* 2004)

CONCLUSIONS

Cultural manipulations have to consider the broader context of agriculture, as any techniques for natural enemy enhancement that conflict with practical farming will remain solely theoretical. It is critical to recognize that farmers have the principal aim of making a profit. This objective may be tempered by secondary aims such as seeking to maximize farm sustainability and alternative income, as well as reducing risk, workload, and environmental impact. Increasing natural enemy density or impact, and even reducing the densities and impact of pests, will be significant only within this far broader frame of reference. In order for cultural manipulation techniques to be put into practice, economic requirements of farmers must be met. For example, if one-third of a crop must be replanted with non-crop floral resources before a significant level of natural enemy enhancement can be achieved, this method (irrespective of its theoretical benefits) will never be utilized. Fortunately, such levels of agronomic disruption are unlikely to be necessary, as improved pest management may require as little as 1 in 20 rows to be planted with floral resources (Grossman and Quarles 1993) or for the crop itself to provide key resources (Hossain *et al.* 1999). Essentially, farmers will be concerned with practical questions such as ‘‘what?’’, ‘‘where?’’, and ‘‘when?’’ By taking an ecological engineering approach, researchers are increasingly able to answer these queries with guidance on issues such as what food plants should be used, where they should be positioned in relation to the crop for maximum benefit, and when to sow or slash the plants to ensure nectar and pollen are available over the desired periods. Such research will require further rigorous empirical studies as the level of interest in conservation biological control grows in farming communities. However, to fully meet the potential for food plants in pest management, the underutilized population modelling and ecological theory approaches need to be developed. This will require researchers to more consistently address the other key questions: ''how?'' and ''why?'' Developing general theories of how floral subsidies affect food webs and why only a minority of cases of natural enemy enhancement translates into reduced crop damage will be critical.

The potential for achieving control of pest damage by manipulating the physical and biological environment of the crop is enormous. However, the potential for causing unintended effects on crop yields is similarly large. Scientific research has investigated the mechanisms by which new and traditional cultural methods of control influence levels of pest populations. This provides an understanding which provides the basis for predicting whether a practice is likely to achieve the desired level of pest control. There is still much to be learned about the complex relationships between the many components of agroecosystems and as our knowledge grows, we will be more and more able to 'engineer' agroecosystems to enhance biological control.

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ROLE OF HABITAT MANAGEMENT TECHNOLOGIES IN THE CONTROL OF CEREAL STEM AND COB BORERS IN SUB-SAHARAN AFRICA

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ABSTRACT

Floral and faunal biodiversity is relevant to pest management in many ways. In the present paper emphasis is given to the use of alternative wild and cultivated host plants as trap plants, mixed cropping and management of soil nutrients through mineral nutrition and use of leguminous cover crops in crop rotation systems for integrated control of maize cob and stem borers in sub-Saharan Africa. Our findings indicate that hydromorphic inland valleys (IVs) are reservoirs for borers and their natural enemies in upland maize fields. Populations of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), the most important borer in Cameroon, were low in IV maize fields, increased and reached high levels during the first and second cropping season in adjacent upland maize fields, while egg parasitism of borers was 40 % higher in the dry compared to the first rainy seasons, in IV and upland maize fields, respectively. Thus, IVs should be targeted for inundative releases in biological control programs. Wild host plants, namely grasses, are highly attractive to ovipositing female moths. However, results from laboratory studies on the survival of immature stages of stem borer on different grass species showed that no *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae) and *Eldana saccharina* (Walker) (Lepidoptera: Pyralidae) larvae pupated on *Pennisetum polystachion* (L.) Schult, indicating the role of wild hosts as trap plants in the vicinity of maize fields. Depending on the wild host plant and borer species, larval densities were reduced by 30-60 % in maize fields surrounded by wild grasses. Consequently up to twofold higher levels of plant damage were recorded in maize without compared to maize with surrounding grasses. Depending on the crop association and planting pattern, intercropping maize with non-host plants reduced egg and larval densities of borers by 52.6-73.7 % and 34.3-51.5 %, respectively, compared to a maize monocrop. Consequently maize yield losses due to stem borers were up to twofold lower in inter- than in monocrops. All intercropping systems had the additional advantage of higher land productivity than the maize monocrop. A maize-cassava intercrop was most efficient in terms of land use, and thus recommendable for land-constrained poor farmers. Average densities of *B. fusca* at 42 days after planting were generally higher after maize-maize and additional nitrogen (N) fertilization of 60 or 120 kg/ha than after a short fallow of leguminous food or cover crops, and higher after legumes than after maize-maize without additional N doses. However, egg-larval mortalities were up to twofold lower in maize-maize compared to legume-maize treatments. As a result, extent of deadhearts did not vary significantly among treatments. The average yield losses due to borers were five times higher in the maize-maize sequence without additional N compared to both a legume-maize sequence and maize-maize and additional N dose treatments, suggesting that an increased nutritional status of the plant enhanced both borer fitness and plant vigor, but with a net-benefit for the plants.

INTRODUCTION

Maize, *Zea mays* L., is an important component of the farming systems in sub-Saharan Africa (SSA), where it is a staple for a large proportion of the population. Food security and human nutritional status of small-scale and resource-poor farmers are directly impacted by losses in quantity and quality of the harvested crop. In some cases, losses due to pests and diseases, both pre- and post-harvest, far outweigh any reasonable hope for increases in productivity through improved germplasm and pre-harvest management. The most damaging field pests of maize in SSA are lepidopterous stem and cob borers belonging to the families Noctuidae, Pyralidae and Crambidae (see overview by Polaszek 1998). Stem and cob borers such as *Sesamia calamistis* Hampson, *Busseola fusca* (Fuller) (both Lepidoptera: Noctuidae), *Eldana saccharina*

(Walker), *Mussidia nigrivenella* Ragonot (both Lepidoptera: Pyralidae) are indigenous to Africa and have moved on to maize after having evolved on native grasses or cereals such as sorghum and millet, and other host plant species. In contrast *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) has been accidentally introduced from Asia (Nye 1960). In recent years maize is increasingly replacing indigenous cereal crops, such as sorghum and millet, as well as wild habitats in SSA; consequently it has become the major host of insect pests.

Yield losses in areas with severe borer problems vary between 10-70% (Bosque-Pérez and Mareck 1991; Cardwell *et al.* 1997; Sétamou *et al.* 2000). In addition, grain damage by lepidopterous borers predisposes maize to pre- and post-harvest infestations by storage beetles, infections by *Aspergillus flavus* Link and *Fusarium verticillioides* (Saccardo) Nirenberg, and subsequent contamination with mycotoxins (Cardwell *et al.* 1997; Sétamou *et al.* 2000). Results from diagnostic surveys indicate that the pest situation in SSA is complex, and that the relative importance of a borer species varies between regions (western vs. eastern Africa), eco-regions within a country, or even within the same eco-region of neighboring countries. In West Africa, the most frequently reported maize pests are *S. calamistis*, *E. saccharina* (Bosque-Pérez and Mareck 1990; Gounou *et al.* 1994; Schulthess *et al.* 1997), and the noctuid *S. botanephaga* (Tams and Bowden) (Endrody-Younga 1968). *B. fusca* is generally of low importance in West Africa but the predominant species across all eco-zones in Cameroon (Cardwell *et al.* 1997; Chabi-Olaye *et al.* 2005a,b; Ndemah 1999; Schulthess *et al.* 1997). The crambid *Coniesta ignefusalis* Hampson, a pest of millet in the Sahelian and savanna regions (Nwanze 1991), is occasionally found on maize in all eco-zones. Other species found in the system are *S. poephaga* Tams and Bowden, mainly a minor pest of sorghum in the Guinea and Sudan savannas (Schulthess *et al.* 1997), and *Chilo* spp. (Moyal and Tran 1991). In East and southern Africa, the most damaging cereal borers are *C. partellus*, particularly in warmer lowland areas (Nye 1960), and *B. fusca* (Overholt *et al.* 1994).

Habitat management strategies, in which available natural resources such as wild hosts and non-host plants of stem borers are used against indigenous stem borer species, can increase the understanding of interactions between pests, their cultivated and wild hosts, as well as their natural enemy fauna on both types of host plants (Khan *et al.* 2000; Ndemah *et al.* 2002; van den Berg *et al.* 2001). In general, wild hosts are believed to be a reservoir for stem borers and responsible for pest outbreaks on crops (Bowden 1976; Sampson and Kumar 1986). However, other authors have argued that grasses harbor natural enemies that prevent stem borers from reaching damaging levels on crops or act as trap plants (Schulthess *et al.* 1997). Recent studies by Chabi-Olaye (unpublished data) indicated that hydromorphic inland valleys (IVs), in which maize is grown during the dry season, maintain carry-over populations of not only *B. fusca* but also of its natural enemies in the humid forest of Cameroon. These finding show that a more complete understanding of the role of wild hosts and IVs in insect pest outbreaks will be useful in generating suitable management strategies for lepidopterous cob and stem borers.

In many regions of SSA maize is traditionally intercropped with various other crops. Generally intercropping allows more efficient land use, and ensures the availability of food throughout the seasons (Mutsaers *et al.* 1993; Vandermeer 1989). The importance of plant biodiversity in agro-ecosystems for reducing crop losses by pests has long been recognized (Baliddawa 1985; Litsinger and Moody 1976; Okigbo and Greenland 1976). A considerable number of studies have shown that pest populations are higher, more frequent and cause greater yield losses in monocrops than in more diverse cropping systems (Altieri and Letourneau 1982; Cromartie 1981; Kareiva 1983; Risch *et al*. 1983). Such a habitat management strategy has also been tested against stem borers in SSA. A considerable reduction in stem borer densities was found when maize was intercropped with non-hosts such as cassava or legumes (overview by van den Berg *et al*. 1998). Mixed cropping systems also have additional advantages such as a higher land productivity and are thus recommendable for land-constrained poor farmers who do not use external inputs such as fertilizer.

It is known that favorable nutrition often improves the ability of plants to withstand pest attack (Chabi-Olaye *et al*. 2005a; Denké 1995; Sétamou *et al*. 1993; 1995). Moreover, surveys by Ndemah (1999) showed a negative relationship between *B. fusca* densities and potassium (K) content of soil, suggesting that improvements of soil fertility can complement pest control measures in Africa.

The present paper reports on the development of habitat management technologies against cob and stem borers in SSA, and discusses the implications for their adoption by small-scale farmers in SSA.

MATERIALS AND METHODS

ROLE OF CULTIVATED INLAND VALLEYS

During the dry season in the humid forest zone of Cameroon, maize is the most important cash crop grown in hydromorphic inland valleys (IVs). Such dry season fields, however, may also be reservoirs for pests such as *B. fusca* and its natural enemies, which invade adjacent upland maize fields during the rainy seasons. From 2002 through 2004 we monitored pest and parasitoids in IVs and nearby upland maize fields. Surveys in each year started during the dry season in the IVs and were extended to upland maize fields during the first and second cropping seasons. Depending on the availability of maize, 10-12 IV maize fields were investigated per dry season. During the first and second cropping season, 1-2 up-land maize fields were sampled around each cultivated IV. Fields were visited two times, i.e., at the vegetative stage and at harvest. At each visit, 24 maize plants were sampled destructively. Data on the number of borer eggs, parasitized eggs, larval densities and their parasitism were gathered.

WILD HOST SURVEY

The wild grasses *Sorghum arundinaceum* (Desv.) Stapf, *Panicum maximum* Jacq., *Andropogon gayanus* Kunth, *Pennisetum polystachion* (L.) Schult and *P. purpureum* Moench are known to be the most common alternative host plants of stem borers in SSA (Khan *et al*. 1997; 2000; Gounou and Schulthess 2004; Shulthess *et al*.1997; van den Berg *et al*. 1997), and their abundance is strongly negatively related to borer incidence in maize fields (Cardwell *et al*. 1997; Schulthess *et al*. 1997). Differences in the relative abundance of borers and the survival of their progeny in the different wild host species may provide some clues for the management of stem borers. The data presented here are based on results of surveys carried out in Benin, Ghana, and Cote d'Ivoire (Gounou *et al*. 2004; Schulthess *et al*. 1997). Sampling was carried out through the first and second growing season along roadside fields at 10-25 km intervals.

Additional samples were also taken in IVs. At each sampling site 100-200 grass tillers were randomly sampled. The number of infested tillers was counted, and plants were dissected and borers collected, and counted according to species level. In addition, *S. calamistis* and *E. saccharina* were reared on pieces of stems from the before mentioned five grass species and larval survival was recorded (Shanower *et al.* 1993).

USE OF ALTERNATIVE WILD AND CULTIVATED HOST PLANTS AS POTENTIAL TRAP PLANTS

Wild hosts, i.e., *S. arundinaceum*, *P. maximum*, and *P. polystachion* in Benin and *P. purpureum* in Cameroon, were evaluated as trap plants for stem borers in field experiments. Experiments were carried out during the first and second growing season of 1997 in the humid forest zone of Cameroon, and in the second growing season of 1999 in the derived savanna zone of Benin. 100-144 m^2 maize plots were surrounded by 1 m border rows of grasses. Grass tufts were planted during the first season of 1997 in Cameroon, and the second season of 1998 in Benin. A control treatment of non-surrounded maize was planted away from the maize-grass treatments to reduce interactions between treatments. Each treatment was replicated four times. Maize was planted at 53,333 plants/ha. Two to three weeks after planting maize plants received NPK fertilizer (15:15:15) at a rate of 160-250 kg in Benin and Cameroon, respectively. Fields were kept weed free. 21 days after planting (DAP), 24 maize plants were randomly sampled at two-weekly intervals for assessment of plant damage (% stems bored and % deadhearts), borer abundance and their natural enemies. Five to eight samplings were taken in Benin and Cameroon, respectively.

M. nigrivenella has been frequently reported as a pest of maize (Bosque-Pérez and Mareck 1990; Gounou *et al.* 1994; Moyal 1988; Moyal and Tran 1991) and cotton, *Gossypium hirsutum* L. (Silvie 1990; Staeubli 1977). High infestations of *M. nigrivenella* were also reported from velvetbean *Mucuna pruriens* DC. and jackbean *Canavalia enseiformis* (L.) DC. (Schulthess and Gounou unpublished data). The two leguminous cover plants are green manure crops, introduced to Africa in the last decades for improving soil fertility and controlling weeds (Carsky *et al.* 1998; Vissoh *et al.* 1998;) and are increasingly used by farmers in SSA. A detailed study on the infestation and preference of *M. nigrivenella* on maize, cotton, jack- and velvetbeans was carried out by Sétamou (1999). The experimental design consisted of a randomized block with three replications containing four plots of 25 m x 25 m each. The distance between blocks was 4 m, and that between plots within a block 2 m. Each host plant was planted in early May 1995 at a density of 31,250 and 25,000 plant stands/ha for maize and cotton, respectively, and 16,500 plants/ha for both jack- and velvetbeans. Maize and cotton crops received NPK (15-15-15) fertilizer at a rate of 200 kg/ha, two weeks after sowing. For each crop, sampling started as soon as 50 % of the fruits were formed. The borer populations were monitored at weekly intervals until harvest. The percentage of fruits infested with all stages of *M. nigrivenella* in the sample was calculated for each host plant on each sampling date.

INTERCROPPING

In these experiments, conducted in the humid forest zone of Cameroon, four crop species were used, i.e., a 110-day open pollinated variety of maize (Cameroon Maize Series [CMS]

8704), a late maturing soybean *Glycine max* (L.) Merr. (var. TGX 1838-5E), an erect type of cowpea *Vigna unguiculata* (L.) Walp. (var. Asonten) and a local variety of cassava *Manihot esculenta* Crantz (called 'automatic' by farmers). Maize was grown as a monocrop or intercropped with cassava, cowpea or soybean. In the intercropping treatments, maize was planted 12-14 days after the non-host plants. Two spatial arrangements were used in the intercrops, i.e., (i) a within row arrangement where each maize plant was followed by a non-host plant, and (ii) strip planting in which two rows of maize were followed by two rows of a non-maize crop, with one row of non-host plants as first and last row borders. Each experiment had a control plot with an insecticide treatment to allow an estimation of yield losses due to borer attack. Insecticides were applied to maize 21 and 42 DAP, using carbofuran at ca. 1.5 a.i. kg ha^{-1} by placing the granules in the whorl. The treatments were arranged in a completely randomized block design with four replications. Plots were 6 × 12 m each. The planting patterns were chosen such that maize populations in all intercrops were the same (26,667 plants/ha) except in the case of alternate hill planting with cassava where the plant population was reduced to 20,000 plants/ha. In the monocrops plant densities were chosen to be 'optimal' for the region, i.e., those that produce the highest yield.

During the vegetative stage, 80 and 40 maize plants/plot were checked weekly in the mono- and intercrops, respectively, for stem borer egg batches. Larval densities were evaluated on 24 and 12 randomly sampled plants per plot in mono- and intercrops, respectively. Sampling started 28-35 DAP and was continued at biweekly intervals until maturity of maize cobs. At each sampling date, maize plants were dissected and borer larvae and pupae were identified to species and counted on a per plant/ plot level. Borer tunnel length and maize yields were estimated on four pre-determined sub-plots of 1.5 m x 2 m per treatment at harvest.

IMPROVED PLANT NUTRITION THROUGH MINERAL FERTILIZER AND LEGUMINOUS COVER CROPS

In 2003, field trials were set up in the humid forest zone of Cameroon to assess the effect of maize-legume cropping sequences and continuous maize growing with and without mineral fertilizer on both stem borer infestations, with a special emphasis on *B. fusca*, and maize yield losses. In the long-short rainy seasons sequence (herewith referred to experiment 1), cover crops were planted mid March and left to grow from March to August of the same year, thus covering the long rainy season. The succeeding maize crop was sown on September 5 of the same year. In the short-long rainy seasons sequence (herewith referred to experiment 2), which lasts from September to August in the next year, cover crops were planted on September 15 and the succeeding maize on March 25. The maize-maize cropping system had three levels of mineral fertilizer, i.e., 0, 60 and 120 kg N/ha. Each experiment had a control plot with an insecticide treatment to allow an estimation of yield losses due to borer attack. The treatments were arranged in a completely randomized block design with four replications. Plots were 6 x 6 m each. The cover crops were cut about four to five weeks before planting of the succeeding maize crop, and their biomass retained on the plots without incorporation into the soil. N was applied in form of urea. The two different N-levels (60 and 120 kg/ha) were equally split in two and three dosages, respectively, and were applied 14, 28 and 56 DAP. All maize planting was done at a spacing of 75 cm between rows and 50 cm within rows. Four seeds of 110-days open pollinated maize (cv. Cameroon Maize Series (CMS) 8704) were sown per hill, and the stands were thinned to two plants per hill 14 DAP. Plots were manually kept weed free. Insecticides were applied to maize 21 and 42 DAP, using carbofuran at ca. 1.5 a.i. kg ha^{-1} by placing the granules in the whorl. Twelve plants per plot were sampled destructively every two weeks starting from 21 DAP until harvest. The number of borer eggs and larvae per plant and percentage of plants with dead-heart symptoms were recorded in insecticide-free plots. For each treatment, borer tunnel length and maize yield were estimated on four pre-determined sub-plots of 1.5 m x 2 m at harvest.

STATISTICAL ANALYSES

Differences in plant infestation, pest abundance and damage variables, i.e., % stems bored, dead-hearts and yield losses were analyzed by analysis of variance (ANOVA), using the general linear model (GLM) procedure of SAS (SAS 1997). The t-test with Bonferroni probability adjustment was used to compare the different wild host plants and seasons. The variation in pest abundance in the mixed cropping systems over sampling days was analyzed by ANOVA, using the mixed model procedure of SAS with repeated measures (SAS 1997). Least squares means (LSM) were separated using the t-test. The significance level was set at $P = 0.05$. The effect of host plants on *M. nigrivenella* infestation levels was evaluated using the closed testing procedures (Hochberg and Tamhane 1987). The percentage of fruits infested for each host plant species were ranked within sampling date. The Chi-square test was then applied on the total sum of ranks of each host plant, to evaluate independence of *M. nigrivenella* infestations according to host plants using the PROC FREQ procedure of SAS (SAS 1997). Maize yield losses due to cob and stem borers were assessed on an area basis as follows:

$$100 \times (Yi-Yt)/Yi$$

where Yi and Yt are the mean yields of insecticide-treated and non-treated plots, respectively.

The overall efficiency of intercropping systems was assessed using the land-equivalent-ratio (LER). It is calculated after Mead and Willey (1980) as follows:

$$LER = (Ia/Ma)+(Ib /Mb)$$

where Ia and Ib are the yields of crops a and b, respectively, in intercropping; Ma and Mb are the yields of crops a and b, respectively, in the monocrops. If the LER is > 1, the intercrop is more efficient in terms of land use and if it is < 1 the monoculture is more efficient.

RESULTS

ROLE OF INLAND VALLEYS

The percentage of plants infested and larval densities varied significantly between IV and upland maize fields (Table 1). Percentage plants infested and borers densities did not differ between the first and second growing seasons, and the averages were 3.3 and 5.0 times, respectively, lower than in the dry season/ IV (Table 1). *B. fusca* was the most abundant borer species across seasons and no differences were found in its abundance among seasons (Table 1). However, *Sesamia* sp. and *M. nigrivenella* densities were 14.6 and 3.1 times, respectively, higher in the dry season/ IV than in the first and second growing seasons (Table 1). Few borer larvae and pupae were parasitized. However, levels of egg parasitism were similarly high during the dry/ IV and the second growing seasons, and the average being 1.7 times higher than during the first growing season.

Table 1. Infestation, abundance and parasitism of stem borers in inland valley and up-land maize fields in the humid forest zone of Cameroon.

Variables	Cropping seasons[1]		
	Dry	First	Second
Infested plants (%)	15.0 ± 2.2b	45.6 ± 5.3a	53.3 ± 9.5a
No. of larvae/plant	0.55 ± 0.04b	2.85 ± 0.63a	2.63 ± 0.80a
Species abundance (%)			
B. fusca	70.5 ± 4.2a	90.4 ± 3.0a	89.7 ± 5.4a
Sesamia sp.	18.2 ± 5.5a	1.5 ± 0.5b	1.0 ± 0.3b
E. saccharina	8.3 ± 1.2a	7.2 ± 2.4a	8.3 ± 4.6a
M. nigrivenella	2.9 ± 0.4a	0.9 ± 0.3b	1.0 ± 0.6b
Parasitism (%)			
Egg	43.2 ± 6.0a	26.1 ± 1.0b	43.9 ± 1.6a
Larvae + pupae	3.5 ± 1.8a	4.2 ± 2.0a	5.2 ± 2.3a

[1] The first and second growing seasons last typically from mid March to mid July and from mid August to end of November, respectively. The major dry season starts in the third week of November and lasts through end of February or beginning of March of the following year. Within row means followed by the same letters are not significantly different at P = 0.05 (Bonferroni t-test).

ROLE OF WILD AND CULTIVATED LEGUMINOUS HOST PLANTS

Borer densities did not significantly differ among the most often reported wild hosts (Table 2). However, the percentage of infested tillers was significantly higher in *S. arundinaceum* compared to the other plants (Table 2). *S. calamistis* was most abundant on *P. maximun* and *P. polystachion* and less on *P. purpureum* (Table 2). By contrast, *B. fusca* was more frequently found on *P. purpureum* than on *S. arundinaceum* and no *B. fusca* larvae were colleted on other plants. However, percentage of larvae-pupal survival was < 7 % on all five wild hosts and on *P. polystachion* no *S. calamistis* and *E. saccharina* and on *P. maximum* no *E. saccharina* larvae pupated (Table 2).

Both in the derived savanna of Benin and humid forest of Cameroon, borer densities were significantly reduced in maize surrounded by wild gramineous hosts compared to non-surrounded maize (Table 3). *S. calamistis* and *E. saccharina* densities were reduced by 51.2 % and 34.1 %, respectively, in maize surrounded by wild hosts compared to the non-surrounded one in the derived savanna of Benin. However, *E. saccharina* densities did not differ among surrounded and non-surrounded maize in both the derived savanna of Benin and the humid forest zone of Cameroon. However, in Cameroon, *B. fusca* density was 1.7 times lower in maize surrounded by *P. purpureum* compared to non-surrounded maize (Table 3). Consequently, depending on the grasses the percentage of stems bored was 1.2-2 times in Benin and 2.2 times in Cameroon lower in maize plots surrounded by grasses than in the non-surrounded maize (Table 3). In the derived savanna of Benin the percentage of egg parasitism was 2.0-2.3 times higher in surrounded compared to non-surrounded maize (Table 3).

Table 2. Relative abundance of stem borers, their incidence and survival of progeny on wild host plants investigated in West Africa.

Variables	**Wild host species**				
	Andropogon gayanus	***Panicum maximum***	***Pennisetum purpureum***	***Pennisetum polystachion***	***Sorghum arundinaceum***
Infested tillers (%)	4.9 ± 0.7b	11.0 ± 3.6b	7.1 ± 2.0b	3.2 ± 0.6b	25.2 ± 4.6a
No. of borers/plant	0.23 ± 0.2a	0.46 ± 0.3a	0.91 ± 0.7a	0.29 ± 0.2a	1.15 ± 0.5a
Abundance (%)[1]					
S. calamistis	66.1 ± 2.7b	90.8 ± 2.1a	12.5 ± 5.8c	78.8 ± 3.0ab	74.9 ± 4.8ab
E. saccharina	33.9 ± 2.7a	9.2 ± 2.1b	10.7 ± 4.4b	21.2 ± 4.0ab	17.7 ± 4.2b
B. fusca	0b	0b	76.9 ± 7.5a	0b	7.4 ± 1.5b
Survival (%)[2]					
S. calamistis	0.3	0.3	1.0	0	6.5
E. saccharina	0.3	0	0.3	0	3.5

[1]Relative abundance of borer species calculated as percentage of density of the species over total borers collected; [2]Data from Shanower *et al*. (1993). Within rows, means followed by the same letters are not significantly different at P = 0.05 (Bonferroni t-test).

Table 3. Least square means of stem borer numbers and plants damaged in maize surrounded and not surrounded by different grass species in Benin:, *Pennisetum polystachion* (Ps), *Sorghum arundinaceum* (Sa) and *Panicum maximum* (Pm); and in Cameroon: *P. purpureum* (Pp).

Variables[1]	**Derived Savanna, Benin**				**Humid Forest, Cameroon**	
	Maize only	**Maize with Pm**	**Maize with Sa**	**Maize with Pm**	**Maize only**	**Maize with Pp**
S. calamistis[2]	0.86a	0.41bc	0.51b	0.34c	-	-
E. saccharina	0.31a	0.27a	0.27a	0.27a	0.06a	0.09a
B. fusca	-	-	-	-	0.50a	0.30b
% stem bored	11.2a	9.5ab	8.1b	5c	7.9a	3.6b
% dead-heart	18.3a	18.1a	9.5c	15.6b	-	-
% egg parasitism	42.6b	85.0ab	95.0a	98.8a	-	-

[1]Data from Ndemah *et al*. (2002).

[2]In number of borers per plant. Within rows, means followed by the same letter per country are not significantly different at P = 0.05 (t-test).

There were significant differences between the sums of fruit infestation ranks (Table 4) of the different host plants ($\chi^2 = 65.33$, df = 6, $P < 0.001$). Infestation of *M. pruriens* pods was significantly higher than that of maize and cotton ($\chi^2 = 13.0$, df = 4, $P < 0.05$), but there were no significant differences among the sums of the infestation ranks of maize and cotton when tested alone ($\chi^2 = 5.6$, df = 3, $P > 0.05$). *C. enseiformis* had significantly higher number of pods infested compared to *M. pruriens* ($\chi^2 = 24.0$, df = 3, $P < 0.001$). Hence, the closed testing procedure revealed that *M. nigrivenella* infestation was significantly highest on *C. enseiformis*, with highest levels at all sampling occasions (Table 4).

Table 4. Sum of weekly ranks of *Mussidia nigrivenella* infestation levels observed on four crops in Benin.

Cultivated Crops[1]	**Sum of Infestation Rank**			
	1	**2**	**3**	**4**
Zea mays L	0	0	5	7
Gossypium hirsutum L.	0	4	5	3
Mucuna pruriens DC	0	8	2	2
Canavalia enseiformis (L.) DC.	12	0	0	0

[1] Data from Sétamou (1999).

INTERCROPPING

Results of the analysis of variances showed that egg batch and larval densities of *B. fusca* were not affected by the crops associated with maize in the intercropping treatments (Table 5). However, the egg batch density differed significantly between strip and within row planting (Table 5). Thus, the egg batch and larval densities, as well as the damage variables were presented per spatial arrangement.

Intercrops of maize with non-host plants significantly reduced the oviposition, infestation and damage due to borers compared to maize monocrop (Table 6). Yet, overall the within row planting reduced the borer egg batches per plant by 73.7 % and larval abundance by 51.5 % compared to sole maize, but treatments did not differ in terms of egg-larval mortality (Table 6). The percentages of stems bored and yield losses did not differ between the two spatial arrangements of the intercrops, and were 5.2 and 2.0 times lower than in the maize monocrop for strip and within row planting, respectively (Table 6).

Table 5. Results of ANOVA on the differences in borer densities between treatments (data pooled across sampling days and seasons).

Source of variance[1]	**Egg batch/plant**			***Busseola fusca*/plant**		
	d.f.	**F**	***P*-value**	**d.f.**	**F**	***P*-value**
Spatial arrangement (SA)	1; 177	23.57	< 0.001	1; 88	0.85	0.368
Crop (C)	2; 177	1.38	0.253	2; 88	0.19	0.830
SA*C	2; 177	1.59	0.207	2; 88	0.04	0.961

[1] Two spatial arrangement, i.e., strip and within row planting. The non-host plants cropped with maize are cassava, cowpea and soybean

The overall efficiency of intercrops is presented in Figure 1. The greater land-equivalent-ratios were obtained when maize was associated with cassava (LER ranged between 1.6 and 1.8). The lowest LER was recorded in maize-legumes with values ranging between 1.15 and 1.45 (Fig. 1).

Table 6. Effect of intercropping on the oviposition, infestation and damage (least square means ± SE) due to *Busseola fusca* in the humid forest of Cameroon.

	Oviposition and infestation			Damage	
Spatial Arrangement	**Egg Batch per Plant**	***B. fusca* per Plant**	**Mortality¹ (%)**	**% Stem Bored**	**% Yield Loss**
Maize monocrop	0.38 0.04a	1.34 ± 0.22a	97.1 ± 0.6a	28.4 ± 2.1a	34.2 ± 5.8a
Maize + non-host plant strip-planted	0.18 ± 0.02b	0.88 ± 0.13b	97.4 ± 0.6a	6.5 ± 1.2b	16.9 ± 3.3b
Maize + non-host plant within-row planted	0.10 ± 0.02c	0.65 ± 0.12b	96.8 ± 1.1a	4.5 ± 1.2b	16.8 ± 3.3b

[1] Egg to larva mortality; within columns, means followed by the same letter are not significantly different at P = 0.05 (t-test).

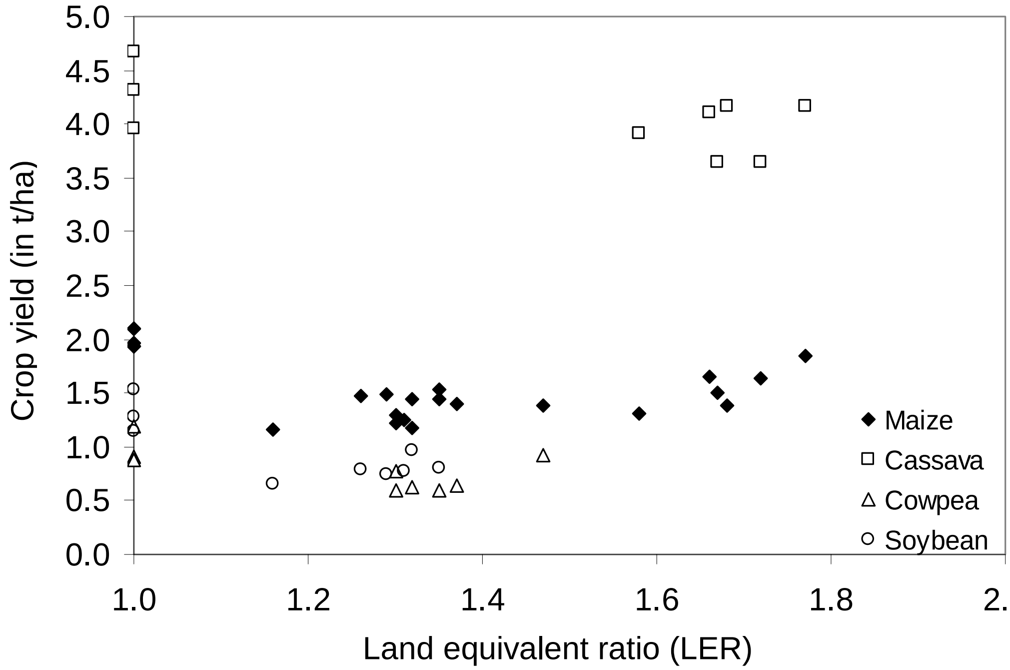

Figure 1. Relationship between land equivalent ratio and crops (maize and associated crops in the intercrops) yields.

PLANT NUTRITION

For both experiments, differences in *B. fusca* larval densities were significant at 42 DAP, while no differences were found among treatments at 63 DAP (Table 7). Average densities of *B. fusca* at 42 DAP and egg-larval mortalities were generally higher following maize-maize with 60 or 120 kg N/ha than following legumes, and higher after both leguminous plants than after maize-maize without additional N (Table 7). Data on stem tunneling and yield losses differed significantly among treatments while no such differences were found in both experiments in the percentages of dead-hearts (Table 7).

In both experiments the greatest extent of stem tunneling was found in the maize-maize and 120 kg N/ha treatment, and no difference was found between the maize after legumes and the maize-maize without additional N treatments (Table 7). By contrast, in both experiments the highest yield losses were found in the maize-maize without additional N treatment. Overall, *B. fusca* densities at 42 DAP and the extent of stems tunneled were 1.1-1.4 and 1-1.8 times, respectively, higher in experiment 1, where maize was planted during the long-short rainy seasons sequence, than in experiment 2, where maize was planted during the short-long rainy seasons sequence.

Table 7. Effect of different fallow and rotation systems on least square means (± SE) of *Busseola fusca*, egg to larvae mortality and damage variables in the humid forest of Cameroon.

Treatments[1]	*B. fusca* per plant		Egg-larva mortality (%)	Stem tunneling (cm)	Dead-hearts (%)	Yield loss (%)
	42 DAP	63 DAP				
Experiment 1						
Maize-maize	1.67 ± 0.3c	1.22 ± 0.1a	45.9 ± 2.5c	22.0 ± 4.8b	3.8 ± 0.3a	25.0 ± 1.1a
Maize-maize + 60 kg ha-1	4.25 ± 0.5a	1.11 ± 0.2a	88.2 ± 4.8a	24.7 ± 4.9b	4.1 ± 0.3a	6.1 ± 1.0b
Maize-maize + 120 kg ha-1	4.58 ± 0.3a	1.19 ± 0.1a	91.5 ± 5.1a	59.8 ± 4.7a	4.0 ± 0.3a	3.7 ± 1.1b
Maize-soybean	2.62 ± 0.3b	1.13 ± 0.1a	70.3 ± 3.2b	14.8 ± 4.1b	3.7 ± 0.2a	4.9 ± 0.9b
Maize-mucuna	3.33 ± 0.2b	1.10 ± 0.1a	69.2 ± 3.8b	16.9 ± 4.2b	4.1 ± 0.2a	4.6 ± 1.0b
Experiment 2						
Maize-maize	1.39 ± 0.2d	1.03 ± 0.2a	38.4 ± 3.1c	19.5 ± 1.9b	2.8 ± 0.3a	17.4 ± 1.0a
Maize-maize + 60 kg ha-1	3.03 ± 0.1ab	1.33 ± 0.2a	85.8 ± 3.5a	20.4 ± 2.0b	2.9 ± 0.4a	2.6 ± 1.1b
Maize-maize + 120 kg ha-1	3.22 ± 0.2a	1.41 ± 0.1a	88.2 ± 4.2a	32.0 ± 1.8a	3.0 ± 0.3a	1.8 ± 1.0b
Maize-soybean	2.46 ± 0.1bc	1.21 ± 0.1a	63.3 ± 3.4b	15.7 ± 1.6b	3.2 ± 0.2a	4.1 ± 0.9b
Maize-mucuna	2.67 ± 0.2c	1.19 ± 0.1a	58.5 ± 2.8b	16.2 ± 1.8b	3.6 ± 0.2a	3.2 ± 0.9b

[1] Experiment 1 was conducted during the long and short rainy seasons sequence and Experiment 2 during the short and long rainy seasons sequence. Within columns, means followed by the same letter are not significantly different at P = 0.05 (t-test).

DISCUSSION

Results of the countrywide surveys on stem and cob borers in West Africa so far showed that borers oviposited heavily on wild host plants but their relative importance, both on maize and wild grasses, varied between regions, eco-zones and within the same eco-zone (Schulthess *et al*. 1997). *S. calamistis* and *E. saccharina*, the most frequently reported maize borers in West Africa (Bosque-Pérez and Mareck 1990; Gounou *et al*. 1994; Schulthess *et al*. 1997), were found in several grasses, but *S. calamistis* was seven times more abundant on *P. maximun* and *P. polystachion* than on *P. purpureum*, while *E. saccharina* was equally abundant on the three surveyed grasses. However, depending on the grass species *S. calamistis* abundance was 1.5-10 times higher than that of *E. saccharina*. *B. fusca*, the predominant borer in the humid forest of Cameroon (Chabi-Olaye *et al.* 2005b; Ndemah 1999; Schulthess *et al*. 1997), where wild grasses are scarce (Ndemah *et al*. 1999), was 10.3 times higher on *P. purpureum* than on *S. arundinaceum*. Given the geographic distribution of stem borers and the role of wild host plants, Schulthess *et al.* (1997) argued that the differences in relative importance of species may be due to differences in human population densities. Increasing population pressure and the concomitant expansion of agricultural areas often result in deforestation and displacement of wild habitats of borers, which probably affect the population dynamics of both borers and their natural enemies.

In the humid forest of Cameroon, lower densities of *B. fusca* were found in the IVs compared to up-land maize fields during the first and second cropping seasons. However, its abundance compared to other borer species in the area did not vary considerably among seasons. By contrast, *S. calamistis* density was > 90 % higher in IVs than in up-land maize fields. Chabi-Olaye *et al*. (2001), using eggs of *B. fusca* and three *Sesamia* spp. as hosts, showed that all four hosts yielded similar levels of parasitism by the sceliotid *Telenomus isis* (Polaszek), egg emergence and sex ratios. In the present study, egg parasitism was up to twofold higher in IVs than in the upland maize fields. These findings suggest that IVs planted with maize during the dry season maintain carry-over populations of not only *B. fusca,* but also of its natural enemies, as well as of alternative minor hosts such as *S. calamistis*. Thus, if crops grow concurrently in IVs and upland fields in an area, the chances of emerging borer females to encounter a suitable host plant, e.g., maize, for oviposition and survival of their progenies, and ensuing overall pest densities in an area increase. IVs therefore should be targeted for inundative releases of egg parasitoids against *B. fusca* with the aim of reducing yield losses in adjacent up-land maize fields.

Results from different field trials in Benin and Cameroon where grasses were grown as border rows around maize plots lead to reduced borer densities in such maize fields compared to non-surrounded maize stands (Ndemah *et al*. 2002). Oviposition and development studies with *S. calamistis* and *E. saccharina* carried out in Benin, using a range of grass species, showed that borers oviposited heavily on grasses (Sekloka 1996; Semeglo 1997; Shanower *et al*. 1993), but larval mortality was nearly 100 % (Shanower *et al*. 1993). Thus, these grasses acted as trap plants and hence can provide natural control for stem and cob borers. Promising grass species in SSA are among others sudan grass *Sorghum vulgare* var. *sudanense* Hitchc., a commercial fodder grass, molasses grass *Melinis minutiflora* Beauv., a non-host forage plant, and silverleaf desmodium *Desmodium uncinatum* (Jacq.) DC (Khan *et al*. 1997; 2000). In

West and Central Africa the most reported grass species are *P. purpureum* and *P. maximum* (Innes 1977; Ndemah *et al*. 2002; Schulthess *et al*. 1997).

Recent work in western Africa showed, that maize intercropped with cassava or grain legumes considerably reduced maize yield losses due to *S. calamistis* (Schulthess *et al*. 2004) and *B. fusca* (Chabi-Olaye *et al*. 2005b), as a result of reduced oviposition of adult moths. The value of such cropping systems has been extensively reviewed by Baliddawa (1985) and van Emden and Dabrowski (1994). Overall, these authors concluded that intercropping contributes to the diversity of agro-ecosystems and can reduce population build-up of insect pests. However, not all attempts to control pests through mixed cropping have been successful. Especially the choice of the associated crops and the spatial arrangements for the intercrops is of outmost importance. In our study higher reduction in *B. fusca* oviposition was observed in within row planting compared to strip planting, though both arrangements did not differ in terms of their land-equivalent ratios. Combined maize-cassava crops yielded a higher land-equivalent ratio than maize-legumes. In addition, a study by Sétamou (1999) showed that *M. nigrivenella* preferred jack- and velvetbeans than maize. Thus, with the increasingly popular practice of using cover crops in maize production systems in SSA to improve soil fertility, *M. nigrivenella* populations could greatly increase on these plants, thereby endangering the following maize crop. However, clever timing can produce the opposite effect, i.e., if the emergence of the maize tassels coincides with pods formation on the legumes, as then the attractive pods will cause *M. nigrivenella* to oviposit principally on jack- and velvetbeans (Sétamou 1999).

In our experiments in the humid forest of Cameroon, the leguminous fallow systems and the maize-maize and additional N treatments had significantly higher stem borer densities. However, the borer-induced grain yield losses were considerably lower than in the continuous maize cultivation without additional N fertilization. These results confirm previous findings by Sétamou *et al.* (1995) who hypothesized that an increased nutritional status of the plants enhance both borer fitness and plant vigor, but with a net benefit for the plants. Thus, improving soil fertility can effectively complement pest control.

Results from the presented studies provide an increased understanding of the role of IVs on the population dynamics of maize cob and stem borers in SSA. Moreover, if properly managed, increased crop-plant diversity can considerably reduce the build-up of pest populations and increase the yield of maize, thus becoming an interesting land-use strategy for resource-poor and land-constrained farmers in SSA.

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THE POTENTIAL ROLE OF INSECT SUPPRESSION IN INCREASING THE ADOPTION OF COVER CROPS

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ABSTRACT

We used a survey of attendees of an organic farming conference in Minnesota, U.S.A., to determine whether farmers would be more likely to adopt a fall-seeded winter rye cover crop preceding soybeans if doing so could aid in the suppression of soybean aphid, *Aphis glycines* Matsumura (Homoptera: Aphididae). Of the 24 soybean growers that responded to our survey, 22 indicated that they would be more likely to either adopt or retain this cover cropping practice if it could aid in soybean aphid suppression. Insect pest suppression may therefore be an effective way to augment adoption of cover cropping practices.

INTRODUCTION

Fall-seeded winter cover crops have the potential to improve soil organic matter, reduce soil erosion, manage excess nutrient loads, and provide for weed and insect management. While the benefits of cover crops to soil fertility, nutrient scavenging, and erosion and weed control are well-documented, research showing improvement in insect control has lagged behind in many cover-cropping systems. To illustrate this, we did a literature search on the AGRICOLA database to compare the number of scientific citations associated with cover crops and terms associated with 'soil', 'weeds' and 'insects'. There was a clear deficit of citations associated with the term 'insects' and 'insect control' when paired with 'cover crop' (Table 1).

Table 1. Number of references recovered by AGRICOLA searches for the period 1984-2004 when 'cover crops' (CC) was entered along with one other term.

Terms Entered	Number of References
CC and soil	803
CC and soil fertility	181
CC and organic matter	125
CC and weeds	159
CC and weed control	253
CC and insects	26
CC and insect control	41

Indeed, judging by this brief search, it would appear that only two scientific articles per year have been published on the effects of cover crops on insect control, compared with more than 12 per year on the effects of cover crops on weed control.

Despite all of the documented benefits of cover crops, they have seen relatively low adoption rates in midwestern corn/soybean production areas in the U.S. (DeBruin *et al.* 2005; Stoskopf 1985). Reasons for low adoption include perceived logistic difficulties in the establishment of fall-planted cover crops and apprehension that yields may suffer in cover-cropped systems. We hypothesize that the adoption rate of cover crops would be increased by a clear demonstration that their use could result in improved insect pest control.

THE RYE SYSTEM

We are testing the effect of a fall-seeded winter rye (*Secale cereale* L.) cover crop following corn and preceding soybeans to aid in the suppression of the soybean aphid, *Aphis glycines* Matsumura (Homoptera: Aphididae). Rye is a well-adapted fall-seeded cover crop for the northern U.S.A., exhibiting exceptional winter hardiness as well as the ability to emerge and grow at low temperatures under a wide soil moisture and soil fertility range. Rye seeds are drilled into corn residue to produce an overwintering rye stand that resumes growth in spring (Porter 2004). Soybeans are drilled into the rye stand at approximately the same time as soybeans are typically planted by organic producers in the Upper Midwestern U.S. (late May – early June). At this point, the rye can be up to three feet in height. The rye is mowed using a flail mower or stalk chopper when soybeans can be up to 6 inches tall (V1, V2 stage).

A number of lines of reasoning suggest that fall-seeded rye cover crops would reduce soybean aphid pressure in soybeans. First, work in numerous cropping systems other than soybeans has demonstrated reduced densities of pest aphids in the presence of various forms of increased vegetational diversity (reviewed by Gurr *et al.* 2000). Second, studies in China have shown that soybeans intercropped with corn at a very small spatial scale have lower densities of soybean aphids than monoculture soybeans (Wu *et al.* 2004). And lastly, some organic soybean growers in Minnesota experimenting with fall-seeded rye cover crops have reported reduced soybean aphid densities and increased densities of soybean aphid predators in cover-cropped fields (Porter 2004). Winter rye could aid in soybean aphid suppression either by reducing colonization of soybean by soybean aphid in the early season and/or by enhancing natural enemies (predators, parasitoids and pathogens) of soybean aphid (Pickett and Bugg 1998). We have preliminary data showing that winter rye harbors aphid natural enemies including aphid parasitoids, coccinellid beetles, and syrphid flies, in the spring during the time that soybean aphid colonization typically occurs (Table 2).

Table 2. Aphids and aphid natural enemies collected from overwintered rye at the University of Minnesota Agricultural Experiment Station, St. Paul, Minnesota, in 2004.

Date	Aphid Species	*Natural Enemy*
5/26/2004	*Rhopalosiphum padi* *Sitobium avenae* *Schizahis gramium*	*Coccinella septempunctata* *Harmonia axyridis* *Ahpidius* sp. (reared from *S. graminum*)
6/4/2004	*R. padi* *S. avenae* Unidentified aphid sp.	*C. septempunctata* *C. trifasciata* *H. axyridis* *Coleomegilla maculata* *Aphelinus* sp. (adult) Unidentified Syrphidae

Coccinellid beetles are the most important predators of soybean aphid in the midwestern U.S. (Fox *et al.* 2004; Rutledge *et al.* 2004), as well as in China (Liu *et al.* 2004; Wu *et al.* 2004). At one of our field sites, the native *Coleomegilla maculata* is present in soybean fields early in the season (i.e., when grain aphids would be present on winter rye), and the exotic *Harmonia axyridis* is present later in the season (Fig. 1). Entomopathogenic fungi are also present in soybean fields, but they tend to become active in July or August, which is after the winter rye is mowed in our system (Fig. 2). It remains to be seen whether the altered microclimate associated with winter rye will encourage earlier epizootics of entomopathogenic fungi in soybean aphid populations.

THE SURVEY

We used a one-page written survey of attendees of the 2005 Minnesota Organic and Grazing Conference and Trade Show, held in St. Cloud, MN, on Jan. 21 – 25, 2005, to evaluate the hypothesis that soybean growers would be more likely to utilize fall-seeded winter rye as a cover crop preceding soybean if this practice could aid in soybean aphid suppression. We were particularly interested in the opinions of organic growers because they cannot use conventional insecticides to control the soybean aphid. To our knowledge, no organically-approved sprays have been shown to be effective against the soybean aphid. The survey was aimed at soybean growers, and we asked respondents whether they farmed organically or conventionally, whether they had ever had problems with soybean aphid, and whether they

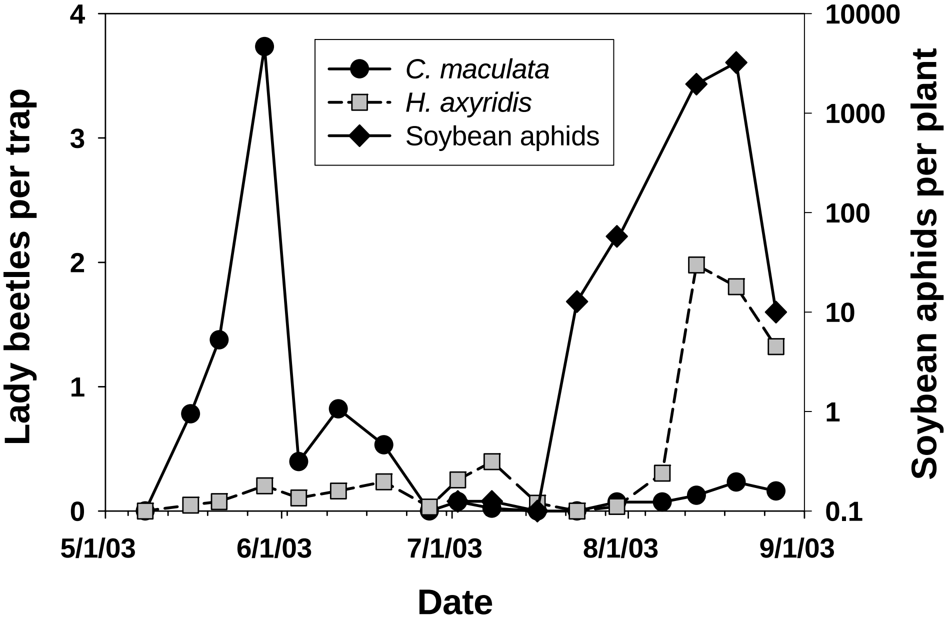

Figure 1. Average numbers of two species of lady beetles (*Coleomegilla maculata* and *Harmonia axyridis*) and soybean aphids (log scale) in soybean fields in 2003 in Lamberton, MN, U.S.A.

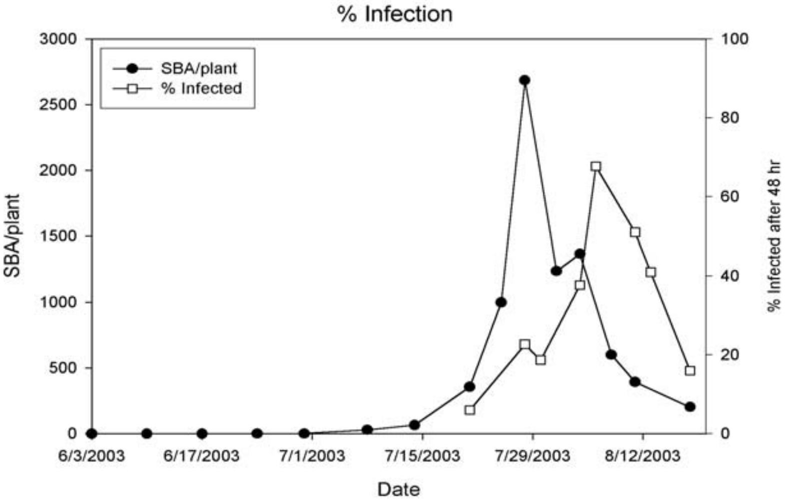

Figure 2. Soybean aphid population, 2003 and prevalence of entomopathogenic fungi in a 48h bioassay of field collected aphids, Rosemount, MN.

had ever used a fall-seeded rye cover crop preceding soybeans. For those growers that had problems with soybean aphids, we asked what management tactics they used (if any) to suppress them, and for those growers that reported having planted a winter rye cover crop, we asked for the primary reason for the use of this tactic. Finally, we asked whether respondents would be more likely to either use the fall-seeded rye cover crops for the first time, or continue using them, if they could aid in the suppression of soybean aphid. The surveys were passed out at a luncheon during the conference and at a symposium presentation on insect pests. The conference had 277 registered attendees, and we received 25 completed surveys.The results of the survey are presented in Table 3.

Table 3. Summary of grower survey results*: rye cover crops and soybean aphid (SBA) management.

Questions	Organic growers	Conventional growers	Growers using both practices
Q1) Have you had problems with SBA?			
A) Yes	9	1	2
A) No	9	3	0
Q2) If so, what management tactic was used for SBA?			
A) None	3	0	0
A) Organic insecticide	4	0	1
A) Non-organic insecticide	0	1	0
Q3) Do you use fall-planted rye cover crops?			
A) Yes	13	1	1
A) No	5	3	1
Q4) If so, why do you use the rye cover crops?			
A) Weed management	9	0	0
A) Soil management	2	0	0
A) Weed and soil management	2	1	1
Q5) If not already using rye cover crops, would you be more likely to do so if it could aid in suppressing SBA?			
A) Yes	4	1	1
A) No	0	1	0
Q6) If already using rye cover crops, would you be more likely to continue doing so, if it could aid in suppressing SBA?			
A.) Yes	12	1	1
A.) No	1	0	0

*Of the 25 growers who responded, 24 grew soybeans (18 organic growers, 4 conventional growers, and 2 growers using both practices).

Out of the 24 respondents that grew soybeans, 18 used exclusively organic methods, four were conventional growers, and two used both organic and conventional practices. Overall, one-half of the respondents reported having had problems with soybean aphid, and of the organic growers with soybean aphid problems, about half did nothing to manage them, and half used organically-approved insecticides. Overall, more than 60% of the soybean growers (15/24) reported using fall-planted rye cover crops. We believe this to be an overestimate of the state-wide adoption of rye winter cover crops. We suspect that growers that use cover crops are more likely to attend the organic farming conference and/or more likely to respond to a survey with the term ‘cover crop’ in the title. It appears from our results that organic farmers are more likely to use the rye cover crop than conventional growers are (72% vs. 25%), but the number of respondents (especially conventional growers) is too small to assess this question properly. The main reason cited for the use of winter rye was weed management, but soil management issues (organic matter augmentation, erosion control) were cited as well.

Our survey showed very clearly that adoption of winter rye cover-crops would likely increase if this practice could aid in the suppression of soybean aphid. Of seven respondents that had never used winter rye, six indicated that they would be more likely to use rye if it could aid in the suppression of soybean aphid. Similarly, of 15 respondents that had already been using rye cover crops, 14 indicated that they would be more likely to continue using rye if it could aid in the suppression of soybean aphid.

Thus, cover crop-associated insect suppression (either via reduced pest colonization and/or improved biological control) is a potential means to increase adoption of cover cropping practices, which themselves produce benefits that are felt beyond the farmers' fields, such as nutrient scavenging and reduced soil erosion.

ACKNOWLEDGEMENTS

We thank Meg Moynihan and Mary Hanks for facilitating our survey activities at the conference and Glen Borgerding for handing out surveys at his presentation. This work was funded by an award from the USDA Integrated Organics Program and the Minnesota Agricultural Experiment Station.

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USING SYNTHETIC HERBIVOR-INDUCED PLANT VOLATILES TO ENHANCE CONSERVATION BIOLOGICAL CONTROL: FIELD EXPERIMENTS IN HOPS AND GRAPES

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ABSTRACT

The potential of using synthetic herbivore-induced plant volatiles (HIPV) as a cultural tool to enhance conservation biological control of insects and mites is being researched in hops and grapes in Washington State. Results to date indicate that a number of natural enemy species in the families, Chrysopidae, Hemerobiidae, Anthocoridae, Geocoridae, Miridae, Coccinellidae, Syrphidae, Braconidae, Empididae and Mymaridae, are attracted to sticky traps baited with aqueous methyl salicylate (MeSA), hexenyl acetate, farnesene or octyl aldehyde. Hop yards and grape blocks baited with controlled release sachet (CRS) dispensers of MeSA recruit larger populations of some insect predators (e.g., *Stethorus punctum picipes* Casey (Coleoptera: Coccinellidae), *Orius tristicolor* White (Hemiptera: Anthocoridae), *Chrysopa nigricornis* Burmeister (Neuroptera: Chrysopidae) than unbaited blocks, resulting in some instances, in improved biological control of spider mites and aphids. CRS dispensers of methyl salicylate, methyl jasmonate and hexenyl acetate increased populations of two parasitic wasp genera (*Anagrus*, *Metaphycus*) in grapes, possibly by 'signalling' to the plants to produce HIPVs. The use of synthetic HIPVs/plant-signalling compounds as 'Herbivore-Induced Plant Protection Odors' (HIPPOs) has the potential to provide a novel yet practical strategy for improving the efficacy and reliability of conservation biological control in a variety of agricultural ecosystems.

INTRODUCTION

The use of conservation biological control (CBC) as a component of integrated pest management in agriculture is a strategy that is increasing in importance and popularity (Barbosa 1998). Concurrent with the increasing use of CBC in agriculture has been a realization that 'generalist' natural enemies (i.e., those that have a broad prey preference) can often play a major role in pest suppression (Symondson *et al.* 2002). Thus CBC as a strategy that enhances guilds or communities of both specialist and generalist natural enemies is now viewed as a pest management strategy, very likely to improve crop protection. Another factor that has encouraged and enhanced the use of CBC in many crop systems is the availability and use of pesticides that are narrow-spectrum and safe to many beneficial insects and mites (James 2002; 2003d; 2004).

CBC research in many crop systems is focused on improving reliability by strengthening the natural enemy community both in terms of population density and species diversity (Cardinale *et al.* 2003). Inevitably there are two aspects of this problem that need to be addressed; 1) attraction of beneficial arthropods to the crop during early cropping phases and 2) maintenance of these populations throughout the life of the crop. Kean *et al.* (2003) identified 'spatial attraction' of natural enemies as the best way of enhancing CBC. Their results suggested an almost linear relationship between natural enemy attraction and prey equilibrium. The use of semiochemical attractants (e.g., host/prey-derived chemicals) to increase recruitment and retention of beneficial arthropods in crop ecosystems, is an area of opportunity for enhancement of CBC.

Herbivore-Induced Plant Volatiles (HIPV) offer the best potential for developing effective and practical semiochemical-based strategies for manipulating natural enemy populations. Plants attacked by herbivores emit specific chemical signals. These are the 'words' of a complex language used to 'warn' other plants of impending attack and to recruit predatory/parasitic arthropods for 'bodyguard' services. Such plant 'bodyguards' respond to plants in distress, and benefit from the food/host resources available (Sabelis *et al.* 1999). A voluminous body of literature now exists on this phenomenon, first demonstrated in a series of elegant laboratory investigations based on a bean plant-spider mite-predatory mite system (Sabelis and Dicke 1985; Sabelis and Van de Baan 1983; Sabelis *et al.* 1984). The qualitative and quantitative characteristics of HIPV can vary according to the herbivore involved, the plant species and even genotype (Takabayashi *et al.* 1994; Turlings *et al.* 1993). HIPV may function as direct attractants and/or as plant signals. Airborne or topically applied methyl jasmonate (MeJA) can act as a plant signal by causing the emission of volatiles in some plants mimicking those produced in response to herbivore damage (Hunter 2002). There is some evidence that methyl salicylate and hexenyl acetate may also function as plant signals (Engelberth *et al.* 2004; Ozawa *et al.* 2000; Shulaev *et al.* 1997). The use of HIPV as signalers or elicitors of 'correct' and complete blends of natural enemy attracting emissions, is an attractive possibility for manipulating predator and parasitoid populations in pest management.

