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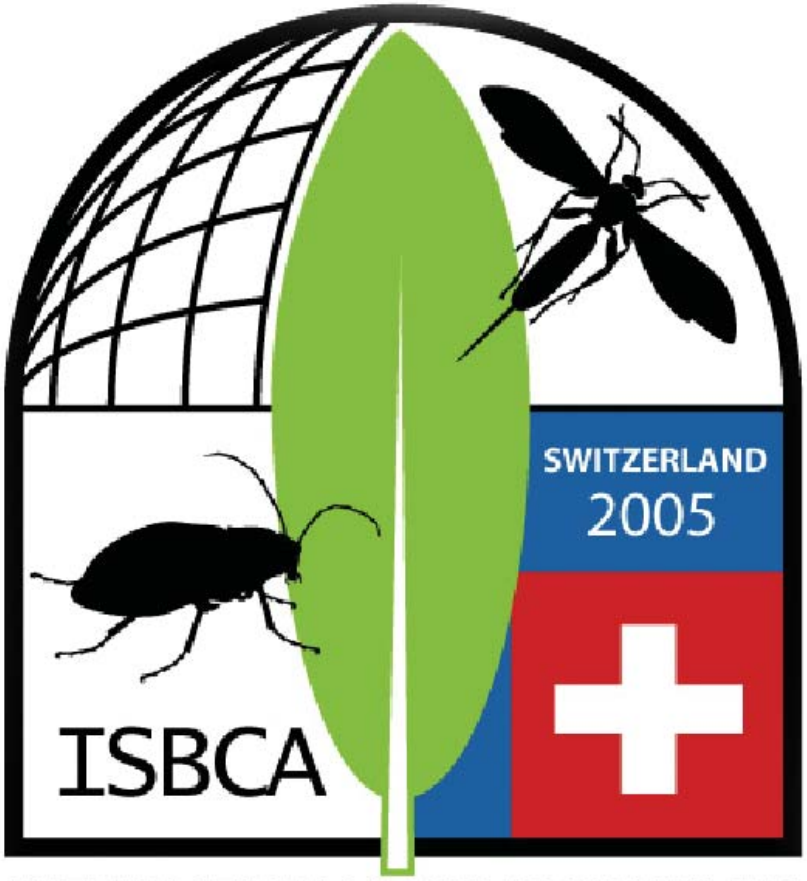
Biological Control



INTERNATIONAL SYMPOSIUM ON BIOLOGICAL CONTROL OF ARTHROPODS

September 12-16, 2005

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Second International Symposium on Biological Control of Arthropods

Davos, Switzerland
September 12-16, 2005

USDA Forest Service
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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.

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SCIENTIFIC SESSION ORGANIZING COMMITTEE MEMBERS

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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.

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CONFERENCE PROCEEDINGS

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Volume II

INTERACTIVE-WEB OF FACTORS GOVERNING EFFECTIVE NATURAL ENEMY FORAGING BEHAVIOR: OVERVIEW OF FOOD RESOURCES AS A CRITICAL COMPONENT

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ABSTRACT

Effective biological control of pests is determined by the abundance, retention and searching efficacy of natural enemies. To assure their reproductive fitness, natural enemies such as predators and parasitoids must effectively balance competing resource needs such as an adequate frequency of encounter with prey and hosts for reproduction, requirements of food other than prey and hosts, and other needs such as shelter and mates. The other food requirements consist primarily of short-term nutritional needs and are often separate from the target pest, such as plant nectar in the case of parasitoids. The appropriate quality, adequate availability, and detectability of these non-mutually exclusive requirements in the target area, strongly affect the natural enemy's retention and pest foraging efficacy. We present a conceptual model of factors determining eventual foraging behavior of parasitoids that would guide empirical studies of the resource needs of parasitoids and other insects. An increased understanding of the interplay of the resource web with the habitat would allow us to leverage this information to design habitat management practices that allow the use of natural enemy species for biological control in a consistent and reliable manner.

THE IMPORTANCE OF ADULT FOOD FOR PARASITOIDS

The importance of adult food for natural enemy species such as predators and parasitoids has been recognized for decades. Parasitoid species (species that lay their eggs on or in other insect species, eventually killing them) are often used as models of natural enemy foraging behavior because of the relatively direct link between their foraging behavior and reproductive fitness when compared with predator species. Numerous laboratory studies have shown that suitable food sources can substantially increase longevity and fecundity of adult hymenopteran and dipteran parasitoids (reviews in Heimpel *et al.* 1997; Lewis *et al.* 1998). It is now appreciated that the consumption of non-host food can influence many other aspects of parasitoid biology such as egg viability, diapause in progeny, foraging decisions, searching

efficiency, the onset and rate of egg resorption, primary sex ratio of progeny, flight initiation, and timing of flight. As a consequence non-host food can affect parasitoid and host dynamics, competitive interactions and niche partitioning among parasitoid species, productivity in laboratory cultures, and the probability of parasitoid establishment in classical biological control (Jervis 1998).

Parasitoids can be separated into four broad categories in terms of adult feeding requirements: (1) Pro-ovigenic species where adult feeding is needed for maintenance but not for egg production (e.g., Jervis and Kidd 1996). Very few examples exist of truly pro-ovigenic species (Jervis *et al.* 2001). (2) Synovigenic species that do not host-feed but feed on non-host food for maintenance and egg production (e.g., *Microplitis croceipes* Cresson, Hymenoptera: Braconidae) (Takasu and Lewis 1993). (3) Synovigenic species whereby females host-feed for egg production and both males and females non-host feed for maintenance (e.g. *Ooencyrtus nezarae* Ishii [Hymenoptera: Encrytidae]) (Takasu and Hirose 1991), (4) Synovigenic species whereby females host-feed for both maintenance and egg production (e.g., *Bracon hebetor* Say, Hymenoptera: Braconidae) (Jervis *et al.* 1994; Takasu unpublished). Thus, the basic drive for adult food sources will vary with the particular parasitoid species, which is determined by their genetic traits. The mouthparts and the size of the parasitoid as determined by their genetics are also important in their ability to access both host and non-host food sources. For host-feeding species, the host materials are mainly obtained directly from the opening of the puncture wound caused by ovipositor insertion, or for some species, through production of so-called feeding tubes that allow them to host-feed on less accessible hosts (e.g. Heimpel *et al.* 1997). Host materials mainly provide parasitoids with protein, vitamin and salt resources for reproduction, whereas plant nectars and honeydew provide energy resources mainly from the sugars present, although amino acids are also present (Harborne 1993). Several taxa have specialized mouthparts, referred to as a 'concealed nectar extraction apparatus' (CNEA), for reaching floral nectar (Jervis 1998; Quicke 1997). The CNEA's of parasitoids vary in length and are primarily utilized to extract nectar contained in long or deep tubular flower corollas that are not accessible to larger sized parasitoids or those lacking a CNEA. Those species lacking a CNEA appear capable of everting their labiomaxillary complex far enough to exploit nectar contained in very short, narrow, tubular flower corollas or for host feeding from the ovipositor puncture wound (Jervis 1998). Therefore, the morphology of parasitoid mouthparts and parasitoid size will influence the accessibility of both host and non-host food sources for parasitoid species.

RANGE OF RESOURCE NEEDS

In addition to adult food, parasitoids also need hosts, shelter and mates throughout their life cycle, and they must balance these needs by effectively responding to stimuli associated with each of these resources. The need for each of these resources may be more important at different times and/or seasons which would depend on the life cycle, and informational and physiological state of the parasitoid. Although little is known about the distribution of parasitoids with respect to their resource needs, several studies have shown innate and directed search by a range of species in response to food-related signals (Patt *et al.* 1997; Stapel *et al.* 1997; Wäckers and Swaans 1993). These responses can be similar to those towards host-related signals shown

by some parasitoids (e.g., Lewis *et al.* 1990), but may also be specific to the task of food foraging (Olson *et al.* 2003; Wäckers *et al.* 2002). Learning also plays a significant role in the search for food as well as hosts, and parasitoids are able to use different visual and olfactory cues in accordance with their physiological state and previous experience (Iizuka and Takasu; Lewis and Takasu 1990; Takasu and Lewis 1996; 1999; Tertuliano *et al.* 2004; Sato and Takasu 2000; Wäckers and Lewis 1994). Learning can be very useful as the quantity and quality of food resources often varies across plants or within the plant. This variation may be caused by factors such as the presence of other nectar-feeding species, the spatial and temporal secretion of nectar, and the nutritional value, repellency, or toxicity of different nectars (Jervis *et al.* 1993). Tertuliano *et al.* (2004) found that females that had learned to associate a particular odor with food rewards will continue to elicit food-searching behaviors after several unrewarding experiences with the odor when they are very hungry, whereas females that were less hungry ceased to respond to the learned odor after only two unrewarding experiences. Interestingly, food-searching responses of the less hungry females were recovered after a single exposure to the odor with a food reward (Tertuliano *et al.* 2004). Adult parasitoids are, therefore, predicted to respond to resource stimuli that are more strongly associated with their current needs and in accordance with prior experience.

The sources of variation discussed above are not mutually exclusive; rather they overlap extensively, even within a single individual. Therefore, it is important that we have a means of clearly delineating the sources, roles, and interacting effects of the variations. The conceptual model of Lewis *et al.* (1990) for collectively describing the various foregoing factors and their sum effect on foraging behavior of parasitoids are presented in Fig. 1. The three major sources of intrinsic variability in the behavior of foraging female parasitoids are represented: (1) genetic diversity among individuals, (2) phenotypic plasticity within individuals because of experience, and (3) the parasitoids's physiological state relative to other needs. The behavior manifested is also dependent on the foraging environment, so the final foraging effectiveness of a parasitoid is determined by how well the parasitoid's net intrinsic condition as a result of these three components is matched with the foraging environment in which it operates.

In Fig. 1, suppose there is a hypothetical parasitoid species and three foraging environments: EA, EB and EC. Under genotypic diversity the response of two representative individual genotypes, G_1 and G_2 are shown. This response potential consists of the genetically fixed maximum range of usable foraging stimuli and ultimate level with which the parasitoid could respond to the stimuli (the total darkened area plus shaded area). This maximum level of response to the array of stimuli is shown as a curve, which indicates that the maximum response level varies with different stimuli in its range. As reflected by the different range and curve configurations for G_1 and G_2, the response potential may vary substantially among individuals within a population (Hoy 1988; Olson and Andow 2002; Prévost and Lewis 1990). The activated response potential of G_1 and G_2 (darkened area) that could be realized at any given time is somewhat less than their overall potential and depends on the experience of the individual. The balance of the response potential that is not currently activated due to the experience of the individual is the latent response potential (shaded area). In the case of naive individuals, the active response potential is that portion that is inherently activated and this does not require experience before it can be manifested. The stimuli of the three representative foraging environments, EA, EB and EC are all within the range of population P1; fur-

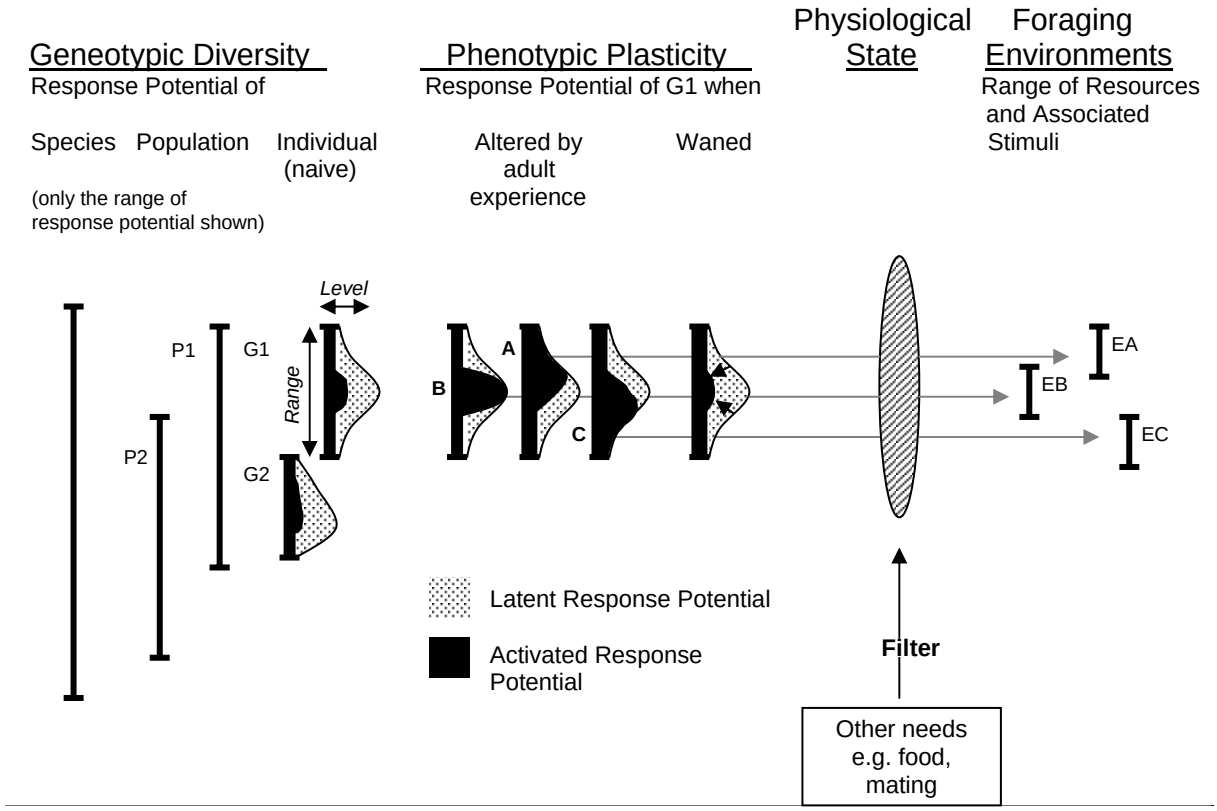

Figure 1. Factors determining eventual foraging behavior of a parasitoid. From Lewis *et al.*1990.

thermore, the response ranges of individuals with representative genotype G_1 are best aligned with these environments, but the inherent preference of the genotype G_1, as indicated, is for environment EB.

As stated previously, a parasitoid's physiological state relative to other needs such as food, mating and hosts can strongly influence their foraging behavior. Thus, as shown in Fig. 1, the physiological state of the parasitoid relative to other needs can be considered as a gateway that filters the detection and responses to foraging stimuli based on priority of the needs.

MATCHING PARASITOIDS WITH THEIR RESOURCE NEEDS

The range of needed resources of parasitoids that often differ in time and space suggests that habitats managed year-round to foster efficiency in the appropriate interplay of resource acquisition would ensure that the basic requirements are met. The resources must have quality and be adequately available (Fig. 2). Mediating cues of the resource are also needed to ensure detectability (Fig. 2). Plants may help parasitoids to increase availability, accessibility and detectability of resources needed by parasitoids. Many plants have traits that help to guide parasitoids to their hosts through chemical signaling in response to herbivory, and parasitoids have been shown to use the plant chemical signaling together with host derived chemicals and visual cues to orient to these plants (Turlings and Wäckers 2004; Wäckers 1994; Wäckers and Lewis 1994) at time in a very host-specific manner (DeMoraes *et al.* 1998). Cot-

ton (*Gossypium herbaceum* L.) and castor bean (*Ricinus communis* L.) plants not only emit volatiles to attract parasitoids but also increase their production of extrafloral nectar when attacked by herbivores (Wäckers *et al.* 2001).

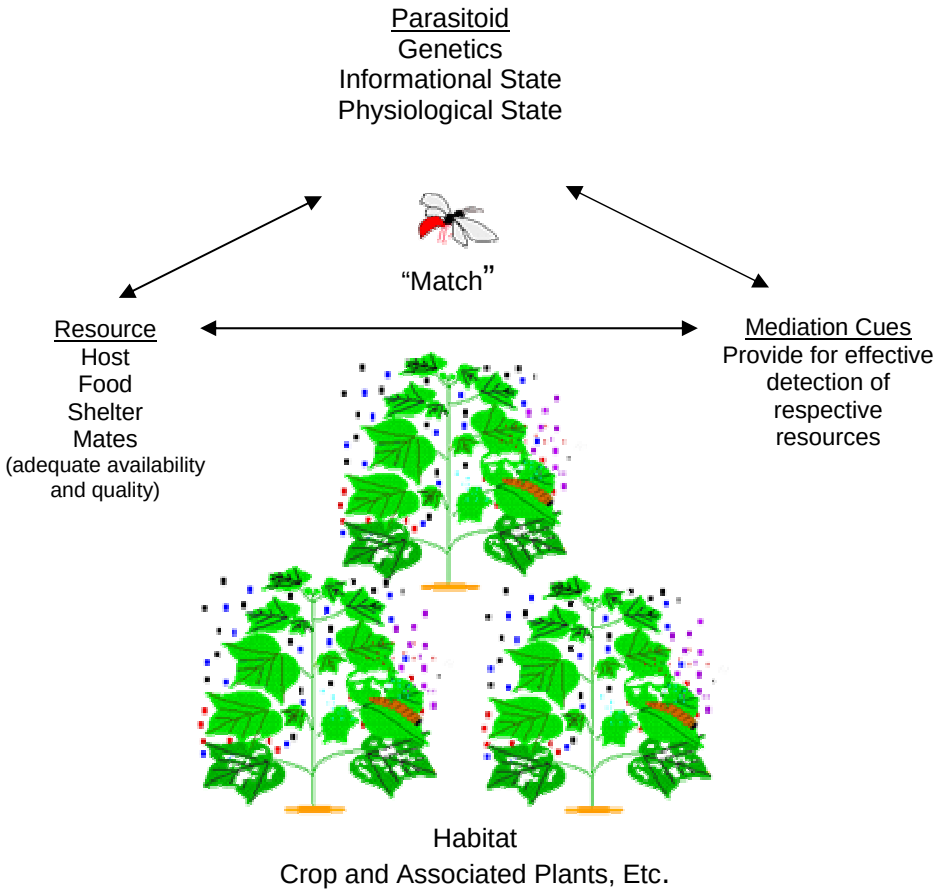

Figure 2. Model of retention and effective performance of biological control agents.

These plants provide parasitoids with the chemical signaling needed to locate the plants and both the host and food resources that they need. Stapel *et al.* (1997) found that hungry parasitoids had higher retention times within a cotton patch when both food and hosts were present than when only hosts were present. Furthermore, parasitoids can improve their rate of food and host location through learning from prior experience (Olson *et al.* 2003; Takasu and Lewis 1993). At larger spatial scales (e.g., kilometers) little is known about how parasitoids locate needed resources (food, hosts, mates and shelter). However, it is likely that having resources available in relative close proximity would provide the most efficiency in their acquisition, especially for species that move only short distances. Thus, the designs of the individual plants supporting the various needed resources are important in foraging efficiency and retention as well as their spatial distribution in the landscape and the latter would depend on the movement behavior of particular species.

Although some crop plants supply more than one of the needed resources of parasitoid species, many requirements must be obtained elsewhere. These associated plants (Fig. 2) may be other crop plants, or vegetative patches within the landscape (e.g., woodlots, hedgerows, fencerows). In a recent study, Wäckers and Steppuln (2003) were able to demonstrate that parasitoids collected adjacent to a flowering field border had higher sugar levels as compared to individuals collected in control fields. Moreover, between 55% and 80% of the collected parasitoids contained honeydew-specific sugars, indicating the prevalent use of this sugar source. In another study of field borders, Olson and Wäckers (unpublished data) were able to show that the larval parasitoid, *Meteorus autographae* Muesebeck (Hymenoptra: Braconidae) captured in naturally regenerated field edge habitat constructed for Bob White Quail habitat along the edge of a cotton field had levels of sugar in their guts that were about equal to those found in non-fed (control) females, whereas those captured in a Cahaba White Vetch experimental plot at the same time of year had about four times the levels found in the Quail and control samples. These samples were taken early in the season prior to the cotton plant's secretion of nectar or when crop plant sugar sources were very limited. In addition, the crop plant at this stage is very small and the microclimate in the field often harsh for many insect species. The early growing stage of crops can include conditions of high heat and low relative humidity which precludes many insect species from early colonization (e.g., Dyer and Landis 1996). These results indicate that having appropriate associated plants available near the crop plant can be crucial to providing several of the parasitoid's needed resources.

CONCLUSIONS

A conceptual model of factors determining eventual foraging behavior of parasitoids helps to guide empirical studies of the resource needs of parasitoids and other insects. Understanding the interplay of the resource web with the habitat allows us to leverage this information to design habitat management practices that allow the use of natural enemy species for biological control in a consistent and reliable manner. Year-round provisioning of resources is needed to account for the range of resource needs of species throughout their lifetime. Understanding the mechanisms involved in the various resource needs of parasitoids and other insect species and their effective acquisition would enable practitioners of biological control to ensure that species-specific resource needs are met.

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IMPACTS OF SUGAR FEEDING ON PARASITOID BEHAVIOR IN THE FIELD

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ABSTRACT

Diversifying agroecosystems with floral habitats has the potential to conserve natural enemies and enhance pest control. In the laboratory, many adult parasitoids readily utilize nectar sources that have substantially increased their longevity and parasitism rates. However, in the field, does the floral habitat retain parasitoids locally so they exert greater control on pests? We studied the post-feeding and aggregation behavior of *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae), an abundant parasitoid of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). First, sugar-fed and hungry *D. insulare* were compared for retention inside cabbage plots (12 x 20 m in 2003; 9 x 15 m in 2004). Sugar-fed and hungry wasps were marked, released inside the plot and recaptured outside the border over 1-3 days. Sugar feeding did not appear to affect dispersal behavior of *D. insulare* in the field. Second, we determined whether sugar sources attracted/retained parasitoids in the crop field by monitoring the abundance of *D. insulare* inside cabbage plots (12 m x 20 m) bordered by 3 m wide buckwheat strips and cabbage plots devoid of floral habitat. For three summers, *D. insulare* were monitored within plots using sticky traps, and the number of adults captured in plots with and without floral borders did not differ. Neither experiment showed evidence that buckwheat flowers increased retention of *D. insulare.*

INTRODUCTION

Establishing nectar-producing floral habitats within or near crop fields can provide adult parasitoids with sugar and reduce risks and energetic costs of commuting between food and host sources (Lewis *et al.* 1998). Parasitoids orient towards nectar odors (Patt *et al.* 1999; Wäckers 2004) and floral colors (Wäckers 1994). Thus, the presence of sugar sources in a host patch should retain parasitoids locally. Host patches of five cotton plants with extrafloral nectar and sucrose have retained *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) for 31.5-34.5 minutes whereas host patches without sugar retained parasitoids for 14 minutes (Stapel *et al.* 1997). Not surprisingly, parasitoids have been found to aggregate more among crops with floral vegetation (Berndt *et al.* 2002; Irvin *et al.* 2000; Stephens *et al.* 1998) or sugar

sprays (Jacob and Evans 1998) than crops without such resources. Also, parasitoid abundance (Platt *et al.* 1999) and parasitism rates (Baggen and Gurr 1998; Tylianakis *et al.* 2004) have been observed to decline in the crop as distance from a floral border increased.

Contrary to expectations, an increase in parasitism rates does not always occur in the presence of floral nectar (Berndt *et al.* 2002; Irvin *et al.* 2000). The expectation that supplementary nectar improves biological control, may not apply if parasitoids have sufficient sugar sources without supplemental floral nectar, parasitoids do not feed from the nectar, or if parasitoid longevity and fecundity are not improved with nectar feeding (Heimpel and Jervis 2005). The parasitoid *Diadegma insulare* has been studied for some of these criteria; it attacks diamondback moth larvae *Plutella xylostella* on cruciferous plants. Presence of supplementary floral nectar sometimes increased feeding by *D. insulare* (Lee *et al.* in prep), increased longevity and the number of eggs laid per female per hour (Lee and Heimpel in prep), but had little impact on resulting parasitism rates (Lee and Heimpel in review). The lack of correlation between feeding and parasitism puts into question whether the behavior of *D. insulare* following sugar feeding may differ from expected.

While sugar-fed parasitoids may search for hosts immediately near the sugar source, parasitoids may eventually disperse to other host patches. Feeding provides ample carbohydrate reserves (Fadamiro and Heimpel 2001; Lee *et al.* 2004; Olson *et al.* 2000) that fuel flight and may induce dispersal and not retention. Some studies support increased flight activity with sugar feeding. In flight chambers, *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae) given honey showed a small but significant 6% increase in the propensity to fly than starved females (Forsse *et al.* 1992). Whether this would reflect a tendency for dispersal flight is not known. In a field study, *Hyposoter* sp. (Hymenoptera: Ichneumonidae) wasps were marked with the trace element Rubidium if they fed on marked floral nectar sources; and fed wasps were captured more frequently at 75 m than at 6 m or less from marked plants (Freeman-Long *et al.* 1998).

Parasitoids may also disperse to increase their fitness. First, parasitoids that cannot discriminate between hosts that have been parasitized may leave a patch early than risk superparasitism and wasting eggs (Rosenheim and Mangel 1994). *D. insulare* appears to lay eggs randomly among hosts in the field without avoidance of superparasitism (Lee and Heimpel 2004). Or, *D. insulare* may be dispersing to other patches to avoid inbreeding since this species can have severe inbreeding depression due to its single-locus complementary sex determination (CSD) (Butcher *et al.* 2000). Parasitoids may also disperse to avoid positively density-dependent hyperparasitism (Ayal and Green 1993). Or parasitoids may 'spread the risk' in case of widespread mortality occurring in a single host patch, but the conditions for using risk spreading over space are rather stringent (Hopper 1999).

While sugar feeding can benefit parasitoids including *D. insulare*, the impacts that floral sources have on longer-term parasitoid behavior are not known. Our objectives were to determine how sugar feeding influences *D. insulare*'s dispersal in and out of a host patch at a greater spatial and temporal scale: 12 x 20 m or 9 x 15 m cabbage plot for 8 hours or longer. Also, we compared the number of *D. insulare* in cabbage plots with/without floral borders for evidence of enhanced attraction/retention.

MATERIALS AND METHODS

FIELD PATCH STUDY

We conducted a mark-recapture experiment on sugar/nectar-fed and hungry *D. insulare* to study retention in a field plot. A 12 x 20 m cabbage plot with 12 cabbage rows was planted with seedling transplants on 5 June 2003 within a soybean field at the Rosemount Field Station. Three mark-recapture trials were started on 9, 15 and 23 September 2003. Experiments were conducted late in the season since natural populations of *D. insulare* had declined, and possible sugar sources such as honeydew from soybean aphids in the surrounding field were not available. Four large sticky traps were set up along each border of the plot, 16 traps total, during the 1st trial. Seven sticky traps were set up per side, 28 total, during the 2nd and 3rd trials (Fig. 1).

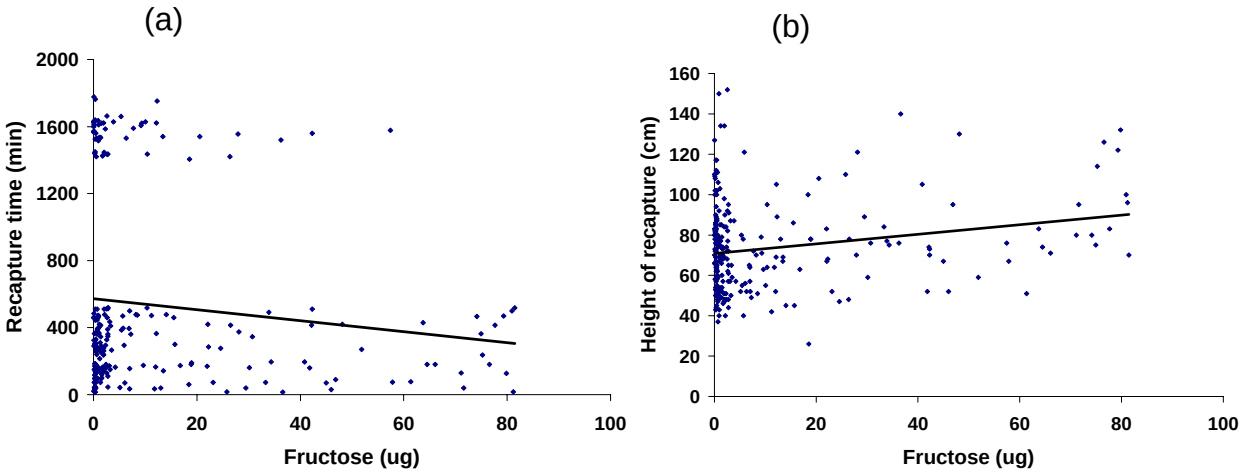

Figure 1. Field plot in experimental soybean field at the Rosemount Field Station.

Sticky traps were 1 x 0.91 m in size composed of grey window screening (mesh size 1 x 2 mm). Traps stood about 40-50 cm aboveground and ~2 m from the plot edge to increase the probability of catching dispersing wasps rather than wasps foraging low for hosts or seeking shelter. Screens were stapled to 4 x 4 x 183 cm wooden stakes pounded into the ground. At 8 am, an aerosol formula of Tangle-Trap® was sprayed onto the screening. Tangle-Trap® was reapplied at noon and at the end of the day.

In the following year (2004), a 9 x 15 m cabbage plot with 9 cabbage rows was handplanted with seedlings on 3 June 2004 in the St. Paul Agricultural Experimental Field. Field corn and soybeans were the predominant surrounding vegetation. Weeds were removed by hand and obvious floral nectar sources were not apparent. Mark-recapture trials were started on 18, 24 and 31 July 2004 before natural populations of *D. insulare* had built up. Seven sticky traps were set up along the length of the plot and six along the width, 26 traps total.

In 2003, we used *D. insulare* from our laboratory colony (1st-3rd generation) started in August 2003 from wild populations. In 2004, we used laboratory reared colonies from both Minnesota and Apopka, Florida. To recruit large numbers of wasps, all wasps emerging 1-5

days prior to the release date were randomly assigned to a 30.5 cm^2 mesh cage (Bug Dorm, BioQuip®) designated as the hungry or fed treatment. Fed wasps were given buckwheat flowers, 20% sucrose or 20% honey solutions *ad libitum*. Since *D. insulare* die in 1-2 days without food (Lee *et al.* 2004), hungry wasps were initially maintained on the same sugar foods until 20 h before release when only water was provided. *D. insulare* fed buckwheat nectar metabolize most of their gut sugars when starved for 12-36 h (Lee 2004). Wasps emerging within 20 h of release were also used, those placed in the hungry treatment did not have any opportunity to feed. Before release, wasps were sexed and aspirated in groups of 50 into 1 oz. plastic vials. To mark wasps, they were first chilled for 5 minutes at -10° C, transferred into a 1 oz. plastic cup with 6 mg of pink or yellow fluorescent powder (Day-Glo Color Corp.) and tumbled. This procedure moderately dusts *D. insulare* without affecting their longevity, and the powder remains visible in their thoracic crevices 13-16 days later despite the wasps grooming themselves (Lee 2004).

Wasps were released in the center of the cabbage plot at 9 am. Some overdusted wasps died or moved little. We counted dead/inactive wasps at the release site at 10 am and subtracted this number to estimate the number of wasps released. About 500 diamondback larvae were sprinkled onto cabbage plants to ensure hosts for females. Sticky screens were monitored every half hour after release of wasps, marked *D. insulare* were collected and frozen with the time, trap number, and height from ground recorded. For the 1st trial in 2003, *D. insulare* were monitored from 9:00-17:00 on day 1 only. For the 2nd trial, *D. insulare* were monitored from 9:00-18:00 on day 1, 10:30-15:00 on day 2, and 12:00-14:00 on day 3. During the 3rd trial, monitoring occurred from 9:00-18:00 on day 1, and 10:00-12:00 on day 2. In trial 1 of 2004, wasps were monitored from 9:00-18:00 on day 1, and 9:00-10:00 on day 2; in trial 2 from 9:20-18:00 on day 1, and 8:20-15:00 on day 2; in trial 3 from 9:00-19:00 on day 1, and 8:00-10:00 on day 2. Dead wasps collected during the morning of the second and third day had likely been captured the previous evening because *D. insulare* are not active at night or early morning (Idris and Grafius 1998). We therefore estimated that they had been caught by 8 pm of the previous day, which is the latest time that *D. insulare* have been reported active (Idris and Grafius 1998). Wind speeds exceeding 8.5 m/s prevented us from monitoring wasps for a longer duration during trials 1 and 3 in 2003. Collected wasps were frozen at -80° C until egg load determination and biochemical analyses could be done for lipid, glycogen, fructose and total sugar levels as described in Lee *et al.* (2004). Prior to biochemical analyses, the Tangle-Trap® was removed from wasps using the following protocol. Each wasp was vortexed for 30 s in a 1 oz. plastic cup with 0.5 ml of De-Solv-it® degreasing solvent and then vortexed in 2 ml of distilled water for 30 s and again with new water. Next, each wasp was transferred into a clean cup and vortexed with distilled water twice and blotted dry on a Kimwipe®.

The proportion of recaptured water-fed and sugar-fed wasps were analyzed by trial in a Chi-square analysis. The effect of treatment (water or sugar) on height and time of recapture were compared using an ANOVA. Effect of treatment on egg load, lipid, glycogen, fructose and sugar levels of wasps were tested in an ANCOVA with wing length as a covariate. Impact of feeding as measured by fructose levels (independent variable) on time and height of recapture and remaining egg load was tested with linear regressions. Analyses were conducted in JMP® (SAS Institute 1995).

AGGREGATION NEAR BUCKWHEAT

We monitored the abundance of *D. insulare* in 12 x 20 m cabbage plots with and without 3 m wide borders of buckwheat *Fagopyrum esculentum* (Moench) as described in Lee and Heimpel (in review). In 2001, four buckwheat and four control plots were at least 67 m apart from each other and embedded in a soybean field. Another four buckwheat and control plots were spaced at least 800 m apart, embedded in separate soybean fields. These plots are referred to as nearby and isolated. In 2002 and 2003, eight cabbage plots were planted at least 800 m apart and in separate soybean fields. Cabbage plots were not treated with insecticide, and planted in new sites each year.

In 2001, four yellow sticky traps (Pherocon®AM) were set up randomly per plot. Traps were 30 cm aboveground and between two cabbage plants to collect *D. insulare* as they moved along a cabbage row. Traps were collected after one week in the field on 16, 23, 30 August 2001 from nearby plots and 14, 21, 28 August from isolated plots. In 2002-2003, six yellow sticky traps were set up per plot at random points and in the field for one week. Traps were collected on 22, 28 July, 5, 12, 19, 26 August and 2 September in 2002, and on 14, 21, 28 July and 4, 12, 18, 26 August in 2003. We tested the effects of treatment, year, and treatment x year interactions on the total number of *D. insulare* captured per trap using ANOVA on square-root transformed data. Since trap collections occurred at different times each year, only traps collected during the last three weeks of August were included in the three-year analysis. Male and female *D. insulare* were distinguished in 2002 and 2003. Average numbers of females captured per weekly trap over 7 weeks were tested in a similar ANOVA described earlier.

RESULTS AND DISCUSSION

FIELD PATCH STUDY

The field patch study did not support the prediction that feeding would lead to either greater retention or dispersal. Water- and sugar/nectar-fed wasps did not have different nutrient levels in the 1st and 2nd trial in 2003 and in 3rd trial in 2004 (Table 1) to adequately test treatment effects. In trial 2 of 2003, more water-fed than sugar-fed *D. insulare* were recaptured outside the plot but these wasps did not differ in their lipid, glycogen, total sugar and fructose levels. Since there was no physiological difference between water- and sugar-fed wasps, we cannot conclude that feeding enabled wasps to remain in the patch longer. When water and sugar-fed wasps differed physiologically in the 3rd trial of 2003, and 1st and 2nd trials of 2004 (Table 1), there were no significant differences in recapture rates, time and height of recapture, and number of eggs remaining in ovaries. Marginal differences were observed twice. Water-fed wasps had marginally greater egg loads than sugar-fed wasps ($p=0.062$) in trial 1 of 2004. This may suggest that feeding enabled more ovipositional activity, but the analysis was based on only four females. Sugar-fed wasps were recaptured marginally later than water-fed wasps in 3rd trial of 2003 ($p = 0.099$). This could indicate that being fed enables wasps to remain longer in a host patch (Stapel *et al.* 1997), but can also occur if sugar-fed wasps simply lived longer and were recaptured later.

Table 1. Proportion of recaptured sugar/nectar-fed versus water-fed *D. insulare*, average time of recapture, height of capture, number of eggs in ovaries, lipid, glycogen, total sugar and fructose levels of wasps.

	Trial 1-2003		Trial 2-2003		Trial 3-2003		Trial 1-2004		Trial 2-2004		Trial 3-2004	
Recapture—Chi-square test												
Water	1.4%	3/221	8.45%	41/485	9.3%	20/215	2.19%	8/365	8.13%	33/406	6.22%	25/402
Sugar	2.0%	4/201	4.56%	16/351	10%	35/350	1.91%	7/366	9.24%	28/303	6.60%	32/485
	X^2=0.26	p=0.61	X^2=5.08	p=0.02	X^2=0.07	p=0.79	X^2=0.07	p=0.79	X^2=0.27	p=0.60	X^2 =0.05	p=0.82
Time of recapture (min)—ANOVA												
Water	121.3 ± 102.8		648.5 ± 109.0		451.5 ± 92.1		112.5 ± 45.5		689.3 ± 105.9		251.7 ± 55.2	
Sugar	71.75 ± 42.4		1131.3 ± 174.4		645.5 ± 69.6		184.0 ± 48.7		686.5 ± 116.2		293.6 ± 41.8	
	$F_{1,5}$=0.25	p=0.64	$F_{1,55}$=5.5	p=0.02	$F_{1,53}$=2.8	p=0.099	$F_{1,13}$=1.2	p=0.30	$F_{1,59}$<0.01	p=0.99	$F_{1,55}$=0.38	p=0.54
Height of recapture (cm)—ANOVA												
Water	105 ± 11.1		85.3 ± 5.1		85.3 ± 5.1		59.3 ± 3.7		64.2 ± 2.9		57.6 ± 2.5	
Sugar	113.8 ± 9.0		91.3 ± 3.8		91.3 ± 3.8		60.4 ± 5.2		65.1 ± 3.1		59.5 ± 3.3	
	$F_{1,5}$=0.39	p=0.56	$F_{1,55}$=0.13	p=0.72	$F_{1,53}$=0.90	p=0.35	$F_{1,13}$=0.04	p=0.85	$F_{1,59}$=0.05	p=0.82	$F_{1,55}$=0.18	p=0.67
Eggs in ovaries—ANCOVA wing length as covariate												
Water	47, female n=1		17.4 ± 3.04, n=17		14.1 ± 3.9, n=7		31.5 ± 11.5, n=2		33.0 ± 5.7, n=9		25.0 ± 3.4, n=6	
Sugar	17 + 3, n=2		17.5 ± 8.9, n=2		8.5 ± 3.1, n=11		28.0 ± 5.0, n=2		38.7 ± 9.7, n=7		28.4 ± 5.01, n=8	
	*no analysis		$F_{1,15}$=0.05	*p=0.82	$F_{1,14}$=0.31	*p=0.59	$F_{1,1}$=103	p=0.06	$F_{1,12}$=0.61	*p=0.45	$F_{1,11}$=0.25	p=0.63
Lipid (µg)—ANCOVA wing length as covariate												
Water	43.4 ± 2.3		30.7 ± 1.8		30.4 ± 3.0		17.1 ± 2.1		20.5 ± 5.2		22.3 ± 1.5	
Sugar	34.1 ± 4.5		27 ± 2.0		27.3 ± 1.8		16. 9 ± 2.2		19.0 ± 1.5		23.4 ± 0.9	
	$F_{1,4}$=2.04	p=0.23	$F_{1,49}$=0.12	*p=0.74	$F_{1,46}$=4.1	*p=0.05	$F_{1,12}$<0.01	p=0.93	$F_{1,52}$=0.04	*p=0.84	$F_{1,52}$=0.01	*p=0.92
Glycogen (µg) —ANCOVA wing length as covariate												
Water	15.0 ± 8.4		8.3 ± 0.8		6.6 ± 1.0		5.4 ± 0.6		5.9 ± 0.84		6.4 ± 1.1	
Sugar	17.1 ± 1.4		7.0 ± 1.0		14.3 ± 2.4		11.0 ± 3.8		7.3 ± 1.20		6.0 ± 0.7	
	$F_{1,4}$=1.53	p=0.28	$F_{1,49}$=0.12	*p=0.74	$F_{1,46}$=3.7	*p=0.06	$F_{1,12}$=3.9	p=0.07	$F_{1,52}$=2.4	*p=0.13	$F_{1,52}$=0.36	*p=0.55
Total sugar (µg)—ANCOVA wing length as covariate												
Water	39.1 ± 35.7		16.5 ± 4.8		16.6 ± 5.4		9.8 ± 3.8		18.7 ± 4.4		12.4 ± 3.1	
Sugar	69.4 ± 9.2		10.1 ± 6.5		43.2 ± 7.8		28.0 ± 13.0		29.6 ± 7.1		15.0 ± 4.5	
	$F_{1,4}$=6.04	p=0.07	$F_{1,49}$=0.61	*p=0.81	$F_{1,46}$=4.0	*p=0.05	$F_{1,12}$=2.5	p=0.14	$F_{1,52}$=3.0	*p=0.09	$F_{1,52}$=0.07	*p=0.79
Fructose (µg)—ANCOVA wing length as covariate												
Water	27.9 ± 26.6		9.0 ± 2.8		7.9 ± 2.9		3.2 ± 1.5		5.8 ± 1.7		5.1 ± 1.5	
Sugar	30.4 ± 5.1		5.4 ± 3.5		30.4 ± 5.8		17.6 ± 7.4		13.5 ± 4.1		8.1 ± 2.8	
	$F_{1,4}$=0.92	p=0.39	$F_{1,49}$=0.06	*p=0.81	$F_{1,46}$=5.9	*p=0.02	$F_{1,12}$=7.1	p=0.02	$F_{1,52}$=5.7	*p=0.02	$F_{1,52}$=0.27	*p=0.61

*Dead wasps and wasps without wing measurements were not included in the ANCOVA analysis of nutrients and eggs.

Linear regression of wasps from all trials revealed that fructose levels marginally impacted time of recapture ($p = 0.06$) (Fig. 2a). A negative slope suggests that wasps with more fructose were caught earlier in the experiment, contrary to our previous finding that sugar-fed wasps were recaptured marginally later than water-fed wasps in trial 3 of 2003. This might reflect fructose levels having declined more in wasps caught at later times, particularly the next day. Next, *D. insulare* with higher fructose levels were recaptured higher on the traps (Fig. 2b) suggesting that feeding correlates with flying higher aboveground. If a higher flight level indicates dispersal behavior compared to foraging behavior, this study might support the hypothesis that feeding leads to more dispersal. However, the distinction between dispersal and foraging flight is not known. Lastly, there was no relationship between fructose levels and the number of eggs remaining in ovaries ($F_{1,72} = 0.50$, $p = 0.48$). Egg load is influenced by ovipositional activity and egg maturation rate. For *M. croceipes*, fed wasps oviposited more than did unfed wasps in host patches devoid of food (Takasu and Lewis 1995). Our results did not show *D. insulare* to be as amenable to sugar provisioning for improving biological control as other species. Yet, our experiment may have a limited scope since only wasps moving outside the plot were monitored. Wasps that remained within the host patch might have exhibited different behaviors based on their nutritional state but they were not monitored.

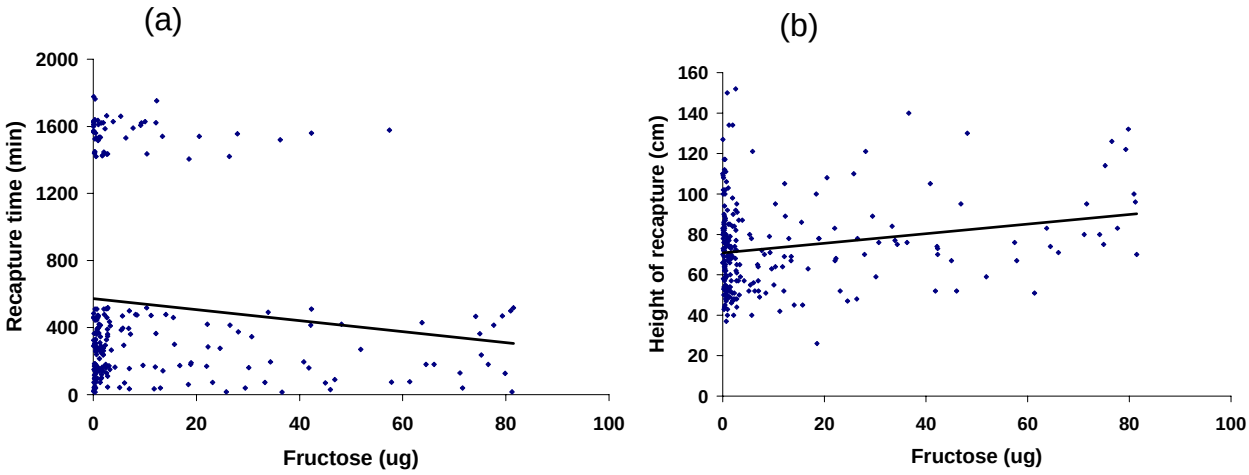

Figure 2. Linear regression of fructose levels of all *D. insulare* by (a) time of recapture, $F_{1,239} = 3.56$, $p = 0.06$, $y = 572.4 - 3.28 \times$ fructose, $r^2 = 0.011$; and (b) height of recapture, $F_{1,239} = 12.1$, $p = 0.0006$, $y = 70.8 + 0.24 \times$ fructose, $r^2 = 0.044$.

AGGREGATION NEAR BUCKWHEAT

From 2001 to 2003, captures of *D. insulare* per weekly trap during a 3-week period in August did not differ by treatment ($F_{1,26} = 0.73$, $p = 0.40$) nor by the treatment x year interaction ($F_{2,26} = 0.51$, $p = 0.61$) (Fig. 3a). Captures varied significantly by year ($F_{2,26} = 29.6$, $p = 0.0001$) with the highest captures in 2003. In 2002-03, females were distinguished from males on traps and traps were placed in the field for a longer period of time. Females captured per trap per week over a 7-week period did not vary by treatment ($F_{1,26} = 0.50$, $p = 0.49$) nor by the treatment x year interaction ($F_{1,26} = 0.94$, $p = 0.35$) but varied significantly by year ($F_{1,26} = 52.4$, $p = 0.0001$) (Fig. 3b).

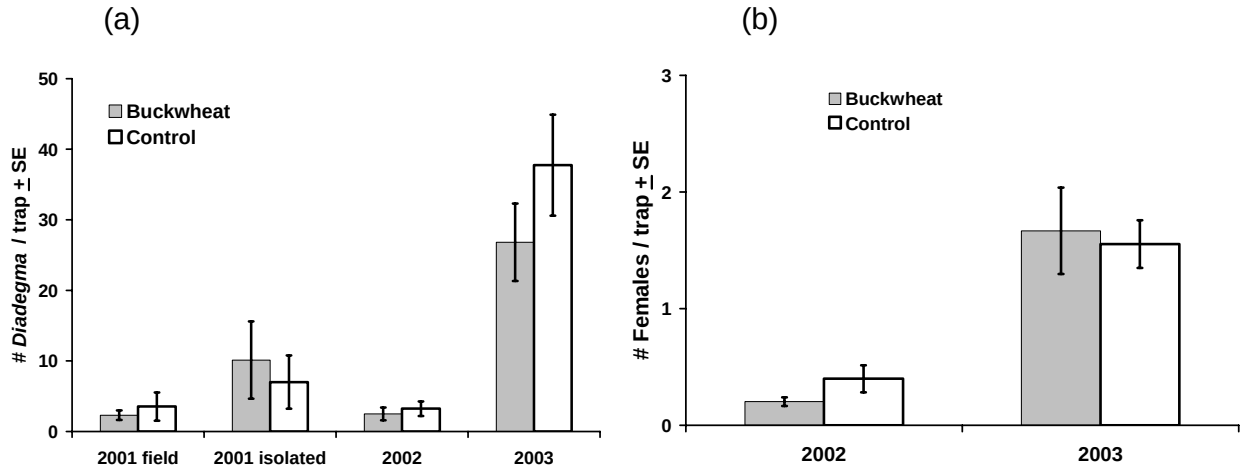

Figure 3. Average number of (a) *D. insulare* wasps collected per week per trap during three weeks of peak activity, 14-30 August 2001, 12-26 August 2002, and 12-26 August 2003. (b) Average number of females collected per week per trap during seven weeks, 22 July - 2 September 2002, and 14 July - 26 August 2003.

Over three years, buckwheat borders did not enhance aggregation of *D. insulare* within cabbage plots. This differs from previous studies with buckwheat (Berndt *et al.* 2002; Irvin *et al.* 2000; Stephens *et al.* 1998), although English-Loeb *et al.* (2003) only found more parasitoids near the crop edge next to the buckwheat but not in the crop interior. *D. insulare* might not have responded to buckwheat borders since other sugar sources were present in vegetation surrounding cabbage plots, such as honeydew produced by soybean aphids, *Aphis glycines* Matsumura, that have recently invaded Minnesota soybean fields. However, floral nectar can have more attractive odors to parasitoids than aphid-infested leaves (Wäckers and Swaans 1992). Buckwheat flowers are white, a color that may elicit more responses by parasitoids (Begum *et al.* 2004). Also, female *D. insulare* live three-fold longer on buckwheat nectar than soybean aphid honeydew (Lee *et al.* 2004). Given the superiority of buckwheat flowers to other common foods in the field, we might still expect to find a numerical increase of *D. insulare* in buckwheat versus control plots. We did not observe such an increase suggesting that *D. insulare* was not attracted or retained by buckwheat flowers. Recent olfactometers studies confirm this, both fed and unfed *D. insulare* showed a little if any response to buckwheat floral odors compared to buckwheat foliage without flowers (Heimpel and Zimmermann, unpublished). An alternative interpretation is that buckwheat may increase local aggregation of *D. insulare* but feeding also reduces their activity levels such that no differences would be observed in the amount collected in the traps.

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GUSTATORY ACCEPTANCE, LONGEVITY, AND UTILIZATION OF NECTAR AND HONEYDEW SUGARS BY *ANAPHES IOLE*, AN EGG PARASITIOD OF *LYGUS* BUGS

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ABSTRACT

Habitat management is a component of conservation biological control that aims to improve the availability of resources required by natural enemies. Access to non-host foods is a critical requirement for many natural enemies, and one that can be manipulated via habitat management. Food sources, usually in the form of nectar (floral or extrafloral), pollen, and honeydew supply natural enemies with energy for maintenance and reproduction. These food sources have different chemical compositions, and studies on parasitoid acceptance, survival, and longevity have helped identify the compounds most important to parasitoids, and therefore, habitat management. While pests may also exploit food sources intended for natural enemies, recent studies have shown that careful selection of food sources can reduce this possibility. Therefore, detailed knowledge of the biology of the pests and natural enemies present in the agroecosystem in question is crucial for selection of appropriate habitat management strategies.

The suitability of naturally occurring carbohydrates and a commercial food source was determined for *A. iole*. In a gustatory response study wasps responded to all 15 of the sugars at the highest concentration tested (2 M). At this concentration, sucrose, glucose, maltose, melezitose, fructose, trehalulose, and erlose all elicited >90% acceptance. Raffinose, trehalose, mannose, galactose, melibiose, rhamnose, stachyose, and lactose led to <50% gustatory response by the wasps at 2 M. Eliminade™ a commercial food supplement, was readily accepted (92%) by *A. iole*. With respect to gustatory response to nectar and honeydew sugars, *A. iole* differs markedly from other hymenopterans that have been studied in that this parasitoid accepted all the naturally occurring sugars with which it was tested. Moreover, for many of the sugars tested, this parasitoid had lower acceptance thresholds than other hymenopterans. Wasp survival varied depending on food source and temperature. Provision with sucrose led to the greatest increase in longevity over controls. Honeydew sugars were highly variable in their effect on survival. Results from sugar digestion trials were consistent with

those from gustatory discrimination and longevity trials, and suggested the presence of invertase in *A. iole* guts.

The broad and sensitive range of gustatory perception, coupled with enhanced longevity afforded by some sugars, might be helpful in the development of a food source for *A. iole* that is not exploited by *Lygus*.

INTRODUCTION

Many adult parasitic wasps require food to satisfy energy needs (Quicke 1997). Nectar (floral and extrafloral) and honeydew excreted by homopteran insects are rich sources of carbohydrates that satisfy energy and maintenance requirements (Jacob and Evans 1998; Jervis *et al.* 1993; Longley and Jepson 1996; Rogers 1985). Provisioning parasitoids with carbohydrates generally increases longevity and subsequent rates of parasitism (Azzouz *et al.* 2004; Baggen and Gurr 1998; Fadamiro and Heimpel 2001; Stapel *et al.* 1997; Wäckers 2001). Therefore, provisioning parasitoids with an adequate food source is an important component of habitat management strategies aimed at enhancing the effectiveness of biological control agents (Berndt and Wratten 2005; Evans and Richards 1997; Landis *et al.* 2000; Wratten and Gurr 1999).

Successful foraging by parasitoids depends on the availability of a suitable food source at the time of foraging. Most parasitoids readily accept sucrose, fructose, and glucose (Jervis *et al.* 1993; Jervis *et al.* 1996), the most common components of most nectar and honeydew (Baker and Baker 1983a). However, other carbohydrates occur in nectar and honeydew as well (Baker and Baker 1983b; Davidson *et al.* 1994; Hendrix and Wei 1994; Koptur 1994). Spatial and temporal variability in the sugar composition, i.e. suitability, of nectar and honeydew can limit successful foraging by parasitoids. With the exception of the predominant carbohydrates, little is known about the suitability of most sugars present in nectar and honeydew for parasitic Hymenoptera. The gustatory response of *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) wasps exposed to individual nectar and honeydew sugars varied greatly (Wäckers 1999), and was positively correlated with longevity (Wäckers 2001). Romeis and Wäckers (2000, 2002) demonstrated differences in sugar utilization patterns between *C. glomerata* and its host, *Pieris brassicae* L. (Lepidoptera: Pieridae). The parasitoid utilized several sugars to which the host did not respond. These findings have practical relevance for pest control, because they suggest that certain natural or artificial food sources might be used to benefit natural enemies to a greater extent than the pest.

In insects, ingested sugars are hydrolyzed into monosaccharide units, after which oxidation via glycolysis occurs. If not used immediately, carbohydrates in insects are sometimes stored as trehalose or glycogen. Boevé and Wäckers (2003) demonstrated that the rate of sugar digestion by *Myrmica rubra* (L.) (Hymenoptera: Formicidae) varied depending on the sugar ingested, and that metabolic suitability of sugars was correlated with gustatory acceptance.

Lygus bugs (Heteroptera: Miridae) are important pests of many crops in North America (Wheeler 2001). In cotton, *Gossypium hirsutum* L., annual losses due to *L. lineolaris* (Palisot de Beauvois) and *L. hesperus* Knight can exceed $75 million (Williams 1999). Historically,

Lygus populations in cotton have been controlled largely by broad-spectrum insecticides aimed at several pests. However, the acceptance of transgenic cotton and the success of the boll weevil eradication program (Hardee *et al.* 2001) might lead to an overall reduction in insecticide use in the cotton belt of the United States. In turn, this may create a scenario in which biological control has greater potential for controlling *Lygus* in cotton (Ruberson and Williams 2000).

Anaphes iole Girault (Hymenoptera: Mymaridae) is an egg parasitoid that attacks *Lygus* and other mirids in North America (Huber and Rajakulendran 1988; Udayagiri *et al.* 2000). *Anaphes iole* is pro-ovigenic and adults do not require a food source in order to mature eggs (Jervis *et al.* 2001). Nevertheless, foraging by adult *A. iole* is important because increased longevity would allow the wasp more time to search for and parasitize hosts, thus leading to a possible increase in realized fecundity. An increase in longevity afforded by sugar foraging is especially important when host densities are low and parasitoids must spend considerable time searching. Adult *A. iole* does not host feed, and field observations of feeding by this tiny wasp are lacking. Under laboratory conditions, longevity of *A. iole* wasps is limited to <3 days in the absence of food, but can exceed 10 days when honey is provided (Jones and Jackson 1990). However, nothing is known about the suitability of individual sugars for *A. iole*. A better understanding of nutritional ecology of *A. iole* may facilitate the development of natural or artificial food sources that confer greater benefit to this parasitoid than to *L. lineolaris*.

Our objectives were to characterize and describe the effect of carbohydrate food sources on gustatory discrimination, longevity, and utilization by *A. iole*.

METHODS AND MATERIALS

INSECTS

Anaphes iole used in this study were obtained from a laboratory colony maintained on *L. hesperus* eggs at the USDA-ARS Biological Control and Mass Rearing Research Unit, Mississippi State, MS. Wasps were held in Plexiglass cages (26 x 26 x 20 cm) at 27±1°C, 65-85% RH, and 14:10 L:D photoperiod until experimentation.

GUSTATORY RESPONSE AND ACCEPTANCE THRESHOLD

Acceptance thresholds for the following 15 sugars were determined for *A. iole*: sucrose, fructose, glucose, maltose, melezitose, erlose, trehalulose, raffinose, trehalose, mannose, galactose, melibiose, rhamnose, stachyose, and lactose. With the exception of lactose, all the sugars tested are known to be associated with plants (e.g., nectar) or insects (e.g., honeydew or bee honey). Lactose was included as a control sugar that *A. iole* is unlikely to encounter in nature. A 2 M concentration of each sugar was prepared with distilled water. This concentration approximates that found in nectar and honeydew (Baker and Baker 1983a). Serial dilutions were then made from the stock solution in a geometric progression (i.e., 1 M, ½ M, ¼ M, etc.) for each sugar.

Gustatory response by *A. iole* was also assessed for a commercial food supplement, Eliminade™(Entopath, Easton, PA). Eliminade™was developed as part of a conservation

biological control program to enhance the effectiveness of parasitoids of the southern pine beetle, *Dendroctonus frontalis* Zimmerman (Coleoptera: Scolytidae) (Hanano 1996; Mathews and Stephen 1999). For this study, Eliminade™was prepared as recommended by the manufacturer.

Bioassays were setup in the following manner. Wasps used in the gustatory discrimination experiments were <3 day old females that were water-satiated (provided with distilled water *ad lib* via saturated absorbent matting and frequent misting), appeared healthy, and were assumed mated. Under the existing laboratory conditions, the longevity of food and water deprived *A. iole* is about 3 days (Williams, unpublished). All gustatory discrimination experiments were conducted between 0800-1700 h CST. Water-satiated wasps were placed individually into a 0.5 dram glass shell vial containing a 5 ml drop of the treatment (sugar solution or Eliminade™ in the bottom of the vial. Wasps were observed at 50x for 5 min. 'Acceptance' was recorded if the wasp fed for more than 5 seconds upon contact with the droplet, or if the total time spent feeding surpassed 5 seconds. Otherwise, the encounter was scored as 'rejection'. Each sugar-concentration combination was presented to 25 wasps in a completely randomized design, and sugar-concentration combinations were replicated 3-8 times. Fresh sugar solutions were prepared for each replicate. For the bioassays using Eliminade™ 50 wasps were included in each of two replicates. Laboratory conditions during the study were 24±3°C and 17-52% RH.

LONGEVITY

Longevity of newly-emerged wasps was assessed after provision with a 1 M solution of the following sugars presented singly: sucrose, trehalulose, maltose, melezitose, trehalose, and rhamnose. Eliminade™was included as an additional treatment. Two controls, distilled water only and no food or water, were also included. Bioassays were setup in a randomized complete block design with six replicates. Each experimental unit consisted of 15 parasitized host (*L. hesperus*) eggs placed into a 28 ml glass scintillation vial. These host eggs contained parasitoids ready to emerge within 12 h. Organdy was placed over the top the vial and two pipet tips (30 ml) were inserted into two small holes cut in the fabric. One pipet tip contained distilled water, and the other was filled with the treatment solution. Separate trials were run concurrently at 20 and 27°C, 65-85% RH, and 14:10 L:D photoperiod. Wasp survival was assessed daily with the aid of a dissecting scope, at which time pipet tips were replaced with fresh water and treatment solutions. Survivorship of wasps was analyzed as a function of time.

CARBOHYDRATE UTILIZATION

Wasps used in this study were 1 day old and were food and water deprived. About 300 wasps were placed in a 9.5 cm-diam glass Petri dish containing two discs of filter paper (Whatman no. 1, Whatman International Ltd., Maidstone, Kent, U.K.), one in the bottom of the dish and one on the top. Each piece of filter paper was saturated with a 1 M solution of sucrose, maltose, or melezitose. Controls were prepared using distilled water only. Petri dishes were then held at 25±1°C for one of three different time intervals; 15 min, 12 h, or 24 h. This time series allowed us to determine the digestion rate for the different sugars. At the appropriate

time, wasps were killed in 70% ethanol. Thirty individuals of each gender were placed in together in 1 ml microcentrifuge tubes with 70% ethanol and held at -20°C until analysis. Prior to analysis, wasps were rinsed in ca. 400 ml distilled water, and were macerated for 10 min in 300 ml distilled water with glass beads (0.4 ml, 400-600 mm diam) using a dental amalgamator.

High-performance anion-exchange chromatography analysis with pulsed amperometric detection (Beach *et al.* 2003; Byrne *et al.* 2003; Hendrix and Wei 1994; HPAEC) was used to identify and quantify the major carbohydrates present in *A. iole*. One hundred microliters of each sample was analyzed by HPAEC. Since detector response varies between individual sugars (Larew and Johnson 1988), peaks were identified and quantified by comparing the time of retention and peak areas of known sugar standards with unknown sugars. Based on daily calibration of the system, peak areas were determined using Dionex PeakNet software.

RESULTS

GUSTATORY RESPONSE AND ACCEPTANCE THRESHOLD

All 15 of the sugars tested elicited a response by *A. iole*, although marked differences were observed. At the 2 M concentration gustatory response partitioned into two groups. Sucrose, glucose, maltose, melezitose, fructose, trehalulose, and erlose evoked >90% acceptance at this concentration. These sugars were considered to be 'highly stimulatory'. The acceptance threshold for these sugars was 1/256 M, with the exceptions of glucose, maltose, and trehalulose, which was 1/16, 1/512, and 1/1024 M, respectively. Response curves of these sugars declined in a relatively linear manner.

The remaining sugars (raffinose, trehalose, mannose, galactose, melibiose, rhamnose, stachyose, and lactose) led to <50% gustatory response by the wasps. These sugars were categorized as 'moderately stimulatory sugars'. The acceptance threshold for these sugars did not exceed 1/4 M, except for raffinose, which was 1/256 M. Response curves for these sugars were also relatively linear, with the exception of raffinose, which displayed an irregular response and never exceeded 30% gustatory acceptance between 2 and 1/512 M. Eliminade™ a commercial food supplement, was readily accepted (92%) by *A. iole*.

LONGEVITY

Wasp survival varied depending on food source and temperature. For the trial conducted at 27°C, average survivorship was lowest (ca. 3 days) when wasps were held without food or water, or were provisioned with only water, or with rhamnose. Provision with trehalose or melezitose increased survival only to a slight degree (ca. 4 days). However, wasps provided with sucrose, trehalulose, maltose, and Eliminade™had the greatest longevity (maltose, ca. 8 days; sucrose, ca. 15 days). Temperature was also an important factor; longevity of wasps was significantly greater at 20°C than at 27°C. For example, average survival of wasps fed sucrose was nearly 2x at 20°C than at 27°C.

CARBOHYDRATE UTILIZATION

Results of HPAEC analysis indicated that trace amounts of glucose and fructose were found in control wasps fed only distilled water. Sugars fed to wasps could be clearly detected within 15 min. However, the rate of digestion appeared to vary depending on the sugar that was ingested. For example, within 15 min of sucrose ingestion, glucose, fructose, trehalose, and sucrose were detected. These sugars were also present at 12 h, with the exception of sucrose. However, at 24 h sugar levels were comparable to controls. In contrast to sucrose, melezitose was still detected 24 h after ingestion. Trace amounts of other carbohydrates were also present in some samples.

DISCUSSION

Female *A. iole* wasps responded to all the carbohydrates tested. The parasitoids were most sensitive to seven sugars present in nectar (sucrose, glucose, and fructose), honeydew (glucose, trehalulose, melezitose, erlose, and fructose), and bee honey (maltose and erlose). Of these sugars, *A. iole* was most sensitive to sucrose, and it is interesting that this parasitoid was significantly more sensitive to sucrose than to its components, glucose and fructose. These sugars are usually the primary components of nectar (Baker and Baker 1983a), an important food source for parasitoids (Jervis *et al.* 1993). Sucrose is the most widely found disaccharide in nature, and is the primary form in which fixed carbon and energy are translocated in plants. The acceptance thresholds for sucrose and other highly stimulatory sugars were much lower than the concentrations (10-50% w/v) at which they naturally occur (Baker and Baker 1983a). High sensitivity to these carbohydrates by *A. iole* would enable this wasp to exploit sources with low concentrations of these sugars. The remaining sugars were moderately stimulatory (<50% acceptance at 2 M). These sugars are found in bee honey (mannose, trehalose, raffinose, and melibiose), honeydew (trehalose, raffinose, and stachyose), plant seeds (raffinose, galactose, stachyose, and rhamnose), and phloem sap (raffinose, melibiose, and stachyose) (Baker and Baker 1983a,b; Donner 1991; Hendrix *et al.* 1992; Kuo *et al.* 1988; Nakajima *et al.* 1980; Wei *et al.* 1996). Our results suggest that *A. iole* can detect a wide range of potential food sources, some of them at very low concentrations. In particular, it appears that nectar and honeydew are natural sources of sugars that *A. iole* can perceive, even if the sources have been diluted by precipitation or dew.

Similar feeding studies conducted with other hymenopterans allow us to put our results with *A. iole* into perspective. For half of the sugars tested, *A. iole* exhibited lower acceptance thresholds than for any hymenopteran tested to date (see Beach *et al.* 2003). Moreover, *A. iole* was the only hymenopteran tested that accepted all the sugars. Differences in sensitivity and range of carbohydrates detected by parasitoids may be a function of the insect's reproductive physiology. Like many egg parasitoids, *A. iole* is pro-ovigenic and produces eggs that rely on the host egg's protein for nourishment (Quicke 1997). Conversely, the parasitoid studied by Wäckers (1999), *C. glomerata*, is synovigenic, meaning that female wasps emerge with a limited number of nutrient-rich eggs (Quicke 1997). When provided with a protein-

rich diet, synovigenic wasps can mature additional eggs. In the absence of food, these wasps sometimes resorb their eggs and utilize the resources for self-preservation. Therefore, protein may not be an important component in the diet of a pro-ovigenic wasp that emerges with a full complement of matured eggs. Carbohydrates, which provide a source of quick energy for locomotion, may play a more important role in the nutritional ecology of *A. iole* than proteins. Nevertheless, *A. iole* readily accepted Eliminade™ a protein-rich commercial food supplement developed for synovigenic parasitoids. Its acceptance by *A. iole* suggests that this wasp is capable of utilizing complex food sources that include proteins and other compounds that are not required for its survival. Reliance on carbohydrates may explain the high sensitivity and range of perception to sugars by *A. iole*. Future studies with other pro-ovigenic and synovigenic parasitoids are necessary to better understand the relationship between gustatory response and reproductive physiology in parasitoids.

Our studies demonstrate the importance of food source and temperature on the longevity of *A. iole*. Several food sources were not suitable for wasp survival (rhamnose, trehalose, and melezitose), while others were beneficial (Eliminade™, maltose, trehalulose, and sucrose). Sucrose is common in nectar, suggesting that plant resources are important for *A. iole* survival in the field. However, rhamnose, another component of nectar, was unsuitable for *A. iole*, and acted as a feeding deterrent to this wasp when mixed with maltose (Beach *et al.* 2003) and to *C. glomerata* when mixed with glucose (Wäckers 2001). These findings indicate the importance of understanding the chemical composition of nectar and its effects on beneficial insects when developing habitat management strategies for biological control. The three honeydew sugars tested (trehalose, melezitose, and trehalulose) had variable effects on survival of *A. iole*, suggesting that honeydews differ in their suitability to this wasp. Increased longevity at the lower temperature, 20°C, may be a function of reductions in behavioral activity and metabolism. Longevity of *A. iole* was relatively consistent with results from gustatory discrimination. The exception was melezitose, which was readily fed on (ca. 75% acceptance at 1 M concentration), but was a poor source of nutrition.

Results from the HPEAC analysis suggested that *A. iole* hydrolyzes some sugars more efficiently than others. This was clearly observed in the comparison between sucrose, which was readily metabolized, and melezitose, which was not. These results are consistent with those in the gustatory discrimination and longevity studies. Our results suggest the presence of invertase, which hydrolyzes sucrose, in the gut of *A. iole*. However, this does not preclude the presence of other enzymes, and further studies of sugar digestion by *A. iole* are underway.

The ability of *A. iole* to detect and utilize a broad range of food sources has practical implications for the development of a food supplement for this parasitoid. However, it must be remembered that *L. lineolaris* might also utilize a food supplement intended for *A. iole*. The presence of nectar can benefit pest herbivores (Adjei-Maafo and Wilson 1983; Belcher *et al.* 1984) as well as their natural enemies (Bugg *et al.* 1989). Therefore, knowledge of the nutritional ecology of *L. lineolaris* as well as *A. iole* is critical for the development of a selective food source. Use of foods that benefit the biological control agent to a greater extent than the pest herbivore may have broad potential in other biological control programs (Cortesero *et al.* 2000; Lewis *et al.* 1997).

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THE VEGETARIAN SIDE OF CARNIVORES: USE OF NON-PREY FOOD BY PARASITOIDS AND PREDATORS

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ABSTRACT

Predaceous and parasitic arthropods can play an important role in the regulation of herbivore populations. However, the majority of predators and parasitoids also use plant-derived foods as a source of nutrients. This vegetarian side of the menu may include various plant-provided substrates, such as nectar, pollen, fruits or foods indirectly derived from plants (e.g., honeydew or pycnial fluid of fungi). Predators and parasitoids may either use plant-derived food as a supplement, or they may strictly depend on these foods during part of their life.

Despite the obvious importance of non-prey food, little is known about the extent to which particular categories of plant-derived foods contribute to the diet of predators and parasitoids under field conditions. To the foraging insect the potential value of a given food source will depend on its availability, detectability, accessibility and nutritional composition. Plant-provided foods can have a dramatic impact on longevity, fecundity, and distribution of predators and parasitoids. As each of these parameters affects the local number of carnivores, the availability of suitable plant-derived food can have a major impact on mass-rearing programs, as well as on herbivore-carnivore dynamics in the field.

OVERVIEW OF RESOURCE USE

NON-PREY FEEDING BY CARNIVOROUS ARTHROPODS

Predators and parasitoids are usually identified by their carnivorous lifestyle. Due to this bias, we easily overlook the fact that the majority of these “carnivores” also require plant-derived foods as a source of nutrients.

The level in which predators or parasitoids depend on primary consumption varies. (Wäckers and van Rijn 2005) distinguish between the categories of ‘life-history omnivores’, ‘temporal omnivores’ or ‘permanent omnivores’. Life history omnivores include those natu-

ral enemies that are strictly dependent on plant-derived food during part of their life cycle, such as hoverflies and many parasitoids. Temporal omnivores supplement their carnivorous diet during part of their life (e.g., host-feeding parasitoids), whereas permanent omnivores retain an assorted diet throughout their lifecycle (e.g., predatory mites and ladybirds).

WHAT'S ON THE MENU? NON-PREY FOOD ITEMS USED BY PREDATORS AND PARASITOIDS

Predators and parasitoids may feed on various substrates. Their fare may include carbohydrate-rich foods such as such as floral nectar, extrafloral nectar, fruits, plant sap, gall secretions, honeydew, Lycaenid dorsal gland secretions, and fungal fluids as well as lipid- or protein-rich sources such as pollen, food bodies, and elaiosomes (Wäckers 2005). In some cases predators may also feed on plant productive tissue, which would classify them as potential herbivores (Coll and Guershon 2002; Eubanks and Styrsky 2005). A few predators exploit a broad range of the above-mentioned food items. This applies especially to ants, which have been the driving force in the evolution of many food-mediated mutualisms (Beattie 1985). The majority of predators and parasitoids restrict their diet to one or a few alternative foods. Most parasitoid species are restricted to feeding on sugar-rich solutions such as nectar and honeydew. Many predators like hoverflies, lacewings, anthocorid bugs, ladybeetles, and predatory mites feed on pollen as well as nectar/honeydew (Wäckers and van Rijn 2005).

EFFECTS ON LONGEVITY AND FECUNDITY

Plant-provided food can have a strong effect on life-history parameters of predators and parasitoids. Temporal or permanent omnivores can use foods like (extra-) floral nectar, pollen or honeydew as an alternative to prey. This diet extension therefore allows them to bridge periods of low prey availability (Limburg and Rosenheim 2001). When combined with predation, nectar and pollen feeding can increase predator fitness over prey feeding alone (Porter 1989; van Rijn and Sabelis 2005). Life-history omnivores, on the other hand, fully depend on non-prey food, usually during their adult stage. Their longevity and fecundity are often seriously compromised in the absence of these food sources. An example of the latter category is the large category of parasitoids that do not engage in host-feeding. At the time of adult emergence, their energy reserves often cover no more than 48 hours of the individual's energetic requirements. Sugar feeding can increase a parasitoid's lifespan considerably; up to 20-fold under laboratory conditions for several hymenopteran parastoids (Fadamiro and Heimpel 2001; Jervis *et al*. 1996; Wäckers 2001), and 2-3-fold for the phorid fly, a dipteran parasitoid of imported fire ants (Chen *et al.* 2005; Fadamiro *et al*. 2005). In addition, sugar feeding can benefit a parasitoid's fecundity, not only through an increase in reproductive lifespan, but also through a positive effect on the rate of egg maturation (Jervis *et al.* 1996). This means that parasitoids that fail to replenish their energy reserves through sugar feeding will suffer severe fitness consequences.

DIFFERENCES IN SUITABILITY

Not all potential food sources are suitable for a given predator or parasitoid. There is substantial variation between and among food categories with regard to their availability, apparency,

accessibility and chemical composition (Olson *et al*., this issue; Wäckers 2005). Whereas food sources may vary widely, consumers may show an even broader variation in foraging behavior, mouthpart morphology and physiology. An effective exploitation of food sources requires that there is a good fit between consumer attributes and food source characteristics (Olson *et al.*, this issue). Identifying and quantifying mechanisms that allow or obstruct successful food source exploitation is not only essential if we want to understand the functioning of food supplements in plant-insect and insect-insect interactions, it also has direct implications for the use of food supplements in biological control programs.

CONSEQUENCES FOR BIOLOGICAL CONTROL

Biological control workers have regularly suspected that the scarcity of sugar- and/or pollen sources in agriculture could impose a serious constraint on the effectiveness of natural enemies in the field (Hocking 1966; Illingworth 1921). Hocking (1966) pointed out that lack of food availability could also hamper the establishing of natural enemies in classical biological control programs. We still have little data on the nutritional status of natural enemies under field conditions (Casas *et al.* 2003; Lee and Heimpel 2003), but recent studies indicate that natural enemies can indeed be food-deprived in the absence of (suitable) flowering vegetation (Olson and Wäckers unpublished data; Wäckers and Steppuhn 2003). Thus, adding suitable food sources to agro-ecosystems could be a simple and powerful tool to enhance the effectiveness of biological control programs. Three types of approaches have been proposed to alleviate the shortage of food in agricultural systems.

1. Diversification of agro-ecosystems. Food sources can be provided by enhancing plant diversity in agro-ecosystems, either through the use of non-crops in undergrowth or field margins (Gurr *et al.* 2005; Landis *et al.* 2000; van Emden 1965), or by growing crops devoid of alternative food alongside crops featuring flowers or extrafloral nectaries. However, not all plant-provided food is suitable as a food source for parasitoids and predators. Flowers may not be perceived by (some) natural enemies, or can be unattractive or even repellent (Wäckers 2004). Other flowers may be attractive, but hide their pollination rewards within constricted floral structures that prevent those natural enemies with unspecialized mouthparts to exploit these food sources.

2. Artificial food supplements. An alternative to the use of (flowering) plants is the use of artificial food supplements such as food sprays (Hagen 1986). Artificial food supplements typically consist of a carbohydrate solution in combination with a source of protein/amino acids. Insects that utilize honeydew as food source may be especially adapted to exploit this 'artificial honeydew'. Many studies have identified short term increases in numbers of natural enemies such as parasitoids, lady beetles, lacewings, and predatory bugs as a result of these food supplements. The impact of food supplements on pest insects has rarely been investigated (Rogers and Potter 2004).

3. Crop-provided food. Some crops produce suitable food supplements themselves. Many crops flower during part of their growing period. In crops grown for their seeds or fruits (e.g., cereals, citrus, beans) this flowering period may coincide with the period that the plant is specifically vulnerable to pest attack. Some crops, such as peppers and tomatoes, even flower during a large part of the growing season, thereby maintaining populations of

predatory mites and anthocorid bugs, that can effectively suppress thrips pests (Van den Meiracker and Ramakers 1991). A number of crops also provide nectar outside the flowering period. These so-called 'extrafloral nectaries' may be found on leaves, stems or fruits. Examples of extrafloral nectar producing crops include Prunus spp. (e.g., cherry, plum, peach, and almond), cassava, faba bean, zucchini, pumpkin, cashew and cotton. Extrafloral nectaries are generally believed to have evolved as a mechanism for plants to attract sweet-toothed carnivores and to benefit from their protective services (Turlings and Wäckers 2004). The fact that extrafloral nectaries have evolved numerous times shows that food supplements are a successful method to enhance biological control under natural conditions. The extrafloral nectar trait is also found in a number of crops and can be a useful element in biological pest control. The crop-produced nectar may suffice as food sources for predators and parasitoids. In other cases, there may be room for plant breeding to improve the timing, quantity and quality of nectar production, to better match the nutritional needs of biological control agents.

Whereas the concept of enhancing biological control through the use of alternative food might seem self-evident, the anticipated effects are not necessarily realized under field conditions (Heimpel and Jervis 2005). In their contribution Lee and Heimpel (this issue) investigate whether food provision has an impact on parasitoid retention under actual field conditions. In a series of experiments they studied dispersal behavior of the parasitoid *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae), in response to nutritional state or the presence of nectar flowers. No evidence for the hypothesis that dispersal behavior is affected by sugar feeding was found. Using a modeling approach, Sabelis and van Rijn (this issue) review the conditions under which alternative food enhances pest suppression by biological control agents as well as the conditions where no effects are expected. Regarding the nutritional value and life stage affected by the alternative food, they show that alternative food can bring pest locally to extinction only when it is substitutable with prey, rather than complementary.

A FLY IN THE OINTMENT: WHEN HERBIVORES BENEFIT FROM FOOD SUPPLEMENTS

Whereas the provision of food supplements is potentially an effective method to enhance biological pest control, the indiscriminate addition of nectar or pollen sources to agro-ecosystems may also backfire. Many arthropod pests are dedicated flower feeders as well (Romeis *et al.* 2005), and some are more effective in flower exploitation than their natural enemies. When herbivores, rather than their antagonists, gain profit from the available nectar or pollen sources, the net impact on pest control could be negative. This potential problem can be avoided by screening flowers with respect to their suitability for biological control agents (Patt *et al.* 1997; Wäckers *et al.* 1996) as well as herbivores (Baggen *et al.* 1999; Winkler *et al.* 2003).

POSSIBILITIES FOR SELECTIVE USE OF FOOD SUPPLEMENTS

We have seen that nectar and pollen sources vary substantially with regard to their suitability as food for particular arthropods. To optimize the impact of food provision in biological control, feeding requirements of both natural enemies and herbivorous pests should be con-

sidered when selecting food supplements. Differences in food ecology between both groups can be exploited in selecting flowers that cater for biological control agents, while being unsuitable for herbivores (Baggen *et al.* 1999; Baggen *et al.* 2000; Wäckers 1999; Winkler *et al.* 2003). The fact that nutritional requirements of natural enemies often differ considerably from those of pest insects can also be used to develop selective food sprays, i.e. food sprays that sustain biological control agents without providing a nutritional benefit to the pest insect (Romeis and Wäckers 2002; Wäckers 2001; Winkler *et al.* 2005).

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WHEN DOES ALTERNATIVE FOOD PROMOTE BIOLOGICAL PEST CONTROL?

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ABSTRACT

That alternative food, whether or not provided by a plant or introduced artificially, promotes biological pest control via its effect on the predators, is not immediately obvious. On the one hand it enhances survival, reproduction and searching. On the other hand it may reduce the rate of predation, which is the case when alternative food and prey are substitutable – as opposed to complementary – food sources. Moreover, it is not immediately obvious how the impact of alternative food on the outcome of biological pest control differs depending on the type of dynamics (e.g., equilibrium vs. transient dynamics), the type of predator (e.g., stage-related consumption and life history effects of alternative food), the spatial structure of the environment (e.g., source-sink, metapopulation) and food web structure (presence of hyperpredators or intraguild predators). We review the conditions under which alternative food can lead to either prey/pest extermination, to a decline of the prey/pest towards a positive, asymptotic density or to no effect on prey/pest density at all.

INTRODUCTION

Carnivorous arthropods such as predators and parasitoids supplement their diet with plant-provided food (PPF), such as nectar or pollen. Depending on the arthropod taxa under consideration, this type of omnivory may occur in all or only in specific feeding stages. It usually stimulates survival, development and/or reproduction, thereby promoting the numerical response of the carnivore to the density of prey or host. Whether this numerical effect on the carnivore also translates in reduced prey/host densities, is not immediately obvious because consumption of PPF may negatively affect consumption of prey, because prey consumption and PPF consumption vary with life history stage, because prey and PPF vary in spatial distribution and because prey and PPF may be eaten by other members of the food web. Assessing the conditions, under which PPF reduces density at the second trophic level via its impact

on the third, is of crucial importance to designing strategies for biological control of crop pests.