Compared with the abundance of laboratory studies on HIPV, there is a dearth of field-based studies (Hunter 2002). The first demonstration of the impact of HIPV in the field came from research on biocontrol of psyllids in pear orchards in the Netherlands (Drukker *et al.* 1995), which showed that densities of predatory bugs (Anthocoridae) increased with the density of caged psyllids. Shimoda *et al.* (1997) recorded more predatory thrips on sticky cards near spider mite-infested bean plants than on traps near uninfested plants. Bernasconi *et al.* (2001) trapped more natural enemies near plants damaged and treated with caterpillar regurgitant, than near undamaged, untreated plants.

The first direct evidence for the potential of synthetic HIPV as field attractants for beneficial insects came from this research group (James 2003a,b,c) which demonstrated attraction of a number of insect species and families to methyl salicylate (MeSA) and (Z)-3-hexenyl acetate (HA) in Washington hop yards. Insects attracted to MeSA included *Chrysopa nigricornis* Burmeister (Chrysopidae), *Geocoris pallens* Stal. (Geocoridae), *Stethorus punctum picipes*

(Casey) (Coccinellidae) and species of Syrphidae. Three species were attracted to HA, a predatory mirid, *Deraeocoris brevis* (Uhler), an anthocorid, *Orius tristicolor* (White) and *S. punctum picipes*. Subsequent synthetic HIPV/trapping studies revealed at least 13 species or families of beneficial insects responded to one or more synthetic HIPV (James 2005). Thirteen HIPV attracted one or more species/family of beneficial insect.

Evidence for recruitment and retention of beneficial insects in grapes and hops using controlled-release (CR) dispensers of MeSA, was presented by James and Price (2004). In a replicated experiment conducted in a juice grape vineyard, sticky cards in blocks baited with MeSA captured significantly greater numbers of five species of predatory insects (*C. nigricornis*, Hemerobius sp., *D. brevis*, *S. punctum picipes*, *O. tristicolor*) than unbaited blocks. Four insect families (Syrphidae, Braconidae, Empididae, Sarcophagidae) were also significantly more abundant in the MeSA-baited blocks, as indicated by sticky card captures. Monitoring conducted in a MeSA-baited hop yard indicated development and maintenance of a beneficial arthropod population that was nearly four times greater than that in an unbaited reference yard. The large population of predatory insects in the MeSA-baited hop yard was associated with a dramatic reduction in spider mite and aphid numbers, and sub-economic populations were maintained for the rest of the season. The evidence presented in James and Price (2004) is highly suggestive that the use of controlled-release MeSA in a crop could increase recruitment and residency of populations of beneficial insects. Here, we report additional data from field experiments in 2004 on the use of synthetic HIPVs to enhance CBC.

MATERIALS AND METHODS

RECRUITMENT OF BENEFICIAL INSECTS TO HOP YARDS AND VINEYARDS

CR dispensers containing MeSA (5 g, 98%, Chem-Tica International, Costa Rica) were deployed in four hop yards and three vineyards (one juice grape, two wine grape) in south-central Washington State during May-September 2004. A control yard with similar characteristics (size, variety etc) was also established,1-2 km from each MeSA site. Dispensers were stapled to supporting posts (~ 0.5 m above ground) in the yards. Dispenser deployment density for the vineyards was A – 336, B – 432 and C – 642, and for the hop yards, A – 180, B – 447, C – 516 and D – 556 per hectare. Beneficial and pest arthropod populations were monitored weekly at each site (including controls) by examining leaf samples, conducting canopy shake sampling and by retrieving/replacing four yellow sticky cards stapled to poles (see James and Price 2004) for full sampling methodology). Insecticide and miticide applications were kept to a minimum at all sites and where possible, chemicals known to have minimal effect on beneficial arthropods were used. Sticky cards were positioned randomly within each hop yard or vineyard and separated by at least 10 m. After collection, they were examined in the laboratory and all beneficial insects identified and counted. Trap data were analyzed using either the Mann-Whitney Rank –Sum Test, or the Kruskal-Wallis ANOVA on ranks.

ATTRACTION OF PARASITIC WASPS TO HIPV-BAITED GRAPES

A field experiment was conducted in an unsprayed 10 ha juice grape (cv. Concord) vineyard in south central Washington State during May-September 2004, to determine the attraction of synthetic formulations of three HIPV, MeSA, methyl jasmonate (MeJA) and (*Z*) – 3 – hexenyl acetate (HA)) to parasitic wasps. CR dispensers containing 5 g (MeSA), or 1 g (HA, MeJA) were prepared and supplied by Chem-Tica Internacional (Costa Rica). The dispensers for each HIPV were deployed in three 8 X 30 m blocks distributed in a completely randomized design with three unbaited blocks in the vineyard. Each block contained 65 grapevines (5 rows of 13 vines) and blocks were separated by a minimum of 100-m. In the HIPV blocks, 14 dispensers were deployed on April 29 with half stapled at the base of supporting posts and the remainder attached to trellis wires at approximately 1.5 m above the ground. Both deployment types were evenly and alternately distributed in each block giving an approximate density equivalent to 586/ha. Dispensers were replaced on July 15. Populations of parasitic wasp species were monitored using three yellow sticky cards per block, equally spaced (8 m apart) along the center row. Cards were collected and replaced weekly. Parasitic wasps (Mymaridae, Encyrtidae) were identified to genus (*Anagrus* and *Metaphycus*) and counted in the laboratory under a stereomicroscope. At two weekly intervals small samples (~25 individuals) of trapped *Anagrus* spp. were identified to species using a compound microscope and the key of Triapitsyn (1998). Trapping data were analyzed using either the Kruskal-Wallis ANOVA on ranks (KW), with means separated by Dunn's method, or the Mann-Whitney Rank-Sum Test (MW).

RESULTS

RECRUITMENT OF BENEFICIAL INSECTS TO HOP YARDS AND VINEYARDS

Hop yards. All of the unbaited control yards were treated with at least one miticide (for twospotted spider mite, *Tetranychus urticae* Koch) and/or insecticide (for hop aphid, *Phorodon humuli* Schrank). The control yard for site B was treated with abamectin and imidacloprid, pesticides known to be harmful to some beneficial insects (James 2001; James and Vogele 2001), thus this pair was not used in this analysis. The remaining control yards were treated with bifenazate and/or pymetrozine, both of which are considered safe to most beneficial arthropods in hops (James 2002). MeSA sites A and D were not treated with any insecticide/miticide, while site C received one application of the aphicide, pymetrozine and another of *Bacillus thuringiensis*. The MeSA-baited hop yards developed larger populations (3-5 X) of predatory insects than corresponding unbaited yards (Figs. 1-2). Predatory insect species that were significantly more abundant in MeSA-baited yards than unbaited yards included *O. tristicolor*, *G. pallens*, *D. brevis* and *Stethorus* spp (Fig. 2). Other species and families recorded and quantified but pooled here as 'predatory insects' included lacewings (Chrysopidae, Hemerobiidae), hover flies (Syrphidae), aphidophagous lady beetles (Coccinellidae), predatory thrips (Thripidae) and damsel bugs (Nabidae). Numbers of the predatory hemipterans, *O. tristicolor*, *G. pallens* and *D. brevis* were combined and were six times more abundant in the high deployment rate MeSA yards than in the corresponding control yards (Fig. 2). The

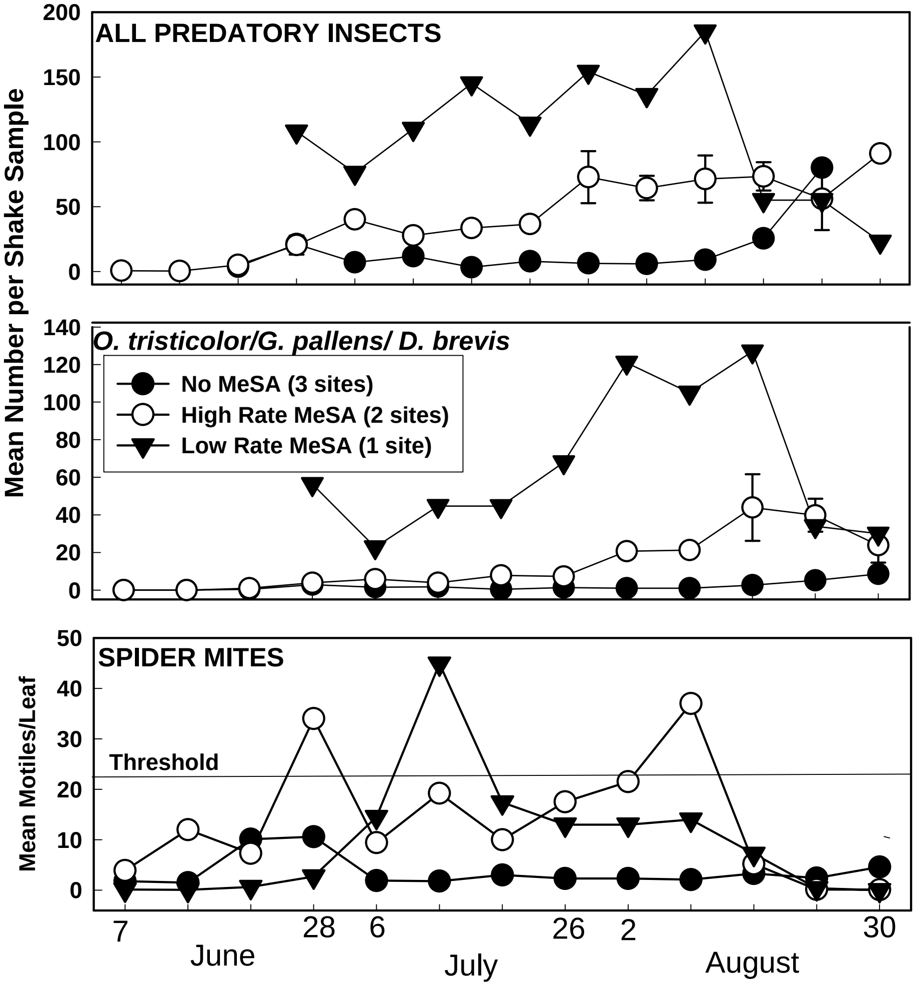

Figure 1. Mean (± SE) abundance and phenology of predatory insects and spider mites in hop yards baited with low (180 dispensers/ha) and high (516/556 dispensers/ha) deployment rates of MeSA or left unbaited.

difference was even greater between the low deployment rate yard and corresponding control yard (21.5 X). Similarly, mite-eating lady beetles (*Stethorus* spp.) were 23.5 X more abundant in the low rate MeSA yard than in the control yard (sticky card data) (Fig. 2). Predatory insect abundance was greater and earlier in establishment in the low rate yard than in the high rate yards (Fig. 1). Spider mite populations in the MeSA-baited yards, briefly exceeded the recommended miticide spray threshold (Fig. 1).

In the low rate MeSA yard spider mites exceeded 40 motiles/leaf for a week in early July but stayed below the threshold for the rest of the season. Similarly, populations in the high rate yards briefly climbed above 30 motiles/leaf in late June and again in mid-August. However, hop cone yield and quality were not affected in the MeSA yard and were comparable to those obtained in the non-MeSA yards. Large populations of predatory insects remained in the MeSA-baited yards during August despite the relatively low numbers of spider mites and aphids (e.g., in the low rate MeSA yard there was a mean of 4.9 ± 2.1 mites/aphids per leaf/ week in August). Other arthropod prey (e.g., thrips) were also present and may have helped sustain the generalist predator community.

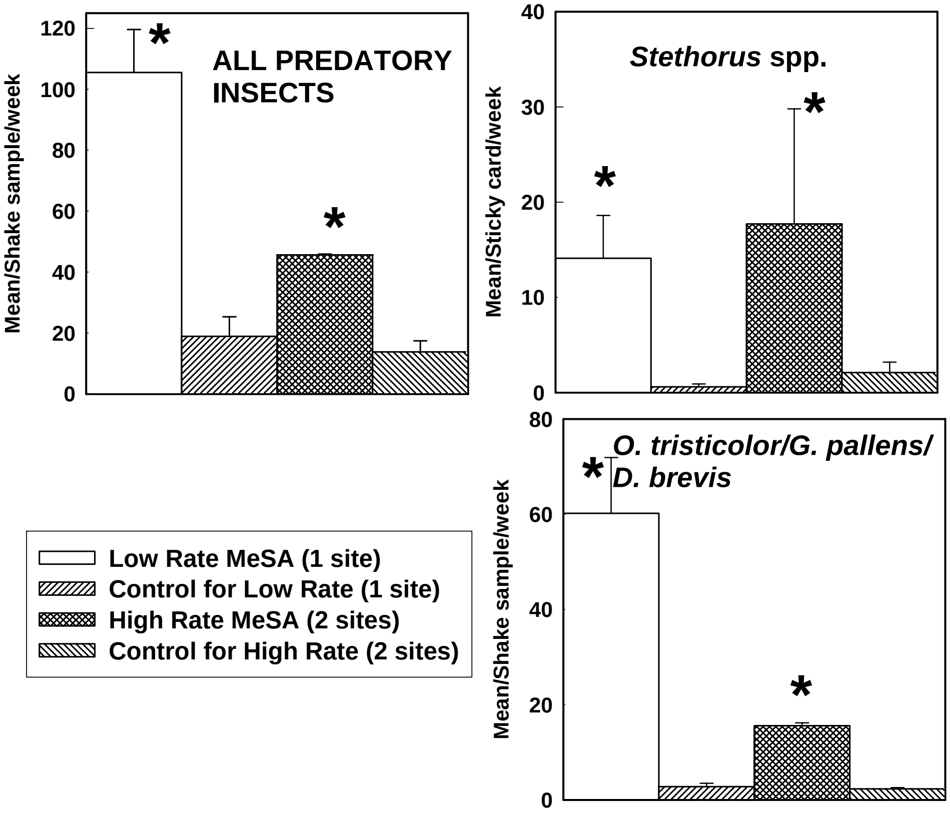

Figure 2. Mean (± SE) abundance of predatory insects (all taxa combined), *Stethorus* spp., *O. tristicolor, G. pallens* and *D. brevis* in MeSA-baited and unbaited hop yards during May-September 2004. Columns marked with asterisks are significantly greater than the corresponding control column (P < 0.05).

Vineyards. Predatory insect populations in vineyards were much smaller than in hop yards. However, significant differences in the abundance of some species or families (e.g., Chrysopidae, *D. brevis, Stethorus* spp.) between MeSA-baited and unbaited sites were indicated by sticky card captures (Fig. 3).

ATTRACTION OF PARASITIC WASPS TO HIPV-BAITED GRAPES

Only two genera of Hymenoptera occurred commonly; *Anagrus* spp. (Hymenoptera: Mymaridae) and *Metaphycus* sp. (Hymenoptera: Encyrtidae). *Metaphycus* sp. appeared to be a parasitoid of scale insects, primarily *Parthenolecanium corni* (Bouche), present on vines in the vineyard. *Anagrus* spp. trapped in this study represented at least three species (*A. erythroneurae* Triapitsyn and Chiappini, *A. daanei* Triapitsyn, *A. tretiakovae* Triapitsyn), all important in biological control of grape leafhoppers in Washington State (Storm 2002). Leafhopper and scale insect populations appeared to be evenly distributed throughout the vineyard. Analyses conducted for the entire sampling period showed that significantly greater numbers of *Metaphycus* sp. were trapped in MeSA, MeJA and HA-baited blocks than in unbaited blocks (Fig. 4) ($P < 0.001$, H =63.68, df = 3, KW). Greater numbers were trapped in MeJA and HA-baited blocks than in MeSA-baited blocks ($P < 0.001$, T = 3531 (MeJA), T =

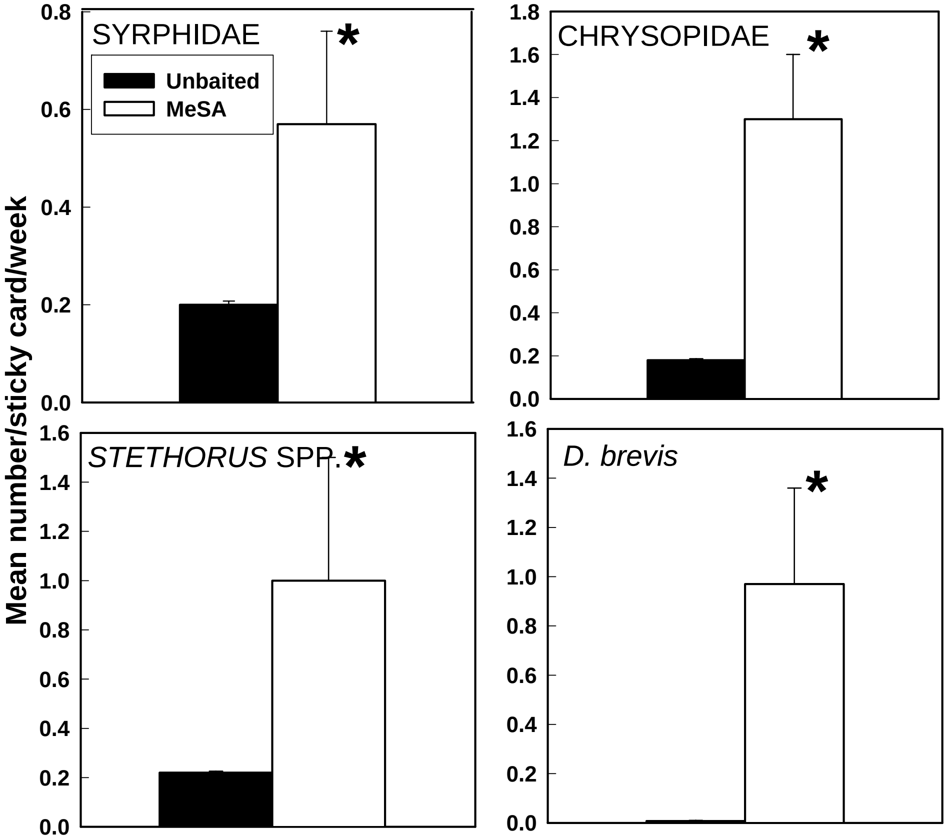

Figure 3. Mean (± SE) abundance of Syrphidae, Chrysopidae, *Stethorus* spp. and *D. brevis* in MeSA-baited and unbaited vineyards during May-September 2004 as indicated by sticky card captures. Columns marked by an asterisk significantly different from corresponding unmarked column ($P < 0.05$).

3449 (HA), MW), with no difference between MeJA and HA-baited blocks ($P = 0.33$, $T = 3819$, MW).

Greater numbers of *Metaphycus* sp. were trapped in MeJA and HA than in unbaited blocks in all months (May $P < 0.001$, $H = 21.30$, df = 3; June $P < 0.005$, $H = 12.99$, df = 3; August $P = 0.001$, $H = 53.82$, df = 3; September $P = 0.001$, $H = 32.41$, df = 3, KW) except July when wasp abundance was low ($P = 0.47$, $H = 2.55$, df = 3, KW) (Fig. 5). Numbers of *Metaphycus* sp. trapped in MeSA blocks were significantly greater than in unbaited blocks during August and September only (Fig. 5).

Numbers of *Anagrus* spp. trapped were not significantly different between baited and unbaited blocks when analyzed over the whole trapping period (Fig. 4) ($P = 0.22$, $H = 4.39$, df = 3, KW). However, when analyzed on a monthly basis, significantly greater numbers of *Anagrus* spp. were trapped in MeSA-baited and MeJA-baited blocks than in unbaited blocks during August-September (August $P = 0.05$, $H = 10.68$ September $P = 0.001$, $H = 35.38$ df = 3, KW) (Fig. 6). Traps in HA-baited blocks caught more *Anagrus* spp. than unbaited blocks during September only ($P < 0.001$, $T = 176$, MW) (Fig. 6).

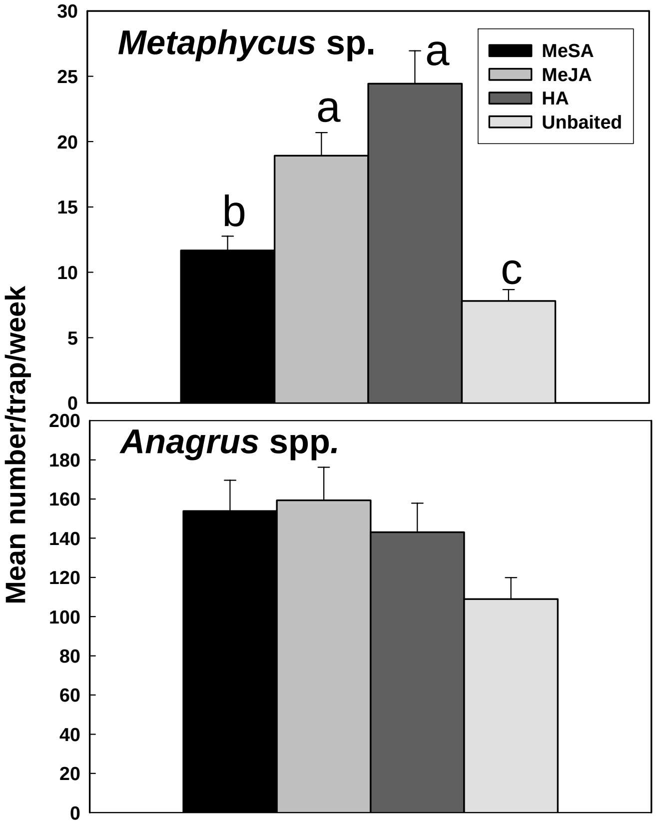

Figure 4. Mean (± SE) abundance of *Metaphycus* sp. and *Anagrus* spp. on sticky cards in methyl salicylate (MeSA), methyl jasmonate (MeJA), (*Z*) – 3 – hexenyl actetate (HA)-baited or unbaited grape blocks during May-September 2004. Different letters on columns denote significant differences for *Metaphycus* sp. ($P < 0.001$). No significant differences for *Anagrus* spp. ($P = 0.22$).

DISCUSSION

The potential of CR dispensers of MeSA for enhancing the recruitment and retention of predatory insects in crops (James and Price 2004), is further supported by the field data presented here. In addition, the attraction of parasitic wasps (not attracted to MeSA, MeJA or HA-baited sticky cards) to blocks of grapevines containing CR dispensers of MeSA, MeJA and HA, raises the possibility that these synthetic HIPV may also have practical application for signaling plants to produce their own HIPV.

Hop yards and vineyards baited with CR dispensers of MeSA at deployment rates of 180-642/hectare harbored larger populations of predatory insects than nearby and comparable yards without dispensers, as indicated by canopy shake sample, sticky card and leaf sample data. The data presented here are only a portion of the total data sets collected for each yard, but aretypical of the results obtained. Species that appeared to respond most strongly to MeSA included *Stethorus* spp., *O. tristicolor*, *G. pallens*, and *D. brevis*. During August up to 200 *Stethorus* spp. per sticky card/week were recorded from MeSA-baited hop yards, and 100-150 predatory bugs (*O. tristicolor, D. brevis, G. pallens,* Nabidae) could be shaken from <u>each</u> hop plant sampled. The largest populations of predators occurred in the hop yard baited with the lowest number of MeSA dispensers (180/ha), suggesting that the higher deployment rates used (447-642) may have been sub-optimal. It is possible that the atmospheric concen-

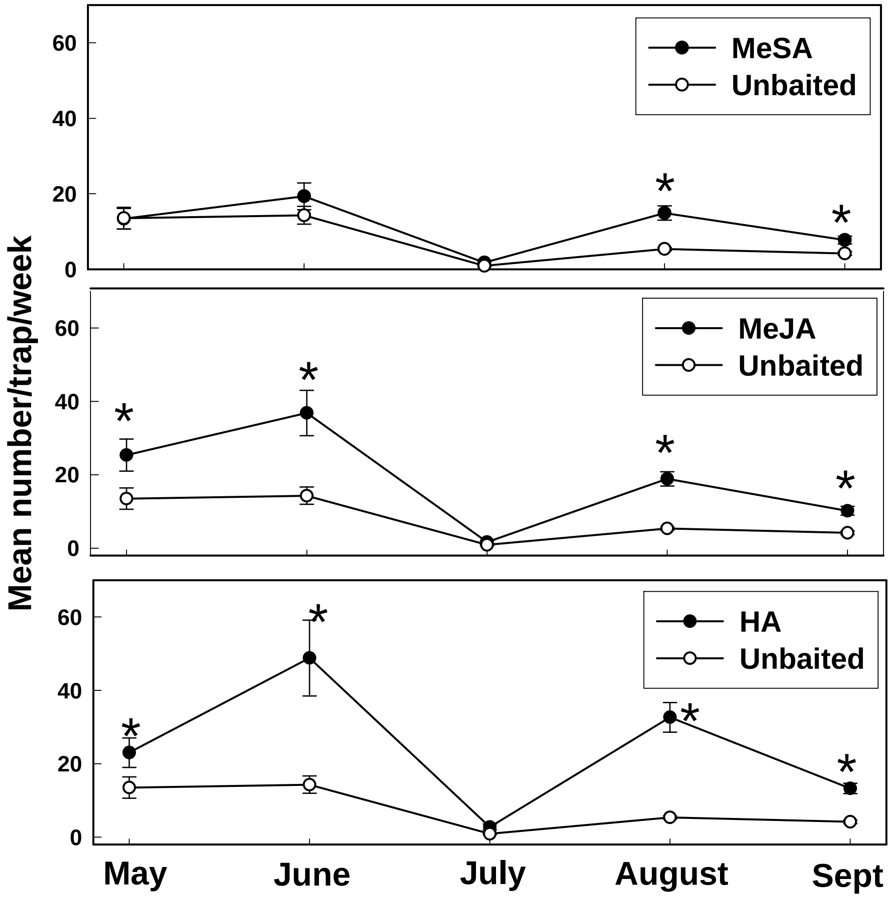

Figure 5. Monthly mean (± SE) abundance of *Metaphycus* sp. on sticky cards in methyl salicylate (MeSA), methyl jasmonate (MeJA), (*Z*) – 3 – hexenyl actetate-baited or unbaited grape blocks during May-September 2004. Asterisks denote significant differences from unbaited blocks ($P < 0.05$).

tration of MeSA in these yards, particularly during spring and early summer, was too high for optimal predator attraction. Predator populations in these yards during July, although higher than in non-MeSA yards, were not as high as in the low deployment rate yard. None of the MeSA-baited hop yards were treated with a miticide and despite short-lived increases in mite populations above the spray threshold, acceptable commercial outcomes in terms of hop cone yield and quality were achieved. The best result was achieved in the low deployment rate yard where mite numbers remained below the spray threshold for all of the cone maturation period (late July-September). The slightly larger populations of spider mites permitted to develop in the MeSA-baited yards (compared to the miticide-treated unbaited yards) may have aided predator recruitment during the summer by direct (numerical aggregation) or indirect means (natural production of HIPV). However, early season spider mite populations in baited and unbaited yards were similar, but the size of predator populations was already differing by late June. The majority of predatory insect species/families attracted to MeSA-baited hops and grapes were generalist feeders. This community of predators will develop and maintain populations even if target prey (e.g., mites), are not abundant as long as alternative prey are available. Another possible advantage of not attracting specialist predators is avoiding selection against responding to synthetic MeSA if nutritional rewards are inadequate.

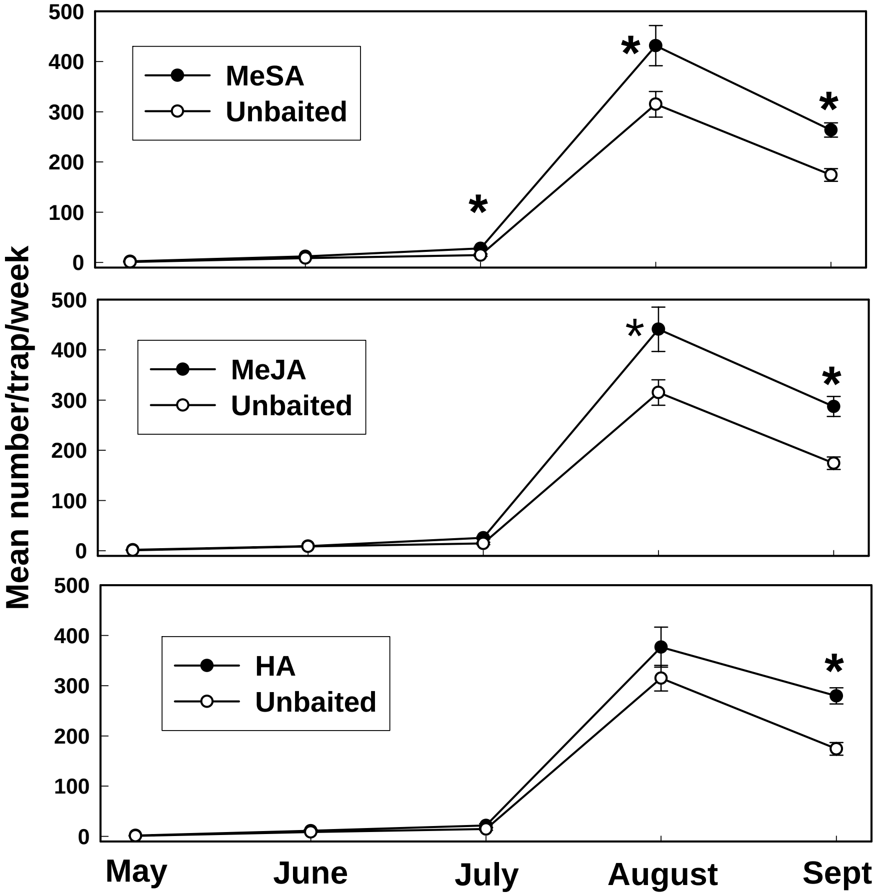

Figure 6. Monthly mean (± SE) abundance of *Anagrus* spp. on sticky cards in methyl salicylate (MeSA), methyl jasmonate (MeJA), (*Z*) – 3 – hexenyl actetate-baited or unbaited grape blocks during May-September 2004. Asterisks denote significant differences from unbaited blocks ($P < 0.05$).

This study also provided evidence for attraction of two genera of specialist parasitic wasps to grapevines baited with MeSA, MeJA or HA. James (2005) reported micro-Hymenoptera (primarily the families Scelionidae, Encyrtidae, Mymaridae (excluding *Anagrus* spp.)) were attracted to MeSA, indole and *cis*- 3- hexen – 1 – ol –baited traps. However, *Angarus* spp. and *Metaphycus* sp. were not attracted to MeSA, MeJA or HA-baited traps in James (2005) or James (2003 b). In the current study, blocks of grapevines baited with controlled-release dispensers of MeSA, MeJA or HA, recruited significantly larger populations of *Metaphycus* sp. and *Anagrus* spp. than unbaited blocks, as judged by their incidence on sticky yellow cards. The effect was strongest for *Metaphycus* sp. with significant responses to the three compounds in all months that these wasps were common. MeJA and HA were more attractive than MeSA. The response by *Anagrus* spp. to baited blocks was confined to late summer and early autumn and greatest responses were seen in the MeSA and MeJA-baited blocks. Numbers of trapped *Anagrus* spp. were small during May-July (means of 1-20 individuals/trap/week) and may have obscured any differences during this period. There was also a shift in species abundance during the season with *A. erythroneurae* and *A. tretiakovae* dominating during May-July (65%) and *A. daanei* dominating during August-September (70%).

Anagrus spp. and *Metaphycus* sp. do not respond to sticky traps baited with MeSA, MeJA or HA (James 2003; 2005), although *A. daanei* was attracted to traps baited with *cis* -3 –hexen – 1 – ol, farnesene or octyl aldehyde (James, 2005). James and Price (2004) using controlled-release dispensers of MeSA in the same vineyard used here, noted no difference in *Anagrus* spp. abundance between baited and unbaited blocks. However, almost twice as many MeSA dispensers were used in each block. There is laboratory evidence for a repellent effect of high rates of MeSA on predatory mites (De Boer and Dicke 2004) and it is possible that *Anagrus* spp. were repelled or at least inhibited in the James and Price (2004) study as well as in the field screening work reported in James (2003 b) and James (2005) when undiluted MeSA was used to bait sticky traps.

Given the earlier non-responsiveness of *Anagrus* spp. and *Metaphycus* sp. to MeSA, MeJA and HA-baited sticky cards, it is possible that the positive responses to grapevine blocks baited with these HIPV reported here, may have been mediated by plant-signaling. Dispersion of MeSA, MeJA or HA volatiles in the vineyard may have signaled the plants to produce their own HIPV blend(s) which attracted *Anagrus* spp. and *Metaphycus* sp. James and Price (2004) suggested that the attraction of a wide variety of insect families to synthetic MeSA deployed in controlled-release dispensers in a vineyard experiment (Chrysopidae, Hemerobiidae, Coccinellidae, Geocoridae, Anthocoridae, Miridae, Syrphidae, Braconidae, Empididae, Sarcophagidae), was also perhaps a consequence of gaseous MeSA acting as a plant signal. Complete blends of natural HIPV from plants would be expected to attract a wider spectrum of pest natural enemies than synthetic MeSA alone.

Evidence of a signaling function for MeSA, MeJA and HA in helping plants to recruit ‘bodyguards’ does exist. Rodriguez-Saona *et al.* (2001) in laboratory studies showed that cotton plants exposed to gaseous MeJA emitted elevated levels of volatiles similar but not identical, to those produced by plants exposed to herbivore attack. Shimoda *et al.* (2002) provided evidence that gaseous MeSA elicits the production of volatiles from bean leaves that are attractive to a predatory thrips species. Arimura *et al.* (2001) demonstrated that gaseous HA induced the expression of defense genes in bean leaves and Engelberth *et al.* (2004) presented evidence of a ‘priming’ role of green leaf volatiles (GLV) incuding HA, which enabled corn seedlings to produce greater amounts of HIPV after herbivore attack, than seedlings not previously exposed to GLV. No published studies have shown signaling effects of gaseous MeSA, MeJA or HA in the field, but this is clearly a promising area of research. The current study while raising the possibility of plants stimulated to produce HIPV in the presence of synthetic MeSA, MeJA or HA, does not provide the evidence needed to confirm this. Measuring and analyzing the volatiles produced by grapevines and hops exposed or not exposed to synthetic HIPV should provide definitive evidence for the existence or not of this mechanism and such studies are planned.

The possible use of synthetic HIPV either as direct or indirect enhancers of natural enemy population levels in crops (Herbivore-Induced Plant Protection Odors: HIPPO), is an exciting prospect. Recent studies (James, 2003 a,b; 2005; James and Price 2004) as well as the present work have provided evidence for the potential use of synthetic HIPV as aids to enhancing conservation biological control in crop ecosystems. However, many questions surrounding the use of these materials in integrated pest management remain to be answered.

For example, what are the ecological consequences of providing synthetic HIPV to predators and parasitoids in the absence (or relative absence) of their prey? Will this 'misinformation' result ultimately in non-response by natural enemies to HIPV? As noted above, most if not all of the predatory insects attracted to synthetic MeSA are generalist-feeding species (James 2003 a,b; James 2005; James and Price 2004) and the misinformation issue may not be as important with these species as it might be to specialist parasitoid species like *Anagrus* spp. which only parasitize leafhoppers. Defining and understanding the mechanism(s) of attraction and recruitment of predatory and parasitic insects by synthetic HIPV, will be of paramount importance in the effective use of these materials in crop pest management. The data in this study suggest that using synthetic HIPV to signal plants to produce their own HIPV blends is a possibility, but more extensive laboratory and field experimentation is required before this can be confirmed. Optimal deployment (release rates, dispenser density) of synthetic HIPV for natural enemy recruitment and retention, will require a good understanding of the precise mechanisms mediating attraction of predators and parasitoids. Comprehensive studies are planned and will be reported in due course.

ACKNOWLEDGEMENTS

We thank the Washington Hop Commission, Hop Research Council, Wine Association of Wine Grape Growers, Washington State Concord Grape Association and the Washington Commission for Pesticide Registration assistance in funding this project. We also thank the hop and grape growers who participated in this research and the many summer casual employees who helped with collecting and processing samples.

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BIOLOGICAL CONTROL IN THE NEOTROPICS: A SELECTIVE REVIEW WITH EMPHASIS ON CASSAVA

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INTRODUCTION

Today, there is ample biological control (BC) research in the Neotropics. Moreover, many integrated pest management (IPM) projects in crops such as potatoes, cotton, soybeans, maize, vegetable crops and fruits include BC as a key component. Cassava cultivation is a good example of where BC has had an important role in managing the main pests, not only in the Americas but also in other continents such as Africa.

BIOLOGICAL CONTROL RESEARCH IN THE NEOTROPICS

In a literature review (1995-2003), a large number of publications on BC research in various countries of the Neotropics was found. Of these publications, the following synthesis can be made: (1) much of the research on BC is primarily on arthropod pests (60%); but there is also considerable research on plant pathogens (30%), as well as nematodes (6%) and weeds (4%). (2) With respect to the BC of arthropods in South America, 50% of the articles reviewed (578 total) report on research done in Brazil; 25% in Colombia, 11% in Chile and 9% in Argentina. In North and Central America most of the publications are from Mexico (169) and only 23 articles are produced in the other countries of the region. (3) The crops of major economic importance on which BC research is being done in South America are cotton, tomatoes, soybeans, maize, cereals, potatoes, coffee, fruits, vegetable crops, sugarcane, cassava and legumes (Table 1).

In general, the group of pests that was target of the highest number of BC projects was Lepidoptera (>40%), followed by Coleoptera (20%) and Homoptera/Hemiptera (19%) (Table 2). The complex of Lepidoptera species is quite numerous and includes genera such as *Spodoptera, Diatraea, Heliothis* and *Anticarsia* (Table 3). With respect to Coleoptera, the pests where most BC research was done were the white grubs (Melolonthidae), the coffee berry borer and the cotton boll weevil. Other BC research includes mites and fruitflies (particularly in Brazil and Colombia), and aphids and whiteflies (Table 4).

Table 1. Articles on BC of Arthropod pests, by crop, in South America (1995-2003).

Crop	**Articles**	**Principal Pest**
Cotton	42	Cotton boll weevil, leaf-eating caterpillar
Tomatoes	40	Whiteflies, leaf miners
Soybeans	36	Velvetbean caterpillar (Anticarsia sp.), especially in Brazil
Maize	33	Fall armyworm
Cereals	32	Lepidoptera/Aphids
Potatoes	29	Potato moths
Coffee	26	Coffee berry borer, especially in Colombia
Pastures	25	Orthoptera/ants/spittlebugs
Fruits	25	Aphids/Lepidoptera
Citrus fruits	23	Fruitflies/scales
Vegetable crops	20	Aphids, whiteflies/Lepidoptera
Forests	19	Lepidoptera/Coleoptera
Sugarcane	17	Sugarcane borer
Cassava	16	Mites, mealybugs
Stored products	12	Grain moth
Common beans	10	Leafhoppers, whiteflies
Legumes	10	Aphids
Ornamentals	7	Mites/thrips
African palm	6	Coconut weevil
Others (grapes, olives, bananas)	18	Various
Total	**446**	

Sources: Agricola, Agris and CAB databases, 1995-2003.

Table 2. Biological control of arthropod pests in South America (1995-2003).

Order	**Articles**	**%**
Lepidoptera	212	41.5
Coleoptera	103	20.2
Homoptera/Hemiptera	99	19.4
Acari	31	6.1
Diptera	21	4.1
Hymenoptera	18	3.5
Orthoptera	16	3.1
Isoptera	8	1.5
Thysanoptera	3	0.6
Total	511	100.0

Sources: Agricola, Agris and CAB databases, 1995-2003.

Table 3. Lepidopteran species pests that have been target of BC in South America (1995-2003).

Principal Pest	Common Name
Spodoptera frugiperda	Fall armyworm
Spodoptera spp.	Cutworms
Diatraea saccharalis	Sugarcane borer
Heliothis virescens	Tobacco budworm
Helicoverpa zea=Heliothis. zea	Corn earworm, tomato fruitworm
Anticarsia gemmatalis	Velvetbean caterpillar
Tuta absoluta	Tomato leafminer
Plutella xylostella	Diamond-back moth; leaf-eating caterpillar
Alabama argillacea	Cotton leafworm
Tecia solanivora	Guatemalan potato moth
Phthorimaea operculella	Potato tuber moth

Sources: Agricola, Agris and CAB databases, 1995-2003.

Table 4. Principal pests reported in articles on BC in South America (1995-2003).

Order	Principal Species	Common Name
Homoptera/Hemiptera	*Myzus* spp. and *Aphis* spp.	Aphids
	Bemisia tabaci	Whiteflies
	Trialeurodes vaporariorum	Whiteflies
	Empoasca spp.	Leafhoppers
	Aeneolamia spp.	Spittlebugs
	Mahanarva spp.	Spittlebugs
Coleoptera	Melolonthidae (Scarabaeidae)	White grubs
	Hypothenemus hampei	Coffee berry borer
	Anthonomus grandis	Cotton boll weevil
	Epicaerus spp.	Potato grub
	Tribolium spp.	Granary weevil
Diptera	*Anastrepha* spp.	Fruitflies
	Liriomyza sativae	Leaf miners
Acari	*Mononychellus tanajoa*	Cassava green mite
	Tetranychus spp.	Red mites

Sources: Agricola, Agris and CAB databases, 1995-2003.

The organisms most studied and used in BC were entomopathogens (about 40% of the articles), parasitoids (35%) and predators (18%). Within the group of entomopathogens, the most researched were fungi, followed by bacteria (primarily *Bacillus thuringiensis*) and baculoviruses. The fungi evaluated most frequently belonged to the genera *Metarhizium*, *Beauveria* and *Lecanicillium* (*Verticillium*). The most studied parasitoids were in the families Trichogrammatidae (40% of the articles) and Encyrtidae (Hymenoptera). The predators studied the most were Chrysopidae and Phytoseiidae (predators of mites).

In Mexico and Central America, the crops in which there was a greater concentration of BC research were fruits, vegetables, maize, coffee, cotton and tomato. Lepidoptera and Coleoptera were the groups of insect pests where there were more studies; and within the Homoptera, whiteflies. The BC organisms studied the most are parasitoids and entomopathogens, especially *B. thuringiensis*.

BIOLOGICAL CONTROL IN THE NEOTROPICS: CASE STUDIES

Biological control is the most important IPM component in tropical and subtropical zones. Although the potential for using BC is high, the use of chemical pesticides continues to increase (Yudelman *et al*. 1998), especially in developing countries. The use of these products above all, their abuse has had adverse effects on both natural and applied BC (Van Driesche and Bellows 1996). In many cases pesticide use has destroyed the natural enemies of the secondary pests, resulting in severe outbreaks of insects that do not normally cause economic levels of damage (yield losses and quality reduction) to crops. In the case of vegetable crops and fruits for exportation, there is a need to reduce or eliminate the toxic residues of the chemical pesticides so that they meet international market requirements (Peña 2002). To extend the use of BC in pest management, there is a need to increase the level of research and funding in the same. The literature review showed that there is increased interest in BC in various countries of the Neotropics. Some cases of success can be cited:

BIOLOGICAL CONTROL WITH BACULOVIRUSES

Baculoviruses have been successful in controlling important pests of various crops, especially soybeans, potatoes and cassava. In the case of potatoes, research at the International Potato Center (CIP) in Peru has led to good control of the potato tubermoth, *Phthorimaea operculella* Zeller (Alcazar *et al*. 1993). Research on the use of baculoviruses to control the cassava hornworm, *Erinnyis ello* L Linnaeus) (Lepidoptera: Sphingidae), and their implementation in the field by CIAT in Colombia are documented below. In soybeans, the use of baculoviruses to control the velvetbean caterpillar (*Anticarsia gemmatalis* Hübner) is one of the most successful examples of BC in the Neotropics (Moscardi 1999). *Anticarsia gemmatalis* can cause severe damage and reduction of soybean crop yields. Research done by EMBRAPA (Brazilian Agricultural and Livestock Research Entity) indicated that the baculoviruses had good potential for controlling *A. gemmatalis*, resulting in the development of a commercial product, which first came into use in 1980. In 1983-1984 applications were done on approximately 20,000 ha and progressively increased until 1.2 million ha in 1997-1998 (Moscardi 1999); in 2001-2002, applications were done on up to 1.5 million ha (Moscardi pers. comm.). This project has had many benefits for the soybean growers. The cost of using baculoviruses is 20-30%

lower than the cost of applying insecticides. The cost per ha is only US$7, which meant a savings of US$10 million in 2001-2002. Up to 2002, the baculoviruses had been applied to 17 million ha, for a total savings of US$120 million. In addition, it is estimated that the use of insecticides has decreased by 1.7 million lt, a benefit for both the environment and human health (Moscardi pers. comm.).

BIOLOGICAL CONTROL IN COTTON

Managing pests in cotton has had a long history in Colombia and illustrates the difficulties of combining BC with the use of insecticides. During the 1960s and 70s, up to 26 applications of insecticides were made per cycle, primarily for the tobacco budworm *Heliothis virescens* (F.)(Lepidoptera: Noctuidae). The insecticides were applied according to a pre-established schedule, without determining the levels of economic damage. Despite the high number of applications, cotton yields declined. By 1977, *H. virescens* had developed resistance to the available insecticides, particularly to methyl parathion (FEDEALGODON 1988). The production of cotton declined, the costs rose, and the crop was abandoned in some zones. In 1980, ICA (Colombian Agricultural and Livestock Institute) and FEDEALGODON (National Federation of Cotton Growers) began research on IPM to lower the use of insecticides. Levels of economic damage were established, and a sampling program to measure the levels of pest populations was implemented. The program was based on BC, especially the increased releases of the hymenopteran parasitoids *Trichogramma* sp. and *Apanteles* sp., lowering the populations of *H. virescens* dramatically. The use of insecticides was reduced to only 2-3 applications, and the yields of cotton rose (Bellotti *et al.* 1990). This program was a good example of the potential of IPM and BC (Smith and Bellotti 1996). This system worked well up to the 1990s when the boll weevil *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) was introduced to Colombia (Díaz 2003). During the period 1991-2002, Colombia experienced a reduction of 83% in the area planted to cotton (Rodríguez and Peck 2004). The 2002-2003 harvest included only 46,514 ha in the two cotton-growing regions of Tolima-Valle and the Atlantic Coast-Meta (DANE 2004). One aspect that has greatly influenced the loss of area planted to cotton in Colombia is the high incidence of pests. The greatest losses are caused by the boll weevil, which affects 89% of the growing area in the provinces of Córdoba, Cesar and Tolima, causing 15% loss of flower heads. The tobacco budworm affects 100% of the cotton-growing area of Colombia, causing damage to 15-20% of the flower heads and bolls. Some 10% of the cultivated area is additionally affected by the Colombian pink bollworm (*Sacadodes pyralis*, Lepidoptera: Noctuidae) and whiteflies (Homoptera: Aleyrodidae).

Control of these pests is largely based on extensive use of agrochemicals, which represent 23% of the direct costs of the crop for the Colombian producer. In the Atlantic Coast, there was an average of 26 applications of pesticides per crop cycle, with 69.2% of those directed toward the control of lepidopterans. In the Cauca Valley, the number of applications has been reduced 73%, to an average of 7 applications per crop cycle, with 57.1% directed towards the control of lepidopterans (CIAT 2004). The apparent solution for this problem is to use transgenic varieties with *Bacillus thuringiensis*. Recent research indicates that the use of the transgenic varieties makes it possible to lower insecticide applications to 8-9 or even less. The use of transgenic varieties combined with BC offers a good opportunity for lowering insecticide applications (Díaz 2003).

BIOLOGICAL CONTROL IN COFFEE

The coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) is one of the world's major pest problems in the coffee crop. Major damage is caused by the larvae penetrating the coffee berries and tunneling in the beans, causing fruit drop. Infested berries are the sources of future attacks (Baker *et al*. 1992). The pest is well adapted to the coffee agroecosystems, and once established, is very difficult to eradicate. Yield losses can range from 5 to 24%, depending on pest infestation, and losses as high as 50% have been reported (Ramirez and Mora 2001).

Hypothenemus hampei was originally reported from Africa and introduced into Colombia in 1988. The Colombian Institute of Coffee Research (CENICAFE) initiated an IPM program, based on BC, to reduce or manage damage by this pest (Bustillo *et al*. 1998). Biological control of coffee berry borer in Colombia has concentrated on the combination of parasitoids and entomopathogens. Since *H. hampei* originated in Africa, several parasitoid species were introduced from that continent. These included *Heterospilus coffeicola*, Schneideknecht *Prorops nasuta*, Waterson *Cephalonomia stephanoderis* Betren and *Phymastichus coffea* La Salle (Borbon 1991). *Prorops nasuta* has been introduced into several countries of the Americas (Mexico, Guatemala, Brazil, Colombia, Honduras and others). Parasitism rates by *C. stephanoderis* have been recorded as high as 65% in Mexico (Barrera *et al*. 1990). Parasitism rates of *P. coffea* on *H. hampei* in Colombia reached 77.6 and 85%, 90 and 150 days respectively, after introduction (Jaramillo *et al*. 2002).

In Colombia, the coffee berry borer is infected with native strains of *Beauveria bassiana* and *Hirsutella eleutherathorum* (Bustillo 1998). Field results with applications of *B. bassiana* in Colombia and other countries have been variable, ranging from 48% to levels above 75% (Bustillo 2002). Present strategy for *H. hampei* control includes the combination of cultural and biological control practices, including the periodic release of parasitoids and the applications of entomopathogens (Bustillo 1998).

BIOLOGICAL CONTROL IN CASSAVA

Cassava (Euphorbiaceae: *Manihot esculenta*) is a perennial shrublike plant that has a 1-2 year cropping cycle. It is usually cultivated on small farmers' fields in tropical and subtropical regions of the world, where it is often intercropped or planted in cycles that overlap with other crops. These and other agronomic characteristics contribute to the diversity of arthropod pests that feed on cassava and to the complex of natural enemies associated with them.

The cassava crop originated in the Neotropics; consequently, there is a great diversity of arthropods that have been recorded attacking the crop in the Americas (Bellotti *et al*. 1999; 2002). Almost all the principal pests of cassava are found on this continent (Table 5). The accidental introduction of the cassava green mite *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) (CGM) and the mealybug *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) from the Americas into Africa has caused considerable losses throughout the African cassava belt and has been the object of a massive BC effort.

In the Neotropics an ample complex of natural enemies exercises a certain level of control on the crop's principal pests (Table 6). There are more than 250 species of natural enemies, including parasitoids, predators and pathogens associated with the pests in the cassava

Table 5. Global distribution of the arthropod pests of importance in the cassava crop, adapted from Bellotti (2002).

Pest	Principal Species	Americas	Africa	Asia
Mites	*Mononychellus tanajoa*	X	*X*	
	Tetranychus urticae	X		
Mealybugs	*Phenacoccus manihoti*	X	*X*	
	Phenacoccus herreni	X		
Whiteflies	*Aleurotrachelus sociales*	X		
	Aleurothrixus aepim	X		
	Bemisia tabaci	X	*X*	
Cassava hornworm	*Erinnyis ello*	X		
	E. alope	X		
Lacebugs	*Vatiga illudens*	X		
	V. manihotae	X		
Burrower bugs	*Cyrtomenus bergi*	X		
Thrips	*Frankliniella williamsi*	X	*X*	
	Scirtothrips manihoti	X		
Scales	*Aonidomytilus albus*	X	*X*	
Fruitflies	*Anastrepha pickeli*	X		
	A. manihoti	X		
Shootflies	*Neosilba perezi*	X		
	Silba pendula	X		
Gall midges	*Jatrophobia* (Eudiplosis) *brasiliensis*	X		
White grubs	*Leucopholis rorida*	X	*X*	X
	Phyllophaga spp.	X	*X*	X
	Others	X	*X*	X
Termites	*Coptotermes* spp.	X	*X*	X
	Heterotermes tenuis	X		
Stemborers	*Chilomima* spp.	X		
	Coelosternus spp.	X		
	Lagochirus spp.	X	*X*	X
Leaf-cutting ants	*Atta* spp.	X		
	Acromyrmex spp.	X		
Root mealybugs	*Pseudococcus mandioca*	X		
	Stictococcus vayssierei		*X*	
Grasshoppers	*Zonocerus elegans*	X	*X*	
	Zonocerus variegatus			

Table 6. Reports of natural enemies of some of the principal pests of cassava, (adapted from Melo 2002).

Pests	**Parasitoids**	**Predators**	**Pathogens**
Cassava green mite		60	2
Cassava hornworm	18	15	15
Whiteflies	17	5	6
Mealybugs	25	46	2
Borers			
Chilomima clarkei	5	2	5
Lagochirus sp.	2		
Burrower bugs		1	5
White grubs	2	1	3
Lacebugs		1	
Thrips		1	
Scales	4	9	2
Total	**73**	**141**	**40**

crop (Melo 2002). Sixty-two species of natural enemies are associated with mites, 48 with the cassava hornworm, 73 with mealybugs and 28 with whiteflies.

Biological control is one of the components in an IPM program, in which varietal resistance (genetic component) and cultural practices (agronomic component) also play an important role. The use of chemical pesticides in traditional agroecosystems of cassava is minimal, due to their high cost and adverse effects on natural enemies, human health and damage to the environment. In addition it has been shown that in some cases, as with whiteflies, the use of pesticides is not economically viable for the small farmers (Holguín and Bellotti 2004).

RECENT ADVANCES IN BIOLOGICAL CONTROL OF MAJOR CASSAVA PESTS

Applied BC has had a major role in managing certain harmful pests of cassava. A brief description of this research, the results and accomplishments follow. Emphasis is on mites, mealybugs, the cassava hornworm, whiteflies, the burrower bug and white grubs.

Cassava green mite. Mites are considered a universal pest of cassava because they cause crop losses in both the Americas and Africa. The CGM (*Mononychellus tanajoa*) is the most important species, especially in lowland tropical regions with prolonged (3 to 6 months) dry seasons. It is native to the Americas, possibly from northern South America or Northeast Brazil, where it was reported for the first time in 1938. The mite attacks young leaves and meristems, preferably feeding on the underside of the leaves, which develop a mottled to bronzed appearance in the form of a mosaic with chlorotic spots until the leaves become deformed. *Mononychellus tanajoa* was introduced accidentally to the African continent during the 1970's, where it caused 13-80% yield loss (Yaninek and Herren 1988).

Research on the control of CGM has been based on two principal strategies: varietal resistance (VR) and BC. Research on VR has identified low-to-moderate levels of resistance in cassava clones. Programs at CIAT, IITA (International Institute of Tropical Agriculture) and EMBRAPA/CNPMF incorporate this resistance to cultivars. As VR is highly complementary with BC, a great deal of emphasis has been placed on evaluating the role of natural enemies. In order to develop a BC program to combat the CGM, explorations, evaluations and taxonomic recognition were carried out at more than 2,500 sites in 17 countries of the Americas (Bellotti *et al.* 1987; Bellotti 2002). An ample complex of the predator mites (Phytoseiidae) were found preying on mite pests. In cassava 66 species of Phytoseiidae were collected, of which 25 were new for science and 13 were very common in other crops. *Typhlodromalus manihoti* (Moraes) was collected most frequently, being found in over 50% of the fields sampled. It is followed by *Neoseiulus anonymus,* Chant and Baker *T. aripo,* De Leon *Galendromus annectens,* (De Leon) *G. helveolus* (Chant) and *Amblyseius aerialis,* (Muma) among others (Fig. 1). *Typhlodromalus aripo, T. manihoti* and *N. idaeus* play an important role in the control of *M. tanajoa* in Africa, where they were introduced from Brazil during the 1980s and 1990s. *Typhlodromalus aripo* has proven to be the most promising species. Field evaluations in Africa indicated that *T. aripo* can reduce the CGM population from 30-90%, bringing about a 30-37% increase in cassava production (Table 7) (Yaninek *et al.* 1993).

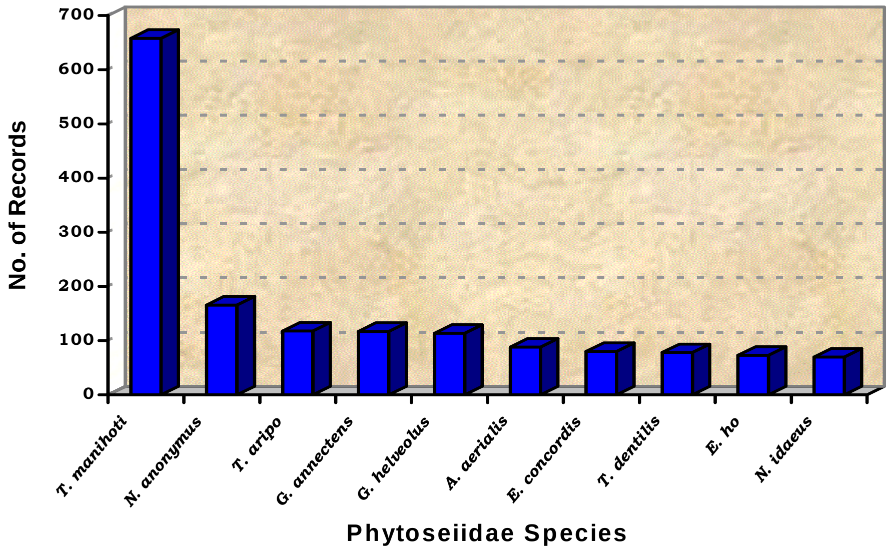

Figure 1. Species of Phytoseiidae reported on the cassava crop in the Americas.

Results of field experiments in Colombia showed the importance and the effect of the diversity of Phytoseiidae species associated with the CGM. In Colombia the production of fresh cassava roots was reduced by 33% when the natural enemies were eliminated; whereas, applications of acaricides did not increase the production, which shows the important role of BC (Braun *et al.* 1989). Explorations also found some insect predators of the CGM, especially the staphylinid *Oligota minuta* and the coccinellid *Stethorus* sp. *Oligota minuta* Cameron has been catalogued as an important predator of *M. tanajoa* populations. In research done at CIAT and in Uganda, *Oligota* populations were found between the fifth and eighth leaves,

Table 7. Establishment of Phytoseiidae species in Africa.

	N. idaeus	*T. manihoti*	*T. aripo*
Year of First Release	1989	1989	1993
No. of countries where established	2	4	11
Rate of dispersion (km/year)	0.01	2.5	12.5
Region occupied (km^2)	< 10	1300	150 000
Reduction in CGM (*M. tanajoa*) population	0%	50%	30-90%

Source: IITA (1995, 1996).

coinciding with the places where the highest populations of the pest are found. In the larval stage they can consume 49-70 mites and 44-61 eggs; in the adult stage they consume 97-142 eggs and adults in 7-16 days. *Stethorus* sp., on the other hand, is mostly found in association with *Tetranychus urticae* Koch. In severe attacks of this mite, 98% of the predators were *Stethorus* and only 2% *Oligota* (CIAT 1982). In laboratory and field observations, the predator *Chrysopa* sp. (Neuroptera) has proven to be very effective, consuming different stages of the pest.

Other natural enemies of mites are the pathogenic fungi belonging to the genera *Neozygites* (Zygomycetes: Entomophthora) and *Hirsutella* (Hyphomycetes: Monilia). The former is a pathogenic fungus that appears sporadically in Colombia and Northeast Brazil (*Neozygites cf floridana)*, causing up to 100% mortality of the CGM in 1-2 wk (Delalibera *et al.* 1992). Some strains are specific to the genus *Mononychellus* (Moraes and Delalibera 1992). In evaluations done in Africa, *Hirsutella* sp. has proven to be very effective controlling mite populations (Odongo *et al.* 1990; Yaninek *et al.* 1996) (Table 8).

Table 8. Natural enemies of the CGM *Mononychellus tanajoa* (Acari: Tetranychidae).