Here, we summarize the results of theoretical exercises with consumer-resource models presented elsewhere (Van Rijn and Sabelis 2005). First, we consider the lessons from simple consumer-resource models that ignore stage or spatial structure. Second, we discuss results from stage-structured consumer-resource models to investigate how the impact of PPF on herbivory may depend on the life history and feeding requirements of the carnivorous arthropods. We compare parasitoids and predators, as well as predators with different types of omnivory. Third, we consider spatial structure and ask how the distribution of PPF and prey relative to each other matters to reducing herbivory. Finally, we step beyond consumer-resource interactions and ask when food web complexities (omnivory, hyperpredation, intraguild predation, competition) alter the predictions from simple consumer-resource models. All this will be discussed with a keen eye for how the theory can be applied to reduce crop damage by herbivores.

WELL-MIXED, UNSTRUCTURED CONSUMER-RESOURCE MODELS

To understand how PPF – through its effect on the carnivore – affects herbivore abundance, consider a system where a predator population directly controls a herbivore population, where individuals of each species are identical (e.g., no stage structure) and populations are well mixed (no spatial structure). These conditions apply to Lotka-Volterra or Rosenzweig-MacArthur models. The addition of PPF will initially result in an increase of the predator population, simply because there is more food available. This increase will come to a halt at equilibrium, i.e., when births exactly compensate for deaths. At this point, the herbivore population has decreased to an extent that compensates for the supply of PPF. Thus, adding food will lead to a decrease in the herbivore population via the consumers they share. This effect of a non-reproducing food source is very similar to the case where adding a second, reproducing prey species causes a decline of the first via the predator they share (Holt and Lawton 1994; Van Baalen *et al.* 2001; Van Rijn *et al.* 2002). The effect of one prey species on the other looks like competition, but in fact it is only apparent, because the mechanism is predator-mediated. This is why Holt (1977) termed it apparent competition, to create a contrast with resource and interference competition.

From the equilibrium equations of the one-predator-two-prey or predator-PPF-prey models, some counterintuitive conclusions emerge. If prey and PPF are substitutable food sources for the predator (Tilman 1982), addition of PPF will reduce equilibrium prey density, even when per capita consumption of PPF leads to a decrease in per capita consumption of prey. This insensitivity of the herbivore equilibrium to per capita prey consumption arises because equilibrium implies that the predators will increase to larger densities to achieve an overall prey mortality rate that compensates the overall prey birth rate. As long as the per capita predation rate exceeds zero, increasing the amount of PPF can even lead to extinction of the prey. At prey extinction, the predator population is maintained only by PPF. This prediction of prey extinction by adding PPF does not hold when food and prey are not substitutable, but complementary. Two food types are thought to complement each other when

they influence different components of the predator's life history. As an extreme example, consider the case where prey affects reproduction and PPF affects mortality. Then, increasing the amount of PPF also reduces herbivore density, but can never lead to extinction of the prey. This is because herbivore density declines asymptotically to a fixed level determined by background (= minimum) predator mortality.

As long as assimilation of PPF and prey will have a positive effect on predator reproduction and survival (which is why PPF should be eaten by the predator anyway!), PPF will reduce equilibrial prey densities irrespective of its effect on the prey consumption rate. Thus, it does not matter whether the predator switches to PPF at low prey densities or whether it becomes satiated for PPF at another level of ingested biomass than for prey (Van Baalen *et al.* 2001; Van Rijn, unpublished data). It even does not matter whether the herbivore consumes PPF and therefore survives, reproduces or develops faster (Van Rijn *et al.* 2002)! At equilibrium, the enhanced herbivore performance due to PPF will be compensated by predation from a larger predator population.

The equilibrium approach holds when environmental conditions, such as climate, availability of PPF to predators and plants to herbivores, remain unchanged over a sufficiently long period. How long the conditions need to be constant to approximate the equilibrium depends on the initial densities of the interacting populations, their generation times, and other traits of predator and prey that determine the dynamics around the equilibrium. For carnivorous mites and herbivorous thrips with generation times of about 3 weeks, populations were already within the 10% range of their equilibrium level after 12 weeks following their introduction in a cucumber crop (Van Rijn *et al.* 2002). After this period the impact of a regularly supplied food source on mite and thrips populations can adequately be predicted from equilibrium equations only. Arthropods larger than mites and thrips generally have longer generation times and their populations require more time to settle around the equilibrium (Sabelis 1992). For insects with only one or two generations per year and with food sources available only during part of the year, an equilibrium approach is unlikely to hold. In that case, one should rather focus on the dynamics displayed before the system approaches its equilibrium state (so-called 'transient' dynamics). Models of such systems require proper representation of developmental delays and age-dependent reproduction and this may make them mathematically less tractable. Although numerical techniques are available to simulate transient dynamics (Caswell 1989; De Roos and Persson 2001; Nisbet, 1997), obtaining transparent insight requires simplification.

We reduced complexity by focusing on the first generation after predator release (Van Rijn and Sabelis 2005). We assumed predators to be subject to a constant (i.e., herbivore-independent) per capita mortality rate, whereas the per capita prey mortality depends on predator density. Solving the integral over the first generation of the predators yields an expression relating prey density to background (= predator-independent) prey mortality, predation rate and predator mortality. If prey and PPF are complementary food sources, PPF may reduce predator mortality without affecting the predation rate. Then, reducing predator mortality by adding PPF translates into reduced prey density. If, however, PPF and prey are substitutable, then adding PPF may reduce predator mortality as well as predation rate. To make herbivore density go down requires that PPF reduces predator mortality more than it

reduces the predation rate. If PPF is not only utilized by predator, but also by prey, adding PPF as a complementary food to prey will only reduce herbivore density when its reducing effect on predator mortality is disproportionally larger than that on background prey mortality. This demand will be even more extreme when substitutability of foods is assumed and therefore consumption of PPF will likely go at the expense of that on prey. Thus, for prey density to go down in the first generation after predator release adding PPF is subject to stringent conditions. It is then critically important to know whether foods are substitutable or complementary and whether herbivores utilize PPF as well. These conclusions are quite complex, yet they are intuitively much more obvious, than the simple general conclusion drawn for the case of equilibrium conditions stating that: PPF always reduces herbivore density irrespective of its effect on predation and irrespective of its utilization by the herbivore.

STAGE-STRUCTURED CONSUMER-RESOURCE MODELS

Real-world predator–prey and parasitoid–host interactions differ in the life stages that are affected by prey (or host) density and/or PPF. In general, three scenarios can be observed that differ in whether (A) adult performance (survival, attack, oviposition), (B) juvenile performance (survival, development) and (C) both adult and juvenile performance are affected by prey density. The different types of PPF (nectar, pollen) can also have three different effects as it may (1) promote survival, (2) provide fuel (when rich in sugars as in nectars) for searching and (3) enhance assimilation processes (when rich in amino acids as in pollen), and thereby development and oviposition. As defined above, PPF and prey can be substitutable or complementary food sources and they are called essential if their absence causes the carnivore population to decline even at the highest abundance of the other food source (i.e., reproduction does not compensate mortality or the basic reproduction ratio $R_0 < 1$). Assuming the prey/host is always essential, PPF is never essential when substitutable, but when complementary it can be either essential or not.

Scenario A applies to parasitoids. Here, the larva is carnivorous. It feeds in or on a single host. So there is no need to search for hosts. The adult female searches for hosts and she makes decisions on whether to lay eggs in hosts. Usually the adult female feeds on PPF, but some species also feed on hosts. Thus, host density affects the oviposition rate of the adult parasitoid, but not the survival or development of the larvae. Scenario B comes close to hoverflies. Here, the purely carnivorous larvae actively search for prey, whereas the adult females feed on PPF and lay eggs near areas with prey. Thus, prey density will here most strongly affect juvenile performance. Scenario C is best illustrated by ladybeetles, predatory bugs, earwigs and predatory mites, where carnivory and search for prey occurs in all active stages. Here, prey density will affect the juvenile, as well as adult performance. Lacewings have always actively searching larvae, but some species are carnivorous as adults and other are not. Thus, they represent either scenario B or C. If the oviposition rate of hoverflies strongly depends on how much prey the area harbours, then they are more close to scenario C than to B. Scenario C also becomes more applicable to parasitoids when they kill and feed on hosts to obtain nutrients essential for egg maturation.

For each of the three scenarios on stage-related prey density dependence models were developed that incorporate the effect of substitutable or complementary PPF affecting (1) survival, (2) searching or (3) development and reproduction, plus any combination of 1, 2 and 3. Parameters ranges were based on literature data. From the equilibrium equations of those models we derived how equilibrium prey density changes with an increase in PPF. Such calculations are particularly meaningful when populations return to the equilibria after perturbation (i.e., equilibria are stable). This is likely when not all prey stages are vulnerable to predator attack (Murdoch *et al.* 1987), which holds for many arthropod predator-prey systems (Sabelis 1992; Sabelis and Van Rijn 1997). These calculations show that increasing PPF – whether substitutable or complementary, essential or not essential – causes equilibrium prey density always to decline under all scenarios, but the mode and quantitative details of the decline depend on the scenario under consideration. Extinction above a critical level of PPF availability can only be achieved when the predators eat PPF and prey as substitutable foods in all life stages (and are thus true omnivores) whereas both mortality and reproduction are affected by PPF. In all other cases increasing PPF can never drive the prey population to extinction. Instead, prey density will asymptotically approach some positive value set by the level of background (= minimum) predator mortality. Under each of the three scenarios (A, B and C) the strongest decline in prey density is achieved when PPF is substitutable (and hence non-essential) and when PPF promotes both survival and reproduction of the predator; effects of PPF on survival alone come second in prey suppression efficiency and effects of PPF on searching alone come third. When PPF is essential (and hence complementary), there is a minimum amount of PPF required for the predator population to persist and thereby to suppress the prey population.

SPATIALLY STRUCTURED CONSUMER-RESOURCE MODELS

The models discussed above are based on the assumption that predators, herbivores as their prey, and food plants of the herbivores are well mixed and therefore do not take the spatial component of predator-prey interactions into account. This assumption is valid as long as the grain size of the spatial heterogeneity is well below the average foraging range of the consumers. In many cases, however, this assumption does not hold. When herbivores as prey and their food plants co-occur only at spatial scales that are beyond the search range of individual predators, new mechanisms may come into play that are absent in fine-grained environments: predator aggregation, spatial subsidies, and metapopulation dynamics.

Suppose there are two types of sites, plants with herbivores as prey and plants providing alternative food (PPF). When the two plant types occur sufficiently close together, or even merged into one plant species harbouring sites with PPF and sites without, predators may disperse fast enough to achieve a distribution proportional to the amount of food (prey and PPF) on those sites (so called Ideal Free Distribution). Suppose Rosenzweig-MacArthur models govern predator-prey dynamics on each of the two sites and the predators distribute themselves ideal free over the two sites (Van Rijn *et al.* 2002). Then, for a constant PPF supply the predator distribution stabilizes at equilibrium. The more PPF, the stronger the predators

aggregate at the PPF site and the stronger the suppression of herbivores at this site, whereas the suppression in the PPF-free site is weak. Decreasing the area of the PPF site relative to the total area intensifies the impact on herbivore density in this area, but leaves the herbivore density in the non-target area unaffected (Van Rijn and Sabelis 2005). Thus, target sites or plants can be protected against herbivory by arresting predators with the aid of alternative food (PPF). The mode of decline in herbivore density (linear decline to extinction or asymptotic decline to constant level) will be much like those observed in the models of well mixed populations and thus depend on whether PPF and prey are substitutable or complementary, essential or non-essential foods. This local indirect effect of PPF on the herbivores via dispersal of the predator is comparable with 'apparent (predator-mediated) competition', but as it results from behavioral rather than life-history responses, it acts already on time scales shorter than a generation, and has therefore been termed 'short-term apparent competition' (Holt and Kotler 1987). Clearly, this short-term effect of PPF will work most effectively when large areas of (semi)-natural habitat surround agricultural fields and have low numbers of potential pest organisms and some redundancy in the carnivorous species feeding on the pest organisms.

When plants with PPF and those without are further apart, the plants with PPF may subsidize the predator population on PPF-free plants, and help to suppress herbivore numbers there. This represents a source-sink system at the landscape scale (Dunning *et al.* 1992; Polis *et al.* 1997; Pulliam 1988) and can be modelled by two Rosenzweig-MacArthur, predator-prey models, one for the source and one for the sink, that are coupled by dispersal. The impact of PPF via the predators on herbivores on the distant PPF-free plant will vary depending on whether PPF and prey are substitutable or complementary, essential or non-essential. If PPF in the source habitat is complementary and essential yet absent in the sink habitat, it may contribute to suppression of herbivores in the sink habitat (even though here – following the definition of a sink – predator reproduction does not cancel out mortality). When PPF is non-essential, the predator population can persist anyway and by definition the PPF-free habitat cannot be a sink. The habitat with PPF will harbour fewer herbivores and more predators, causing dispersal into the PPF-free habitat. Here, the impact on herbivore density is less pronounced, however, than if PPF is essential. When PPF and prey are substitutable, herbivore density is reduced in the habitat with PPF to a level that exactly compensates for the amount of PPF available. Hence, predator density in that habitat remains unaltered and there will be no net migration into the PPF-free habitat and, hence, no change in prey density. If, however, PPF availability is such that it just supports the predator population and drives prey extinct, any further increase in PPF availability will no longer be compensated by a decrease in prey density, but will translate directly into a larger carnivore population. Predators dispersing into the PPF-free habitat will now cause herbivore density to decrease.

For many real-world systems of arthropod predators and their prey, equilibria may not be feasible. Sources may turn into sinks and vice versa depending on the season or local predator-prey dynamics is intrinsically unstable. In some acarine predator-prey systems in orchards PPF (e.g., pollen) emerging early in the growing season plays a critical role in building up a predator population large enough to suppress the prey population later in the year. In other acarine predator-prey systems predators tend to overexploit their prey and then disperse aerially to find new prey patches (e.g., Pels and Sabelis 1999; Pels *et al.* 2002; Sabelis and

Van der Meer 1986). Here, PPF may either alter the outcome of transient dynamics in local predator-prey populations or it may provide indispensable fuel for dispersal. According to metapopulation models of the patch-occupancy type (Levins 1969) increased rates of dispersal due to PPF will result in a decrease of the number of prey patches. If, however, PPF promotes the within-patch per capita growth rate of the predators this is likely to result in earlier prey extermination and lower numbers of predators that disperse from a patch. This implies a lower dispersal predator dispersal rate and hence an increase of the number of prey patches in the metapopulation. Thus, to understand the metapopulation consequences of PPF it is critical to assess how it influences between-patch predator dispersal as opposed within-patch predator-prey dynamics.

DISCUSSION: BEYOND CONSUMER-RESOURCE MODELS

In this article we provided a review of the conditions under which PPF gives rise to herbivore suppression via a shared consumer. In particular, we considered how the impact of PPF is modified by stage- and space-related interactions. With few exceptions, the overall pattern is that PPF somehow promotes herbivore/prey suppression. The underlying assumption was that the system consists of one species at the third trophic level, one species at the second trophic level and PPF, as an influence from the first trophic level. In reality, herbivore and carnivore are part of a much more complex food web of species interacting with each other, (Polis and Strong 1996). How will these interactions affect the conditions under which PPF leads to herbivore suppression?

Consider first the presence of a fourth trophic level. Carnivores may have their own suite of (hyper-)predators, (hyper-)parasitoids, and pathogens (Rosenheim 1998; Sullivan and Volkl 1999). Trophic cascade models predict that the top-carnivore will at equilibrium control the primary carnivore, so that the herbivore is released from top–down control (Oksanen *et al.* 1981). The equilibrium density of the primary carnivore would be determined by the traits of the top-carnivore, whereas the herbivore would grow to a density where it is limited from bottom up. This implies that at equilibrium, food provided to the primary carnivore would no longer affect the density of the primary carnivore, nor that of the herbivore! In some cases, PPF can (also) be used by the top-predator or hyperparasitoid (Chang *et al.* 1994). PPF will now likely reduce the density of the primary carnivore, and consequently have a negative rather than a positive impact on biological control of the herbivores. Thus, the presence of a fourth trophic level may dramatically alter the predictions for the impact of PPF on herbivore suppression.

At the third trophic level, competition for herbivores as prey and intraguild predation may alter the species composition and thereby the impact on herbivore suppression (Polis and Holt 1992; Polis *et al.* 1989; Rosenheim 1998). PPF may change the outcome of competition and intraguild predation by promoting one species more than others (e.g., Evans and England 1996). If PPF supports the species that in absence of PPF is a worse competitor but a good intraguild predator, PPF may reduce the density of the better competitor and promote herbivore density (Briggs and Collier 2001; Holt and Polis 1997; Hunter *et al.* 2002; Mylius *et al.* 2001; Rosenheim 2001; Rosenheim *et al.* 1995; Snyder and Ives 2001). However, beyond the PPF level that results in exclusion, PPF will have the same effects as predicted from simple

carnivore-herbivore models. Thus, rather restrictive conditions are required for competition and intraguild predation to alter the predictions for the impact of PPF on herbivore suppression obtained from simple predator-prey models.

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OVERVIEW OF THE ROLE OF GENERALIST PREDATORS IN BIOLOGICAL CONTROL

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SESSION 9 INTRODUCTION

The subject of generalist predators in biological control is rich, diverse, and stimulating. It is also frustrating, providing ample grounds for enthusiasm for their potential as significant agents of pest population suppression, along with well documented examples of near successes and patent failures. On the basis of ecological theory and extensive meta-analyses of the literature, generalists are apt to be, and have been *found* to be, significant biocontrol agents in many situations (Murdoch *et al.* 1985; Change & Kareiva 1999; Greenstone & Sunderland 1999; Symondson *et al.* 2002). Nevertheless the devil is in the details of habitat, crop phenology, interspecific interactions, and weather, and we are still trying to work out the conditions for success in employing generalist predators for biocontrol.

The broad selection of papers in this session nicely illustrates some of the challenges facing us as we struggle to discover the determinants of such success. Kindelmann and colleagues (this volume), who some might consider to have crashed the party by discussing a group of predators that are more narrowly stenophagous than most of those under discussion, show by means of a removal experiment that two coccinellid species do not reduce the peak numbers of their aphid prey, reinforcing what is becoming a depressing consensus that coccinellids are not effective regulators of pest populations. Furthermore, Harwood & Obrycki (this volume) find that one of those two species, *Harmonia axyridis* (Pallas), is more fit on a pure aphid diet than a mixed lepidopteran one. Being thus averse to using alternate prey as part of a "lying-in-wait" strategy until aphids arrive, coccinellids may be predisposed to avoid crops until aphids are too abundant to control, another blow to their potential effectiveness as pest population regulators.

On the other hand Harwood & Obrycki (this volume) find that the linyphiid spider *Erigone autumnalis* (Emerton) is unable to survive to adulthood on a pure pest (aphid or leaf hopper) diet, requiring a mixed diet including collembolans and flies to reach maturity. It is thus well suited to the lying-in-wait strategy, able to subsist on a variety of other insects until

pests arrive. However, because it prefers non-pest alternate prey to pests, it is probably less effective than it might otherwise be in regulating pest populations. This is exacerbated by the fact that in the alfalfa system studied by Harwood and Obrycki, pests and alternate prey tend to covary in abundance, rising or falling simultaneously. This is different from the verbal model that biocontrol practitioners like to use to describe the lying-in-wait strategy, in which high alternate prey numbers early in the season sustain predators until pest populations build up later.

Spiders are iconic generalists. In their study of the influence of landscape on spider species richness and biocontrol, Schmidt *et al*. (this volume) find that wheat field spider species richness is more strongly affected by the proportion of perennial non-crop habitats in the surrounding landscape than by the presence of directly adjoining non-crop habitats. The proportion of non-crop habitats in the surrounding habitats also influenced spider *densities*, with the most important spatial scale being smaller (0.19 – 0.53 km) for lycosids, which tend to walk, than for linyphiids (up to 3.0 km), which are more apt to balloon to and from overwintering sites. Knowledge of the influence of the surrounding landscape is important, because spiders may significantly depress aphid densities in this system; unfortunately, since spider densities fluctuate greatly from year to year, they may not be dependable regulators of aphid populations.

But as anyone who has followed coccinellids around a crop field or counted cereal aphids in linyphiid webs knows, even predators that show up too late to an infestation to singlehandedly control a pest infestation, or that would rather eat detritivores than pests, can still dispatch phenomenal numbers of pests. They may therefore make significant contributions to pest control despite a few disappointing attributes. Pfannenstiel (this volume) remind us to think about the entire assemblage of generalists rather than focusing on particular groups of predators. He finds that there is strikingly little overlap in the nocturnal and diurnal predator assemblages of lepidopteran eggs in annual crops in the southern USA. He also discovers that spiders are prominent among the nocturnal assemblage, accounting for almost a quarter of egg mortality by a diverse suite of arachnid and insect predators operating at night. Besides showing that generalist arthropod predators impose very high mortality on lepidopteran eggs, Pfannenstiel (this volume) reminds us that there is a great deal that we still do not know about diel periodicity in predators and the relative importance of diurnal vs. nocturnal predation. Greenstone & Roberson (this volume) show that we also know almost nothing about the role of immature predators.

Snyder & Straub (this volume) find that the assemblage of predators attacking *Myzus persicae* (Sulzer) in potatoes is very diverse. In an experiment designed specifically to determine whether there is more or less intraguild complementarity or interference in assemblages made up of different specific predator species, they find that complementarity or interference effects do not affect aphid suppression. However, the identity of predators does, with a coccinellid being more effective than a thomisid spider in suppressing aphids.

Generalist predators are diverse and abundant, and it is critical that we define their role in both agricultural and natural systems. Predation events tend to happen cryptically and infrequently, and there have been significant obstacles to our ability to quantify and characterize predation in usually complex trophic webs. In this necessarily short collection of pa-

pers, we have been unable to highlight any of the exciting work on molecular gut analysis that is transforming our ability to study and understand the role of predators in biological control (Zaidi *et al.* 1999; Chen *et al* 2000; Hoogendoorn & Heimpel 2001; Harper *et al.* 2005). Molecular techniques, in combination with the kinds of carefully designed experiments (Kindelmann *et al*.; Snyder & Straub) and exhaustive direct observation (Pfannenstiel) illustrated in this session, will facilitate our understanding of predator impact and ecology, and improve our potential for successfully manipulating them for biological control.

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FIELD TEST OF THE EFFECTIVENESS OF LADYBIRDS IN CONTROLLING APHIDS

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ABSTRACT

Some experiments indicate the ability of coccinellids to significantly suppress aphid abundance. Exclusion of predators by caging aphid-infested plants has repeatedly resulted in higher aphid populations and greater aphid population growth rates. However, aphidophagous coccinellids have never proved effective in controlling aphid populations in the field. To resolve this apparent contradiction, a field experiment was used to determine the effectiveness of two coccinellids, *Coccinella septempunctata bruckii* and *Harmonia axyridis* in suppressing populations of the aphid, *Aphis gossypii*, on shrubs of *Hibiscus syriacus* under natural conditions. Instead of caging some of the shrubs, the effect of each species of coccinellid on aphid population dynamics was estimated by direct counts and a manipulative experiment, in which all the eggs of *C. septempunctata bruckii* were removed from 8 shrubs, all those of *H. axyridis* from another 8 shrubs, all those of both species from an additional 12 shrubs, and no eggs were removed from 6 control shrubs. The predators did not have a negative effect on the peak numbers of their prey. This is in full accord with the GTR hypothesis, according to which long-lived predators cannot be effective in controlling a short-lived prey.

INTRODUCTION

Aphidophagous coccinellids are probably the most abundant generalist predators of aphid populations. Some experiments indicate they significantly suppress aphid abundance. Exclusion of predators by caging aphid-infested plants has repeatedly resulted in signiûcantly higher aphid populations (Brown 2004; Chambers *et al*. 1983; Michels *et al*. 2001) and greater aphid population growth rates (Elliott and Kieckhefer 2000), indicating that coccinellids markedly reduce aphid abundance. However, aphidophagous species of ladybirds have never proved effective in controlling aphid populations (e.g., van den Bosch and Messenger 1973).

The apparent contradiction of the results of the exclusion experiments and attempts to use coccinellids in the large-scale biocontrol of aphids may be explained as follows: when access of predators to aphids is excluded by caging the aphid-infested patches, aphids cannot react to their own increasing local density by emigration, which causes large aphid density in caged patches. Thus, there are more aphids in caged patches because they cannot leave the patch, not because predators reduce aphid numbers in non-caged patches. To test this hypothesis, field experiments were used to determine the effectiveness of *Coccinella septempunctata bruckii* Mulsant (Coleoptera: Coccinellidae) and *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) to suppress populations of the aphid *Aphis gossypii* Gloven (Homoptera: Aphididae) on small shrubs of *Hibiscus syriacus* L. under natural conditions. Instead of caging infested shrubs, the effect of each species of ladybird on aphid population dynamics was estimated by direct counts on naturally infested shrubs and in a manipulative experiment, in which the eggs of one or both predators were removed from the shrubs.

METHODS

The study site was on the Yamagata University farm (Tsuruoka, Yamagata pref., Japan, 38^{o} 43' N, 139^{o} 49'E). It consisted of about 100 shrubs *of Hibiscus syriacus* L., which is the primary host of the aphid *Aphis gossypii* Glover. This aphid is attacked by two coccinellids: *Coccinella septempunctata bruckii* and *Harmonia axyridis*.

In the years 1993-1996, a total of 30 shrubs naturally infested with aphids and their predators were monitored from mid May to mid July. The numbers of coccinellid eggs, larvae, and of aphids were counted daily. To evaluate the effect of the number of aphids at the beginning of the season, x, the number of egg batches of *C. septempunctata bruckii*, c, and the number of egg batches of *H. axyridis*, h, on the peak number of aphids, Y, a stepwise regression, descending method, was applied to the data from 1993-1996. As aphids multiply exponentially, and therefore linear dependence of the logarithm of the peak on other variables was expected, the same methodology was applied to the data set with log(Y+1) instead of Y.

It is difficult to identify coccinellids at the egg stage because they are often similar in size, color and number in a batch. In 1993, eggs were identified to species using the larvae that hatched from them. In the following years, a few eggs were removed from each egg mass and placed in Petri dishes at 25 $^{\circ}$C and a 14L:10D photoperiod in the laboratory, and identified when the larvae hatched. Eggs reared in the laboratory hatched earlier than those left on the shrubs, which enabled the removal of the eggs before they hatched.

In 2000 and 2001, the effect of the absence of each coccinellids on aphid population dynamics was estimated. For this an additional 34 shrubs were selected for a manipulative experiment. After identification to species but before hatching, all the eggs of *C. septempunctata bruckii* were removed from 8 shrubs, those of *H. axyridis* from another 8 shrubs, eggs of both species from an additional 12 shrubs, and on the remaining 6 control shrubs no eggs were removed. Sticky bands were placed at the bottom of each shrub in order to prevent colonization by larvae from other shrubs. The shrubs were monitored from mid May to mid July. The numbers of coccinellid eggs, larvae and aphids were counted daily.

RESULTS

In the model with the peak number of aphids, Y, as the dependent variable, no independent variable demonstrated a significant effect (Table 1). If ln(x + 1) was used instead of x and ln(Y+1) instead of Y, as the aphids are expected to grow exponentially, at least at the beginning of the season, the equation for the reduced model was: $\ln(Y+1) = 4.6 + 0.32*\ln(x+1) + 0.055*h$. The selected independent variables explained 33.26% of the variability of ln(Y+1). There is a 4.82% risk of rejecting the hypothesis that a constant model would be better, so the selected variables make a significant contribution to the model.

The variable that explained the most of the variation was the intercept (Table 1). In neither of the models did the abundance of either predator species significantly affect the peak aphid numbers (Table 1). As there is a lot of unexplained variability in this system, a manipulative experiment was carried out in 2000-2001. On average, 12.9 egg batches of *H. axyridis* and 13.9 of *C. septempunctata bruckii* were laid per shrub during 2000 and 2001. Almost no parasitism was observed. The resulting peak numbers of aphids are shown in Fig. 1. Predators did not significantly affect the peak numbers of aphids (one-way ANOVA gives F = 3.71, P = 0.67 in 2000 and F = 3.24, P = 0.37 in 2001).

DISCUSSION

There has only been one attempt until recently to account for the low effectiveness of insect predators: Kindlmann and Dixon (1999; 2001) proposed that the ratio of generation time of insect predators to that of their prey (generation time ratio, GTR) determines their effectiveness in suppressing prey. Kindlmann and Dixon (1999) assume that on a large spatial scale, at any instant, herbivore populations exist as patches of prey, associated with patches of good host plant quality. Predators exploit these patches, which vary greatly in number of prey both spatially and temporally (Kareiva 1990). GTR in insect predator-prey systems is often large – the developmental time of insect predators often spans several prey generations and is similar to the duration of a patch of prey (Dixon 2000). Cannibalism is common in insect predators (Agarwala and Dixon 1993; Fox 1975) and is adaptive, as eating conspecific competitors will increase the fitness of their larvae (Dong and Polis 1992). Mortality during larval stages reaches about 99% (Hironori and Katsuhiro 1997; Kindlmann *et al.* 2000; Kirby and Ehler 1977; Matsura 1976; Osawa 1993; Wright and Laing 1982). Because of the enormous larval mortality, the life history strategy of these predators is likely to be selected to maximize the probability of survival of their offspring, rather than maximize the number of eggs laid. In major-

Table 1. Results of stepwise regression, descending method, on the effect of the number of aphids at the beginning of a season, *x*, the number of egg batches of *C. septempunctata bruckii*, *c*, and the number of egg batches of *H. axyridis*, *h*, on the peak number of aphids, *Y*, and on its logarithm, log(*Y*). Statistically significant values indicated by asterisks (*means 5%, ** means 1% significance level).

		DF	SS	MS	Fisher's F	Pr > F
Model with Y		2	5753648	2876824	2.83	0.091
Residuals		15	15264871	1017658		
Total		17	21018519			
Model with log(Y)		2	20.38	10.19	3.74	0.048*
Residuals		15	40.90	2.73		
Total		17	61.28	6.00		
			Value	Std dev.	Student's t	Prob.
Model with Y	Intercept		544.3	349.2	1.56	0.14
	Initial # aphids		1.21	0.71	1.70	0.11
	C. septem-punctata bruckii		104.4	86.7	1.20	0.25
Model with log(Y)	Intercept		4.60	0.68	6.75	0.00**
	ln(Initial # aphids+1)		0.32	0.19	1.74	0.10
	H. axyridis		0.055	0.043	1.27	0.22

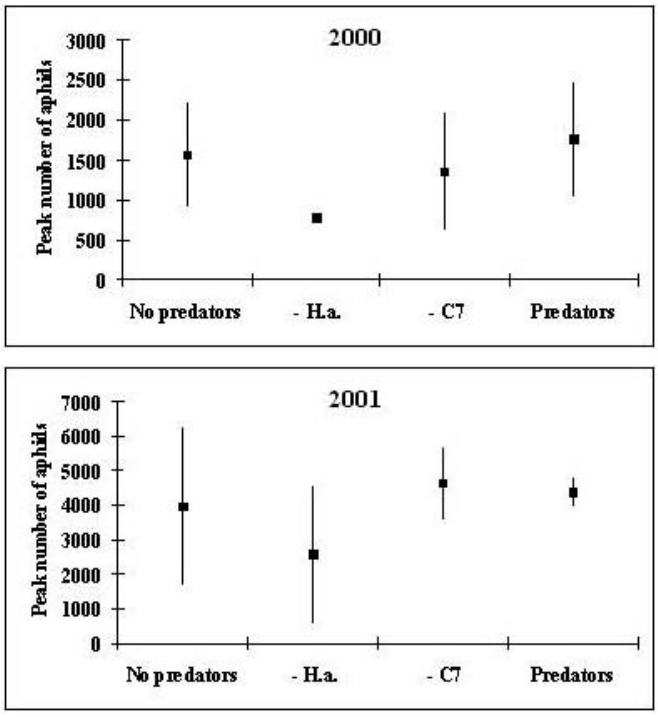

Figure 1. Peak numbers (± SD) of aphids on shrubs from which eggs of all predators were removed (no predators), only *C. septempunctata bruckii* eggs were removed (- C7), only *H. axyridis* eggs were removed (- H.a.) and no eggs were removed (predators), in years 2000 and 2001.

ity of cases, the adults are winged and can easily move between patches, whereas the immature stages are confined to one patch throughout their development, and their survival is associated with the quality of the patch of prey in which they were born. Therefore, the fitness of most predators (especially those feeding on highly aggregated and ephemeral prey patches such as aphid colonies, like aphidophagous ladybirds and hoverflies), measured as the number of offspring that survive to reproductive age, is likely to be more closely associated with oviposition strategy (the choice of patch for laying eggs), than the trophic interactions commonly used in models of prey-predator population dynamics.

When GTR is large and cannibalism is common, eggs laid by predators late on in the existence of a patch of prey are highly likely to be eaten by larvae of predators that hatch from the first eggs to be laid. In addition, because of the large GTR, there is insufficient time for the larvae that hatch from late laid eggs to complete their development. Thus cannibalism and the ephemeral existence of patches of prey pose such constraints that females that can assess the age of a patch of prey gain an advantage. As a consequence, females oviposit in young patches ("egg window hypothesis", Dixon 2000). The short "egg window" during which it is advantageous to lay eggs in a patch of prey in large-GTR systems reduces the number of eggs laid per patch. Incidence of cannibalism is likely to be proportional to the probability of encountering another predator, i.e., to the relative abundance of predators to prey ("meet and eat hypothesis", Kindlmann and Dixon 2003). If this is true, then even if predators are abundant and therefore many eggs are laid in a patch of prey during the egg window, strong density dependent cannibalism greatly reduces the abundance of the predators (Mills 1982). Therefore, no matter whether abundant or not, insect predators have little impact on prey population dynamics, when GTR is large ("GTR hypothesis", Kindlmann and Dixon 1999). A simple dynamic model published by Kindlmann and Dixon (1993) demonstrates why the verbal logic presented here is correct.

Laboratory experiments and field observations provided the foundations on which the GTR and egg window hypotheses were built. Several insect predators have evolved mechanisms that enable them to oviposit preferentially early in the development of a patch of prey and avoid patches that are already being attacked by larvae (Hemptinne *et al.* 1992; 1993; 2001). This leads to eggs being laid during the "egg window" and may lead to low effectiveness of these predator species in suppressing the numbers of their prey. However, there has not been a field test of the effectiveness of these predators.

In the coccinellid – aphid system studied here, the GTR is close to 3, and thus the GTR hypothesis would predict a low effect of predators on aphid abundance. In this study the predators did not have a negative effect on the peak numbers of their prey. On the contrary, the peak number of aphids in the control (with both predator species present) was larger, although not significantly so, than on the shrubs from which one or both predator species were removed. These conclusions only apply to predator prey systems with a large GTR and especially to predators feeding on highly aggregated and ephemeral prey patches such as aphid colonies, like aphidophagous coccinellids and syrphids. It does not follow that all insect predators are ineffective in controlling their prey as is well illustrated by the outstanding success of *Rodolia cardinalis*.

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SPIDERS IN SPACE: HOW LANDSCAPE-WIDE MOVEMENT OF GENERALIST PREDATORS INFLUENCES LOCAL DENSITY, SPECIES RICHNESS, AND BIOCONTROL

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ABSTRACT

Arthropods are mobile, and often move beyond the classical bounds of ecological field studies. We have done landscape analyses to explore the influence of the surrounding landscape on spiders in winter wheat fields and on their potential to control cereal aphids. The densities of many spider species were enhanced by high percentages of non-crop habitats at scales ranging from 95 m to 3 km radius, suggesting that complex landscapes with rich populations of natural enemies are favorable for aphid control.

INTRODUCTION

Local arthropod communities can be influenced by the surrounding landscape, either through short-term dispersal events or the dynamics of long-term population establishment and extinction (Kareiva and Wennegren 1995; Ricklefs 1987; Tscharntke and Brandl 2004). Recent studies have demonstrated that ecosystem services such as pollination and biocontrol by arthropods depend on landscape patterns at scales up to several kilometers (Kremen *et al.* 2004; Roland and Taylor 1997; Thies and Tscharntke 1999; Thies *et al.* 2005). Landscape effects on local pest-natural enemy interactions may be particularly strong in arable crops because of the necessity of annual recolonization (Schmidt *et al.* 2004a).

Spiders are important predators of various insect pests (Marc *et al.* 1999; Nyffeler *et al.* 1994). As they overwinter predominately outside of arable fields in Central Europe, the colonization of crops during spring should be related to the availability of perennial non-crop habitats in the surrounding landscape (e.g., Lukzak 1979; Schmidt and Tscharntke 2005a; Topping and Sunderland 1994). The circumference around a field in which the landscape is relevant should further depend on the movement capacity of each species. It may thereby provide a measure for the effective dispersal range of a species, which is otherwise hard to determine.

We studied spider communities in winter wheat in relation to the surrounding landscape and local farming system, and conducted field experiments on the relative importance of spiders and other natural enemies for cereal aphid control.

MATERIALS AND METHODS

The studies started in 2001 in two regions in Germany. Eighteen landscape sectors were selected around the city of Göttingen (Southern Lower Saxony), which had moderate to high percentages of arable land (25-85% at a scale of 1.5 km). In the Lahn-Dill Bergland (Central Hesse), 20 landscapes were selected in which the percentages of arable land were lower (7-61% at a scale of 1.5 km), and percentages of various non-crop habitats correspondingly higher than around Göttingen. In each of the 38 landscape sectors, one or two fields of winter wheat were studied. Landscape composition was calculated for 11 scales between 95 m and 3 km radius around the study fields (Fig. 1). Spiders were sampled with pitfall traps and web abundance with a distance method, and species richness and density were related to local management and to landscape features.

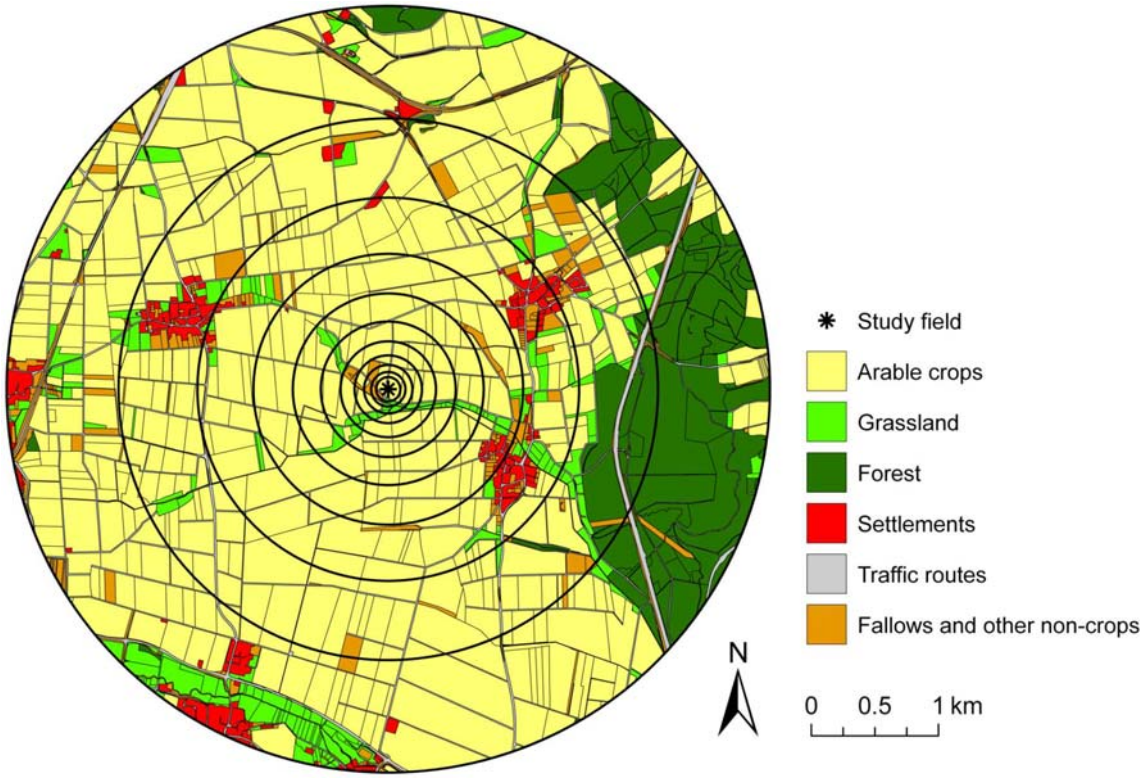

Figure 1. One of the 38 landscape sectors, with a relatively high percentage of arable crops (68.7%), but little grassland (5.6%), forest (12.5%) and other non-crop habitats. The circles represent the eleven spatial scales of 95, 135, 190, 265, 375, 530, 750, 1060, 1500, 2120 and 3000 m radius around the study field, at which landscape composition was calculated.

RESULTS AND DISCUSSION

SPIDER SPECIES RICHNESS

Overall, 37,303 spiders were determined, which belonged to 139 species. Surprisingly, local species richness was influenced more strongly by the composition of the surrounding landscape than by the presence of directly adjoining non-crop habitats, and this relation was consistent across both study regions (Fig. 2). The correlation between species richness and the percentage of non-crop habitats in the surrounding landscape was strongest at 1-1.5 km radius.