Predators	**Pathogens**
Neuroptera *Chrysopa* sp. Coleoptera *Stethorus* sp. *Oligota* spp. Acari (114 strains) *Typhlodromalus manihoti* *T. aripo* *Neoseiulus idaeus* Others	Fungi *Neozygites floridana* *Hirsutella thompsonii* Virus Not identified (found in Colombia, unpublished information)

Cassava mealybugs. More than 15 species of mealybugs have been found feeding on cassava in the Americas, Africa and Asia. The two most important species are *Phenacoccus manihoti* and *P. herreni* (Hemiptera: Pseudococcidae), which, cause significant reductions in cassava yield. Both species are of Neotropical origin. The former is found in Paraguay, certain areas of Bolivia and in the State of Mato Grosso in Brazil, but causes no economic damage in these regions. When *P. manihoti* was inadvertently introduced into Africa at the onset of the 70s, it dispersed rapidly, causing considerable losses in yield (up to 80%) (Herren and Neuenschwander 1991). *Phenacoccus herreni* is distributed in northern South America (primarily in Colombia and Venezuela) and in Northeast Brazil, where high populations can cause considerable losses. The damage produced by both species is similar: feeding of the nymphs and adults causes yellowing, curling of the leaves, formation of rosettes on the growing points, necrosis, defoliations, distortion of the stem, and death of the shoots (Bellotti 2002).

Management of mealybugs is a well-documented example of classical BC, especially in Africa, where *P. manihoti* is being controlled successfully by the parasitoid *Apoanagyrus lopezi* De Santis, which IITA introduced to Africa from Paraguay. Although *P. herreni* is distributed in northern South America, it causes serious yield losses only in Northeast Brazil (Bellotti, *et al.* 1999) (up to 80% yield reduction reported). Thus *P. herreni* can be an exotic species in this region, probably coming from Colombia and Venezuela (Williams and Granara de Willink 1992).

Numerous species of parasitoids, predators and entomopathogens of *P. herreni* have been identified in Colombia and Venezuela. Various parasitoids have shown a specialty or preference for *P. herreni*. Three Encyrtidae (*Apoanagyrus diversicornis* Howard, *Aenasius vexans* Kerrich and *Acerophagus coccois* Smith) have been evaluated as parasitoids (Van Driesche *et al.* 1988; 1990). All three species were observed having higher percentages of parasitism on *P. herreni* in laboratory studies in Colombia than on *P. madeirensis* (Table 9). Through the combined efforts of CIAT and EMBRAPA (Brazil), the three species were exported from CIAT and released by EMBRAPA/CNPMF (National Center of Research on Cassava and Fruits) in Northeast Brazil, primarily in the States of Bahia and Pernambuco from 1994-1996. More than 35,000 individuals of the three species were released. Although all three species became established, *A. diversicornis* and *A. coccois* had the most rapid and extensive dispersion (Fig. 2). Observations indicate that the mealybug populations have been reduced substantially and that the cassava crop has returned to areas that had been abandoned due to *P. herreni* infestations (Bento *et al.* 1999; 2000). *P. herreni* outbreaks have not been observed in Northeast Brazil in recent years (Farias pers. comm.)

Table 9. Parasitism (%) of three parasitoids (Encyrtidae) on two mealybug species (*Phenacoccus herreni* and *P. madeirensis*) under laboratory conditions.

Parasitoids	***P. herreni***	***P. madeirensis***
Acerophagus coccois	32	27
Apoanagyrus diversicornis	32	16
Aenasius vexans	38	2

Figure 2. Dispersion of three parasitoid species of the cassava mealybug (*P. herreni*) in Bahia, Northeast Brazil (Bento *et al.* 2000).

The cassava hornworm. *Erinnyis ello* is one of the most important cassava pests in the Neotropics. The species is not reported from Africa or Asia. The migratory capacity of the adults, their broad climatic adaptation and range of hosts contribute to their extensive distribution throughout the cassava-growing zones of the Americas and their sporadic attacks. In addition to its migratory capacity, the explosive appearance of *E. ello* occurs because of its great reproductive potential. A female can lay up to 1,800 eggs (avg of 800/female). Given the foregoing, many plantations have suffered severe defoliations for various cycles until reestablishing the balance between the pest and its natural enemies.

The hornworm's life cycle has a duration of 32-49 days (25-30°C). The larva passes through five instars in its development. The larval stage, which has a caudal horn (thus its name), lasts from 12-15 days and is responsible for the damage to the cassava plants, causing complete defoliation with up to 60% losses in yield when consecutive attacks occur. The voracity of the larva is such that it can consume up to 1100 cm^2 of leaf surface, 75% of which is consumed during the last (fifth) instar (Arias and Bellotti 1984).

Resistance to *E.* ello has not been identified in landrace varieties of *M. esculenta*; however, there are numerous natural enemies with some 40 species of parasitoids, predators and pathogens identified. Several have been evaluated extensively for the egg, larva and pupa stages of *E. ello* (Table 10). The effectiveness of this complex of natural enemies is limited, probably due to the great flight capacity and migratory ability of *E. ello*, which acts as a defense against the effectiveness of the natural enemies (Bellotti *et al.* 1992).

Among the entomopathogens, *B. thuringiensis* has been used successfully when applied to young larvae (first to third instar). From the onset of the 70s, CIAT identified a granulosis virus (Baculoviridae) attacking *E. ello* in cassava crops. Pathogenicity studies in the lab and field gave almost 100% mortality of hornworm larvae. The infected larvae can be collected in the field, blended, filtered through gauze, made into a solution with water, and applied in

Table 10. Principal natural enemies of the cassava hornworm (*Erinnyis ello*), adapted from Melo (2002).

Parasitoids		**Predators**		**Entomopathogens**	
Trichogramma spp.	(E)[1]	*Chrysopa* spp.	*(E,L)*	*Bacillus thuringiensis*	(L)
Telenomus sphingis	(E)	*Podisus nigrispinus*	*(L)*	Baculoviruses of *E. ello*	(L)
Cotesia americana	(L)	*P. obscurus*	*(L)*	*Metarhizium anisopliae*	(L)
Cotesia sp.	(L)	*Polistes carnifex*	(L)	*Beauveria bassiana*	(L)
Euplectrus sp.	(L)	*P. erythrocephalus*	*(L)*	*Paecilomyces* sp.	(L)
Drino macarensi	(L)	*P. canadensis*	*(L)*	*Nomuraea rileyi*	(L)
Drino sp.	(L)	*P. versicolor*	*(L)*	*Cordyceps* sp.	(P)
Euphorocera *sp.*	(L)	*Polybia emaciata*	(L)		
Sarcodexia innota	(L)	*P. sericea*	*(L)*		
Thysanomyia sp.	(L)	*Zelus nugax*	*(L)*		
Belvosia sp.	(L)	*Zelus* sp.	*(L)*		
Forcipomyia eriophora	(L)	*Calosoma* sp.	*(L)*		
		Spiders (Tomicidae, Salticidae, others)	*(L)*		

[1] E=egg; L=larva; P=pupa.

fields attacked by the hornworm (Bellotti *et al*. 1992). Baculoviruses have also been used successfully to control *E. ello* in southern Brazil (Santa Catarinha State). In Venezuela the baculovirus replaced insecticides on large plantations where the hornworm is endemic. In 2003, Biotropical, a Colombian firm, formulated, in collaboration with CIAT, a commercial product (Bio-virus) for the BC of *E. ello* that is presently being used by cassava producers.

Whiteflies. As a direct feeding pest and vectors of viruses, whiteflies cause significant damage to the cassava crop in the Americas, Africa and Asia. There is a large complex in the Neotropics, where 11 species have been recorded feeding on cassava (Table 11). The most important species is *Aleurotrachelus socialis* Bondar, which is widely distributed in northern South America: Ecuador, Colombia and Venezuela (Trujillo *et al*. 2004). Attacks of 1, 6 and 11 months have resulted in 5, 42 and 79% yield losses, respectively, in field trials in region of the Tolima Province, Colombia.

Aleurothrixus aepim, which primarily attacks cassava, but has additional hosts, is found in high populations, causing yield losses in Northeast Brazil (Farias 1994). *Bemisia tuberculata* Bondar and *Trialeurodes variabilis* (Quaintance) are reported in low populations from Brazil, Colombia, Venezuela and several other countries (Bellotti 2002).

Research on cassava whitefly management in the Neotropics initially emphasized varietal resistance. Diverse sources of VR to *A. socialis* have been identified. Clone MEcu 72 has consistently expressed a high level of resistance so it was included in a cross with MBra 12, which resulted in various high-yielding hybrids and moderate levels of resistance to *A. socialis* (Bellotti and Arias 2001). As a result of this work, the Colombian Ministry of Agriculture and Development released the whitefly-resistant hybrid Nataima-31 in 2003.

Table 11. Whiteflies associated with the cassava crop in Northeastern South America.

Species	**Colombia**	**Ecuador**	**Venezuela**	**Brazil**
Aleurotrachelus socialis	X	X	X	X
Aleurodicus dispersus	X	X	X	
Aleurothrixus aepim				X
Aleuroglandulus malangae	X			
Aleuronudus sp.	X			
Bemisia tabaci		X		
Bemisia tuberculata	X	X	X	X
Paraleyrodes sp.	X			
Tetraleurodes sp.	X	X		
Tetraleurodes ursorum	X			
Trialeurodes variabilis	X	X	X	X

Source: Adapted from Trujillo (2004).

A. socialis is not limited to dry season attacks; in the last decade damaging populations are found throughout the crop cycle. In research done with chemical insecticides, it was found that this control alternative decreased whitefly populations in the field; but for farmers with small areas of the crop, it was not the most viable alternative given that the high pesticide costs make the repeated applications needed for adequate control, uneconomical (Holguín and Bellotti 2004). These results confirm the need for finding more economic alternatives such as BC for controlling whiteflies in cassava.

In recent field explorations carried out in the Neotropics, especially in Colombia, Venezuela, Ecuador and Brazil, a considerable number of natural enemies associated with the whitefly complex in cassava have been identified. The most representative group is that of the microhymenopteran parasitoids. The richness of species in Colombia, Venezuela and Ecuador is primarily represented by the genera *Encarsia, Eretmocerus* and *Amitus*, frequently associated with *A. socialis* (Table 12) (Trujillo *et al*. 2004).

Gaps in the knowledge on the complex of natural enemies associated with the different whitefly species have limited the utilization and determination of their effectiveness in biological control programs. Consequently, there is little knowledge on levels of parasitism, rates of parasitism by species, specification of the host and its effect on the regulation of whitefly populations.

More than 20 species of entomopathogens have been reported infecting whiteflies, including *Aschersonia* sp., *Lecanicillium* (*Verticillium*) *lecanii*, *Beauveria bassiana* and *Paecilomyces fumosoroseus*; however, a careful selection of the species is required, as well as the identification and evaluation of native isolates of entomopathogen fungi. Greenhouse experiments at CIAT with isolates of *L. lecanii* resulted in 58-72% *A. socialis* nymphal mortality (depending on nymphal stage) and 82% egg mortality (Aleán *et al*. 2004). At present *L. lecanii* is being formulated into a commercial product that should be available to cassava growers

Table 12. Parasitoids of whiteflies collected from cassava in diverse agroecosystems of Colombia, Ecuador and Venezuela.

	Colombia				**Ecuador**		**Venezuela**
Species	Caribbean	Andean Zone	Inter-Andean Cauca Valley	Inter-Andean Magdalena River Valley	Coast	Sierra	Plains
Amitus sp.					X		
Eretmocerus sp.	X	X	X	X	X	X	X
Encarsia sp.	X		X	X	X	X	
E. híspida	X	X	X				X
E. pergandiella	X	X					X
E. bellotti	X	X	X				
E. sophia	X		X				X
E. luteola	X		X				
E. cubensis							X
E. americana					X		
E. strenua	X						
Encarsia sp. prob. *variegata*	X						
Metaphycus sp.	X						X
Euderomphale sp.		X			X		X
Signiphora aleyrodis		X		X	X		X

in Colombia during 2005. An integrated strategy for *A. socialis* management based on host plant resistance, the release of parasitoids and predators, and applications of entomopathogens is now being implemented in selected regions of Colombia.

Cassava burrower bug. *Cyrtomenus bergi* Froeschner, a polyphagous insect found in a subterranean habitat, is considered one of the principal pests of diverse crops such as cassava, onions (*Allium* strain), sugarcane (*Saccharum officinalis*), asparagus (*Asparagus officinalis*), sorghum (*Sorghum vulgare*), peanuts (*Arachis hypogaea*) and forage peanuts (*A. pintoi*). Since its appearance feeding on cassava at the onset of 1980, basic studies have been conducted on its biology, behavior, population dynamics and feeding preferences. Trials have been conducted on chemical, cultural and BC with fungi and entomopathogenic nematodes (EPNs). The potential of BC of *C. bergi* is presently being researched. Recent studies with entomopathogenic fungi and EPNs indicate that they have a potential importance in a BC program; however, this research has only been done in the lab and glasshouse so field studies are needed before recommending the most acceptable technology.

Steinernema carpocapsae 'All strain' was the first EPN species evaluated to control *C. bergi.* Caicedo (1993) reports that the adult stage was susceptible to all nematode doses evaluated with 60% parasitism and very low mortality, while the youngest instars were less susceptible, with 3-17% parasitism.

Evaluations of native species (*Heterorhabditis* sp.) and *Steinernema* sp., found in field samples in Colombia, together with exotic strains from the USA and UK, on fifth instar and adults under lab conditions, showed that both *C. bergi* stages were parasitized by all entomopathogenic nematode species. *Steinernema* sp. SNI 0100 was the species that showed the highest parasitism in the fifth instar and adult stage of *C. bergi* with 77 and 100% parasitism respectively. *Heterorhabditis* sp. HNI-0198 resulted in 28 and 49% parasitism in the fifth instar and adult stage respectively, 10 days after inoculation. Although the highest mortality (22%) occurred in the fifth instar, no correlation with parasitism (77%) was observed. The lowest mortality was observed with *Heterorhabditis* sp HNI-0198 with only 4% (Caicedo *et al.* 2004).

There were no significant differences among all the nematode species and doses evaluated in greenhouse studies against *C. bergi* adults. When adults were exposed to 1,000 nematodes of *Steinernema carpocapsae*, *Steinernema* sp. SNI 0100 and *Heterorhabditis* sp. HNI-0198, the parasitism was 21, 18 and 10% respectively and mortality was not observed. The parasitism and mortality caused by *S. carpocapsae* and *Heterorhabditis* sp. HNI-0198 was increased with the dose of 25,000 nematodes to 55 and 45% parasitism and 29 and 9% of mortality respectively. The adults exposed to 100,000 nematodes showed an increase in the mortality caused by *Steinernema riobrave, Steinernema* sp SNI0100 and *Heterorhabditis* sp. CIAT of 33, 28 and 26% respectively. These low mortalities suggest that it could be possible that *C. bergi* is showing immune response against all six nematodes species evaluated (Caicedo *et al.* 2004b).

Work with fungal entomopathogens, primarily *Metarhizium anisopliae*, was done in lab and glasshouse studies for three years. The most successful strains were evaluated in the field, where the best strain was selected, based on its mortiferous capacity, which reached 61% for the fifth nymphal instar of *C. bergi*. Thus this BC agent was selected for its potential management of this pest. At this time there is a specific commercial product, whose active ingredient is the strain evaluated at CIAT that is available to cassava producers. Positive results with *C. bergi* control on asparagus have been reported.

OTHER PESTS OF CASSAVA

Rhizophagous white grubs. *Phyllophaga* spp., *Anomala* sp., *Plectris* sp. and others are soil pests that feed directly on cassava roots and stem cuttings. Strains of fungi, bacteria and EPNs, which cause high mortality to the white grub larvae in the lab, are being identified (CIAT 2003) (Table 13).

Scales. *Aonidomytilus albus* Cockerell and *Saissetia miranda* (Cockerell and Parrott) are the two species that are frequently found feeding on cassava. There is natural BC for both species due to numerous parasitoids. The misuse of pesticides can, however, eliminates this advantage and results in increased scale populations.

Diptera. For some pests such as the fruitfly (*Anastrepha* spp.), shootflies (*Neosilba perezi* (Romero and Ruppell), *Silba pendula* (Bezzi)) and gall midges (*Jatrophobia brasiliensis* Rubsaaman), BC agents have not been identified. Fortunately under normal circumstances these pests do not cause economic damage to the cassava crop.

Stemborers. Especially *Chilomima clarkei* (Amsel), and the lacebugs (*Vatiga* spp.) can cause losses in cassava yield in serious attacks. To date, effective natural enemies have not been identified (Table 13).

Table 13. Other pests of cassava and their natural enemies.

Species	Parasitoids	Predators	Pathogens
White grubs *Plectris* spp. *Phyllophaga* spp. *Anomala* spp.	Diptera Tachinidae Asilidae	Coleoptera Elateridae	Fungi *Metarhizium anisopliae* *Beauveria bassiana* Bacteria *Bacillus popilliae* Bolentimorbus *Serratia* spp. Nematodes *Heterorhabditis* spp. *Steinernema* spp.
Stemborers *Chilomima clarkei* *Lagochirus* spp.	Hymenoptera *Bracon* sp. *Apanteles* sp. *Brachymeria* sp.		Fungi *Spicaria* sp. Bacteria *Bacillus thuringiensis* Virus Unidentified
Lacebugs *Vatiga manihotae*		Hemiptera *Zelus nugax*	
Thrips *Scirtothrips manihoti*		Acari *T. aripo*	

CONCLUSIONS

Biological control has been successful against certain cassava pests, especially introduced species of mites and mealybugs in Africa. Natural enemies have been used to reduce populations of the cassava hornworm (baculovirus), the mealybug in the Americas and Africa (parasitoids), and mites (ample complex of Phytoseiidae predators). The success of natural enemies depends to a great extent on the minimal use of pesticides, which can destroy the effectiveness of the BC.

In general pesticide use in traditional cassava agroecosystems is minimal, primarily due to their high cost. Farmers in the Neotropics can, however, respond with pesticides to pest population explosions. Given that the production of cassava is changing to larger plantations, the tendency to apply more pesticides for controlling these pest outbreaks has increased. There is considerable potential for replacing the use of chemical pesticides by biopesticides for managing pests in cassava. Further research is needed to develop biopesticides and methodologies for their effective implementation. This perennial crop has advantages for implementing BC given its long vegetative cycle, cultivars adapted to given agroecosystems, tolerance to drought, profitability, no specific periods of economic damage, and high potential for recovering from the damage produced by some of these important pests. The use and success of BC as an important component in an IPM program require a significant initial investment in research and collaboration among scientists, extension agents and farmers if it is to be sustainable. The role of private industry will be of key importance for biopesticides based on entomopathogens and/or botanical derivates before they can be successfully employed in a cassava IPM strategy.

ACKNOWLEDGEMENTS

The authors express their gratitude to Trudy Brekelbaum, Josefina Martínez and Carlos Alberto Ortega for their collaboration in the development and revision of the manuscript; and to Mariano Mejía for his help in searching the literature on BC in Latin America.

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HUNGER, POVERTY, AND PROTECTION OF BIODIVERSITY: OPPORTUNITIES AND CHALLENGES FOR BIOLOGICAL CONTROL

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ABSTRACT

The role and contribution of biological control to hunger and poverty alleviation, and protection of biodiversity are discussed in context of the global development agenda. These linked themes are projected to continue occupying the global development agenda for the foreseeable future. Hunger and poverty alleviation efforts have frequently focused on improving agricultural production *inter alia* with a view to provide adequate but safe food to meet local and export demands especially to northern markets. Such markets have increasingly put stringent requirements on imported food including minimum acceptable pesticide residue levels. Recent years have also seen a rise in demand for organic food, providing more opportunities for export of tropical produce. Implicit in these trends has been the growing need for ecological crop management. From this context, it is inferred that there is a demand for biological control as a tool to manage the large number of native and alien pests. Another area for application of biological control is the management of invasive alien species (IAS) in the context of biodiversity conservation, under article 8h of the Convention on Biological Diversity. An assessment of the trends in biological control research and application suggest that there has been little growth despite increased opportunities and challenges.

INTRODUCTION

Biological control in its various forms has made major contributions to global development especially in dealing with the myriad arthropod pests that affect agricultural production. Pests are a major constraint for instance, Oerke *et al.* (1994) estimated that for eight major crops (rice, wheat, barley, corn, soybeans, potatoes, cotton and coffee), 42 percent of attainable production was lost due to pests. The estimated losses in Africa and Asia were just below 50%. Not surprisingly, mitigation of these losses has been a major preoccupation of many agencies over the years. The 1960s onwards saw the emergence of integrated pest management (IPM) approaches for pest mitigation and with it the important role of biological control as a fundamental component. Since then there have been many striking advances such as the spectacular success achieved in rice systems in Asia where over and misuse of pesticides had led to emergence of *Nilaparvata lugens* Stal (Hemiptera: Delphacidae) as a serious constraint to rice production (Wardhani 1992). The 1980s saw a redirection of IPM efforts especially focusing on technology delivery and this emphasis continues today in many parts of the developing world (Heinrichs 2005; Waage 1996).

Biological control has also been a central feature of the fight against the increasing spate of invasive alien species. Introduced alien species (IAS) have the potential to cripple crop production. For instance, in the 1970s, two cassava feeding arthropod pests native to South America, the cassava mealybug, *Phencacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudoccidae) and the cassava green mite, *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) were accidentally introduced to Africa. Populations built up quickly causing crop losses ranging from 35-40% and as high as 80 % in some parts. The two pests were successfully brought under control through the introduction of specialist natural enemies (Zeddies *et al.* 2001). Similar successes have continued to be achieved as with the recent control of the hibiscus mealybug, *Maconellicoccus hirsutus* Green (Hemiptera: Pseudoccidae) in the Caribbean, Central America, northern South America and Florida (Kairo *et al*. 2000). The pest attacks a wide range of fruit, vegetables, ornamentals and forest trees.

In recent years, the importance of IAS as a threat to biological diversity has also come to the forefront (McNeely *et al.* 2001). While preventative measures are more cost effective, it is almost certain that some species will escape and become established, requiring mitigation. Classical biological control is one of the main tools available to deal with such species (Wittenberg and Cock 2001).

The important role of biological control in global development can therefore not be understated. This paper begins by looking at some key issues driving the global development agenda with particular reference to areas where biological control can play an important role. Next, it examines the demands for biological control. Given the issues and demand, this paper then examines the growth of biological control research with a view to identify how the technology can be more effectively applied.

GLOBAL DEVELOPMENT AGENDA

On 8th September 2000, 187 world heads of state and government gathered for the 55th session at the United Nation's Headquarters in New York made a number of bold declarations in response to Agenda 60 (b) (United Nations 2000). The declaration was organized around eight main themes including: values and principles, peace and security, human rights, democracy and good governance, protecting the vulnerable, meeting the special needs of Africa, strengthening the United Nations, development and poverty alleviation, and protection of the environment. The following year at the 56th session, the UN Secretary General outlined a series of strategies for action towards meeting the goals (United Nations 2001).

Among the areas addressed by the declaration were global development and poverty eradication, familiar to most as the 'Millennium Development Goals' (MDGs) as well as protection of the environment. The goals were time bound with the anticipation that by 2015 significant milestones towards addressing extreme poverty in its many dimensions including income poverty, hunger, disease, lack of adequate shelter, and exclusion, while promoting gender equality, education, and environmental sustainability would have been achieved. Thus it was targeted that by 2015, the proportion of people who suffer from hunger would have been halved. The environmental components of the Millennium Declaration spoke to issues such as global warming, forestry, the Convention on Biological Diversity (CBD), Convention to Combat Desertification, and water use among others. In July 2002, the UN Secretary General launched the 'Millennium Development Project' with a view to prepare strategies to help countries achieve the various goals (United Nations 2002). A number of task forces were set up to address the various goals and one focused on hunger. This task force made seven recommendations and while several of these have relevance to biological control, one was particularly about increasing the agricultural productivity of food for insecure farmers. This recommendation identified among other things, the need to improve soil health (mainly through access to organic and inorganic fertilizers, access to better seeds and crop diversification including a focus on crops such as vegetables. Although not mentioned as a key recommendation, the management of pests will have to be an integral component if success is to be realized.

THE DEMAND FOR BIOLOGICAL CONTROL

HUNGER AND POVERTY

Poverty and hunger are inextricably linked. At the global level, 852 million are chronically hungry and this is a slight reduction from the 1990 levels (UN Millennium Project 2005a). Agriculture is the largest economic activity for the estimated 75% of the world poor (Majid 2004). 204 million people in sub-Saharan Africa are hungry and this number is rising. Among the causes for hunger are poverty and low food production. The management of pests and by extension, use of biological control, forms part of the technical suite of solutions.

Biological control offers technical solutions to secure food production against indigenous as well as non-indigenous pests. In addition to the obvious cost advantages, biological control within an IPM framework offers a way to minimize requirements for expensive pesticides. More importantly, it increases the scope for market access in countries with the increasingly stringent requirements for minimum acceptable residues (MRLs) of pesticides on food.

In addition to traditional exports of tropical produce, new niche markets are opening up with potential for higher income to farmers. For instance, during the last years of the 1990s, sales values of organic products grew by 20-30% following major food scares such as bovine spongiform encephalopathy (BSE) (FAO, 2001). While it is not envisaged that the high growth rates will persist, nevertheless, the demand is projected to grow, and land under organic produce has continued to expand (Yussefi, 2005). Organic agriculture brings with it numerous challenges for biological control.

BIOLOGICAL DIVERSITY

Over the last couple of decades, the importance of IAS on biological diversity has become increasingly recognized (UNEP 2003). The Convention on Biological Diversity explicitly recognized the risk posed by IAS in Article 8h. This article specifically calls on Parties to "as far as possible and as appropriate: prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species." In 2002, the sixth Conference of the Parties (COP 6), adopted a set of guiding principles on how to develop effective strategies to minimize the spread and impact of IAS and a program of work for the implementation of Article 8(h). One of the principles addressed control of IAS and noted that effective control will often rely on a range of integrated management techniques, including mechanical control, chemical control, biological control, and habitat management.

TRENDS IN BIOLOGICAL CONTROL AND IPM RESEARCH

ANALYTICAL APPROACH

Given the demand for biological control and as part of the process of assessing the opportunities and challenges, the trends in knowledge generation and application based on published material abstracted for the CAB International Database (CAB abstracts) of global research in applied life sciences including, agriculture, forestry and the environment among other subjects were analyzed. Over 220,000 new records are added to the database each year, from over 6,000 academic journals and 3,500 other documents, including other serials, books, 'grey' literature and conference proceedings. The database has an international coverage, including research from over 140 countries in 50 languages.

The analysis itself was kept simple and essentially focused on number of abstracts referencing specific search terms. Firstly we examined the number of publications on 'biological control or biocontrol.' These were further categorized on basis of geographic regions. For each region 3-4 representative countries were selected based on size, history of biological control research or presence of research institutions working in the area. The regions/countries were as follows:

- Africa - Benin, Kenya, Nigeria and South Africa
- Asia - China, India and Indonesia
- South America - Brazil, Colombia and Chile
- Caribbean and Central America - Cuba, Costa Rica, Jamaica and Trinidad and Tobago
- U.S.A.

The number of publications referencing particular biological control approaches (classical and augmentation) on a global basis was also determined. This was also done for other terms which were directly relevant to biological control namely: integrated pest management, farming systems research (incorporates participatory research) and 'invasive and species'. For comparison, an assessment of the number of publications on biotechnology, being a relatively new field was also done.

It is acknowledged that the analyses might have precluded relevant material which did not reference the specific search terms used or particular geographic areas. Additionally some search terms especially invasive or species may include publications unrelated to invasive species. Additionally biases in abstracting or inherent inefficiencies or gaps might introduce further complexities. Nevertheless, it is felt that the trends generated are sufficiently robust and indicative of the real situation.

THE OUTCOMES

During the period 1995-2004, the number of publications which directly reference biological control or biocontrol has ranged between 3405 and 4530 well below the 1990 number of 4856 (Fig.1). However, the general trend appears to be one of little growth, even decline, in 2001-2004, especially when compared to the trends in other fields such as biotechnology which rose from less141 publications in 1980 to 11, 878 in 1990 and over 12,000 1995-2000 (Fig. 2). After 2000, the number of publications in biotechnology also appears to have undergone a dramatic decline.

The number of publications which make specific reference to particular country groupings ranged between 19-37% of the global total for each year (average 22%). An analysis of these by geographic areas is given in Fig. 3. For Africa, this number was 37 rising to a high of 99 in 1990. Between 1995-2004 the number ranged between 48-76 but generally there was no growth. The highest number for the Caribbean and Central America was 40 in 1985 and 2002. Between 1985 and 2003, the number fluctuated between 18-40 with the lowest being recorded in 2004. The general trend in recent years has been one of decline. South America saw a growth between 1980-1996, rising from 63 to 106. The ensuing period was characterized by inconsistent growth with a maximum of 143 being attained in 2002 followed by a decline. In Asia, there was growth from 151-445 between 1980-85 followed by a decline (1985-95). This was followed by growth over the period 1995-2000 which flattened out in subsequent years. In the U.S.A., the period 1980-85 was characterized by growth from 307 to 654 publications but subsequently there has been a general decline.

Fig. 4a gives the number of publications referencing augmentation and classical biological control specifically. For augmentation biological control, the number has remained relatively low with little growth, fluctuating between 3-26 publications. Although there was growth (2-53) in the early period (1980-90), the number for classical biological control has varied widely in subsequent years (22-60) with no clear trend. For IPM, there has generally been growth ranging from 129-1025 with a maximum being recorded in 2002 (Fig. 4b). 1980-1990 saw a growth of farming systems research publications from 20-97 followed by a relative flattening (1990-2000) and a subsequent decline. The new area of IAS has seen a consistent increase from 6-520 in 2004 (Fig. 4b).

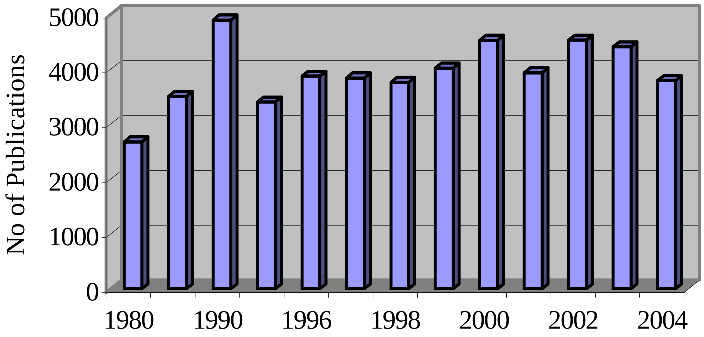

Figure 1. Publications on biological control over the period 1980-2004.

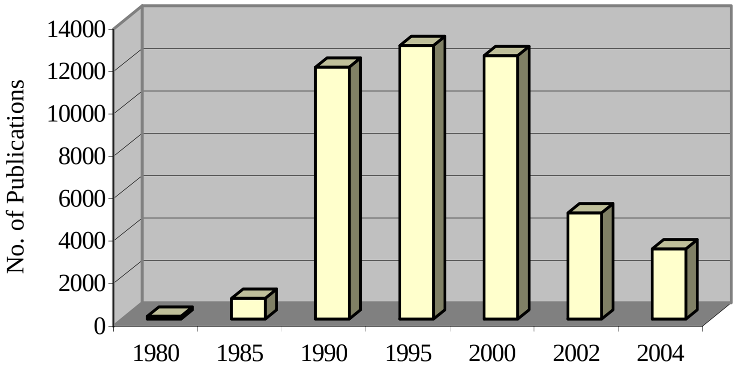

Figure 2. Publications on biotechnology over the period 1980-2004.

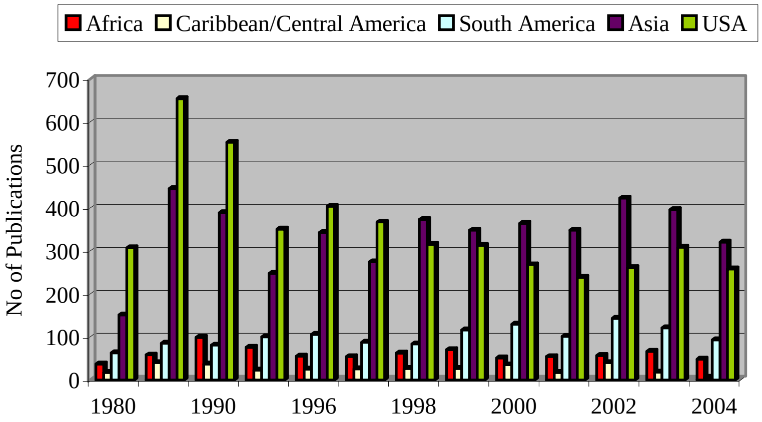

Figure 3. Distribution of publications referencing particular geographic areas.

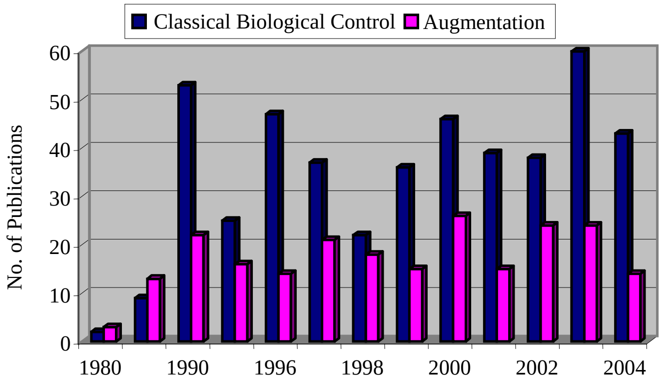

Figure 4a. Global publications on classical and augmentation biological control.

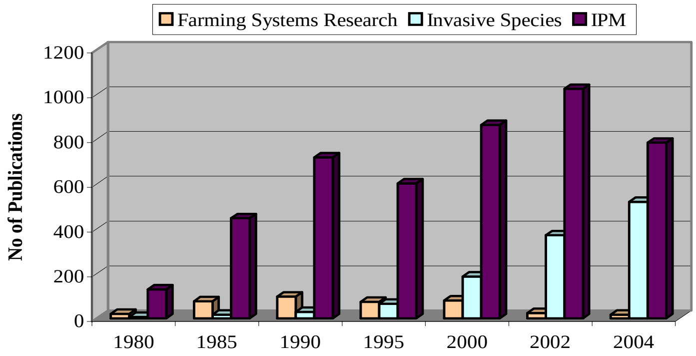

Figure 4b. Publications referencing 'farming systems research', invasive species and IPM.

DISCUSSION

Solving the problems associated with hunger and poverty or protection of biological diversity is a complex matter. Solutions will require an integrated multi-sectoral approach comprising of policy and technical imperatives. The first five years of implementation of the MDGs have elapsed. A recent assessment of progress showed that while some regions had made progress many others were largely off-track (UN Millennium Project 2005b).

Accepting that biological control is a small but nevertheless a very important component, intuitively one would expect to see increased activity as evidenced by published works. The global trends could perhaps best be described as stagnant. This pattern is also reflected in the geographical analysis and in some cases such as the U.S.A., a decline. A comparison of biological control with biotechnology, a relatively new field shows dramatic contrast.

The number of articles on classical biological control varied considerably over the study period, perhaps reflective of the opportunistic nature of such research. Overall, the number was surprisingly low while at the same time the number of articles on invasive species increased. There has been little growth in augmentation biological control over the years yet the potential for exploitation of this approach is recognized even by industry (Guillon, 2004). Indeed it is unfortunate that tremendous successes such as those achieved in the development biological pesticides against locusts have not been duplicated (Lomer *et al.* 2001). Overall, the results suggest that supporting research has not grown. There has been steady growth in IPM over the research period. While much emphasis has been placed on participatory approaches for transfer of ecological pest management strategies captured in CAB abstracts as farming systems research, there appears to have been little growth in published works on the subject.

It has been argued that much Development work is not amenable to publication in forms such as those abstracted in CAB abstracts. Notwithstanding this argument, the historical development of human endeavor in science and development has included published material on the generation as well as application of knowledge. We therefore argue that growth in a particular field should also be reflected in the published literature. This is clearly reflected in the case of biotechnology.

While diminished funding for research has been a constraint across the globe, the renewed interest in fulfilling the MDGs provides an opportunity for applying biological control. Challenges such as increased regulation for classical biological control will need to be surmounted. Overall however, the prognosis is not good and the challenge will be for biological control practitioners to ensure that the immense potential benefit from the approach is brought to bear on the pressing problems facing the world at the moment.

ACKNOWLEDGEMENTS

I acknowledge use of the CAB International CAB Abstracts Database and wish to thank Halina Dowson and Marion Doy for helpful suggestions on the analyses. I also wish to thank Mark Hoddle and Rebecca Murphy for editorial assistance.

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CLASSICAL BIOLOGICAL CONTROL OF CITRUS PESTS IN FLORIDA AND THE CARIBBEAN: INTERCONNECTIONS AND SUSTAINABILITY

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ABSTRACT

Beginning in 1993, Florida's citrus industry has been invaded by citrus leafminer (*Phyllocnistis citrella* Stainton, Lepidoptera: Gracillariidae), brown citrus aphid (*Toxoptera citricida* Kirkaldy, Homoptera: Aphididae), and the Asian citrus psylla (*Diaphorina citri* Kuwayama, Homoptera: Psyllidae). The source(s) of these pests remain unknown but other countries in the Caribbean, as well as Central and South America, also have suffered invasions by these pests. Brown citrus aphid and Asian citrus psylla are vectors of serious citrus diseases (citrus tristeza virus and greening disease, respectively), while citrus leafminer damage provides openings for invasion of the citrus canker pathogen into the foliage. All three pests were considered suitable candidates for classical biological control. Dr. Ru Nguyen (Division of Plant Industry, Gainesville, Florida) and I have collaborated on importing, evaluating, rearing and releasing parasitoids for each pest into Florida's 860,000 acres of citrus between 1993 and the present. Two parasitoids (*Ageniaspis citricola* Logvinovskaya, Hymenoptera: Encyrtidae and *Cirrospilus quadristriatus*, which was subsequently determined to be *C. ingenuus* Gahan, Hymenoptera: Eulophidae) of the citrus leafminer were imported from Australia, Thailand, and Taiwan with the assistance of several scientists. Both parasitoids have established in Florida, and *A. citricola* has become the dominant parasitoid while *C. ingenuus* has had no apparent effect. *Ageniaspis citricola* has been supplied to colleagues in the Bahamas, Bermuda, Brazil, Chile, Mexico, Honduras, and several other countries from our rearing program. In all cases, *A. citricola* was provided free of charge along with information on rearing methods, as well as the risk assessment that we developed prior to obtaining release permits from the Florida Department of Agriculture and Consumer Services and the U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS). Such information assisted the recipients in obtaining local release permits, thus reducing the costs of importation and release for these agencies.

Two other parasitoids were imported for control of the Asian citrus psylla: *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Shafee, Alam and Agarwal) (Hymenoptera: Encyrtidae). The parasitoids were obtained through the kind assistance of colleagues in Taiwan. Again, we have made both parasitoids available to coun-

tries in the Caribbean, upon request, along with rearing methods and our risk assessment data.

Finally, the parasitoid *Lipolexis scutellaris*, which was later designated *L. oregmae* Gahan (Hymenoptera: Aphidiidae), was imported from Guam for a classical biological control program directed against the brown citrus aphid. This parasitoid and our data have been provided upon request from colleagues in several locations (Hoy and Nguyen 2000c).

Classical biological control historically has had an ethos that fostered cooperation, interconnections, and sharing of resources and knowledge. This ethos must be maintained if classical biological control is to be sustained as a viable pest management tactic. A few governments recently have behaved as if their natural enemies are national resources that require extensive financial remuneration; this attitude will threaten the sustainability of classical biological control. We must share information and resources in order to win our struggle to manage invasive pests.

INTRODUCTION

The objective of this paper is to provide an overview of three classical biological control projects directed against invasive citrus pests in Florida. In addition, I will provide a personal perspective on several issues limiting the sustainability of classical biological control, and make a plea that communication needs to be improved if classical biological control is to be sustainable in the region.

Beginning in 1993, Florida's citrus has been invaded by three significant pests: the citrus leafminer (*Phyllocnistis citrella*), the brown citrus aphid (*Toxoptera citricida*), and the Asian citrus psylla (*Diaphorina citri*). These invasions have created serious disruptions to the integrated pest management program, which is based on biological control of scale insects, mealybugs, mites, and whiteflies (Browning and McCoy 1994; Hoy 2000; McCoy 1985). The majority of citrus pests prior to 1993 were under substantial biological control and Florida citrus growers could manage diseases and most arthropod pests with the use of oil and copper sprays once or twice a year, especially if their crop was destined for juice production (because cosmetic damage is not an issue).

PEST STATUS OF INVADERS

After each new invasion, the introduced pests multiplied and spread rapidly throughout Florida's citrus, causing economic damage. For example, the citrus leafminer colonized 860,000 acres within a year after its detection (Heppner 1993; Hoy and Nguyen 1997). Population densities were often extremely high, despite the presence of generalist natural enemies such as spiders, lacewings, ants, and eulophid parasitoids (Browning and Peña 1995). Densities of the citrus leafminer were so high that fruits and stems, in addition to foliage, were attacked (Fig. 1) (Heppner 1993). Growers repeatedly sprayed their trees, especially nursery trees and young groves, in a futile effort to suppress the leafminer populations. Subsequently, the citrus leafminer has been implicated as exacerbating the spread of citrus canker in south Florida, where this disease is the target of an eradication program (Gottwald *et al.* 2001).

Figure 1. Citrus leafminer damage on citrus foliage (left) and fruits (right). An operational economic injury level is estimated to be less than 1 leafminer per leaf. UGA1390033, UGA1390034

The brown citrus aphid can be a direct pest of tender new citrus foliage (= flush) (Fig. 2), causing shoot deformation and production of sooty mold. The aphid completes one or two generations before the flush hardens off and then alate aphids are produced. However, the concern over the invasion of the brown citrus aphid was the fact that this aphid is a very efficient vector of *Citrus tristeza virus* and accentuated by the knowledge that approximately one-fourth of Florida's citrus was planted on rootstock susceptible to the disease caused by the virus (Yokomi *et al.* 1994). This acreage has had to be replanted on tristeza-tolerant rootstock at great expense.

Figure 2. Brown citrus aphids develop on tender new shoots of citrus. The ephemeral aphid populations make it difficult to sample for parasitoids. UGA1390035

The Asian citrus psylla is a vector of the bacterium that causes greening, one of the most serious diseases of citrus in Asia (Gottwald *et al.* 2001; Halbert *et al.* 2000; Knapp *et al.* 1998; Whittle 1992). Psyllids also can cause direct feeding damage to young shoots (Fig. 3). The pest apparently invaded Florida without the greening pathogen (Hoy *et al.* 2001), but Florida's citrus is vulnerable to the disease now that the insect vector is well established (Knapp *et al.* 1998).

Figure 3. Asian citrus psylla: orange eggs on tender flush (left) and adults feeding on mature foliage (right). Adults can survive over the winter on mature foliage, which leads to a lag in populations of their host-specific parasitoid, *T. radiata*, in Florida in spring. Psyllid nymphs, which are hosts for the *T. radiata*, can develop only on tender new growth. UGA1390036, UGA1390037

The citrus leafminer, Asian citrus psylla, and the brown citrus aphid all feed on tender new growth (flush), which can potentially reduce tree growth or yield, although economic injury levels for these pests have not been determined for all citrus cultivars in Florida. Because Florida citrus receives rainfall all year, management of pests that attack the flush is especially difficult because populations can be high between March and October each year due to the production of four or five major flush cycles.

HOW DID THESE PESTS INVADE?

The method by which these pests invaded Florida remains unknown, although it is likely that the increased trade and tourism has made invasions more frequent (Enserink 1999; Frank and McCoy 1992). It appears that Florida, and other tropical and subtropical regions are especially vulnerable to invasions and the apparent inability of quarantines and regulatory agencies to stem the flow of pest arthropods into new regions from around the world will continue to create new opportunities for classical biological control (York *et al.* 2005). Because the IPM program in Florida's citrus is so heavily dependent on biological control, I believe we are on a 'biological control treadmill', rather than the more common 'pesticide treadmill', because new pests need to be controlled in a compatible manner with the long-established biological control of our exotic pests (Hoy 2000).

CLASSICAL BIOLOGICAL CONTROL

All three invaders were considered suitable candidates for classical biological control and Dr. Ru Nguyen (Division of Plant Industry, Gainesville, Florida) and I have collaborated on importing, evaluating, rearing and releasing parasitoids for each pest into Florida's 860,000 acres of citrus from 1993 to the present.

CITRUS LEAFMINER

Two parasitoids (*A.* and *C. quadristriatus*, now *C. ingenuus*) of the citrus leafminer were imported from Australia, Thailand, and Taiwan (Hoy and Nguyen 1997, Hoy and Nguyen 2003). The first collections were made possible through the kind assistance of Dan Smith, of the Queensland Department of Primary Industries in Australia. Both parasitoids had been imported into Australia and undergone risk assessment there (Neale *et al.* 1995). Because the climate of Queensland matches that of Florida relatively well, we chose to collect parasitoids there first. Dan Smith generously provided me with field assistance and data that facilitated our efforts to obtain rapid permission to release *A. citricola* in Florida.

The release of *A. citricola* in Florida may have achieved a record for least time from importation into quarantine until release; I returned from Australia on a Monday (April 25, 1994) with large numbers of adults and pupae of *A. citricola*, and Dr. Nguyen and I recognized that we would 'waste' many of these adults due to a lack of space and hosts in our quarantine facilities. Because we had written a draft request to release *Ageniaspis* prior to my travel to Australia, based in part on the information provided by Australian scientists from their risk analysis, we were able to submit our request to release *A. citricola* to the Division of Plant Industry for review on Tuesday, which immediately submitted it to the USDA-APHIS for review. Permission to release *A. citricola* was facilitated by John LaSalle at the British National Museum, who confirmed the identity of the parasitoid after we sent specimens to him by overnight shipment. The Division of Plant Industry of the Florida Department of Agriculture and Consumer Services assisted in a rapid review, as did the USDA-APHIS, and Dr. Nguyen and I had permission to make the first releases of adults of *A. citricola* into populations of citrus leafminers by Friday (April 29) (Hoy and Nguyen 1997).

Ageniaspis citricola pupae are produced within the pupal chamber of the citrus leafminer; this encyrtid is polyembryonic and females typically deposit two eggs per oviposition event, one of which develops into a male. The second egg twins, producing two daughters (Zappalà and Hoy 2004); this reproductive strategy may contribute to its success when host populations are low. *Ageniaspis citricola* and *C. ingenuus* have both established in Florida, with *A. citricola* now the dominant parasitoid of the citrus leafminer (Hoy and Nguyen 1997; Hoy *et al.* 1995; Hoy *et al.* 1997; Peña *et al.* 1996; Pomerinke and Stansly 1998; Smith and Hoy 1995; Villanueva-Jimenez and Hoy 1998a; Villanueva-Jimenez *et al.* 2000) (Fig. 4).

Cirrospilus ingenuus has had no apparent effect in reducing citrus leafminer densities, although this eulophid has established in south Florida (LaSalle *et al.* 1999). In retrospect, however, Dr. Nguyen and I regret releasing this ectoparasitoid because we discovered, after the release, that it could hyperparasitize *A. citricola* (Hoy and Nguyen 1997).

Figure 4. *Ageniaspis citricola* pupae.
UGA1390038

Ageniaspis citricola has many of the attributes of an effective natural enemy (Rosen and Huffaker 1983). It is host specific (Neale *et al.* 1995), able to locate low-density leafminer populations and to discriminate between previously parasitized hosts (Edwards and Hoy 1998; Zappalà and Hoy 2004), although it is not able to perform well in regions with low relative humidity (Yoder and Hoy 1998) and lags behind citrus leafminer populations in the spring in Florida (Villanueva-Jimenez *et al.* 2000). Citrus leafminer populations decline to very low densities over the winter when there is no new flush and typically only a very few citrus leafminers are found in the first flush cycle in spring. Since *A. citricola* is host specific and polyembryonic, populations of *A. citricola* increase from very low densities to detectable levels by the second flush cycle in Florida and, if not disrupted by drought or pesticide applications, become the dominant parasitoid, capable of parasitizing up to 100% of the leafminer pupae by the fall, which decreases the number of citrus leafminers able to overwinter (Villanueva-Jimenez *et al.* 2000; Zappalà *et al.*, unpublished). A second population of *A. citricola* was imported from Taiwan, and this population appears to be a cryptic species (Alvarez and Hoy 2002; Hoy *et al.* 2000). Although it was released in Florida, we have no evidence of its establishment (Alvarez and Hoy 2002).

During 2000 and 2001, Florida suffered a drought that was especially serious in the spring, leading to a greater lag between populations of *A. citricola* and the citrus leafminer than before. This led us to consider release an additional parasitoid that would have the potential to suppress citrus leafminers early in the season when *A. citricola* densities are very low and a long list of potential candidates was reviewed (Heppner 1993; Schauff *et al.* 1998). Such a parasitoid ideally would tolerate lower relative humidities than *A. citricola* and might have an alternative host on which it could overwinter. With the assistance of Dr. G. Siscaro of the University of Catania in Italy, we imported the eulophid *Semielacher petiolatus* Girault (Hymenoptera: Eulophidae) (Fig. 5) for evaluation in quarantine (Hoy *et al.* 2004). This parasitoid had established in citrus in the Mediterranean and promised to have a greater tolerance of low relative humidities (Ateyyat 2002; Lim *et al.* unpublished). It was also reported

Figure 5. *Semielacher petiolatus* female.
UGA1390039

to use alternative hosts, including a dipteran leafminer in the genus *Liriomyza* (Massa *et al.* 2001), which could provide hosts for *S. petiolatus* during the winter when citrus leafminer populations are extremely low in Florida.

After importing *S. petiolatus* into quarantine we demonstrated that it could develop on the citrus leafminer, but that it often superparasitized (Lim and Hoy 2005). Additional research confirmed that *S. petiolatus* does not discriminate between unparasitized and parasitized hosts with its own progeny or with the endoparasitoid *A. citricola* and could potentially disrupt the substantial control provided by the host-specific *A. citricola* (Lim *et al.* unpublished). Also, it did not parasitize *Liriomyza trifolii* Burgess (Diptera: Agromyzidae), a common and abundant leafminer pest of vegetables during the winter in Florida (Lim *et al.*, unpublished). After this risk analysis in quarantine, we recommended against releasing *S. petiolatus* in Florida because of the information previously mentioned and also because there was no evidence that it would provide control of the citrus leafminer during the spring when populations of *A. citricola* lag behind those of its host. Although it is difficult to predict with any certainty the outcome of potential releases of *S. petiolatus* in Florida, the potential benefits do not appear to justify the potential risk. In regions where *A. citricola* is not an effective parasitoid, it is possible that releases of *S. petiolatus* are appropriate, but independent risk analyses should be conducted in each country.

Ageniaspis citricola has been supplied to colleagues in the Bahamas, Bermuda, Brazil, Chile, Mexico, Honduras, and several other countries (including Morocco, Italy, Spain) from our rearing program (Hoy and Jessey 2004; Villanueva-Jimenez *et al.* 1999). In all cases, *Ageniaspis* was provided free of charge along with information on rearing methods (Smith and Hoy 1995), studies of its biology and susceptibility to pesticides (Alvarez and Hoy 2002; Edwards and Hoy 1998; Hoy *et al.* 2000; Villanueva-Jimenez and Hoy 1998b; Yoder and Hoy 1998; Zappalà and Hoy 2004) and the risk assessment data that we developed prior to obtaining release permits from the Florida Department of Agriculture and Consumer Services and the U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS). Such information was intended to assist the recipients in obtaining permission to make releases, thus reducing the costs of importation, evaluation and release for local regulatory agencies.

ASIAN CITRUS PSYLLA

Two host-specific parasitoids were imported for control of the Asian citrus psylla: *T. radiata* and *D. aligarhensis* (Fig. 6) (Hoy and Nguyen 1998). Both parasitoids were obtained through the kind assistance of P. K. C. Lo of the Taiwan Agricultural Research Institute and had shown efficacy in Taiwan and on Reunion Island (Aubert and Quilici 1984; Chien 1995; Chien and Chu 1996; Chu and Chen 1991). Before we could obtain permission to release these parasitoids we had to 'prove a negative', namely that they did not harbor the greening pathogen. This led us to develop a polymerase chain reaction (PCR) test with a known level of sensitivity for the greening pathogen (Hoy and Nguyen 2000a; Hoy *et al.* 1999; 2001). Both parasitoids appear to be host specific and were mass reared and released throughout Florida, where *T. radiata* is now widely distributed (Hoy *et al.* 2000; Hoy *et al.* unpublished; Skelley and Hoy 2004). The status of *D. aligarhensis* is unclear because only a few recoveries have been made (Hoy *et al.*, unpubl.).

Again, we have made both parasitoids available to colleagues in the Caribbean, upon request, as well as our rearing methods, information on the parasitoid's biology (McFarland and Hoy 2001; Skelley and Hoy 2004) and our risk assessment data.

Figure 6. Asian citrus psylla nymphs parasitized by *Tamarixia radiata* (left) and *Diaphorencyrtus aligarhensis* (right). Exit holes for *T. radiata* and *D. aligarhensis* are on the thorax and abdomen, respectively, making it easy to discriminate parasitism by the two parasitoids in the field. UGA1390040, UGA1390041

BROWN CITRUS APHID

The parasitoid *Lipolexis scutellaris*, which was later designated *L. oregmae* by Miller *et al.* (2002), was imported with the assistance of Ross Miller in Guam for a classical biological control program directed against the brown citrus aphid (Hoy and Nguyen 2000b,c). Petr Stary provided taxonomic identifications and other information, and Susan Halbert, of the Florida Department of Agriculture and Consumer Services, provided expert advice on preparing the application to release *L. scutellaris* in Florida (Hoy and Nguyen 2000c).

This parasitoid was easy to rear on the brown citrus aphid on citrus trees after the discovery of its unusual behavior of causing parasitized aphids to walk off the tree to mummify in the soil at the base of the trees (Hill and Hoy 2003). We treat the soil in the potted trees with a 2-3% sodium hypochlorite solution prior to exposing the trees to aphids and parasitoids to control fungal pathogens of the parasitoid mummies (Hill and Hoy 2003, Persad and Hoy 2003a,b; Walker and Hoy 2003b).

Laboratory analyses indicated that *L. oregmae* and *Lysiphlebus testaceipes* (Cresson), a parasitoid already established in Florida and a natural enemy of the brown citrus aphid, are not intrinsically superior to each other (Persad and Hoy 2003a). Beginning in 2000, releases were made throughout the state over several years, and *L. oregmae* seems to have established (Hoy *et al.* unpublished; Persad *et al.* 2004). However, populations of *L. oregmae* are low in Florida, perhaps because this parasitoid is preyed upon by abundant red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), in citrus groves (Hill and Hoy 2003; Persad and Hoy 2004; Walker and Hoy 2003). Red imported fire ants will feed on mummies in the soil and also will climb into the tree to remove parasitized aphids, leaving behind the unparasitized pests (Persad and Hoy 2004). A PCR test that allows us to sample aphids and assay them for the presence of either *L. oregmae* or *L. testaceipes* allowed us to obtain qualitative data on distribution and spread of *L. oregmae* in Florida (Persad *et al.* 2004). This technique is sufficiently sensitive that we could grind up 500 aphids of which only one was parasitized by *L. oregmae*, yet get a positive PCR product??. Once we know that *L. oregmae* is present in a grove, additional samples can be taken to ascertain the relative abundance of *L. testaceipes* and *L. oregmae*.

Because *L. oregmae* attacks black citrus aphid (*T. aurantii* Boyer de Fonscolombe), spirea aphid (*Aphis spiraecola* Patch), cotton aphid (*Aphis gossypii* Glover), and cowpea aphid (*Aphis craccivora* Koch*)*, on citrus and other crops in Florida, it has alternative hosts that can sustain it when brown citrus aphid populations are low (Hoy and Nguyen 2000c). These aphids also are imported pests of citrus in Florida so there was reduced concern about the nontarget effects of *L. oregmae*.

Releases of *L. oregmae* were also made in Bermuda during the July of 2002, but its establishment has not yet been confirmed. Shipments of *L. oregmae* have been requested by scientists in CARDI for release in Jamaica and permits have been issued by the Jamaica Department of Agriculture.

CONSTRAINTS TO CLASSICAL BIOLOGICAL CONTROL IN THE REGION

Biological control is, in my opinion, at a turning point in its development as a discipline. It could become a more important component of pest management programs if we are able to resolve concerns about potential risks to biodiversity (Howarth 1991; Simberloff and Stiling 1996). If we are unable to resolve those concerns, there could be less classical biological control conducted in the future, rather than more. Several constraints need to be eliminated or reduced.

INTERNATIONAL COOPERATION

International cooperation is crucial to the success of classical biological control programs (FAO 1997). Such cooperation will become even more important in the future because we lack sufficient resources to conduct classical biological control projects in isolation. Scientists in Australia, Taiwan, Thailand and Guam were instrumental in our ability to respond rapidly to the three invasive species in Florida's citrus. They provided assistance, information, and resources that enabled us to respond rapidly to the threat of these invaders. Historically, classical biological control has depended on such generous international cooperation and it needs to be maintained. The belief that natural enemies are national resources that should be sold is detrimental to the continued success of classical biological control. Indeed, biological control scientists may wish to become even more proactive about cooperating in classical biological control of citrus pests and begin sharing information about the natural enemies of potential invaders in advance, perhaps using websites as a repository of information.

THE FUTURE OF CLASSICAL BIOLOGICAL CONTROL

It is ironic that, just when there is an increased focus on and potential role for biological control of arthropod pests, serious concerns about biodiversity could restrict its use. Current constraints also include the deployment of relatively few resources, at least compared to those available to develop new pesticides or transgenic crops. Most of the funding for classical biological control is obtained from public sector sources, which have not had sufficient increases in their budgets to meet the current and potential demand.

The history of biological control of arthropod pests is filled with outstanding examples of successes and a remarkably low number of ecological problems (Frank 1998; Funasaki *et al.* 1998). Despite this, we will have to embrace increased oversight and consideration of ecological issues. The question then becomes: how best can we achieve appropriate oversight without hampering the benefits of biological control?

One solution for biological control practitioners might be to focus more frequently on natural enemy species that are narrowly host- or prey-specific. Scientists working on biological control of weeds already have accepted this constraint, and undergo external reviews of the biology, behavior, and host specificity of the natural enemies they wish to release. It also will be useful to have more thorough scientific peer review before natural enemies are released for classical biological control of arthropod pests (Ewel *et al.* 1999). Despite increased peer review, it may be impossible to eliminate all risk concerns.

Risk analyses are neither simple nor easy. Blanket criticisms of biological control are of little constructive value in the absence of comparative data on the alternatives, including doing nothing (Thomas and Willis 1998). Furthermore, biological control has numerous public benefits, including relatively inexpensive and long-term control, and reduced pesticide applications, which can result in reduced negative effects on ground water, nontarget species, human health, and worker safety.

RECOMMENDATIONS

- Sharing of information is essential if classical biological control is to be cost effective; providing information on risk assessments, unpublished data on biology and ecology, and copies of hard-to-find literature on web sites would be an efficient method of sharing key information that will allow scientists and governmental agencies to evaluate potential introductions of natural enemies for classical biological control in other countries. At present, this form of sharing occurs on an *ad hoc* basis. The University of Florida has provided resources and technical support to assist us in providing information in this manner, but it may be useful to consider developing a centralized and international site where practitioners of classical biological control can deposit such information.

- If possible, scientists and organizations should provide colonies of natural enemies upon request to others at the lowest possible cost. Reimbursements for shipping and rearing costs are appropriate, but tying the request for natural enemies to large-scale funding for the donor could delay or preclude the introduction of key natural enemies in a timely fashion.
- Funding for post-release evaluations is particularly difficult to obtain because most funding is provided for collection, importation, rearing and release. Sharing of information and colonies would produce savings that could be used to obtain needed data on the effects of the imported natural enemies on the target pests subsequent to their establishment. Such studies should occur after equilibrium has developed between the pest and its natural enemies in the new environment. In addition, funding needs to become available for evaluating the impact of key importations on nontarget species. Again, this type of funding remains relatively rare, but is essential if we are to develop the data to understand the long-term costs and benefits of classical biological control.

CONCLUSIONS

Our collective responses to these challenges will determine how effectively classical biological control is maintained as a viable discipline. We have valuable new tools, including molecular genetic methods, which will allow us to answer previously intractable questions in systematics, ecology, behavior and quality control. The use of pesticides no doubt will decline and the ones used may be less hazardous to the environment. The demand for classical biological control could increase in the 21st century, especially if we respond effectively to concerns regarding potential negative environmental consequences attributed to biological control. When risks and benefits are compared appropriately, classical biological control should fare very well in comparison to the risks and benefits associated with other pest management tactics such as chemical control, cultural practices, host plant resistance (including the use of transgenic crops), and genetic control.

The potential risks and benefits of classical biological control must be calculated in a realistic manner because it is not possible to manage pests without any risk. As pointed out by Lubchenco (1998), our world is changing and we now live on a "...human-dominated planet. The growth of the human population and the growth in amount of resources used are altering Earth in unprecedented ways." Lubchenco (1998) concluded that the role of science now includes "...knowledge to reduce the rate at which we alter the Earth systems, knowledge to understand Earth's ecosystems and how they interact with the numerous components of human-caused global change, and knowledge to manage the planet". This change in perception of the status of ecosystems must become widespread among scientists and others if appropriate policy decisions are to be made. To increase awareness of this change in perception, perhaps a new term should be coined to describe our role and responsibilities as 'planet ecosystem management' or 'PEM' (Hoy 2000). Humans are, in fact, remodeling the entire global ecosystem.

Classical biological control historically has had an ethos that fostered cooperation, interconnections, and sharing of resources and knowledge. This ethos must be maintained if classical biological control is to be sustained as a viable pest management tactic. A few governments recently have behaved as if their natural enemies are national resources that require extensive financial remuneration; this attitude will threaten the sustainability of classical biological control. We must share information and resources in order to win our struggle against invasive pests.

ACKNOWLEDGEMENTS

I am especially grateful to Ru Nguyen, Florida Department of Agriculture and Consumer Services, Gainesville, for his collaboration on these classical biological control projects and his outstanding skills in rearing the natural enemies in quarantine. I thank the many people who have assisted during collecting trips or shipped material to us (especially Rut Morakote, P. Nanta, P. K. C. Lo, Dan Smith, Dan Papacek and G. Siscaro), the much-appreciated scientists who provided taxonomic identifications (John LaSalle, John Noyes and Petr Stary), students and postdoctoral scientists who assisted in the molecular genetic analyses (and especially A. Jeyaprakash) and those in my laboratory who helped to rear and release these natural enemies in Florida. Several graduate students or postdoctoral scientists, including Juan Villanueva-Jimenez, Shane Hill, Alison Walker, Juan Manuel Alvarez, Un Taek Lim, Lucia Zappalà, Lucy Skelley, Anand Persad, Clint McFarland and Owain Edwards, have contributed by studying the biology of the various natural enemies. J. A. Yoder conducted a comparative analysis of water balance in the citrus leafminer and *Ageniaspis citricola*. Colleagues in Florida (and their staff), including Harold Browning, Jorge Peña, Phil Stansly, David Hall and Joe Knapp, found release sites and released our shipments throughout the very large citrus-growing area. Finally, I appreciate the support provided by the Davies, Eckes and Fischer Endowment in Biological Control that has helped make this work possible, as well as financial support from the Florida Citrus Production Research and Advisory Committee and TSTAR. This is University of Florida Agricultural Experiment Station publication R-10802.

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CONSERVATION AND ENHANCEMENT OF BIOLOGICAL CONTROL HELPS TO IMPROVE SUSTAINABLE PRODUCTION OF BRASSICA VEGETABLES IN CHINA AND AUSTRALIA

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ABSTRACT

Brassicas comprise a major group of vegetable crops in Zhejiang Province, China and southeast Queensland, Australia. In Zhejiang, heavy reliance on chemical control to manage insect pests in brassica vegetable production has resulted in insecticide resistance, increased costs of pest control and insecticide residues hazardous to human health. In southeast Queensland, reliance on chemical control has also resulted in increased cost of pest control, control failures due to insect resistance and reduced profits. To improve sustainable production of brassica vegetables in the two regions, a group of Chinese and Australian scientists have undertaken a joint project to develop practical integrated pest management (IPM) strategies for these crops.

In both regions, major efforts have been made to evaluate the complexes of endemic natural enemies under different pest management practices, and to conserve and enhance these natural enemies as the central elements of effective management programs. In Zhejiang, field trials were conducted across crops, seasons and localities to test and improve an IPM system that emphasized the use of proven action thresholds for different crop growth stages and strategic application of selective insecticides to promote the impact of natural enemies. Compared with conventional methods, IPM practices were associated with substantially higher natural enemy activity, a 20-70% reduction in input of insecticides, and no yield loss. The improved IPM system has been implemented to various degrees in major vegetable production areas in Zhejiang, and has improved the safety and profitability of production.

In southeast Queensland, as an important part of the IPM development and implementation effort, a three-year experimental field study was conducted to evaluate the impact of endemic natural enemies on independent farms practicing a range of pest management strategies. Natural enemy impact was greatest on farms adopting IPM and least on farms practicing insecticide intensive conventional pest control strategies. On IPM farms, the contribution of natural enemies to pest mortality permitted the cultivation of marketable crops with no yield loss but with an average of 70% less insecticide inputs compared to conventional farms.

The field studies and IPM implementation in China and Australia indicate that naturally occurring biological control can be substantially enhanced to form the central element of effective IPM programs and improve vegetable production. Demonstration of the effectiveness of biological control in the two regions through an international joint effort not only made the evidence more convincing but also promoted the adoption of the improved IPM strategies by farmers.

INTRODUCTION

Brassicas constitute a major group of vegetables in China. Depending on the region, brassicas account for 35-45% of all vegetable crops. In Zhejiang province, the proportion of brassica vegetables has decreased in recent years due to an increase in other vegetable crops, but they still account for approximately 30% of all vegetables and a total area of 235,000 ha was cultivated in 2004 (calculated on single crops). Brassica vegetables are mostly grown by small landholders (<0.5 ha) around urban centers, and in specialized production areas where farms can be much larger. The crop systems are complex and erratic, revolving around intercropping practices (growing more than one crop on a small piece of land at the same time) throughout the entire year. In Zhejiang, a complex of insect pests attacks brassica vegetable crops. The major species include the diamondback moth (DBM), *Plutelle xylostella* L. (Lepidoptera: Plutellidae), the cabbage white butterfly, *Pieris rapae* L. (Lepidoptera: Pieridae), the cluster caterpillars, *Spodoptera litura* F. (Lepidoptera: Noctuidae), the beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae), the green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae), and the turnip aphid *Lipaphis erysimi* Kaltenbach (Hemiptera: Aphididae) (Liu *et al.* 1996).

The brassica industry in Queensland grows a total of 2,300 ha of crops per annum (Heisswolf *et al.* 1997). The major production region is the Lockyer Valley, a river system about 100 km inland from Brisbane and farm sizes range from 40-100 ha. Planting of crops begins in February (later summer) and weekly or fortnightly plantings are made until September (early spring), final harvests are collected in late spring/early summer. On many farms, regular weekly plantings used to be continuous and crops were grown all year around. A suite of lepidopterous pests attack brassica vegetable crops in the region. These include DBM, cabbage white butterfly, centre grub, *Hellulla hydralis* Guenee (Lepidoptea: Pyralidae), *Hellulla undalis* F. (Lepidoptea: Pyralidae), cabbage cluster caterpillar, *Crocidolomia pavonana* F. (Lepidoptea: Pyralidae), cluster caterpillar, and *Helicoverpa* spp. (Lepidoptera: Noctuidae). DBM has been the most difficult pest to manage, largely due to its resistance to a range of commonly used insecticides (Heisswolf *et al.* 1997).

In Zhejiang, the control of insect pests on brassica vegetable crops has relied heavily on the use of chemical insecticides since the 1970s, resulting in insecticide resistance, increased costs of pest control and insecticide residues hazardous to human health (Liu and Yan 1998; Liu et al. 1996). In southeast Queensland, reliance on chemical control in the 1970s and 1980s also resulted in increased cost of pest control, control failures due to insect resistance and reduced profits (Heisswolf et al. 1997). To improve sustainable production of brassica vegetables in the two regions, a group of Chinese and Australian scientists undertook a joint project to develop practical integrated pest management (IPM) strategies for these crops (Zalucki and Liu 2003).

A JOINT VENTURE IN IMPROVING BRASSICA IPM

This project was started in 1995 to build on existing studies to develop sound, sustainable brassica IPM strategies that significantly reduce pesticide hazards, and are acceptable to the growers in Zhejiang and Shanghai, east China, and Queensland, Australia. The project involved five institutes in China, working in close collaboration with two institutes from Australia (Liu *et al*. 1996; Zalucki and Liu 2003). The working strategy consisted of three overlapping and ongoing phases: problem definition, research and development, and implementation. Structured problem definition workshops, involving all groups of stakeholders and in particular farmers and extension workers, were organized at the start of the project to promote information flow, determine priority issues, address priority needs, and propose action plans (Liu *et al.* 1996). Work has since concentrated on the following five, interacting components: (1) survey and evaluation of natural enemies?, (2) rational application of insecticides, in particular promoting use of biological insecticides, (3) development of action thresholds, (4) development of management strategies through season-long in-field IPM trials, and (5) IPM implementation activities.

RESEARCH, DEVELOPMENT, AND IMPLEMENTATION IN EAST CHINA

SURVEY AND EVALUATION OF ARTHROPOD NATURAL ENEMIES

Regular sampling in both farmers' fields and unsprayed fields in Hangzhou showed that a range of parasitoids attack each of the major pests. For example, DBM is attacked by at least 8 species of parasitoids, of which *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae), *Oomyzus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae) and *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae) are the major larval, larval-pupal and pupal parasitoids respectively (Liu *et al.* 2000). The cabbage white butterfly is attacked by a suite of at least 7 species of parasitoids, of which *Cotesia glomeratus* (L.) (Hymenoptera: Braconidae) and *Pteromalus puparum* L. (Hymenoptera: Pteromalidae) are often most abundant.

Insect parasitoids are active in fields despite the heavy use of chemical insecticides in the crop systems over the years. For example, in fields that have not been heavily sprayed during a growing season, parasitoids usually achieved 10-60% parasitism of DBM larvae and pupae during June to early July and September-November each year when DBM was most abundant (Liu *et al*. 2000). IPM field trials demonstrated that both parasitoids and arthropod predators were several-fold more abundant in fields that were sprayed with selective insecticides, than in fields that were sprayed with wide-spectrum chemical insecticides (Lin *et al.* 2002; Yu *et al.* 2002; Zhang *et al.* 1999).

EVALUATION OF BIOLOGICAL AND SELECTIVE INSECTICIDES

Biological and chemical insecticides were bio-assayed in the laboratory and tested in the field. A number of Bt and NPV products were shown to have high efficacy in killing the target pests with no side effects on the beneficials (Shi and Liu 1998; Shi *et al.* 2004). Other insecticides showing selectivity include abmectin, avermectin, spinosad and fipronil against DBM and *P. rapae*, chlorfluazuron and chlorfenapyr against *S. litura* and *S. exigua*, and imidacloprid against aphids (Guo *et al.* 1998; Guo *et al*. 2003; Zalucki and Liu 2003).

DEVELOPMENT OF ACTION THRESHOLDS

Laboratory and greenhouse trials demonstrated that several cultivars of common cabbage and cauliflower could endure some defoliation without reduction of head weight at harvest. There was evidence of over-compensation for defoliation at the pre-heading stage. However, the plants were more sensitive to defoliation at the cupping stage. For example, 10% defoliation of common cabbage (cultivar Jin-Feng No.1) at the pre-heading, cupping or heading stages respectively resulted in mean head weights at harvest 9.8% heavier, 4.3% lighter and 3.3% heavier than undamaged controls (Chen *et al.* 2002; Liu *et al.* 2004). These data were used to assist in developing action thresholds for practical application (Table 2). Of particular value was the characterization of crop growth stages sensitive to insect damage. Thus, farmers and extension officers were asked to monitor the insect pests more closely at both the seedling and cupping stages.

IPM FIELD TRIALS

Based on the findings of studies of various components and information from literature, management strategies were formulated and tested in the field to evaluate the effects of different management strategies on pest and natural enemy populations and to develop practical IPM guidelines and protocols. The major components in the IPM strategy included use of action thresholds in decision-making and strategic use of biological and selective insecticides (Tables 1 and 2). In each location, a field trial with a crop of approximately one ha was divided into 2-3 plots. Each plot was managed by an IPM or a conventional, insecticide intensive, approach for an entire season. Regular sampling was conducted through the season and pest control action was taken according to the guidelines in Tables 1 and 2. At the end of each trial, crop yield and quality, input of insecticides and levels of natural enemy activities of different plots were compared (Table 3). Field IPM trials with common cabbage were conducted in Hangzhou from 1996 to 2000 and in 2000 trials were conducted at five sites in Zhejiang and Shanghai (Lin *et al.* 2002; Liu *et al.* 2004; Yu *et al.* 2002; Zhang *et al.* 1999). In 2001 and 2002, field IPM trials with cauliflower, broccoli or Chinese cabbage were conducted at three sites in Zhejiang and Shanghai (Zalucki and Liu 2003). The results showed that biological and selective insecticides could offer effective control of all the insect pests and that the activities of natural enemies were promoted (Table 3). Compared with conventional practice, IPM practice could reduce insecticide input by 20-70%, with no risk of crop loss (Fig. 1; Zalucki and Liu 2003).

Table 1. Summary of designs of field IPM trials in China.

Treatment	Description	Application of insecticides
IPM	Use of action thresholds, apply biological and selective insecticides	Spray Bt for control of DBM and Pieris rapae, spray chlorfluazuron and NPV for control of *Spodoptera* spp. and spray imidacloprid for control of aphids
Conventional	Simulation of typical practice by farmers, or recording of farmer's practice	Basically calendar sprays with mixtures of broad-spectrum chemical insecticides such as chlorpyrifos, fenvalerate, methomyl, fipronil, and methamidophos

Table 2. Action thresholds (mean number of insects/plant) used in IPM treatment in China.

	Cabbage Growth Stages			
Pests	**Transplants**	**Pre-heading**	**Cupping to Early Heading**	**Heading to Mature**
Lepidoptera[a]	0.5	1.0	1.0	4.0
Aphids	5	500	500	2000

[a] Number of lepidopteran larvae were converted to "standard" insects by the following formula: 1 standard insect = 1 *Pieris rapae* = 1 *Spodoptera exigua* = 0.5 *Spodoptera litura* = 5 *Plutella xylostella*.

Table 3. Examples of results of field trials including plots managed with IPM or conventional (Con) pest control strategies in Hangzhou, China, in autumn 1998 and autumn 2000.

	1998		2000	
Assessments[a]	**IPM**	**Con**	**IPM**	**Con**
Mean head weight (kg)	1.23 a	1.11 a	1.18 a	1.02 a
% marketable heads	94.4 a	88.0 b	95.6 a	91.1 a
% heads without insect damage	52.5 a	16.7 b	76.7 b	96.7 a
Number of sprays[b]	7(8)	8(23)	3(5)	5(8)
Cost of insecticide application per ha (RMB Yuan)	2,700	3,780	680	1025
Mean % parasitization of DBM larvae	19.4 a	2.0 b	35.2 a	7.1 b
Mean % parasitization of DBM pupae	32.6 a	1.3 b	18.8 a	13.0 a

[a] Figures in the same row of the same year followed by the same letter do not differ ($p>0.05$, Student-t test).

[b] In the IPM treatment, usually one insecticide and only rarely a mixture of 2 insecticides was used per spray, while in the conventional treatment, usually a mixture of 2-3 insecticides was used per spray. Figure in brackets indicate the relative amount of insecticide input calculated on the basis of one insecticide in one spray at the recommended rates.

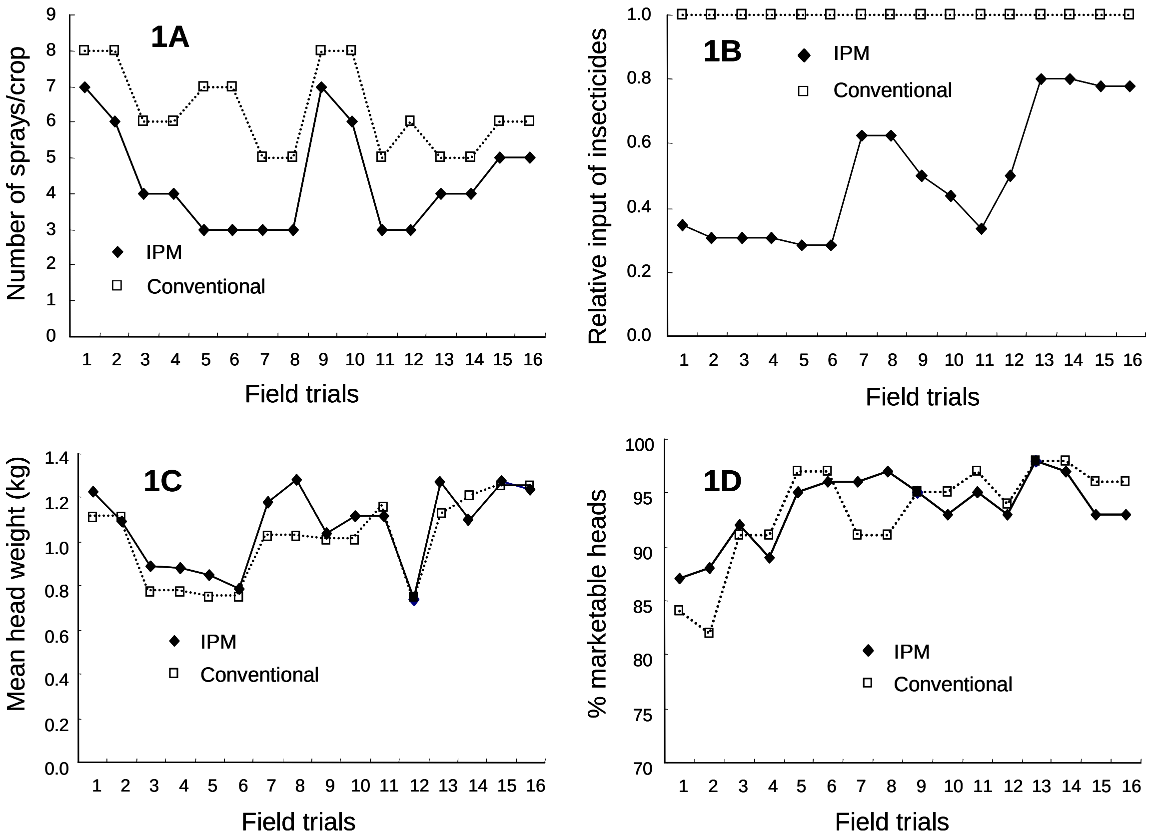

Figure 1. Comparison of insecticide input and crop yield between field plots managed by IPM or conventional approaches at each of 16 field trials at various locations in Zhejiang and Shanghai, China, from 1999 to 2001. 1A: number of insecticide applications per crop; 1B: relative quantity of insecticide input with that of conventional approaches set as unity (see footnote of Table 3 for further explanation); 1C: mean head weight in kg at harvest; and 1D: % of marketable heads.

IMPLEMENTATION

Implementation activities included grower involvement in field trials, field days and participatory workshops, frequent dissemination of fact sheets, as well as short training courses for extension officers and growers (Liu *et al.* 1996; Zalucki and Liu 2003). An independent project evaluation in the project areas showed substantial improvement in farmers' knowledge, attitude and approaches towards IPM (Liu and Qiu 2001). For example, by 2001, 36% of the growers in the project areas conducted regular monitoring of insect pests on their crops and usually tried to use biological or selective insecticides if required, compared with only about 20% in the non-project areas; growers in the project areas had more frequent contact with extension officers than growers in the non-project areas (Liu and Qiu 2001). An extensive survey by the agricultural departments in Zhejiang and Shanghai in late 2002 showed that in 10 major, project-associated production areas, which involved some 50,000 farming families and produced some 2 million tons of brassica vegetables in a year, input of chemical insecticides was reduced by 30-60% in a period of five years. Legally excessive pesticide residues on brassica vegetables from August to October (the season of the year when insecticides are mostly applied) were reduced steadily from 20-40% in the mid 1990s to 0-10% (0% in the central project areas) in 2002 (Zalucki and Liu 2003).

RESEARCH, DEVELOPMENT, AND IMPLEMENTATION IN SOUTHEAST QUEENSLAND, AUSTRALIA

DEVELOPMENT AND IMPLEMENTATION

The effort to develop an IPM approach for the control of insect pests in brassica vegetable crops in Queensland began in late 1980s when many growers encountered frequent spray failures with chemical insecticides. In many cases control failures were so severe that crops failed completely. A resistance management strategy was implemented in 1988 with widespread support of the industry. This strategy included a summer production break, improved spray application, an understanding of insecticide resistance and the need for insecticide rotation on farms (Heisswolf *et al.* 1997; Niemeyer 2004).

In the early 1990s, development work to reduce the reliance on conventional insecticides began by focusing on the crop system level of pest management and introducing Bt into the emerging IPM system. Research and extension activities involved a series of demonstration plantings at the local research station and on commercial farms. Data on pest activity, abundance of natural enemies, yields and quality of harvested products were collected. Results were then shared with growers and used to recommend improvements to management regimes with particular emphasis on spray decision making (Heisswolf *et al.* 1997; Niemeyer 2004).

Following the start of the joint brassica IPM project between Australian and Chinese scientists in 1995, more fields trials were conducted to focus on issues such as protocols for monitoring pests and parasitoids, action thresholds, insecticide spray coverage, and development of decision-making tools (Deuter and Liu 1999; Heisswolf *et al.* 1997; Zalucki and Liu 2003). Insect identification workshops were held for growers and field days were organized for growers to view the field trials and discuss the implications for improving pest management on their farms. Many growers started to appreciate the principles of IPM and recognized the potential impact of natural enemies and the capacity of crops to tolerate some damage particularly at the pre-heading stage. Seeing the benefits of IPM and the value of information exchange between growers and extension and research scientists, about 30 growers in the Lockyer Valley formed the Brassica Improvement Group in February 1998. This group met once a month during the growing season each year to share and exchange information with researchers, industry and other growers. These research and extension activities promoted the acceptance of IPM concepts and more and more growers gradually shifted from reliance on regular sprays of broad spectrum chemical insecticides to a reasonably integrated strategy, which included a combination of a summer production break, regular crop scouting, threshold-based decision making, strategic application of selective insecticides, and conservation of natural enemies (Deuter and Liu 1999; Furlong *et al.* 2004a; Zalucki and Liu 2003). One of the key elements in the IPM systems is always to start a growing season with a “soft approach”, that is to spray a selective insecticide only if needed, to ensure conservation of natural enemies and to aid the promotion of their activities later in the season (Niemeyer 2004).

ON-FARM EVALUATION OF THE IMPACT OF NATURAL ENEMIES ON THE SUCCESS OF IPM

Adoption of IPM programs is usually gradual and slow (Trumble 1998), and the brassica IPM program in the Lockyer Valley has not been an exception. Despite the intensive development and implementation effort and wide support from the industry, growers varied in their perception and approaches to the alternative pest management strategies. By 2000, a wide spectrum of pest management practices, ranging from the conventional calendar sprays to reasonably sophisticated approaches, was observed on different farms (Furlong *et al.* 2004a). As many farms in the valley grow a comparable range of vegetable crops and the general features of the ecosystem (climate, soil type and non-crop vegetation) are similar throughout the area, the wide spectrum of pest management strategies on different farms offered a unique opportunity to measure the effect of pest management practices on pest and natural enemy populations and crop production at the farm level. Mechanical exclusion with cages and life table analysis were used as the major techniques in this on-farm evaluation study, and the major pest DBM was used as the target pest (Furlong *et al.* 2004a,b).

This on-farm experimental study was conducted on 10 independent farms between 2000 and 2002. Individual farms, each of an area of 45-80 ha, were assessed and the management practices (production breaks, conservation of natural enemies, regular crop scouting, threshold based decision making, use of broad-spectrum insecticides, number of insecticide applications per crop, and tank mixes of insecticides) were scored and summed to produce a management index (Furlong *et al.* 2004a). For example, as regards threshold based decision making, a farm scored -2 for no action or +3 if decisions were based on the population density of pests as well as parasitoids. Farms with an overall score of >5.2 were categorized as IPM, farms with a score <0 as conventional practices, and those in between as intermediate. Each farm operated independently and thus formed a somewhat independent crop ecosystem. Such an approach allowed the long-term management practices to be included as a single variable in the analysis, and the effects of adopting different strategies on the efficacy of natural enemies could be evaluated (Furlong *et al.* 2004a).

During the study, three species of larval parasitoids *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae), *Apanteles ippeus* Nixon (Hymenoptera: Braconidae) and *O. sokolowskii* and two species of pupal parasitoids *D. collaris* and *Brachymeria phya* Walker (Hymenoptera: Chalcididae) attacked immature DBM. *Diadegma semiclausum* was the only parasitoid abundant over the course of the study (Furlong *et al.* 2004a; also see Wang *et al.* 2004).The most abundant groups of predatory arthropods caught in pitfall traps were Araneae (Lycosidae) > Coleoptera (Carabidae, Coccinellidae, Staphylinidae) > Neuroptera (Chrysopidae) > Formicidae. On crop foliage, Araneae (Clubionidae, Oxyopidae) > Coleoptera (Coccinellidae) > Neuroptera (Chrysopidae) were most common. The abundance and diversity of natural enemies was greatest at sites that adopted IPM, correlating with greater DBM mortality at these sites. Over the course of the study, the mean mortality of immature DBM caused by the natural enemy complex was 73% of the original test cohorts at IPM sites but only 20% of the original cohort at conventionally managed sites (Fig. 2). At IPM sites the contribution of natural enemies to pest mortality permitted the cultivation of marketable crops with no yield loss (Fig. 3) and a substantial reduction in insecticide inputs.

On average the number of sprays per crop was 8.6 on conventionally managed farms and 2.3 on IPM farms, an impressive more than three fold difference. Furthermore, these 2.3 sprays on IPM farms were almost all Bt formulations or selective insecticides (Furlong *et al.* 2004a).

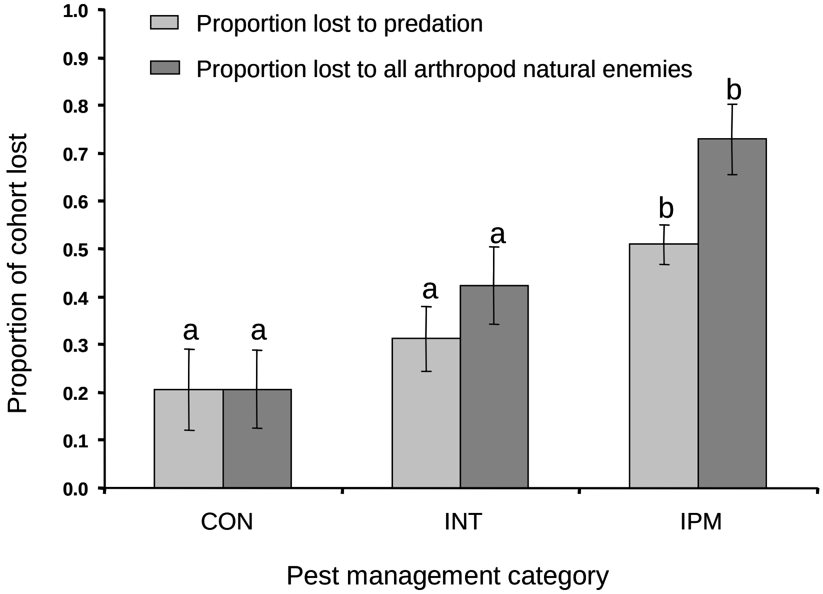

Figure 2. Estimated mean proportion (± SE) of original *Plutella xylostella* cohorts lost to predation and lost to the combined effects of the endemic arthropod natural enemy complex at sites practicing conventional (CON), integrated (IPM), and intermediate (INT) approaches to pest management (2000-2002) in the Lockyer valley, southeast Queensland, Australia. Columns of the same color marked by different letters are significantly different (LSD; $P < 0.05$) (adopted from Furlong *et al.* 2004a).

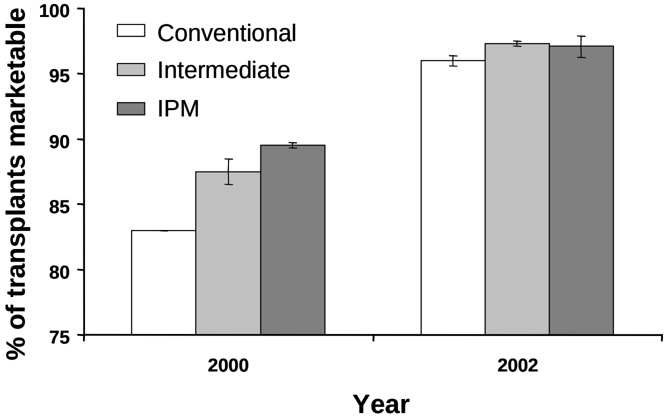

Figure 3. Cabbage yield at sites practicing conventional (CON), integrated (IPM) and intermediate (INT) approaches to pest management in 2000 and 2001 in the Lockyer valley, southeast Queensland, Australia (adopted from Furlong *et al.* 2004a).

DISCUSSION AND CONCLUSIONS

The history of pest control in the last century has repeatedly shown that sustainable pest management can only be achieved by utilizing endemic biological agents as part of a total ecosystem approach to crop management (Lewis *et al.* 1997). Many modern agricultural practices, which often reduce the ecological complexity of habitats and rely extensively on chemical pesticides, require revision. In this international cooperative project on brassica IPM, joint efforts were made to carry out research, development and implementation in two regions in Australia and China. While the brassica crops in the two regions share some of the same major pests, the crop ecosystems differ in many ways (see Introduction), and on-farm evaluation of the impact of natural enemies required different experimental setups in the two countries.

In Zhejiang and Shanghai, China, field trials were conducted for single seasons on a rather small scale, using plots within the same field, although extensive effort was made to repeat the same trials in different locations and years (Lin *et al.* 2002; Liu *et al.* 2004; Yu *et al.* 2002; Zhang *et al.* 1999). In such circumstances movement of natural enemies between treatments can confound results and the effectiveness of the natural enemy complex at the important agro-ecosystem level cannot be addressed. In the Lockyer Valley, brassica crops are grown on relatively large (50-100 ha) independent farms responsible for making their own pest management decisions. As the continuum of pest management practices included in the field study evolved over a course of approximately 10 years, the comparative experimental analysis between farms reflected the outcomes from different pest management strategies at the realistic crop ecosystem level over time (Furlong *et al.* 2004a; Heisswolf *et al.* 1997). Despite the differences in crop ecosystems between the two regions and the differences in experimental methods, the results indicate that in both regions naturally occurring biological control can be substantially enhanced to form the central elements of effective IPM programs and improve vegetable production.

One of the major features of this cooperative project has been the frequent interchange of visiting studies by both sides and frequent exchange of information. Experimental results and information on recent developments in IPM implementation in both regions were delivered to all team members through annual reports and project review meetings. Effort was made to convey the information to the growers in various extension activities (Deuter and Liu 1999; Zalucki and Liu 2003). Data on the effectiveness of naturally occurring biological control, as affected by pest management practices, in both geographic regions helped the extension scientists and growers to build up their confidence for a shift from chemical control to an IPM strategy. There is ample evidence that the improvement in pest management achieved through this joint project has promoted the sustainability of the brassica industry in the two regions of China and Australia (Zalucki and Liu 2003).

ACKNOWLEDGEMENTS

This work has been supported by the Australian Centre for International Agricultural Research (Projects: CS2/1992/013, CS2/1998/089), and the Department of Science and Technology, Zhejiang Province, China (Projects: 959218, 001106124). About 20 scientists from

China and 10 scientists from Australia contributed substantially to the achievements and many others participated in activities at various stages. Their names are not listed here because of space.

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BIOLOGICAL CONTROL OF FRUIT PIERCING MOTH (*EUDOCIMA FULLONIA* [CLERCK]) (LEPIDOPTERA: NOCTUIDAE) IN THE PACIFIC: EXPLORATION, SPECIFICITY, AND EVALUATION OF PARASITOIDS

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ABSTRACT

Adult fruit piercing moths (Noctuidae) are common pests of ripening fruit over much of tropical and subtropical Southeast Asia, Australia, and the western Pacific islands. *Eudocima fullonia* (Clerck), a target for classical biological control, occurs in that region including Papua New Guinea where it is not a pest and where it is thought to be controlled by natural enemies. Surveys conducted in Papua New Guinea revealed that two abundant egg parasitoids, *Telenomus lucullus* (Nixon) and *Ooencyrtus* sp. (*Papilionis*, species- group, Encyrtidae) were contributing up to 95% mortality of moth eggs. The host specificity of both parasitoids was studied in the laboratory by exposing them to eggs of related Noctuidae. *T. lucullus* was found to be specific to *Eudocima* spp. in the laboratory but *Ooencyrtus* sp. oviposited and developed on several non-target noctuid species in the presence of the moth host's food plants. *T. lucullus* and *Ooencyrtus* sp. were assessed as adequately host specific for release in Samoa, Tonga, Fiji and the Cook islands. However, the parasioids were not assessed with the non-target *E. iridescens* (T.P. Lucas), a rare species from northern Australia unavailable for testing. The two egg parasitoids were released on Samoa, Tonga, Fiji, and the Cook Islands but were not released in Australia due to the inability to demonstrate adequate host specificity. *T. lucullus* and *Ooencyrtus* sp. both became established in Tonga and Fiji but only *T. lucullus* became established in Samoa and the Cook islands. After establishment of parasitoids increased levels of egg parasitism and declines in the abundance of target eggs occurred in Samoa and Tonga, and decreases in the abundance of the moths and its damage to fruit were observed in Fiji and Cook Islands. The methods for conducting surveys, host specificity testing and field evaluations are described.

INTRODUCTION

Fruit piercing moths (*Eudocima* spp. [= *Othreis* spp.], Noctuidae: Catocalinae) are serious pests of ripe and ripening fruit in many subtropical and tropical countries including parts of

Africa, Southeast Asia and western Pacific countries (Waterhouse and Norris 1987). The most widespread pest species, *Eudocima fullonia* (Clerck) occurs in Australia and western Pacific countries (Waterhouse 1997) including New Caledonia (Cochereau 1977). Although cosmopolitan in the Pacific, Waterhouse and Norris (1987) suggested that the Indo-Malaysian region is the most likely area of origin of *E. fullonia*.

Both sexes of adult fruit piercing moths puncture fruit with their long, stout proboscis which is adapted to penetrate the rind of firm, intact fruit allowing moths to feed on fruit juice and pulp. Secondary invasions by micro-organisms spread into damaged tissues causing rot and premature fruit-fall (Sands *et al*. 1993). There are two different biotypes of *E. fullonia*. In Papua New Guinea (PNG) and on most Pacific islands, larvae of *E. fullonia* feed on several *Erythrina* spp. (Fabaceae) as well as vines of the family Menispermaceae, whereas in Australia, Southeast Asia, and Africa, the larvae feed only on Menispermaceae (Sands and Chan 1996; Sands & Schotz 1991).

In eastern Australia the moths migrate annually in warmer months from the tropics, to temporarily colonise the temperate regions (Sands *et al*. 1991) and their abundance varies from year to year (Mosse-Robinson 1968) with climatic variation. In New Caledonia, outbreaks mainly follow prolonged periods of drought (Cochereau 1977). In western Pacific countries, including New Caledonia, indigenous natural enemies do not prevent the build up of moth numbers that invade orchards and cause serious damage (Cochereau 1977). However, *E. fullonia* is not abundant or a pest in Papua New Guinea, where its abundance is thought to be reduced by parasitoids (Sands and Broe 1991).

In early attempts to control *E. fullonia*, a larval parasitoid *Winthemia caledoniae* Mesnil (Diptera: Tachinidae) from New Caledonia, (Cochereau 1977) was relocated within the region but it failed to become established (Kumar and Lal 1983; Waterhouse and Norris 1987). Very few other parasitoids of larvae of *Eudocima* spp. are known. However, *Euplectrus maternus* Bhatnagar from India and *E. melanocephalus* Girault from northeastern Australia have been considered to be potential biological control agents (Jones and Sands 1999).

Two egg parasitoids from PNG, *Telenomus lucullus* Nixon (Hymenoptera: Scelionidae) (= *Telenomus* sp., LPL 530 in Sands *et al*. 1993) and an *Ooencyrtus* sp. (Hymenoptera: Encyrtidae) (*papilionis* Ashmead, species-group), were recently introduced into the western Pacific (Sands and Liebregts 1992; Sands *et al*. 1993) in attempts at biological control of *E. fullonia*. The exploration, evaluation, and the release of these egg parasitoids, the introduction into Tonga of another egg parasitoid, *O. crassulus* from Samoa, and the reasons for not releasing egg parasitoids from Papua New Guinea in Australia, are discussed. Preliminary evaluation of *E. melanocephalus* from Australia, as a possible agent for the Pacific islands is also discussed.

MATERIALS AND METHODS

Exploration for parasitoids in Papua New Guinea. Surveys for parasitoids of *E. fullonia* were conducted in Papua New Guinea (PNG) in 1987 and 1988, at the edge of coastal rainforests and on roadside vegetation near Madang, northern PNG, near Vudal, New Britain, at Tep Tep in the Finisterre Ranges (alt. 2000 m), and at the edge of mesophyll vine thickets near Port

Moresby, southern PNG. In a search for any alternative hosts of *Ooencyrtus* sp. or *Telenomus lucullus* , eggs of Noctuidae (other than *E. fullonia*) were collected opportunistically near Madang, PNG and incubated in the laboratory until egg parasitoids emerged.

The host plants of *E. fullonia* were examined and any immature stages located were returned to the laboratory for rearing. Immature stages of the moth from individual eggs and egg masses deposited on leaves of the food plant, *E. variegata* var. *orientalis* L., and occasionally from vines (Menispermaceae) were collected from localities close to sea level, whereas at a high altitude (2,000 m) locality, Tep Tep, Morobe Province, stages of *E. fullonia* were collected from the menisperm vine, *Stephania japonica*.

Leaf portions of *E. variegata* or menisperm vines with single eggs and egg masses were excised and incubated in ventilated plastic containers for up to 28 days until parasitoids or larvae eclosed. Parasitoids that emerged were maintained by feeding with honey droplets smeared on wax paper. Moth larvae were provided with fresh leaves of appropriate food plants until they appeared to be parasitised, or if they pupated, until moths or parasitoids eclosed, or unparasitised pupae died. Percent parasitism of each host stage was calculated for each field locality and food plant based on the numbers of immature stages that developed fully, died or produced parasitoids. Parasitised larvae of *O. fullonia* were occasionally recovered from food plants in rainforest in PNG but none were successfully reared or positively identified. These parasitoids were thought to be a *Euplectrus* sp. (Eulophidae) (Sands unpublished).

TESTING THE HOST SPECIFICITY OF PARASITOIDS

Cultures of egg parasitoids *T. lucullus* and *Ooencyrtus* sp. (*papilionis* species-group) were established in the laboratory in Madang, PNG to provide material suitable for consignment to Australia. Parasitoids were reared in PNG through one generation using moth eggs obtained from a caged culture of *E. fullonia*. Parasitised eggs of *E. fullonia* were then separated from leaf substrates for subsequent packaging and consignment to Australia. All host specificity tests were conducted in a quarantine facility in Brisbane, Australia, where cultures of both PNG egg parasitoids were established using eggs of *E. fullonia* (Australian biotype) as hosts. Parasitoids were reared and tested in large (14 x 3 cm) ventilated plastic tubes containing a card smeared with honey as food.

Representatives of non-target, indigenous Australian Noctuidae were exposed to parasitoids for specificity tests. They were selected for testing on the basis of their taxonomic relatedness to the target genus, *Eudocima* (Noctuidae: Catocalinae), their known life histories, and the availability and practicability of obtaining fertile eggs or larvae. To obtain eggs of all species, gravid moths were held in cages and induced to oviposit on organza using the method described by Sands and Schotz (1991).

In a first group consisting of other *Eudocima* spp., eggs of *E. salaminia* (Cramer), *E. materna* (Linn.), *E. aurantia* (Moore), *E. iridescens* (T.P. Lucas) and *E. cocalis* (Cramer) were nominated for exposure to *T. lucullus* and *Ooencyrtus* sp. In a second group, eggs of less closely-related Catocalinae, species of *Ophiusa* spp., *Dasypodia* spp., *Achaea* sp., *Phyllodes imperialis* , *Donuca* sp., *Erebus terminitincta* (Gaede) and an *Anomis* sp. were tested. Immature stages of two species *Helicoverpa armigera* (Hübner)(Heliothinae) and *Spodoptera litura*

(Fab.)(Acronictinae), representating other subfamilies as their life histories well known. Cultures of these were obtained from the University of Queensland, Brisbane.

The host specificities of *Ooencyrtus* sp. and *T. lucullus* originally from PNG, were evaluated for their suitability for introduction into western Pacific islands and mainland Australia. The host specificity of the Australian *E. melanocephalus* was determined as preliminary for its proposed introduction into Fiji and Samoa, countries where the temperature and humidity were predicted to be most favourable (Jones and Sands 1999).

Egg parasitoids from PNG were tested for their host specificity by exposing to eggs of selected non-target species attached to gauze: (i) without plant material and (ii) with leaf portions of plant hosts of *E. fullonia* (*S. japonica* and *E. variegata*) to test for any different (tritrophic) responses to the eggs (Table 1).

INDIGENOUS NATURAL ENEMIES IN AUSTRALIA AND THE PACIFIC

Prior to introducing an exotic agent, the indigenous natural enemies were surveyed in each proposed receiving country, to: (i) ensure that the agent species was not already present, (ii) identify indigenous natural enemies and distinguish them from the proposed agent, and (iii) quantify impacts by each indigenous species on the target host. Information from the literature (e.g., Sands *et al*. 1993; Waterhouse and Norris 1987) and a co-ordinated program focussed on indigenous and introduced parasitoids (Table 2) of *E. fullonia* in the western Pacific.

The most abundant indigenous parasitoids of eggs that needed to be distinguished from species proposed for introduction from PNG included: *O. crassulus* Prinsloo and Annecke (Hymenoptera: Encyrtidae) and *Trichogramma* spp. in Samoa; *O. cochereaui* Prinsloo and Annecke, *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) and *Telenomus* sp. (Hymenoptera: Scelionidae) in New Caledonia (Cochereau 1977; Maddison 1982).

The impact on eggs by an important predator of eggs, *Germalus samoanus* China (Hemiptera: Lygaeidae), was quantified during the assessment of egg parasitism in Samoa.

Specimens of parasitoids reared from *E. fullonia* were retained in the Australian National Insect Collection, Canberra and others were submitted to the Natural History Museum, London for identification.

LARVAL PARASITOIDS

On the Pacific islands very low levels of parasitism were recorded from larvae during the reported study. In Australia, egg and larval parasitoids (Huber 1999) were reared from immature stages of *Eudocima* spp.. *Euplectrus melanocephalus* Girault and an unidentified *Euplectrus* sp. were identified as larval parasitoids from northeastern Queensland, but they were only abundant during the warm, humid months each year (Huber 1999). Parasitised larvae of *Eudocima* spp., mostly instars 1 and 2, were collected from menisperm vines near Cairns, northern Queensland. Using methods described by Jones and Sands (1999) they were maintained with leaves of the food plant until they pupated, died, or parasitoids developed. The suitability of *E. melanocephalus* as a biological control agent was evaluated in a secure facility in Brisbane. The effects of temperatures on immature development times were

Table 1. Host specificity tests: parasitoids of *E. fullonia* exposed to eggs of Noctuidae.

Parasitoid	Host[a]/Non-target Host[b]	Stage of Host	Pars. Oviposition	Pars. development
E. melanocephalus	*E. fullonia*	2nd, 3rd inst. larva	+	+
"	*E. materna*	2nd, 3rd inst. larva	+	+
"	*E. salaminia*	2nd, 3rd inst. larva	+	+
"	*E. aurantia*	2nd, 3rd inst. larva	+	+
"	*Erebus terminitincta*	2nd, 3rd inst. larva	-	-
"	*Spodoptera litura*	2nd, 3rd inst. larva	-	-
Ooencyrtus sp.	*E. fullonia*	egg	+	+
"	*E. materna*	"	+	+
"	*E. salaminia*	"	+	+
"	*E. aurantia*	"	+	+
"	*Erebus terminitincta*	"	+/- *	+/- *
"	*Dasypodia* spp.	"	+/- *	+/- *
"	*Phyllodes imperialis*	"	+/- *	+/- *
"	*Ophiusa* sp.	"	+/- *	+/- *
"	*Achaea* sp.	"	+/- *	+/- *
"	*Donuca* sp.	"	+/- *	+/- *
"	*Spodoptera litura*	"	+/- *	+/- *
"	*Helicoverpa armigera*	"	+/- *	+/- *
Telenomus lucullus	*E. fullonia*	"	+	+
"	*E. materna*	"	+	+
"	*E. salaminia*	"	+	+
"	*E. aurantia*	"	+	+
"	*Erebus terminitincta*	"	-	-
"	*Dasypodia* spp.	"	-	-
"	*Phyllodes imperialis*	"	-	-
"	*Ophiusa* sp.	"	-	-
"	*Achaea* sp.	"	-	-
"	*Donuca* sp.	"	-	-
"	*Spodoptera litura*	"	-	-
"	*Helicoverpa armigera*	"	-	-

*oviposition and development only in presence of *Erythina variegata* and *Stephania japonica*.

Table 2. Introductions of parasitoids for biological control of the fruit piercing moth, *Eudocima fullonia*.

Natural Enemy	Country of Origin	Country Released	Host Stage	Established	Reference
Winthemia caledoniae Mesnil	New Caledonia	Fiji (1983-84)	larva	-	Kumar and Lal 1983
Winthemia caledoniae Mesnil	New Caledonia	Tonga (1979)	larva	-	Waterhouse and Norris 1987
Ooencyrtus cochereaui	New Caledonia	American Samoa	egg	-	"
Ooencyrtus cochereaui	New Caledonia	Samoa	egg	-	"
Ooencyrtus crassulus	Samoa	Tonga (1992)	egg	+	Sands and Liebregts 1992
Ooencyrtus sp. (*papilionis* group)	Papua New Guinea	Samoa (1989)	egg	-	Sands *et al.* 1993
Ooencyrtus sp. (*papilionis* group)	Papua New Guinea	Fiji (1992)	egg	+	Sands *et al.* 1993
Ooencyrtus sp. (*papilionis* group)	Papua New Guinea	Tonga (1992)	egg	+	Sands 1996
Ooencyrtus sp. (*papilionis* group)	Papua New Guinea	Cook Islands (1996)	egg	+	Sands and Liebregts unpubl.
Telenomus lucullus	Papua New Guinea	Samoa (1989)	egg	+	Sands and Liebregts 1992
Telenomus lucullus	Papua New Guinea	Fiji (1992)	egg	+	Sands *et al.* 1993
Telenomus lucullus	Papua New Guinea	Tonga (1993)	egg	+	Sands 1996
Telenomus lucullus	Papua New Guinea	Cook Islands (1996)	egg	+	Sands and Liebregts unpubl.

determined to predict its adaptability to the tropical environments of the Pacific islands (Jones and Sands 1999). The suitability of the unidentified *Euplectrus* sp. was not evaluated.

MEASURING ABUNDANCE AND PARASITISM OF EGGS OF *E. FULLONIA*

To monitor the abundance of moth stages some variation in methodology was applied in each country, where the immature stages, mostly eggs of *E. fullonia* on the host plant *Erythrina* spp., were sampled monthly for more than 12 months, before and after release of PNG parasitoids in Samoa, Fiji, and Tonga.

Eggs and egg masses on leaves of the food plant (mostly *E. variegata* var. *orientalis* (L.) Merrill, but also *E. subumbrans* (Hask.) in Fiji and Samoa) were collected to calculate percent parasitism by indigenous egg parasitoids (before release of PNG parasitoids). Only one pre-release survey for parasitoids was carried out in Rarotonga, Cook Islands.

Low trees of *Erythrina* spp. on properties, road boundaries or fence posts were selected for sampling sites when supporting the immature stages of *O. fullonia*. After each sampling event, trees were pruned to approximately 3 m to encourage lateral and terminal growth suitable for re-sampling. From each site each month, 100 terminal or lateral stems with leaves attached were cut from each of 20 *Erythrina* plants. Leaves were removed from terminals and all attached eggs and egg masses containing living stages (moth embryo or parasitoid) were recorded, returned to the laboratory and incubated in vials until moth larvae or parasitoids emerged. If a minimum of 30 eggs or masses was not recovered each month additional leaves were collected until 30 eggs or egg masses were retrieved. From the eggs recovered, egg abundance, egg mortality and identity of the egg parasitoids were recorded. Percent parasitism of single eggs and egg masses were calculated separately.

In the receiving countries for the egg parasitoids, *Ooencyrtus* sp. and *T. lucullus,* methods for post-release studies on eggs of *E. fullonia* were based on those to monitor pre-release parasitisation and egg abundance. The appearance of parasitised and post-parasitised stages allowed estimates to be made of parasitism in the field and were applied to the sampling methods. For example, eggs parasitised by *T. lucullus* were identifiable by markings on the chorion of eggs, and *Ooencyrtus* sp. and *Trichogramma* spp. were identified by the colour of the egg, eggshell and meconium. The abundance of eggs, levels of parasitisation by indigenous parasitoids and the release dates in each country for *Ooencyrtus* sp. and *T. lucullus* were recorded as follows:

Samoa. Single eggs as well as egg masses were abundant. An indigenous *Trichogramma* sp. ranged in abundance from 4-16% of host eggs parasitised and eggs parasitised by *O. crassulus* averaged 28-35% on the islands of Savai'i and Upolu. The PNG *Ooencyrtus* sp. and *T. lucullus* were released on both islands in 1988.

Tonga. Single eggs were abundant and egg masses uncommon. An indigenous *Trichogramma* sp. varied greatly in abundance from 6-85% of eggs parasitised on Tongatapu island and from 0-53% on the island Eua. An indigenous *Telenomus* sp. was uncommon with parasitism ranging from 0-5% on Tongatapu. The Samoan egg parasitoid *O. crassulus*: was released on Tongatapu between December 1992 and June 1993, and on Eua in November 1993. The PNG *Ooencyrtus* sp. was released in August 1992 on Tongatapu and *T. lucullus* on Tongatapu and on Eua in November 1993.

Fiji. Single eggs were abundant and egg masses uncommon. *Trichogramma* sp. parasitised 2-16% of eggs and a rare indigenous *Telenomus* sp. parasitised less than 2% of eggs. The PNG *Ooencyrtus* sp. was released in October 1990 on the island Viti Levu and *T. lucullus* on Vanua Levu and Viti Levu islands in October 1993.

Roratonga, Cook Islands. Single eggs predominated over egg masses. *Trichogramma* sp. and an indigenous *Telenomus* sp. together parasitised less than 2% of eggs. The PNG *Ooencyrtus* sp. and *T. lucullus* were released in October 1996.

RESULTS

CLIMATIC SUITABILITY OF PARASITOIDS

The PNG egg parasitoids, *Ooencyrtus* sp. and *T. lucullus*, were confirmed to be well suited to tropical climates, and less suited to sub-tropical or temperate climates of the receiving countries. After they were released *Ooencyrtus* sp. and *T. lucullus* were recovered from the receiving islands, except from Samoa where only *T. lucullus* became established, and Cook Islands where only *Ooencyrtus* sp. became established. Although predicted to be suitable for release in most Pacific inland countries (Jones and Sands 1999), based on climatic and host range suitability, the Australian larval parasitoid *E. melanocephalus* was not released due to the lack of opportunity to culture it and monitor its establishment.

HOST SPECIFICITY TESTS WITH NON-TARGET NOCTUIDAE

In PNG, *Ooencyrtus* sp. or *T. lucullus* was reared only from field-collected eggs of *Eudocima* spp., and on no occasions were they recovered from eggs (35 spp. mostly unidentified) of non-target Noctuidae. Several parasitoids of the same genera emerged but their specific identities were not determined.

After the PNG parasitoids became established in Fiji, eggs of other Noctuidae and some unrelated moths with eggs of similar size to the target, *E. fullonia*, were sampled close to release sites in an attempt to find any evidence of attack on non-target species. In the Pacific, there was no evidence (monitoring discontinued in 1997) from samples of Noctuidae eggs, that *Ooencyrtus* sp. and *T. lucullus* had crossed over to attack eggs of any non-target moth species. On several occasions a similar *Telenomus* spp. were recovered from eggs including a hawk moth (probably *Agrius* sp.) but the parasitoid proved to be a species different to *T. lucullus* (W. Liebregts unpubl.).

In Australia, *Eudocima* spp. available for testing in the laboratory were confirmed suitable hosts for the complete development of the PNG egg parasitoids *Ooencyrtus* sp., *T. lucullus* and the Australian *E. melanocephalus* (Jones and Sands 1999). Eggs of other related moths (Catocalinae) failed to support complete development of the parasitoids. However, when testing eggs of Noctuidae in the presence of leaves of the hosts (*Erythrina variegata, Stephania japonica*) of *E. fullonia*, *Ooencyrtus* sp. (but not *T. lucullus*), oviposited in the eggs of all non-target species and some, or complete parasitoid development occurred. When eggs of the same Noctuidae attached to gauze, without leaves were exposed to *Ooencyrtus* sp., no non-target species attracted oviposition by this parasitoid.

The inability to obtain immature stages of the rare *E. iridescens* for testing, a species closely-related to the target pest species, influenced the decision not to release the PNG egg parasitoids *Ooencyrtus* sp., *T. lucullus* in Australia.

RELEASE AND ESTABLISHMENT OF EGG PARASITOIDS

Samoa. *Ooencyrtus* sp. from PNG failed to become established in Samoa. *T. lucullus* released at the same time, became established and was first recovered in Samoa in October 1988. After the establishment of *T. lucullus* on Savai'i, total egg parasitism of *E. fullonia* increased from 62% to 79% for single eggs, and from 56% to 80% of egg masses.

Tonga. *O. crassulus* became established on Tongatapu and was recovered in October 1993 and December 1994. *Ooencyrtus* sp. was recovered on the same island from 1993 with egg parasitism reaching an average of 30% in 1996. *T. lucullus* was recovered on Tongatapu in 1994 where total egg parasitism increased from 19% to 27% in 1996. On Eua total egg parasitism increased from 22% in 1994 to 69% in 1996 after release of *T. lucullus*.

Fiji. *Ooencyrtus* sp. was recovered on the island Vanua Levu from September 1992 and *T. lucullus* was recovered from both islands in October and November 1993. Quantitative data on egg parasitism after parasitoids became established were not available.

Roratonga, Cook Islands. The PNG *Ooencyrtus* sp. and *T. lucullus* were released in October 1996. Only *Ooencyrtus* sp. was recovered in April 1997. In the Cook Islands quantitative data were not collected and sampling was discontinued after establishment of the parasitoids was confirmed.

DISCUSSION

The procedure for testing exotic parasitoids with non-target species highlighted some of the difficulties in obtaining the appropriate stages of species for testing and the need to avoid testing non-target species in the presence of the certain plants to avoid 'false positive' results (Sands and Van Driesche 2000). In this example, the parasitoid *Ooencyrtus* sp. oviposited in eggs of a range of non-target hosts when portions of the food plants of *E. fullonia* were present but did not do so when the plant material was withheld. Identified also were the difficulties of making decisions about whether or not, to release an agent, when these anomalous results are obviously obtained and when the risks of releasing an agent could potentially affect a rare species closely related to the target, when it could not be obtained for testing.

Although field data show increases in total parasitism of eggs of *E. fullonia*, and decreases in the 'hatch' (moth larvae) of eggs in all countries wherever *Ooencyrtus* sp. and *T. lucullus* became established, the resulting declines in adult moth density were not easily demonstrated. However, levels of damage to fruit were reported to have decreased in all countries. For example, in Fiji in1997 levels of damage to oranges and mangoes were noted by orchard managers and agricultural research staff, to have decreased when compared with earlier years. Damage to fruit was lower since monitoring began in the early 1990's, 5 years after the parasitoids had become established (S. Lal pers. comm.). In Samoa a decline in damage to firm fruit (e.g., citrus), but not soft fruit (e.g., carambola) was noted in 1997 (unpublished data). In Rarotonga, Cook Islands, a marked decrease in moth abundance occurred after egg parasitoids had become established (M. Poschko pers. com.). Clearly more attempts are needed to quantify levels of parasitism to eggs of *E. fullonia* and damage to fruit, to determine if the introduced egg parasitoids have had a permanent beneficial impact on horticultural production in those countries.

ACKNOWLEDGEMENTS

We thank Sada Lal, Maja , Pila Kami, Martina Schotz, Rose Broe and Veronica Brancatini for their contributions to this project. The project was supported by Australian Centre for International Agricultural Research.

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IMPACT OF EDUCATING FARMERS ABOUT BIOLOGICAL CONTROL IN FARMER FIELD SCHOOLS

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ABSTRACT

An Integrated Pest Management (IPM) Farmer Field School (FFS) is a field-based activity that lasts a full cropping season. A typical FFS meets once a week and 25-30 farmers participate in an FFS. In addition to group dynamics activity and a special topic session, an FFS emphasizes scientific learning through experimentation. Biological control is discussed and confirmed by carrying out insect zoo studies. A common insect zoo in rice IPM FFS involves placing a lycosid spider among field collected Brown Plant Hopper (BPH) in a clear bottle. Seeing is believing and perhaps for the first time, the concept of biological control is understood by resource-poor farmers. This process of educating farmers about biological control can be further enhanced by simple “exclusion cage” experiments.

Learning about biological control was also achieved in Dalat, Vietnam, leading to an understanding of the selective action of the microbial insecticide *Bacillus thuringiensis* in managing the diamondback moth on cabbage. With the information from experiments and pilot studies, farmers were able to organize village-wide activities to conserve the population of *Diadegma semiclausum* (Hellen), a key larval parasitoid of *Plutella xylostella* (L.).

Similar impacts were observed in cotton fields in China, India and Pakistan where populations of pests were kept in check when FFS graduates did not carry out early sprays. Preliminary studies indicated that the species diversity was higher in IPM plots as compared with plots regularly treated with insecticides. In India, the number of species was 48 in IPM plots and 31 in non-IPM plots. For Bangladesh, the study showed 49 species in IPM plots compared with 36 species in non-IPM plots. Combined with an increase in biological control knowledge (FFS farmers scoring 16.9 points for recognizing natural enemies as compared to 2.3 for non-IPM), there is a concomitant reduction in use of insecticides (43% for IPM farmers versus 34% for non-IPM). With the skills acquired at FFS, farmers have increased incomes, as farmer education activities help consolidate the impact of biological control in farmers’ pest management decision making (34% increase in FFS farmers as compared to 10% for non-FFS farmers).