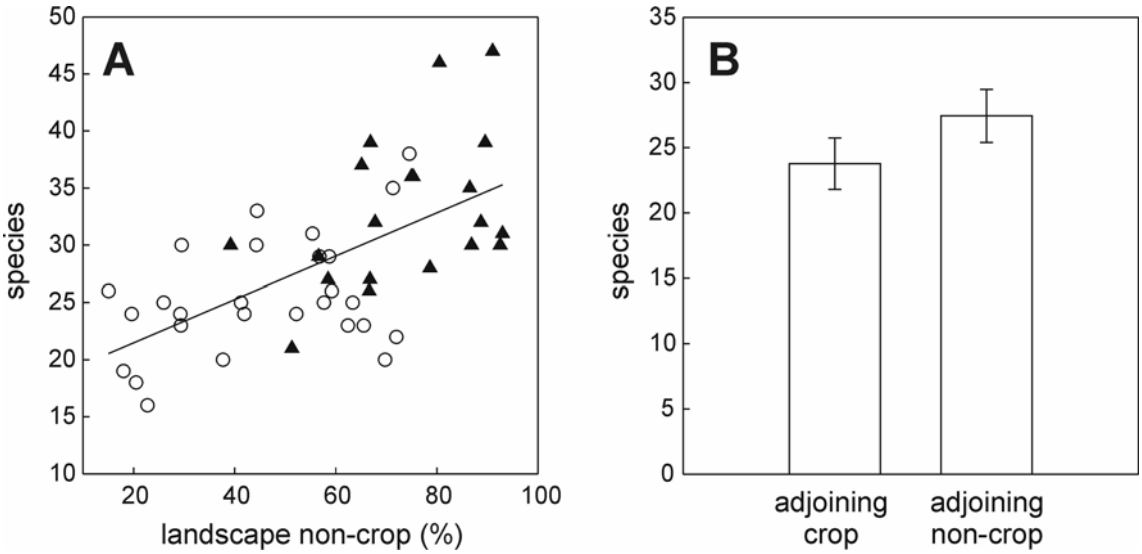

Figure 2. Landscape effects on the species richness of spiders. **A:** Correlation between local species richness and the percentage of non-crop habitats 1.5 km around fields. Open circles: Southern Lower Saxony; Solid triangles: Lahn-Dill Bergland (Hesse). GLM: non-crop: $F_{1,43} = 9.8$, $p < 0.001$; region: $F_{1,43} = 0.1$, $p = 0.8$; non-crop × region: $F_{1,43} = 0.6$, $p = 0.4$. **B:** Effect of adjoining non-crop habitats. n = 9 pairs of fields, each within one landscape sector. t-test for matched pairs: $t_{1,8} = -3.2$, $p = 0.01$.

SPIDER DENSITY

Out of the 64 most common spider species, 34 were locally enhanced by high amounts of non-crop habitats in the surrounding landscape. Wolf spiders (Lycosidae) were influenced by landscape composition at smaller scales (mostly between 190-530 m radius) than the more ballooning Linyphiidae (up to 3 km radius). In contrast, directly adjoining non-crop habitats increased the densities of only two out of 64 spider species, which cannot be considered statistically significant when accounting for the multiple species tested. According to web densities, the abundance of sheetweb spiders (Linyphiinae) rose with the percentage of non-crop habitats in the surrounding landscape, e.g. from 18-130 webs per m^2 in late May 2001 (Schmidt and Tscharntke 2005b). A similar positive relationship between web abundance and landscape composition was also present in other years, and plainest between scales of 1 km and 3 km around the study fields.

In 2002, we compared the effects of landscape to the effects of local organic versus conventional management in twelve pairs of organic and conventional fields along a landscape gradient. Thereby, organic management increased overall density of ground-dwelling spiders by 62%. In contrast, species richness was determined by landscape, only (Schmidt *et al.* 2005). Overall, densities of *Oedothorax apicatus* were affected mostly by management, *Pardosa* species by both landscape and management, and other species mostly by landscape. This shows that enhancement of certain generalist predators can only be effective when the landscape is considered.

BIOLOGICAL CONTROL

In supplementary field experiments, we demonstrated how ground dwelling-predators reduce aphid infestation in winter wheat. Cereal aphid densities increased by 40-55% when ground-dwelling predators were excluded, most likely due to reduced predation by spiders (Schmidt *et al.* 2003; 2004b). The differences in spider density between control and exclusion were in the range of the differences that could be observed between landscapes with high and low percentages of non-crop habitats. Therefore, aphid suppression by spiders can be expected to be stronger in landscapes with high percentages of non-crop habitats. However, sheetweb spider densities in 2002-2004 were less than one third of those in 2001, when the aphid control experiments were carried out. Therefore, an influence of spiders on aphid populations may be inconsistent not only among landscapes, but also among years.

CONCLUSIONS

Spiders in wheat fields are strongly influenced by the surrounding landscape, which could lead to a significant increase of aphid control in landscapes with high amounts of perennial non-crop habitats. This underlines that a purely local orientation of biological control is not always sufficient. Similar effects of landscape-wide dispersal by pests and their natural enemies can be expected in many situations worldwide, offering an exciting field for biocontrol research.

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THE ROLE OF ALTERNATIVE PREY IN SUSTAINING PREDATOR POPULATIONS

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ABSTRACT

Generalist predators are widely acknowledged to contribute valuable levels of biological control in agroecosystems throughout the world. Although their feeding habits can result in the rejection of target pests in favor of preferred and often more nutritious non-pest prey, these natural enemies are capable of colonizing habitats prior to the arrival of pests by subsisting on alternative sources of food. The effect of consuming non-pest species on rates of pest predation by a generalist predator can be twofold; feeding upon these nutritious food items generally enhances fecundity thus improving their population growth, but the presence of alternative prey, especially during times when pest regulation is required, can result in reduced levels of pest consumption per individual predator. However, an increased density of natural enemies can counteract this reduction in pest consumption and exert significant levels of biological control.

The role of alternative prey in sustaining predator populations has been widely reported in laboratory studies and field trials examining the fecundity, feeding behavior and growth rates of species subjected to diets of varying quality. Recently, the application of monoclonal antibody and molecular technology to study predation rates in the field has revealed the extent to which many predator communities rely on alternative prey before, during and after the immigration of pests into crops. In this study we examine the role of key species of alternative prey to generalist predators and discuss their impact in the context of biological control. The importance of these prey items to sustaining linyphiid spider and coccinellid communities will also be examined. Microsite sampling of arthropod populations in alfalfa indicated that the overlap in availability of pests (*Acyrthosiphon pisum* and *Empoasca fabae*) and alternative prey to linyphiid spiders is likely to reduce the ability of these generalist predators to restrict the growth of pest populations.

INTRODUCTION

Generalist predators, as part of a complex community of natural enemies, can make significant contributions to the biological control of many pests (Obrycki and Kring 1998; Sunderland *et al*. 1997; Symondson *et al*., 2002). Although they readily consume target pests, their polypha-

gous feeding habits can result in alternative non-pest food resources constituting a significant component of their diet. Furthermore, the availability of these alternative food items can affect pest consumption rates in the field (Harper *et al*. 2005; Harwood *et al*. 2004) and reduce their role in integrated pest management. Despite this interference, these arthropods are capable of impacting upon pests once they arrive in the crop, employing a "lying-in-wait" strategy by subsisting on alternative prey (Chang and Kareiva 1999; Murdoch *et al*. 1985) and impacting upon pests with favorable predator:pest ratios when control is required (Settle *et al*. 1996). However, many species of alternative prey are preferred food items (Toft 2005) and increase growth rates (Mayntz and Toft 2001; Toft 1995), while pests may even elicit aversions from some predators after extended exposure (Toft 1997). This diversion away from target pests thus reduces their capacity for effective biological control (Koss and Snyder 2005; Koss *et al*. 2004; Madsen *et al*. 2004) (Fig. 1). However, simply because pests are a poor quality prey item (Toft 2005) does not necessarily translate to little or no biological control in the field where generalist predators are frequently in a state of hunger (e.g., Bilde and Toft 1998) and readily consume these prey (Harwood *et al*. 2004; 2005).

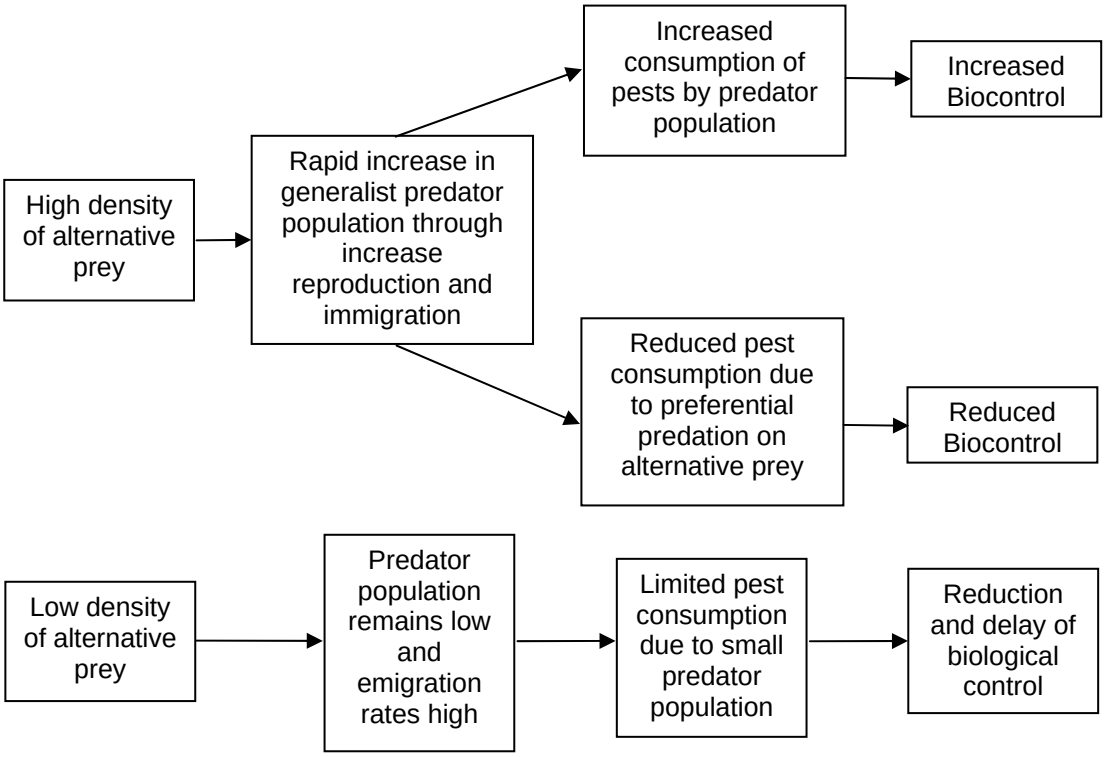

Figure 1. The role of alternative prey in mechanisms of biological control by generalist predator populations.

Many predators build up their populations early in the season by feeding on alternative prey items which are abundant at this time of year (Harwood *et al*. 2001; 2003). This enables them to impact upon pests as soon as they arrive and has been supported by the mathematical modeling of pest populations (Fleming 1980). Murdoch *et al*. (1985) even suggested that generalist predators could be more valuable in biological control than individual specialists acting alone. Early season predation could be extremely important in the control of pests such as the potato leafhopper, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), since control measures are generally required before injury symptoms first appear (Steffey and Armbrust

1991). The presence of a "lying in wait" predator complex could therefore restrict population growth when their densities are low and before specialist natural enemies colonize the habitat.

This study examines the role of alternative sources of food in sustaining populations of two different groups of predator: spiders (true generalists) and coccinellids (aphidophagous predators that exhibit some generalist habits). Field research will focus on the importance of alternative prey to the diet of linyphiid spiders in alfalfa and form a baseline of ecological data for the subsequent molecular analysis of predator feeding habits in the field.

MATERIALS AND METHODS

Adult coccinellids, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), and spiders, *Erigone autumnalis* (Emerton) (Araneae: Linyphiidae), were collected from the University of Kentucky Spindletop Research Station and maintained in the laboratory at 21°C on a 16:8 L:D cycle. Prior to laboratory experiments (below), all individuals were provided with an *ad libitum* supply of isotomid Collembola and Diptera (for spiders) or aphids (for coccinellids).

EFFECTS OF ALTERNATIVE PREY ON *HARMONIA AXYRIDIS*

Adult male and female *H. axyridis* were paired and provided an *ad libitum* diet of *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) and *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) larvae. Eggs were collected, maintained at 21°C on a 16:8 L:D cycle, and upon hatching equal numbers of larvae were systematically assigned to one of five treatments (Table 1). Three parameters were measured; (a) percentage survival to adult, (b) mean development time, and (c) mean weight of adult females at emergence.

Table 1. Invertebrate prey added to each of five treatments. Food was supplied *ad libitum* to all coccinellid larvae.

Treatment	Prey species
A	*Danaus plexippus* (L.) (Lepidoptera: Nymphalidae)
B	*Papilio polyxenes* F. (Lepidoptera: Papilionidae)
C	Mixed diet of *Danaus plexippus* and *Papilio polyxenes*
D	*Aphis glycines Matsumura (Hemiptera: Aphididae)*
E	*Aphis glycines, Danaus plexippus* and *Papilio polyxenes*

EFFECTS OF ALTERNATIVE PREY ON *ERIGONE AUTUMNALIS*

Adult male and female *E. autumnalis* were paired and provided with an *ad libitum* diet of alternative and pest prey (Table 2). Eggsacs were collected and upon hatching, spiderlings were separated and placed into individual Petri dishes with a moist Plaster-of-Paris base to maintain high humidity. Equal numbers of individuals were systematically assigned to one of six treatments (Table 2) after the first molt. Prior to this, small isotomid and sminthurid

Table 2. Invertebrate prey added to each of six treatments. Food was supplied *ad libitum* to all spiderlings.

Treatment	Prey species
A	Isotomid Collembola
B	*Drosophila melanogaster*
C	*Acyrthosiphon pisum*
D	*Empoasca fabae*
E	*Acyrthosiphon pisum and Empoasca fabae*
F	Mixed diet of Isotomid Collembola, *Drosophila melanogaster, Acyrthosiphon pisum* and *Empoasca fabae*

Collembola were provided as prey (large food items were not taken by first instar linyphiid spiderlings). Three parameters were measured; (a) percentage survival to adult, (b) mean development time, and (c) mean weight of new adult females.

INTERACTIONS BETWEEN ALTERNATIVE PREY AND PESTS IN ALFALFA

Quantifying the availability of pest and non-pest prey to linyphiid spiders was undertaken in alfalfa fields at the University of Kentucky Spindletop Research Station. Linyphiid spiders were collected weekly from May until August and immediately frozen in separate Eppendorf tubes (for subsequent molecular analysis of gut-content). The availability of prey was monitored by mini-sticky traps following protocols described elsewhere (Harwood *et al*. 2001; 2003). These small (7.5 cm^2) sticky traps were left *in situ* for 24 h and were designed to monitor activity-density of all prey entering the web-site over time (total n = 420). Thirty web-sites were sampled per week (throughout three cutting cycles of alfalfa).

RESULTS

EFFECTS OF ALTERNATIVE PREY ON *HARMONIA AXYRIDIS*

Larvae of *H. axyridis*, a "generalist" aphidophagous predator, fed with a single-species diet of Lepidoptera had longer development times ($F_{4,27}$ = 29.02, P<0.001) and reduced weight at emergence ($F_{4,21}$ = 13.70, P < 0.001) compared to the mixed Lepidoptera or aphid-containing diets (Table 3). However, these parameters were statistically similar between the mixed lepidopteran diets and those consisting of aphids (either as single species or part of a mixed diet with Lepidoptera) (Table 3). The only parameter reduced in the absence of aphids was survival (<50% survived to adult on Lepidoptera-only treatments).

EFFECTS OF ALTERNATIVE PREY ON *ERIGONE AUTUMNALIS*

No spiderlings survived to adult on single-species diets of *A. pisum* or *E. fabae* although spiderlings consuming *E. fabae* lived significantly longer than those feeding on *A. pisum* (t_{37} =

5.37, $P < 0.001$). However, a mixed diet of the two poor quality pests produced a significant increase in survival parameters (20% survived to adult). Alternative prey (Collembola and Diptera) provided as a single-species diet or part of a mixed diet enabled most spiders to survive to adult. Interestingly, development time from hatching to adult did not vary between treatments (aphid-only and leafhopper-only diets excluded from analysis because no individuals survived beyond the third molt) ($F_{3,67} = 3.52$, $P = 0.065$) but adult weight of female spiders was significantly lower in the mixed pest-only diet (Treatment E) compared to those treatments containing alternative prey ($F_{3,31} = 9.45$, $P < 0.001$) (Table 4).

Table 3. Mean (± SE) development time and weight at emergence of *Harmonia axyridis* subjected to feeding regimes of different quality.

Treatment	Development (days)	Adult weight (mg)
A (*D. plexippus*)	31.2 ± 3.8	21.3 ± 3.8
B (*P. polyxenes*)	38.1 ± 3.4	17.9 ± 4.8
C (*D. plexippus* + *P. polyxenes*)	23.4 ± 2.9	27.1 ± 3.1
D (*A. glycines*)	20.9 ± 3.1	*26.5 ± 2.8*
E (All of above prey)	21.2 ± 2.1	28.6 ± 2.4

Table 4. Mean (± SE) development time and weight at emergence of *Erigone autumnalis* subjected to feeding regimes of different quality.

Treatment	Development (days)	Adult weight (µg)
A (Collembola)	34.1 ± 6.4	68.1 ± 4.3
B (*D. melanogaster*)	39.8 ± 8.0	71.4 ± 9.1
C (*A. pisum*)	n/a	n/a
D (*E. fabae*)	n/a	n/a
E (*A. pisum* + *E. fabae*)	41.7 ± 10.4	39.6 ± 7.2
F (All of above prey)	38.3 ± 8.8	66.8 ± 5.0

INTERACTIONS BETWEEN ALTERNATIVE PREY AND PESTS IN ALFALFA

Spiders captured in alfalfa were dominated by the linyphiid sub-families Erigoninae ($n = 293$) and Linyphiinae ($n = 201$). More spiders were captured than web-sites sampled ($n = 420$) because, occasionally, more than one spider occupied a single web-site. The total number of potential prey captured at web-sites of linyphiid spiders are presented in Fig. 2. Collembola (and other alternative non-pest prey) were an important food resource to these spiders, but pests represented a significant proportion (21%) of their potential diet.

Although alternative prey can improve growth parameters and biological control by spiders (Fig. 1), many of these non-pest food items are preferred by generalist predators (e.g., Bilde and Toft 1994) and can detract biocontrol agents from feeding on pests if populations overlap temporally and spatially. Activity-density of prey in alfalfa indicated a highly significant, and positive, correlation between the availability of *E. fabae* and alternative prey to linyphiid spiders at web-site locations (Fig. 3).

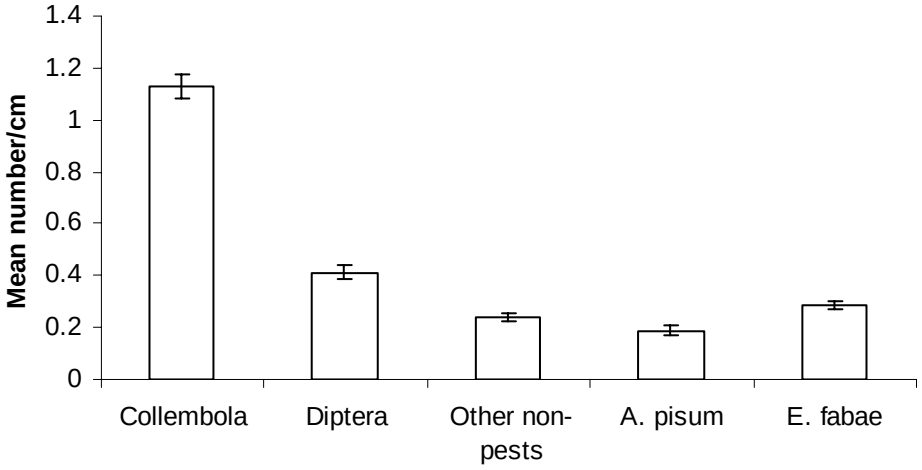

Figure 2. Mean number (± SE) of potential prey captured at web-sites of linyphiid spiders in alfalfa.

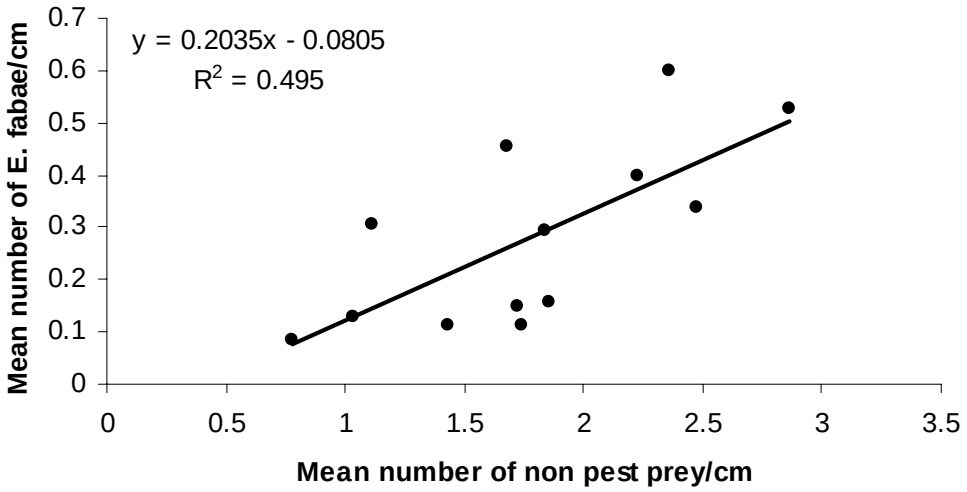

Figure 3. Correlation between availability of *Empoasca fabae* and alternative, non-pest prey in alfalfa.

DISCUSSION

Given the concerns associated with the use of insecticides, it is possible that significant levels of biological control can be provided through the conservation and enhancement of natural enemies. This could be particularly important in crops such as alfalfa which are tolerant to a limited incidence of pests without reducing their yield or quality (Obrycki and Harwood 2005). The aim of this research was to address a key, but poorly understood, component of predator-prey interactions within agroecosystems – the role of alternative prey in sustaining (or disrupting) predator populations from regulation of two pests of alfalfa, *A. pisum* and *E. fabae*. Although conservation biological control may enhance predator growth rates by providing an abundant and nutritionally balanced diet, it is feasible that predators will divert feeding efforts towards non-pest food items, thus reducing biological control. Evidence from manipulative experiments suggests that alternative prey interfere with mechanisms of biological control (Koss *et al*. 2004; Madsen *et al*. 2004). However, molecular evidence and the monitoring of predator population densities are required to accurately quantify the feeding behavior of generalist natural enemies in the field. This may (or may not) implicate alternative prey as a causative factor in the disruption of pest consumption by predator populations. To date, molecular evidence tends to suggest that non-pest prey constitute a significant proportion of diet of many generalist predators (Agustí *et al*. 2003; Harper *et al*. 2005), but even though pest predation rates per individual predator may decline in the presence of these alternative sources of food, feeding activity by the population as a whole may lead to improved levels of control.

Alternative prey has the ability to sustain generalist predators when pest density is low. However, the development of some coccinellids is lengthened and sub-optimal when allowed to feed on such food items (Kalaskar and Evans 2001; Wiebe and Obrycki 2002) and their reproductive output declines on single-species non-aphid diets (Evans *et al.* 2004). Despite these sub-optimal feeding conditions to more specialized aphidophagous predators, true generalists (such as spiders) tend to exhibit increased reproductive output and population growth on alternative non-pest sources of food (Toft 2005). The laboratory studies reported here support these conclusions and indicate that although single-species lepidopteran diets are unlikely to maintain coccinellid populations over significant periods of time, increased diversity of alternative prey could be sufficient to sustain *H. axyridis* (and possibly other coccinellids) until the arrival of favored aphid pests. The ability to employ this lying-in-wait strategy, sustaining themselves on non-pest food resources, would be especially important given that generalist predators are most likely to impact on these pest species early in the year (Chang and Kareiva 1999; Chiverton 1986).

While growth parameters of coccinellids were maximal on aphid diets, the true generalists, spiders, exhibited the opposite effect when fed a diet of alternative prey. Pest-only diets resulted in no hatchling spiders reaching adult, conclusions reported in other spiders (Bilde and Toft 2001). The alternative, non-pest prey items (which consisted of Collembola and Diptera) maximized population growth of these important predators and clearly allowed the juvenile population to be sustained. This ability to subsist (and maximize growth) on alternative prey implicates spiders as particularly valuable biocontrol agents of major pests of agroecosystems. However given that spiders prefer alternative prey, if the availability of non-

pest food overlaps with pests, their potential value in the control target arthropods may be reduced due to diverting their feeding efforts towards alternative prey.

The field-monitoring of arthropod populations in alfalfa supported this hypothesis. There were clear trends indicating that pest and non-pest prey exhibited a strongly positive correlation in their availability to linyphiid spiders. Probably a result of the cyclical nature of cutting, populations of pest and non-pest prey were synchronous such that both occurred in high numbers at the same time. Such synchrony is likely, in the case of true generalists, to compromise their ability to restrict pest population growth given the impact of alternative prey on feeding rates of pest species in the field (Harwood *et al*. 2004). It is clear that while alternative prey items are capable of sustaining generalist predator populations (and in some cases enhancing population growth), the reliance on individual predators in biological control is likely to be ineffective against many agricultural pests. Alternative prey, rather than sustaining predator populations, could reduce the ability of generalists to control crop pests in the field. It is therefore important to maximize the diversity of natural enemies to counteract the interference caused by alternative prey to true generalists such as spiders. This will enable effective levels of control to be exerted by the community as a whole (Sunderland *et al*. 1997; Symondson *et al*. 2002), rather than individual natural enemies acting alone. Ultimately, molecular detection of prey remains (using monoclonal antibodies and/or DNA-based technology) in predator guts and the parallel monitoring of predator population densities will enable the true role of alternative prey in sustaining predator populations to be quantified. Such information can be modeled with prey availability to determine the capacity of different groups of predators in the biological control of arthropod pests and reveal potential interference caused by increased availability of alternative non-pest prey.

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NOCTURNAL PREDATORS AND THEIR IMPACT ON LEPIDOPTERAN EGGS IN ANNUAL CROPS: WHAT WE DON'T SEE DOES HELP US!

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ABSTRACT

Predation is often a key factor maintaining insect populations below pest status in annual crops. However, in many cases, the predators causing significant mortality to particular pests in the field are not well understood. In particular, the complex of nocturnally active predators feeding on pest species is usually unknown.

The predator complexes attacking lepidopteran eggs in cotton, corn and soybean in south Texas, U.S.A., were determined with the goal of characterizing diurnal and nocturnal predator complexes, determining the role of nocturnal predators in lepidopteran pest mortality, and quantifying diel patterns of predation. The evaluations reported here focused on the predator complexes feeding on *Helicoverpa zea* (Boddie) and *Spodopteran exigua* (Hübner), (Lepidoptera: Noctuidae). This work has been conducted using direct observation to accurately measure and identify predation of lepidopteran eggs while removing the bias towards day-active predators.

Egg predation was typically high in all crops in all years, although it ranged from 25 to 99% in any 24 h period. Nocturnal predation was a significant component of this mortality in all crops. The diurnal and nocturnal predator complexes observed feeding on eggs differed between crops. The relative importance of nocturnal predation varied among dates, but on average was similar to diurnal predation in cotton, corn and soybean in 2002 and soybean in 2003. Mortality due to nocturnal predation was >50% higher than diurnal predation in cotton in 2003. Predators observed feeding at night constituted nearly 72% of all observations in south Texas cotton, 52% in corn and 49% in soybean. Nocturnal predators of particular importance included a group of cursorial spiders responsible for nearly 25% of all observations of predation in cotton as well as the formicids (primarily *Solenopsis invicta* Buren) Of the four spider species most frequently observed feeding on eggs, only one had previously been reported as an important predator in agricultural settings (*Cheiracanthium inclusum* [Hentz]). Few predators were commonly active during both day and night. Nocturnal observations revealed both predators that were previously suspected to be important mortality factors as well as those that had not been perceived to be important (e.g., cursorial spiders).

In summary, nocturnal predation was significant in all crops and was usually similar to diurnal predation in relative impact. However, the predators causing mortality were different between day and night with little overlap of dominant predator species. It is likely that there are nocturnally active predators in many crops that are important yet are not perceived as such. Whether nocturnal predation is important in all crops and in all environments is unknown, however these studies demonstrate that there is much to be learned from the study of nocturnal predation. Future research on predation as a component of biological control should incorporate studies of nocturnally active predators.

INTRODUCTION

Predation is often a key factor maintaining populations of lepidopteran pests at a level that prevents injury to annual crops. Studies in cotton (Nuessly and Sterling 1994; Pfannenstiel 2004; Sansone and Smith 2001) and soybean (Anderson and Yeargan 1998) and soybean and corn (Pfannenstiel and Yeargan 2002) have demonstrated that predation on lepidopteran eggs can be consistently high. Studies have attempted to identify predators of Lepidoptera using a variety of techniques, including visual observation (e.g., Whitcomb and Bell 1964), autoradiography (e.g., McCarty *et al*. 1980) and molecular techniques (e.g., Ruberson and Greenstone 1998; Sisgaard *et al*. 2002). These studies have produced widely varying results and it is unclear whether the variation is due to regional/yearly variation in predator abundance or variation in methodology. Buschman *et al*. (1977) obtained estimates of the predator complexes feeding on eggs in soybean that varied depending on the use of diurnal visual observations or autoradiography. One possible explanation for the variation between techniques could be the degree to which they sampled predators that are active nocturnally, something that was almost never explicitly controlled for. If some effort was made to evaluate nocturnally active predators, the effort was only a fraction of that expended evaluating diurnal predation. To explicitly address this, Pfannenstiel and Yeargan (2002) carefully used visual observation to evaluate diel patterns of predation. In almost all circumstances we have no knowledge of the relative contribution of nocturnal mortality, nor much information on the predators that might be causing nocturnal mortality.

During 2001-2004, I evaluated the predator complexes feeding on the lepidopteran pests (*Helicoverpa zea* [Boddie] and *Spodoptera exigua* [Hübner] [Lepidoptera: Noctuidae]) in cotton, corn, and soybean in south Texas, U.S.A. This work has been conducted using carefully conducted direct visual observation to accurately measure and identify predation of lepidopteran eggs, remove biases towards day-active predators, and accurately characterize nocturnal predation. Initial studies indicated that nocturnal predation could be consistently high, predator complexes varied between crops, and that arthropods observed feeding on eggs at night were different from those seen during the day (Pfannenstiel and Yeargan 2002). In that study, several predators that were important were previously unreported as predators of lepidopteran eggs. Other predators were determined to be primarily nocturnal, whereas they were previously considered diurnal. Here, I will present further research results on predation of lepidopteran eggs in annual crops and directly address the relative importance of nocturnal predation in annual crops.

MATERIALS AND METHODS

Predation on lepidopteran eggs was evaluated in cotton, corn, and soybean in south Texas during 2001-2004. The results presented here come from several different studies and correspondingly plot size and arrangement varied. Egg mortality and the predators responsible were quantified using the methods of Pfannenstiel and Yeargan (2002) as modified in Pfannenstiel (2004), but will be summarized here. For all studies, stations within each crop planting were established with flags at 3 to 5 m intervals in each of 3 different rows in each plot or field. At each of these stations, fresh sentinel lepidopteran eggs (*H. zea* or *S. exigua*) were placed and monitored over the next 24 h.

Sentinel eggs were obtained by allowing *H. zea* and *S. exigua* moths to oviposit onto green florist paper that was place as a lining in 3.8 l ice cream cartons. Paper on which eggs had been laid was collected daily and placed into a refrigerator at 4°C to stop development until used or discarded after 4 d. These sheets were cut into small (3 to 20 cm^2) sections containing either 10 *H. zea* eggs or one *S. exigua* egg mass each and re-placed into the refrigerator until use. All eggs in each *S. exigua* egg mass (range 20 to 200 eggs/mass) were counted and recorded before placement into the cotton field. *H. zea* eggs were used for studies in all three crops; *S. exigua* eggs were used only in studies in cotton.

Eggs were attached to plants at 3:00 PM by stapling the eggs to the top of a leaf about 55 - 70% of the distance from the ground to the top of the plant and this relative location was maintained as the plants grew during the season. In corn, eggs were attached to the small leaves on the terminal end of the ear. Pests of field crops often deposit their eggs on the foliage of the middle to upper parts of the plant (Terry *et al.* 1987; Sappington *et al.* 2001; R.S.P. pers. obs.) although often on the undersides of leaves. Placing the eggs on the top of leaves was done to facilitate observation. Neussly and Sterling (1994) found no differences in predation on *H. zea* eggs between the upper and lower leaf surfaces in cotton in central Texas. *H. zea* and *S. exigua* eggs typically take 2.5 d or more to develop in the field and would be available to predators throughout this time (R.S.P. pers. obs.).

Egg groups were observed at three-hour intervals (6:00 PM, 9:00 PM, 12:00 Midnight, 3:00 AM, 6:00 AM, 9:00 AM, 12:00 noon, and 3:00 PM CDT) for the following 24 h. This distribution of sampling times results in four day (9:00 AM, 12:00 Noon, 3:00 PM and 6:00 PM) and four night samples (9:00 PM, 12:00 Midnight, 3:00 AM, and 6:00 AM CDT). Sunrise occurred as the 6:00 AM sample was being finished and sunset occurred just before the 9:00 PM sample was initiated, allowing for equal numbers of day and night samples despite a photophase lasting about 14 h. At each observation period, predators observed feeding on the eggs were identified or collected for subsequent identification. All observations of predation could be assigned to day (9:00 AM, 12:00 Noon, 3:00 PM and 6:00 PM) or night (9:00 PM, 12:00 Midnight, 3:00 AM, and 6:00 AM). Eggs of each species were replaced when all eggs on the sheet had been consumed allowing accurate estimation of egg mortality (24 h). *H. zea* eggs were counted at each 3 h period to allow for accurate estimation of mortality for this species at shorter time intervals (3h, or day vs. night). Evaluations of predation were conducted from 8 to12 times per year from 2001-2004. Observations were initiated in late April/ early May and continued at two- to four-week intervals through late August. Not all crops were sampled on each date because of differences in crop development and senescence or the

focus of a particular experiment. Cotton was the focus of several of the studies from which data was obtained therefore sample sizes for cotton are larger than those for corn and soybean. The null hypothesis entering the study was that there would be no difference in the frequency of observed predation events or predator complexes between day and night.

RESULTS

Egg mortality was consistently high, although this varied both within and between seasons with no obvious, consistent pattern. Predation rates on any one date (24h) ranged from 30 to 87% in cotton, 35 to 99% in corn, and 25 to 89% in soybean from 2001 - 2003. On dates where all three crops were evaluated predation was often highest in corn, followed typically by cotton and with soybean having a slightly lower rate of predation. Seasonal mean predation rates were similar between day and night for all crops in 2002 and soybean in 2003. However, mortality due to predation in cotton was significantly higher during the night in 2003 than during the day (Fig. 1).

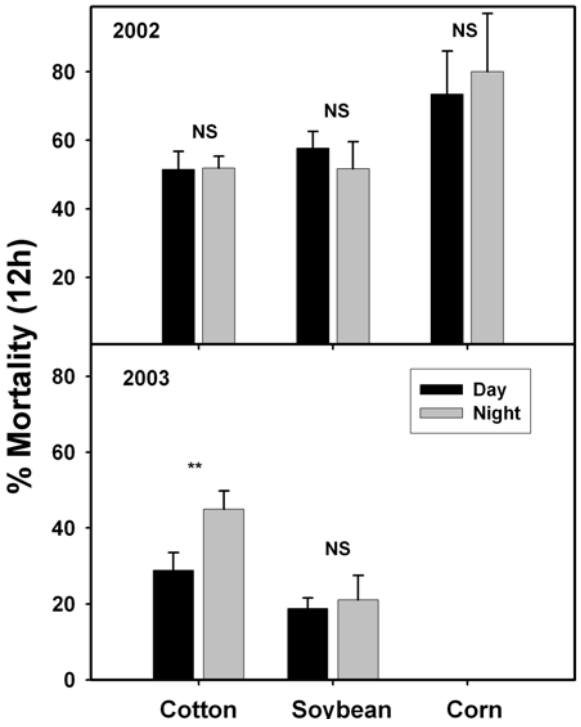

Figure 1. Diel Predation of *H. zea* eggs in cotton, soybean and corn in 2002 and 2003. Data are presented as mean percentage of eggs consumed per 12 h (Day vs. Night) ± SE. Because eggs were replaced when consumed, summed 12-h predation rates exceed 24-h predation rates. Means are compared using a Paired t-test of arcsin(Square Root[X]) transformed proportion data; significance (**) is $P \leq 0.05$; NS = Not Significant $P > 0.05$.

For all crops except cotton in 2003, there was no apparent pattern to the variation in predation by diel period and it appears that the relative contribution of diurnal vs. nocturnal predation balanced out through the season. In 2003, when nocturnal predation was higher, it was consistently higher throughout the season. In 2002, there was no obvious pattern to the variation in diel predation (Fig. 2.)

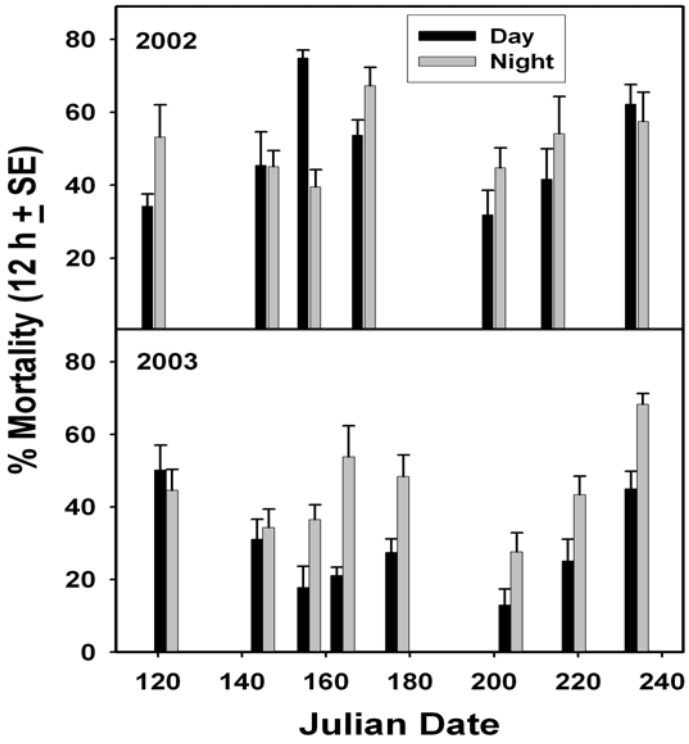

Figure 2. Diel Predation of *H. zea* eggs in cotton by date for 2002 and 2003. Data are presented as mean percentage of eggs consumed per 12 h (Day vs. Night) ± SE.

Predation on eggs was observed >1500 times during these studies. The percentage of observations of nocturnal predation relative to the total in each crop was 72% in cotton (n=1228), 52% corn (n=142), and 42% in soybean (n=195). The predators responsible for the observed predation varied between crop and between diel period (Table 1). Only two predators made up more than 5% of the predators observed feeding in both the day and night periods in any crop. These were the formicids (predominantly *Solenopsis invicta* Buren) and the omnivorous mirid, *Pseudatomoscelis seriatus* (Reuter), which made up > 5% of the observed predation events in cotton during both day and night. In corn, the formicids were also observed during day and night. Both of these predators are most active at night, but will continue to forage during the day. The 11 other predators that contributed >5% of the observed predation events in either diel period were primarily observed during the day or night, but not both. In soybean, there was no overlap in the predators responsible for >5% of the observed predation in either diel period.

Table 1. Predators most frequently observed feeding on *H. zea* and *S. exigua* eggs in cotton, corn and soybean during 2001-2004. For each crop, the predators are ranked by the most frequently observed during day or night; predators with observations constituting < 5% of the total are not reported. Data are presented as the proportion of observations attributable to a predator taxon during each diel period in each crop.

Crop	Rank	Day		Night	
		Predator Taxa	**% of Observed**	**Predator Taxa**	**% of Observed**
Cotton	1	*Geocoris* spp.	24.5	Cursorial spiders	23.7
	2	Formicidae	19.9	Mites	12.1
	3	*Pseudatomoscelis seriatus*	15.0	Formicidae	11.3
	4	*Hippodamia convergens*	9.5	*Pseudatomoscelis seriatus*	9.7
	5	*Collops* sp.	7.8		
Corn	1	*Coleomegilla maculata*	35.3	Formicidae	50.0
	2	Formicidae	25.0	Elateridae	10.8
	3	*Orius* spp.	19.1	Cursorial spiders	8.1
	4	*Hippodamia convergens*	10.3	Dermaptera	5.4
Soybean	1	*Geocoris* spp.	53.0	Cursorial Spiders	28.4
	2	*Collops* sp.	21.0	Formicidae	13.7
	3	*Coleomegilla maculata*	6.0	Nabidae	10.5
	4			Dermaptera	10.5
	5			Elateridae	10.5

Cursorial spiders and ants were consistently among the most important nocturnal predators of lepidopteran eggs in all crops. The cursorial spider complex was dominated by 4 species; the anyphaenids *Hibana futilis* (Banks) and *Hibana arunda* Platnick, the lyniphiid *Grammonota texana* (Banks) and the miturgid *Cheiracanthium inclusum* (Hentz). Two species of geocorids, *Georcoris lividipennis* Stål and *Geocoris punctipes* Say, were the most frequently observed diurnal predator of eggs in soybean and cotton. The coccinellid *Coleomegilla maculata* DeGeer was the most frequently observed diurnal predator in corn.