From experiences in implementing IPM FFS in rice, vegetables and cotton in Asia, it was evident that educating farmers about biological control result in farmers using less chemical insecticides and becoming more efficient in their production activities. Even in a crop that is not subjected to regular use of insecticides, such as coconut, teaching biological control is just as critical to farmers to keep pests in check. For example, teaching farmers about how baculovirus and *Metarrhizium anisopliae* kill the rhinoceros beetle, *Oryctes rhinoceros* (L.), help them better comprehend the nature of rhinoceros beetle damage and encourage them to reduce breeding sites for the pest. Outbreaks of invasive pest species, such as *Brontispa longissima* (Gestro) on coconut, provide additional opportunities to use the Farmer Field School to educate coconut farmers about biological control.

INTRODUCTION

Biological control usually refers to "the action of parasitoids (parasites in the original definition), predators and pathogens in maintaining another organism's density at a lower average than would occur in their absence" (DeBach 1964). Implicit in this definition is the desire to understand how these parasitoids, predators and pathogens act on their prey. However, in the last century, this empirical approach was replaced with a desire to discover effective parasitoids and predators for possible introduction into a new area where a pest has been transported. This approach is called classical biological control. It drew much support from successes with the cottony cushion scale in California (DeBach 1974) and control of the coconut leaf moth in Fiji (Tothill *et al*. 1930). Another field of interest in biological control is the rearing of millions of natural enemies for release into the field, often at regular intervals. This inundative biological control has been viewed as too expensive and probably unnecessary in a tropical setting.

Besides classical and inundative biological control, there is a rich and dynamic array of natural enemies that help keep pests in check under most agro-ecosystems in Asia. These have been reported by Kenmore *et al*. (1984), Ooi (1986), and Wood (1973). This paper will examine the education of farmers using the Integrated Pest Management (IPM) Farmer Field School (FFS) approach (Dilts and Hate 1996; Pontius *et al.* 2002). Lessons learnt in programmes involving rice, vegetables, cotton and coconut will be analysed to identify the impact of biological control education on implementation of IPM by farmers.

REVISITING FARMER FIELD SCHOOL

Over the last decade, the IPM Farmer Field School (FFS) has emerged as a robust approach to educate poor farmers to manage their crops more efficiently, not just in Asia but also in Africa and South America. The defining principles of an FFS have been clearly described by ter Weel and van der Wulp (1999) and Pontius *et al*. (2002). The FFS is the primary learning approach piloted in Indonesia (Dilts and Pontius 2000). An IPM Farmer Field School (FFS) is a field-based activity that lasts a full cropping season. A typical FFS meets once a week and 25-30 farmers participate in an FFS. In addition to group dynamics activity and a special topic session, an FFS emphasizes scientific learning through experimentation. In each FFS, field plots would be set up to compare an ecological approach versus an existing practice. Each week, farmers would observe both plots and collect data on plant development and population trends of insect pests and their natural enemies. The data collected enable farmers to learn and practice agro-ecosystem analysis and farmers will acquire the skills to make a "science-informed" decision. The process of agro-ecosystem analysis involves stepping into the field to collect data on crop growth and population of pests and natural enemies (Fig. 1). Upon returning to the meeting room, farmers learn to sort out the insects collected (Fig. 2).

Following discussion farmers present their results in an agro-ecosystem drawing (Fig. 3). The decisions made by the group are presented to the class and actively discussed and opportunities for follow up activities identified (Fig. 4). In this way, male and female farmers are introduced to the complex concept of biological control.

Discussions of conditions of the field plots are based upon farmers' analysis of field collected data. The Field School uses a participatory learning process. The process emphasises taking decisions and actions based on an open discussion of ideas which is free from the domination of any individual. These decisions are tested in the field laboratory. The FFS process, besides its emphasis on field ecology, provides participants with an opportunity to examine human social dynamics. As a result, FFS participants not only learn about the cause and effect relationships that exist in the field, they also acquire a greater understanding of human relationships.

The analytical processes employed in the FFS enhance farmers' capacities to examine the conditions, in which they live and work. Participants, having completed their FFS, are able to take decisions and take actions that would improve those conditions. The increased understanding of participants regarding human social dynamics enables them to develop collaborative efforts to ensure that planned actions are implemented.

Even after the FFS, farmers continued to experiment and in some cases worked in groups. For example, farmers in the village of Kalensari in Indonesia continued to study a non-pesticide method to control the white stemborer (Warsiyah *et al*. 1999). Examples of follow-up studies by farmers are reflected in Ooi (1998), Ooi (2000), Ooi *et al.* (2001) and van den Berg *et al*. (2004). This confirms that farmers are able to carry out experiments and develop innovative IPM (Chambers *et al*. 1989).

Figure 1. Collecting field data from a sub-plot in a rice field in Thailand.

Figure 2. Sorting out insects collected from the rice field.

Figure 3. Making an agro-ecosystem drawing.

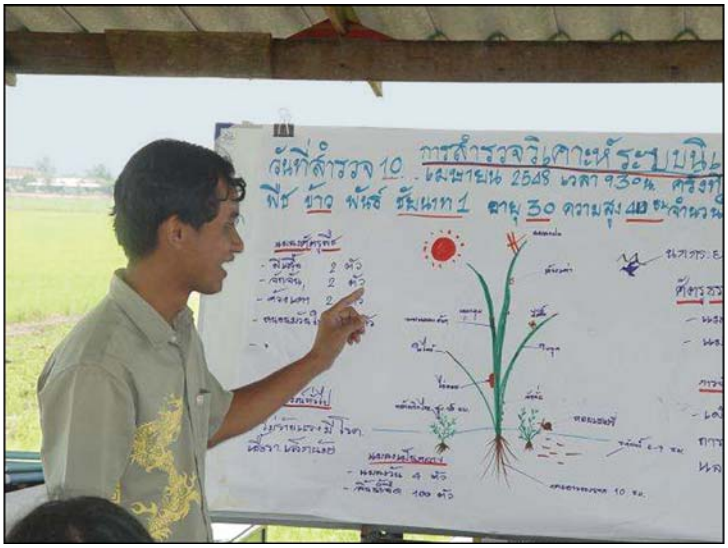

Figure 4. Using the agro-ecosystem drawing to explain the decisions arrived by the farmer group.

ROLE OF BIOLOGICAL CONTROL IN IPM

When severe outbreaks of leaf-eating caterpillars were reported in the late 1960s at the time when oil palm was encouraged in a diversification to rubber planting in Malaysia, Wood (1973) determined that these outbreaks followed the use of broad-spectrum insecticides. This was proven using an insecticide check technique in large blocks sprayed recurrently with dieldrin. Wood (1973) thus concluded that the bagworm, *Metisa plana* Walker (Lepidoptera: Psychidae) was made to increase by the destruction of its natural enemies. The successful identification of biological control as the main factor in keeping insect pests in check in oil palm led to management practices in most estates which avoided use of insecticides.

Field studies in the late 1970s and early 1980s showed that biological control is the core of IPM in an annual crop such as rice (Kenmore *et al.* 1984; Ooi, 1986). That biological control is central to the development of IPM in rice was shown from insecticide check experiments similar to the one conducted in oil palm. This suggested that regular use of insecticides can lead to pest outbreaks in rice in the tropics.

Recent literature reviews further confirmed the importance of indigenous natural enemies in rice in the tropics (Ooi and Shepard 1994; Shepard *et al.* 1987; Way and Heong 1994), but ironically, farmers did not know about the role of these natural enemies, particularly of predators in rice fields. Farmer education was important to help farmers understand the need to conserve these natural enemies (Kenmore 1996; Matteson *et al.* 1994).

Arguably, the introduction of *Diadegma semiclausum* (Hellen) (Hymenoptera: Ichneumonidae) and its successful establishment on cabbage with diamondback moth had demonstrated a positive impact in the cooler highlands of Indonesia (Sastrosiswojo and Sastrodihardjo 1986), Malaysia (Ooi 1992), Philippines (Poelking 1992; Ventura 1997), Vietnam (Ooi *et al.* 2001) and Taiwan (Talekar *et al.* 1992). In Dalat, Vietnam, in order to enhance the establishment of *D. semiclausum*, farmers in two communities carried out studies to better understand the role of Bt (Fig. 5) in conserving this parasitoid. Through this effort, the impact of *D. semiclausum* was realized within six months when it was not possible for the parasitoid to establish despite two years of continuous releases before the study (Ooi *et al.* 2001).

In cotton, as in rice, biological control has been shown to be fundamental in the development of IPM (Ooi *et al.* 2004). All the key pests in cotton in Asia are native and indeed have a wide range of parasitoids, predators and pathogens that help keep pest populations in check. Similarly, the focus of coconut IPM has been on the role of biological control in keeping key pests such as *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae) in check (APCC 2005). While the baculovirus and *Metarhizium anisopliae* occur naturally in its centre of origin in Southeast Asia, the rhinoceros beetle occasionally escape from natural biological control. This is often attributed to poor sanitation where organic materials that serve as breeding grounds for the beetle are carelessly left in the open. In this particular case, farmer education about the diseases that kill *O. rhinoceros* larvae, will convince coconut farmers to remove potential breeding materials of the beetle. Recent outbreaks of the coconut leaf beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae) in Asia and the successful biological control with *Asecodes hispinarum* Boucek (Hymenoptera: Eulophidae) offers yet another opportunity to educate farmers about biological control (FAO 2004).

Figure 5. Farmers carrying out bioassay of *Bt* using DBM larvae in Vietnam.

HOW TO TEACH FARMERS BIOLOGICAL CONTROL

Biological control, whether classical or conservation, relies on the recognition, understanding and appreciation of the action of natural enemies. Implicit in this statement is the necessity to educate farmers. The IPM-FFS has emerged as a means to educate farmers about the complex ecological principles in their agro-ecosystems. As biological control is the key component of IPM, it is natural that farmers are introduced to ecology through an understanding of biological control.

It has been argued that in classical biological control, there is no need to involve farmers. This may be true in the case of the coconut moth in Fiji (Tothill *et al.* 1930) where interventions by coconut farmers were not common. However, lessons learned in the biological control of the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), suggest that cabbage farmers should be made aware of and participate actively to enhance establishment and maintenance of the key parasitoid, *D. semiclausum*. (Ooi 1992).

That farmers can understand ecological concepts in general and biological control in particular have been reported (Bentley 1992; Ooi 1998). It is not surprising that using the FFS approach, farmers are introduced to biological control using the insect zoo approach. Often, the insect zoo is used to help farmers discover the predatory behaviour of natural enemies found in the field, thus helping them increase their understanding of ecological principles in their agro-ecosystem (Pontius *et al.* 2002). Questions about biological control events are discussed and confirmed by carrying out insect zoo studies. For example, in rice, the biological control of the brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is convincingly demonstrated by placing a lycosid spider among field collected BPH in a clear bottle (Fig. 6). Seeing is believing and perhaps for the first time, the concept of biological control is understood by resource-poor farmers.

Figure 6. *Lycosa pseudoannulata* feeding on brown plant hopper. UGA1390042

To prove that generalist predators are important, exclusion cage experiments are usually set up to demonstrate the importance of these predators in keeping the brown planthopper (BPH) population in check. In this experiment, several large cages are placed over young rice seedlings and over the week, the seedlings are cleaned of any arthropods. BPH adults are introduced and when the population of BPH are established, half of the cages are opened to allow general predators in. Both types of cages are monitored weekly and the results are usually astounding, hence confirming the learning of biological control (Kenmore *et al*. 1984; Ooi 1996).

Armed with the skills to discover biological control, farmer graduates have been able to discover the impact of nuclear polyhedrosis virus (NPV) of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (van den Berg *et al*. 2004). Similarly, farmers in Dalat, Vietnam have been able to carry out studies to better understand the action of Bt to replace the use of chemical insecticides and hence give an opportunity for *D. semiclausum* a chance to impact on the populations of DBM (Ooi *et al*. 2001).

In all FFS, insect zoos form part of the farmer education process and through this, many farmers come to appreciate the role of biological control in keeping pest populations in check. Some exercises to teach rice farmers about biological control are suggested by Ooi *et al*. (1991) and Shepard and Ooi (1991).

To facilitate learning of biological control, IPM Facilitators are themselves educated first in season long Training of Facilitators programme. IPM Facilitators are taught in a way that will enable them to use a similar way to teach farmers in FFS.

RESULTS OF TEACHING FARMERS BIOLOGICAL CONTROL

In discussing the impact of IPM in general and the teaching of farmers in biological control, the outputs from the FAO-EU IPM Programme for Cotton in Asia are used. The impact studies evaluated pre- and post-FFS data from FFS graduates, from non-FFS farmers (exposed) in the same village and a separate control group. It compared farmer practices in the year before farmer field school training with those in the year after. As soon as FFS groups were formed in the selected study sites, a sample of participating farmers was interviewed about their previous season's cotton cultivation and other related background information. The survey was repeated in the year after the FFS when the participants were by themselves again and were no longer guided by an FFS facilitator. The post-training data collection was conducted several times over the entire crop cultivation season in order to minimize errors from recalling information. In China and India the studies covered the years 2000 and 2002, while in other countries the years 2001 and 2003. Selected results from the studies are presented to reflect the result of farmer education.

In a study of 287 farmers in India and Pakistan, it was shown that cotton farmers who graduated from FFS had 16.9 point scores for recognition of natural enemies as compared to 2.3 points for non-FFS (exposed) farmers (Fig. 7).

This enhanced knowledge and skill can be translated to incomes in a concomitant study to evaluate average gross margins. A comparison of 1,060 farmers across five countries in Asia where the EU funded and FAO implemented Cotton IPM project was implemented showed that the average gross margins increased by $228 per hectare (+34% relative to control) for FFS farmers and $67 (+10%) for exposed farmers (Fig. 8); thus demonstrating the potential of educating farmers in biological control for reducing rural poverty.

The gains made could be attributed in part to savings in reducing use of insecticides by the farmers as illustrated in Fig. 9 based on the same farmers studied above. Insecticide use was reduced by 6.0 kg per hectare (-43%) for FFS farmers and 5.0 kg (-34%) for exposed farmers. The results suggest diffusion of knowledge from FFS farmers to non-FFS farmers.

This again may be translated into a healthier agro-ecosystem in a separate study of the species found in IPM and non-IPM fields. This study was undertaken as part of a study to encourage IPM Facilitators to teach farmers about agro-biodiversity in cotton fields. In both sites in India and Bangladesh, there was a higher number of species in IPM fields (48 and 49 for India and Bangladesh respectively) as compared to non-IPM fields (31 and 36 respectively) (Fig. 10). Predators and parasitoids contributed to the higher biodiversity recorded in IPM fields.

Lessons learnt from the implementation of IPM in rice, vegetables and cotton suggest that farmers need to be educated in the science of biological control if they are to benefit from the impact of this renewable resource (Ooi 1996; Ooi *et al*. 2001; 2004).

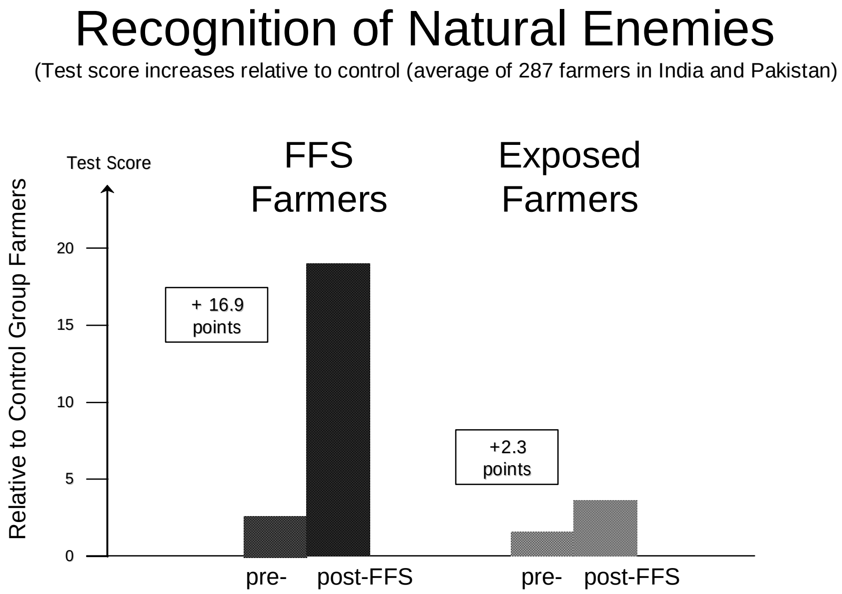

Figure 7. Recognition of natural enemies. Test scores increase relative to control (average of 287 farmers in India and Pakistan).

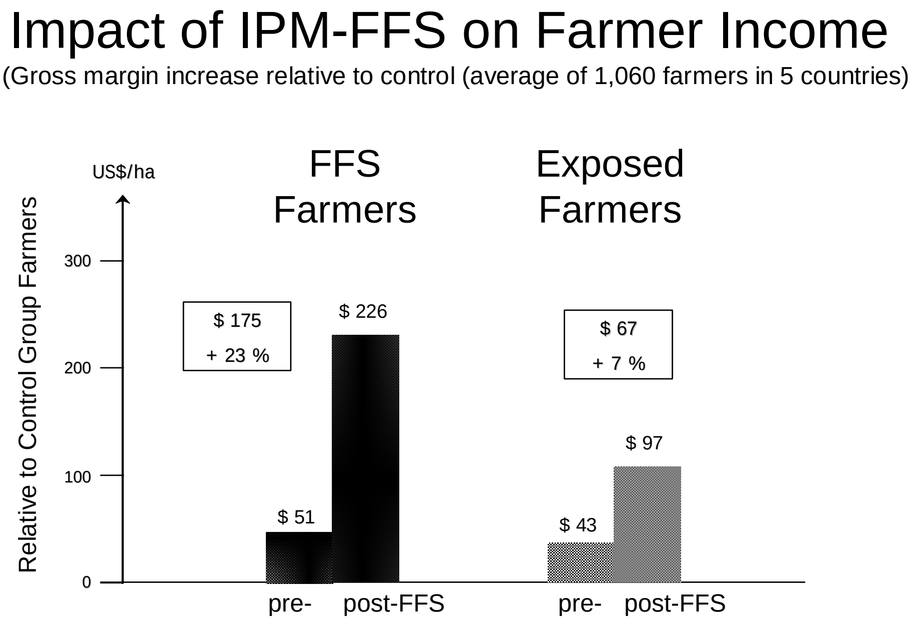

Figure 8. Impact of IPM-FFS on farmer income. Gross margin increase relative to control (average of 1,060 farmers in five countries – Bangladesh, China, India, Pakistan and Vietnam).

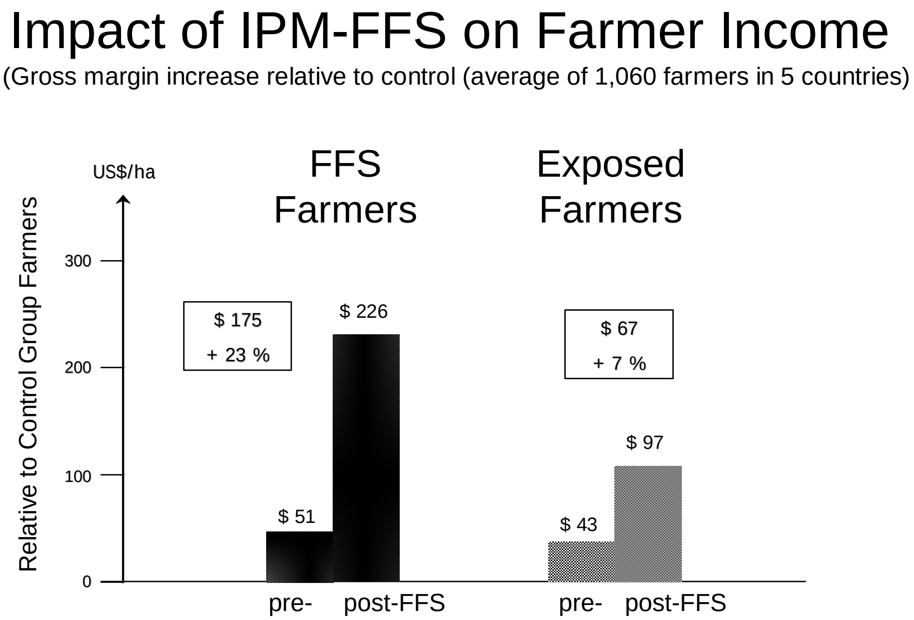

Figure 9. Impact of IPM-FFS on pesticide reduction. Gross margin increase relative to control (average of 1,060 farmers in five countries – Bangladesh, China, India, Pakistan and Vietnam).

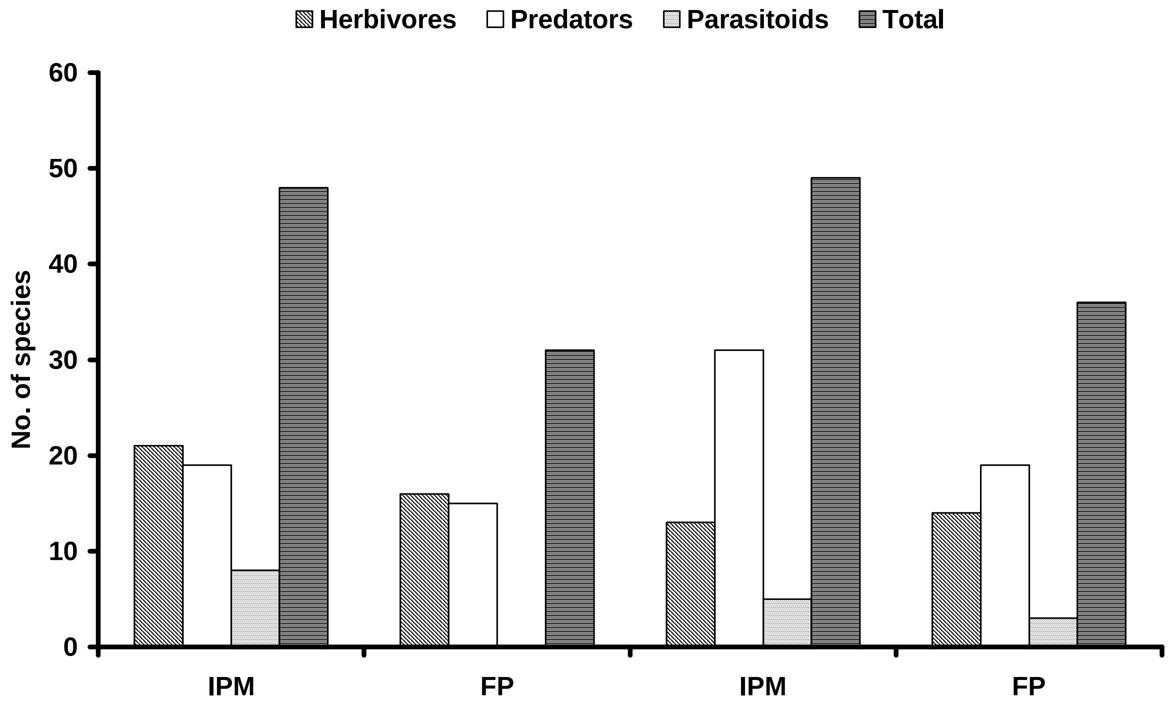

Figure 10. Comparison of number of species sampled from IPM and non-IPM cotton fields in Andhra Pradesh, India and Jessore, Bangladesh, 2003.

ACKNOWLEDGEMENTS

The authors are grateful to the organizers of ISBCA 2005 for the kind invitation to share their experiences in educating farmers in biological control. Results of the impact studies came from the effort of colleagues from the different countries and co-ordinated by Gerd Walter-Echols and Hermann Waibel. We wish to thank them for the use of some of the data. We wish to extend our gratitude to the farmers, IPM facilitators and national IPM programs for their support of the IPM activities. The support of EU and ADB for the cotton IPM program and to Australia, Netherlands and Norway for rice and vegetable IPM, we wish to thank them for joining us in educating farmers to enhance skills and incomes of resource poor farmers.

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THE ROLE AND SIGNIFICANCE OF FARMER PARTICIPATION IN BIOCONTROL-BASED IPM FOR BRASSICA CROPS IN EAST AFRICA

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ABSTRACT

Few attempts on biological control of arthropod pests on annual crops in sub-Saharan Africa have been successful. This is because of (1) inadequate taxonomic information on potential biocontrol agent(s), target pest and preferred host plants; (2) insufficient adaptation of potential agent(s) to bio-ecological conditions; (3) lack of consideration of the total pest complex of the target crop and farming systems; and (4) poor involvement of farming communities and extension personnel in information dissemination. The ICIPE-led diamondback moth (DBM) biocontrol project for East and Southern Africa has been addressing these issues since its inception in 2000.

Diadegma semiclausum, an exotic DBM parasitoid, was released in Ilkiding'a-Arusha/ Tanzania pilot area in October 2002. Hands-on farmer training was conducted before the release. The establishment, spread and impact of the parasitoid was monitored through surveys. Parasitism rates increased from 10% before release to 36.2% and 66% (10 months and two years after release, respectively). Farmers reduced spraying frequency and changed increasingly to Bt-based products, many stopped spraying for DBM control completely. Aphid control measures changed from area to spot application. In consequence, DBM population and damage was significantly reduced. Evidence from neighbouring areas, where the parasitoid had spread to but where farmers were not trained, indicated that farmers were unaware about the presence of the parasitoid. They continued routine spraying with broad-spectrum insecticides. Consequently, the level of parasitism was much lower and damage very high.

The lessons from this experience and their implications for wider use are discussed in this presentation.

INTRODUCTION

Crucifers, particularly cabbage, *Brassica oleracea* L. var. *capitata*, kale, *B. oleracea* L. var. *acephala*, cauliflower, *B. oleracea* L. var *botrytis*, Ethiopian mustard, *B. carinata*, Chinese cabbage, *B. campestris* L. var. *pekinensis/chinensis* and broccoli, *B. oleracea* L. var. *italica*, are among the major crucifer vegetables grown for home consumption and for cash in many parts of East Africa, notably in the highlands. They are a valuable source of vitamins and minerals. About 90% of the crop is produced by small holders on ¼ to one-acre land holdings. The bulk of the produce is sold in urban centers where it has high demand as a relish.

In East Africa, crucifers are grown in a wide range of agro-ecological conditions, and therefore, the pest complex and intensity, and management strategies practiced by farmers vary within and between farmers and locations.

A wide range of pests (insects and diseases) attacks the crop (Varela *et al.* 2003). The diamondback moth, *Plutella xylostella* (L.), aphids, *Brevicoryne brassicae* L., *Lipaphis erysimi* (Kaltenbach) *and Myzus persicae* (Sulzer), the webworm, *Crocidolomia binotalis* Zeller, the sawfly, *Athalia* sp. and cutworms, *Agrotis* spp, are the major insect pests. Although DBM was identified as the key pest of brassica crops in Eastern and Southern Africa in a workshop conducted in 1995 (Nyambo and Pekke 1995), the pest status of the cabbage aphid, *B. brassicae*, is becoming increasingly a major threat to brassica crops in East Africa. Its attack is associated with the transmission of the tulip mosaic virus (TuMV) disease, which can be devastating to the crop. Black rot, *Xanthomonas campestris* pv *campestris* (Pammell) Dawson, downy mildew, *Peronospora parasitica* (Pers.) Fr., and the tulip mosaic virus, TuMV, are the key diseases limiting production. Thus, any pest control strategy recommended to growers must consider its implications to the total pest complex within the prevailing production system. In East Africa, mixed cropping is common and pesticides recommended for one crop are used on a number of crops in the production system (Macharia *et al*., 2005; Nyambo, pers. obs.).

Until recently, application of synthetic pesticides was the preferred method of controlling pests of crucifers in East Africa, and testing of pesticides remained the major research activity (Löhr *et al*. 1998). In Kenya, the tests conducted in 1995/96 showed that organophosphates, carbamates and pyrethroids were no longer giving effective control of DBM compared to new products such as growth regulators, phenyl pyrazole and Bt-aizawai-based products (Kibata 1996). More recent studies confirmed that Karate, the most commonly used insecticide, is not only ineffective against the DBM; its use had negative economic returns in four separate trials (Macharia *et al*. 2005). There has also been an increase in complaints from farmers and extension workers about the loss of effectiveness by the majority of commonly used insecticides against crucifer pests, particularly the DBM, in Kenya and Tanzania in recent years (Macharia *et al*. 2005).

Due to the existing pest complex in crucifer crops and the status of DBM as indicated above, effective pest control approaches that emphasize integrated pest management at farmer level, are imperative in the East African production systems. Thus, recent studies have emphasized biocontrol-based IPM for crucifer crops in the region. The main objective has been to identify and develop IPM compatible components that can be used by the majority of the small-scale farmers in the region and a delivery vehicle that can ensure sustainable uptake. This involved a two-pronged approach (1) development of biocontrol-based IPM approaches and (2) participatory dissemination of new knowledge generated.

DEVELOPMENT OF BIOCONTROL-BASED IPM APPROACHES IN EAST AFRICA BIOCONTROL OF DIAMONDBACK MOTH

Since it was becoming increasingly difficult to control DBM with the commonly used synthetic pesticides, it became necessary to investigate use of natural enemies, a practice successfully used in Asia. The identification of suitable DBM natural enemies for integration in pest management strategies for East Africa has been a process that started with (1) inventory of indigenous natural enemies (2) assessment of the effectiveness of local natural enemies, (3) proper taxonomic studies though molecular techniques of local natural enemies, and (4) search for more effective natural enemies for introduction into the region.

EFFECT OF INDIGENOUS NATURAL ENEMIES ON DBM

In surveys conducted in Kenya, Tanzania, and Uganda in 2000/2001, parasitism rates were shown to be below 15% (Löhr and Kfir 2002). In this study, the most frequent parasitoids were *Diadegma mollipla* (Holmgren) (Hymenoptera: Ichneumonidae) and *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae). Entomopathogens, notably *Zoophthora* sp., *granulosis* virus (PlxyGV) and an unidentified bacterial diseases were also recorded in Kenya and Tanzania but their impact on DBM was well below 2% (Cherry et al. 2004; Nyambo pers. obs.; Oduor et al. 1997).

PROBLEMS OF PROMOTING BIOLOGICAL CONTROL OF DBM IN EAST AFRICA

Taxonomic confusion. Before publication of the review of Diadegma parasitoids of diamondback moth (Azidah et al. 2000), all Diadegma spp collected from DBM in African countries were considered as D. semiclausum and/or simply Diadegma spp. Biocontrol practitioners challenged this as D. semiclausum has been successfully used for the control of DBM in Asia (Amend and Basedow 1997; Singh et al. 1993; Talekar et al. 1989) and there was no obvious reason why it should not perform in the East African highlands where conditions are very similar. Azidah et al. (2000) grouped all African Diadegma parasitoids of DBM under D. mollipla. This was confirmed by Wagner et al. (2002), using molecular taxonomy techniques in the ICIPE led DBM project. Henceforth, the indigenous Diadegma spp attacking DBM in East and Southern Africa is now commonly known as D. mollipla (Wagner et al. 2002), a parasitoid that is more effective on the potato tuber moth, Phthorimaea operculella (Zeller). This scientific revelation on the true taxonomic status of the African Diadegma species provided the justification to import and introduce D. semiclausum from Asia to the East Africa highlands. The parasitoid was introduced to Kenya, Tanzania and Uganda following the existing national biocontrol regulations all of which are based on the FAO code of conduct.

Insufficient adaptation of potential agent(s) to bio-ecological conditions. Ecological adaptation and efficiency of biotypes is yet another constraint that had to be addressed to ensure optimization of identified agents. Some populations of Cotesia plutellae are recognized as efficient control agents for DBM, while others are very poor (Amend and Basedow 1997; Talekar, pers. comm.). The C. plutellae biotype of South Africa is a highly efficient parasitoid as compared to the very rare ecological homologue Apanteles sp. (misnamed C. plutellae) in East Africa (Löhr and Kfir 2002). Therefore, the South Africa strain was chosen for mass rearing and introduction in semi-arid areas of East Africa.

Lack of consideration of the total pest complex of the target crop and farming systems. There have been few successful attempts to control arthropod pests of annual crops using biocontrol agents. This is partly due to the fact that perennial crops offer a more stable environment where both the pest and its natural enemy can co-exist for a long time. In annual crops, success has been achieved in systems where continuous cropping and harvesting is practiced, e.g. the cassava mealybug (Neuenschwander 2003; Nyambo pers. obs.) and/or where alternative host plants provides refugia for the pest and its natural enemy. This could be the real reason for the success of the cereal stem borer biocontrol programme in Eastern and Southern Africa and the successful suppression of bean fly maggot in some parts of Ethiopia by Opius phaseoli Fischer and Sphegigaster brunneicornis Ferrière (Abate 1995; Nyambo pers. obs.).

The single pest approach, which ignores other important and/or potential pests, and is adopted in many situations, has contributed to the failure of many other biocontrol attempts (Abate 1995). This is because farmers will continue to apply pesticides to control the other major crop pests for which no alternative control measures are known. This not only threatens the survival and effectiveness of biocontrol agents, but also brings into question the rationality of attempting biocontrol on a single pest within a crop pest complex (Nyambo 1995). The attempted biological control of the potato tuber moth, *Phthorimaea operculella* Zell in Zambia 1979-82 using two introduced parasitoids, *Copidosoma koehleri* Blanchard and *Bracon greeni* Ashmead failed in some areas because farmers continued to apply broad spectrum insecticides to control aphids and the spread of viral disease in the crop. However, where farmers stopped spraying, the parasitoids were well established and brought the pest under control (Mingochi *et al.* 1995). The same fate applied to the attempt to control *Helicoverpa armigera* Hübner in tomato, eggplants, okra and tobacco in 1980 using *Trichogrammatoidea armigera* Nagaraja and *Apanteles ruficrus* Haliday (Mingochi *et al.* 1995). This must have happened because farmers were neither informed nor knowledgeable about their role in enhancing effective biocontrol of arthropod pests. A single method approach to pest control will also not be efficient and sufficient for the brassica production systems of East Africa due to the existing pest complex. Based on this background, it became necessary to investigate the possibility of combining biocontrol with other compatible options that will facilitate optimization of introduced biocontrol agents.

Lack of biocontrol compatible alternatives. The situation reported in Zambia (Mingochi et al. 1995) is not unique and could be repeated in many other production systems if not well addressed. To optimize the benefits of the biocontrol-based IPM programme in East Africa, it was deliberately planned in the ICIPE led DBM project to investigate and integrate all possible strategies that would reduce over-dependency on synthetic pesticides.

BOTANICAL PESTICIDES

In recent years, many farmers in East Africa started to use plant extracts, often home extractions from a wide range of plants, for pest control in a wide range of crops. This has partly been because synthetic pesticides are costly, and, as indicated above, they are ineffective in controlling key pests. However, not all plant extracts are compatible with biocontrol agents. Some could also have negative effects on non-target organisms, human and the environment, and this has to be avoided. Fortunately, the use of neem-based pesticides has been greatly researched worldwide including at ICIPE (Schmutterer and Ascher 1984; 1987), and commercial formulations are available in local shops.

The use of botanicals to control major insect pests of crucifers and their compatibility with arthropod natural enemies was studied in Kenya (Akol *et al*. 2002; 2003; Okoth 1998) and South Africa (Charleston *et al*. 2003). In the work done in East Africa, extracts from the neem tree, *Azadirachta indica* A. Juss, were found effective against the DBM and had low negative effects on *D. mollipa*, the indigenous DBM parasitoid, and therefore, could be used as alternatives to synthetic pesticides where they are available. Neem-based pesticides were also tested elsewhere and found to be compatible with arthropod natural enemies of DBM (Haseeb *et al*. 2004; Leeson 2001). As a result, neem-based products are being promoted as alternatives to the commonly used synthetic pesticides among crucifer growers in the region.

BIOPESTICIDES

Microbial control agents with potential against DBM have been recorded in East Africa as indicated above. However, their contribution in regulating DBM populations is low. Some of these, e.g. the granulosis virus, PxlyGV, is a potential agent that could be produced, formulated and applied (Cherry *et al*. 2004; Grzywacz *et al*. 2002). Dudutech, a private company based in Kenya, has taken up this challenge and the work is on going. Moreover, some strains of *Bacillus thuringiensis* (Bt) have been shown to give effective control of DBM and other lepidopteran pests, and, with low negative effects on potential arthropod natural enemies of DBM (Amend and Basedow 1997; Haseeb *et al*. 2004; Kibata 1996; Kok and Acosta-Martinez 2001; Krishnamoorthy 2002; Ng *et al*. 2002). Based on these findings, *Bt*.-based products, such as dipel, thuricide, xenthari, all available from local pesticide dealers, are suggested as an option where spraying lepidopteran pests becomes necessary.

AGRONOMIC PRACTICES

This is the basis for sound IPM development and promotion (Varela et al. 2003). It encompasses selection of varieties with some tolerance to key pest problems when and where available, e.g. the Danish types of cabbages are known to be tolerant to mosaic virus. Use of certified disease free seeds are highly recommended as a strategy for controlling black rot. Mulching the crop with dry grass in the nursery and field has been shown to (1) minimize attack by aphids and therefore the build up of tulip mosaic viruses (Achieng et al. 2003) (2) provide attractive environment for ground dwelling natural enemies and (3) conserve ground moisture for better plant growth, and (4) reduces splash and hence early black rot infestation. Other measures include plant nutrition, water management, e.g. when and how to use overhead or furrow irrigation, field sanitation and time of planting, proper selection and application of pesticides based on frequent (at least once a week) crop scouting and making use of the observations for decision making.

Many growers still have to spray against the cabbage aphid, and so guidance on which insecticide to choose and how to spray for effective control of aphids have to be made clear to the end users. The current best practice is spot application on infested plants only. This has given satisfactory results in smallholder cabbage production.

PARTICIPATORY DISSEMINATION OF RESEARCH OUTPUTS

Good research results are useful if they are properly disseminated to end-users. Many biological control attempts in sub-Saharan Africa ignored the role and contribution of national extension services and farmers, which contributed to failures and lack of sustainability (Mingochi *et al.* 1995; Nyambo 1995). The ICIPE-led DBM project attempted to address this issue by involving and engaging the national research and extension programme of the collaborating countries in project formulation (Nyambo and Pekke 1995; Seif and Löhr 1998), research and dissemination of results. Workers of the national research and extension departments in each respective country conducted the surveys on indigenous natural enemies. National programmes largely handled parasitoid release and monitoring activities (Fig. 1).

DISSEMINATION OF THE RESEARCH OUTPUTS TO FARMERS

To ensure sustainable uptake and dissemination to farmers, it was deemed necessary to capacitate the national extension workers in each respective country (Fig.1). This was done through an intensive one-week hands-on training course in biocontrol-based IPM for master trainers in each collaborating country. The course targeted the district subject matter specialists (horticulture and crop protection). These course participants would be responsible for down streaming the information through their national extension systems until it reaches the farmers. The national biocontrol units were given a two-week training course on how to rear the natural enemies and carryout field releases. The two national units were to collaborate in terms of planning effective field releases in major crucifer growing areas.

CASE STUDY: ILKIDING'A, ARUMERU DISTRICT, TANZANIA

The multi-practice approach was field tested at Ilkiding'a, Arumeru District Tanzania in October 2002, a coffee-vegetable based production system. Two of the master trainers from the Tanzanian Ministry of Agriculture and Food Security (MAFS) identified the release site in an area where cabbage growing is taking off as a cash crop among small-scale farmers. Baseline data were collected for a year before the planned field releases. The level of parasitism in northern Tanzania was about 10% before releases. Farmers were already spraying twice a week from transplanting to harvest using either selecron, endosulfan, decis or karate (alone or in mixtures), all recommended for use on coffee, and yet the cabbage produce was of extremely poor quality. The DBM pressure on the cabbage crop in the area was causing a crisis situation. This being a highland area, *D. semiclausum* was the preferred agent. A release permit was obtained from the Plant Health Service, MAFS. On the day of the release, a hands-on farmer training was conducted. It involved a demonstration of the parasitoid and a question and answer session to educate the farmers about *D. semiclausum* (what it is, how it works) and the role of farmers in its preservation. About 20 farmers and the village extension worker from the locality participated in the training. Farmers were shown how to release the agents and participated actively in the release exercise. Approximately 700 female and a similar number of male parasitoids were released. Thereafter, the farmers were left alone. It was anticipated that the agent would get established and propagate itself in the area while the know-how would be spread by word of mouth between farmers. The event was captured on the local TV and radio stations and broadcast during the week of field release.

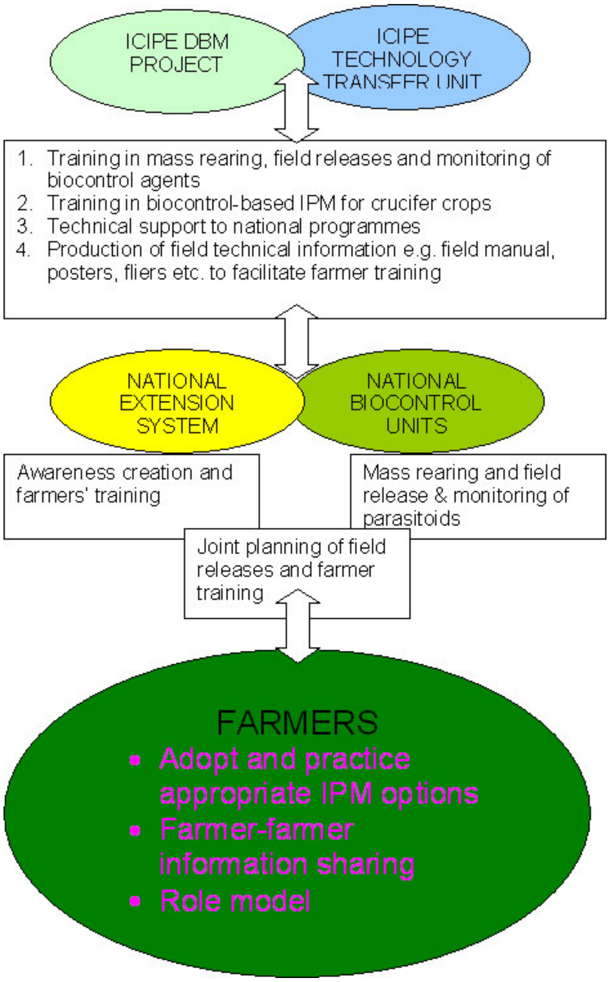

Figure 1. Capacity building for dissemination of biocontrol-based IPM program.

RESULTS

Ten months after first releases, the level of DBM parasitism had increased to 36.2%, this increasing to 66% after two years at the release site (Fig. 2). At the release site, farmers reduced spraying frequency and changed increasingly to Bt-based products and many stopped spraying for DBM control completely. Aphid control measures changed from area to spot application. In consequence, DBM population and damage was significantly reduced from 32 DBM/plant pre-release period to 4.0 DBM/plant in two years. This is a very significant improvement considering the fact that spraying has been reduced to a maximum of two per season and the crop quality improved. Evidence from neighbouring areas, where the parasitoid had spread to but farmers were not trained, indicated that farmers were unaware about the presence of the parasitoid. They continued routine spraying with broad-spectrum insecticides. Consequently, the level of parasitism was much lower, with an average of 51% and 1.8 damage score per plant (Fig. 2).

Following these results, a refresher course for the Tanzania master trainers was organized in collaboration with Ilkiding'a farmers. The Ilkiding'a cabbage growing area became a classroom for the extension workers and the course facilitators in February 2005.

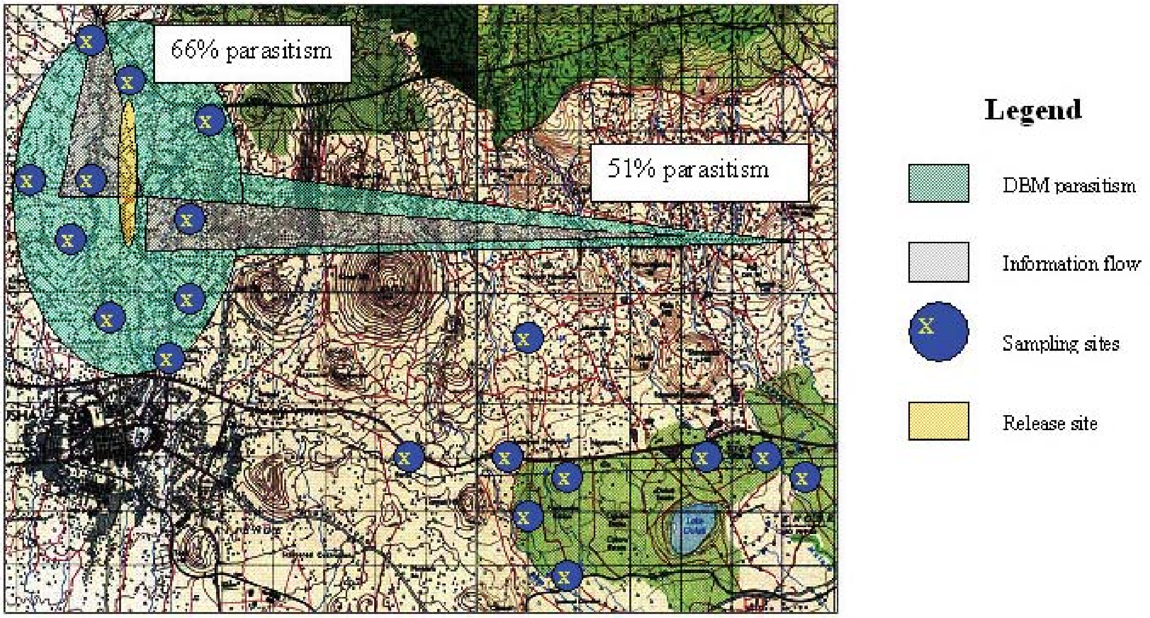

Figure 2. Ilkiding'a release site: Level of parasitism, parasitoid spread and information flow from release site after two years: October 2002 to October 2004.

LESSONS FROM THE ILKINDING'A RELEASE SITE

1. Participatory research and extension, that emphasizes production systems and pest complex, should be the way forward to enhance and facilitate sustainable uptake and effectiveness of identified arthropod biocontrol agents
2. Capacitating farmers to take active participation in biological control initiatives is of utmost importance to ensure successful establishment of the agent, its propagation and effective control of target pest.
3. Field releases of biocontrol agents and farmer training has to be planned in such a way that they occur simultaneously.
   a. I see I remember
   b. I touch I remember
   c. I hear, I see, I touch, I remember more

It is important to emphasize to farmers the fact that, unlike pesticide sprays, which give results within a short time, biocontrol takes time and the results may not be immediate. The Ilkiding'a group realized the effects of the agent in the September-October 2003 cabbage crop, a year after the first release. To them, this was the real convincing point, which marked the start of the success of the initiative. They learned by doing it themselves.

4. Farmer training should be supported with other forms of ICT to reinforce the message and also to raise awareness among other growers in similar growing zones. It should not be a one-off activity.
   a. Although *D. semiclausum* spread to over 10 km from the release point in two years, the knowledge about it and how to enhance its effectiveness remained concentrated

at the release point (Fig. 2). As a result, crucifer growers in other villages continued to use the broad-spectrum pesticides and damage on the crop persisted despite the presence of the parasitoid.

b. The radio and TV programmes were a motivation to the farmers at the release point to practice what they learned. It also served to raise some awareness at the district level, and as a result, other growers made some enquiries wanting to learn from the farmers at Ilkiding'a.

c. The TOT course participants need to visit successful release sites to learn from growers for them to conceptualize and internalize the value of biocontrol-based IPM practice.

CONCLUSIONS AND THE WAY FORWARD

Thoroughly researched and carefully implemented classical biological control is an important tool for integrated pest management. Equally important are the consideration of the whole pest complex of the crop and the participation of farmers and extensionists in research and implementation. This ensures full understanding of the introduced changes and allows the natural control factors to play their role. The ICIPE-led DBM biocontrol-based IPM for brassica crops could become a role model for other biocontrol attempts in Africa.

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CASE STUDY ON ORGANIC VERSUS CONVENTIONAL COTTON IN KARIMNAGAR, ANDHIRA PRADESH, INDIA

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ABSTRACT

Cotton (*Gossypium hirsutum* Linn..) yields, profits and pest incidence at fields of farmers partaking in an export oriented organic cotton production program are compared with yields of conventional cotton production in the same village during a bad cotton season (2004). Late season drought reduced actual yield by 42% compared to the estimated yield in October 2004 and usual average yields. Organic cotton yielded on par at 232 Kg seed cotton /acre against conventional cotton at 105 Kg/acre. Organic cotton was more profitable at plus Rs 559/acre (approx. US $ 13) (1 US$ = Rs. 44) versus minus Rs 1307/acre (minus US$ 30) in conventional cotton and had significantly less problems with *Helicoverpa armigera* (Hubner) Lepidoptera, Noctuidae and *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). Pest control in organic cotton was about Rs. 220 (US $ 5) per acre (5% of total production costs of organic cultivation) as against Rs. 1624 (US $ 37) per acre (30% of total production costs of conventional cultivation) in conventional cotton. Pest management in organic cotton was based on prevention: balanced nutrient management, intercrops and early spray of HaNPV.

Thirty-four farmers, part of a large organized group (over 200 farmers), volunteered to test organic cotton on part of their farm, allotting 79 acres for organic farming though owning about 296 acres. For certification purposes a contiguous area of about 40 acre should go organic. The 34 farmers were organized in two groups for training, credit and savings, maintenance of certification administration and marketing purposes. Farmer Field School sessions (FFS) were conducted on weekly basis during the season but also after the season to deal with

post harvest handling and marketing. As a result of this year's experience all participating farmers will bring their total cotton under organic management, another 70 farmers will join and 10 neighboring villages are interested, but have been asked to wait because of lack of training manpower. Farmers of the old and new groups will be trained to become farmer trainers.

Packages used for training are based on the FAO IPM- FFS and long term experience of ETC India and its staff in cotton cultivation in Southern India. Linkages are maintained with CIPMC, national cotton research programme and universities. The latter mainly for the selection of varieties. Inputs are purchased from the private sector. Yearly a meeting will be organized in which representatives of farmers from the whole organic cotton program (240 in 2004) will interact with researchers, input suppliers, banks, ginners and spinners. This is meant to create synergy in the whole chain.

INTRODUCTION

Profitability of cotton production systems under rainfed conditions in Andhra Pradesh in India has drastically come down due to loss of soil and soil fertility, imbalanced nutrient application, lack of soil organic matter, and finally, indiscriminate pesticide application. Cotton production in India involves about 9 million hectares (5.5% of arable land) and 4 million marginal, small and large farmers. The production level is about 13 million bales (lint, 170 Kg/ bale) per year (GOI) or about 20% of global production. The major problems in cotton production in India are low productivity, mixing of varieties, low profitability, lack of adequate knowledge at farm level, indebtedness of farmers due to high interest rates at the hands of private moneylenders (up to 85% per annum) and finally contamination of cotton with non-cotton materials both at the field level and off the field. According to the Central Institute of Cotton Research (CICR, Nagpur, India), cotton productivity in Andhra Pradesh (AP) has been declining steadily from about 265 kg seed cotton per acre in 1995 to about 162 kg seed cotton per acre in the year 2003.

FAO and ETC implemented IPM, Non Pesticidal Management (NPM) and organic cotton programmes from 1997 onwards. Then it was observed that these methods of cotton production are usually more profitable for farmers growing rain-fed cotton with seed cotton yields up to about 1.2 tonnes/hectare Organic cotton production entails the use of cultural preventative methods (like intercropping, border cropping, drainage, variety selection), use of natural fertilizers (Farm Yard Manure (FYM), compost, bio-fertilizers, poultry manure, etc..) and biological controls (NPV, Trichogramma, Trichoderma, etc…) rather than synthetic fertilizers and pesticides/fungicides.

When ETC was asked by Solidaridad (that promotes the Made-by fashion label in the Netherlands: guaranteeing fair trade and organic products), to implement an organic cotton programme it accepted the challenge and decided that detailed data collection should be done at organic and conventional farms. The main objective of data collection was (1) To be able to compare the yield, income and profitability of organic and conventionally grown cotton and judge whether organic cotton production is a viable proposition. (2) To find out about the effectiveness of organic nutrient and pest management methods, among others the use of

NPV for managing *Helicoverpa armigera.* (3) To compare the quality parameters of cotton lint with management regimes of individual organic farmers. (4) To identify issues for further detailed studies needed.

MATERIALS AND METHODS

The study comprises of 34 farms/farmers, from 2 villages (Arapally and Repaka) in the district of Karimnagar in the state of Andhra Pradesh in India, who form a part of the larger group of 239 farmers involved in cotton cultivation in an export oriented organic cotton production programme. The local NGO (KRUSHI) was working in these villages for quite some years prior to the introduction of the organic cotton programme. This made the selection of farmers within the village easier. Farmers volunteered after a series of introductory meetings in the concerned villages. In these meetings the concept of organic farming was explained, risks and advantages as well as conditions (contiguous area of 40 acres organic farming, certification, data collection and recording, group sales of cotton…). Farmers feared most loss of production due to complete elimination of chemical fertilizers. The elimination of pesticides was considered less of a problem by the farmers, partially due to the fact that they had been exposed to IPM technologies to a certain extent. Project staff provided an alternative cropping system and a comprehensive outline of crop management, which showed that the same levels of nutrient application could be achieved through organic means and that various tested organic options for disease and pest management existed. This convinced farmers that they would not end up in loss.

Land for cotton production was from a contiguous area of approximately 40 acres in each of the two villages. Cotton was grown on 79 acres of land out of a total of 273 acres of land allotted for organic cultivation. Within the contiguous area 49.5 acres land came out of a long-term fallow (> 3 years) and 28.5 out of a short-term fallow (<1 year). Soils were very light black cotton soils

Farmers were organised in groups of about 20 farmers along the lines of Self Help Groups (credit and saving groups). These groups received an interest free revolving fund equal to Rs 4,000 per member either in kind or cash. This had to be repaid by individual farmers to the group with an interest of 17% as decided by the group (compared with 86% when obtained from money lenders). They mobilized inputs as a group based on the nutrient management package worked out by ETC. The groups would also receive FFS training on a weekly basis. After harvesting, they would store harvested cotton in a common facility and market their cotton produce as a group.

Seeds were procured locally by farmers from their regular commercial seed sources. Cotton seeds were treated and pelletted with nitrogen fixing bacteria Azospirillum, Azotobacter and Phosphorus Solubilizing Bacteria and an antagonistic fungus - *Trichoderma viride* mixed in fresh cow dung slurry before sowing. A comprehensive intercropping package was proposed to control pests. One of them was intercropping with pulses. All farmers chose to grow soybean but only a few farmers agreed to let it grow till maturity (soybean was not proposed as the preferred intercrop by the ETC team). The main reason that they decided not to let the soybean mature was that they had never intercropped cotton and feared a negative influence of the intercrop on the yield of cotton, also they felt that inter-cultivation, which they consider important for enhancing cotton yield, was impeded by the intercrop of soybean. Nutrient management practices were worked out for a yield level of 6 quintals of seed cotton per acre and adjusted later on during the season based on weather conditions. Farmers generally decided not to apply the recommended nutrient management package, due to lack of availability but also due to costs involved, particularly with regard to the topdressing with poultry manure. Table 1 gives the nutrient management practices that were suggested to farmers during the year, while Table 2 gives the actual quantity of different nutrient sources applied based on actual field conditions. Fig. 1 shows mixing of bio-fertilizers with seed.

Table 1. Manure requirement for a yield projection of 6 quintals of cotton per acre.

Organic Manure*	N**	P	K	Ca	Mg	S	Zn	Fe
Requirement	48	6	24	18	5	9	0.2	0.9
FYM 4 MT	4	4	14.4	22	16	2.8	0.02	0.06
Enriched FYM (300 kg)	0.36	2.23	1.3	1.6	1.17	0.74	0.001	0.003
Bio-fertilizers (Azotobacter, Azospirillum, each 1 Kg/acre)	10	0	0	0	0	0	0	0
Poultry Manure 2 MT	24	5	18	44	Traces	10	0.1	0
Wood Ash (100 kg/ac)	0.15	0.53	6.5	20	1.25	1	0.0233	0.85
Inter crops	10	0	0	0	0	0	0	0
Total Applications	48.51	11.76	40.2	87.6	18.42	14.54	0.14	0.91

*Data out of compiled database owned by ETC. From various sources: Internet, books, own chemical analysis.
**All nutrients in kilograms

Figure 1. Mixing bio-fertilizers with cotton seeds.

Table 2. Actual quantity of manures applied by all farmers for 79 acres.

Manures	Suggested (kg)	Actually Applied (kg)	% of Suggested
Farm Yard Manure, 4 MT per acre	316,000	14,525	5
Enriched Farm Yard Manure, 300 kg per acre	23,700	7,200	30
Azospirillum	79	86	108
Azotobacter	79	83	105
PSM	79	83	105
Trichoderma viride	39.5	42	106
Poultry Manure, 2 MT per acre	158,000	47,905	30
Wood Ash	7,900	7,650	97

From Table 2, it can be concluded that organic manure was applied at a far lower rate than advised. It was agreed by the farmers that they would take part in the weekly Farmer Field School (FFS) during the cropping season. FFS was conducted for 20 sessions from pre sowing till the commencement of harvesting. Crop management decisions (pest and nutrient) were based on weekly Cotton Eco System Analysis (CESA), which includes monitoring of plant growth parameters, pest predator ratio and local weather conditions. Fig. 2 shows the setting within which the FFS took place.

Figure 2. The setting of a Farmer Field School (FFS).

Table 3 provides the generalized pest management options provided to farmers prior to sowing. Individual pest management practices were modified based on Cotton Ecosystem Analysis (CESA) and farmers' capabilities. Fig. 3 provides a view of how the border crop was actually planted in organic cotton fields.

Table 3. Pest and disease management strategies in organically grown cotton fields.

S. No.	Activities
1.	Seed treatment with Trichoderma viride, for root rot and wilt
2.	Intercropping of short duration pulses - cotton : pulses 1:2
3.	Border crop of Maize or Sorghum 5 - 6 rows
4.	Trap crop of Bhendi 50 plants per acre against Earias vitella
5.	Trap crop of Marigold sown randomly against Helicoverpa armigera
6.	Trap crop of Castor sown randomly against Spodoptera litura
7.	Delta pheromone sticky trap against Pectinophora gossypiella
8.	Bird perches within the fields
9.	250 LE of HaNPV (UV stabilized) applied after noticing egg laying
10.	Yellow Sticky traps smeared with castor oil against white fly
11.	Blue sticky traps smeared with castor oil against thrips
12.	Hand picking wherever possible
13.	Detopping after 15 - 17 sympodial nodes

Figure 3. A border crop of maize in organic farmer's field.

The internal control system (ICS) required for organic certification demands that all operations by farmers are documented: the type and quantity of inputs used, the sources, the costs, the labour required, cotton harvested per picking, quality of the cotton, etc.. These data were documented by the farmers under close supervision of ETC and NGO staff, cross checked by an independent internal inspector and shared with the certifier (SKAL). In addition data collected during CESA were documented. These two sources, ICS and CESA, were used for making the analysis we present in this paper.

Farmers were encouraged to compare cotton grown organically in their fields with cotton grown conventionally within their village at every stage during the entire crop growth period. Their impressions were recorded. However, no systematic CESA was implemented in conventional fields.

The 2004 monsoon in Karimnagar was abnormal (total of about 60% of long term average rainfall): heavy rains end of May and early June, a drought from 10 June till early July and cessation of the rains end of October. Sowings commenced from the first week of June 2004 with the earliest sowing done on 6th June 2004. Sowings continued till the end of July 2004 as and when local rainfall and soil moisture permitted. Bulk of planting was done during the month of July, almost a month later than the normal date of sowing. Temperatures were normal during the growing season especially the early and the mid season. Delayed planting combined with late season drought made 2004 a trying year for cotton cultivation in Karimnagar district.

Some farmers (3 out of 34 cotton growers) had a ratoon crop of cotton. The data of these farmers have been excluded from the analysis, as their yields were substantially lower. One organic farmer used critical sprinkler irrigation and obtained significantly higher yields than average. This farmer too was excluded from the analysis. Then there is one farmer who grew a relatively unknown variety and who had very low yields. We attributed that to the variety and excluded the farm from the analysis. So in all a data set of 29 organic farmers is compared with a data set of 11 conventional farmers. The conventional cotton plots were selected near the organic cotton plots based on similarities in soil conditions and varieties grown.

RESULTS AND DISCUSSION

VARIETIES AND YIELD

Farmers used many different, mostly hybrid, varieties. We checked whether any indication existed that varieties were yielding differently. There are insufficient data to test the hypothesis that there is no difference. The fact that yields of different varieties planted at the same day are similar suggests that yields did not differ because of variety issues. Between organic and conventional farmers, similar varieties were used. Thulsi, Bunny and Dyna are most frequently used varieties by both groups of farmers. Thulsi is planted by 30% organic farmers and 40% of the conventional farmers. The other 2 varieties make up another 20 to 30%. The rest of farmers grow a bouquet of varieties, of which Sundeep (grown only by organic farmers) appears to be promising due to high yields that are observed at organic farms.

DATE OF SOWING AND YIELD

Organic cotton plots were on average sown three weeks later (12-07-2004) than the plots of conventional cotton (25-06-2004). One would expect yield to be affected by date of sowing, especially under rainfed conditions, resulting in lower yields due to late planting. We tested this for organic plots and found an insignificant correlation. We also tested the effect on date of sowing on yields of conventional cotton and found again a weak correlation. Thus we concluded that we could use the whole population of organic and conventional cotton fields for analysis.

ORGANIC COTTON YIELDS ON FIELDS AFTER LONG TERM FALLOW (> 3 YEARS) COMPARED WITH SHORT TERM FALLOW (< 1 YEAR)

Analyzing the data we noticed that a number of farmers, especially in Arapally, seemed to have lower than average yield. When checking the background data, we observed that these farmers had decided to try out organic cotton on fields they had not used for quite some time: from 3 to 15 years. When we tested whether the difference in yield was significant, we found that to be not the case. We also tested the hypothesis that yields of ST fallows were significantly different from the yields of conventional fields and found that yields are not significantly different.

However, we concluded that income of organic cotton on short term and long term fallow fields are significantly higher (p=0.05) than the income of conventionally grown cotton (Table 4). The reduction in cost of cultivation of organic farms is the main factor contributing to the higher net-income.

Table 4. Comparative table of yield, income and profitability of short and long term fallow (within organic farms) with conventional farms.

	Organic fields			
Particulars	**L.T. fallow (n=12)**	**S.T. fallow (n=17)**	**Conventional (n=11)**	**Remarks**
Seed cottonYield, Kg/acre	224**	240 **	205	Thus income from cotton alone not significant different
Total income per acre, Rs/acre	4617**	5233**	4105	Income of cotton and intercrop
Total costs, Rs/acre	4212 * ($p < 0.005$)	4517 * ($p < 0.04$)	5412	
Net income, Rs/acre	402* ($p < 0.035$)	716* ($p < 0.008$)	-1307	

* Significant; ** Not Significant

This leads to the next question as to which aspect of cotton cultivation takes up the major chunk of cost. Table 5 provides the break up of costs of cultivation.

Table 5. Cost of cultivation per acre (Rs/ac) for organic and conventional cotton cultivation.

Parameters	Organic LT (n=12)	Organic ST (n=17)	Conventional (n=11)
Yields	225	240	205
Seeds	626**	581* ($p < 0.014$)	720
Fertilizer/manure	1204* ($p < 0,04$)	1285**	1566
Ploughing	972* ($p < 0.0001$)	917* ($p < 0.0002$)	482
Weeding	526**	704* ($p < 0.04$)	360
Inter-cultivation	293	391	290
Pest Management	203* ($p < 2.16E-07$)	237* ($p < 3.54E-09$)	1624
Harvesting cost	390	403	371
Total costs	4214* ($p < 0.005$)	4518* ($p < 0.04$)	5413

* Significant; **Not Significant

There is no significant difference between the costs of fertilizers between organic and conventional farms, though conventional farmers spent more than those organic farmers who used land that came out of a long term rotation. On the other hand, cost of ploughing (primary and secondary) is significantly higher in organic farms. The cost of weeding shows a mixed picture. One would have expected the fields that come out of a long term fallow to have the higher costs, but they are significantly higher in plots that have not been under long fallow. There is no significant difference between the cost of intercultivation between organic and conventional farms. The cost of pest management is significantly higher in conventional farms. While in organic farms, the cost of pest management was an average of Rs. 220 per acre, the cost of pest management in conventional farms has been as high as Rs. 1624/ acre.

EFFECT OF RATE OF NITROGEN APPLICATION ON YIELD

We assumed that yields would increase with higher levels of nitrogen application. This hypothesis was tested within organic cotton data set but also between organic and conventional farming. Within the organic cotton data set, only a very weak positive correlation could be established. In conventional farms the nitrogen application level is 2.5 times higher than in organic farms (52 against 20 Kg N/acre, Table 6). Even then, we do not find any significant difference in seed cotton yield between organic and conventional farms. The fact that rains stopped so early, could have caused that the higher nitrogen availability could not be expressed in the final yield. Water was apparently a more serious constraint than nitrogen.

NUTRIENT MANAGEMENT AND YIELD

For high productivity of cotton good nutrient management is necessary. We were worried that organic cotton would under perform due the very low doses of Farm Yard Manure applied. Thus nutrient management regimes were compared between organic and conventional farms. Nutrient management was calculated purely based on applied nutrients. Soil testing was not done for the first year. Table 6 gives the nutrient management regimes for organic as well as conventional farms. Nutrients have been calculated based on the source of nutrients actually applied as indicated in Table 2 on a per acre basis.

Table 6. Applied nutrients (kg per acre) at organic and conventional farms.

Nutrients	Organic kg/acre	Conventional kg/acre	Remarks
Nitrogen	19.82	52.11*	P < 0.0001
Phosphorus	9.11	40.76*	P < 0.001
Potassium	18.12	23.47**	
Calcium	52.48	0	
Magnesium	5.24	0	
Sulphur	6.65	0	
Zinc	0.25	0	

* Significant; **Not Significant

Nitrogen and Phosphorus application was significantly higher in conventional farms than in organic farms ($p < 0.001$ and $p < 0.001$ respectively). There was no significant difference in potassium application between organic and conventional farms.

A simple calculation of nutrient balance (theoretical removal by full crop compared with total nutrients applied, not measuring anything and not taking into account mineralization, fixation, leaching or vaporization) was done comparing conventional and organic farms. Table 7 provides the average estimated nutrient balance of all the farms in conventional and organic.

Conventional farms appear to have a positive nutrient balance of Nitrogen, Phosphorus and Potassium, while there is a negative balance of secondary and micronutrients. (Ca, Mg. S and Zn). Organic farms have a negative balance on Nitrogen only. Interpretation of these figures is hazardous, but it can be safely assumed that in organic farming more attention needs to be given to nitrogen application and in conventional farming to secondary and micro-nutrients. We can also conclude that the application levels by the organic farmers have been sufficient for the yields realized mainly because water availability was a problem.

Table 7. Comparison of nutrient balance between organic and conventional fields (kg/ac).

Nutrients	Conventional Balance left in soil (kg/acre)	Organic Balance left in soil (kg/acre)
Nitrogen	35	-2.5
Phosphorus	39	4.62
Potassium	14	7.84
Calcium	-7	39
Magnesium	-2	1.56
Sulphur	-3	3.11
Zinc	-0.07	0.11

* Significant; **Not Significant

INTERCROP EFFECT

Soybean was sown (at various dates after sowing cotton) as an intercrop by most of the farmers in both villages involved in the programme. Some farmers have gone in for 2 rows of soybean and some farmers for a single row of soybean. Fig. 4 shows how intercropping was done.

Figure 4. Intercropping with soybean.

A superficial analysis suggests no influence of growing a soybean crop. However, on closer analysis, it appears that those organic farmers in Repaka who harvested soybean have a significant lower yield than the farmers who plowed their soybean into the soil after some time of growth. (119 kg seed cotton/acre as against 356 kg seed cotton per acre, p= 0.017). Other factors are similar (plant density, N applied, varieties, date of sowing). In Arapally such effect could however not be established because only two farmers harvested the soybean and date of planting of the cotton varied much with the other farmers (one early July, the other end of July).

PEST MANAGEMENT

The major pest problems faced during the season were the boll worms, *Pectinophora gossypiella* and *Helicoverpa armigera*. For pink boll worms, delta sticky pheromone traps were used. In the villages of organic cotton, some farmers had gone in for ratoon cotton. It was expected that pink boll worm infestation would be higher in ratoon crop than other fields. Accordingly enumeration was done randomly to find out the severity of pink boll worm vis a vis the distance from the ratoon crop (Table 8). These observations weren't done systematically and therefore the data presented should be looked at as indicative only.

Pink Boll Worm infestation was about 30% in ratoon cotton, in the organic cotton field used for FFS no Pink Boll Worm infestation was noticed.

Table 8. Enumeration of Pink Boll Worm (PBW).

			Counting of PBW adult moths (Nos.)		
Date of installation	Date of observation	Period (days)	Ratoon crop	Near and adjacent fields	Far away from ratoon crop
12 - 18 Sept., 2004	First week of October	15 days	138	84	68

USE OF HANPV AND NEEM SEED KERNEL EXTRACT (NSKE) FOR MANAGEMENT OF *HELICOVERPA ARMIGERA*

During the season, infestation of *H. armigera* commenced by the first week of September. Based on the Farmers Field School's (FFS) CESA, farmers decided to go in for spraying of HaNPV. Table 9 provides the details of Ha NPV application as soon as the eggs and first instar larvae were noticed in cotton. Fig. 5 shows the preparations towards HaNPV application.

During the season, farmers were encouraged to visit conventional farms also in order to assess the comparative advantage of organic methods especially the use of Ha NPV over synthetic chemicals for managing pests in particular *H. armigera*. Conventional farmers were using costly chemicals, including synthetic pyrethroids, for the control of *H. armigera*. Common beneficial (predatory) insects observed in organic cotton plots by farmers during FFS are presented in Table 10. Fig. 6 shows that also birds liked to have their nests in the organic cotton fields.

Organic farmers who visited conventional fields observed very low levels of natural predators in those fields.

Table 9. Management of *Helicoverpa armigera* using HaNPV and NSKE.

Distribution of Ha. N.P.V to the farmers	10.09.2004
Farmers started HaNPV spray	11.09.2004 (approximately 63 days after sowing)
Farmers completed the spray	30.09.2004
Dose	50 m.l/ acre (5 tanks x 10 lit.) (10 ml HaNPV/ tank)
Time of application	Early morning: 5.00 a.m and Late evening: After 5.00 p.m
Weather condition	On the whole weather conditions were highly favorable after Ha NPV application for epizootic conditions
5% NSKE application	5% NSKE was applied after 6-15 days gap after Ha. N.P.V spray
Ha. N.P.V. Incidences	Observed Ha. N.P.V affected larvae on 21.09.2004 onwards. (i.e., 5th day after spraying)
Mortality rate	High percentage of mortality observed

Figure 5. Preparations towards HaNPV applications.

Table 10. Common beneficial (predatory) insects observed by farmers in organic plots.

Date(s)	Predator Insects on Organic Plots
10.08.2004 to 21.08.2004 (45 - 50 DAS)	*Geocoris* spp (Lygaedae. Hemiptera) *Coccinella novemnotata* (Coccinellidae. Coleoptera): Lady beetle adults, pupae, grubs and eggs *Syrphus* spp (Syrphidae. Diptera)
14.09.2004 to 28.09.2004 (75 - 80 DAS)	*Cicindela* spp (Cicindellidae. Coleoptera): Tiger beetle adults *Zelus bilobus* (Say) Reduviidae. Hemiptera: Reduvid bug), *Orius* spp. (Anthocoridae. Hemiptera) *Chrysoperla* sp. (Chrysopidae, Neuroptera): Chrsoperla adults, *Pantala flavescens* (Fabricius) (Libelludae, Odonata): Dragonfly, Lestus sp. (Lestidae, Odonata): Damselfly
07.10.2004 to 14.10.2004 (> 85 - 90 DAS)	Podosus maculoventris (Pentatomidae. Hemiptera): Spined soldier bug

Figure 6. A birds' nest in an organic cotton plot.

QUALITY OF LINT

Of organic cotton was tested by CIRCOT (Central Institute for Research on Cotton Technology) in Nagpur. The average lint length of the samples was 29.1 mm, micronaire 3.1 and tenacity 22 (3.2 mm (g/tex)). No clear correlation can be found between the varieties grown by the farmers and lint quality. It can be concluded that the cotton quality is satisfactory, though the micronaire clearly shows the effect of emergency ripening (we would have preferred between micronaire 3.5 and 4.5).

CONCLUSIONS AND RECOMMENDATIONS

These conclusions are based on one, bad cotton season. All conclusions are thus to be considered with caution. Different rainfall patterns and quantities can lead to very different results. It must be recommended to compare organic and conventional production for a number of years.

In the year 2004 organic cotton yielded generally at par with conventional cotton. In the case of organic cotton grown on fields that came out of a short term fallow, yields were higher than yields of conventional cotton. Profitability of organic cotton was significantly higher than conventional cotton. The main contributing factor to higher profitability was the reduced expenditure on pest management.

We did not ask farmers how much money they borrowed from money lenders. Assuming them borrowing about Rs 4,000 per acre, their additional costs would have been Rs 180-200 per acre (assuming 87% interest rate against 17% interest in the SHG and 9 months of borrowing). When a premium will be paid for organic cotton, farmers will earn an additional Rs 400 per acre over conventionally produced cotton. It can thus be concluded that organic cotton production appears to be financially feasible and attractive.

Quality parameters of organic cotton are good and appear to be similar between the different Hybrids used by the farmers. It would be required however to compare the quality parameters with conventionally grown cotton. This year that was not done because conventional farmers sold their cotton before samples could be taken. More detailed sampling on a larger scale would be required to arrive at final conclusions regarding the influence of varieties on lint quality parameters.

Organic farming requires high doses of organic manure. Farmers did not apply the required levels. Still they performed better than farmers who used only mineral fertilizers. The recommended nutrient management package was based on a yield projection of 6 quintals per acre whilst the average yield was only 2.5 quintals. It is quite possible that if the rains would not have stopped early, the lack of nitrogen (under present yield levels on average already short with 2.5Kg per acre) would have expressed itself in poor maturation of bolls and thus low yields. More work is needed to improve the availability of organic manures, particularly on manures with high nitrogen content like poultry, pig or sheep manure.

Intercropping with soybean and harvesting the produce seems to have a negative influence on yield of cotton. This effect could be caused by simultaneous high demand for nitrogen by both cotton and soybean (even when properly inoculated) or (in this case, 2004) it could be caused by competition for water. Farmers might conclude from the results that weeding and inter-cultivation are important to boost yields. Systematic testing of intercrop versus mono-crop, various intercrops and intercropping methods (1 row or more) and inter-cultivation should be undertaken to arrive at definite conclusions.

Pest load in 2004 was relatively low, due to the prevailing climatic conditions. Conventional farmers were facing more severe infestation of cotton bollworm than organic farmers according to their own, organic farmers' and our staff observations. Two factors might have saved the organic farmers. One, the predator population in organic fields was high. Second, early (when few first instar larvae were spotted) and proper spraying of HaNPV (early morning or evening, conditions of high humidity) on a large scale (all organic farmers did it) created an epizootic condition in the fields which controlled the development of the pest. These factors that controlled development of cotton bollworm could only be established because of the FFS approach, which educated farmers properly.

Ratoon crops of cotton are a source of pink bollworm as became evident from our limited study. Further study into the effect of ratoon crops on establishment of a pink bollworm population should be undertaken.

CASE STUDY: KNOWLEDGE TRANSFER IN CABBAGE IPM THROUGH FARMER PARTICIPATORY TRAINING IN DPR KOREA

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ABSTRACT

Yield losses in DPR Korean cabbage production are serious due to the main brassica insect pests, diamondback moth (*Plutella xylostella* L.) and small white butterfly (*Pieris rapae* L.). Traditional chemical pesticides have a limited impact on these pests because the diamondback moth has developed pesticide resistance. A new Integrated Pest Management (IPM) approach was therefore implemented in 2003. A preliminary IPM trial on five Cooperative Farms (Co-Farms) proved to be very successful and the feedback from participating farm managers was so positive that project partners decided to expand the area of IPM implementation. However, preparing for a large-scale shift in agricultural practices requires effective capacity building at each level of organization in the DPR Korean agricultural structure as well as a sustainable system for knowledge transfer within and between these levels. In 2004, a knowledge transfer concept was developed for the organization of training activities based on the experience from previous years. The scheme is adapted to the DPR Korean agricultural system and meets the requirements of an increased IPM implementation area. The thorough training of the Cabbage IPM Focus Group, a core group of scientists at the Plant Protection Institute, was continued. The knowledge about developing, implementing and monitoring IPM systems is thereby anchored in a scientific DPR Korean Institution, encouraging further independent initiatives for sustainable agriculture. At the same time these scientists were trained to become Master Trainers for the IPM implementation through the national extension service. Training material for the dissemination of IPM ideas was jointly developed by the project partners. A set of farmer participatory exercises was adapted to the DPR Korean context and evaluated at several Co-Farms. From the experiences gained with the training at the Co-Farm level and with the input of the Focus Group members, “A Farmer’s Manual for Cabbage IPM in DPR Korea” was developed. This comprehensive information compilation on cabbage IPM will support the knowledge transfer to the practitioner in the cabbage field.

With these measures the stage is set for a successful large-scale implementation of cabbage IPM leading towards strengthening food security in DPR Korea through sustainable production of healthy food.

INTRODUCTION

Cabbage crops are of high importance in the traditional diet and local economy of DPR Korea. The amount of cabbage distributed yearly within DPRK varies between counties and ranges from 60 to 400 kg per family. Cabbage is particularly important as a food source during winter, as it is made into kimchi, a long lasting pickle. The high nutritional value is not the result of a high caloric level but rather the content of vitamins and trace elements. Cabbage is a good source of vitamin C and B as well as iron. Processing cabbage into kimchi by lactic acid bacterial fermentation is very effective in preserving vitamin C and increasing levels of vitamin B. In DPRK, regular kimchi consumption is indispensable for a balanced diet, especially in winter. It is therefore not surprising that the demand for kimchi raw material, cruciferous vegetables, is consistently high. Particularly in urban centres of DPRK, this represents a real challenge for the vegetable farms. One of the consequences of this is that large areas in close proximity to cities are used for continuous brassica cultivation. As a result, problems arise such as decreasing soil fertility, the build-up of soil borne diseases, insect pest outbreaks, and a general negative impact on bio-diversity in the agro-ecosystem.

Surveys during the past years showed that in DPRK, extensive problems arise because of the damage from agricultural insect pests, particularly from the diamondback moth, *Plutella xylostella* Linnaeus (Lepidoptera: Yponomeutidae), and the small white butterfly, *Pieris rapae* L. (Lepidoptera: Pieridae). This is exacerbated by the occurrence of insecticide resistance in the former species. The acquisition of pesticide resistance by diamondback moth is a wellknown phenomenon and leads to pest control failures in cabbage crops throughout the world (Talekar and Shelton 1993; Waterhouse 1992). In some areas, economic production of cabbage has become impossible (Talekar 1992).

Integrated Pest Management (IPM) is one of the remaining strategies available to achieve sustainable and profitable cabbage production. Over several years, applied research, capacity building, and knowledge transfer for the development and implementation of cabbage IPM were conducted in close collaboration with Cooperative Farm (Co-Farm) managers, executives and workers. Different methods, tools and techniques were tested in the field and a strategy was developed (Fig. 1.) that met with general approval. The core components of the strategy are 1) transplanting clean seedlings to delay insect pest population build-up, 2) replacing chemical pesticides with bio-pesticides and thereby enhancing the impact of the natural enemy community, 3) releasing natural enemies, and 4) following the recommendations of a monitoring and damage threshold model for pesticide applications. The area of implementation of this strategy was gradually increased and the results achieved on the five Co-Farms involved have proven to be very promising compared to the traditional chemical pest management approach.

Capacity building through knowledge transfer plays a major role within this strategy for the implementation of cabbage IPM. During the first project years, training activities were aimed at forming a core group of scientists, called the “Cabbage IPM Focus Group”, at the Plant Protection Institute (PPI) Pyongyang.

1. Seedlings are **covered** with a synthetic fleece to prevent early pest damage; a **selective insecticide** is applied

→ the transplanting of clean seedlings delays the population build-up of pest insects

2. Application of the Swiss **monitoring and damage threshold model** to decide whether the field needs spraying or not

→ unnecessary sprays are eliminated, the impact of native natural enemies is strengthened on both target pest species

3. A **selective bio-pesticide** will be used (*Bt* product) to replace chemicals

→ pest populations are suppressed without killing natural enemies of the diamondback moth and the small white butterfly

4. Release of the **parasitic wasp** *Diadegma semiclausum* (natural enemy augmentation)

→ to enhance the suppression of the diamondback moth

Testing of the Pest Management strategy at five Co-Farms in DPRK

Bt **32'000 IU Switzerland**	***Bt*** **IU unknown DPRK**	**Traditional Chemical Pesticides**	**Untreated + releases of natural enemies**

	Bt **Switzerland**	***Bt*** **DPRK**	**Chemical**	**Untreated + NE**
Seedbed	● Seedbeds are covered with a layer of synthetic fleece to suppress flea beetle and aphid attack and additional two layers for protection against cold temperatures ● If there are still insect pest problems, seedlings must be treated with the product *Audienz* 0.03% prior to transplanting ● Treatment of seedlings must be similar to make sure that plant quality at the transplanting date is comparable; seedlings must be free of pests			
Transplanting and Cultivation Period	● The pest density in the field is assessed weekly with the damage threshold model ● The *Bt* product Delfin is applied following the recommendations of the model ● Formulation: 500g in 300 litres per ha (max. 4-5 treatments)	● The pest density in the field is assessed weekly with the damage threshold model ● The local *Bt* product is applied following the recommendations of the model ● Formulation: According to the producer (PPI)	● The monitoring strategy applied is defined at the beginning of the growing season ● A chemical product (e.g. *Deltamethrin*) is applied following the recommendations of the Ministry of Agriculture	● The pest density in the field is assessed weekly with the damage threshold model ● No pest management action by the farmer ● Formulation: 500 to 1000 natural enemies per ha (min. 2 releases)
	● Standardized farming practises (weeding, irrigation, fertiliser applications) are carried out in all the field plots throughout the growing season			
	● PPI Focus Group will carry out studies about pest density levels on a per plant basis and will determine the incidence of natural enemies			
At Harvest	● Yield per area will be estimated for each field plot individually (several replicates within each field plot). Care has to be taken to make sure that results from differently managed plots are not mixed up.			

Figure 1. Integrated pest management strategy for cabbage in DPRK.

Knowledge transfer for the Focus Group included various aspects of developing and implementing IPM in the model crop cabbage. Scientific aspects were covered during this time like the development of monitoring and damage threshold models, experimental design and analysis of IPM related field studies as well as technical aspects like the rearing of a diamondback moth parasitoid. At the same time, pilot farmer training activities started at five Co-Farms. Since the field testing of the newly developed IPM strategy was conducted at these farms, the cooperating farmers had to be trained in order to implement IPM in the test fields. The first on-farm training sessions focused on rather technical aspects like using the monitoring and damage threshold model and the application of a Bt bio-pesticide.

The overall aim for the training remained the same during the entire project activities: to build up a sustainable system for the knowledge transfer in DPRK with competent trainers transferring knowledge at the Co-Farm level. In this paper we describe the four prerequisites identified by the project partners for a successful, scaled up implementation of IPM in DPRK: 1) the development of a *knowledge transfer concept* on an institutional level in order to meet the requirements of an increased implementation area; 2) the continuation of *capacity building for trainers*; 3) the development and evaluation of *training exercises* for the transfer of basic ideas behind IPM in *Farmer Participatory Training (FPT)* and 4) the preparation of *didactic materials for knowledge transfer,* like e.g. tailor-made information in an adapted language and illustrations such as high quality pictures, for the on-farm implementation.

MATERIALS AND METHODS

COLLABORATION AND IMPLEMENTATION AREA

A joint initiative from the Plant Protection Institute (PPI) of the Academy for Agricultural Sciences (AAS) Pyongyang together with CABI Bioscience Switzerland and the Swiss Agency for Development and Cooperation (SDC) addressed the above mentioned problems in DPRK, with the aim of achieving a sustainable improvement of brassica production through the biological control of key pests in an Integrated Pest Management approach. The work concentrated on three different regions of DPRK: Pyongyang City, the Miru Hills area, and the South Hamgyong Province in the northern highlands (see Fig. 2.)

KNOWLEDGE TRANSFER CONCEPT

For the development of a knowledge transfer concept, the organizational and socio-political structure of DPRK agriculture had to be considered. In DPRK, a cooperative farming system is established where 1000 to 2000 people are living and working together on a farm covering an area of approximately 500 hectares (in the case of vegetable production). Crops that have to be grown are defined by the governmental planned quota, and usually more than 90% of the yield is fed into the public distribution system. Co-Farms, led by a manager and a chief engineer, are partitioned into work teams and sub-work teams with their respective leaders and engineers. Each work team specializes in the cultivation of a certain crop (a vegetable in the case of vegetable farms). Access to Co-Farms is limited and subject to permissions issued by the Ministry of Agriculture (MoA) for each farm and visit, respectively. The main project partner, the Plant Protection Institute, is one of the research institutes of the Academy of

Figure 2. Collaborating Cooperative Farms for the farm-wide implementation of the cabbage IPM strategy.

Agricultural Sciences (AAS), the latter being active in various fields of agricultural sciences. The traditional pathway for knowledge transfer in this system is a top down approach via the extension service of the MoA. Extension officers (one per Co-Farm) act as intermediaries of MoA and the farm. PPI traditionally is the advisory body for the MoA. It was necessary to develop a knowledge transfer structure that was adapted to these conditions.

CAPACITY BUILDING FOR TRAINERS

The capacity building for the Cabbage IPM Focus Group had to be continued during the 2004 project phase. Members of the Focus Group must, on the one hand, become experts in IPM, understanding the complex interactions in the agro-ecosystem and being able to cover scientific requirements for IPM implementation. On the other hand, they have to acquire didactic concepts and the pedagogic background to transfer their knowledge in an appropriate way, based on principles of adult education. The training in 2004 for the cabbage IPM Focus Group reflected these two parts. During the more scientific part, further training was provided to the Cabbage IPM Focus Group for the rearing of natural enemies of the diamondback moth. The Focus Group attended a one week training course “Statistics and Threshold Models” covering important aspects of experimental design and data analysis in the context of IPM implementation. As in previous years, the experimental design of the field testing, the monitoring program during the field season and the data analysis were jointly planned and carried out. Training of Trainers (TOT) for the Focus Group included the transfer of previously consolidated knowledge about crop rotation and, as a main activity, the planning, preparation, implementation and analysis of the “Introductory Training for the Implementation of Cabbage IPM" at the Co-Farm level. The IPM Focus Group implemented this training course in collaboration with a facilitator from CABI Switzerland on the four Co-Farms close to Pyongyang and independently on the highland Co-Farm.

FARMER PARTICIPATORY TRAINING (FPT)

The first steps of Farmer Participatory Training (FPT) during the first project phase focused on the transfer of basic project ideas about IPM and technical skills for the implementation of the strategy such as the introduction of the "Monitoring and Damage Threshold Model". Since the area managed for IPM in 2004 was extended and more farmers were involved in its implementation, the basic training was repeated for new participants and further training sessions were created. The first new session, carried out in spring, concentrated on crop rotation. The focus of these participatory learning sessions was on the importance of soil-born diseases and their impact on cabbage production. In summer 2004, the FPT field exercises were planned and evaluated at the five project Co-Farms. FPT focused first on the recognition of cabbage insect pests and the natural enemy complex controlling them. In a next step, the impact of using a broad-spectrum chemical insecticide compared to a specific *Bt* biopesticide was investigated. This knowledge is required for a better understanding of the IPM concept that is to be implemented. Courses were based on the following principle of adult education (see also Pontius *et al.* 2002): adults learn best from direct experience. Learning by doing adds to farmers' knowledge and experience, and improves their capacity as farm managers in a way that passive experience, like listening to extension messages, can not. Therefore, the most important components in the training were the exercises, where a logical sequence of small experiments, carried out by the farmers, supported the knowledge acquirement.

DIDACTIC MATERIAL FOR KNOWLEDGE TRANSFER

With respect to a broader dissemination and implementation of the cabbage IPM strategy, a manual was developed to provide a concise information compilation on cabbage IPM for the DPRK context in order to support the knowledge transfer. Step by step, inputs from all sides, farmers and scientists, and material adapted from already existing sources (Praasterink 2000; Van Mele *et al.* 2002; Vos 1998) were put together with the aim of developing a booklet that meets the needs of the practitioner in the cabbage field. The intended final product was a portable, weather-resistant booklet with all information necessary for cabbage IPM implementation to make sure that the knowledge can be transferred to where it is needed

RESULTS

KNOWLEDGE TRANSFER CONCEPT

The plan developed for knowledge transfer in DPRK (Fig. 3.) includes a pilot phase, which focuses on the thorough training of a core group of scientists at the PPI. The main aim is to anchor the capacity of developing, implementing and monitoring IPM systems in a scientific DPRK institution, thereby making sure that further IPM activities in other crops could be developed and implemented independently in the future. Parallel to the scientific capacity building, a TOT is run and subsequently PPI scientists gain their first experience in facilitating FPT at the Co-Farms. Over the course of the first phase and with the appropriate training, these scientists become IPM specialists and Master Trainers.

For large-scale dissemination of the IPM approach in cabbage, the farm extension officers are involved and will be responsible for the knowledge transfer on their farms. At the same time, county extension officers from the MoA join as trainers. Each county extension officer will later become a Master Trainer transferring knowledge to the farm extension officers in the respective county. In the beginning, the TOT is carried out by the core group of PPI scientists together with a CABI extension specialist with the aim that the latter makes her-/himself redundant.

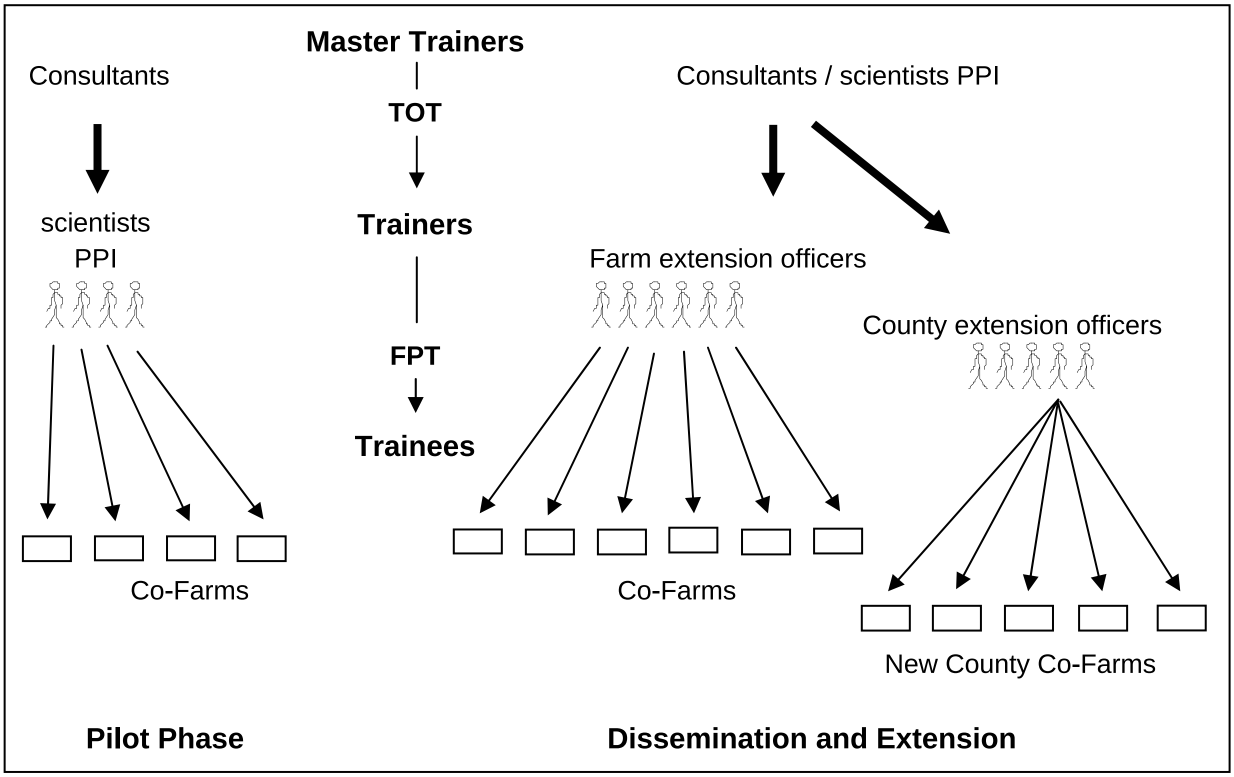


Figure 3. IPM knowledge transfer concept for DPR Korea.

CAPACITY BUILDING FOR TRAINERS

To assure that the Cabbage IPM Focus Group meets the requirements in this knowledge transfer concept, capacity building was continued on two levels: concerning scientific contents and FPT. The main training units, which were in the context of IPM implementation in 2004, are summarized in Table 1.

Extended CABI visits to DPRK in 2004 set the foundation for continuous knowledge transfer to the Cabbage IPM Focus Group and made it possible to discuss problems and IPM-specific questions. In addition to this continuous process, specified training activities were planned. The topic “Crop Rotation” was subject of discussion and dealt with in depth. Further training was provided for the important IPM component of rearing natural enemies.

Table 1. Topics and methods of the main training units in 2004 for the capacity building of the IPM Focus Group in chronological order. SCI = scientific topics; FPT = Farmer Participatory Training topics.

	Topic	**Methods**
SCI	Crop rotation: theoretical background.	Lectures and discussions as a preparation of FPT
FPT	Crop rotation: On-Farm Information sessions based on current Farm practices	Theoretical sessions with a participatory approach at all participating Co-Farms
SCI	"Statistics and Threshold Models"	One week training course in Pyongyang
SCI	Rearing of natural enemies	Practical work in greenhouse and rearing lab; working out guidelines and management practices during a one month consultancy
SCI	Design of the experimental set-up 2004 for the Co-Farm areas of IPM implementation	Discussion and deduction of the program, monitoring plan
FPT	Basic training for the cabbage IPM implementation at Co-Farms, training for the threshold model and technical training	Participatory training sessions at all Co-Farms carried out by the Focus Group
FPT	Implementation of the experimental set-up on at Co-Farm level	On-site support and backstopping, weekly at all Co-Farms
SCI	International Plant Protection Conference Beijing	Poster presentation at an International Congress, international contacts and exchange of experiences
FPT	Monitoring, the application of bio-pesticides and yield measurements	On-site support and backstopping, weekly at all Co-Farms
FPT	"Introductory Training for the Implementation of Cabbage IPM" on the 4 lowland Co-Farms	Training unit with participatory exercises; hands-on training, discovery learning
FPT	Introduction of the strategy, technical backstopping and FPT unit at the highland Co-Farm	Methods as above, carried out by the Focus Group
SCI	Data compilation, analysis and interpretation	Preparation of the data set 2004 for the presentation at the National Information Day

During a 10 day training course “Statistics and Threshold Models”, the Focus Group together with 16 other participants were able to improve their statistics skills. Other topics covered were an introduction to hypothesis testing and experimental design, and the knowledge gained could be applied to the IPM program by the Focus Group. Finally, attending the International Plant Protection Congress in Beijing facilitated international contacts with researchers world-wide. Moreover, four future Master Trainers from DPRK had the opportunity to exchange experiences in the field of IPM.

The main FPT activities for the Focus Group were directed towards the “Introductory Training for the Implementation of Cabbage IPM” to be carried out at the Co-Farms. The main steps of knowledge transfer were discussed and the didactics and pedagogical concepts behind the exercises were clarified. Together with the Focus Group, the training was prepared in terms of logistics and material. After the implementation in the Pyongyang area together with the consultant, the Focus Group had the opportunity to consolidate the acquired skills by implementing the training themselves at the highland Co-Farm.

FARMER PARTICIPATORY TRAINING (FPT)

The first new unit of FPT in 2004 concentrated on crop rotation at four Co-Farms (Hwasong, Changchon, Dangsan, and Mangyongdae). All the crops grown at the Co-Farms in one season were compiled with their respective areas and yields. Farmers identified soil-born diseases causing problems on their farms. Adverse effects like yield losses due to these diseases and due to the degradation of soil fertility associated with continuous cabbage cultivation were explained. The method of crop rotation was presented as a means to solve production problems. It became clear that, at the present time, the implementation of a three or four year crop rotation on a large scale is not feasible. Especially in autumn, the production of cabbage and turnip occupies almost the total surface area available for crop rotation in order to respond to the high cabbage demand for kimchi production. Nonetheless, Co-Farms are highly interested in the basic principles of crop rotation. As an important first step for further activities in Integrated Crop Management (ICM), all the Co-Farms will implement and test a small-scale, three-year rotation of vegetable crops.

The main FPT activity in 2004 was the “Introductory Training Unit for the Implementation of Cabbage IPM”, compiled and implemented with the trainers from the IPM Focus Group at the five project Co-Farms. At each Co-Farm, 15-30 participants, both farmers and work-team leaders, attended the training sessions. Training focused first on the recognition of cabbage insect pests and the natural enemy complex controlling them (Table 2). This was done with activities around a so-called “insect zoo”: insects collected in the field were identified and in a discovery learning approach (Fig. 4.) were dealt with to improve the farmers’ understanding of the cabbage arthropod community. Important steps of this part of the training were:

- The identification of “good” and “bad” insects (farmers’ friends and foes)
- The direct observation of predators killing pests
- The direct observation of parasitoids attacking their host

In a next step, the impact of using a broad-spectrum chemical insecticide compared to a specific bio-pesticide, *Bacillus thuringiensis kurstaki*, was shown. Important steps of this part of the training were:

- The effect of a chemical on the pest/natural enemy
- The effect of a Bt product on the pest/natural enemy
- Implications of the findings on the use of a damage threshold model

Table 2. Activities and objectives of the FPT, implemented at partner Co-Farms in 2004.

Activities	Objectives
Introductory Training for the Implementation of Cabbage IPM	
DAY 1	
Exercise 1: Insect zoo: collection and identification of insects	
Different insects are collected in the cabbage field. They are sorted and identified.	Curiosity about the arthropod community in the cabbage ecosystem is stimulated. Participants acquire basic skills in handling and identifying pests and beneficial insects.
Exercise 2: Insect zoo: studying predators	
Experiments are set up in order to find out about qualitative (who is eating whom?) and quantitative aspects (how much do they eat?) of predation.	Participants recognize predators and discover the importance of these beneficial insects in the cabbage field.
Exercise 3: Insect zoo: studying life cycles of pests	
Experiments are set up in order to observe the entire life cycles of lepidopteran pests. They are discussed after having reared different field collected instars.	Participants observe egg laying and subsequent development of pest instars in order to understand life cycles and phenology of these insects. This is an important prerequisite to anticipating pest problems.
Exercise 4: Insect zoo: studying life cycles of parasitoids	
Parasitoids are directly observed when laying eggs. The possible impact of this behavior is assessed in experimental caging.	Participants gradually become acquainted with different aspects of parasitism, starting with a general life cycle of a model parasitoid (Diadegma).
DAY 2	
Theory part 1: Discussion of the results from Ex2 / 3	
Results from the experiments are reported to the group. Examples of pest insect life cycles and pest - predator interactions are summarized. Additional theoretical background information is provided.	With short presentations by the participants and the subsequent discussion including theoretical inputs by the facilitator, new findings about pests and predators are structured and consolidated.
Exercise 5: Comparison of biological and chemical pesticides used in caterpillar control	
The action of chemical broad - spectrum pesticides on pest insects is compared with the action of a specific Bt product. In cage experiments, Plutella and Pieris larvae feed on leaves treated with a) Bt, b) a chemical insecticide, or c) nothing.	Based on the previously acquired knowledge about pest - natural enemy interactions, participants can imagine to what extent "natural" control is decreased, if broad-spectrum chemical insecticides are used. They are motivated to conserve natural enemies.
Exercise 6: Effects of pesticides on natural enemies	
The set-up from Ex 5 is used to assist the discovery of the survival of different natural enemies when Bt is used.	As above (Ex 5).
Exercise 7: Parasitoids on the small white butterfly and the diamondback moth	
Pest pupae (and mature Piers larvae) and parasitoid cocoons are collected and identified. Parasitoid life cycles are repeated. The parasitism level in the field is assessed for both pests, and its impact is analyzed.	Participants become more familiar with the most prevalent parasitoid species of Plutella and Pieris. They learn more about parasitism and its effect on the two main pest species in cabbage.
Theory part 2: Brief analysis of Ex 5 / 6	
First results are discussed and experiments are assigned to participants for further observations.	

Table 2. Activities and objectives of the FPT, implemented at partner Co-Farms in 2004 (continued).

Introductory Training for the Implementation of Cabbage IPM	
Activities	**Objectives**
DAY 3	
Theory part 3: Discussion of the results from Ex 5 / 6	
The outcome of the experiments is reported to the group and discussed.	The initiated dialogue amongst participants creates awareness about pesticide associated problems.
Exercise 4: Evaluation of the caging experiment	
Parasitized and unparasitized diamondback moth pupae are counted and the influence of parasitism on the Plutella population is discussed.	Participants discover the efficacy of a parasitoid and find out about possibilities to conserve adult parasitoids in the cabbage field
Theory 4: Implications on the use of the damage and threshold model	
Based on the new knowledge acquired during the previous training days, factors which influence the extent of damage done in the field by a certain number of pests are listed. Theoretical examples are given on how to consider these factors (parasitism, predation, crop stage, pest stage) and the weather situation into a model-based decision making process.	Participants consolidate their knowledge by its application in the new context of threshold model implementation. They are motivated to translate a refined threshold model approach into practice

Figure 4. Distinguishing between "good" and "bad" insects through discovery learning at the Co-Farm level.

The Cabbage IPM Focus Group facilitated the training unit helped to successfully implement this training component. It became obvious that the Focus Group does not only have a broad knowledge about IPM and its principles, but is also capable of transferring the acquired knowledge in IPM to other people. The participants showed high interest and commitment during the courses. From the remarks and questions made by the participants it became clear, that with this training, a vital support for IPM was induced.

DIDACTIC MATERIAL FOR KNOWLEDGE TRANSFER

During the cabbage IPM implementation, the project partners realized that, despite the wealth of information available for IPM and cabbage, there was a need for a manual that provides concise information on cabbage IPM, is written in the Korean language and adapted to the local context. Descriptions and illustrations of major cabbage insect pests and diseases had to be included, as did ideas on how knowledge in IPM needs to be transferred. The first step in the manual's development was a joint decision about its contents. Considering that IPM as a plant protection strategy is new for most cabbage producers in DPRK, an introduction into this approach and its tools was seen to be essential. Farmers should recognize major cabbage insect pests, natural enemies and diseases in the field. Therefore, a section of fact sheets was considered to be indispensable for the manual. It was also decided that a segment covering the discovery-learning exercises should be included in order to facilitate knowledge transfer. When the first draft of the English version of the manual was written, the Cabbage IPM Focus Group reviewed it and adapted it to the local context. Pictures of cabbage pests and diseases, as well as pictures from participatory training, were taken during the whole season. In order to illustrate the IPM component, an artist from the AAS made the drawings. After reviewing the English text version once more, it was translated into Korean while the layout for the English version of the manual was completed.

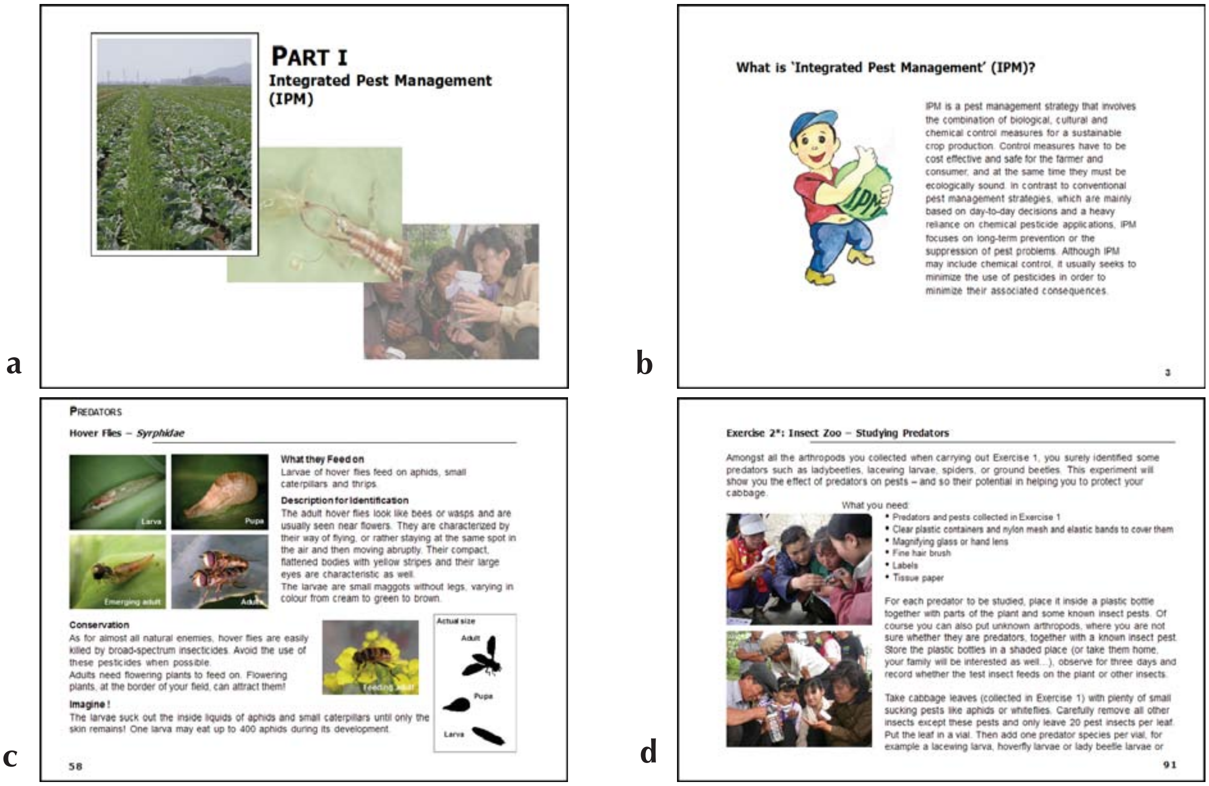
The English version of "A Farmer's Manual for Cabbage IPM in DPRK" is now available. It consists of 120 pages in a loose leaves system in a ring binder. It has a handy C6 format and the water-repellent paper and print allow farmers to take it to the field (some extracts are printed above).

Part one (Figs. 5a + b.) gives a rather general overview of the IPM approach and its methods and tools. Specific advice is included for the cultivation of cruciferous vegetable crops in DPRK. These different ideas can be implemented and tested in the cabbage field according to the prevailing situation.

The successful implementation of IPM requires fundamental skills and understanding of the relatively complex interactions of organisms in the agro-ecosystem. This in turn demands knowledge about the components of the interactions. Part two (Fig. 5c.) is therefore dedicated to the identification and understanding of pests, diseases and natural enemies in the cabbage field.

Part three (Fig. 5d.) takes into account that the knowledge transfer of a complex matter like IPM is challenging, but nevertheless essential for its implementation. The manual provides a training curriculum based on discovery learning exercises for the introduction of cabbage IPM at the Co-Farm level. The unit was carried out and evaluated on five Co-Farms in 2004 and proved to be very successful. As with the IPM approach itself, the knowledge transfer of its contents has to be flexible and adaptable to specific situations. Therefore some additional exercises were compiled for trainers and farmers covering topics such as the spread and effects of pathogens, plant compensation studies and cage exclusion of natural enemies.

Within the framework of the National Information Day for Cabbage IPM Implementation in DPRK, the English version of the manual was presented to the audience. Options are currently being explored to print the Korean version in Pyongyang and joint efforts will ensure that a high-quality manual in the Korean language will be available in the future for the dissemination of cabbage IPM on a large scale in DPRK.



PART I
Integrated Pest Management
(IPM)

What is 'Integrated Pest Management' (IPM)?

IPM is a pest management strategy that involves the combination of biological, cultural and chemical control measures for a sustainable crop production. Control measures have to be cost effective and safe for the farmer and consumer, and at the same time they must be ecologically sound. In contrast to conventional pest management strategies, which are mainly based on day-to-day decisions and a heavy reliance on chemical pesticide applications, IPM focuses on long-term prevention or the suppression of pest problems. Although IPM may include chemical control, it usually seeks to minimize the use of pesticides in order to minimize their associated consequences.

3

PREDATORS

Hover Flies – *Syrphidae*

What they Feed on
Larvae of hover flies feed on aphids, small caterpillars and thrips.

Description for Identification
The adult hover flies look like bees or wasps and are usually seen near flowers. They are characterized by their way of flying, or rather staying at the same spot in the air and then moving abruptly. Their compact, flattened bodies with yellow stripes and their large eyes are characteristic as well.
The larvae are small maggots without legs, varying in colour from cream to green to brown.

Conservation
As for almost all natural enemies, hover flies are easily killed by broad-spectrum insecticides. Avoid the use of these pesticides when possible.
Adults need flowering plants to feed on. Flowering plants, at the border of your field, can attract them!

Imagine !
The larvae suck out the inside liquids of aphids and small caterpillars until only the skin remains! One larva may eat up to 400 aphids during its development.

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Exercise 2*: Insect Zoo – Studying Predators

Amongst all the arthropods you collected when carrying out Exercise 1, you surely identified some predators such as ladybeetles, lacewing larvae, spiders, or ground beetles. This experiment will show you the effect of predators on pests – and so their potential in helping you to protect your cabbage.

What you need:

- Predators and pests collected in Exercise 1
- Clear plastic containers and nylon mesh and elastic bands to cover them
- Magnifying glass or hand lens
- Fine hair brush
- Labels
- Tissue paper

For each predator to be studied, place it inside a plastic bottle together with parts of the plant and some known insect pests. Of course you can also put unknown arthropods, where you are not sure whether they are predators, together with a known insect pest. Store the plastic bottles in a shaded place (or take them home, your family will be interested as well...), observe for three days and record whether the test insect feeds on the plant or other insects.

Take cabbage leaves (collected in Exercise 1) with plenty of small sucking pests like aphids or whiteflies. Carefully remove all other insects except these pests and only leave 20 pest insects per leaf. Put the leaf in a vial. Then add one predator species per vial, for example a lacewing larva, hoverfly larvae or lady beetle larvae or

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Fig. 5. Extracts from “A Farmer’s Manual for Cabbage IPM in DPRK”.

CONCLUSIONS

The experimental implementation of the cabbage IPM strategy in DPRK showed promising results. White cabbage yield has been increased by up to 40% compared to the traditional chemical pest management approach. Unnecessary chemical treatments were avoided in Chinese cabbage since IPM was adopted. In 2004 the IPM strategy was extended to nearly 150 ha representing the majority of the white cabbage cultivation at the five Co-Farms. For the transition from an experimental to a field-testing scale and ultimately to common agricultural practice, capacity building through knowledge transfer is of highly important.

- A *knowledge transfer concept* was developed and adapted to the local agricultural system. A core group of PPI scientists will become Master Trainers through appropriate training and will then facilitate the central TOT involving farm and county extension officers as trainees. The option will also be available for county extension officers to become Master Trainers for county-based TOTs.

- To assure that the core group of PPI scientists meets the requirements in this knowledge transfer concept, *capacity building* was intensified. Through the planning and implementation of training units at Co-Farms, this group acquired the necessary background for FPT and subsequently acting as Master Trainers in the national extension service. Training continued at the same time for scientific aspects of developing, implementing and monitoring IPM systems, encouraging further independent initiatives for sustainable agriculture.
- Training material for the dissemination of IPM ideas through *FPT* was jointly developed by the project partners. A set of FPT exercises has been designed, adapted to the DPRK context and evaluated at several Co-Farms. The participants showed high interest and commitment during the courses. From the remarks made and questions asked by the participants it became clear that this training stimulated awareness and support for IPM.
- Based on training experience at the Co-Farm level and with additional input from the Focus Group and consultants, “A Farmer’s Manual for Cabbage IPM in DPRK” was developed. This didactic material will be used to support knowledge transfer to field-level personnel.

These measures have created the framework for a successful large-scale implementation of cabbage IPM leading towards strengthening food security in DPRK through sustainable production of healthy food.

ACKNOWLEDGEMENTS

The authors wish to thank the farmers, work team leaders and managers of Mangyongdae, Dangsan, Hwasong, Changchon and Pujon Co-Farms (DPR Korea) for their support during all project phases. Wade H. Jenner (Carleton University, Ottawa, Ontario, Canada) is acknowledged for reviewing the manuscript. The work presented in this paper has been funded by the Swiss Agency for Development and Cooperation

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IMPLEMENTATION OF BIOLOGICAL CONTROL IN GLASSHOUSE HORTICULTURE IN THE NETHERLANDS

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INTRODUCTION

The use of biological control in Dutch glasshouses has increased tremendously in the second half of last century. Integrated pest management (IPM) is practiced on a large scale in all main vegetable crops. In glasshouse ornamentals IPM is more complicated, but at the end of last century biocontrol was applied in more than 10% of the area with ornamental crops (LTO Nederland, vakgroep Glastuinbouw 2003; van Lenteren 2000). The expansion of the glasshouse area subjected to biocontrol has, however, now come to a halt. In some crops, like gerbera, the number of biocontrol species released is even declining seriously. In general growers mention the following reasons for discontinuing biocontrol: disappointing results with natural enemies, new pesticides which made biocontrol 'unnecessary', the lack of selective pesticides against new pests and the restriction of other selective pesticides.

There are many different factors determining the degree of success of biocontrol measures and the composition of an IPM strategy. Implementation of IPM is complex not only in technical, but also in socio-economic sense (for an overview of motives for growers whether or not changeover to IPM, see de Buck and Beerling, in press). Hence, custom-made IPM strategies are required.

The traditional co-operation between Research, Extension and Education took care of the development and implementation of (new) knowledge, but this so-called triptych fell apart in the nineties due to changes in the market (see de Buck and Beerling, in press). Stakeholders are now following their own strategies and there is a lot of disagreement between for instance growers, environmental organizations and supply chains. This hampers the transition to a sustainable production system.

The traditional 'trend-setter model' is not helpful in the diffusion of complicated innovations without a clear value to growers, such as biocontrol and IPM. A new system of knowledge transfer is needed that meets the interests, visions and strategies of the stakeholders.

Recently in the Netherlands two types of networks have been developed based on the principle of collaboration of all parties: 'growers' networks' and 'socio-technical networks' (STNs). Both types of networks aim to generate interactive knowledge and are formed in order to speed up the innovation process. These networks are discussed hereafter, but first the role of the Dutch government in the transition to sustainable horticulture is described.

LEGISLATION

The Dutch government aims to make crop protection more sustainable: by 2010 the environmental 'burden' should be reduced by 95% when compared to 1998. The government regards IPM as the approach to achieve this reduction and proposes that all growers have switched to IPM by 2010. She has taken on the responsibility to promote knowledge on and implementation of IPM (Dutch Ministry of Agriculture, Nature and Food Quality 2004).

By funding a research program the government facilitates the development and implementation of IPM. This program comprises fundamental and applied research, in which not only solutions to single pest problems are sought, but also interactions of control measures and the integration into complete control strategies are taken into account (see e.g., Dik *et al*. 2004; Pijnakker *et al*. in press). Furthermore, much attention is given to the implementation of (new) knowledge and to the process of transition to sustainable agriculture, for which growers' networks and socio-technical networks have been developed (see hereafter).

GOOD CROP-PROTECTION PRACTICE

In 2003 the government, the growers' organization (LTO), the association of crop protection suppliers (Agrodis), the association of the Dutch agrochemical industry (Nefyto), and organizations for drinking water (VEWIN) and water boards (UvW), reached an agreement whereby they all will be working on reducing the environmental pollution caused by pesticides with at least 95 per cent by 2010 (Agreement on Crop Protection). As a consequence, a Royal Ordinance on the principles of IPM was drafted, which determines that all growers should work according to the principles of 'good crop-protection practice' and that the use of pesticides is reduced to the very minimum necessary to control pest populations below the economic-damage threshold (Besluit beginselen geïntegreerde gewasbescherming 2004). The definition of good crop-protection practice depends on the feasibility of crop-protection measures for 80-90% of the growers of a particular crop, and may change in time. Growers working according to EUREP-GAP guidelines of the European retailers and their suppliers will meet the demands of the Ordinance without difficulty.

Insight into measures of good crop-protection practice must be given in a crop-protection plan and a logbook. The crop-protection plan should address measures with respect to prevention, establishment of the necessity of control, non-chemical control measures, and chemical control measures. Deviations to the plan should be written down in a crop-protection logbook. The plan and logbook are mandatory from 2005 onwards, but at present growers are not yet forced to comply with the crop-protection plan or implement specific crop-protection measures. The aim of a crop-protection plan is to raise consciousness and induce behavioral change in growers.

BEST CROP-PROTECTION PRACTICE

Due to new knowledge and understanding the transition into an even more sustainable crop protection should be a continuous process. To stimulate this process, the government requested researchers to draw up so-called 'best practices' of crop-protection (for glasshouse horticulture: Dik and De Haan 2004). 'Best practices' are the most important crop protection measures that will potentially contribute to a reduction in the environmental burden. Examples are the use of natural enemies for pest control, more efficient pesticide application techniques and screening windows to keep pests out. 'Best practices' are not yet generally implemented and practical experience is often lacking. Almost all 'best practices' face obstacles that need to be removed before implementation is possible, or need further study. Therefore, 'best practices' are not mandatory for the growers, but this set of potential measures is a guide for research funding organizations (like the government) and growers' organizations. Both 'good practices' and 'best practices' will change over time due to advancing possibilities and understanding, thus accomplishing a stepwise improvement of IPM.

IMPLEMENTATION OF IPM BY NETWORK FORMATION

Recently in the Netherlands two types of networks have been developed based on the principle of collaboration of all parties: 'growers' networks' and 'socio-technical networks' (STNs). Both networks mobilize all decisive stakeholders for the implementation of sustainable horticulture. These parties include growers themselves, suppliers and buyers, knowledge workers (from Wageningen University and Research) and advisors (private extension service and crop protection suppliers), sector organizations, producers' organizations and government. Growers' networks have a practical approach and are focused on the changeover to IPM and the awareness of the necessity to implement the latest feasible 'best practices'. The socio-technical networks aim at a practical implementation of an innovation agenda for sustainable development. This agenda is fully decided on by growers and other stakeholders, without a specific focus beforehand.

GROWERS' NETWORKS (FARMING WITH FUTURE)

The heart of the network. The heart of the growers' network (project 'Farming with future') is formed by a group of 6 to 8 growers who meet several times a year (Fig. 1). These groups are lead by researchers (crop protection specialists), trained in managing processes of change. At the moment there are five crop-related networks: for cucumber, for tomato, for rose, for chrysanthemum and for potted-plants. Each group consists of different types of entrepreneurs, *i.e.* growers with different attitudes towards biocontrol and choice of crop protection strategy, but with a common awareness of the need to change to IPM. The growers are from different regions of the country and are an authority within their crop, although not only trend-setters are chosen. The choice of growers is made in consultation with the growers' organization LTO. Within the group discussions about 'best practices', (new) control measures and strategies are stimulated, giving special attention to biocontrol and natural pesticides. In this way growers learn from each other and also get acquainted with new strategies. The flow of information is not directed in one way, *i.e.* to the grower, only. The growers'-networks project ('Farming with Future') is embedded within the governmental research program mentioned before, which facilitates feedback to research. Questions and information on obstacles for 'best practices' for example, flow back to research institutions, thus stimulating new research and demonstration projects.