DISCUSSION

Studies of egg predation in annual crops in the southern USA, particularly cotton and soybean, have yielded consistently high estimated predation rates. McDaniel and Sterling (1982) observed an average of 77% daily predation rates of *Heliothis virescens* (F.) eggs in cotton in central Texas. In another, more detailed study, Neussley and Sterling (1994) demonstrated average total (~ 72 h) predation rates > 80% on *H. zea* eggs. Clearly, predation on lepidopteran eggs in these crops can vary, but frequently is quite high. Despite these and other studies that document the impact of predation, we have discovered only a portion of the predators causing this mortality. Although predation of lepidopterans on cotton and soybean in the USA has been relatively well studied in historical terms, very little information exists on the role of nocturnal predation. Recently, a study by Diaz *et al.* (2004) evaluated nocturnal predation of *S. exigua* eggs in relation to *S. invicta* populations, but they did not include diurnal observations.

There were similar levels of predation intensity during the day and night in 2002. Nocturnal predation was essentially equivalent in importance to diurnal predation in cotton, corn and soybean. In 2003, nocturnal predation in cotton was more than 50% greater than was observed during the day. There was no concurrent increase in nocturnal predation in soybean. At the same time, there was little overlap in the predators that are active during the day in comparison with those nocturnally active (2 taxa out of 12). Few studies have directly addressed nocturnal predation and some that may have detected nocturnal predation using molecular techniques did not control sampling intervals in a way that might have accurately identified the diurnal and nocturnal predator complexes. Studies of predation on *H. zea* eggs in corn and soybean in Kentucky (Pfannenstiel and Yeargan 2002), exhibited similar results to those described here. Predation was high during the day and night and most predatory taxa exhibited activity patterns that were primarily diurnal or nocturnal, not both.

Many arthropod species contributed to the high egg mortality rate. However, daylight observations would have correctly identified only a few of the important predators in these crops. The composition of the predator complex observed feeding on lepidopteran eggs at night was different from that observed during the day. Diurnal observations would not have correctly identified other predator groups such as the cursorial spiders, which appear to be particularly important in south Texas cotton. To accurately characterize the predators attacking a particular pest species, it is critical to carefully investigate predation during nighttime as well as daytime hours. Studies using diurnal visual observations alone would not identify a significant proportion of the important predators. In a previous study, Pfannenstiel and Yeargan (2002) also identified unusual taxa such as phalangids (Opiliones) as common predators of lepidopteran eggs. The important predators identified in this study, particularly the cursorial spiders, should be further evaluated to improve our understanding of their role as biological control agents in these crops and to determine if they can be manipulated to increase their impact.

These studies of the predator complexes feeding on lepidopteran eggs in south Texas, as well as the previous studies by Pfannenstiel and Yeargan (2002), demonstrate that nocturnally active predators are important in several annual crop systems in the southeastern USA. Although cotton and soybean have two of the better characterized predator complexes among cropping systems in the USA, evaluation of nocturnal predator activity is reshaping our perception of the predator complexes attacking lepidopteran pests in these crops. It is critical that future research incorporates greater consideration of the role nocturnally active natural enemies play in biological control of crop pests.

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EXPLORING THE RELATIONSHIP AMONG PREDATOR DIVERSITY, INTRAGUILD PREDATION, AND EFFECTIVE BIOLOGICAL CONTROL

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ABSTRACT

In conservation biological control, we seek to make agricultural systems more hospitable to natural enemies, in an attempt to increase enemy abundance and diversity. However, it is unclear whether the effectiveness of biological control actually increases with growing natural enemy diversity, in communities including many species of generalist predators. Studies have shown that suppression of herbivores may be either enhanced or disrupted by adding predator species to a community, but these studies do not distinguish between the effects of predator diversity and the effects of predator abundance, identity, and composition. Here, we first demonstrate that a diverse community of natural enemies, dominated by generalist predators, attacks the green peach aphid, *Myzus persicae*, in potato fields in Washington State. Second, in a large-scale field experiment, we experimentally isolate the effect of predator diversity on aphid biological control. We show that increasing predator diversity does not affect prey exploitation; overall there is no strong, net complementarity or interference among predators that alters the strength of aphid suppression. However, our experiment revealed strong effects of predator species identity, because predators varied dramatically in their per capita consumption rates. Because of these strong species identity effects, green peach aphid biological control will improve with growing predator diversity, because particularly effective aphid predators will be more likely to be included within diverse communities. However, our results do not suggest any benefits to biological control of natural enemy diversity *per se*.

INTRODUCTION

Agricultural systems frequently display explosive herbivore outbreaks, while less-disturbed natural communities rarely do. This has led to the suggestion by agroecologists that restoring some elements of biodiversity to agricultural systems may improve natural pest control (Pimentel 1961). However, much recent work in the predator ecology literature suggests that increasing natural enemy diversity increases the risk of adding intraguild predators, such that herbivore suppression might actually decline as predator diversity increases (Rosenheim *et al.* 1993; Snyder and Ives 2001; Snyder and Wise 2001). But few experimental studies have

explicitly examined the relationship between predator diversity and herbivore suppression, in part due to the lack of logistically manageable experimental designs for examining interactions within complex (> 2 predator species) natural enemy communities (but see Finke and Denno 2004).

The growing body of biodiversity-ecosystem function ("BEF") literature provides experimental approaches that may be useful to predator ecologists (Ives *et al*. 2005). BEF studies typically have demonstrated that ecosystem function, for example, net productivity for plant communities, improves as species diversity increases (Tilman *et al*. 1996; 1997). However, interactions among multiple trophic levels have almost never been considered in the BEF literature (Duffy 2002; Ives *et al*. 2005; Wilby and Thomas 2002). BEF studies share design traits that are unfamiliar to most predator ecologists: treatment levels are the number of species present, with species drawn from a predetermined pool of possible species, and substitutive rather than additive designs are used, so that the total predator densities are constant across diversity treatments (Ives *et al*. 2005).

We have been studying the community of natural enemies attacking the green peach aphid (*Myzus persicae*) in Washington State potato (*Solanum tuberosum*) fields. Our presentation is in two parts. First, we discuss a compilation of taxonomic surveys within potato fields in Washington, demonstrating this crop's high natural enemy species diversity. Second, we summarize the results of a large-scale field experiment wherein we experimentally constructed natural enemy communities that varied in their natural enemy diversity, and compared the impacts of these communities on green peach aphid.

MATERIALS AND METHODS

DIVERSITY OF NATURAL ENEMIES IN WASHINGTON POTATO FIELDS

In the northwestern United States, insect pests of potatoes have traditionally been controlled using applications of broad-spectrum insecticides (Ruffle and Miller 2003). However, the specter of loss of these chemicals to changes in federal regulations has led some conventional growers to experiment with newer selective pesticides, and organic potato production is growing rapidly in the region. In the 2001-2003 growing seasons we intensively sampled the arthropods in 15 production potato fields under three pest management regimes: conventional fields treated with broad-spectrum pesticides (Hard), conventional fields treated with selective pesticides (Soft), and certified organic fields (Organic) (Koss *et al*. 2005). All fields were within the Columbia Basin of Washington State, a desert region where crops are typically grown under center-pivot irrigation. We sampled arthropods using three techniques: D-vac suction sampling, pitfall trapping, and visual searching (Koss *et al*. 2005).

EXPERIMENTAL MANIPULATION OF PREDATOR BIODIVERSITY

We have conducted a series of experiments wherein we adopted, and somewhat modified, a BEF experimental approach to examine the role of natural enemy species diversity in modifying the control of the green peach aphid (Straub and Snyder, in review). Here, we use one of these experiments to demonstrate our experimental approach and representative results.

In a large-scale field experiment, we experimentally created communities of natural enemies that varied in diversity (either 1 or 3 natural enemy species present), while keeping total predator density constant, and compared the abilities of these communities to control aphids. Our experimental arenas were large, 2m x 2m x 2m field cages, in the field enclosing 4 large potato plants. Cages were first de-faunated using a D-vac suction sampler followed by extensive hand-removal, after which aphids and then predators (according to diversity treatments as described below) were re-added; we then followed the impact of these predator manipulations on aphid population dynamics through time (Straub and Snyder, in review).

In this experiment our species pool included the following five taxa: the predatory bugs *Nabis* spp. and *Geocoris* spp. bug, *Coccinella* and *Harpalus* spp. beetles, and the spider *Misumenops lepidus*. The diversity of taxa (called species here for simplicity) in this predator community has the potential to enhance or disrupt green peach aphid biological control. The considerable variation in foraging behavior among these predators could lead to complementary resource-use and thus a positive relationship between predator diversity and aphid suppression (Ives *et al*. 2005; Wilby and Thomas 2002). However, intraguild predation is also common among these taxa (Brodeur and Rosenheim 2001; Raymond 2000; Snyder and Wise 2001). Such intraguild predation has the potential to lead to a negative relationship between predator diversity and aphid suppression (Finke and Denno 2004; Polis *et al*. 1989; Rosenheim *et al*. 1995). Thus, we had no *a priori* expectations regarding the value of predator diversity in this system. Each of these natural enemy species was present in monoculture, each replicated four times, together comprising the Low Diversity treatment. The High Diversity treatment included 3 predator species, with each of the ten unique combinations of 3 taxa from the pool of 5 replicated once. Thus, our experiment was designed to minimize any influence of species identity, and to isolate any influence of predator species diversity *per se* upon aphid control (Ives *et al*. 2005; Straub and Snyder in review). Ten No Predator control cages were also included, for a total of 40 cages across the experiment. Aphid densities were recorded at 0, 5, and 10 days following predator release.

RESULTS

DIVERSITY OF NATURAL ENEMIES IN WASHINGTON POTATO FIELDS

Geocoris spp. and *Nabis* spp. bugs, and web building tetragnathid and linyphiid spiders, were the most abundant predators in plant foliage, and ground beetles and linyphiid spiders dominated the community on the ground (Table 1). At least 3 parasitoids were common (Table 1). Determining the total number of species that exist in highly disturbed systems like potato fields is difficult. Many species occurring in these fields are immigrants that move in from surrounding vegetation (Wissinger 1997). Rather than attempting to compile and compare complete species lists for fields under each management regime, we took the approach of comparing predator biodiversity using functional groups of taxonomically related species to examine one component of biodiversity, equitability, in our field samples. Overall, equitability scores did not consistently differ between fields receiving hard or soft pesticides, or those under organic management ($P > 0.5$). However, there was a great deal of variability between individual fields (Fig. 1). Some fields had fairly even species distributions, while others were strongly biased towards certain taxa.

Table 1. Common natural enemy taxon groups in Washington potato fields. Data are presented as overall relative abundance of predators, pooling fields across Soft, Hard and Organic management regimes.

Taxon	Common Name	%*	Notes	Functional Group?
In D-vac samples of the foliage (data from Koss 2003)				
Geocoris spp.	Big-eyed bugs	44	*Geocoris* spp. are active hunters with good vision. They are primarily insectivores, but also do some plant feeding. Adults ca. 5 mm in length.	Foliar Active
Nabis spp.	Damsel bugs	7	*Nabis* spp. are also active hunters in the foliage, that like *Geocoris* will do some plant feeding. Adults can be over 1 cm in length.	Foliar Active
Linyphiidae	Sheet web spiders	20	These are tiny spiders (<5mm in length) that build webs to trap prey on the soil surface and lower in the plant canopy.	Trapping
Tetragnathidae	Long-jawed spiders	11	Larger (> 1 cm adult length) spiders that use webs, constructed in the foliage, to capture prey.	Trapping
Other	NA	Each <5	Predatory flies; Orius bugs; lacewings; coccinellid, staphylinid, and carabid beetles; mantids; other spiders. None > 5% of the total.	
In pitfall trap samples (data from Koss 2003)				
Bembidion spp.	Sm. ground beetle	38.5	*Bembidion* spp. are smaller ground beetles (< 1 cm adult length) active hunters, often diurnal, and sometimes observed in plant foliage	Ground Active
Harpalus fraternus	Lg. ground beetle	16.0	*H. fraternus* is a larger ground beetle (> 1.5 cm adult length), and active hunter that is usually nocturnal. Less frequently in foliage?	Ground Active
Linyphiidae	Sheet webspiders	13.1	see above	Trapping
Other	NA	32.4	Other carabid spp., staphylinid beetles; other spiders. No single taxon made up greater than 5% of the total.	
In collections of parasitoids emerging from field-collected M. persicae (Data from Pike 2002)				
Aphidius matricariae	none	61.3	Solitary koinobiont, attacks nymph (pref. 3rd instar), emerges from adult or last instar nymph host	Parasitoid
A. ervi	none	15.3	"	Parasitoid
Diaeretiella rapae	none	12.7	"	Parasitoid
Other parasitoids	NA	10.7	A diverse group of other parasitoids; no single taxon > 5% of the total.	Parasitoid

* percentages are proportion of total predator community that taxon represents, across treatments and fields.

EXPERIMENTAL MANIPULATION OF PREDATOR BIODIVERSITY

We measured predator diversity and abundance at the end of the experiment and found that it had changed little. The High diversity treatment remained more species-rich and more diverse than the Low diversity treatment (richness, $t_{28} = 2.544$, $P < 0.05$; Simpson's diversity index, $t_{28} = 2.735$, $P < 0.05$; Straub and Snyder, in review). There was no difference in predator abundance between diversity treatments ($t_{28} = 0.886$, $P > 0.10$), suggesting that overall rates of predator interference were not different under Low versus High predator diversity (Straub and Snyder, in review). There was no evidence that predator diversity impacted aphid suppression: aphid densities were consistently lower in treatments including predators, compared to No Predator controls [Predator addition (High diversity + Low diversity) vs. Control; Exp 1: $F_{1,38} = 10.442$, $P < 0.01$], but aphid densities were indistinguishable in Low and High diversity cages (treatment x time Wilks' lambda = 0.846, $F_{2,27} = 2.454$, $P > 0.10$; diversity $F_{1,28} = 1.542$, $P > 0.10$) (Straub and Snyder, in review). We then asked if species identity might be a better predictor of herbivore suppression. Using Paine's interaction strength index (Paine 1992) to quantify the per-capita impact of predators, we found that species identity had a strong effect on herbivore suppression ($F_{4,15} = 7.028$, $P < 0.01$), with *Coccinella* beetles provided stronger, and thomisid spiders weaker, suppression than in the High diversity treatment (Straub and Snyder, in review).

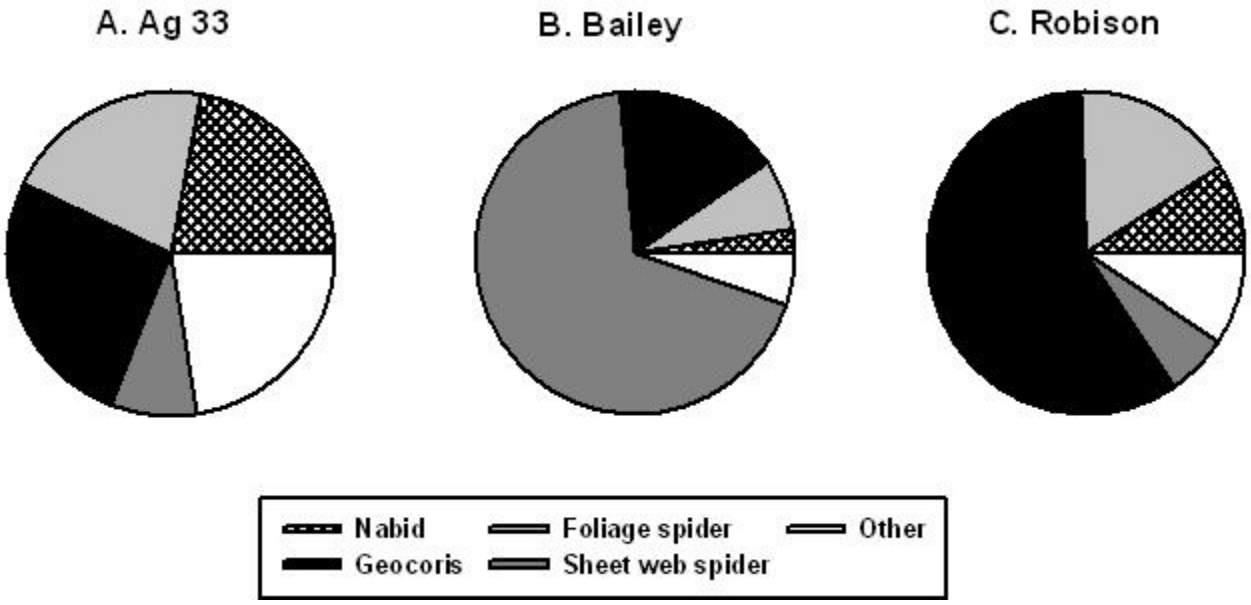

Figure 1. Predator community makeup in three fields in 2001. A) Ag 33 was a Hard field; B) Bailey was a Soft field; and C) Robison was a Hard field. These fields represent the three equitability patterns we saw in the field. Ag 33 has a high equitability score, because the major taxa are similar in abundance. Bailey and Robison have uneven taxa abundances, yielding low equitability scores. However, while their equitability scores are similar, species composition is not – Bailey is dominated by the Trapping functional group, while Robison is dominated by Foliar Active predators.

DISCUSSION

The community of natural enemies attacking green peach aphid locally is diverse, including many species of generalist (and thus likely intraguild) predators. Our predator community includes >20 common species. Therefore, functional diversity of the type necessary for species complementarity, and thus a positive relationship between natural enemy diversity and more complete resource exploitation (Naeem and Wright 2003), likely is present in this community. However, despite our attempts to include representative functional diversity within our field experiment, we found that varying predator diversity *per se* had no effect on the strength of aphid control. It is perhaps surprising that there was no evidence for species complementarity leading to an increase in the strength of herbivore suppression. Our predator species were chosen to span a range of hunting strategies, including active hunters in the foliage, active hunters on the ground, and sit-and-wait hunters in the foliage. Also, we intentionally included one pair of taxa, *Coccinella* and *Harpalus*, which constitute one of the best-documented cases of predator facilitation in a terrestrial system (Losey and Denno 1998). Nonetheless, we recorded no increase in the efficiency of aphid exploitation by more species-diverse natural enemy communities.

Intraguild predation also appeared to be a weak force in our experiment. This result appears in stark contrast to experiments that have shown strong disruptive effects of intraguild predation on herbivore suppression (e.g., Finke and Denno 2004; Rosenheim *et al*. 1993; Snyder and Ives 2001). The lack of intraguild predation in this study is unlikely to be entirely due to an inherent reticence towards intraguild predation within our communities, as many of the included taxa have been shown to feed on one another. For example, *Nabis* and *Geocoris* feed on one another (Raymond 2000), *Harpalus* eats *Nabis* (Snyder and Wise 2001), and most of the predators feed on parasitoids (Brodeur and Rosenheim 2000). One interesting explanation for the difference between the results of this and other studies is that we used a substitutive, rather than an additive, experimental design. Additive designs have often shown strong, disruptive intraguild predation among species (Finke and Denno 2004; Rosenheim *et a*l. 1993; Snyder and Ives 2001). However, compared with substitutive designs, additive designs may deflate intraspecific interference and inflate interspecific interference. This is because, in additive designs, predator encounter rates and competition for prey will be higher in treatments including multiple predator species because these treatments also include higher overall predator densities. Regardless, our results suggest that greater predator diversity does not generally weaken pest suppression, as might be surmised from the numerous studies emphasizing the negative effects of predator interference among species (e.g., those reviewed in Polis *et al*. 1989; Rosenheim *et al*. 1995). This is good news given that sustainable agricultural practices such as organic farming often lead to greater on-farm diversity (Hole *et al*. 2005).

The finding that predator identity is a better determinant of pest suppression than predator diversity also has implications for biological control. It implies that, for the biological control of any one pest species, conservation strategies that target particularly effective predator species will be more effective than those targeting predator diversity more broadly. This result supports the common-sense view that conservation biological control practitioners should strive to identify and manage for "the right kind of diversity", rather than managing for greater biodiversity itself (Landis *et al*. 2000).

In summary, our results suggest that predator diversity *per se* has little effect on the strength of aphid suppression. This result is in contrast with BEF work at other trophic levels, which has consistently revealed a positive relationship between rising consumer biodiversity and the efficiency of resource utilization (Cardinale *et al*. 2002; Naeem and Wright 2003; Tilman *et al*. 2001). Thus, pest suppression may be less sensitive than other ecosystem services to biodiversity loss, provided that key predator species are conserved.

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ARTIFICIAL DIET FOR REARING *TRICHOGRAMMA* WASPS (HYMENOPTERA: TRICHOGRAMMATIDAE) WITH EMPHASIS ON PROTEIN UTILIZATION

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ABSTRACT

Trichogramma wasps are tiny hymenopterous egg parasitoids widely used in biological control programs worldwide. The huge quantities of insects necessary for inundative releases are mainly produced on factitious hosts like *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae), or on silkworms. In order to simplify production, increase its flexibility, and potentially reduce cost, studies on artificial media for the development of the parasitoids have been ongoing for many years. Some successes were obtained, mainly with artificial media containing insect extracts such as pupal hemolymph from Lepidoptera. To define new artificial media devoid of insect components or improve the performances of existing ones, a better knowledge of parasitoid nutrition would be useful. Proteins are key components in artificial media, and research was conducted on *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) to better understand the nutritional value of proteins by investigating to what degree they are assimilated by the insect.

A method was developed for studying the assimilation of these nutrients by the preimaginal stages of *T. pretiosum* based on adding a mixture of free 14C-radiolabelled amino acids to the medium to be tested. The basic composition of the medium already included proteins, and proteins to be tested were also added. Amino acid analyses were performed on medium (for free and protein amino acids) and on *T. pretiosum* grown in the medium (for protein amino acids). For each radiolabelled amino acid, comparison of the specific activity in total amino acids in *T. pretiosum* pupae with the specific activity in free and protein amino acids in the medium, allowed us to determine the degree and the means by which the protein was utilized.

We showed that the proteins included in the hemolymph-based medium, as well as casein added at final concentrations of 1.6 or 3.2 %, were completely assimilated. This protein, incorporated into the hemolymph-based medium to increase its protein content, led to im-

proved body composition and some development parameters of *T. pretiosum*. Even media containing hemolymph could be improved by protein addition because of the relatively low content of proteins in the hemolymph. The addition of 3.2% casein increased the protein content of *T. pretiosum* pupae by 25% and normal adult emergence yield by 40%.

INTRODUCTION

Oophagous Hymenoptera of the genus *Trichogramma* are used in many countries in biological control programs to regulate pest populations (mainly lepidopteran species) (Li 1994; Parra and Zucchi 2004). These parasitoids are generally reared in factitious host eggs, the most common belonging to Lepidoptera like *Ephestia kuehniella* Zeller, *Corcyra cephalonica* (Stainton) (Pyralidae), *Sitotroga cerealella* (Olivier) (Gelechiidae) or silkworms, but their multiplication on a large scale remains expensive. This limitation to their use can be overcome by the possibility of artificial rearing systems. Studies have been conducted in different countries on *in vitro* rearing of egg parasitoids for many years. Presently, different kinds of artificial media are available enabling immature development of many species of *Trichogramma*. The best results have been obtained with media mainly composed of insect-derived elements such as hemolymph, body, or egg juices, but media without insect additives have also been tested with some success (Consoli and Parra 1997; Grenier 1994; Grenier *et al.* 1995; Thompson 1999; Thompson and Hagen 1999). In these latter media, one of the main concerns is protein supply, and this is true even with artificial media containing insect hemolymph, which is usually poor in protein content compared to lepidopteran eggs.

This work was conducted in order to define artificial diets for *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) that are more suitable for the development of this oophagous parasitoid, based upon a better knowledge of the nutritional value of proteins and of their utilisation by larvae. The assimilation of the proteins was evaluated by adding a mixture of radiolabelled amino acids to the medium. In addition, a hemolymph-based medium, also supplemented with proteins, was tested for *Trichogramma* development. Assimilation and development tests were performed with the basic medium and with casein supplementations.

MATERIALS AND METHODS

Stock cultures of a thelytokous strain of *T. pretiosum* originating from Uruguay, were maintained on *E. kuehniella* eggs killed by UV irradiation. Adults were fed on a diluted honey solution (30% in water). For experiments, rearing was conducted in 1 litre-glass jars (10 cm diameter, 16 cm high) with the proportion of one female for 10 host eggs glued on cardboard. Climatic conditions were 23 ± 0.5 °C, 75 ± 5 % R.H., and a 16:8 h light-dark regime.

The method of investigation was based on the adding of a mix of 14C-labelled amino acids (aa) to the artificial medium in which the *Trichogramma* larvae were grown. The specific activity of each labelled aa is defined as the radioactive activity of the aa in counts per min / mg (cpm/mg) divided by the concentration of the aa in nmol/ mg. We analyzed free and protein aa in the medium, but only the protein aa in the insect body, considering that i) if the

Trichogramma larvae do not digest and assimilate the proteins in the medium (utilization of free aa only), the specific activity of the aa in the insect body will be the same as the specific activity of the free aa of the medium, ii) if the *Trichogramma* larvae completely digest and assimilate the proteins in the medium, the specific activity of the aa in the insect body will be the same as the specific activity of the total aa of the medium, iii) if the *Trichogramma* larvae partly digest and assimilate the proteins in the medium, the specific activity of the aa in the insect body will be intermediate between the specific activity of the free and total aa of the medium.

Artificial host eggs made of a polyethylene film (15 µm thick) in the form of hemispherical cupules were filled with artificial medium (about 5 µl) used as the diet for larval development. Each rearing device contained 30 cupules arranged as a 6 x 5 matrix. The experiments were conducted under aseptic conditions as described earlier (Grenier 1994; Grenier and Liu 1990; Grenier *et al.* 2002). Climatic conditions were the same as for the stock culture.

The basic artificial medium contained pupal hemolymph from *Mamestra brassicae* L. (Lepidoptera: Noctuidae) (40%), hen's egg yolk (20%), semi-skimmed cow's milk (20%), Neisenheimer salt solution (10%) and distilled water (10%). Besides this medium, two other media enriched with casein (BDH) at two concentrations (final concentrations in the medium of 1.6 or 3.2%) were used for investigating protein assimilation.

The experimental process consisted of incorporating into the media a radiolabelled aa solution of a 14C-protein hydrolysate containing Ala, Arg, Asp, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Tyr, and Val (Sigma). This labelled medium was distributed in 4 out of 30 cupules of each matrix, the remaining cupules being filled with the same medium without radiolabelled aa. Analyses were performed on the medium (free and total aa). *T. pretiosum* females were allowed to lay eggs for 24 hours. After the larvae had completed development, the pupae were analysed for total aa. Each experimental condition was replicated three times.

For total aa analysis of media and pupae, all samples were hydrolysed under nitrogen in HCl vapour at 120°C for 24 hours using a Pico-Tag work station (Waters, St. Quentin Les Yvelines, France). Along with 2-(beta)-mercaptoethanol (4%) to preserve sulphur-containing aa, 200 µl of 6N HCl were placed in the hydrolysis tank. After hydrolysis, 10 nmol of glucosaminic acid per mg of sample were added as an internal standard. The samples were dried under vacuum in a Speedvac apparatus (Savant Instrument Inc., Farmingdale, New York) and taken up with 0.05 M lithium-citrate buffer (pH 2.2). Samples were submitted to ion exchange chromatography in an automatic amino acid analyser (Beckman 6300, Roissy, France). Amino acids were detected by the ninhydrin reaction, identified by their retention time and wavelength ratio, and quantified by their absorption at 570 nm (440 nm for proline). For each condition, 3 to 5 replicates were analysed. Free aa of media were analysed by the same procedure without hydrolysis, but after precipitation of the proteins by TCA (trichloro acetic acid, final concentration 5%) followed by the elimination of TCA and lipids by chloroform extraction. Again, 3 to 5 replicates were analysed.

Biological (parasitism, adult emergence rate, normal adult rate) and biochemical data (pupal body composition in aa) of *Trichogramma* reared in the different media were compared. The diets were prepared and the experiments were performed as described above, but

no radiolabelled aa were added. The degree of parasitism was measured by the number of eggs laid per cupule. The percentage of emergence was evaluated by dividing the number of cupules per box producing adults by the total number of cupules x 100. The percentage of normal adults was calculated by dividing the number of adults with normal wings and abdomen by the total number of adults per box x 100. The compositions in aa were expressed in nmol/mg of fresh pupal weight.

RESULTS AND DISCUSSION

ASSIMILATION

The quantity of labelled aa represented 1% of the quantity of the free amino acids in the medium, and thus was not intended to modify the original balance in aa. The external contamination of the pupae grown in labelled medium, checked by washing them several times, was negligible. The feces, rejected just after the emergence by the adults obtained from *E. kuehniella* eggs, were collected on a glass tube and analysed for aa presence. They contained mainly ammonium and only very small quantities of aa (0.52 nmol / *Trichogramma* vs. 10-30 for body content according to their size and consumed food).

In the three media, the specific activity for all free aa was quite high (up to 15000 cpm/nmol), while the specific activity for total (free and protein) aa in the pupal body was lower (less than 1000 cpm/nmol) (Fig. 1). The specific activities of the aa in the pupal body were quite similar to the specific activity of the total aa of the medium for most of the amino acids, mainly essential ones. The lower amounts of labelled total aa observed in pupal body compared to media, for some aa (Thr, Ser, Glu, Gly, and mainly Pro and Ala) could be explained by the importance of the intermediate metabolism in which these energetic aa are implicated. These differences were greater in control medium and lower in medium with 3.2% of casein, showing a better efficiency of protein utilisation in the latter medium. For essential basic aa (Lys, His, Arg), the proteins did not seem to be completely assimilated, because the values for total aa content of pupae were slightly higher than those for the media. Nevertheless, the differences were very small.

In the control medium as well as in media with casein added, all the proteins present were almost completely digested and assimilated. Subsequently, the effect of adding casein was tested on biological and biochemical parameters.

DEVELOPMENT IN MEDIA

Female wasps readily laid eggs inside artificial host eggs (Fig. 2). The parasitization rate, measured as the mean number of eggs laid per artificial host egg (cupule) was not significantly modified when 1.6% casein was added to the control medium (139.1 vs. 146.9), but was significantly reduced with 3.2% casein (111.6). Free aa are usually known as egg laying stimulants (Xie *et al.* 1991), thus this lower parasitization rate was possibly due to a reduction of the relative concentration in aa resulting from the addition of pure casein. Larvae successfully developed and after excreting a black substance turned into pupae (Fig. 2).

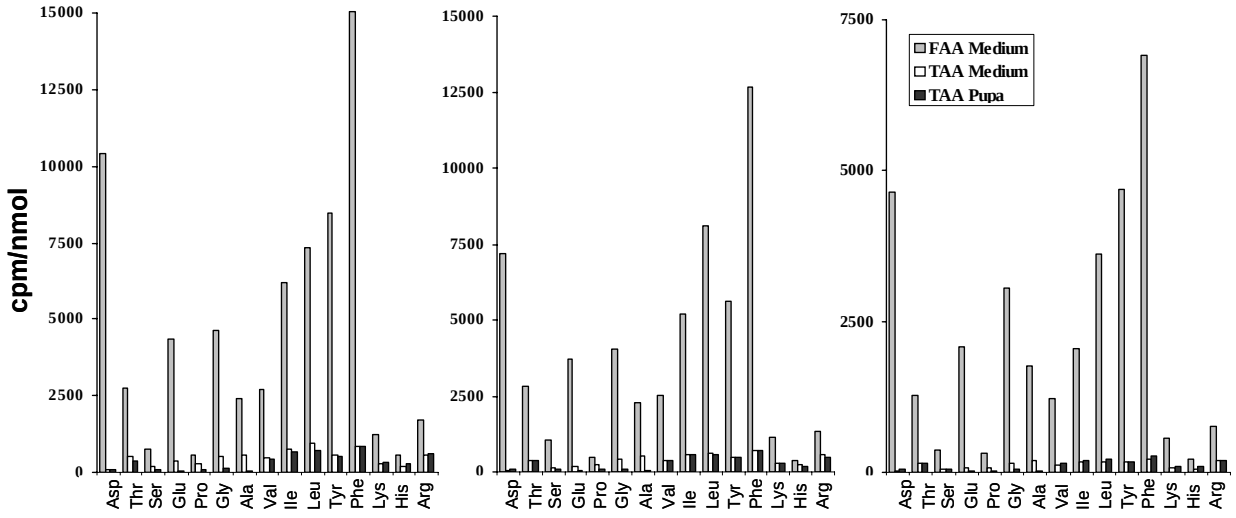

Figure 1. Specific activities (cpm/nmol) of free (FAA Medium) and total amino acids (TAA Medium) in the basic artificial medium as control, in this basic medium supplemented with 1.6 or 3.2% of casein, and of the total amino acids (TAA Pupa) in *Trichogramma pretiosum* pupae grown in these three media.

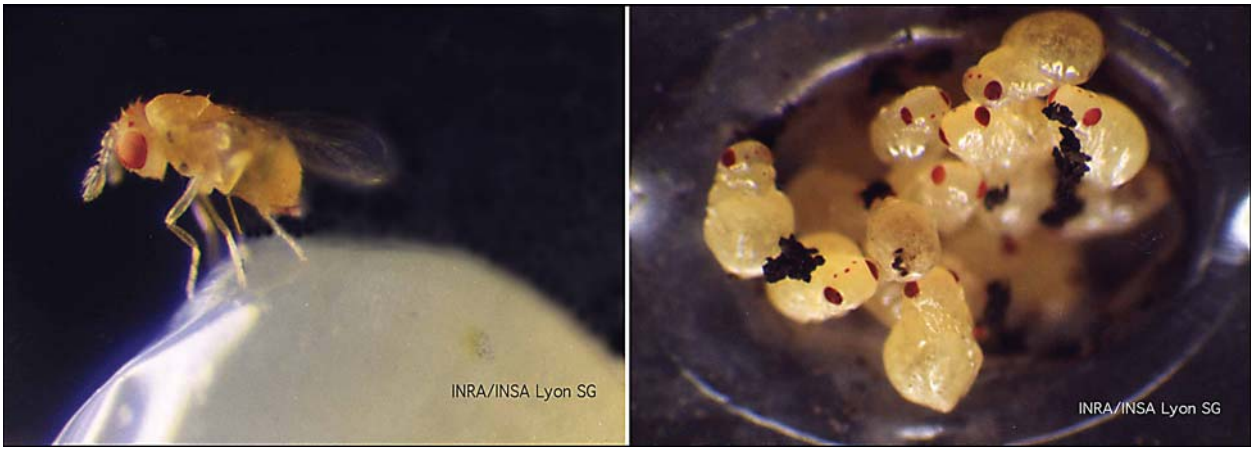

Figure 2. *Trichogramma* female laying eggs inside an artificial host egg (left); *Trichogramma* pupae grown in an artificial host egg (right). Photos: INRA/INSA de Lyon, Simon Grenier. UGA1390018, UGA1390019

Adult production (emergence rate or normal adult rate) was increased when casein was added either at 1.6 or 3.2% (Fig. 3). The lower emergence rate with 3.2% casein compared to 1.6% casein could be explained by the lower parasitization observed in the 3.2% casein medium: if the number of larvae in a cupule is too low, the larvae will become bloated and no further development can occur (Grenier *et al.* 1995). The percentage of normal adults was the highest in medium with 3.2% casein, probably in correlation with a higher amount in aa content of the pupae. The total aa content was 672.3 ± 38.0, 729.3 ± 28.0, and 839.6 ±36.4 nmol/mg for pupae grown in basic medium, and in medium with 1.6% or 3.2% of casein, respectively. The highest value for total aa content of pupae obtained in medium with 3.2% casein was lower than the control values obtained with pupae grown in *E. kuehniella* eggs (88.7 vs. 118.4 expressed in ng/µg), and also than the value (128.1 ng/µg) found for *Trichogramma dendrolimi* Matsumura grown in *E. kuehniella* eggs (Grenier *et al.* 1995).

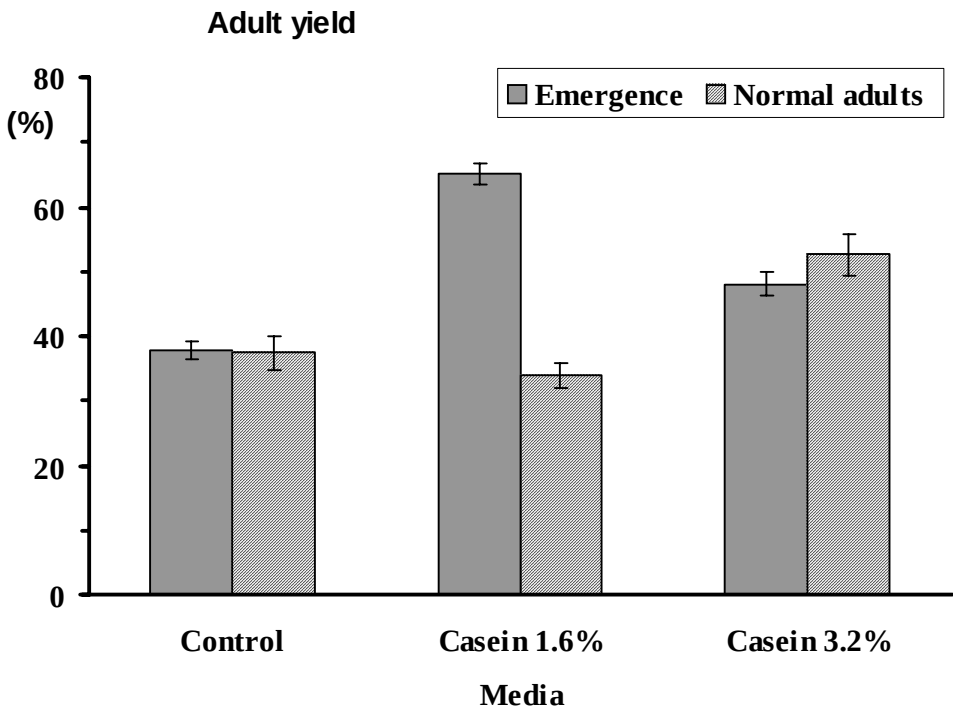

Figure 3. Percentages of emergence and normal adult rates of *Trichogramma pretiosum* on the basic artificial medium, and on the basic artificial medium supplemented with 1.6 or 3.2% casein. Means are given with their SE.

CONCLUSIONS

It was demonstrated that the principle of studying the assimilation rate of proteins can be applied successfully to tiny endoparasitoid insects such as *Trichogramma* species (pupal weight around 30 µg). The results revealed a complete utilisation of the proteins for essential aa, and showed the high level of implication in intermediate metabolism for the other aa.

The methodology, although quite complex and difficult to perform, was shown to be efficient. Through several experiments it appeared that the *Trichogramma* larvae completely assimilate all the proteins present inside the basic medium. Also the casein added into the medium was completely assimilated at the tested concentrations of 1.6 or 3.2%. Thus, casein could be used in artificial media to increase the protein content and improve the performance of the basic medium.

For further experiments, different proteins should be tested at various concentrations to enlarge the spectrum of the components to be used in artificial media. Experiments using this method could also be conducted on *Trichogramma* strains harbouring or not *Wolbachia*, a symbiont inducing thelytokous parthenogenesis in *Trichogramma*, to elucidate the possible role of this symbiotic rickettsia in the digestive physiology of the host. Artificial media could be used not only for production purposes, but also as a powerful tool to study the physiology of immature parasitoids, particularly endoparasitoids, by simplification of their environment (Grenier 2000), as shown again in this study.

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LARGE-SCALE AUGMENTATIVE BIOLOGICAL CONTROL OF ASIAN CORN BORER USING *TRICHOGRAMMA* IN CHINA: A SUCCESS STORY

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ABSTRACT

Asian corn borer, *Ostrinia furnacalis* (Guenée), is the most destructive pest of corn in China. It causes 6 to 9 million ton yield loss in an average year. Biological control using releases of *Trichogramma* has increased greatly since *T. dendrolimi* Matsumura can be successfully mass reared on eggs of the Chinese oak silkworm, *Antheraea pernyi* Guérin-Méneville. The process and technique for mass production and releasing of *Trichogramma* has been greatly improved in recent years. A series of machines and devices for mass rearing the *Trichogramma* with the eggs of oak silkworm has been developed, which promote *Trichogramma* production and make application for control of the Asian corn borer more practical and efficient. Asian corn borer control by release of *T. dendrolimi* on a large scale has been the key pest management technique in North China. In *T. dendrolimi* release areas parasitism of corn borer eggs ranged from 73.4% to 87.8%, with a 92.5% decrease of stalk-boring. Overall, augmentative releases have been made on 4.1 million ha of corn from 1990 to 2002 in Jilin Province with good pest control effects. *T. dendrolimi* and *T. chilonis* have been successfully produced by means of artificial host eggs and releases of these have had similar effects to the same species reared from factitious host eggs. Field application techniques also have been greatly improved. Large ecological and economic benefits have been achieved in the area where *Trichogramma* have been released continuously for many years. In Miyun County of Beijing, where *Trichogramma* have been released for more than 20 years, populations of natural enemies in cornfields have increased, which allow natural control of other insect pests without application of pesticides. Parasitism due to natural *Trichogramma* increased from 1% and 79.3% in 1980 to 33% and 92% in 1991 for first and third generation of Asian corn borer eggs, respectively. In Gongzhuling City, Jilin Province, the mean borer holes and number of larvae per hundred stalks decreased by 73.66% and 75.93%, respectively, where the *Trichogramma* were released from 1990 to 1996. In recent years *Trichogramma* releases for control of Asian corn borer cover 1 to 1.3 million ha annually, and have become one of the key techniques for IPM of corn pests in China.

INTRODUCTION

Corn (*Zea mays* L.) is playing a very important role in grain production in China. Among the grain crops grown in China, corn ranks second after rice in planting area, total yield and average yield. The average annual planting area is 24 million ha, total yield is 125 million ton, the average yield was 4,839 ton/ha. China is also the second largest corn production country in the world. The Asian corn borer, *Ostrinia furnacalis* (Guenée), is distributed in East and Southeast Asian countries, such as China, Japan, Korea, Thailand, The Philippines, Indonesia, Malaysia, and some islands in the Pacific Ocean (Nafus and Schreiner 1991). It causes serious damage to corn, sorghum, millet and cotton. It remains the most significant economic insect pest of corn in China. Estimated average annual losses in China due to this insect range from 6 to 9 million tons. These losses can be much greater in an outbreak year (Zhou and He 1995).

In China, the Asian corn borer is distributed in most corn growing areas. It goes through one to seven generations a year from the far northern Heilongjiang Province to the southern Hainan Province, according to different latitudes and elevations (All China Corn Borer Research Group, 1988). Among these, one- to three-generation areas are of greater economic importance, owing to the extensive cultivation of corn in these regions. The generations that occur in whorl stage corn cause more serious direct reduction in yield than those that occur in the silking/pollen-shedding stages (Zhou and He 1995). However, the indirect yield loss caused by the generations occurring in later crop stages is much greater than that in whorl stage because the larvae feed on silk and kernels inducing ear and kernel rot which result in contamination of corn grains by mycotoxins produced by fungi, such as *Aspergillus*, *Fusarium*, and *Penicillium*.

Since the early 1950s, a comprehensive study of utilization of the egg parasitoid *Trichogramma* has been conducted for controlling Asian corn borer. Shandong Academy of Agricultural Sciences successfully produced *Trichogramma dendrolimi* Matsumura on the Chinese oak silkworm, *Antheraea pernyi* Guérin-Méneville in the 1960s (Wang 2001). As the eggs of *A. pernyi* were used as host for mass rearing *Trichogramma* in 1970s, research and application of *Trichogramma* have expanded in China and it has been widely used in the successful biological control of many insect pests, especially the Asian corn borer in North China (Gou 1986).

Since 1983, the Chinese government has funded National IPM Technique Research Projects as one of the State Key Research Programs in four successive State Five-year Plans. Biological Control Technique Research is one of those research projects. Since then, there have been improvements in process and technique for mass production and releasing of *Trichogramma*, especially for the Asian corn borer.

TRICHOGRAMMA SPECIES USED FOR ASIAN CORN BORER IN CHINA

There are 12 *Trichogramma* species identified from parasitized Asian corn borer eggs throughout China. Among them, *T. dendrolimi*, *T. chilonis* Ishii, *T. ostriniae* Pang et Chen, and *T.*

evanescens Westwood are distributed throughout the country, and *T. leucaniae* Pang et Chen, *T. poliae* Nagaraja, *T. closterae* Pang et Chen, *T. pintoi* Voegele, *T. ivelae* Pang et Chen, *T. exiguum* Pinto and Platner, *T. forcipiformis* Zhang and Wang, and *T. tielingensis* Zhang and Wang are distributed in some regions. *T. ostriniae* is the dominant species attacking the Asian corn borer in most corn growing regions of China, comprising from 72.2% to 100% of the *Trichogramma*. However, *T. dendrolimi* comprises 97.3%, 28.9% and 45.1% of the total *Trichogramma* in Heilongjiang, Jilin, and Liaoning Provinces in Northeast China, respectively, and *T. chilonis* comprises 88.9% of all *Trichogramma* in Guizhou Province in Southwest China. *T. ostriniae* accounts for up to 90% of the total parasitized Asian corn borer eggs in Beijing (Zhang *et al.* 1990).

Although *T. ostriniae* is the dominant species attacking Asian corn borer eggs in China and it is more effective for corn borer control than *T. dendrolimi*, the cost for mass rearing *T. ostriniae* is higher and the production efficiency is lower. This species can only be mass reared on small eggs, such as the rice grain moth eggs, *Corcyra cephalonica*, and not use oak silkworm eggs or artificial host eggs. As a result the application of *T. ostriniae* is limited in practice (Feng 1996). *T. dendrolimi* and *T. chilonis* are the two *Trichogramma* species which can be mass reared on the eggs of *A. pernyi* and artificial host eggs. Some field releases showed that *T. chilonis* provided better control for Asian corn borer control in some areas than *T. dendrolimi* (Feng *et al.* 1999; Tan 1999; Wu *et al.* 2001).

In northeastern China, the Chinese oak silkworm is reared on oak tree as sideline occupation in forest regions. The Chinese oak silkworm cocoons are harvested in autumn and transported to biological stations throughout the country, and then stored in cool room for *Trichogramma* mass rearing the following year. The cocoons of oak silkworm can be stored under -5°C for 5 months. In the early summer, the cocoons are hung in the emerging room before mass rearing begins. The eggs squeezed from abdomens of female moths are better for parasitization of *Trichogramma*. These eggs are obtained by squeezing female moth abdomens 1 or 2 days after adult emergence, where upon they are washed and dried. Each female moth can produce 200 eggs and between 50-262 wasps emerge from each egg. A number of 60 per egg is optimal and more than 80% of emerged adult parasitoids are females (Liu *et al.* 2000).

Procedures and equipment for mass production of *T. ostriniae* by using *Sitotroga cereallela* (Olivier) eggs have been developed and *T. ostriniae* will be available for improved Asian corn borer control in the near future (Jia *et al.* 2002; Zheng *et al.* 2003)

IMPROVEMENT OF MASS PRODUCTION OF *TRICHOGRAMMA* USING CHINESE OAK SILKWORM EGGS

Given the large size of an oak silkworm egg, adjustments must be made in the ratio of female wasps to host eggs as well in the exposure time to avoid superparasitism and degeneration. For oak silkworm eggs, the optimum ratio between the number of parent female wasps and host eggs is 2:1. The optimum time of exposure is shorter than 24h. The parasitism of fresh eggs usually reaches 90% (Liu *et al.* 2000).

At least 5 different production components have been developed, which are basically composed of (1) collection of *Trichogramma* from the field that are then cultured on host eggs and reserved as founder population for the following year; (2) selection of host eggs and their storage, and (3) mass propagation (Piao and Yan 1996). The selection of female cocoons which will produce host eggs and treatment of host eggs have been mechanized. A set of machines and devices has been designed, which includes machines for collecting emerged silkworm moths, squeezing of female moths, washing and drying host eggs, preparing egg cards, and parasitizing eggs (Liu *et al.* 1980; Liu *et al.* 1991; Song *et al.* 1994). Equipment for separating immature eggs from mature eggs was also developed. An automatic production line, with the capacity of producing 40 billion *Trichogramma* annually was established in Jilin (Song *et al.* 1994).

The procedure for *Trichogramma* mass production has been simplified. *Trichogramma* spp. are reared simply by the method of releasing wasps in a small empty room with the egg cards on glass windows or on hanging screens (room-rearing). Sometimes the parasitized host eggs (before emergence of *Trichogramma*) are mixed with fresh unparasitized host eggs on wooden trays. When the wasps emerge, they parasitize the host egg directly. Every day in such biological stations 800-1000 million wasps are produced (Liu *et al.* 2000).

To maintain high quality of *Trichogramma* reared on oak silkworm eggs, an instrument for selecting healthy parasitized host eggs was designed. It can distinguish parasitized from unparasitized host eggs, and healthy from infected host eggs based on the elasticity of parasitized host eggs (Wang *et al.* 1999). In addition, the processes of mass production, quality control and field release are standardized in Jilin, Liaoning and Beijing in North China (Piao and Yan 1996).

Technical regulations for *T. dendrolimi* mass production using *A. pernyi* eggs were standardized in 2004 and await approval. This will regulate procedures for *Trichogramma* production and improve the quality of the parasitoid using *A. pernyi* eggs in China.

TRICHOGRAMMA SUCCESSFULLY MASS REARED ON ARTIFICIAL HOST EGGS

Research conducted since 1975 in China has resulted in successful rearing of *Trichogramma* in vitro on artificial host eggs. Breakthroughs have been made on the rearing of *T. dendrolimi* and *T. chilonis* by means of artificial eggs and further research has shown that their efficacies were similar to the same species reared from factitious eggs. Oviposition synergists that improved oviposition by *T. dendrolimi* and *T. chilonis* were selected (Han *et al.* 1994). With the addition of tricosane in a polyvinyl alcohol hydrophilic colloid, parasitism and pupation of *T. dendrolimi* on artificial host eggs reached 100% and 81.25%, respectively (Zhang 1993). The system closest to commercial production is that developed for *T. dendrolimi* on a basis of insect hymolymph. This diet has been packaged in plastic host egg-cards. Mechanized production of *T. dendrolimi* and *T. chilonis* with artificial host eggs has been successful. A model GD-5 automatic machine for mass production of artificial host egg cards was successfully created in 1995, and the technological process of *Trichogramma* produced with artificial host egg cards was developed. A computer controlled machine automatically completes all five

processes for egg production including setting-up the synthetic membrane, forming the "egg shells", injecting the artificial media into the shells, sealing the double-layered membrane, and separating into egg cards. Operating rules for mechanized production of artificial host eggs for *Trichogramma* and techniques for propagating parasitoids, quality control, and releasing were formulated (Dai *et al.* 1996; Liu *et al.* 1996). Two artificial host egg production lines for *Trichogramma* were set up in Guangzhou and Beijing in the late 1990s. The parasitoids from in vitro rearing have been used on more than 150,000 ha with parasitism and control effects equal to parasitoids from natural host eggs (Wang 2001). Field experiments showed that the egg parasitism was 65.44% to 68.16%, when using *T. dendrolim*i and *T. chilonis* reared on artificial host eggs to control summer corn borer. In comparison with chemical control, the percentage of tunnels and broken tassels was reduced by 66.67% to 70.37%, and 73.33% to 80.00%, respectively. *T. chilonis* also showed good control of corn earworm, *Helicoverpa armigera,* on corn with 71.1% control, significantly better than that of *T. dendrolimi* (20% control) (Feng *et al.* 1999). China is the first country to make use of in vitro rearing of *Trichogramma* for commercial production and use on a large scale (Wang 2001).

IMPROVEMENT OF FIELD APPLICATION TECHNIQUES FOR *TRICHOGRAMMA* AND CONTROL EFFICACY

Field application techniques have been greatly improved since the 1980s. Release sites have decreased from 90 to 45 per ha based on the dispersal distance of *T. dendrolimi* in the field. The frequency of release has decreased from 3 to 2 and the number of *Trichogramma* released has increased from 135,000 wasps to 150,000-300,000 wasps/ha. Release timing is determined by monitoring of Asian corn borer pupation rate. When the pupation rate of the overwintering generation is 10%, the first release is made 10 days later. The second release is usually done seven days after the first release. Parasitoid releases have shown consistent levels of 60-85% parasitism, with reductions in damage of 65-92% (Piao and Yan 1996). Meanwhile, long-period egg cards were exploited for corn borer control by mixing different stages of *Trichogramma* development on a card, thereby staggering emergence, so that only one or a few releases need to be made. This approach ensures that there are always some females actively searching throughout host oviposition (Zhang *et al.* 1993).

The mean parasitism of Asian corn borer egg masses was 76% compared with 12% in the uncontrolled area on a 72,400 ha scale trial in 1988 in Yushu City, Jilin Province. The parasitism of corn borer eggs by *T. dendrolimi* ranged from 73.4% to 87.8%, with a 92.5% decrease of the stalk-bore rate (Liu *et al.* 2000). Overall, releases were made in 4.1 million ha of corn from 1990 to 2002 in Jilin Province with good control efficacy. In two-generation areas, additional *Trichogramma* release was needed when the egg masses of the second generation were observed, leading to an average reduction of 46.3-73.6% for the overwintering population of Asian corn borer. The strategy for controlling the Asian corn borer in two-generation areas consists of inundative release for the first generation, and inoculative release for the second generation. This strategy has been exploited on a large-scale in Liaoning Province where it has resulted in sustainable management of Asian corn borer (Cong *et al.* 2000). Where large pest outbreaks occurred chemical insecticide granules and *Bacillus thuringiensis* were applied in the late whorl stage.

Large ecological and economic benefits have been achieved in areas where *Trichogramma* have been released continuously for many years. In Miyun County of Beijing, where *Trichogramma* have been released for more than 20 years, the populations of natural enemies in corn fields have increased. This helps keep other insect pests under control without application of pesticides. Parasitism due to natural *Trichogramma* increased from 1% and 79.3% in 1980 to 33% and 92% in 1991 for first and third generation eggs of the Asian corn borer, respectively (Shi 1996). The number of overwintering larvae was reduced to 5.6 larvae per hundred stalks with a yield of 7500 kg/ha in Xifeng County, Liaoning Province, where *Trichogramma* was released continuously on a large scale for over 30 years, compared with 193.6 larvae per hundred stalks and 5250 kg/ha in other surrounding counties when the Asian corn borer outbreak occurred in 1997 (Cao and Sun 2002). In Gongzhuling City, Jilin Province, mean bores and number of larvae per hundred stalks decreased by 73.66% and 75.93%, respectively, where the *Trichogramma* were released from 1990 to 1996.

Trichogramma release for control of the Asian corn borer has become one of the key techniques of IPM of corn pests in China (Wang *et al.* 2003). It has been commonly adopted by the farmers in the northeastern provinces in China because of its easy use and good control efficacy. *Trichogramma* releases to control Asian corn borer comprise 1 to 1.3 million ha each year. With the Chinese government paying attention to grain production and environmental protection, the technique has been expanded to the Huang-Huai-Hai summer corn region and the Northwestern corn region in recent years. The Jilin, Liaoning and Heilongjiang provincial governments have provided some subsidies for controlling the Asian corn borer with *Trichogramma* in recent years. This has expanded the *Trichogramma* release area to 2 million ha in 2004.

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EFFECTIVE AUGMENTATIVE BIOLOGICAL CONTROL – IMPORTANCE OF NATURAL ENEMY DISPERSAL, HOST LOCATION, AND POST-RELEASE ASSESSMENT

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ABSTRACT

Augmentative biological control in outdoor cropping systems is often considered to be ineffective. High release rates are often needed for effective control and may be so frequently required that they become prohibitively expensive, especially when natural enemies are purchased from commercial suppliers. Natural enemies released argumentatively may provide control levels that are considered too low to be economically viable. Other germane issues are the selection of appropriate natural enemy species or strains for specific crops, and protocols related to timing and density of releases relative to crop phenology and other pest management strategies.

There are indeed cases where effective augmentative programs have been implemented in outdoor crops. This paper addresses grounds for the effectiveness of these programs, with special reference to the use of *Trichogramma ostriniae* in sweet corn and field corn, where low-density inoculative releases can be highly effective. The importance of understanding dispersal capacity and host location behavior of the biological control agents is examined. Host-seeking behavior of parasitoids in different crop habitats is considered and expanded upon as an aspect of central importance in ensuring effectiveness of augmentative biological control.

This is compared to less successful efforts at developing augmentative biological control in other crops with other parasitoid species (Trichogrammatidae and Scelionidae), in an attempt to identify key characteristics of a potential augmentative agent that are likely to result in success or failure.

Appropriate post-release assessment procedures are also considered. Measurement of the impact that augmentative releases have on integrated pest management systems are explored, to determine whether current approaches to measuring success of augmentative releases are reasonable and adequate. Measuring success of augmentative biological control releases as a component of a holistic IPM program, rather than in isolation, is considered with emphasis on reduced dependence on insecticides.

INTRODUCTION

Augmentative biological control of insect pests in outdoor cropping systems is an attractive option for IPM programs. Augmentative releases of biological control agents have promise as environmentally safe applications of biological control, and as an approach that should be compatible with the application of appropriate pest monitoring and economic injury levels. However, the effectiveness and economic value of augmentative biological control options is questionable in many cases – 64% of augmentative control projects are failures, and in many cases the costs associated with these programs are as high or higher than insecticides (Collier and van Steenwyk 2004). The generally low success rate is attributable to unfavorable environmental conditions, compensatory mortality, enemy dispersal, host refuges from released natural enemies, and predation of released agents (Collier and van Steenwyk 2004). Situations in which augmentative control may be particularly valuable include IPM systems that include pesticides that disrupt natural enemies periodically and crops with moderate to high economic injury levels. Both inundative and inoculative release approaches have the potential to be effective.

Natural enemy dispersal and host location are among the most important components identified by Collier and van Steenwyk (2004). These characteristics of biological control agents have long been recognized as essential components of classical biological control (e.g., Caltigirone 1981).

In spite of the recognized importance of these aspects of the ecology of augmentative biological control agents, they have receive scant attention. In this paper, we discuss some case studies illustrating the importance of understanding dispersal and host location, and the need for post-release assessment. We emphasize the importance of understanding searching behavior and dispersal in specific habitats, and the implications for effective augmentative biological control. Dispersal is defined here as the organism "moving from a point of release, to the place where they reproduce" (*sensu* Caughley 1980). This is an essential aspect of the effectiveness of parasitoids as biological control agents – although they might move throughout a habitat quickly, they must be able to locate and parasitize the target host to be effective.

SOME CASE STUDIES

A SUCCESSFUL AUGMENTATIVE BIOLOGICAL CONTROL PROJECT

While there are many cases of augmentative biological control that are considered ineffective, there also are success stories. Here we examine a system with which we are intimately familiar, and then compare this with another effort at augmentative biological control that has been less successful.

Trichogramma ostriniae Pang et. Chen (Hymenoptera: Trichogrammatidae), released augmentatively against European corn borer (*Ostrinia nubilalis* (Hübner), Lepidoptera: Crambidae) in sweet corn (*Zea mays* L.) is an example of an augmentative biological control agent with great potential. After initial efforts to use this wasp in a classical biological control program failed, an interest was developed in augmentative releases, particularly inoculative releases. This was based on field observations by M.P. Hoffmann and colleagues, which indicated that *T. ostriniae* seemed to have pronounced dispersal characteristics and appeared to establish effectively for a season following low-density release early in the season. Subsequent work on *T. ostriniae* demonstrated that this insect is indeed an excellent candidate for inoculative augmentative biological control. Hoffmann *et al.* (2002) showed that *T. ostriniae* does establish effectively in sweet corn fields in the northeast USA, and can survive insecticide applications at certain times. The wasp demonstrated a Type-I functional response under field conditions, and was thus able to maintain a consistent rate of parasitism across the range of *O. nubilalis* egg mass densities typically encountered in the northeastern U.S. (Hoffmann *et al.* 2002). Further work demonstrated that following low density (70,000 females per hectare), early season release, *T. ostriniae* contributes substantial and significant indispensable mortality to *O. nubilalis* populations, increasing pest mortality from ~60% to more than 95% (Kuhar *et al.* 2002). This mortality level was adequate to consistently reduce damage to ears of corn by ~50%, and the costs of conducting these releases were minimal, based on rearing costs for mass production of the wasps (Wright *et al.* 2002). *Trichogramma ostriniae* has indeed since been made commercially available. The effectiveness of *T. ostriniae* in augmentative biological control releases is attributed largely to its remarkable dispersal and host-location abilities, and the considerable indispensable mortality it is able to contribute as a result. Wright *et al.* (2001) showed that *T. ostriniae* could disperse throughout a large area (~10 ha) within less than seven days, and were able to effectively locate *O. nubilalis* egg masses during their dispersal. Laboratory work in Y-olfactometers showed that *T. ostriniae* females are attracted to the scales of female *O. nubilalis*, presumably to kairomones emitted from these, and field-deployed sentinel egg masses were indeed more attractive to the wasps when lightly sprinkled with fresh wing scales from moths (M. Wright and S. Pitcher, unpublished data).

Further investigation into the ecology of *T. ostriniae* showed that the wasps were substantially more effective at locating and parasitizing hosts in corn fields than in other habitats. When released in broad-leaf vegetable crops, they were relatively ineffective unless released at high density (Kuhar *et al.* 2004). When released in forest habitat, they were less than 10% as effective as in adjacent corn fields, with equal release densities (Wright *et al.* 2005). It was also evident from work done to measure dispersal of *T. ostriniae* out of corn fields and into adjacent habitat, that the wasps prefer to remain within cornfields unless the plants are shorter than about 50 cm (Wright *et al.* 2005). When plants are shorter than this the wasps appeared to readily disperse from the release field (M. Wright, unpublished data).

In summary, factors that make *T. ostriniae* an effective augmentative biological control agent are: effective dispersal; effective host location in the target crop; habitat fidelity; and persistence within the release field.

In addition to the above considerations, it is clear that the selection of an appropriate species of natural enemy is of cardinal importance. For example, attempting to use *T. ostriniae* for the control of an orchard pest is unlikely to be effective, considering the searching behavior demonstrated.

A LESS THAN SUCCESSFUL AUGMENTATIVE BIOLOGICAL CONTROL PROJECT

Nezara viridula (Hemiptera: Pentatomidae) is a perennial pest of macadamia nuts in Hawaii (and many other crops) (Jones 2002). A number of natural enemies have been introduced to control *N. viridula* in Hawaii, including adult parasitoids (*Trichopoda* spp., Diptera: Tachinidae) and an egg parasitoid *Trissolcus basalis* (Hymenoptera, Scelionidae). While *T. basalis* is considered to be a landmark success story in classical biological control in many areas (Jones 1995), it shows variable effectiveness in Hawaii. Parasitism levels may exceed 95% of *N. viridula* eggs on some islands (e.g., Oahu), yet be less than 5% in other areas (southern regions of the Big Island, Hawaii). This variability prompted an investigation into the possibility that augmentative biological control using *T. basalis* may be useful in areas where it has limited effectiveness as a classical agent (Wright *et al.* 2003). The dispersal capacity and host location abilities of *T. basalis* were investigated within macadamia orchards and in adjacent weedy habitats, to determine effective augmentative biological control release sites (Wright *et al.* 2004). The results from numerous releases of 5,000 female *T. basalis* within orchard areas of 5 ha have been uniformly disappointing – low parasitism rates were recorded, and dispersal was sporadic (Wright *et al.* 2004). Other work has shown that *T. basalis* probably contribute negligible indispensable mortality to *N. viridula* in Hawaii (Johnson *et al.* 2005; Jones 1995), at least in tree-habitats. Jones (1995) showed that parasitism by *T. basalis* was minimal within trees in orchards (up to 2.5%), but considerably higher in weed-infested orchard boundaries (up to 13.8%).

The effectiveness of *T. basalis* as an augmentative parasitoid of *N. viridula* eggs appears to be limited by ineffective host location and choice of release site within macadamia orchards and weedy areas. Local climatic conditions may also play an important role, with minimal parasitism resulting even after augmentative releases in dry areas, but high parasitism in areas with predictably high humidity levels.

POST RELEASE ASSESSMENT

Assessment of effectiveness in augmentative biological control programs is probably as important as releasing the natural enemies. Comprehensive life table studies show the extent of indispensable mortality attributable to a specific natural enemy, and can be used to measure the expected impact on the target pest. An understanding of expected yield gains attributable to natural enemies is also a useful measure that may be used in deciding whether to employ augmentative biological control. This approach will also allow the development of a meaningful measure of effectiveness, viz. to what extent a natural enemy reduces dependence on chemical or other pest management options.

CONCLUSIONS

The many failed attempts at augmentative biological control are primarily attributable to a poor understanding of the natural enemy's ability to locate hosts in specific crops after release. This is also identified as an important constraint by Collier and van Steenwyk (2004) in their comprehensive review of success and failures in augmentative biological control. A lack of knowledge of the expected dispersal behavior of a natural enemy influences the decision on release rates and the crop system targeted for augmentative biological control. Work on *T. ostriniae* has shown that low density, early-season releases are effective in corn (Wright *et al.* 2002), yet in solanaceous crops, release rates have to be orders of magnitude higher to achieve even moderate parasitism levels (Kuhar *et al.* 2004). This will clearly impact the benefit-to-cost ratio of using the same species in different crop systems.

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REMOVAL OF A PREDATORY BUG FROM A BIOLOGICAL CONTROL PACKAGE FACILITATED AN AUGMENTATIVE PROGRAM IN ISRAELI STRAWBERRY

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ABSTRACT

The demands of export and domestic markets have led growers to adopt a biological control-based integrated pest management program in low-tunnel strawberry fields in Israel. The program consists of the mass release of the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae) against red spider mites and of the parasitic wasp *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) against the cotton aphid. A study was launched to assess the potential use of *Orius laevigatus* (Fieber) (Heteroptera: Anthocoridae) to control the western flower thrips (WFT), Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae), in strawberry. After first developing economic thresholds for WFT in strawberry, we investigated (i) the ability of *O. laevigatus* to reproduce on vegetative and reproductive plant parts, (ii) the potential damage to fruits caused by *O. laevigatus* feeding and oviposition, and (iii) the species composition of the naturally-occurring WFT predator complex in strawberry fields.

ORIUS REPRODUCTION

Laboratory experiments show that *O. laevigatus* females prefer to deposit most of their eggs in reproductive parts of strawberry plants, including flowers, green, white and ripened fruits, and their petioles. Inspection of strawberry plants collected from commercial fields revealed a similar distribution pattern of *Orius* eggs. A similar egg deposition pattern was found on field-collected strawberry plants. The egg deposition pattern corresponded with egg hatch: hatching rate was significantly higher for eggs deposited in flowers than in those deposited in leaf tissues.

ORIUS-INFLICTED DAMAGE

To test whether *Orius* feeding and oviposition cause damage to strawberry fruits, we confined 10 female *O. laevigatus* on intact flowers, green fruits and white fruits for 72 hrs. After

removing females, we allowed the fruits to develop and recorded their quality at harvest. Inspection of the flowers and fruits revealed an extremely high density of *Orius* eggs imbedded in plant tissues. Nonetheless, no *Orius*-inflicted damage was visible on the harvested fruits as compared to controls. *Orius* feeding and oviposition thus do not inflict appreciable damage to strawberry fruits even at extremely high and un-realistic densities.

PREDATOR POPULATIONS IN STRAWBERRY FIELDS

The predominant WFT predators found in strawberry flowers were *O. albidipennis*, *O. niger* and predaceous thrips of the genus *Aeolothrips*.

CONCLUSIONS

In light of the established thresholds, the natural abundance of *Orius* predators in strawberry fields in Israel, their spatial and temporal co-occurrence with WFT, and their ability to reproduce successfully in this crop, *O. laevigatus* could be excluded from the commercial biological control package. This step made the package much more economically attractive to growers and accelerated its implementation, so that more than 80% of the strawberry acreage in Israel is now under a biologically-based integrated management program.

INTRODUCTION

The IPM/biocontrol program in Israeli strawberries was initiated as a direct result of the Western European export market's demand for significantly lower chemical input in plant protection. During the season of 1998/99, 15 ha. of commercial strawberries were designated as a pilot/demonstration field. Since then, the area encompassed by the program has increased steadily, reaching 300 ha. in the 2004/05 season (Fig. 1), which is ca. 80% of the total strawberry acreage grown in Israel. The majority of the crop is produced under low tunnels on Israel's coastal plain between the months of September and May. About 120 growers currently participate in the program.

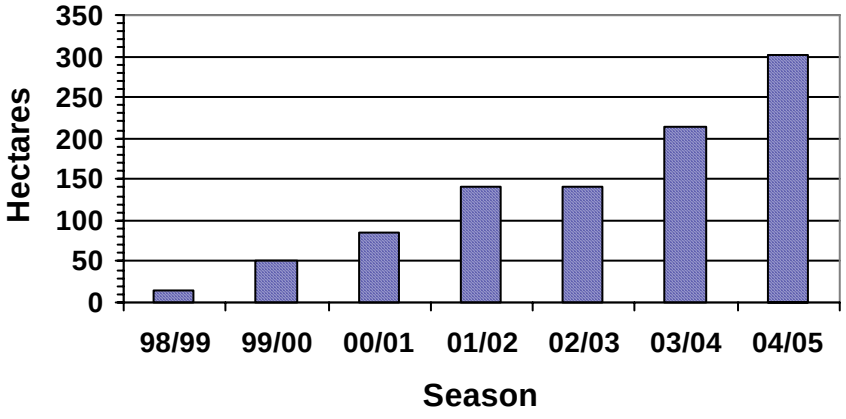

Figure 1. Area of the Israeli strawberry crop under IPM/biocontrol program.

From the onset, the IPM/biocontrol program for Israeli strawberries has been financially supported by the export marketing companies, growers' association and the Ministry of Agriculture. The technical implementation of the program is conducted by Bio-Bee Sde Eliyahu Ltd, the sole commercial producer of natural enemies for biological pest control in Israel. Professional scouts, supervised by Bio-Bee's technical advisory service, monitor the IPM/biocontrol plots on a weekly basis. They provide the grower with detailed reports on the status of pests and natural enemies, as well as recommendations for biological or chemical control action.

We report herein on the major biological components of the biologically-based IPM program for Israeli strawberries. Special emphasis is placed on the predatory bug *Orius laevigatus* (Fieber) (Heteroptera: Anthocoridae) and the rationale behind its exclusion from the commercial biocontrol package.

DEVELOPMENT OF THE BIOLOGICAL CONTROL COMPONENT OF THE PROGRAM

USE OF THE PREDATORY MITE *PHYTOSEIULUS PERSIMILIS* ATHIAS-HENRIOT (ACARINA: PHYTOSEIIDAE) AGAINST THE RED SPIDER MITE

In most plots, *P. persimilis* is introduced in early November, when plastic mulch is in place. The red spider mite is present in the majority of the fields at that time. During the last two seasons, the release rate of *P. persimilis* has stabilized at 20-24 predatory mites per m^2, a dramatic decrease from the 1999/2000 season when an average of 86 predatory mites were released per m^2 (Fig. 2). The continuous reduction in predatory mite release rate can be attributed to experience gained by the growers and scouts during the course of the project regarding both timing and mode of introduction of the predatory mites, and to economic considerations: during the last two seasons, growers have paid for *P. persimilis* on the basis of product used, rather than a lump sum paid in the past for a "biocontrol package" including an almost unlimited supply of natural enemies. In addition, since the 2003/04 season, the new acaricide 'bifenazate' has been applied with *P. persimilis*. 'Bifenazate' is harmless to the predatory mites or to any other natural enemies in the system. Hence, it is an ideal chemical for use against the red spider mite in this system, where needed. 'bifenazate' is mainly effective against the adult spider mites, allowing *P. persimilis* to sustain itself on the immature stages (eggs, larvae and nymphs). In this case the biological and the chemical agents act synergistically. During the 2003/04 season, 35% of the 67 participating IPM/biocontrol plots did not correct with 'bifenazate' at all, 23% used one application, and 42% corrected selectively in hot spots.

USE OF THE PARASITOID *APHIDIUS COLEMANI* VIERECK (HYMENOPTERA: APHIDIIDAE) AGAINST THE COTTON APHID

A. colemani is released following a single application of 'imidacloprid' or 'thiamethoxam' at the beginning of fruit-set. During the last two seasons, the average release rate of *A. colemani* has ranged from 0.7-1.0 parasitoids per m^2. As with *P. persimilis*, this rate also reflects a sharp decrease in the number of parasitoids released per m^2, from 13 per m^2 in the 1999/2000 season (Fig. 3). The reasons for this trend are the same as discussed regarding *P. persimilis*, i.e., experience, economics and availability of compatible aphicides.

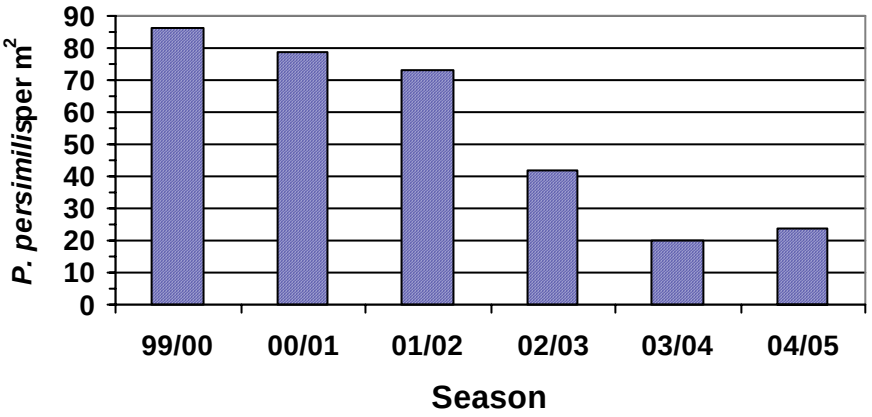

Figure 2. Average release rate of *P. persimilis* (number of predatory mites per m^2) in IPM/biocontrol strawberry fields in Israel during six growing seasons.

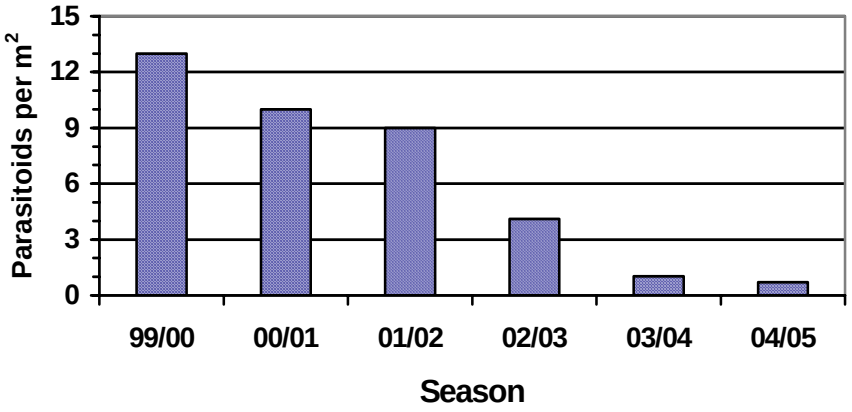

Figure 3. Average release rate of *A. colemani* (number of parasitoids per m^2) in IPM/biocontrol strawberry fields in Israel during six growing seasons.

USE OF THE PREDATORY BUG *ORIUS LAEVIGATUS* (FIEBER) (HETEROPTERA: ANTHOCORIDAE) AGAINST WESTERN FLOWER THRIPS

During the 1999/2000 winter growing season, an average of 3.5 predatory *O. laevigatus* bugs were introduced per m^2 of strawberry. There was no significant recovery of this species from the release fields. During the spring of the 2000/01 growing season, an average of 0.8 predatory bugs was released per m^2. Again, no recovery was recorded of the released bugs. As a result of an intensive research effort (see below), no commercial applications of *O. laevigatus* bugs were made on the subsequent growing seasons in strawberry fields.

ASSESSMENT OF THE PEST STATUS OF THE WESTERN FLOWER THRIPS AND JUSTIFICATION FOR *ORIUS LAEVIGATUS* RELEASES

BACKGROUND

The first report of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (WFT), in Israel dates back to 1987 (Argaman *et al.* 1989). This species is reported to be the dominant thrips species on strawberries in Israel (Shouster 2003) and is thought to be a key pest of this crop elsewhere (Allen and Gaede 1963; Tommasini and Maini, 1995). It has been credited for causing serious damage, mainly through flower drop and fruit distortion. Yet the pest status of WFT in strawberries and the nature of the damage it inflicts are the subject for much debate in many parts of the world.

The few published studies provide contradictory reports regarding WFT damage to strawberry. Damage to the flowers is typically caused by feeding punctures (Tommasini and Maini 1995) that lead to browning and premature withering of the stigmas and anthers, occurring after fertilization (Ribes 1990; Zalom *et al.* 2001). This damage can result in malformation of fruits, sometimes called cat-facing or monkey-facing (Allen and Gaede 1963; Buxton and Easterbrook 1988), which is unacceptable to consumers (Houlding *et al.* 1995). It has been suggested that thrips inject toxic saliva into the plant tissues, which also results in fruit deformation (Buxton and Easterbrook 1988). However, Allen and Gaede (1963), Easterbrook (2000) and Schaefers (1966) reported that various thrips species did not cause fruit malformation through their feeding but instead sometimes caused fruit discoloration. Damage to styles and stigmas may also lead to irregular fertilization and consequent failure of some achenes to develop. WFT may therefore be responsible for uneven ripening and yield loss (Parker 2004). Feeding by thrips on fruit surface and underlying cells often results in discoloration, sometimes accompanied by a silvery sheen caused by air filling the emptied cells (Lyth 1985). Hancock (1999) suggested that thrips feeding on developing seeds and the tissues between seeds results in damaged, small fruit with a seedy, dull or bronze-colored surface, and unevenly developed berries. Views on WFT-inflicted damage in strawberry thus remain ambiguous. Determining the extent and nature of the damage inflicted by WFT to strawberry flowers and fruits was therefore our first step toward the development of a thrips management program in this crop. Specifically, we (i) characterized damage symptoms, (ii) established WFT thresholds, and (iii) monitored pest population densities and compared them to the established threshold levels.

The second stage of this research involved assessing the possible use of *Orius* predators (Heteroptera: Anthocoridae) for the biological control of WFT in strawberry. Predatory bugs of this genus, such as *Orius laevigatus* (Fieber), are known to be effective natural enemies of WFT and are currently used for its control in a number of agricultural systems (Riudavets 1995). Towards this end, we (iv) investigated the ability of *O. laevigatus* to establish itself and reproduce on strawberries, and (v) determined the natural occurrence of *Orius* predators and other natural enemies of WFT in strawberry fields.

WESTERN FLOWER THRIPS AS A STRAWBERRY PEST IN ISRAEL

To characterize WFT damage and determine the vulnerable stage of fruit development, we confined 20 WFT adults for three days on flowers and on white, green and pink fruits. The fruits were then allowed to develop to maturity. At harvest, we compared the weight, size, shape, and coloration of fruits from the different treatments (i.e., time of exposure to WFT) to those of uninfested control fruits. In an additional experiment, we varied the number of adult WFT confined for four days on pink fruits (0 to 25 adults per fruit) and assessed the WFT density-fruit damage relationship.

A significant reduction in fruit fresh weight was recorded only when WFT infestation occurred at the green and pink fruit stages. Fruits in these treatments weighed approximately 40% less than controls. Bronzing was the only type of fruit damage attributable to WFT infestation, and this symptom appeared only when thrips fed on pink fruits. Thrips feeding resulted in punctures around the achenes and the appearance of silvery spots. At low WFT densities, light spotting and slight browning of the calyx were visible. At higher densities, fruit damage was characterized by bronzing, surface russeting and feeding punctures on the fruit surface. WFT-inflicted damage was clearly visible on the fruit surface beneath the calyx; these brown spots due to WFT feeding were particularly apparent at high densities (25 thrips per fruit). No fruit deformation was recorded in any of the treatments and no fruit damage was visible when WFT infestation occurred at the flowering stage. Field experiments, in which thrips populations were kept low in half of the plots but allowed to attain high densities in the others, showed similar results. The field experiments also suggest that WFT may play an important role in flower drop: a tendency toward higher flower drop was recorded in the high-WFT plots in the field. WFT feeding on strawberry blossoms was characterized by brown and withered stigmas and anthers. Necrotic spots were detected on the calyx of the flowers at high thrips densities and flower receptacles were significantly smaller at thrips densities greater than 10 per flower, compared to uninfested control.

These results were used for the establishment of economic thresholds for WFT in strawberry. Two thresholds were established, one for fruits grown for winter export between December and February, and the other for fruits for the local market (March-May). Thresholds for WFT sampling in strawberry flowers were set according to density-damage relations on the fruits, and the recorded ratio of 1:3 of WFT found on fruits and in flowers, respectively. Our calculations indicate that the economic threshold for WFT for exported fruits is 10 adults and second instars per flower. The threshold for the local market was set at 25 adults and second instars per flower.

Weekly sampling of strawberry flowers showed that WFT appears in strawberry fields during the winter, but populations become well established only in early spring. WFT numbers per flower rarely exceed the above thresholds. Typically, an average of 2-7 adult and second instar thrips were found per flower at peak population densities, with high variability among fields and years. WFT density on strawberry flowers began to decrease in April, and the population level remained low until the end of the season (an average of < 2 individuals per flower). Based on our field monitoring, it therefore appears that WFT populations rarely exceed the economic thresholds and, usually, no control measures are warranted against thrips in strawberry.