Before the start of the crop (or a year) the grower, assisted by his regular crop protection advisor (private extension service or crop protection supplier) and using input of the latest knowledge from the researcher, designs a crop protection plan. The crop-protection strategy and corresponding plan remain the choice of the grower and will therefore differ between growers. At the end of the cropping season (or a year) the plans are evaluated individually and within the group. To help the evaluation of the chosen strategy, growers register the input of chemical and natural pesticides, natural enemies, and also costs involved (in time and money), as well as output, *i.e.* yield. Using these figures the researcher calculates the environmental impact and the economic results. For the following year, a new plan is made, based on the experiences of the previous year and with new input from research and consultants, thus accomplishing a stepwise implementation of 'best practices'.

Other growers. Next to coaching the individual growers and the networks, much effort is put into the dissemination of results to other growers and convincing them to also implement the strategies that prove to be feasible. For this purpose co-operation (in communication) is sought with stakeholders surrounding the growers (see Fig. 1), thus creating a solid basis for the implementation of new knowledge. Focus is on distribution of technical information as well as on increasing acceptance.

Communication with growers outside the networks occurs in numerous ways and often in co-operation with the extension division of the National Sector Organization 'LTO', which started a communication project called 'Strategist' for IPM in glasshouse ornamental crops. Communication involves leaflets with information about the major pests and diseases for each crop, publications and interviews in growers' magazines, an internet site, presentations at national and regional meetings organized by growers' association, and excursions to participating growers.

As stated before, the implementation of IPM is complex. Straightforward facts, like the efficacy of a (microbial) pesticide, are picked up easily by growers and find their way quickly via study groups and other contacts with and between growers. Knowledge about natural enemies, and more particularly IPM strategies, are never straightforward and require guidance when implemented. In the first place, this means that stakeholders surrounding the growers, in particular the advisors should acquire knowledge. For the large group of 'followers' amongst the growers, crop advisors are even the main knowledge providers in crop protection and play an important role in the crop-protection strategy the grower chooses. The advisors may be independent (e.g., the privatized extension service 'DLV'), but more often they represent a crop-protection supplier. These companies vary in state of knowledge and have their own - more or less sophisticated - IPM strategies. A complicating factor is that the natural aim of these companies is to sell as many products (biological or chemical) as possible to as many customers as possible.

Participation of crop-protection suppliers in this innovation process is sought in several ways (Fig. 1). Advisors from different companies advice the growers within the network. These advisors are directly involved in the compilation and evaluation of the crop-protection plan of 'their' grower. Also, bilateral meetings of research and crop-protection suppliers and other companies involved in advising growers are organized to discuss strategies and research results. The advantage of this one-to-one approach is that the companies then discuss their strategy with the researchers more openly than when competitive companies are present. Awareness of these important stakeholders of the necessity and feasibility of IPM enhances the adoption of biocontrol and a custom-made IPM strategy.

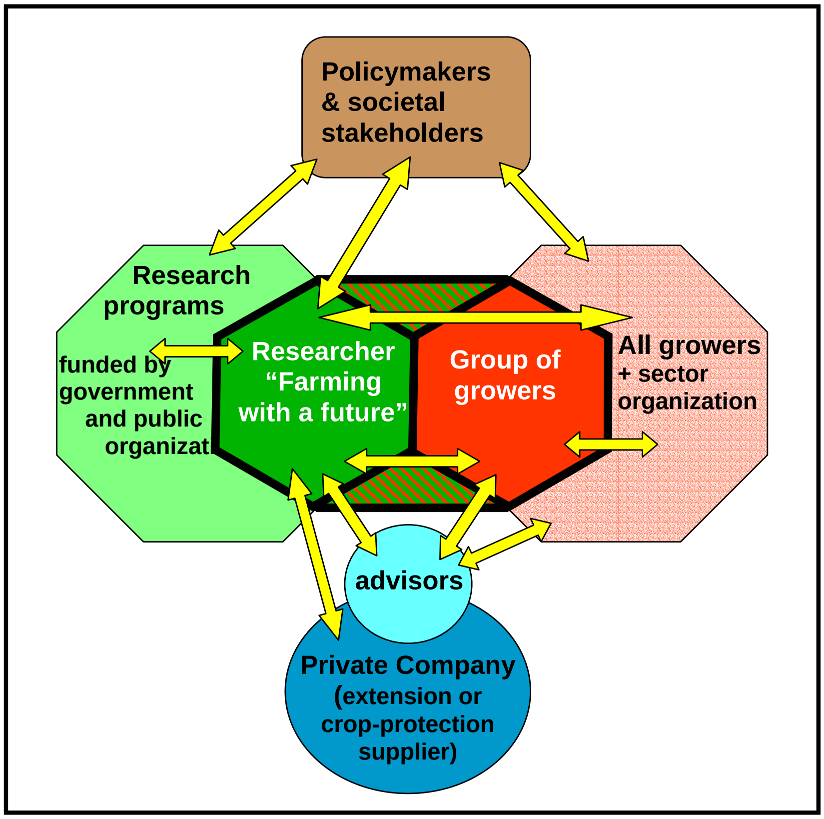

Figure 1. Schematic presentation of a growers' network in glasshouse horticulture and the direct and indirect interactions between the project 'Farming with Future' and stakeholders.

Policymakers and societal stakeholders. Policymakers and societal stakeholders also play an important role in the changeover to a more sustainable crop protection because they can stimulate the changeover, set the goals and determine the framework in which it should take place. In a low-lying country full of waterways and lakes like The Netherlands, regional water boards, drinking water companies and environmental organizations highly influence the present regional and national policy on crop protection. Policy officials and politicians are also influenced by discussions with growers' organizations and organizations of biocontrol producers, chemical industries and suppliers, for instance as in the Agreement on Crop Protection.

The project 'Farming with future' aims to provide policymakers and societal stakeholders a realistic view of the present and future (im) possibilities of biocontrol and IPM and to stimulate discussion among the stakeholders. For this purpose policymakers and societal stakeholders regularly receive a newsletter and also bilateral meetings as well as round-table discussions are organized.

SOCIO-TECHNICAL NETWORKS

A socio-technical network (STN) is another method to speed up an innovation process by collaboration of stakeholders. The aim of an STN is 1) to intelligently use the forces of sustainability (also called 'People, Planet and Profit') for speeding-up the innovation process to sustainable plant production, and 2) better utilize 'surrounding partners' to induce entrepreneurship. The 'technical part' of a STN consists of one or more specific innovations in the field of technical, knowledge, (consumer-) product or sector development. In addition to Profit, the innovations should improve the aspects of Planet and People.

A STN is primarily based on the capacity of growers to innovate. Growers and stakeholders can be activated by meeting their interests, strategies and visions. The participants formulate a common vision on sustainable development of the sector and the problems that they want to work on themselves. They decide on an innovation agenda for sustainable development, without a specific focus beforehand. Hence, in a STN, the development (for instance of knowledge) is driven by demand.

Secondly, a STN aims at a consensus within the intermediate groups, such as producers' organizations, NGO's and government. Without consensus of intermediates from the start, there is an evident risk that the development and the dissemination of the innovation will become frustrated.

A methodology has been developed to create a STN (Buurma *et al.* 2003; De Buck and Buurma 2004). It comprises three consecutive steps: 1) interviewing stakeholders, 2) identifying potential coalitions between stakeholders, and 3) composing a collaboration agreement. These steps are explained hereafter and illustrated with the case of formation of a STN in the second largest cut-flower sector in the Netherlands: the cut-chrysanthemum sector.

Interviewing stakeholders. A STN requires participation of supporters of values that are related 1) with market (to generate Profit), 2) with society (to care for People and Planet) and 3) with human resource (to induce entrepreneurship and innovative power). A value triangle (Fig. 2) is a tool to identify the mutual positions of the stakeholders. Firstly, stakeholders professionally involved in the innovation are identified for each of these values. These stakeholders are interviewed in-depth, focusing on four items: 1) the values of the respondents, 2) their position in the professional environment, 3) their vision on strategic development and the relevance for themselves and 4) the barriers that hamper its implementation. The interviews do not just focus on a specific theme, *i.e.* IPM, but address the inter-relationships with other important issues as well.

From the interviews of stakeholders within the cut-chrysanthemum sector and during a workshop (see later) four developmental pathways for transition towards sustainable production were apparent, which were visualized in a mind landscape (Fig. 3). Adherents of development 1 urged on the transition from chemical pest control to biocontrol and IPM.

Further knowledge has to be developed on IPM strategies suitable for cut-chrysanthemum. Pest control practices need to be revised, as organisms increasingly become resistant. The decrease in the number of registered pesticides is a result of severe government regulations with respect to environmental protection, combined with the relatively small market demand for pesticides in Dutch glasshouse horticulture as a whole.

Another group believed that cropping systems on mobile benches in artificial substrate are indispensable for a sustainable chrysanthemum sector (development 2). Firstly, the new system increases production efficiency and secondly the use of artificial substrate would eliminate problems with soil-borne pests and diseases. The use of mobile benches offers possibilities for pest management and product development (small, separately manageable units). Results (a better productivity) should be available on the short term, as economic continuity of the chrysanthemum sector is at stake.

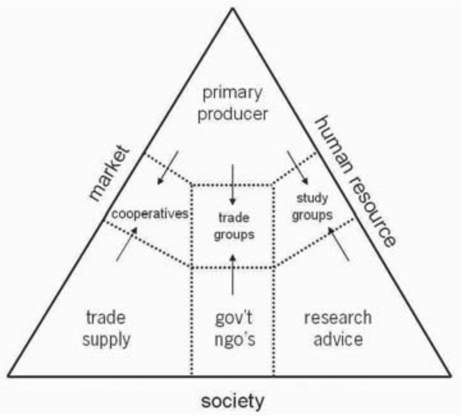

Figure 2. Value triangle: the position of stakeholder groups in the agricultural sector between values that are related with market, human resource and society.

Some stakeholders urge the necessity of more collaboration in the knowledge system: the private companies, research and extension organizations and sector organizations need each other to develop and disseminate IPM in the chrysanthemum sector. This point of view can be considered as network development (development 3).

Adherents of development 4 believe that the market position of the product (the chrysanthemum flower) needs to be improved. The negative image of chrysanthemum as a 'poisonous flower' and its character of cheap mass produce hamper this.

Identifying potential coalitions. Based on the interviews, the next step is the identification of potential coalitions in the mind landscape. Some conditions for a successful coalition are: compatibility of individual strategic solutions, innovative power and a balanced set of individuals' values. The coalition is formed around a central person (like the formation of a cabinet, headed by a Prime Minister) with authority, goodwill, having the willingness and the ability to co-operate. This central person has the mandate of intermediate groups.

In the cut-chrysanthemum sector, changing over to a cropping system in artificial substrate on mobile benches looked promising for development towards profitability and ecological sustainability. Representatives of this developmental pathway operated with confidence, had innovative power and found a link with IPM knowledge development (1 in Fig. 3) evident. Moreover, there were already serious research efforts on development of an IPM strategy for Dutch cut-chrysanthemum production, with involvement of several stakeholders. Therefore, a STN around system development (2 in Fig. 3) and not directly around IPM knowledge development was initiated (De Buck and Buurma 2004).

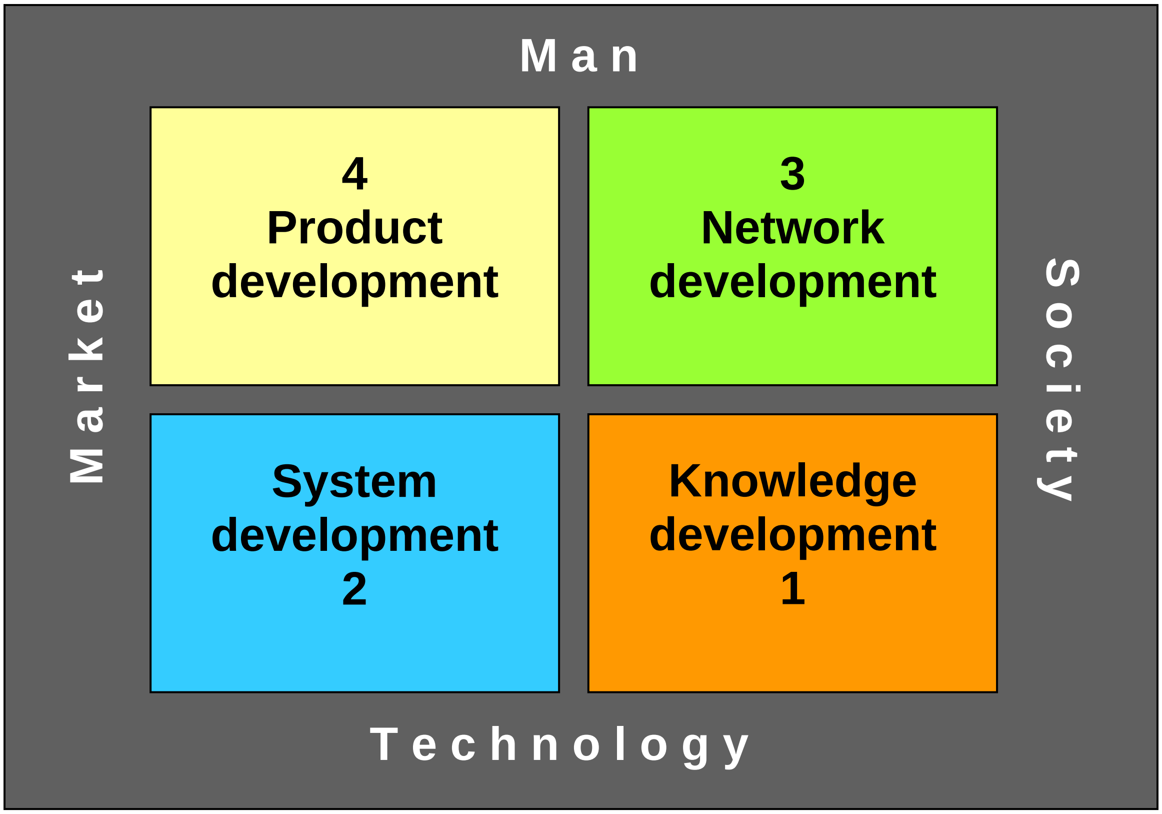

Figure 3. Mind landscape: the four developmental pathways for system innovation in chrysanthemum.

The chairman of the National Crop Committee (in Dutch: Landelijke Gewascommissie Chrysant, an NGO), a chrysanthemum grower himself, was appointed as the central person of STN. Through his position as chairman and grower, he was able to create support for the innovation throughout the sector. As a first activity of the STN a meeting was organized with all leaders of IPM initiatives in cut-chrysanthemum, including ‘Farming with future’ (chrysanthemum growers’ network), ‘Strategist’ (communication project), a crop-protection producer and its supplier (carrying out a trend-setting IPM project), and a researcher involved in fundamental and applied aspects of IPM in chrysanthemum. This meeting has contributed to a close collaboration between all current projects on IPM in the chrysanthemum sector. In fact, this initiative can be considered as a first step in network development (3 in Fig. 3).

Composing a collaboration agreement. In the final step a collaboration agreement is composed, reflecting the intentions and commitment of the participants in this STN to implement a specific innovation development. An appropriate action for this is a workshop with all interviewed stakeholders in which future images are outlined and a plan is designed, necessary to reach one or more of these desired future images.

Concerning the STN in the chrysanthemum sector, a strategic document on sector development on behalf of the National Sector Organization for Horticulture was drafted (De Buck and Buurma 2004). This document elaborates sustainable development as a combination of the four developmental pathways. For the approval and funding of RandD proposals in a specific sector in horticulture the National Crop Committee (representing the sector; LTO) advises the National Sector Organization for Horticulture (in Dutch: Productschap Tuinbouw, an NGO). Both organizations require support from the sector for their decisions. The sector will support those decisions that lead to sustainable sector development in terms of Profit as well as People and Planet.

As a conclusive step, a workshop was held for the stakeholders who had been interviewed. In this workshop, the participants agreed upon the four developmental pathways required for sustainable horticulture (Fig. 3). There was full support for the fact that IPM should be incorporated in the development of the new production system as soon as possible. The participants were aware of the need for support from the whole sector for such extensive changes (system innovation) in cut-chrysanthemum production. Furthermore, the participants concluded that better expertise in pest control is necessary, but acknowledged that this was covered by recent initiatives, *i.e.* the projects ‘Strategist’ and ‘Farming with future’. Finally, the transition to a new production system and IPM should be used to enhance product and market development of chrysanthemum (development 4 in Fig. 3).

CONCLUSIONS

The growers’ network – for example those of the project ‘Farming with future’ - is an appropriate method for participative and stepwise learning, and enables the implementation of complicated knowledge about IPM and biocontrol. A Socio-technical network (STN) appears to be a useful tool and an appropriate method for stakeholders to decide on an innovation agenda for system innovation, such as the implementation of biocontrol and IPM. It is activated by the innovative capacity and common interests, strategies and visions of growers.

Socio-technical networks and growers’ networks mobilize all decisive stakeholders for the implementation of sustainable horticulture. The interrelationship between the two types of networks on a specific crop is evident. In the case of the cut-chrysanthemum sector, the Growers’ network on IPM stands for the dimension of knowledge development of the STN on sustainable sector development. The Growers’ network enhances the STN as it is driven by stakeholders rather than by researchers. Hence, these networks contribute to a new knowledge system as a successor for the traditional triptych of Research, Extension and Education in the Dutch agricultural sector. Briefly, in a modern knowledge system based on these networks, the focus has shifted from critical success factors to critical success actors. The challenge for the coming years is to spread biological control and new IPM strategies that are developed and applied in the networks, towards the rest of the growers in the sector.

ACKNOWLEDGEMENT

This work is funded by the Dutch Ministry of Agriculture, Nature and Food Quality.

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COMPATIBILITY OF INSECT-RESISTANT TRANSGENIC PLANTS WITH BIOLOGICAL CONTROL

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SESSION 7 INTRODUCTION

Insect resistant transgenic crops that express genes derived from the soil bacterium *Bacillus thuringiensis* (*Bt*) are grown on a steadily increasing area worldwide since their first introduction in 1996. In 2004, *Bt*-transgenic plants were grown on 22.4 million ha worldwide (James 2004).

Bt (Cry) toxins are known to have a very specific mode of action and plants commercialized today target either lepidoteran pests, including stem borers in maize and the budworm-bollworm complex in cotton, or coleopteran pests including the Colorado Potato beetle, *Leptinotarsa decemlineata*, and corn rootworms, *Diabrotica* spp. (James 2004 ; Shelton *et al.* 2002;). *Bt*-crops should not be viewed as silver bullets to solve all insect pest problems but should be regarded as just another tool to help manage certain pest populations in an economically viable and environmentally safe manner.

While in some areas of the world, especially in Europe, the debate is focusing on the potential environmental risks that could come with the large scale deployment of *Bt*-transgenic crops, other countries are investing time and efforts to evaluate how these crops can be implemented in integrated pest management (IPM) programs for sustainable pest control. One factor of particular interest in this respect is the impact of *Bt*-transgenic crops on non-target organisms that fulfil important ecological and economic functions within the agricultural system. This includes pollinators and biological control agents such parasitoids and predators that are of importance for natural pest regulation. Since *Bt*-transgenic plants express proteins with insecticidal properties, their effects on non-target arthropods should be assessed within an ecological risk assessment prior to commercialization of the crop (Conner *et al.* 2003; Dutton *et al.* 2003). Research to date on commercialized *Bt* crops indicates that the expressed Cry toxins do not have any direct effect on species belonging to orders other than the target insects (Lepidoptera or Coleoptera) (O'Callaghan *et al.* 2005). This is not surprising given the long history of safe and very targeted use of microbial *Bt* products (Glare and O'Callaghan 2000).

Thus, *Bt*-transgenic crops have the potential to be a viable alternative to conventional insecticides. In cotton fields, broad-spectrum insecticides are generally applied for the control of lepidopteran pests, i.e. the bollworm-budworm complex. Around the globe, deployment of *Bt* cotton has consistently resulted in a 60-80% decrease in insecticide applications in this crop (Fitt *et al.* 2004). Similarly, *Bt* sweet-corn has been found to be a suitable alternative for control of lepidopteran pests (Musser and Shelton 2003). In other crops such as maize, the introduction of the *Bt* gene to control the European corn borer, *Ostrinia nubilalis*, has not lead to substantial insecticide decreases simply due to the fact that this pest is generally not controlled by foliar insecticides so many growers simply did not treat and were resigned to the losses (Phipps and Park 2002).

The published information available to date reveals no detrimental impact of *Bt*-transgenic crops on the abundance or efficiency of biological control agents. In cases where *Bt* crops replaced the use of conventional insecticides (e.g., cotton or sweet-corn), substantial positive effects on the biocontrol fauna have been reported, resulting in increased control of potential secondary pests such as aphids (Reed *et al.* 2001; Wu and Guo 2003). Thus *Bt*-transgenic crops should be regarded as a biocontrol friendly technology that can help promote the conservation of biological control agents for key pests in cropping systems that are currently dominated by insecticide use. Furthermore the replacement of broad-spectrum insecticides by *Bt* crops opens up an opportunity for biocontrol of secondary pests, such as plant and stink bugs in cotton, that were controlled by the insecticides applied against the lepidopteran pest complex (Green *et al.* 2001; Wu *et al.* 2002).

The following session will provide information on the non-target risk assessment conducted by biotech companies as part of the regulatory process (Graham Head). This will be followed by examples from *Bt*-maize (Rick Hellmich) in the U.S. and *Bt* cotton in the U.S. (Steven Naranjo), China (Kongming Wu) and Australia (Gary Fitt) on how *Bt* crops can be implemented in IPM systems.

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ASSESSING THE INFLUENCE OF *BT* CROPS ON NATURAL ENEMIES

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ABSTRACT

Transgenic *Bt* crops expressing proteins derived from *Bacillus thuringiensis* (*Bt*) currently are grown commercially in ten countries on over 20 million hectares. Assessing their environmental safety is a critical part of the regulatory approval process and product stewardship for *Bt* crops. The environmental safety testing process for *Bt* crops follows a standard risk assessment approach, and involves multiple tiers of laboratory and field testing. Lower tier testing is based primarily upon pure protein tests, with testing concentrations keyed off of the maximum possible environmental exposure for a variety of non-target species. Test species are chosen based on considerations of the product and region, and typically include insect predators and parasitoids. These laboratory studies have not found any direct toxic effects of Cry1, Cry2 or Cry3 proteins against any generalist predator or parasitoid. The results of these studies agree with other laboratory and field studies conducted prior to and post-commercialization of *Bt* crops. Collectively, the non-target studies performed to date demonstrate that *Bt* crops do not have any unexpected toxic effects on natural enemy species, as would be predicted from knowledge of the mode of action and specificity of *Bt* proteins.

INTRODUCTION

Two decades of advances in the areas of molecular biology and genetics have led to the creation of exciting new opportunities in agriculture. The use of genetic engineering techniques to transfer traits useful in insect, disease and weed control have provided farmers with a new set of tools to control some old, intransigent problems (James 2004; Schuler *et al.* 1998). Some of the first genetically engineered crops, and some of the most widely used, have been modified to express insecticidal crystalline (Cry) proteins derived from the common soil bacterium *Bacillus thuringiensis* (*Bt*) Berliner (Perlak *et al.* 1991). These so-called *Bt* crops are protected from the feeding of various groups of pest insects. They provide pest control solutions that are highly effective and yet very specific, leading to substantial direct benefits for farmers as well as providing greater flexibility in crop management practices.

Since 1995, various biotechnology companies, including Monsanto, Syngenta, Dow and Dupont-Pioneer, have registered varieties of corn, cotton and potatoes that express *Bt* proteins for commercial use in ten countries. The *Bt* cotton and *Bt* corn products, in particular, are widely accepted and used, with total global adoption exceeding 20 million hectares (James 2004). In cotton, the proteins expressed (Cry1Ac, Cry1F and Cry2Ab) confer protection from a broad array of lepidopteran herbivores, enabling the use of broad spectrum insecticides to be greatly reduced and, in some cases, eliminated. *Bt* cotton (particularly varieties expressing Cry1Ac and sold as Bollgard^{Ò} or Ingard^{Ò}) has been registered for commercial use in Argentina, Australia, China, Colombia, India, Mexico and South Africa. Some of the most exciting possibilities for such a product exist in tropical systems where substantial broad spectrum insecticides would otherwise be used. In areas of Asia, such as India and China, cotton crops may be sprayed more than ten times in a year in the absence of *Bt* cotton in an attempt to control severe lepidopteran pest outbreaks (e.g., Wu and Guo 2005). *Bt* corn, modified to express either Cry1Ab or Cry1F to combat a set of stalk-boring Lepidoptera, or Cry3Bb1 to control feeding by coleopteran *Diabrotica* spp., has similar potential to *Bt* cotton. In 2004, about 12 million hectares of *Bt* corn were planted in the United States, almost 50% of corn acres in Argentina were planted with *Bt* corn, and smaller amounts were planted commercially in Canada, the Philippines, South Africa and Spain.

A critical part of the introduction of such products is to ensure their safety and safe use. This involves comprehensive laboratory and field testing to ensure that the products' characteristics are understood and that they are used correctly. In this paper, I describe the environmental safety assessment process used for *Bt* crops, with particular emphasis on the assessment of impacts of *Bt* crops on natural enemies. I then summarize the results of regulatory and related testing of the impacts of *Bt* crops on natural enemies, and compare the results with what would have been predicted from knowledge of the mode of action of *Bt* proteins.

TESTING AND REGULATION OF *BT* CROPS

The environmental safety of *Bt* crops has been addressed throughout their development process and has involved review by regulatory agencies and scientific experts from the government, academia, and industry. In particular, environmental safety is a criterion in the initial product design, and then is the focus of substantial laboratory and field testing. Regulatory review typically occurs through Ministries of Agriculture and/or the Environment. For example, in the U.S., this primarily involves the Environmental Protection Agency Office of Pesticide Programs (EPA OPP) and, secondarily, the U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS).

TRANSGENIC PRODUCT DESIGN

Proteins being considered for use in insect-protected transgenic crops are screened based on effectiveness and specificity. The aim is to find proteins with high activity against the target pest insects and little or no activity against other taxa. As a consequence of this selection process, proteins that might cause adverse environmental impacts because of either broad toxicity or activity against key non-target groups are eliminated early in the development process. The choice of *Bt* crystalline (Cry) proteins for currently commercialized insect-protected transgenic crops are an illustration of this approach. These proteins must be ingested to be insecticidal. Once ingested, the mode of action of *Bt* proteins is complex and involves:

solubilization, proteolytic stability, binding to the midgut epithelium, formation of ion channels in the midgut cells, and finally lysis of these cells (English and Slatin 1992). These proteins are highly specific in their effects because of this mode of action, particularly compared to other proteins that have insecticidal properties such as lectins and protease inhibitors. Only a few insect groups have the appropriate mid-gut characteristics and binding sites for a particular *Bt* Cry protein to be active. For example, Cry1-type proteins control various Lepidoptera, Cry2- type proteins affect certain Lepidoptera and Diptera, and Cry3 proteins control certain Coleoptera. Unrelated non-target species are unaffected.

Apart from selecting insecticidal proteins based upon the mode of action, efforts also are made to choose proteins with a history of safe use. Where possible, proteins that have been previously used in comparable ways without environmental problems are preferred. This was another reason for the choice of *Bt* Cry proteins. These proteins have been used extensively in foliar sprays for over 30 years. In that form, they also have been scrutinized by regulatory agencies. They have proven to be extremely safe with respect to both human safety and environmental impacts (EPA 2001; McClintock *et al.* 1995).

SAFETY TESTING

The environmental safety testing process for *Bt* crops follows a standard risk assessment approach, and involves multiple tiers of laboratory and field testing (Sharples 1991). The assessment is specific to the product and region, and considers the nature of the trait, crop plant biology, local farming practices, and the local ecological community. The tests used are shaped by the requirements of regulatory agencies (such as the EPA and the USDA-APHIS in the U.S.), as well as by product stewardship considerations (Nickson and Head 2000). The overall environmental risk assessment can be thought of as addressing two basic areas: first, whether the transgenic crop is biologically equivalent to comparable untransformed varieties other than the presence of the *Bt* protein, and second, whether the *Bt* protein has any direct or indirect effects on the ecological community (through toxicity, gene flow, or selection for pest resistance). Potential non-target impacts of *Bt* crops primarily fall into the latter category.

Because *Bt* proteins are chosen for their insecticidal properties, possible impacts on non-target insect species are a particular source of concern. Lower tier (early) testing for such impacts is based primarily upon pure protein tests, with testing concentrations keyed off of the maximum possible environmental exposure for a variety of non-target species. Where appropriate, testing uses relevant plant tissues. Test species are chosen based on considerations of the product and region, and typically include insect predators, parasitoids and pollinators, as well as soil-dwelling and aquatic invertebrates. These species are selected to be representative of different taxa and ecological guilds, and often are economically important species. The results of these tests can be compared to the known properties of the protein (mode of action). Different routes of exposure to the insecticidal protein are assessed, including direct consumption of leaf tissue by herbivores, deliberate or incidental feeding on pollen, and ingestion of plant material that has become incorporated into the soil. Where some hazard is indicated in lower tier testing, or significant uncertainty remains in the hazard and/or exposure assessment, higher tier studies may be initiated. Higher tier tests are more field-based and may be carried out both prior to and after commercialization. In these studies, the product is compared with reasonable agronomic alternatives. After commercialization, work can take place in commercial-sized fields managed with standard grower practices.

Thus far, no unintended adverse ecological impacts have been identified for any commercialized *Bt* crop, despite the comprehensive regulatory assessment in multiple countries, extensive commercial use, and post-commercial monitoring (e.g., Betz *et al.* 2000; EPA 2001; Mendelsohn *et al.* 2003).

REGULATORY TESTS OF NATURAL ENEMIES

Important criteria in choosing suitable natural enemies for testing are comparable to the criteria used for selecting any non-target species: they should adequately represent organisms relevant to the cropping system where the *Bt* crop will be used; they potentially should be exposed to the *Bt* proteins expressed in *Bt* crops; they should be relatively easy to work with in the laboratory; and suitable laboratory colonies must be available.

Potential routes of natural enemy exposure to *Bt* proteins include direct feeding on pollen, nectar or other plant tissues of *Bt* crops, or secondary exposure through feeding on prey species that have themselves fed upon *Bt* plants. *Bt* protein expression in *Bt* crops is highest in actively growing green tissues, lower in older vegetative tissues and reproductive tissues, and lowest or absent in the phloem (Head *et al.* 2001; Raps *et al.* 2001). This suggests that regulatory testing should focus upon those natural enemies that opportunistically feed on pollen or vegetative tissues of crops. Furthermore, direct routes of exposure generally lead to much greater exposure to the *Bt* proteins in *Bt* crops than secondary exposure for several reason. First, the level of *Bt* protein that is present in herbivores that have fed on *Bt* plants is far lower than the level of *Bt* protein present in the plant tissues, presumably because of dilution effects (Dutton *et al.* 2002; Head *et al.* 2001). Second, some insects, particularly phloem feeders like aphids, ingest only minimal amounts of *Bt* protein because little or no *Bt* protein is present in the parts of the plant where they are feeding (Head *et al.* 2001; Raps *et al.* 2001). Thus predators feeding on these different prey species will be exposed to very little *Bt* protein. Third, arthropod predators usually prey upon a variety of species, some or all of which may not be feeding on the *Bt* crop at all. Therefore regulatory testing logically focuses on direct exposure to *Bt* proteins through ingestion of pollen or green tissues.

As described above, several representative natural enemies typically have been included among the lower tier regulatory tests. Indicator organisms tested for currently registered lepidopteran-active *Bt* proteins (e.g., Cry1Ab, Cry1F, Cry1Ac and Cry2Ab) have included lady beetles, the green lacewing, *Chrsoperla carnea* Stephens (Neuroptera: Chrysopidae) and a parasitic Hymenoptera such as *Nasonia vitripennis* Walker (Hymenoptera: Pteromalidae). An additional reason for the choice of these species was their history of testing with microbial pesticides which provides useful comparative data.

Among generalist predators, lady beetles are a logical choice for testing because of their abundance and importance within cropping systems, and particularly corn and cotton agroecosystems. Studies have been conducted with the convergent lady beetle, *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae) and the pink-spotted lady beetle, *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae). Of these two species, *C. maculata* is the preferred species for testing because it is more of a generalist predator and more readily feeds on pollen than *H. convergens* (Lundgren *et al.* 2004).

Other coleopteran generalist predators also may be suitable for regulatory testing. In particular, ground beetles (Carabidae) and rove beetles (Staphylinidae) are logical candidates for lower tier tests, and have been used in assessing the impact of conventional insecticides and *Bt* crops expressing coleopteran-active proteins. These taxa are ecologically and economically important within agro-ecosystems, and fill diverse niches. Many are capable of feeding on pollen. For example, *Pterstichus* spp. and *Amara* spp. are abundant carabids within corn fields, and could be adapted for laboratory testing.

Green lacewings are important generalist predators in many crops, but typically are less abundant and influential in corn and cotton cropping systems than coccinellids and heteropteran predators such as *Geocoris* spp. and *Orius* spp. (e.g., Candolfi *et al.* 2004; Hagerty *et al.* 2005). Furthermore, green lacewings consume little pollen in the field and primarily feed upon on phloem-feeding aphids, and thus their exposure to the *Bt* proteins in *Bt* crops will be limited (Head *et al.* 2001; Raps *et al.* 2001). For these reasons, green lacewings are being used less as test species for *Bt* crops, and instead are being replaced with species like the insidious flower bug, *Orius insidiosus* Say (Heteroptera: Anthocoridae). This species is highly abundant in crop systems, readily feeds on pollen, and also feeds on leaves and other green tissues under certain conditions.

Parasitoids typically will only see limited exposure to the *Bt* proteins in *Bt* crops because their main route of exposure will be through secondary pathways. Egg parasitoids such as *Trichogramma* spp. will not be exposed at all. In addition, the Cry1, Cry2 and Cry3 proteins currently expressed in commercial *Bt* crop varieties are not expected to be directly toxic to Hymenoptera, and the honey bee, *Apis mellifera* L. (Hymenoptera: Apiidae) routinely is used in non-target testing for *Bt* crops in any case. Thus, testing of generalist predators usually has taken precedence over testing of hymenopteran parasitoids. As noted earlier, regulatory testing for *Bt* crops often has included the dipteran pupal parasitoid *N. vitripennis*. However, other species with greater relevance to corn and cotton cropping systems also are being considered (for example, braconids such as *Cotesia* spp.).

RESULTS OF REGULATORY AND POST-COMMERCIAL TESTING

TESTING OF PREDATORS

Natural enemies, and particularly generalist arthropod predators, have been the focus of many studies because of their role in the biological control of various agricultural pests. Based on what is known about the limited spectrum of activity of the *Bt* Cry proteins expressed in currently commercialized *Bt* crops, no direct toxic effects from *Bt* crops would be expected for any of these species. As predicted, the Tier 1 (early tier) laboratory studies that have been conducted by companies as part of the regulatory packages for *Bt* crops have not found any direct toxic effects of Cry1, Cry2 or Cry3 proteins against insect predators for *Bt* protein concentrations at or much greater than maximum possible exposure under natural conditions (for example, see reviews in Betz *et al*. 2000; EPA 2001). Obviously these tests are not meant to mimic natural exposure nor do they test all possible species that could be exposed but they do represent highly conservative tests of possible hazard using carefully chosen surrogate species.

Researchers interested in the fate of particular predatory species have carried out additional laboratory and semi-field tests of potential non-target impacts. These tests have used a variety of designs, with differing degrees of realism in terms of the route and level of *Bt* exposure. Given that many predators feed on some amount of pollen at some point in their life cycle, many of these studies have involved feeding predatory insect species pollen from *Bt* crops and comparable control lines. None of these studies have found any adverse impacts of *Bt* pollen on the survival or development of various insect predators (e.g., Pilcher *et al.* 1997). Comparable studies using *Bt* corn silks with a heteropteran predator also found no effect (Al-Deeb *et al.* 2001)

Obviously the above studies involved direct exposure and, under field conditions, exposure also can occur through secondary pathways with predators feeding upon herbivores that had fed on a *Bt* crop plant. However, secondary exposure of this sort should have relatively little impact on arthropod predators for the reasons outlined above. However, one set of studies has been presented as a possible example of adverse impacts through secondary exposure. Hilbeck *et al.* (1998a,b; 1999) performed a number of laboratory studies with the predatory lacewing *C. carnea*, feeding the larvae on lepidopteran larvae that had fed on *Bt* corn. They found higher mortality and slower development of lacewings exposed to *Bt*-intoxicated insects than for lacewings fed on comparable controls. Subsequent studies by other researchers indicate that these results actually reflected feeding on nutritionally poorer prey rather than any toxic effect of the *Bt* protein (Dutton *et al.* 2002; Romeis *et al*. 2004). Such a situation should have little relevance to the field because other prey sources that are not affected by *Bt* crops will be more available and probably preferred under natural conditions. Furthermore, other tritrophic studies by Al-Deeb *et al.* (2001) with *O. insidiosus* saw no effect when feeding on *Bt*-intoxicated prey. In this case, the results were confirmed with direct feeding studies on *Bt* corn silks and field observations.

Numerous field studies also have focused on generalist predators, particularly *C. maculata*, *C. carnea*, *O. insidiosus*, and guilds of carabids because of their abundance in cornfields and their perceived importance. No adverse effects have been seen for any of these species in these studies or in the broader, community-level studies of *Bt* corn (e.g., Candolfi *et al.*, 2004; Lozzia, 1999; Pilcher *et al.* 1997) and *Bt* cotton (Hagerty *et al.* 2005; Xia *et al.* 1999). The absence of even indirect trophic effects of *Bt* corn and *Bt* cotton in these studies is not surprising because most of these predatory species feed on many different prey species, the vast majority of which are not directly impacted by *Bt* corn e.g., sucking insects like aphids and whiteflies. In contrast, the insecticidal sprays used in conventional corn had clear adverse impacts, at least transiently, on almost all common predators, and particularly those species foraging above ground (Candolfi *et al.* 2004). Similarly, the insecticidal sprays used in conventional cotton also had clear adverse impacts on almost all of the important arthropod predators (Hagerty *et al.* 2005; Wu and Guo 2005; Xia *et al.* 1999).

TESTING OF INSECT PARASITOIDS

As with arthropod predators, no direct toxic effects from *Bt* crops would be expected for any of parasitoid species given what is known about the spectrum of activity of the *Bt* proteins expressed in currently commercialized *Bt* crops. Furthermore, because the larvae of these groups feed solely on other arthropods, larval parasitoids will not face any direct exposure. Adult exposure also will be very limited because of their occasional feeding on pollen or nectar. However, secondary exposure to *Bt* proteins may occur if the parasitoids feed on herbivore larvae that have fed upon a *Bt* crop plant. In addition, indirect effects may occur at the population level if the host species of the natural enemies are a target of the *Bt* crop and are depressed in numbers.

As with predatory species, the Tier 1 laboratory studies have not found any direct toxic effects of Cry1, Cry2 or Cry3 proteins against parasitoids for *Bt* protein concentrations at or much greater than maximum possible exposure under natural conditions (see reviews in Betz *et al.* 2000; EPA 2001). On the other hand, secondary exposure studies indicate that parasitoids that develop on hosts exposed to *Bt* may be adversely impacted. When reared on *Bt*-susceptible insects that had fed on *Bt* corn, the larval development and mortality of the parasitoid *Parallorhogas pyralophagus* Marsh (Hymenoptera: Braconidae) was adversely affected, but the fitness of emerging adults was not impacted (Bernal *et al.* 2002).

It should also be remembered that fundamental differences in how *Bt* plants act relative to conventional insecticides will be a major determinant of the relative impact that these products have on non-target species. With *Bt* plants, having expression of the insecticidal protein only within the plant and preferentially within certain tissues means that many parasitoids will never be exposed to any *Bt* protein.

A number of field studies have looked at impacts on parasitoids or the level of parasitism in *Bt* cornfields. Because of their specificity, species that parasitize the larval stages of target pests of *Bt* crops would be expected to be rarer in fields of *Bt* crops than in comparable fields of conventional crops. As expected, the few specialist parasitoids that parasitize *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) and certain other stalk boring Lepidoptera in corn have been found to be rarer in *Bt* corn than in conventional corn, e.g. *Macrocentrus cingulum* Brischke (Hymenoptera: Braconidae) (Candolfi *et al.* 2004). Similarly, the few specialist parasitoids that parasitize foliage-feeding Lepidoptera like *Helicoverpa armigera* Hübner (Lepidoptera:Noctuidae) in cotton have been found to be rarer in *Bt* cotton than in non-*Bt* cotton (e.g., Xia *et al.* 1999). Of course, it is important to consider these results in the context of alternative practices. As mentioned earlier, the insecticidal sprays used in conventional corn (Candolfi *et al.* 2004) and cotton (Hagerty *et al.* 2005; Wu and Guo 2005; Xia *et al.* 1999) have clear adverse impacts, at least transiently, on these same parasitoid species. Furthermore, any effective pest control practice that decreases the abundance of the host species will have comparable effects.

CONCLUSIONS

Collectively, the non-target studies performed to date demonstrate that *Bt* crops do not have any unexpected toxic effects on natural enemy species, as would be predicted from knowledge of the mode of action and specificity of *Bt* proteins. Because of this specificity, *Bt* crops effectively preserve local populations of various economically important biological control organisms that can be adversely impacted, at least transiently, by broad-spectrum chemical insecticides. The only indirect effects on non-target organisms that have been observed with *Bt* crops are local reductions in numbers of certain specialist parasitoids whose hosts are the primary targets of *Bt* crops. Such trophic effects will be associated with any effective pest control technology, whether it be transgenic, chemical, or cultural, as well as with natural fluctuations in host populations.

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INTEGRATION OF *BT* MAIZE IN IPM SYSTEMS: A U.S. PERSPECTIVE

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ABSTRACT

Bt maize has become increasingly popular with United States (U.S.) growers since it was commercially available in 1996. Yield protection, reduced need for insecticides, improved grain quality, and ease of use are benefits that motivated growers to plant 32 percent of total acres to *Bt* maize in 2004. Rapid adoption of a technology raises many questions concerning product longevity and how the technology will influence the maize agricultural ecosystem. Overuse could result in the development of resistant insects, economic populations of secondary pests, or influence populations of non-target organisms. Grower strategies for using *Bt* maize in the U.S. vary regionally and depend on targeted and secondary pests, cropping practices, and insect resistance management requirements. A challenge for scientists and educators has been to try to keep grower recommendations uniform and grounded in principles of Integrated Pest Management (IPM). The talk will highlight a project funded by United States Department of Agriculture Risk Avoidance and Mitigation Program (USDA–RAMP) that uses site-specific high-resolution information to help merge transgenic technology with traditional insect IPM tools.

INTRODUCTION

The most damaging pests of maize in the United States Corn Belt are the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) and corn rootworm complex, *Diabrotica* spp. (Coleoptera: Chrysomelidae). European corn borer is a chronic pest of maize in the eastern two-thirds of North America. In the U.S., it is annually responsible for over one billion dollars in yield and control costs. Before transgenic maize was available, *O. nubilalis* was managed by planting insect resistant maize and by using rescue treatments of chemical insecticides (Mason *et al.* 1996). Western corn rootworm, *Diabrotica virgífera* LeConte, and northern corn rootworm, *Diabrotica barberi* Smith and Lawrence, also are estimated to cost U.S. growers more than a billion dollars in crop losses and control costs annually. Historically, these pests have been controlled by crop rotation and prophylactic use of soil insecticides. Biocontrol of these pests has not been embraced by most U.S. growers because it is usually not cost effective. There have been limited successes with *O. nubilalis*. Biological sprays of *Bacillus thuringenisis* Berliner (*Bt*; e.g., Dipel) are commonly used by seed producers to protect seed corn and by organic growers. Inundative and inoculative releases of the egg parasitoid, *Trichogramma ostriniae* Pang and Chen (Hymenoptera: Trichogrammatidae), can significantly reduce *O. nubilalis* damage in sweet corn (Hoffman *et al.* 2002; Seaman *et al.* 1996; Wright *et al.* 2002) but is not commercially available. The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin shows potential for controlling *O. nubilalis* under field conditions (Bing and Lewis 1991). Biocontrol of corn rootworm is nonexistent, although there is some indication that certain species of nematodes could be useful (Journey and Ostlie 2000; Nickle *et al.* 1994). In areas in and near Illinois, crop rotation has failed as a cultural control method for western corn rootworm because some rootworm beetles oviposit in soybean fields (Sammons *et al.* 1997). Also, in some areas of Iowa, Minnesota and South Dakota populations of northern corn rootworm have adapted to crop rotation through a two-year or more extended diapause (Krysan *et al.* 1986).

Transgenic maize hybrids with a gene from *B. thuringiensis* are revolutionizing maize insect management. Transgenic maize with resistance to *O. nubilalis* was commercially available in the U.S. in 1996. Use of this maize has steadily increased where in 2004 32% of field maize in the U.S. was *Bt* (USDA–NASS 2004). The perceived value of *Bt* maize tends to increase going from east to west and in areas where univoltine and bivoltine moths overlap (e.g., southern Minnesota and South Dakota). Growers are attracted to *Bt* maize because it protects their yield, simplifies pest management, and in some cases leads to better quality grain (Munkvold *et al.* 1997).

Along with the advancements of molecular biology, there have been improvements in computer processing power. Faster computers allow scientists to evaluate and integrate data sets that were not practical even ten years ago. A collaboration of scientists supported by a grant from USDA–RAMP provides unique approaches to improve field crop IPM by using site-specific high-resolution information to help merge transgenic technology with traditional insect management tools. Maize and *O. nubilalis* development models driven by degree days lay the foundation for these analyses, which allow entomologists to determine when corn borer presence and potential damage are most detrimental to maize development. An economist then uses these data to access where in the Corn Belt *Bt* maize is the most economical. These types of analyses could open doors for improving biocontrol because they also could use degree-day models on large spatial scales to tie together the phenologies of biocontrol agents with those of maize pests.

METHODS

The *Bt* maize Economic Tool or BET program is composed of several models: pest phenology, maize phenology, site-specific weather data and an economic component. The maize and corn borer phenology models, using site-specific (~ 1 km^2 resolution) historical weather data as input, calculate the weather-driven seasonal synchrony of maize growth stage and the period of European corn borer stalk tunneling for every location east of the Rocky Mountains. An economic analysis model calculates the potential yield losses associated with all possible “seasonal” synchronies. The site-specific weather data, which serve as input into both crop and pest models, are derived from either observations or forecasts. Data from observations are the result of interpolating between weather stations; while, data from forecasts are derived from numerical weather prediction models. Site-specific weather data are increasing becoming a popular input choice for local crop and pest models (Magarey *et al.* 2001; Russo 2000). These models extend from collaborative efforts of many scientists, but the chief designers of the BET program are Drs. Dennis D. Calvin, Jeffrey Hyde (Penn State University), and Joseph M. Russo (ZedX, Inc.).

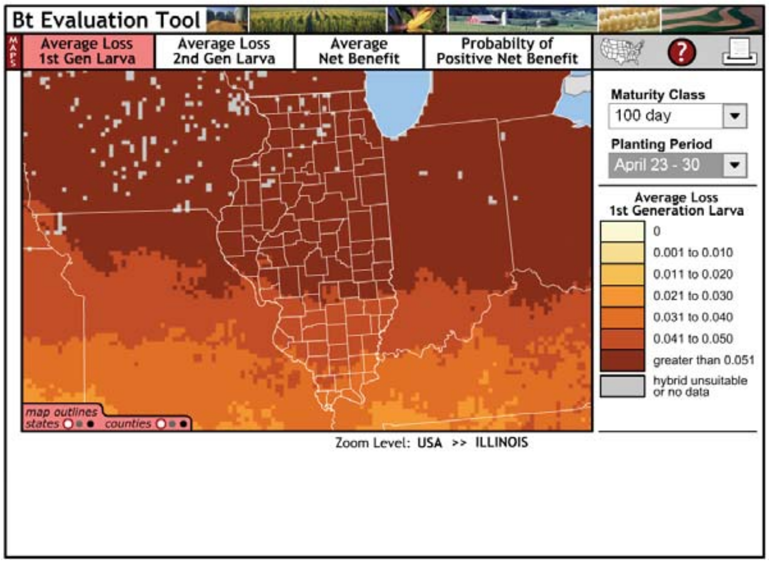
The BET model is available at an interactive website (www.essc.psu.edu/bet/). Grower inputs include seed-maturity class and planting date, and inputs for the economic analysis include first and second generation *O. nubilalis* densities (mean larvae per plant), *Bt*-pest control, seed premium, average yield, planting rate, and maize price. Default values are based on long-term averages. Growers can update these values to reflect local conditions and economics.

RESULTS AND DISCUSSION

The BET model provides growers with a tool that allows them to make decisions whether to plant *Bt* maize based on the probability of positive net benefits. A grower in central Illinois, for example, who traditionally plants a 105-day hybrid the last week in April, could expect first generation *O. nubilalis* to overlap with an attractive stage of maize (Fig. 1). Similar output for second generation *O. nubilalis* suggests potential problems are less (data not shown), primarily because most maize would be past the vulnerable stage when *O. nubilalis* were present. This grower could expect a positive return on *Bt* maize 31 out of 33 years (Fig. 2). This type of information provides a starting point for growers to assess whether the technology is cost effective in their area, which could minimize overuse of the technology. Overuse of *Bt* technology can result in selection for insects that are resistant. Prescriptive use of a pest control instrument is one form of resistance management, which would apply to transgenic crops and any other pest control technology.

A grower also could evaluate planting date possibilities that reduce the overlap of first or second generation *O. nubilalis*. For example, if a grower is most concerned with first generation *O. nubilalis* he or she may want to plant *Bt* maize early and non-*Bt* maize later; and vice versa if second generation borers are a concern. Such information also could be valuable for organic growers who might decide to alter planting dates and seed maturity to avoid peak pest densities. An organic organization also potentially could evaluate places to establish farms that would minimize pest problems.

A visual tool for evaluating the overlap of pest and plant phenologies offers possibilities for evaluating biocontrol agents. Scientists have long puzzled over why effectiveness of biocontrol agents varies spatially and temporally. For example, Clark *et al.* (2001) have found very high levels of parasitism in maize in Nebraska, but other researchers (Bruck and Lewis 1999; Lewis 1982) found consistently low levels over several years in Iowa. Granted many factors contribute to the success of parsitoids, but one could start with a specific parasitoid phenology model and overlay it with maize and *O. nubilalis* phenologies. First-level analyses would entail modeling various planting dates and locations to determine if patterns emerge that provide clues to more efficient use of a particular parasitoid. Second-level analyses could follow whereby other factors are incorporated into the model, such as the phenology of plants that provide shelter, nectar, or other benefits to the biocontrol agent. Ultimately this systems approach to pest management could allow investigators to incorporate unique features of landscapes into the analyses. One could be overwhelmed by the near infinite combinations of pests, biocontrol agents, and environments, but the system outlined here could allow scientists to identify patterns of effective combinations. Bottom line, this approach provides a way to visualize complex interactions and provides a valuable tool toward developing more sustainable approaches to pest management.

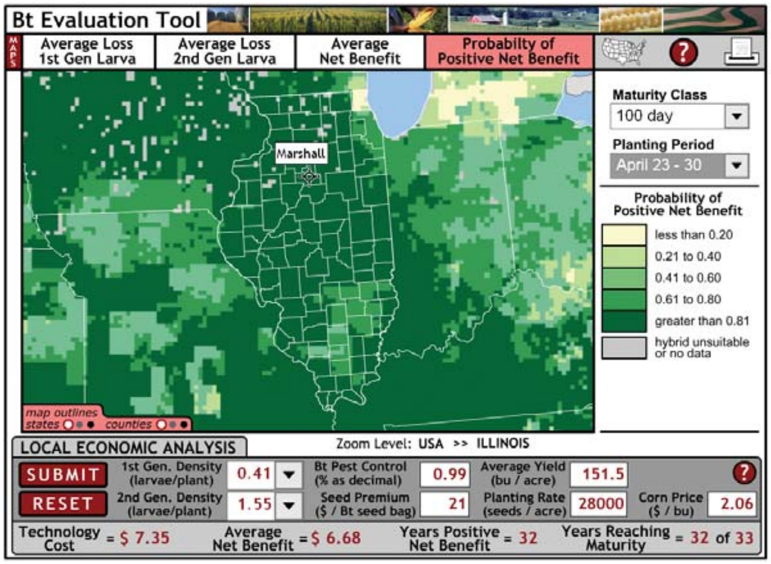


Maturity Class: 100 day

Planting Period: April 23 - 30

Zoom Level: USA >> ILLINOIS

Figure 1. *Bt* Evaluation Tool (BET) model results showing average loss of first generation *O. nubilalis* though Corn Belt states when a 100-day maturity seed is planted the last week in April.



Maturity Class: 100 day

Planting Period: April 23 - 30

Zoom Level: USA >> ILLINOIS

1st Gen. Density (larvae/plant): 0.41

2nd Gen. Density (larvae/plant): 1.55

Bt Pest Control (% as decimal): 0.99

Seed Premium ($ / Bt seed bag): 21

Average Yield (bu / acre): 151.5

Planting Rate (seeds / acre): 28000

Corn Price ($ / bu): 2.06

Technology Cost = $ 7.35

Average Net Benefit = $ 6.68

Years Positive Net Benefit = 32

Years Reaching Maturity = 32 of 33

Figure 2. BET model results showing probability of positive net benefits of using *Bt* technology for controlling *O. nubilalis* though Corn Belt states when a 100-day maturity seed is planted the last week in April. Local economic analysis focuses on Marshall County in Illinois.

ACKNOWLEDGEMENTS

This research was supported by a grant from USDA–CSREES, Risk Avoidance and Mitigation Program (RAMP). Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA, Iowa State University or Pennsylvania State University.

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FIELD ABUNDANCES OF INSECT PREDATORS AND INSECT PESTS ON δ-ENDOTOXIN-PRODUCING TRANSGENIC COTTON IN NORTHERN CHINA

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ABSTRACT

China is one of the largest producers of cotton in the world. Insect pests such as cotton bollworm, cotton aphid, and mirids are the major factors that contribute to a decrease in cotton production. Transgenic cotton that expresses a gene derived from the bacterium *Bacillus thuringiensis* (*Bt*) has been deployed for combating cotton bollworm since 1997 in China, and expanded rapidly to 3.7 million of the total cotton acreage of 5.3 million ha in 2004. Field monitoring on the change of pest status derived from *Bt* cotton commercialization in a large scale were conducted during 1998-2004 in Hebei Province. The results indicated that *Bt* cotton efficiently controls cotton bollworms, while the decrease of pesticide applications allows the build up of high populations of predators, such as lady beetles *Coccinella septempunctata*, lacewings *Chrysopa sinica*, spiders and others in mid-season. Furthermore, planting *Bt* cotton efficiently prevented the resurgence of cotton aphids caused by insecticide use for control of cotton bollworm. However, the investigation of the seasonal dynamics of mixed populations of mirids showed that mirid density increased drastically, probably due to a reduction in the number of foliar insecticide applications in *Bt* cotton fields.

INTRODUCTION

Cotton bollworm, *Helicoverpa amigera* (Hübner) (Lepidopetra: Noctuidae) is a serious pest of cotton, *Gossypium hirsutum* L. in China. To combat the unprecedented *H. armigera* pest pressure in the early 1990s, cotton farmers in China had to apply synthetic pyrethroids, endosulfan and organophosphate insecticides at 2~3 day intervals during critical periods, resulting in more than 20 sprays during the season (Wu and Guo, 2005). In recent years, control of this pest has relied heavily upon commercial cultivation of *Bt* cotton that expresses a gene encoding an insecticidal protein from the bacterium *Bacillus thuringiensis* Berliner, which has been expanded rapidly to 3.7 million of the total cotton acreage of 5.3 million ha in 2004 (James, 2004).

There are numerous arthropods in cotton fields. While the *Bt* protein is toxic to only a narrow spectrum of lepidopteran species, the dynamics of other species may be indirectly affected. Effects on non-target species may be positive due to the removal of disruptive pesticides, or negative due to the effective removal of prey (Fitt, 1994). Because a total insect control strategy in cotton involves both lepidopterous and non-lepidopterous pests, it is necessary to understand the population dynamics of insect predators and insect pests after *Bt* cotton deployment (Pilcher *et al.*, 1997; Riggin-Bucci and Gould, 1997; Wilson *et al.*, 1992). The influences of *Bt* cotton on cotton bollworm, cotton aphid, and mirids have been evaluated in field trials (Wu and Guo, 2003; Wu *et al.*, 2002; Wu *et al.*, 2003). However, previous studies do not provide an insight in the population changes of predators and secondary pests derived from *Bt* cotton commercialization in the long term or on a large scale. Here we report the monitoring results on the chance in abundance of insect predators and insect pests associated with *Bt* cotton planting in northern China.

MATERIALS AND METHODS

COTTON VARIETIES

A transgenic cotton variety expressing the Cry1Ac gene (NuCOTN33B), a transgenic cotton variety (SGK321) expressing Cry1Ac and CpTI genes and its parental line (Shiyuan321) supplied by Monsanto Co. (St. Louis, MO) and Shijiazhuang Academy of Agricultural Sciences (Shijiazhuang, Hebei Province), respectively, were used in the experiments.

EXPERIMENTAL DESIGN

Experiments were conducted from 1998 to 2004 at Langfang Experimental Station of the Chinese Academy of Agricultural Sciences, located in Hebei Province. Experiments consisted of three treatments (two transgenic cotton varieties and one conventional cotton). The field was layed out as a randomized complete block replicated three times. Each plot was about 0.033 hectare and was seeded at the rate expected to produce 45000 plants per planted hectare. No insecticide was sprayed in *Bt* cotton plots and its control plots (normal variety). Cotton was maintained with standard agronomic practices for northern China.

SAMPLING FOR INSECT PESTS AND PREDATORS

Each treatment was sampled every 3-4 d from middle-June to early-September, and each sample consisted of 5 sites with a total number of 100 cotton plants. Field counts consisted of eggs and larvae of cotton bollworm, immature and adult *Lygus pratensis* Linnaeus, *Lygus lucorum* Meyer-Dür, *Adelphocoris suturalis* Jak., *Adelphocoris fasciaticollis* Reuter and *Adelphocoris lineolatus* (Goeze), and predators, such as lady beetles [*Coccinella septempunctata* Linnaeus, *Leis axyridis* (Pallas), *Propylaea japonica* (Thunberg)], lacewings (*Chrysopa sinica* Tjeder, *Chrysopa septempunctata* Wesmael, *Chrysopa shansiensis* Kawa and *Chrysopa formosa* Brauer), spiders (*Erigonidium graminicolum* and *Misumenopos tricuspidata*) and *Orius similis*. Three leaves per plant from upper, middle and lower parts of cotton plant were sampled to estimate densities of cotton aphid, *Aphis gossypii* Glover.

STATISTICAL ANALYSES

Population densities of insects were analyzed using analysis of variance (ANOVA) and means were separated using the protected least significant difference (LSD) test (SAS Institute 1988).