POSSIBLE USE OF *ORIUS LAEVIGATUS* TO CONTROL WFT IN STRAWBERRY

Laboratory experiments demonstrated that the predatory bug *Orius laevigatus* is able to reproduce on strawberries. Most oviposition takes place on plants that are in the reproductive stages of growth, and oviposition was higher on flowers than on leaves. Flowers and both unripe and ripe fruits were the preferred oviposition sites, and significantly fewer eggs were deposited between flowering cycles, when flowers and fruits were not available. *Orius* oviposition did not cause any visible damage to strawberry fruits even under excessive deposition of eggs in fruits and flowers (approx. 70 eggs per plant part). These results indicate that while inoculative releases of *O. laevigatus* could be considered for the control of thrips in strawberry, the bugs should not be released before flowers and fruits appear in the field, or between flowering cycles. The establishment of the bug in the field could be confirmed by examining egg deposition in flowers and fruits.

Our field monitoring indicated that the dominant natural enemies of thrips in strawberry flowers were *O. niger* (Wolff) and *O. albidipennis* (Reuter). Contrary to expectations, *O. laevigatus* was rare in our fields. *Orius* spp. became established in the crop in April and appeared to reduce WFT populations at that time. Other thrips natural enemies that spontaneously occurred in strawberry fields included predatory thrips of the genus *Aeolothrips* and the hymenopteran parasitoid *Ceranisus menes* (Walker).

RECOMMENDATIONS

Taken together, our results indicate that the western flower thrips is not a key pest of strawberries in Israel, and that under most circumstances no steps are needed for its control. WFT is present on the crop mainly during the second half of the growing season (spring), when the market value of the yield is relatively low and the fruit is destined for the local market, which tolerates a moderate level of cosmetic damage. Also, thrips density in flowers is generally kept in check by naturally occurring natural enemies that are abundant in un-sprayed, biological control-IPM fields. The predatory bug *Orius laevigatus* has the potential to serve as an effective biological control agent of WFT in strawberries; it reproduces on the crop, its presence is compatible with standard agrotechnical practices, and it causes no damage to flowers or fruits. In the Israeli strawberry system, however, the release of *O. laevigatus* is not economically justified; other *Orius* species appear spontaneously in high numbers in insecticide-free fields and the cost of *Orius* production is prohibitive.

CONCLUDING REMARKS

Several important lessons could be derived from the biological control-IPM program in Israeli strawberry. First, it is important to address all major pests in the system so that all used control measures are compatible with the employed biological control agents. Second, it is crucial to secure, early on, the financial and strategic support of private and government sectors, to allow the development of a viable and sustainable program. Third, to maximize profits, biological control producers and suppliers must not seek to maximize sales of a particular biological control agent. Rather, they should aim at developing a system-wide program, even at the cost of excluding a particular biological control agent from the package. Finally, stake-

holders often include growers, extension people, natural enemy producers, marketing companies, and retailers that spread across several countries. An international coordinated effort is therefore warranted to match the interest of all parties.

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RESEARCH-POTENTIAL VERSUS FIELD-APPLIED SUCCESS AND USE OF AUGMENTED NATURAL ENEMIES IN NORTH AMERICAN FIELD CROPS

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ABSTRACT

The effectiveness of augmentation programs varies depending on natural enemy species released, targeted pest, and release environment. For example, open-fields, row crops, and orchards present a more difficult environment for successful natural enemy release than protected environments, such as glasshouses. Released natural enemies may disperse from the target site, perform poorly at ambient temperatures, or fall prey to resident predators. Successful programs consider characteristics of the released natural enemy, the target pest, and the release environment before developing commercial release programs. Too often, matching the natural enemy to the target pest and environment is overlooked. To illustrate the impact of natural enemy biology on the success (or failure) of an augmentation program, we present results from research on augmentation programs for the vine mealybug, *Planococcus ficus* (Signoret), obliquebanded leafroller, *Choristoneura rosaceana* Harris, and variegated leafhopper, *Erythroneura variabilis* Beamer.

INTRODUCTION

Three broad categories describe how natural enemies are used in biological control: classical biological control, augmentation and conservation. Augmentative biological control is used when resident natural enemies occur too late in time or too low in number to provide adequate pest control, and includes inoculation - "seeding" natural enemies in the release area, and inundation - mass-releasing natural enemies to overwhelm the pest population (Daane *et al.* 2004). The effectiveness of augmentation programs varies depending on natural enemy species released, targeted pest, and release environment. How are natural enemy species selected for augmentation programs? The requirements for species selection and their successful use may include an ability (a) to rear or collect predictable quantities of natural enemies of high quality, (b) to store, transport, and release natural enemies effectively, and (c) to understand the compatibility of released natural enemies with the target pest(s) and other manage-

ment practices (Daane *et al.* 2002; Tauber *et al.* 2000). Nevertheless, the importance of the natural enemy's biological attributes is often undervalued as compared with advantageous insectary-rearing and shipment attributes.

Information regarding aspects of reproductive development, brood sizes, and dispersion along with culturability, sex ratio, food requirements, and host preference has greatly aided in the interpretations of the dynamics in biological control successes and provide a basis to evaluate natural enemy performance in different areas (Ehler 1990; Legner and Bellows 1999). To illustrate the impact of natural enemy biology on the success (or failure) of an augmentation program, we highlight research results from augmentation programs for *Macrocentrus iridescens* French (Hymenoptera: Braconidae) attacking obliquebanded leafroller, *Choristoneura rosaceana* Harris (Lepidoptera: Tortricidae) (Fig. 1), *Chyrsoperla carnea* Stephens (Neuroptera: Chrysopidae) attacking variegated leafhopper, *Erythroneura variabilis* Beamer (Hemiptera: Cicadellidae) (Fig. 2), and *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) attacking vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) (Fig. 3).

Figure 1. *M. iridescens* pupae near dead OBLR. Photo: Kent Daane. UGA1390002

Figure 2. *C. carnea* feeding on variegated leafhopper. Photo: Kent Daane. UGA1390003

Figure 3. *A. pseudococci* adult on honeydew. Photo: Kent Daane. UGA1390004

OBLIQUEBANDED LEAFROLLER AND *MACROCENTRUS IRIDESCENS*

The obliquebanded leafroller (OBLR) is a polyphagous feeder that can cause economic damage to several different crops over a wide geographic range in North America. In California pistachios, recent high OBLR population densities and resultant crop losses have led farm managers to apply insecticides, most commonly tebufenozide and carbaryl. Additional control tools for OBLR are needed to reduce the dependence on insecticide applications, prevent yield losses, and maximize profits.

A rich complex of more than 45 parasitoid species has been reported attacking OBLR; however, the level of parasitism and the parasitoid species present varies greatly among crops and regions surveyed. *Macrocentrus iridescens* is a polyembryonic parasitoid with a wide host and geographic range in North America. While *M. iridescens* is relatively ubiquitous, being reared from larvae in the Tortricidae, Lasiocampidae, Gelechiidae, Plutellidae, and Geometridae families in surveys from Ontario to California, it has rarely been reported as the dominant parasitoid or a key biological control agent (references in Krugner *et al*., 2005). A recent exception was a survey of California pistachio orchards, where *M. iridescens* was the dominant parasitoid species and it was considered a promising biological control agent for OBLR in this crop and region. We developed a laboratory colony of *M. iridescens* and conducted inoculative field release studies. Because little is known about *M. iridescens* biology or ecology, we conducted a series of laboratory assays of *M. iridescens* biology to determine its potential for mass culture, as well as its impact in an augmentation program.

VARIEGATED LEAFHOPPER AND *CHRYSOPERLA CARNEA*

In San Joaquin Valley (California) vineyards, the variegated leafhopper became the dominant insect pest in the 1980s (Daane and Costello 2000). At high densities, leafhoppers cause chlorotic spotting and defoliation, and their excretion acts as a substrate for sooty molds, resulting in cosmetic damage to fruit. Before the successful development and use of nicotenoid (imidacloprid) insecticides in the mid-1990s, farm managers sought alternative to insecticide applications, and some used inundative releases of green lacewings. Numerous experimental releases of *Chrysoperla* spp. have been tested against a variety of arthropod pests (for reviews, see Daane and Hagen 2000; Tauber *et al*., 2000); however, large-scale *Chrysoperla* spp. release programs for leafhoppers required better guidelines than were currently available. We evaluated green lacewing release impact and release methodologies in vineyards. Here, we present pertinent results from four years of field and laboratory experiments.

VINE MEALYBUG AND *ANAGYRUS PSEUDOCOCCI*

Vine mealybug has become a primary insect pest of vineyards in South Africa, Mexico, and California (Daane *et al.* 2005). When left uncontrolled, vine mealybug infestations result in spoiled, infested fruit. Thick layers of excreted honeydew covering the vine also promote sooty mold growth, which can result in defoliation and reduced yield, and a further reduction in crop quality from sunburn. In California, suggested mealybug insecticide treatments include multiple insecticide applications, often with organophosphates. However, because the vine mealybug can feed on all vine sections, there is often poor insecticide coverage and mealybug control in the more protected areas of the vine, such as under the bark, where mealybugs often reside is difficult (Geiger and Daane 2001). Moreover, repeated insecticide use also has adverse impacts on mealybug natural enemies (Walton and Pringle 1999). For these reasons, the development of effective, species-specific, and environmentally safe control programs is needed to work in combination with or as an alternative to insecticides.

Natural enemies attacking vine mealybug in California vineyards include the encyrtid parasitoids *A. pseudococci*, *Allotropa* nr sp. *mecrida* Walker, and *Leptomastidea abnormis* (Girault); several species of green (*Chrysoperla* and *Chrysopa* spp.) and brown (*Hemerobius* spp.) lacewings, and coccinellid beetles. Of these, *A. pseudococci* is currently the most effec-

tive natural enemy, with percentage parasitism as high as 90% of the exposed mealybugs collected near-harvest-time (Daane *et al*. 2004). *Anagyrus pseudococci* is well-known as a parasitoid of the citrus mealybug, *Planococcus citri* (Risso) (Noyes 1994). A polyphagous parasitoid, it also attacks distantly-related species such as *Pseudococcus comstocki* (Kuwana), *Phenacoccus herreni* Cox and Williams, *Dysmicoccus brevipes* (Cockerell), and *Maconellicoccus hirsutus* Green (Noyes 1994). While *A. pseudococci* has been well-studied as a parasitoid of the citrus mealybug (Islam and Copland 2000; Rosen and Rössler 1966; Tingle and Copland 1989), there are no comparable studies with the vine mealybug. Therefore, along with augmentation trials, we conducted a series of studies with *A. pseudococci* reared on vine mealybug to improve effectiveness of biological control in California vineyards.

MATERIALS AND METHODS

OBLIQUEBANDED LEAFROLLER AND *MACROCENTRUS IRIDESCENS*

Field augmentation. Parasitoids and OBLR were cultured as described by Krugner *et al*. (2005). *Macrocentrus iridescens* females, derived from a laboratory colony, were released in late April to early May in two commercial pistachio fields, near Hanford, California (Kings Co.). Each commercial field (8–20 ha blocks), was split into release and control plots (10 rows x 10 trees) that were separated by »50 buffer rows. Release adults were 1–2 days old, and fed honey and water prior to release. There were from 1,210–2,279 adult *M. iridescens* released in each 100-tree plot, with releases timed to attack the overwintered OBLR larvae (8 April through 9 May). To determine the impact of released parasitoids on OBLR density, we recorded the number of OBLR strikes (infested pistachio leaves) during timed counts (20 trees per plot per sampling date) and made collections of live OBLR (100 per plot per sampling day) to determine percentage parasitism.

Macrocentrus iridescens biology. We report here on two studies that were particularly pertinent to the impact of *M. iridescens* in the augmentation program (described in Krugner *et al.* 2005). First, the ideal temperature range for *M. iridescens* was determined by comparing development and mortality at eight constant temperatures (between 12.6–36.8°C). The upper and lower temperature thresholds, and development rates were estimated by graphing inverse development rates against temperature and fitting a nonlinear curve. Second, the OBLR host stage preferred by *M. iridescens* and the possible range of OBLR host stages that *M. iridescens* can attack were determined in both choice and non-choice tests. In the choice test, all five OBLR instars were placed in an oviposition cage and adult parasitoids added for a 24 hour exposure period. In the non-choice test, each oviposition cage had only one OBLR development stage present. In both experiments the exposed larvae were individually isolated in diet cups and reared to adult parasitoids or OBLR. The experiment was a randomized complete block design with six replicates.

VARIEGATED LEAFHOPPER AND *CHRYSOPERLA CARNEA*

Field augmentation. The effectiveness of commercial *C. carnea* release programs was evaluated in three vineyards located near Madera, CA (Madera Co.) from (1990 to 1993) (described in Daane *et al.* 1996). *Chrysoperla carnea* were released at rates varying from a total of 37,065

eggs per ha over five periods. Leafhopper densities were estimated 7 days before and 14 and 21 days after lacewing releases with counts of leafhopper nymphs on 20 leaves per plot, following sampling guidelines described by Daane and Costello (2000).

Chrysoperla carnea prey-consumption. Results from these field studies brought into question the effectiveness of release methods, such as egg vs. larval release. For this reason, we studied release methodology, and describe here results from one experiment on the impact of varying release rates, which helps highlight the impact of target prey selection for the "generalist" predator, *C. carnea* (described in Daane and Yokota 1997). To test different release rates, we used a vineyard block at the Kearney Agricultural Center, located near Parlier, CA (Fresno, Co.). Individual vines were isolated by pruning canes on either side. Treatments consisted of a no-release control and 10, 50, 100, 250, 500, and 1000 *C. rufilabris* eggs per vine. These rates correspond to 12,350, 61,750, 123,500, 308,750, 617,500, and 1,235,000 eggs per ha, respectively, with the higher release rates clearly uneconomical To determine impact leafhopper nymphs were counted on 15 leaves just before and 14 days after treatment application, as described previously. Treatments were set in a randomized complete block design with nine replicates.

VINE MEALYBUG AND *ANAGYRUS PSEUDOCOCCI*

Field augmentation. Field studies were conducted in five commercial raisin vineyards located near Del Rey, California (Fresno Co.). Treatments were *A. pseudococci*-release and a no-release control, with 0.6 ha treatment plots set in a randomized split plot design, and with each vineyard serving as a replicate. Treatment plots were separated by a buffer zone to minimize dispersion of released *A. pseudococci* into control plots. We released 8,090 *A. pseudococci* per ha on 12 June, 3 July, and 30 July; the release dates were selected based on mealybug movement to exposed locations on the vine. To measure the impact of *A. pseudococci* release, vine mealybug density was determined by a 5-minute search per vine on each of 10 randomly selected vines per plot, as described in Geiger and Daane (2001). Additionally, parasitoid activity was evaluated by collecting 100 mealybugs from each treatment plot (all mealybug stages were sampled). The collected mealybugs were stored in gelatin capsules and held for parasitoid emergence. Crop damage was evaluated at harvest-time by ranking damage of 50 randomly selected vines per treatment plot (five clusters per vine).

Anagyrus pseudococci biology. Our research suggests that *A. pseudococci* overwintering biology and host searching efficiency impacts its success in biological control programs. First, we studied *A. pseudococci* overwintering and spring emergence patterns (for details, see Daane *et al.*, 2004). Briefly, mealybugs were exposed to *A. pseudococci* and then placed at either ambient temperatures (outside) or at room temperatures. The inoculation periods were repeated each month with inoculation dates in October, November, December, January, February, and March. We then recorded the period of adult emergence. Second, we studied the impact of mealybug location on *A. pseudococci* effectiveness (for details, see Daane *et al.* 2005). In commercial vineyards, we collected »100 mealybugs per month per vineyard. Each mealybug was categorized by development stage and location, as "protected" for mealybugs collected under ground, under the bark of the trunk or older canes, or in cavities formed by wood-boring moths, or as "exposed" for mealybugs found on new canes, leaves and clusters. The collected mealybugs were then held for parasitoid emergence.

RESULTS

OBLIQUEBANDED LEAFROLLER AND *MACROCENTRUS IRIDESCENS*

Field augmentation. There were significantly more "old" shoot strikes (plant damage – but no live OBLR) in the control than release plots in the mid-July and late-August surveys (t = 2.54, P = 0.014 and t = 2.59, P = 0.016, respectively) (Fig. 4a). Similarly, there was a significantly higher percentage parasitism in the release treatment in the mid-June period, and derived from the targeted overwintered OBLR larvae (Fig. 4a). Still, there were no significant differences between treatments near harvest-time (Fig. 4a,b) and there were no differences in the number of "new" shoot strike (damaged leaves with OBLR larvae). In summary, we had a significant increase in parasitism in release plots in late-May, just after the parasitoids were released. Unfortunately, this success was short-lived and did not carry over to the next collection periods. Particularly significant is the mid-July reduction in percentage parasitism in the release treatment, suggesting no carry-over between OBLR generations in parasitoid activity.

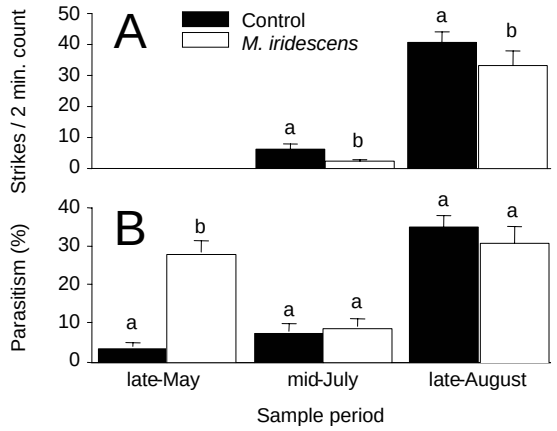

Figure 4. a) Number of OBLR damage leaves (shoot strikes) and b) percentage parasitism by *M. iridescens* in release and no release treatment plots.

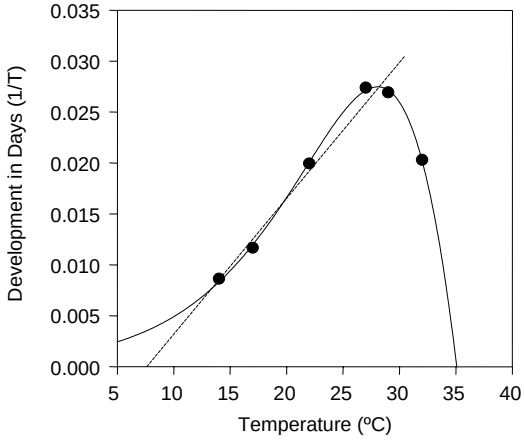

Figure 5. Relationship of temperature and *M. iridescens* development rate at eight constant temperatures.

Macrocentrus iridescens biology. Why was there no season-long impact of the parasitoid release? We believe the answers can be found in the biological data collected in the laboratory. A nonlinear model (Wang *et al*. 1982) gave an excellent fit to the data set (R^2 = 0.998) and suggests optimal and upper development temperatures (Fig. 2). The fastest development time, estimated from the upper asymptote, is 36.36 days at 28°C (Fig. 5, dotted line); the upper temperature threshold is 35°C (Fig. 5, solid line) and a lower temperature threshold was determined to be 7.6°C. Using these data, we found that the development time for *M. iridescens* (in degree days) was longer than that reported for OBLR (Gangavalli and AliNiazee 1985). Therefore, there is only one *M. iridescens* generation to each OBLR generation. This by itself can reduce the effective build-up of the natural enemy population.

We also found the mean number of adults emerging from each OBLR significantly decreased at temperatures above 28.2 31.0°C (F = 12.605, δf = 5, $P \leq 0.001$). Since host larvae were parasitized under the same conditions and randomly exposed to different temperatures,

the only variable assumed to affect the size of the emerging progeny was temperature. Therefore, it is possible to conclude that constant temperatures above 28.2°C reduces the number *M. iridescens* individuals emerging from each OBLR larvae. This suggests that during the hot summer temperatures in the Central Valley there will be a reduction in the number of parasitoids produced per OBLR larva. The sex ratio also became more male biased (data not presented, see Krugner *et al.*, 2005). Furthermore, the parasitoid has clear host preference for second and third stage OBLR larvae and if these are not available its reproductive potential will drop. Such circumstances are more likely to occur early in the season because there is clear overlap of OBLR development stages in late July and August when there is also a naturally high level of parasitism.

VARIEGATED LEAFHOPPER AND *CHRYSOPERLA CARNEA*

Field augmentation. In 9 of 20 trials, leafhopper densities were significantly lower in *C. carnea*-release than no-release plots. Data from all trials were combined to determine possible explanations for the variation in the effectiveness of *C. carnea* releases. Possibilities include differences in release trials, rates, and methods, as well as prey density. The average reduction of leafhoppers in *C. carnea*-release plots, as compared with no-release plots, was only 9.6% in commercial vineyards. A significant, although only weakly positive, correlation was found between release rate and effectiveness. There was also a greater reduction of leafhopper nymphs when lacewings were released as larvae, as compared with eggs. Combining data from all studies, the number and percentage reduction of leafhopper nymphs was related to leafhopper density (Fig. 6). Most importantly, when leafhopper densities were above the suggested economic injury level (15-20 nymphs per leaf), the reduction in leafhopper number was frequently not sufficient to lower the leafhopper density below the economic injury threshold.

Chrysoperla carnea prey-consumption. We tested a wide range of release rates (12,350 to 1,235,000 eggs/ha/generation) with the expectation of generating a dose response. However, no correlation between release rate and leafhopper density was found (Fig. 7). One explanation is that higher release rates resulted in increased cannibalism, which reduced the overall impact of added lacewings. Although lacewing larvae are more likely to cannibalize the egg stage, hungry larvae will attack most soft bodied prey, including conspecifics. Satiated larvae are rarely cannibalistic. However, while there was abundant leafhopper prey in these trials, lacewing prey selection is based, in part, on its ability to capture prey (Daane 2000) and small conspecifics may be easier to capture than large leafhoppers. Moreover, because the lacewing are actively moving in search of prey, while the leafhoppers are relatively sessile while feeding, there may be more chance encounters of lacewing to lacewing than lacewing to leafhoppers.

VINE MEALYBUG AND *ANAGYRUS PSEUDOCOCCI*

Field augmentation. Mealybug season-long density was significantly lower in the *A. pseudococci* release than control treatment (Fig. 8). Cluster damage rating was a significant 57% lower in the *A. pseudococci* release (0.22 ± 0.03) than control (0.51 ± 0.05) treatment (t = 5.522, df = 1, 444, P <0.001). However, we are unable to conclude that the released *A. pseudococci* were solely responsible for this reduction. First, while there was no treatment difference in

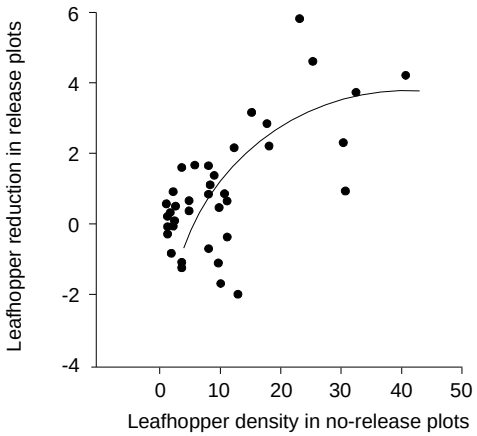

Figure 6. Percentage reduction of leafhopper nymphs in *C. carnea*-release plots plotted against mean number of leafhopper nymphs in associated no-release plots.

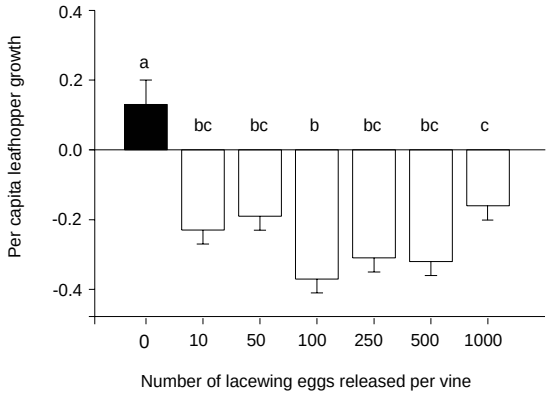

Figure 7. Per capita change (± SEM) in leafhopper density after release of 10 to 1000 lacewing eggs per vine. Different letters above each bar indicate a significant difference (Tukey's P < 0.05)

mealybug density on 27 March (*t*-test = 1.659, *P* = 0.101), when treatment plots were randomly assigned, there were significantly fewer mealybugs on 5 June (*t*-test = 3.701, *P* < 0.001), just before the *A. pseudococci* release. Second, there was no season-long difference in percentage parasitism (Repeated Measures ANOVA: F = 2.114, df = 1, 521, *P* = 0.147), although percentage parasitism is often an unreliable tool to measure natural enemy impact.

Nevertheless, the data provide encouraging information for the commercial use of *A. pseudococci*. From 7,458 mealybugs collected and held in gelatin capsules, 1,978 were parasitized (26.5%) and 1,235 parasitoid were reared to the adult stage. Parasitoids reared were *A. pseudococci*, *L. abnormis*, *Allotropa* sp. and a hyperparasitoid, *Chartocerus* sp. Of the adult parasitoids, *A. pseudococci* was dominant, comprising >93% of all reared parasitoids. Third instar mealybugs were the most commonly attacked, reflecting the host preference of *A. pseudococci*. Most important, there was a significant reduction in crop damage near harvest-time (data not shown, see Daane *et al*. 2005).

Anagyrus pseudococci biology. Earlier studies showed that *A. pseudococci* in California vineyards has an initial period of activity in late May, a result of temperature-dependent development during the overwintering period (Daane *et al*. 2004). For this reason, we believe that early-season inoculation/inundation could dramatically improve parasitism rates. While augmentation with *A. pseudococci* did increase parasitism (Fig. 8) there remained a significant population of the pest in the vineyard. We attribute this resident population to the parasitoids' ineffective host searching attributes for mealybugs located in the more protected locations.

From field collected vine mealybug, we found host size impacted both parasitism and parasitoid gender, as found in earlier studies (Nechols and Kikuchi 1985; Sagarra and Vincent 1999). The percentage of female *A. pseudococci* reared from first and second instar mealybugs was only 2.9 ± 2.9 and 3.6 ± 0.8%, respectively, while from third instar and adult mealybug we reared 95.4 ± 1.1 and 92.9 ± 2.2% females, respectively. More important for parasitoid impact was the great difference in parasitoid effectiveness with respect to mealybug location

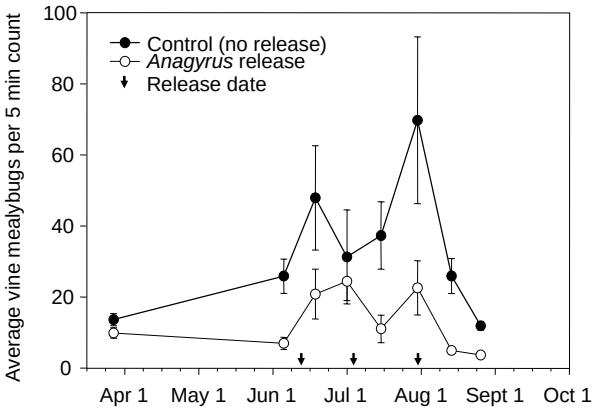

Figure 8. Season-long density (± SEM) of settled vine mealybugs was significantly lower in treatments with *A. pseudococci* release, as compared with no-insecticide control plots (Repeated Measures ANOVA: F=13.27, df=1, 76, $P < 0.001$).

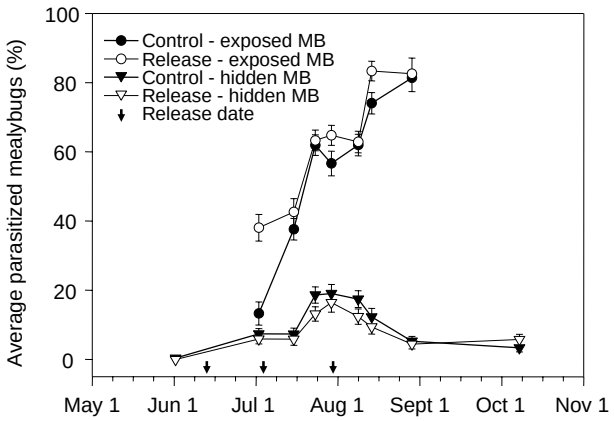

Figure 9. Season-long percentage parasitism (±SEM) of settled vine mealybugs, separated by treatment and location, shows significantly higher in parasitism exposed than hidden locations for both control (F = 247.3, df = 1, 273, $P < 0.001$) and release (F = 501.5, df = 1, 249, $P < 0.001$) treatments.

on the vine. Season-long percentage parasitism, with data separated by date and location of collected mealybugs, show the importance of timing augmentative release after mealybugs have moved from protected locations (Fig. 9). While there was a low season-long percentage parasitism of mealybug collected from hidden locations (e.g., under the bark) never exceeding 20%, there was a consistent season-long rise in parasitism of mealybugs collected from exposed locations (e.g., on the leaf). No mealybugs could be found in exposed locations on the 1 June sampling date, prior to *A. pseudococci* release. After releases began, there was significantly greater percentage parasitism of exposed mealybugs in release than control plots on the initial sample date (Fig. 9). Parasitism rose steadily in both release and control plots, reaching >80% by late August, after which we could find no live mealybugs in exposed locations.

DISCUSSION

The market for biologically based pest controls is potentially great, driven largely by consumers' desire for pesticide-free produce and loss of current pesticides (Parrella *et al.* 1992). Nevertheless, much of the pest control market is directed towards "soft" insecticides rather than commercially reared and released natural enemies. To meet these needs, researchers and the insectary industry are working to develop more efficient programs. In the insectary, the efficiency of mass culture of beneficial insects is highly dependant on improvement of methods to facilitate and accelerate the insectary process. For this, insectary managers must consider the biology of the host and the parasite in order to produce large numbers while maintaining quality of the mass-reared natural enemy. Here, we describe how natural enemy biology also has considerable impact on its field effectiveness, which is often overlooked.

Whenever feasible, early-season, inoculative release is preferred because it requires fewer natural enemies and provides control over a longer period. In the first study reported, we evaluated the inoculative release of *M. iridescens* for OBLR control in pistachios. *Macrocentrus*

iridescens was earlier found to be the most common parasitoid reared from OBLR in California pistachios, and we were able to develop laboratory colonies to conduct release trials. However, well-timed inoculative release against the overwintered OBLR generation did not impact OBLR density near harvest-time. The problem rested in the parasitoids' biological attributes. Parasitoids often exhibit optimum temperatures different from those of their host, and may become ineffective at higher or lower temperatures. For *M. iridescens*, high temperatures reduced its overall reproductive potential and its developmental rate was slightly longer than its host, indicating that there will be a single parasitoid generation for each OBLR generation. Combined with a relatively narrow host stage preference, *M. iridescens* was unable to respond numerically to the increasing host density until late July and August, when the OBLR population age structure presented acceptable hosts throughout the adult's life time.

In the second study reported, we evaluated the commercial use of inundative releases of green lacewing eggs. *Trichogramma*, predaceous mites and green lacewings are some of the most commonly used natural enemies in inundative augmentation programs (Daane *et al.* 2002). Our work on inundative releases with green lacewings illustrates that this generalist predator may not be the best natural generalist predator for all targeted pest species. Released lacewings are subject to predator-predator interactions at the release site (Daane 2000) and information on other predator species may help release decisions. In our studies, the most significant intraguild predation may have derived from lacewing cannibalism.

In the third study reported, we tested what amounted to both inoculative and inundative releases of *A. pseudococci* for mealybug control in vineyards. While we are enthusiastic about the commercial potential of *Anagyrus* to lower economic damage in the grape clusters, we found that augmentation against vine mealybug may be incomplete because mealybugs have protected locations on the vines. In fact, 100% of the live mealybugs found in September and October samples were located in protected locations of the vine and this, we believe, greatly reduces the ability of foraging adult *Anagyrus* to locate and parasitize vine mealybugs that will constitute the overwintering parasitoid population. Furthermore, we reared primarily male *Anagyrus* from first and second instar mealybugs. These results show that *Anagyrus* release should be timed to coincide not only with the presence of mealybugs in exposed locations, but also with the presence of third instar mealybugs. A final problem with the commercialization of this program is the mass-culture of *A. pseudococci*. Currently, vine mealybug is a pest in vineyards only, reducing the demand for this specialized parasitoid and the potential market for insectary production of *A. pseudococci.*

Augmentation in North American field crops has a long history that includes some of the initial research and successful examples (Daane *et al*. 2002; Parrella *et al.* 1992). One of the most successful augmentative release programs has been against California red scale, *Aonidiella aurantii*. Beginning in 1956, mass-production and inoculative releases of *Aphytis melinus* by the Fillmore Citrus Protection District has suppressed red scale populations. One of the first commercially successful uses of augmentation was against spider mites (*Tetranychus* spp.) on strawberries and cotton. Much of this early work helped develop guidelines for the commercial programmes that emerged in the 1980s. Nevertheless, research on the proper use and efficacy of augmentation programmes in field studies often lagged behind concurrent improvements in mass-production methods for parasitoids and increases in their commercial use, especially in glasshouse systems in Europe.

During this past decade, research has once again focused on field-ecology in augmentation programs and, as a result, there have been substantial advances in our understanding of the potential and problems of both inundative and inoculative programs. Future research will include (a) systematic revisions of natural enemy species that make correct identification and evolutionarily-based biological comparisons a reality, (b) improvements in the methodology for mass-production, (c) applying information from chemical ecology and seasonality to conserve and manipulate natural populations, and (d) rigorous experimental evaluation of release methodology (as described for lacewings in Tauber *et al.* 2000).

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EFFECTS OF INTRAGUILD PREDATION AND INTERSPECIFIC COMPETITITON AMONG BIOLOGICAL CONTROL AGENTS IN AUGMENTATIVE BIOLOGICAL CONTROL IN GREENHOUSES

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ABSTRACT

Two natural enemy species are frequently released simultaneously to control one pest species in augmentative biological control in greenhouses. Intraguild predation (IGP) and interspecific competition between natural enemies might affect the biological control. IGP occurs between two parasitoids, between one parasitoid and one predator, and between two predators. Although unidirectional IGP has been found in many studies about IGP between natural enemies used in the biological control of greenhouse pests, no significant effects of IGP on biological control have been recognized. On tomatoes in greenhouses, *Liriomyza trifolii* is usually controlled by the combined release of *Dacnusa sibirica* and *Diglyphus isaea*. *Trialeurodes vaporariorum*, another pest of greenhouse tomatoes, can be controlled by the combined use of *Encarsia formosa* and *Eretmocerus eremicus*. Simulation models incorporating IGP or interspecific competition between these parasitoid species have been constructed for evaluating biological control using two parasitoid species. These simulation models suggested no significant negative effects of IGP or interspecific interactions between two parasitoids on biological control.

INTRODUCTION

The number of biological control agents (BCAs) released in greenhouses has increased greatly. Today, over 125 BCAs are commercially available in Europe (Weintraub and Cheek, 2005).0Thirty-two BCAs were registered as biopesticides by 2004, and some are widely used in commercial greenhouses in Japan.

Biological control agents are frequently used in combination. In some cases, two species which have complementary effects are released simultaneously. In recent release systems, first a less costly species is released preventively to control the target pest. When the pest density reaches a high level, another more expensive species (often generalist predators) may be released curatively to suppress the pest population.

Biological control can be disrupted by direct or indirect interactions such as competition, apparent competition, intraguild predation (IGP), and behavioral interference between natural enemies. Rosenheim *et al.* (1995) reviewed theoretical and empirical evidence to dis-

cuss the significance of IGP in biological control. IGP occurs when two species that share a host or prey also engage in a trophic interaction with each other (parasitism or predation). They hypothesized that IGP by predators is particularly likely to influence the efficacy of biological control.

Brodeur *et al.* (2002) argued the significance of IGP by generalist predators released curatively in greenhouse systems. Generalist predators may disrupt biological control by interfering with natural enemies released preventively. They concluded that IGP by generalist predators is less important in greenhouses than in annual or perennial agroecosystems.

In this article, recent studies about the significance of IGP in augmentative biological control in greenhouses are first reviewed. Then simulation models for evaluating IGP or interspecific competition between parasitoids released to control whiteflies or leafminers in greenhouse tomatoes are described.

INTERACTIONS BETWEEN TWO NATURAL ENEMIES

INTERACTION BETWEEN TWO NATURAL ENEMIES IN BIOLOGICAL CONTROL IN GREENHOUSES

Table 1 shows the list of studies about IGP among arthropod natural enemies used in augmentative releases in greenhouses. Three types of IGP are considered, i.e., IGP between two parasitoids, between one predator and one parasitoid, and between two predators. IGP between predators has been studied for many interactions. There have only been a few studies about IGP between two parasitoids, and IGP of a parasitoid by a predator. Most of the studies are IGP experiments with or without alternative hosts. Thus, the effects of IGP on the population dynamics of both natural enemies and a host or a prey in biological control have been found for only several cases. In most cases listed in Table 1, IGP is unidirectional.

IGP AND INTERSPECIFIC COMPETITION BETWEEN TWO PARASITOIDS

Liriomyza trifolii (Burgess) (Diptera: Agromyzidae), a pest of greenhouse tomatoes, is usually controlled by a combined release of *Dacnusa sibirica* Telenga (Hymenoptera: Braconidae) and *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae). Two whitefly species, *Trialeurodes vaporariorum* (Westwood) and *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae) can be controlled by a combined use of *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) and *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae).

D. isaea is always superior to *D. sibirica* in their interaction.0*D. isaea* adults kill parasitized leafminer larvae by *D. sibirica*. *D. sibirica* adults cannot attack dead larvae killed by *D. isaea*. This interaction can be regarded as IGP. When whitefly larvae were parasitized by both *E. formosa* and *E. eremicus*, *E. eremicus* always survived and *E. formosa* was killed in the direct interference between two species (Mitsunaga, unpublished).

IGP BETWEEN ONE PARASITOID AND ONE PREDATOR

Two types of unidirectional IGP by arthropod predators on parasitoids are recognized. First, predators may prey directly on immature stages of ectoparasitoids or on free-living parasitoid adults. Second, predators may prey on parasitized hosts. Once a host is encountered,

Table 1. Intraguild predation (IGP) among arthropod natural enemies used in biological control in greenhouses.

Study Type	Interaction	IGP Species (E=exploiter; V=victim)	Biocontrol Target	Reference
Laboratory experiment, Simulation	Parasitoid-parasitoid	*Dacnusa sibirica* (V) *Diglyphus isaea* (E)	*Liriomyza trifolii*	This study
Laboratory experiment	Predator- parasitoid	*Anthocoris nemorum* (E) *Aphidius colemani* (V)	*Myzus persicae*	Meyling et al. 2002
Laboratory experiment	Predator- parasitoid	*Aphidoletes aphidimyza* (E) *Aphidius colemani* (V)	*Aphis gossypii*	Enkegaard et al. 2005
Laboratory experiment, Greenhouse experiment	Predator- predator	*Neoseiulus californicus* (E, V) *Phytoseiulus persimilis* (E, V)	*Tetranychus urticae*	Walzer & Schausberger 1999ab; Schausberger & Walzer 2001
Laboratory experiment	Predator- predator	*Orius tristicolor* (E) *Neoseiulus cucumeris* (V)	*Frankliniella occidentalis*	Gillespie & Quiring 1992
Laboratory experiment	Predator- predator	*Orius tristicolor* (E) *Phytoseiulus persimilis* (V)	*Tetranychus urticae*	Cloutier & Johnson 1993
Laboratory experiment	Predator- predator	*Orius majusculus, O. insidiosus* (E) *Neoseiulus cucumeris* (V)	*Frankliniella occidentalis*	Sanderson et al. 2005
Laboratory experiment	Predator- predator	*Orius majusculus* (E) *Iphiseius degenerans* (V)	*Frankliniella occidentalis*	Brodsgaard & Enkegaard 2005
Laboratory experiment	Predator- predator	*Orius majusculus* (E) *Aphidoletes aphidimyza* (V)	*Aphis gossypii*	Christensen et al. 2002
Laboratory experiment	Predator- predator	*Orius majusculus* (E) *Macrolophus caliginosus* (V)	*Frankliniella occidentalis*	Jakobsen et al. 2002
Laboratory experiment	Predator- predator	*Dicyphus tamaninii* (E) *Macrolophus caliginosus* (V)	*Trialeurodes vaporariorum*	Lucas & Alomar 2001, 2002

predators may have different probabilities of attacking unparasitized versus parasitized hosts (Rosenheim *et al.* 1995).

Prey preference between *Aphidius colemani* Viereck (Hymenoptera: Braconidae), parasitized *Myzus persicae* Sulzer (Homoptera: Aphididae) (mummy stage) and unparasitized aphids was evaluated for female *Anthocoris nemorum* L. (Heteroptera: Anthocoridae) in the laboratory. *A. nemorum* preyed readily on the immature parasitoids contained within mummies, and showed no preference for either of the two prey types (Meyling *et al.* 2002).

The intraguild predation between the aphid predator *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) and the parasitoid *A. colemani* was examined in the laboratory. Gallmidge larvae readily killed parasitized but not yet mummified aphids. The predator showed a slight preference for parasitized over unparasitized aphids. Aphid mummies were not predated at all (Enkegaard *et al.* 2005).