RESULTS

PREDATORS

Transgenic *Bt* cotton may affect natural enemies indirectly through the removal of eggs, larvae, and pupae of lepidopteran insects that serve as food sources for predatory arthropods. Considerable reduction in the number of insecticide applications is another important factor that regulates the population dynamics of natural enemies. The field surveys showed that the populations of lacewings (Fig. 1), lady beetles (Fig. 2), spiders and *Orius similis* remained at high densities in *Bt* cotton and conventional cotton through the season. This was likely due to the reduced application of insecticides.

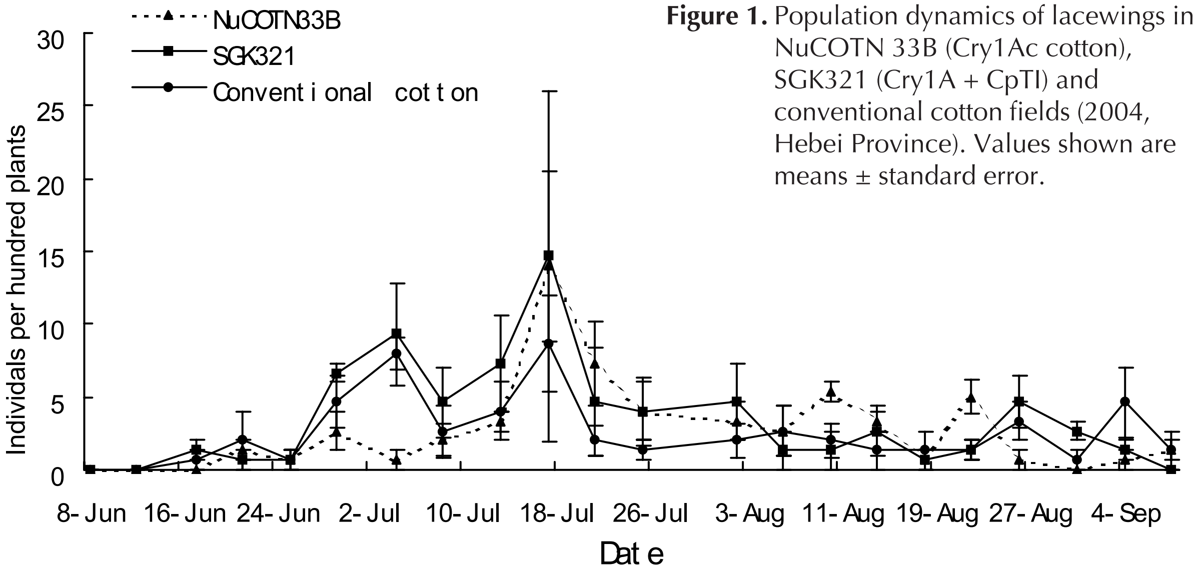

Figure 1. Population dynamics of lacewings in NuCOTN 33B (Cry1Ac cotton), SGK321 (Cry1A + CpTI) and conventional cotton fields (2004, Hebei Province). Values shown are means ± standard error.

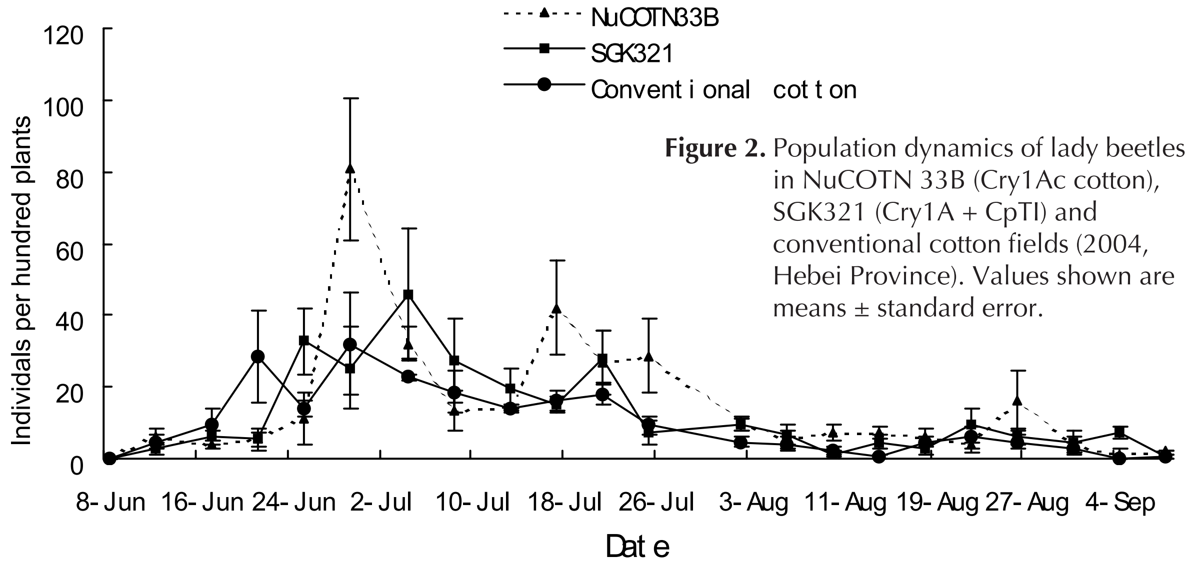

Figure 2. Population dynamics of lady beetles in NuCOTN 33B (Cry1Ac cotton), SGK321 (Cry1A + CpTI) and conventional cotton fields (2004, Hebei Province). Values shown are means ± standard error.

COTTON BOLLWORM

The field evaluations from 1998 to 2004 indicated that *Bt* cotton provided good control of the cotton bollworm. Data for 2004 illustrate this trend (Fig. 3). In this year, the bollworm occurred slightly, and the larval densities on July 8 and August 10 in conventional cotton fields were significantly higher than those on *Bt* cotton ($P < 0.05$). Historically, potentially damaging bollworm larval densities have developed in transgenic cotton under severe egg densities in some years. However, in recent years, the regional occurrence of cotton bollworm in northern China has decreased drastically and this is likely due to the large-scale deployment of *Bt* cotton

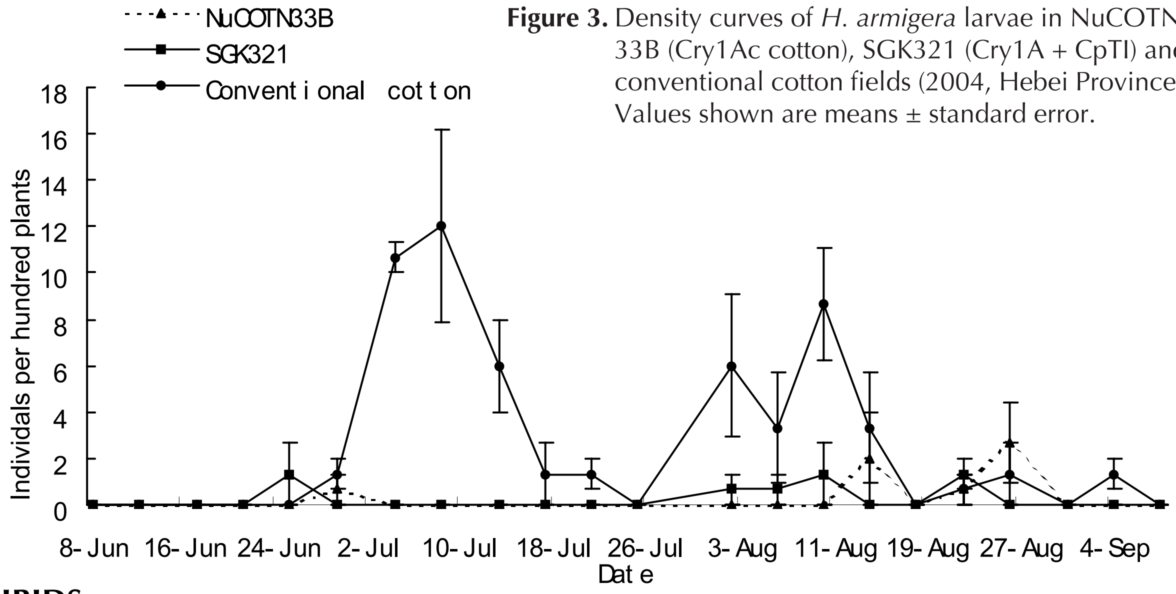

Figure 3. Density curves of *H. armigera* larvae in NuCOTN 33B (Cry1Ac cotton), SGK321 (Cry1A + CpTI) and conventional cotton fields (2004, Hebei Province). Values shown are means ± standard error.

MIRIDS

Investigations on the seasonal population dynamics of a species-complex of mirids in *Bt* cotton fields indicated that mirid density on *Bt* cotton increased drastically, probably due to the reduced number of insecticide sprays (Fig. 4). This suggests that mirids have become key insect pests in *Bt* cotton fields, and their damage to cotton could increase further with the expansion of the *Bt* cotton growing area if no additional control measures are adopted.

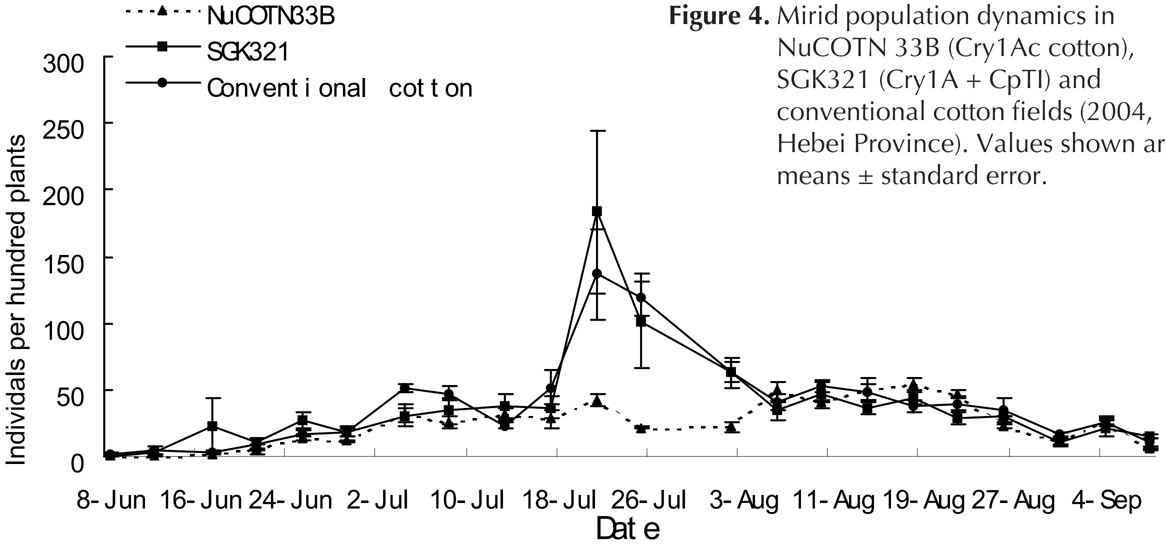

Figure 4. Mirid population dynamics in NuCOTN 33B (Cry1Ac cotton), SGK321 (Cry1A + CpTI) and conventional cotton fields (2004, Hebei Province). Values shown are means ± standard error.

APHIDS

Cotton aphid is one of the most important insect pests after cotton bollworm in cotton production in China. Historically, the period during which the cotton aphid caused yield loss was restricted to the seedling stage of cotton plants. Before the 1970s, aphids could easily be controlled by seed treatment with insecticide. In the mid 1970s, aphids became an important insect pest of cotton due to insecticide-induced resurgence in mid and late season. Since the 1980s, its damage to cotton has become more serious and frequent because insecticide sprays directed against *H. armigera* killed most natural enemies, such as ladybeetle and lacewing that are major predators of cotton aphids. Field experiments on the population dynamics of cotton aphids in *Bt* cotton fields indicated that cotton aphid populations were effectively controlled at a low level, probably due to high densities of ladybeetle and lacewing populations (Fig. 5). This suggests that *Bt* cotton planting could effectively prevent resurgence of cotton aphids caused by insecticide use for control of cotton bollworm.

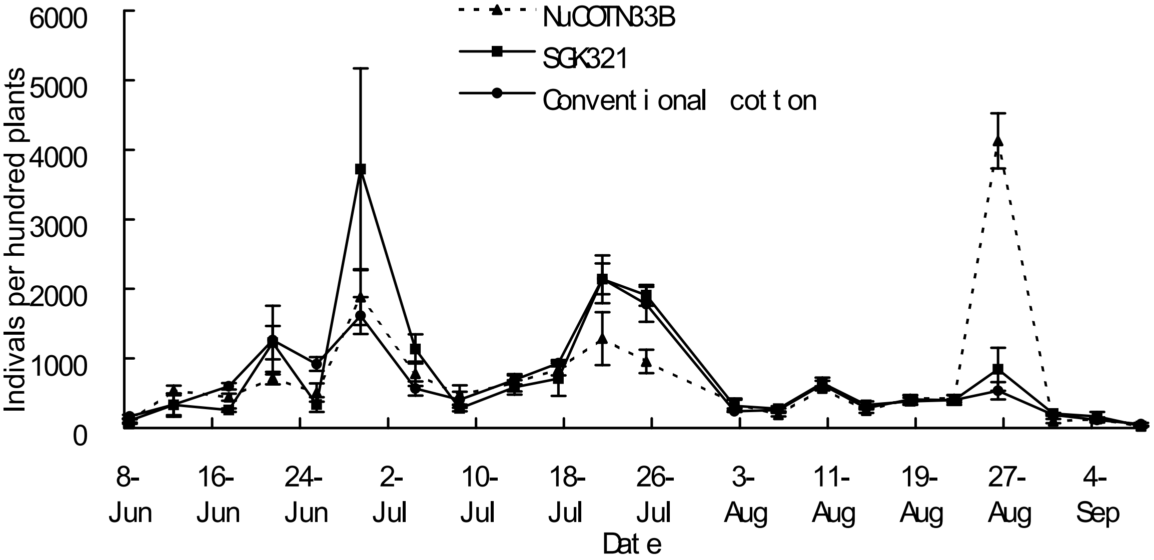

Figure 5. Population dynamics of cotton aphid in NuCOTN 33B (Cry1Ac cotton), SGK321 (Cry1A + CpTI) and conventional cotton fields (2004, Hebei Province). Values shown are means ± standard error.

DISCUSSION

Transgenic cotton, containing *Bt* genes, offers great potential to dramatically reduce pesticide use for control of major lepidopteran pests (Wu and Guo 2005). The greatest threat to the continued efficacy of *Bt* cotton against *H. armigera* is the evolution of resistance (Gould 1998). In addition to current resistance management theory of non-transgenic refuges for the preservation of susceptible alleles, it is also important to consider the prudent use of insecticides, especially late in the season, to reduce overall larval densities in transgenic fields. If late-season survivors in *Bt* fields (under direct selection by the toxin) are reduced by foliar insecticides, the total number of resistance alleles in a region could be reduced. Therefore, it is important that late-season larval density on *Bt* cotton plants be carefully monitored and controlled with effective insecticides.

In addition to the control of Lepidoptera, a control strategy for mirids needs to be developed. In contrast to the greater dispersal ability of the cotton aphid and cotton bollworm, mirids can only fly short distances to adjacent fields. Weeds near cotton fields are their major host plants before moving to cotton, and any action to destroy the weed may decrease the likelihood that the pests will occur and damage the crop. In addition, an alfalfa / cotton system can result in major outbreaks of the mirids in cotton fields. It is thus important to avoid *Bt* cotton planting in fields adjacent to alfalfa and other host plants that mirids prefer (Zhang *et al.* 1986).

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FUNCTIONAL NON-TARGET DIFFERENCES BETWEEN *BT* AND CONVENTIONAL COTTON

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ABSTRACT

A five-year field study was conducted in Arizona to assess the long term impact of transgenic cotton expressing the Cry1Ac dð-endotoxin of *Bacillus thuringiensis* (*Bt*) on population densities of 22 taxa of foliar-dwelling arthropod natural enemies and on the effect of the natural enemy community on key pests in the system. Multi-year analyses of arthropod abundance revealed small, but statistically significant, reductions in five common arthropod predator taxa in unsprayed *Bt* compared with unsprayed non-*Bt* cotton. In contrast, the use of conventional insecticides led to large reductions in 13 predator taxa. Furthermore, functional studies conducted over a three-year period indicated that the small reductions in abundance observed in *Bt* cotton may have little ecological meaning. Sentinel eggs and pupae of *P. gossypiella* experienced the same rates of mortality, primarily from predation, in both *Bt* and non-*Bt* cotton and cohort-based life tables for *B. tabaci* demonstrated that rates of sucking predation, parasitism and dislodgement (chewing predation in part) were unchanged between *Bt* and non-*Bt* cotton. Results demonstrate that long-term and multi-factor studies are required to examine meaningful non-target effects in the field.

INTRODUCTION

Transgenic crops expressing the insecticidal proteins of *Bacillus thuringiensis* (*Bt*) have been commercially available in the U.S. since 1996 and their adoption continues to expand rapidly in the U.S. and other parts of the developed and developing world (James 2004). In 2004 it was estimated that *Bt* cotton represented about 46% of all upland cotton production in the U.S. (USDA 2004). Use rates are much higher in Arizona where *Bt* cotton was grown on 81% of the upland cotton acreage in 2003, most of it (74%) in a stacked configuration with transgenes conferring glyphosate resistance (Tronstad *et al.* 2004). The primary target of *Bt* cotton in Arizona and southern California is the pink bollworm, *Pectinophora gossypiella* (Saunders), a caterpillar that feeds within the cotton fruit and is difficult to control with conventional insecticides (Henneberry and Naranjo 1998). *Bt* cotton is extremely effective in controlling this pest (Flint and Parks 1999).

As with any new technology, both benefits and risks are associated with transgenic crops in agricultural production systems. The use of transgenic crops have led to significant reductions in conventional, broad-spectrum insecticides, improved suppression of target pests, improved yields, reductions in production costs leading to increased profitability, and increased opportunities for biological control (Cannon 2000; Edge *et al.* 2001; Federici 2003; Shelton *et al.* 2002). Some of the potential risks include outcrossing through pollen drift, horizontal transfer of transgenes to other organisms, food safety, loss of susceptibility to *Bt* toxins in target pests, and effects on non-target organisms and biodiversity (Cannon 2000; Conner *et al.* 2003; Marvier 2001; Shelton *et al.* 2002; Wolfenbarger and Phifer 2000). Despite the long history of safety associated with the topical use of *Bt* endotoxins (Federici 2003; Glare and O'Callaghan 2000) the season-long expression of these toxins in crop plants has prompted research to address potential ecological concerns.

A growing number of studies have examined non-target effects in both the laboratory and field (see reviews by Glare *et al.* 2001, Lovei and Arpaia 2005; Pilson and Prendeville 2004; O'Callaghan *et al.* 2005; Schuler *et al.* 1999) with most concluding that *Bt* crops are highly selective. Laboratory studies have tended to focus on defining the effects of direct exposure or indirect exposure via trophic interactions to *Bt* toxins on the biology of non-target species while most field studies have focused primarily on changes in abundance and diversity of non-target taxa. Relatively few studies have examined predator/prey or host/parasitoid interactions, especially in the field (Bourguet *et al.* 2002; Orr and Landis 1997; Sisterson *et al.* 2004) and all have been relatively short-term in duration and have examined only a few of the potential interactions that may occur between natural enemies and their prey or hosts in transgenic crops.

Cotton hosts a rich diversity of parasitoid and arthropod predator species (van den Bosch and Hagen 1966; Whitcomb and Bell 1964), and these natural enemies are known play an important role in regulating pest herbivore populations (e.g., Eveleens *et al.* 1973; Naranjo and Ellsworth 2005; Stoltz and Stern 1978). Conventional cotton production relies heavily on the input of insecticides which typically have broad toxicity to both pests and their natural enemies. The pattern of insecticide use in cotton is one of the most severe constraints to realizing the potential of natural biological control in this system. Improving the compatibility between chemical and biological control depends on minimizing the effects of insecticides on natural enemies through reductions in use of broader-spectrum materials and adoption of more selective compounds (Hull and Beers 1985; Newsom *et al.* 1976). Transgenic *Bt* crops have the potential to contribute to natural enemy conservation through both their selective activity and associated reductions in the broad-spectrum insecticides they replace. However, longer-term and more inclusive studies are needed to define any potential unintended effects of transgenic crop production.

A five-year field study was conducted in Arizona to assess the long term effects of *Bt* cotton expressing the Cry1Ac dð-endotoxin on natural enemy abundance and on the potential impact of the natural enemy community on pest populations. The objectives were to compare: 1) populations of a large group of common natural enemy taxa and several key target and non-target pests between *Bt* and non-*Bt* cottons and to contrast any potential effects relative to conventional production practices using an array of selective and broad-spectrum insecticides, and 2) rates of natural enemy-induced mortality on two key pests between unsprayed *Bt* and non-*Bt* cotton using sentinel prey and field life table studies.

MATERIALS AND METHODS

STUDY SITE AND EXPERIMENTAL DESIGN

Cotton plots were established at the University of Arizona, Maricopa Agricultural Center, Maricopa, Arizona between 1999-2003. All plots were planted in early April of each year and grown according to standard agronomic practices for the area. Each year included a contrast between Deltapine NuCOTN 33B, a transgenic cultivar expressing the Cry1Ac insecticidal protein of *B. thuringiensis*, and its non-transgenic parent cultivar Deltapine 5415. A randomized complete block design with four replications was used in all years; plot size varied from 0.12-0.17 ha. Studies in 2001 and 2002 included positive control treatments which consisted of split plots of *Bt* and non-*Bt* main plots that were spayed for *P. gossypiella*, other lepidopteran pests, *Bemisia tabaci* (Gennadius) and *Lygus hesperus* Knight based on established action thresholds (Ellsworth and Barkley 2001; Ellsworth *et al.* 1996; University of California 1996). Applications were made on 12 and 20 July and 2 August in 2001, and 12 and 25 July and 16 and 28 August in 2002 consisting of organophosphates, pyrethroids, carbamates and insect growth regulators.

ARTHROPOD NATURAL ENEMY AND PEST DENSITY

Studies in all years tracked the density of a consistent, selected complex of 22 taxa of foliage-dwelling arthropod natural enemies, primarily predators, along with densities of various key pests including *P. gossypiella*, *B. tabaci* and *L. hesperus*. Most arthropods were sampled using a standard sweep net (38-cm diameter). Two sets of 25 sweeps were collected weekly in each plot between early June and mid-September each year. Densities of immature aphelinid parasitoids attacking *B. tabaci* (*Eretmocerus* spp. and *Encarsia* spp.) were estimated by weekly leaf samples (20-30 per plot) from the seventh mainstem node below the terminal. Densities of *B. tabaci* nymphs and adults were estimated weekly from early July through mid September each year using standard methods (Naranjo and Flint 1994; 1995). Densities of *P. gossypiella* larvae were estimated by counting all larvae inside 100 hostable green bolls per plot every two weeks from early July onward. Adult moth density was monitored weekly with beginning in June with pheromone traps. The abundance of other larval lepidopterans as well as *L. hesperus* were estimated from sweep net samples.

PEST MORTALITY STUDIES

Mortality of *P. gossypiella* and *B tabaci* was examined in unsprayed plots of *Bt* and non-*Bt* cotton from 2001-2003. To examine mortality of *P. gossypiella* eggs, small cards containing 20 eggs (1 d old) obtained from the USDA-ARS rearing facility in Phoenix, AZ were pinned under the bracts of cotton bolls that were approximately 20 day old to simulate oviposition by female moths. Twenty cards (one per plant) were placed in each plot and left exposed for 24 hours after which they were examined under magnification in the laboratory for evidence of predation. Intact eggs that remained were then held at 27°C for an additional 6 d to evaluate parasitism. The experiment was repeated 3-4 in 2001-2003. The mortality of *P. gossypiella* pupae (USDA-ARS rearing facility in Phoenix, AZ) were studied on four dates each in 2002-2003. Individual pupae were placed at the base of 20 cotton plants in each plot and left exposed for 24 hours. This simulated a type of pupation site used by larvae exiting bolls. Pupae that remained were returned to the laboratory and examined under magnification for evidence of predation. Intact pupae were held for three weeks to evaluate parasitism. Appropriate controls were run for both sentinel egg and pupae. Morality of *B. tabaci* nymphs was examined using an *in situ* life table approach (Naranjo and Ellsworth 2005). Briefly, the method involves marking the location of individual settled 1st instar nymphs ($\geq$ 50 per plot) with a non-toxic felt pen on the underside of leaves and then repeatedly observing these nymphs every 2-3 d until death or adult emergence. Mortality due to dislodgment (from wind, rain and chewing predators), sucking predation, parasitism, and unknown causes was recorded for each of the four nymphal instars. Life table studies were repeated twice each year between mid-July and early September in 2001-2003.

ANALYSES

Yearly analyses were conducted for all arthropod taxa (Naranjo 2005a) but only multiyear analyses will be highlighted here. These analyses were conducted by calculating seasonal mean densities for all taxa for each replicate plot in each year and entering block and year as random effects. Arthropod counts were transformed by $(x+0.5)^{0.5}$ or $ln(x+1)$ throughout as necessary to achieve normality and homoscedasticity before analyses; untransformed means are presented. The response variable for egg and pupal mortality of *P. gossypiella* was the proportion missing, eaten or parasitized. Marginal mortality rates were calculated from *B. tabaci* life table data based on apparent mortality using the methods outlined by Elkinton *et al.* (1992) (see Naranjo and Ellsworth 2005 for details) to correct for mortality due to contemporaneous agents. Mixed model ANOVA was used to test for treatment effects in each year where block and trial were entered as random effects. Mixed model ANOVA was also used for multi-year analyses with block, year and trial within year entered as random effects. Proportional and marginal mortality values were transformed by arcsin•prior to analyses as needed.

RESULTS

ARTHROPOD ABUNDANCE

Yearly analyses revealed few differences between *Bt* and non-*Bt* cotton in any natural enemy taxa and no differences for the natural enemy community as a whole (Fig. 1). However, multiyear analyses revealed significant ($P < 0.05$) declines in seasonal densities of five predator taxa in *Bt* compared with non-*Bt* cotton including a group of miscellaneous spiders, *Hippodamia convergens* Guérin-Méneville, *Geocoris punctipes* (Say), *Nabis alternatus* Parshley and *Drapetis* nr. *divergens* (Table 1). In general, the changes in density were smaller than those observed in individual years; however, the increased sample size of the analyses improved power considerably allowing smaller changes to be detected. Overall, the mean decline in these five taxa was around 19%. In contrast, the use of insecticides resulted in significant ($P < 0.05$), and larger declines in 13 individual taxa averaging nearly 48% (Table 1). As expected, there were essentially no *P. gossypiella* larvae in *Bt* cotton and densities of other lepidopterans were reduced in most years (Fig. 1). However, populations of two other key pests, *B. tabaci* and *L. hesperus* were similar in *Bt* and non-*Bt* cotton (Fig. 1).

MORTALITY OF PEST INSECTS

Eggs of *P. gossypiella* were readily preyed upon in both *Bt* and non-*Bt* cottons with no significant differences ($P > 0.05$) in rates of predation in each of three individual years or all years combined (Fig. 2). No parasitism was detected in eggs that survived predation in any year. Pupae of *P. gossypiella* placed on the soil surface beneath plants also experienced high rates of mortality and there was no significant difference ($P > 0.05$) in rates of pupal mortality between *Bt* and non-*Bt* cottons in either year or both years combined (Fig. 2). Most pupae disappeared presumably from the action of chewing predators and no parasitism was observed in intact pupae held in the laboratory after field exposure.

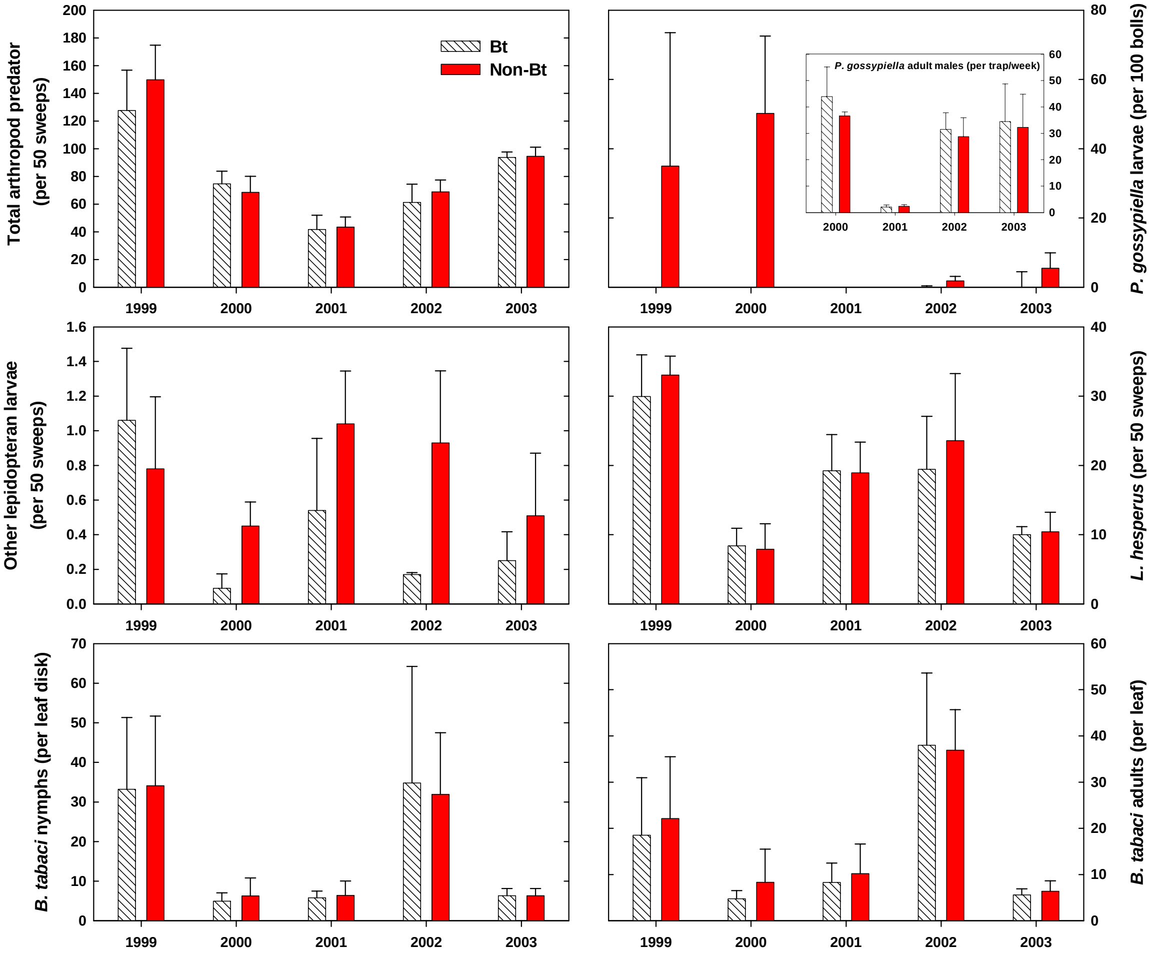

Figure 1. Seasonal mean density of arthropod natural enemies and pests over all sample dates. Error bars are 95% confidence intervals. From Naranjo (2005a).

Table 1. Overall change in mean densities of arthropods (per 50 sweeps) in *Bt* and non-*Bt* cottons (5 years) and in sprayed and unsprayed cottons (2 years), Maricopa, AZ, 1999-2003. Data from Naranjo (2005a).

Taxa	Order: Family	1999-2003		2001-2002	
		Non-Bt density[a]	**Prop. Δ(P)[b]**	**Unsprayed density[a]**	**Prop. Δ(P)[b]**
Dictyna reticulata Gertsch and Ivie	Araneida: Dictynidae	0.62±0.05	0.121 (0.56)	0.62±0.07	-0.443 (0.02)
Misumenops celer (Hentz)	Araneida: Thomisidae	2.59±0.28	-0.038 (0.42)	1.30±0.13	-0.410 (<0.01)
Salticidae	Araneida: Salticidae	0.33±0.07	-0.268 (0.07)	0.11±0.02	-0.143 (0.71)
Other Araneida	Araneida	0.63±0.14	-0.233 (0.02)	0.10±0.04	0.154 (0.73)
Collops vittatus (Say)	Coleoptera: Melyridae	1.65±0.29	-0.062 (0.51)	0.67±0.09	-0.349 (0.02)
Hippodamia convergens Guérin-Meneville	Coleoptera: Coccinellidae	1.20±0.13	-0.189 (0.04)	0.48±0.12	-0.613 (0.03)
Anthicidae	Coleoptera	1.48±0.23	-0.095 (0.33)	0.32±0.10	-0.439 (0.21)
Other Coccinellidae	Coleoptera	0.59±0.18	-0.132 (0.56)	0.20±0.04	-0.538 (0.05)
Geocoris punctipes (Say)	Heteroptera: Lygaeidae	7.30±1.69	-0.176 (0.01)	6.22±0.37	-0.781 (<0.01)
Geocoris pallens (Stål)	Heteroptera: Lygaeidae	4.30±0.79	0.058 (0.38)	2.08±0.25	-0.677 (<0.01)
Orius tristicolor (White)	Heteroptera: Anthocoridae	4.89±0.67	0.054 (0.21)	5.39±0.26	0.270 (0.01)
Nabis alternatus Parshley	Heteroptera: Nabidae	2.53±0.25	-0.238 (<0.01)	1.01±0.17	-0.837 (<0.01)
Zelus renardii Kolenati	Heteroptera: Reduviidae	0.71±0.20	-0.011 (0.77)	0.05±0.02	-0.714 (0.05)
Sinea spp.	Heteroptera: Reduviidae	0.01±0.01	0.370 (0.74)	-	-
Lygus hesperus Knight	Heteroptera: Miridae	18.8±2.23	-0.073 (0.35)	34.5±2.28	-0.557 (<0.01)
Pseudatomoscelis seriatus (Reuter)	Heteroptera: Miridae	10.3±2.33	0.044 (0.98)	1.80±0.20	-0.398 (0.02)
Spanogonicus albofasciatus (Reuter)	Heteroptera: Miridae	2.99±0.53	0.052 (0.38)	0.73±0.10	0.234 (0.65)
Rhinacloa forticornis Reuter	Heteroptera: Miridae	0.26±0.07	-0.160 (0.31)	0.03±0.02	0.000 (0.96)
Chrysoperla carnea s.l. Stephens	Neuroptera: Chrysopidae	2.27±0.21	-0.042 (0.56)	4.08±0.24	-0.105 (0.26)
Drapetis nr. *divergens*	Diptera: Empididae	19.3±4.31	-0.118 (0.02)	17.8±2.35	-0.387 (<0.01)
Aphelinid parasitoids	Hymenoptera: Aphelinidae	6.18±1.26	-0.273 (0.21)	3.64±0.63	-0.317 (0.08)
Other Hymenoptera	Hymenoptera	1.72±0.20	0.039 (0.59)	1.31±0.15	-0.208 (0.13)

[a] Overall means (±SE) based on seasonal means in four replicate main plots in each of five years for Bt and non-Bt contrasts (n=20) and two years for unsprayed and sprayed contrasts (n=8).

[b] Prop. Δ is the proportional change in density in Bt cotton relative to non-Bt cotton or sprayed cotton relative to unsprayed cotton. Numbers in parentheses following prop. Δ are P-values; values < 0.05 are bolded.

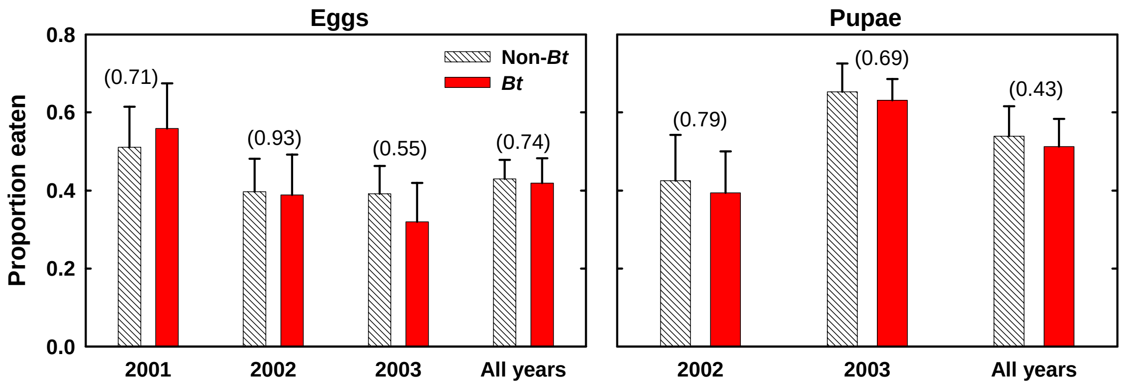

Figure 2. Comparison of natural enemy induced mortality of sentinel *P. gossypiella* eggs and pupae between unsprayed *Bt* and non-*Bt* cottons over a three-year period. Numbers above paired bars are P-values for ANOVAs of each individual year or all years combined. Error bars represent 95% confidence intervals. Results for each individual year based on 3-4 separate experiments. From Naranjo (2005b).

Nymphs of *B. tabaci* were subject to high rates of predation, moderate rates of dislodgement, and low to moderate rates of parasitism over the three years of study (Fig. 3). Marginal rates of predation did not differ significantly (*P* > 0.05) between *Bt* and non-*Bt* cotton in any year or all years combined. Rates of dislodgement, which included the effects of chewing predation and weather (Naranjo and Ellsworth 2005) varied over years but not as a result of the use of *Bt* cotton. Parasitism was generally low but there was no difference (*P* > 0.05) in rates of parasitism between the two cottons.

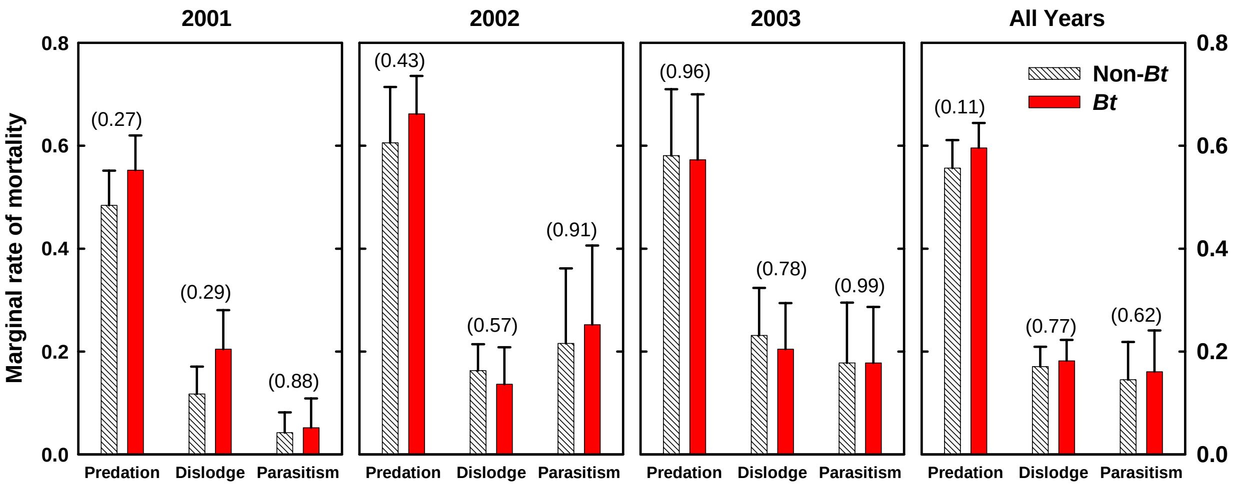

Figure 3. Comparison of natural enemy induced mortality of natural cohorts of *B. tabaci* nymphs between unsprayed *Bt* and non-*Bt* cottons over a three year period. Numbers above paired bars are *P*-values for ANOVAs of each individual year or all years combined. Error bars represent 95% confidence intervals. Results for each individual year based on two separate experiments. From Naranjo (2005b).

DISCUSSION

Although separate analyses each year generally indicated no negative effects, combined analyses across the five years revealed a significant average decline of about 19% in five predator taxa representing four orders including *H. convergens*, *G. punctipes*, *N. alternatus*, *D.* nr *divergens* and a group of miscellaneous spiders. With very few exceptions there was a numerical decline in seasonal population density of all these taxa in *Bt* compared with non-*Bt* cotton in all five years. Combining the data sets simply allowed for a larger sample size and correspondingly greater statistical power to discern smaller changes in density.

The causes for these declines are uncertain but could be associated with sampling error, declines in target or non-target prey abundance, or sublethal effects resulting from exposure to *Bt* toxins. Sampling error seems to be an unlikely cause because populations were consistently lower in *Bt* cotton for the five taxa in the majority of years. There was also no obvious difference in the canopy structure between *Bt* and non-*Bt* cotton that could have affected sampling efficiency. Many stages of *P. gossypiella* are relatively invulnerable to natural enemies (Henneberry and Naranjo 1998) and so it is unlikely that the absence of this prey in *Bt* cotton would have measurable effects on generalist predator populations. *B. tabaci* was the most abundant prey for the affected predators but densities of immature and adult stages were similar in both *Bt* and non-*Bt* cotton. Other potential caterpillar prey occurred at low densities and differed relatively little between *Bt* and Non-*Bt* cotton, however, because all are foliage feeders they are susceptible to predation and reductions in their density may have influenced predator populations. Direct feeding on the plant by *G. punctipes* and *N. alternatus* could expose these predator to *Bt* toxins, however, Armer *et al.* (2000) found no negative effects for *Geocoris* and *Nabis* spp. feeding directly on *Bt* potato foliage. Pollen feeding may be an avenue of exposure to the predaceous bugs as well as *H. convergens* but this has not been examined in these species. *D.* nr. *divergens* largely specialize on adult *B. tabaci* which are phloem feeders and unlikely to possess *Bt* toxins in their bodies. However, Ponsard *et al.* (2002) observed modest declines (H”27%) in longevity of adult *G. punctipes* and *O. tristicolor* (but not *Nabis* spp.) feeding strictly on *Bt* intoxicated *S. exigua* compared with larvae feeding on non-*Bt* cotton in the laboratory. Although, these predators would not feed exclusively on such caterpillars in the field it does suggest a potential explanation that merits further study.

The biological relevance of these declines in *Bt* cotton is also uncertain. Clearly, conventional alternatives to the use of *Bt* cotton, as represented by the positive controls in this study, are many times more damaging to the natural enemy community, causing much large reductions in density and affecting a broader range of taxa. Such disruptions by broad-spectrum insecticides have been shown repeatedly to compromise the natural biological control of cotton pests (e.g., Eveleens *et al.* 1973; Stoltz and Stern 1978). On the contrary, the results of functional studies here demonstrated that the overall contribution of the natural enemy community to mortality of *P. gossypiella* eggs and pupae and *B. tabaci* nymphs was equal in *Bt* and non-*Bt* cotton that received no additional insecticide applications. In addition there was no indication of resurgence by key pests in the system (*B. tabaci* or *L. hesperus*) that might indicate a reduction in natural control. Thus, the small declines in several taxa of natural enemies in *Bt* cotton observed here may not be ecologically meaningful in terms of at least some trophic interactions.

The lack of association of reductions in density of some predator taxa and rates of natural enemy mortality on two key pests in the system may be explained by the general feeding behavior of most of these predators. Reductions in the density and associated activity of any one species in the complex is offset or replaced by the activity of other members of the community. Life table studies with *B. tabaci* revealed that most of the mortality from any one source is replaceable (Naranjo and Ellsworth 2005). Thus, a reduction in predation *G. punctipes* or *N. alternatus*, for example, could be easily replaced by *Orius tristicolor* White or *Zelus renardii* Kolenti. However, there are limits to the amount of mortality that can be replaced by the natural enemy community. Life table studies with *B. tabaci* also have shown that the use of broad-spectrum insecticides which cause large reductions in natural enemy density can significantly reduce the contribution of these natural enemies to pest mortality leading to a situation where the continued use of insecticides are required for pest suppression (Naranjo 2001; Naranjo and Ellsworth unpublished data). In contrast, the use of selective insecticides for *B. tabaci* only slightly reduces the abundance of various natural enemies (Naranjo *et al.* 2004) but allows the complex to continue contributing significant mortality that enables long-term pest suppression in the absence of additional insecticides (Naranjo 2001; Naranjo and Ellsworth unpublished data).

Transgenic *Bt* cotton appears to represent a highly effective and selective technology for lepidopteran pest control. The long-term studies described here suggest that negative effects of *Bt* cotton on non-target arthropods, particularly natural enemies, are minimal and that even small declines in density of some taxa do not appear to be associated with any meaningful changes in the function of the overall natural enemy community. The use of *Bt* cotton and other selective methods of pest control will continue to advance the important role of biological control in cotton IPM in the western U.S. and elsewhere.

ACKNOWLEDGMENTS

I thank Kim Beimfohr, Rebecca Burke, Luis Cañas, Melanie Charney, Scott Davis, Greg Owens and Jeffrey Rivas for expert technical assistance and Peter Ellsworth (Univ. Arizona) for assistance with insecticide applications. This study was funded entirely by public funds appropriated to the USDA-ARS through CRIS project 5344-22620-015-00D.

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INTEGRATION OF *BT* COTTON IN IPM SYSTEMS: AN AUSTRALIAN PERSPECTIVE

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ABSTRACT

Insect pests may have a severe impact on cotton production in Australia. Key pests are *Helicoverpa* spp which are well adapted to exploit cropping systems and often evolve resistance to pesticides. Until recently adoption of IPM has been restricted by a lack of non-disruptive tools.

IPM must be founded on a thorough understanding of the ecology of pest and beneficial species, their interaction with the crop and surrounding non-crop environments. Insect resistant transgenic cottons have proved successful in providing a foundation for more sustainable, economically acceptable IPM with the integration of a range of other non-chemical tactics.

In Australia, *Bt* cottons (tradename INGARD®) expressing the CryIAc endotoxin from *Bacillus thuringiensis* subsp. *kurstaki*, were commercialised in 1996/97 and gradually increased in area under an industry agreed deployment strategy which limited use to 30% of the cotton area. Two gene (Cry IAc/Cry 2Ab) varieties (Bollgard II) have been commercialised from 2004/05 and have now completely replaced Ingard varieties. All *Bt* varieties are grown under a comprehensive management strategy designed to minimise the risk of resistance evolving in *Helicoverpa armigera*, the main target pest.

Commercial use of Ingard cotton varieties has reduced pesticide applications for *Helicoverpa spp* by 60%, providing major environmental benefits. Even greater pesticide reductions now occur with Bollgard II varieties. Pre-release environmental impact assessments demonstrated no significant effect of these *Bt* cottons on natural enemies. Commercial experience with *Bt* cotton crops has now shown a 3-4 fold increases in beneficial insect abundance compared to conventional crops. Co-incidentally several selective insecticides (indoxacarb, spinosad, and emamectin) became available for *Helicoverpa* control on conventional cotton, which further assisted in conserving beneficials. While resistance is the greatest risk for *Bt*

cottons their sustained value in IPM systems also requires focus on the management of secondary pests, which are suppressed in conventional cotton by *Helicoverpa* sprays. Enhanced levels of beneficial species help to partially suppress secondary pests.

Maintaining an appropriate balance and retaining the benefits of *Bt* cottons requires vigilant resistance management, sustained efforts to enhance beneficial species and non-disruptive, short residual pesticides for key sucking pests. Overall the stability of these systems will require mobilization of the whole farm environment and greater understanding of the flows of impacts and services between intensive cropping systems and the surrounding landscape.

INTRODUCTION

Insect pests represent a significant threat to Australian cotton production (Fitt 1994). Key pests include the noctuid moths *Helicoverpa armigera* and *H. punctigera*, spider mites (*Tetranychus urticae*), aphids (*Aphis gossypii*) and mirids (*Creontiades dilutus*), while insecticide resistance in several pests further complicates management.

Pest management using conventional pesticides can be effective but imposes significant economic and environmental costs including disruption of natural biological control agents. Integrated pest management has long been proposed as a more sustainable approach in many situations, however, the adoption of a truly integrated pest management approach has been extremely patchy.

Broadly IPM can be defined as “the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms.” (FAO 2002). In this paper we will illustrate how the adoption of *Bt* cotton varieties has assisted the implementation of IPM in the Australian cotton industry.

Many aspects of IPM have been applied in the Australian cotton industry since the late 1970s when the computer based decision support system, SIRATAC, was released to industry (Hearn and Bange 2002). The minimal IPM approach involved the use of sampling systems and thresholds to better time the use of pesticides. However, today IPM represents a more expansive approach which seeks to minimise pesticide use and include a broader range of tactics such as pest resistant varieties, conservation and augmentation of beneficial insect populations, use of selective and short residual insecticides, recognition of the compensatory capacity of the plant and various cultural control practices which have long been associated with the IPM concept.

These broad principles are captured in the “Integrated Pest Management Guidelines for Cotton Production Systems in Australia” produced for the Australian industry (Deutscher, Wilson, and Mensah, 2004 - http://cotton.crc.org.au/Assets/PDFFiles/IPMGL05/IPMGLFor.pdf). The Guidelines emphasise four principles:

1. conservation and utilization of beneficial insects;
2. preferential use of selective insecticides;
3. an emphasis on both profitability and sustainability, ensuring that both input costs and yield are considered, rather than the traditional emphasis on maximizing yield;
4. integration of all farm management activities, throughout the annual cycle of production, not just during the cotton season.

It is into this milieu that *Bt* cotton varieties contribute significantly by providing effective control of the key pests without disruption of the system so providing opportunities for the enhanced role of naturally occurring biocontrol agents to be recognised, manipulated and managed to achieve more sustainable systems.

GENETICALLY MODIFIED COTTONS IN IPM

Bt cotton varieties expressing the Cry 1Ac protein from *Bacillus thuringiensis* subsp. *kurstaki* were first registered in Australia in1996 (INGARD®) and gradually increased in area under an industry agreed deployment strategy which limited use to 30% of the cotton area. Two gene (Cry IAc/Cry 2Ab) varieties (Bollgard II) have been commercialised from 2004/05 and have now completely replaced Ingard varieties. All *Bt* varieties are grown under a comprehensive management strategy designed to minimise the risk of resistance evolving in *Helicoverpa armigera*, the main target pest. Fitt (2003; 2004) provides an assessment of the impact of *Bt* cotton in Australia over the first six years of commercial use. While efficacy of INGARD cottons is not consistent through the growing season and can be highly variable (Fitt *et al*. 1994; Fitt *et al.* 1998), growers have learned to manage INGARD varieties and substantial reductions in pesticide use on *Bt* cotton have occurred.

Potential non-target impacts of *Bt* cotton were one of the environmental impacts which required pre-release assessment. Cry proteins from *Bacillus thuringiensis* (*Bt*) introduced have been deployed as safe and effective pest control agents in microbial *Bt* formulations for almost 40 years in many developed and developing countries (Glare and O'Callaghan 2000). Potential impacts of *Bt* cotton on non-target species may involve direct or indirect effects and a range of assessment protocols have been proposed. Schuler *et al.* (2001; 2004) and Poppy (2000) outline a comprehensive, hierarchical protocol for assessing non-target effects commencing with laboratory studies to assess direct or indirect impacts on non-targets or their predators and parasitoids – a worst case scenario, through a second tier of semi-field contained population experiments and finally a third tier of field experiments.

In the case of direct effects of Cry IAc and Cry 2Ab proteins on non-target species, the well established specificity of these proteins provides a clear safeguard that greatly reduces risks of direct effects on non-lepidopteran species. Non-targets such as predators which do not feed on the plant are not directly exposed and no evidence exists for a secondary impact through consumption of intoxicated prey. The reported effects of Cry IAb expressed in maize on the survival of lacewing larvae (Hilbeck *et al*. 1998a,b; 1999) were recently shown to be mediated by reduced prey-quality rather than any direct effect of the protein toxin (Romeis *et al*. 2004).

Research in Australia examined the diversity and species richness of invertebrate communities present in unsprayed *Bt* and conventional crops. Across a number of specific invertebrate categories, Fitt and Wilson (2002) found no significant impacts of *Bt* cotton. When analysed as a whole community (Whitehouse *et al.* in press) there were small differences between the communities in *Bt* cotton and conventional cotton, but these differences peaked at different times during the season, and the timing of differences was not consistent between years. Again for the majority of functional groups there was no impact of *Bt* cotton.

Indirect effects on non-target species may be mediated through changes in abundance and diversity of prey. The significance of a reduced density of Noctuid larvae and pupae as food sources for predators or as hosts for parasitoids depends on the importance of *Helicoverpa* life stages in cotton in maintaining local populations of these beneficials. Clearly within transgenic cotton fields, the abundance of some predators and parasitoids may be reduced, particularly those whose survival is closely tied to the abundance of *Helicoverpa,* but this is unlikely to threaten their regional persistence since in the cropping systems where cotton is usually grown a significant proportion of the *Helicoverpa* population is also present on other crops and uncultivated hosts (Fitt 1989; Hearn and Fitt 1992) where parasitoids are also active. None of the known predators that attack Lepidoptera in cotton are specialists; *Helicoverpa* may be only incidental prey items for some key predators whose within-field abundance is maintained by other prey. Other studies have sought further indirect effects of transgenic on parasitoids and non-target herbivores but have generally found no effect of the *Bt* plant itself (e.g., Schuler *et al*. 2001; 2004).

Within-field impacts on non-target insects, even if they do occur, are unlikely to be significant compared to the undoubted impacts from broad-spectrum pesticides. The appropriate control treatment for any experimental comparison of the impact of transgenic cotton must include not only unsprayed non-transgenic conventional cotton, but also conventional cotton with its required management which will usually involve synthetic pesticides (Fitt and Wilson 2002). A similar sentiment has been expressed by EU researchers (http://europa.eu.int/comm/research/fp5/pdf/eag-gmo.pdf). Observations over the past 6 years confirm that the abundance of beneficial invertebrates in commercial *Bt* cotton fields is markedly greater than in conventional fields.

An additional concern with *Bt* cottons is that secondary pests, once suppressed by insecticides applied for *Helicoverpa*, may become significant pests in their own right. The suite of sucking pests (mirids, aphids, stink bugs, cotton stainers etc.) are the group most likely to show such effects. While it is true that sucking pests have become a more significant part of the pest complex in *Bt* crops in some countries (Wilson *et al*. 2004; Wu *et al*. 2002) they have not required additional spraying at levels where the advantage of the *Bt* crop has been significantly eroded. In Australia, an average 60% reduction in sprays applied for *Helicoverpa* was accompanied by no change in sprays for mirids, aphids, mites and thrips (Fitt 2004). Likewise Wu and Guo (2003) report that *Bt* cotton in China help to prevent resurgence of aphid populations. By contrast in the south-eastern USA stink bugs have assumed significant pest status in *Bt* cotton crops (Greene *et al*. 2001).

IMPACTS OF *BT* COTTON IN IPM

Commercial use of Ingard cotton varieties in Australia generated an average reduction in pesticide applications for *Helicoverpa* of 56%, with no significant change in pesticide applications for minor pests (Fitt 2004). With Bollgard II varieties now commercialised in the last two years and completely replacing Ingard varieties in the current (2004/05) cotton season, significantly greater reductions compared to conventional cotton have been achieved. Indeed during the 2004/05 season many Bollgard II cotton crops were not sprayed for pests. Anecdotal evidence also shows that researchers now find difficulty in establishing pest populations in field plots for experimental purposes because of the widespread high abundance of predators and parasitoids. As discussed later there have also been simultaneous reductions in pesticide use on conventional cotton over the last 5-6 years, although to a lesser extent.

Ingard and Bollgard II cotton varieties are not perceived as "magic bullets" for pest control in Australia. Instead they are viewed broadly as an opportunity to address environmental concerns about cotton production and more specifically as a foundation to build IPM systems which incorporate a broad range of biological and cultural tactics (Fitt 2000; Wilson *et al.* 2004). The most consistent "winner" from INGARD® technology has been the environment, with reduced pesticide loads.

Coincident with the adoption of *Bt* cotton varieties has been widespread adoption of an IPM approach, supported by a strong extension campaign (Christiansen and Dalton 2002). This is revealed by a significant change in grower attitudes in addition to significant reductions in pesticide use (expressed as active ingredient) on both conventional and transgenic crops (Fig. 1), achieving environmental gains and enhancing future sustainability of the industry.

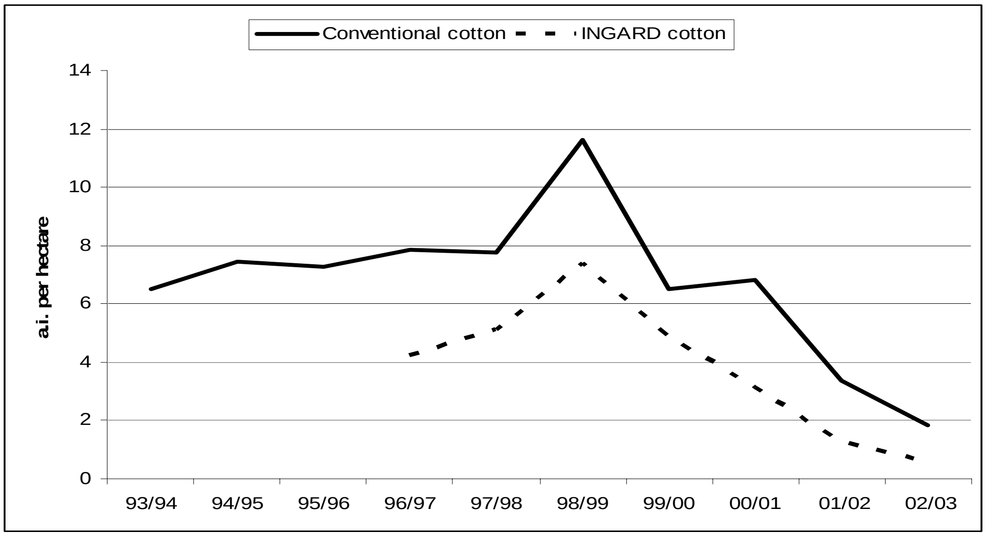

Figure 1. Change in Pesticide Use (active ingredient per hectare) on Australia cotton crops. Significant reductions have been achieved on both conventional and INGARD® cottons over the last 5 years. Source: Cotton Consultants Association Market Audit Survey 2003.

Care must be taken in interpretation of Figure 1 since insecticide use is linked to pest abundance and *Helicoverpa* spp. have been at relatively low densities during the prolonged drought in many cotton areas. Likewise several newer pesticides are active at much lower concentrations than the pesticides they replaced. It also seems unlikely that the reduction in pesticide use on conventional cotton results from the regional impact of *Bt* cotton on *Helicoverpa* abundance since over the period shown the area of *Bt* cotton was limited to a maximum of 30% of the cotton in a region. A number of factors are likely involved here, but one important possibility is that the coincident release of *Bt* cotton and the industry wide extension effort on IPM, allowed many growers to build confidence in the potential for IPM by managing their *Bt* cotton crops. They were able to become more comfortable with seeing a "living" crop, filled with numerous and mostly innocuous or beneficial insects, more attuned the critical importance of managing agronomic inputs, and more willing to work cooperatively with neighbours through the IPM and area wide groups. It will be instructive to view the ongoing management of conventional cotton and the performance and adoption of Bollgard II cotton varieties now that the 30% cap on *Bt* varieties has been lifted. In the 2004/05 season Bollgard II varieties accounted for 70% of the Australian cotton area.

CONCLUSIONS

IPM systems for future production of many broad acre and horticultural crops will, of necessity, be more complex than the pesticide based systems currently in place. In essence IPM reflects a sound interaction of science and pragmatism to achieve productive, viable and sustainable production systems.

As farming systems change the pest complex will also change. The fundamental role of IPM in reducing pest pressure and insecticide use means that it must continue to evolve. This is particularly so in high value, high input production systems such as cotton. For Australian cotton there seem little doubt that *Bt* cotton varieties have brought considerable gains in management of key pests but more importantly have facilitated a broader recognition and adoption of IPM principles, particularly the importance of natural biological control.

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