IGP BETWEEN TWO PREDATORS

Many predators are generalists and consume a broad array of prey. IGP among predators is widespread and both unidirectional and bidirectional IGP appear to be common. The presence of alternative prey is often critical in modulating the occurrence of IGP (Rosenheim *et al.* 1995). The relative size of two predators is crucial in unidirectional IGP. In general, the larger predator exploits the smaller one. Bidirectional IGP often takes the form of late instars or adults of two species feeding on each other during earlier developmental stages.

IGP and the cannibalism of the generalist *Neoseiulus californicus* McGregor (Acarina: Phytoseiidae) and the specialist *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae) were examined. *N. californicus* distinguished con- and heterospecific larvae and fed more by IGP than cannibalism. *P. persimilis* had a higher predation rate by cannibalism than IGP (Walzer and Schausberger 1999a,b). Combined and single species release of *N. californicus* and *P. persimilis* for suppressing *Tetranychus cinnabarinus* Boisduval (Acarina: Tetranichidae) were compared on greenhouse gerbera. The population growth of *P. persimilis* was greater and the population decline steeper in a combined release than a single species release. *N. californicus* grew and declined more gradually in a combined release than in single species one. These differences in the population dynamics of two phytoseiid mites can be attributed to contrasting properties in competition, IGP, and cannibalism (Schauberger and Walzer 2001).

IGP by *Orius* spp. on phytoseiid mites has been studied for many combinations of species (Table 1). *O. majusculus* and *O. insidiosus* showed different preferences for *N. cucumeris* versus *F. occidentalis. O majusculus* showed no preference. In contrast, *O. insidiosus* preferred *N. cucumeris* over thrips (Sanderson *et al.* 2005). *O. majusculus* showed a clear preference for *F. occidentalis* over *Iphiseius degenerans* (Berlese) (Acrina: Phytoseiidae) in choice tests (Brodsgaard and Enkegaard 2005).

O. majusculus preyed on the eggs and larvae of *A. aphidimyza.* However, the extent of IGP was affected by the presence of *A. gossypii* (Christensen *et al.* 2002).

Macrolophus caliginosus (Wagner) (Heteroptera: Miridae) is preyed on by *O. majusculus* and *Dicyphus tamaninii* Wagner (Heteroptera: Miridae) (Jakobsen *et al.* 2002; Lucas and Alomar 2001). IGP by *D. tamaninii* on *M. caliginosus* did not disrupt whitefly predation by *M. caliginosus* in tomato greenhouses (Lucas and Alomar 2002).

SIMULATION STUDIES FOR EVALUATING BIOLOGICAL CONTROL USING TWO PARASITOIDS

STRUCTURE OF THE SIMULATION MODELS

A simulation model has been developed for evaluating the IGP between *D. sibirica* and *D. isaea* for the biological control of *L. trifolii* with these parasitoids. The model comprises the leaf area growth submodel, the Type I functional response model of the parasitoids to the host density, and the IGP submodel between the two parasitoid species. The aging processes in immature stages were described using "the boxcar train method" (Goudriaan and van Roermund 1989).

D. isaea is a synovigenic species and needs host feeding for egg production. The interactions between egg load, oviposition, and the host feeding of *D. isaea* were considered in the model based on the results of laboratory experiments (Ozawa, unpublished). The life history parameters of the leafminer and the two parasitoid species and the parameters of the functional responses were calculated from the results of glasshouse experiments or from the literature (Minkenberg 1990; Ozawa unpublished; Sugimoto unpublished).

A similar simulation model was developed to predict the biological control with the release of *E. formosa* and *E. eremicus* to control *T. vaporariorum* on tomatoes. The model comprises the leaf area growth submodel, the Type I functional response model of the parasitoids to the host density, and the competition submodel between two parasitoid species. The ageing processes in immature stages were described using "the boxcar train method".

PREDICTION FROM THE SIMULATIONS

The simulations of these models suggested no significant negative effects of the interspecific interactions between two parasitoids on biological control. However, the unidirectional interactions between the two parasitoids resulted in the extinction of the inferior species in the later cropping period. When both parasitoid species were released simultaneously at different release ratios, the intermediate ratios resulted in better control than the single species release of one of the two species (Figs.1, 2).

In both cases, the systems could not persist for a long period. That is one of the reasons IGP has less effect on biological control. Actually, pest–natural enemy systems in biological control in greenhouses persist only for a shorter period than in annual or perennial agroecosystems. Brodeur *et al.* (2002) pointed out that the spatial scale of the greenhouse system is small and persists for a short period, which makes the system transient and unstable. Since the model in this simulation study did not have a spatial structure, the effect of spatial scale was not studied.

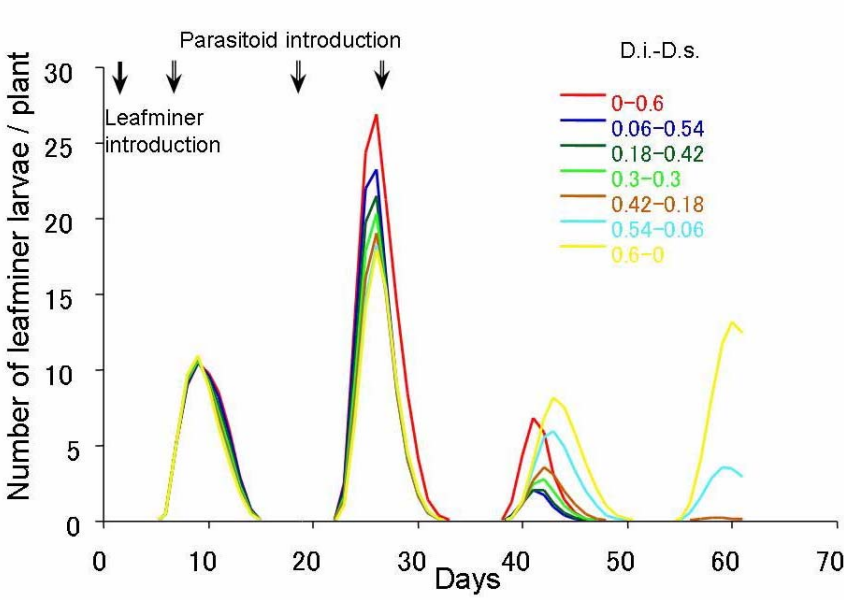

Figure 1. Evaluation of different release ratios of *D. isaea* (D.i.) and *D. sibirica* (D.s.) in the biological control of *L. trifolii*. Total number of released parasitoids was 0.6 female adults / plant per introduction.

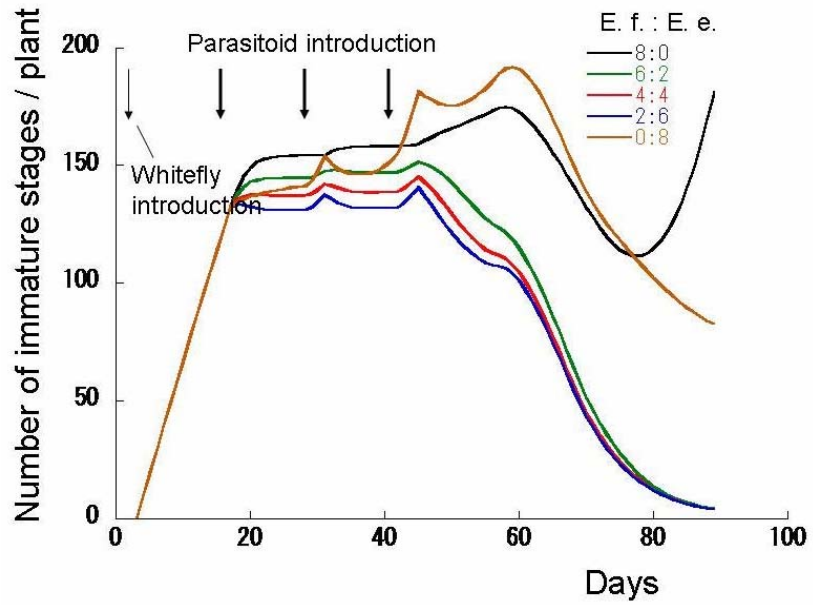

Figure 2. Evaluation of different release ratio of *E. formosa* (E.f.) and *E. eremicus* (E.e.) in the biological control of *T. vaporariorum*. Total number of released parasitoids was 8 female adults / plant per introduction.

CONCLUSIONS

IGP among natural enemies in biological control in greenhouses might commonly occur. Most of the IGP interactions seem to be unidirectional, because two natural enemies for combined use should be different in size and belong to different taxa. Although the effects of IGP on the population dynamics of pests and natural enemies have been studied for only several cases, the effect of IGP is expected to be less important in greenhouses than in annual or perennial agroecosystems.

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IMPLEMENTATION OF BIOLOGICAL CONTROL IN GREENHOUSES IN LATIN AMERICA: HOW FAR ARE WE?

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ABSTRACT

Application of biological control in greenhouse production areas in Latin America is growing. However, there are many factors negatively affecting this development, although there are currently also important positive factors stimulating biological control. This paper discusses the development of biological control in the largest developing greenhouse regions in Latin America as Brazil, Colombia and Mexico, and the factors which are affecting the implementation of such strategies.

INTRODUCTION

The world greenhouse area is currently estimated at approximately 310,000 ha, 40,000 ha of which is covered with glass, 270,000 ha with plastic. Vegetable crops are grown in about 65% of greenhouses, and ornamentals in the remaining 35%. In the past 24 years the surface areas with greenhouse have increased more than 100%, with an increase of 4.4% per year (Bueno 2005; van Lenteren 2000). Production under protected cultivation in Latin America started in the 1970's and now several countries are showing a strong increase in protected areas attracted by cultivation of high-value crops. Ornamentals occupy the largest area under protected cultivation in Latin America.

Pest and disease management form a crucial aspect of greenhouse production. Various insect and mite pests occur in the different vegetable and ornamental crops. Most of the pests are similar to those in the other greenhouse areas of the world. For many years, not enough attention has been paid to exploiting and amending production technology for the integrated management of pests in Latin America, and pest control is still mainly by chemicals. Most Latin America countries produce flowers and vegetables for the local market (with the exception of Colombia), and these products are not subjected to only very limited control regarding pesticides residues. But the situation of the export market (primarily for flowers) is quite different, mainly because of the norms and standards of protocols as EUREPGAP or ISO.

Currently biological control of greenhouse pests is being implemented in several Latin America countries, although application is still limited considering the total area of over 15,000 ha with greenhouses. But several stimuli are pushing growers to use fewer pesticides and

adopt more sustainable ways to protect crops from pests as world markets become more global, and biological control is a corner stone of sustainable production.

The approach for development and implementation of biological control in protected crops in Latin America areas should not be based on mere import and release of commercially produced exotic natural enemies (van Lenteren and Bueno 2003). The first priority is to study which pest species occur in unsprayed plots, and which of these pests are kept under natural control by native natural enemies. In the next phase a good biological control solution should be developed for those pest species that are not kept under reliable natural control, for example by timely introduction of mass produced native natural enemies.

Biological and integrated control programs can then be developed making use of the most effective native natural enemies, which might be supplemented with exotic natural enemies for those pests where native biological control agents are ineffective. Interestingly, in Latin American countries natural control of pests occurs very generally and, therefore, plays an important role. In several countries, like Brazil, Colombia and Mexico, biological control programs exist or are implemented on pilot greenhouse farms. Below, a number of examples are presented from these countries to demonstrate the progress achieved to date. Also, factors that frustrate or stimulate the implementation of biological control are discussed.

EXAMPLES OF BIOLOGICAL CONTROL STRATEGIES IN GREENHOUSE REGIONS IN LATIN AMERICA

COLOMBIA

Colombia was one of the first countries in Latin America starting with the production of ornamentals in greenhouses 35 years ago. This country is now the second largest cut flower exporter in the world after The Netherlands. About 98% of the flowers produced in Colombia are for exportation. The current official figure for cut flowers produced for export in greenhouses is 6,016 ha. A quarantine pest in the case of export flowers is *Thrips palmi* Karny (Thysanoptera: Thripidae).

Over the years the flower industry has experienced many problems and to solve them, Asocolflores (Colombian Association of Flowers Exporters) representing 75% of Colombia's flower production, has in the past years made a tremendous investment in the newest varieties and also in technology to offer the best quality. In 1996, the Florverde® Program (Green Flower) was created by Asocolflores. The program is a code of conduct aimed at sustainable production of flowers involving several areas such as human resources, natural resources, IPM, waste management and landscaping. Florverde promotes the implementation of IPM programs which are based on three principles: (1) use of reliable and timely monitoring systems that provide guidance and support to decision-making efforts; (2) give priority to the use of control strategies other than chemical controls; (3) rational and safe use of pesticides, that is, only at the times they are actually required and only in the required amounts, so as to minimize impact on human health and the environment (Rebecca Lee, pers. comm., Colombia).

Biological control of a range of pests on greenhouse ornamentals occurs on 9 ha of flowers. Biological control of leafminers has been developed and implemented in *Gypsophyla paniculata* L. by introduction and conservation of the parasitoid *Diglyphus begini* (Ashmead) (Hymenoptera: Eulophidae) (Cure and Cantor 2003). However biological control is still very little used due the complicated legislation in Colombia for import and use of exotic natural enemies. The predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) was registered for use as natural enemy a year ago, but still is not used very much in flowers. Local companies have focused on the elaboration of botanical pesticides as well fungal based biological control.

Production of vegetables in greenhouses in Colombia is a more recent development, and takes place in cold climate zones. In tomato crops at altitudes from 1,800 to 2,600 meters natural control of leafminers and aphids has been observed. For control of whiteflies, studies are conducted with species of *Encarsia*, *Eretmocerus* and the native species *Amitus fuscipennis* MacGaen and Nebeker (Hymenoptera: Platygastridae) (De Vis 2001; De Vis and Fuentes 2001; Manzano 2000).

MEXICO

The greenhouse area in Mexico is around 3,000 ha. The first commercial operations of vegetable production in greenhouses started in the 1990's on 50 ha, and they increased to around 2,208 ha today. The main vegetable crops under protected cultivation are tomato, pepper and cucumber. For the largest greenhouse vegetable crop, tomato, Mexico is known to apply biological control on 110 ha. For pepper grown in greenhouses, biological control is used on 30 ha (all information, pers. comm. Mario Steta and Rigoberto Bueno, Mexico).

Mexico, in comparison with other Latin American countries, has imported and released a number of exotic natural enemies. The legislation procedures for importation seem to be clearly defined and more advanced than in other Latin American countries. Natural enemies have been imported for biological control of whiteflies [*Encarsia formosa* Gahan and *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae)]; of leafminers [*Dacnusa sibirica* Telenga, *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae)]; of mites [*Phytoseiulus persimilis* Athias-Henriot, *Amblyseius cucumeris* (= *Neoseiulus cucumeris* (Oudemans) (Acari, Phytoseiidae), *Feltiella acarisuga* (Vallot) (Diptera, Cecidomyiidae)]; of aphids [*Aphidius ervi* Haliday, *Aphidius colemani* Viereck (Hymenoptera: Braconidae, Aphidiinae), *Aphelinus abdominalis* Dalman (Hymenoptera, Aphelinidae), *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae), *Epysirphus balteatus* De Geer (Diptera: Syrphidae)].

BRAZIL

Production under protected cultivation is a relatively recent development in Brazil. The first initiatives took place around 1970's in the South and Southeast region, and nowadays are spreading all over the country. The total greenhouse area is about 2,500 ha and most of this area is used for production of ornamentals (60%). Tomato, lettuce and sweet pepper are among the main vegetables grown in greenhouses. Chrysanthemums and roses are the largest crops grown under protected cultivation for cut flower production. In these two flower crops the major pests are thrips, aphids and mites. Frequent sprays with pesticides (it is not uncommon

to spray three times per week during the whole production cycle) result in quick development of resistance and in killing of the natural enemies, and are now also creating problems for the exportation of the products.

Studies are conducted with aphid parasitoids *Lysiphlebus testaceipes* (Cresson), *Aphidius colemani* Viereck and *Praon volucre* (Haliday) (Hymenoptera: Braconidae, Aphidiinae), and *Orius* species to control aphids and thrips in chrysanthemums and vegetables crops (Bueno *et al.* 2003; Rodrigues *et al.* 2001; Rodrigues *et al.* 2005; Silveira *et al.* 2004). All these species of natural enemies were found in Brazilian agro-ecossystems. We have set the following goals: (1) follow development of the pests and their native natural enemies in commercial greenhouses; (2) studies on biology, behavior and influence of environmental conditions on pests and natural enemies, (3) development of methods of mass rearing of the native natural enemies, and (4) release of natural enemies in commercial crops, including studies on release rates (Bueno 2005; Bueno *et al.* 2003).

For the aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) and the thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), both key pests in chrysanthemum, we have now developed satisfactory biological control. Control of *A. gossypii* populations was achieved by seasonal inoculative releases of the parasitic wasp *L. testaceipes.* The predator *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) showed to effectively control agent thrips in cut chrysanthemum in commercial greenhouses (Bueno *et al.* 2003; Silveira *et al*. 2004).

The development of biological control of lepidopteran pests [mainly *Tuta absolu*ta (Meirick) (Lepidoptera: Gelechiidae)] by seasonal inoculative releases of *Trichogramma pretiosum* (Riley) (Hymenoptera: Trichogrammatidae) is now evaluated in Brazil. Further, the control of mites (*Tetranychus* spp.) by *Phytoseiulus macropilis* (Banks) and *Neoseiulus californicus* (MacGregor) (Acari: Phytoseiidae) is currently tested.

CHILE

The greenhouse area in Chile is around 1,500 ha. Some experimental biological control programs have been developed in tomato crops where greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae), is controlled with several *Encarsia* and *Eretmocerus* species, and a leafmining caterpillar, *Tuta absoluta* (Meirick), with a native egg parasitoid *Trichogramma nerudai* Pintureau and Gerding (Hymenoptera: Trichogrammatidae)

ECUADOR

The area of ornamentals under protected cultivation in Ecuador is about 1,200 ha. Ecuador together with Colombia provide the United States with 80% of its cut flower imports, and 70% of the flowers produced by Ecuador are exported to the USA. However the demand to apply ISO standards is creating problems for flower exportation by Ecuador. An IPM and biological control program of pests has been conducted in roses on about 10ha.

OTHER COUNTRIES

Bolivia has a growing commercial flower production in greenhouses. The greenhouse area in Argentina is around 1,000 ha. In both countries biological control is not yet applied, although development of biological control is being considered.

FACTORS LIMITING APPLICATION OF BIOLOGICAL CONTROL IN LATIN AMERICA

Several problems complicate the implementation of biological control in greenhouses in Latin America. These factors include the following:

1. Lack of commercial availability of natural enemies. There are only some producers and the production is limited to one or a few species of natural enemies.
2. Bureaucratic and time-consuming procedures concerning importation and release (quarantine regulations) of natural enemies that have shown to be effective elsewhere. Often legislation is not ready yet and under discussion.
3. The excessive use of pesticides pushed by aggressive marketing strategies of pesticides dealers, connected with the power of the chemical industry.
4. The wide variety of ornamental crops (> 300 species) and cultivars (can be > 100 per crop species) each demanding specific biological control/IPM programs.
5. Limited greenhouse technology. Greenhouse frames may be constructed of wood, which harbor pests and they are very difficult to clean. There are exceptions such as in Brazil, Colombia and Mexico.
6. Control of microclimatological conditions. Most climate control is limited to opening and closing of the greenhouses, the use of shade screens or whitewashing of the plastic. The mild climate outside enables pests to develop year around and pest pressure is, therefore, very high. Ventilation leads to continuous migration of organisms in and out of the greenhouse.
7. Lack of biological control and IPM technology transfer. An efficient exchange of information between university, institute and grower is often not available, and also extension services are often not well informed about IPM and biological control. Most of the growers in Latin America are often less specialized that those in e.g. Europe, but there are important exceptions such as in Brazil, Colombia and Mexico (van Lenteren and Bueno 2003).

FACTORS STIMULATING APPLICATION OF BIOLOGICAL CONTROL IN LATIN AMERICA

Although there are quite a number of factors frustrating the implementation of biological control in greenhouses in Latin America, there are the following positive factors for its development:

1. The most important stimulating factor is that there are many local natural enemy species available. For example, while doing the first biological control experiments in greenhouses, we found spontaneous invasion of natural enemies into the greenhouse, resulting in good control of the major pests (Bueno 1999; Bueno *et al.* 2003). This may mean that we can control most pests with native natural enemies, and, thus, prevent the problems related to import of exotic natural enemies (van Lenteren *et al*. 2003)

2. Recently, the commercial mass production of a number of natural enemies started in Latin America. With the availability of these natural enemies, biological control becomes a realistic option for pest control (Parra 2002)
3. For small scale farming, the money for chemical pesticides is usually not available, and farmers therefore appreciate the use of biological control.
4. The recent revival of the Neotropical Regional Section of IOBC may stimulate collaboration in this field, which then will result in easier access to and exchange of information about new natural enemies. The formation of an IOBC-NTRS working group on IPM in greenhouses might speed up development of biological control in greenhouses.

CONCLUSIONS

Greenhouses are of very different construction in Latin America, and this strongly affects pest development and control. Some greenhouses are very simple structures with hardly any possibilities for climate management, the growers are only part time involved in production and have other primary professions; the result is poor pest management and no interest in knowledge intensive biological control programs. Other greenhouses are of the same high technological quality as those in Europe, and have professional pest managers. With good education of these managers and growing availability of natural enemies, biological control is a realistic possibility.

The area with greenhouses is strongly growing in Latin America countries. Pest control is still mainly by chemical pesticides and several factors currently limit application of biological control. However, many native beneficial insects occur in Latin America and have proven to be good natural enemies for control greenhouse pests. The next step should be to stimulate research in this area and to develop greenhouse biological control networks in Latin America under the guidance of IOBC, so that the Latin American region can use the excellent knowledge developed earlier in Europe.

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AUGMENTATIVE BIOLOGICAL CONTROL IN GREENHOUSES: EXPERIENCES FROM CHINA

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ABSTRACT

To enhance biological control of insect pests in greenhouses, facilities and procedures for mass production of the parasitoids, *Eretmocerus* sp., *Encarsia formosa*, and *Trichogramma brassicae*, and the predator, *Aphidoletes aphidimyza* were successfully developed in Hengshui, Hebei province, China. Mass production of the aphelinid wasps was achieved by using different plant varieties and host insect species, as well as specific rearing procedures and techniques. Production of *T. brassicae* was greatly enhanced through the design of special devices and improved rearing techniques. Annual production of natural enemies in our institution reached 2 billion individuals. Biological control experiments conducted in sunlight greenhouses and plastic greenhouses allowed innovative techniques to be developed. Inoculative release techniques were established, including preparation before release, appropriate release time, release rate and special measures. Through experimental results and demonstrations, populations of aphelinid parasitoids and cecidomyid predators were able to establish and play very important roles in pest control on tomato, cucumber, and ornamental crops grown in greenhouses. Parasitism of the whiteflies, *Trialeurodes vaporariorum* and *Bemisia tabasi* was as high as 85% to 96%. Natural enemies released also effectively suppressed aphid populations on tomato and cabbage crops. Egg parasitism of the cabbage butterfly, *Pieris rapae*, and the cotton bollworm, *Helicoverpa armigera*, by *Trichogramma* wasps reached 78% to 95% on average. It was shown that natural enemies can suppress populations of target insect pests to below the economic threshold in greenhouse vegetable crops. When these techniques are combined with other non-chemical means of control for diseases and non-target insect pests, such as application of target specific fertilizers, augmentative biological control practices could greatly reduce the utilization of chemical pesticides, making non chemically-polluted vegetable products possible. A great economic benefit was acheived in 11,000 ha of biological control demonstration areas in Hebei, Beijing and Tianjin, by implementing the above augmentation biocontrol techniques from 2001 to 2004.

INTRODUCTION

As the most important method of vegetable production, greenhouses are becoming more and more prevalent in North China, and people are paying more attention to greenhouse pests.

Controlling greenhouse pests using chemical pesticides raises environmental concerns and can result in problems such as the development of resistance in pests. The use of biological control can overcome these problems while still providing adequate pest control.

ARTHROPOD PESTS AND THEIR NATURAL ENEMIES IN GREENHOUSES

The main arthropods that are greenhouse pests in North China are the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), tobacco whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), cabbage aphid, *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae), and several acarid species. There are also other pests in greenhouse, such as *Tetranychus urticae* Koch (Hemiptera: Tetranychidae), *Polyphagotarsonemus latus* Banks (Hemiptera: Hemisarcoptidae), *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) and some coccids, etc. (Cheng 2002; He 1996; Qu *et al.* 2002; Shi *et al.* 1995; Zhang *et al.* 1997) These pests cause significant damage on the vegetables produced in these greenhouses.

There are many species of parasitic wasps that attack whitefly, including 34 from the genus *Encarsia*, 14 of the genus *Eretmocerus*, and several species of *Amitus* and *Metaphycus*. In China there are about 19 species of parasitic wasps which include *Encarsia formosa* Gahan, *Encarsia pergandiella* Howard and *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae). Approximately 114 species (9 orders, 13 families) of whitefly predators are known to exist in China. Some of the most important of these are *Lygus pratensis* L. (Hemiptera: Miridae), *Chrysoperla sinica* Tjeder (Neuroptera: Chrysopidae) and several predatory mites (Zhang *et al.* 2003; 2004).

Some predators of greenhouse aphids were found to be: *Leis axyridis* Pallas, *Propylea japonica* Thunberg, *Coccinella septempunctata* L., *Adonia variegata* Coeze (Coleoptera: Coccinellidae), *Syrphus corollae* F., *Epistrophe balteata* De Geer, *Lasiopticus Pyrastri* L., *Sphaerophoria scripta* L. (Diptera: Syrphidae), *Aphidoletes apidimyza* Rondani (Diptera: Cecidomyiidae), *Eringonidium graminicolum* Sundevall (Araneae: Erigonidae), *Pardosa T-insignita* Boes *et* Str. (Araneae: Lycosidae), *Chrysopa sinica* Tjeder, *Chrysopa septempunctata* Wesmael, *Chrysopa formosa* Brauer (Neuroptera: Chrysopidae), *Hemerobius humuli* Linnaeus (Neuroptera: Hemerobiidae), *Nabis sinoferus* Hsiao, *Nabis stenoferus* Hsiao (Hemiptera: Nabidae), *Orius minutus* L. (Hemiptera: Anthocoridae), and *Deraeocoris punctulatus* Fall (Hemiptera: Miridae). Parasitoids that help control these greenhouse aphids include species from the hymenopteran families: Ichneumonidae, Braconidae, and Chalcidae. As well, a parasitic fungus (Chen 2002; Chinese Academy of Science (Zooscopy Institute) 1978; He *et al.* 1986; Liu 2000; Xia *et al.* 2004).

Non-parasitic natural enemies of phytophagous mites found in China include ladybird beetles, the anthocorid, *Phytoseiulus persimilis* Athias-Henriot (Acariformes: Phytoseiidae), and *Campylomma chinensis* Schuh (Hemiptera: Mirridae). It has been reported that *P. persimilis* successfully controls phytophagous mites both in its native habitat, and in other habitats abroad (Dong *et al.* 1986; Liang 2004; Yang *et al.* 1989).

Worldwide, arthropod natural enemies of thrips include species of Nabidae, Miridae, Anthocoridae, Sphecidae, Eulophidae, Trichogrammatidae, Mymaridae, Coccinellidae,

Syrphidae, Dolichopodidae, Cecidomyiidae, Aeolothripidae, and some predatory mites (Ananthakrishnan 1973; Lewis 1973).

In China, there are few reports about the natural enemies of common thrips. Qing *et al.* (2004) found that predatory arthropods include *Campylomma chinensis*, *Cyrtorhinus lividipennis* Reuter (Hemiptera: Miridae), *Orius simillis* Zheng (Hemiptera: Anthocoridae), *Geocoris pollidipennis* F. (Hemiptera: Lygaeidae), *Scolothrips takahashii* Piesneer (Thysanoptera: Thripidae), some ladybird beetles, spiders, and ants. A total of 10 families and about 20 species of predators; among them, *C. chinensis* are the dominant natural enemies (Qing *et al.* 2004).

The known predatory arthropods of leaf miners include *Propylaea japonica*, *C. septempunctata*, *E. graminicolum*, and *P. T-insignita*. The parasitic wasps include *Opius spp.* and *Dacnusa spp.* (Lu *et al.* 2000); species of *Chrysocharis*, *Dacnusa*, *Diglyphus*, *Opius*, *Neochrysocharis*, *Hemiptarsenus* and *Halticoptera* are some of the more common parasitoids found to control leaf miner (Chen *et al.* 2001).

MASS-REARING OF BENEFICIALS IN CHINA

In recent years, techniques for mass-rearing beneficials have been developed and improved, to efficiently control major arthropod greenhouse pests. Several species can now be produced on a large-scale, and released in greenhouses in China. Beneficials such as *Trichogramma spp.*, *E. formosa*, *Eretmocerus spp.*, *P. persimilis* and *Aphidoletes apidimyza* have been successfully mass-produced by the Hengshui Tianyi Bio-control Company, Dryland Farming Institute.

TRICHOGRAMMA SPP.

In order to rear *Trichogramma spp.* with high selectivity to vegetable pests, *Sitotroga cerealla* eggs were used as host eggs. Several species, including *T. evanescens, T. pretisum, T. brassicae, T. embryophagum,* and *T. cacoaciae* can be mass-produced using this system. For mass-production of *S. cerealla* eggs, new production line and rearing techniques were developed. A specially made egg auto-collection machines were used and over 10 million eggs could be collected in 24 hours, provided there is an ample supply of emerged moths. Other equipment for use in moth rearing and egg purification was also developed by Hengshui Tianyi Bio-control Company in Hebei, China (Zheng 2003; 2004).

ENCARSIA FORMOSA AND *ERETMOCERUS* SP.

It is very important to find a proper variety of fod plants to feed to the insect hosts of both *Encarsia* and *Eretmocerus*. Since tobacco can be perennially cultured in greenhouses, varieties of tobacco were screened for their suitability as host plants for whitefly. Selection of these varieties ensures that sufficient numbers of whiteflies survive for a longer time, offering ample host accessibility to both *Encarsia* and *Eretmocerus*. Wasps oviposit into the young whitefly larvae, and when they develop to their pupal stage they are harvested. A special mass-production procedure of *Encarsia* and *Eretmocerus* has been developed by HTBC in Hebei, China (Zheng 2004).

APHIDOLETES APIDIMYZA

For mass rearing of *A. apidimyza,* insect hosts and their host plants were selected. The HTBC has also developed mass-rearing techniques of *A. Apidimyza* (Zheng 2004).

OTHER BENEFICIALS

Jiexian Jiang studied the mass-rearing and application of *Aphidius gifuensis,* and found that this parasitoid could be used to control the damage caused by aphids (Jiang *et al.* 2003). Although there are many natural enemies of aphids worldwide, only *A. apidimyza* has been reared on a large scale, and used in greenhouses.

It is very difficult to mass-rear ladybird beetles with artificial food. It has been reported however, that an artificial food diet, suitable for a female ladybird beetle to lay eggs on, has been successfully produced in China. An artificial diet for lacewings has also been successfully made, what's more, all stages of lacewing could develop on artificial eggs.

RELEASE OF BENEFICIALS AND BIO-CONTROL IN GREENHOUSES

PREPARATION BEFORE RELEASE

To satisfy the need for a controlled effect, some preparatory measures need to be taken before the release of natural enemies. These measures include: growing clean seedlings for transplanting, cleaning and sterilizing greenhouses for about 15 days and fixing screens on ventilation devices to prevent access by outside insects. The above precautions allow inoculative releases of beneficials to be successfully made after transplanting seedlings into greenhouses.

RELEASE OF *ENCARSIA FORMOSA* TO CONTROL WHITEFLY

These tiny wasps lay eggs inside the scales of developing whitefly larvae. The parasitoids then complete their development inside the whitefly larvae, killing the host in the process. Upon emergence, adults immediately begin to search for other larvae. Parasitized whitefly larvae are easy to recognize, as they will turn black over time.

When the average number of adult whitefly reaches 1000 in one greenhouse (about 0.05ha.), it is time to release *E. formosa*. The ratio of enemy versus adult pests is 3:1 (3000-5000 wasps per house). Wasps are introduced every 7-10 days, and after 3-4 releases, a balance is reached between wasps and whiteflies, and the introduction of the parasitoids to the greenhouse can be stopped. The temperature of the greenhouse containing the wasps should be controlled and maintained between 15-35 °C.

RELEASE OF *APHIDOLETES APIDIMYZA* TO CONTROL APHIDS

To control aphids successfully, *A. apidimyza* was introduced into the greenhouse before the aphid could damage the vegetables. These predators cripple the aphids by quickly injecting a paralyzing toxin, then sucking out the body fluid, leaving a shriveled aphid husk still attached to the leaf. When aphid numbers are high, they may kill many more aphids than they eat. Fully-grown predator larvae leave the plant to pupate in the soil.

If some wheat plants containing wheat aphid are brought into the greenhouse, *A. apidimyza* will survive on these aphids and the aphids will not feed on the greenhouse vegetables. As a result, initial aphid numbers can be controlled at low-density levels. At the first occurrence of aphids, *A. apidimyza* was released in the ratio of 1 larvae for every 20 aphids, and had a controlling effect after 2-3 continual releases.

RELEASE OF *TRICHOGRAMMA* SPP. TO CONTROL PESTS OF LEPIDOPTERA

In greenhouses without screen or ventilation, pests of *Lepidoptera* may seriously damage vegetables. In this case, *Trichogramma* spp. should be introduced. Several days after their introduction into the greenhouse, *Trichogramma* spp. wasps will emerge from parasitized eggs and seek out a new lepidopteran host.

RELEASE OF *PHYTOSEIULUS PERSIMILIS* TO CONTROL PHYTOPHAGOUS MITES

The predatory mite, *P. persimilis*, is a very good natural enemy to control phytophagous mites. To efficiently control these mites, the ratio between *P. persimilis* and phytophagous mites should be about 1:10 to 1:20. *P. persimilis* was released every 7-10 days, and after 3-4 weeks the number of phytophagy mites dropped notably (Dong *et al.* 1986; Li *et al.* 2004).

This predator does not feed on the plant or shrub and is fully dependent on the spider mite and its eggs for food. Generally, only one introduction of *P. persimilis* is required each season, because the predator population remains in low numbers once control is gained. To obtain optimal reproduction rates, the temperature of the greenhouse should be maintained between 21-27°C.

OTHER BIOLOGICAL CONTROL METHODS IN GREENHOUSES

PATHOGENIC FUNGI OF INSECT PESTS

Most pathogenic fungi used for the control of whitefly are Hyphomycetes including species of *Paecilomyces*, *Verticillium*, and *Aschersonia*. *Aschersonia aleyrodis* Webber (Sphaeropsidales: Sphaeriodaceae) is an important pathogenic fungus of whitefly and coccids, and much attention was given to *Paecilomyces fumosoroseus* Wize and *Verticillium lecanii* Zimmermann (Moniliales: Moniliaceae) (Xiao 2002; Zhang 2003; 2004).

There are 37 species of pathogenic fungus that can be used for the control of aphids. These are included within 9 genera of Entomophthorale and 7 genera of Hyphomycetes; among these, *Beauveria bassiana* Balsamo (Moniliales: Moniliaceae) and *V. lecanii* can also be used to control common thrips (Li *et al.* 2005; Qin *et al.* 2001).

BIOLOGICAL PESTICIDES

The main biological pesticides used in greenhouses today include *Bacillus thuringiensis* (Berliner) [*Bt*], abamectin, Azadirachtin and Polynactin. Athough pheromones were used to control pests during the 1960's, there are few reports on this topic. Due to the closed conditions within a greenhouse environment, kairomones that are produced by insect pests are not useful to many natural enemies. Some plants can produce metabolites such as terpene, alkene,

alkaloid, lignin, steroid, flavone and polysaccharide, which can then be used to control greenhouse pests. Naturally occuring pesticides such as plecocidin, which is developed from plants, can be used in greenhouses to control pests and will not lead to environmental problems.

YELLOW BOARDS

The use of yellow boards within a greenhouse environment can efficiently monitor the effects of biological control efforts. Approximately 20 yellow boards are sufficient in one house, and when hung properly in greenhouses, can attract whiteflies, aphids and leaf miners.

GREENHOUSE CONDITIONS IN CHINA

Currently, the total greenhouse area in China is over 2 million ha. These all fall within three different categories:

Glasshouse. The glasshouse is the style of greenhouse that provides the optimal conditions for use with natural enemies. There are about 1300ha of glasshouse in China, making up no more than 0.1% of the total greenhouse area. The main advantage to using this type of greenhouse is the control one has over the environmental conditions through the use of heaters, fans and other devices. The temperature can be maintained above 15°C during the cold season and below 35°C during the hot season, and the humidity in these glasshouses can also be reduced or raised to an optimal level. Optimal control can easily be reached after the release of the beneficials into the glasshouse; however, much attention should be paid to monitoring the development of the insect pests while different crops with different growing seasons are harvested in the same house.

Cold plastic house. One of the most extensively used greenhouse styles in China is the cold plastic house. These are covered only by plasticand crops cannot be grown during the wintertime; instead crops are produced during two growing seasons. For the first season, crops are planted in spring and harvested in summer. Since pests are not a serious problem in spring, farmers usually neglect to control them at the beginning of planting. Farmers also pay little attention to the pests in the summer, since the vegetables are beginning to be harvested. For the second season, crops are planted in summer or the beginning of autumn, and it is at this time when high populations of pests occur. Most farmers grow seedlings without using effective pest prevention methods; as a result, many pests are easily transported from outside into the plastic house when vegetables are transplanted. These high populations of pests make control much more difficult when releasing natural enemies.

Warm plastic house. Another style of greenhouse, used most extensively in China, is the warm plastic house. A thick wall built on the north side of the house prevents penetration of the strong wind during cold winters, and allows crops to grow year-round. During most of the year, throughout each growing season temperature and humidity are satisfactory to release beneficial arthropods. It is only during the wintertime, because there is generally no heating temperature and humidity levels are unfavourable and the use of natural enemies is not possible.

In China, most of the greenhouses used are made of plastic, and are either a warm house or a cold house. To obtain efficient control of arthropod pests after the release of beneficials, we strongly suggest that farmers grow clean seedlings and use screen on the ventilation systems of their greenhouse, before applying biological control techniques.

With the improving demand for green food and the increasing greenhouse area, bio-control in greenhouses will have a more important place with regards to pest control and safe-food production. Improving bio-control and rearing measures will provide more efficient control over greenhouse pests.

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COMPATIBILITY CONFLICT: IS THE USE OF BIOLOGICAL CONTROL AGENTS WITH PESITICIDES A VIABLE MANAGEMENT STRATEGY?

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ABSTRACT

Biological control or the use of natural enemies is an alternative pest management strategy for dealing with arthropods. However, natural enemies may not always provide adequate control of plant-feeding insects and mites in greenhouses. As a result, research has assessed the concept of using natural enemies in conjunction with pesticides and the potential compatibility when both pest management strategies are implemented together. There are a variety of factors that influence the ability of using natural enemies with pesticides, these include whether the natural enemy is a parasitoid or predator, natural enemy species, life stage sensitivity, rate of application, timing of application, and mode of action of a particular insecticide or miticide. Pesticides may impact natural enemies by affecting longevity (survival), host acceptance, sex ratio, reproduction (fecundity), foraging behavior, percent emergence, and development time. In our studies, we have found a number of pesticides to be compatible with the natural enemies of the citrus mealybug, *Planococcus citri* and fungus gnats, *Bradysia* spp. For example, we have demonstrated that foliar and drench applications of the insecticides novaluron and pyriproxyfen, and the fungicides fosetyl-Al and mefenoxam to be compatible with the predatory mite, *Stratiolaelaps scimitus*. We have also shown that the insecticides azadirachtin and pyriproxyfen are compatible with the citrus mealybug parasitoid, *Leptomastix dactylopii*. Additionally, the insecticides buprofezin, pyriproxyfen, and flonicamid were not harmful to the adult stage of the mealybug destroyer, *Cryptolaemus montrouzieri*. Despite the emphasis on evaluating the compatibility of natural enemies with pesticides, it is important to assess if this is a viable and acceptable pest management strategy in greenhouses.

INTRODUCTION

Biological control or the use of natural enemies such as parasitoids, predatory mites, predatory bugs, and/or beneficial bacteria, fungi, and nematodes is an alternative strategy to manage greenhouse pests (Van Driesche and Heinz 2004). However, the sole use of biological control may not always be sufficient to control plant-feeding insect or mite populations in

greenhouses (Medina *et al.* 2003). As a result, research within the last 5 to 10 years has investigated the possibility of using so-called "biorational" or "reduced risk" insecticides or miticides in conjunction with biological control agents (=natural enemies) to determine if there is compatibility when both management strategies are implemented together. Those insecticides and miticides that are classified as biorational or reduced risk include insect growth regulators, insecticidal soaps and horticultural oils, and microbials including beneficial bacteria and fungi, and related compounds.

If a given insecticide or miticide kills a particular target pest or pests, why would it not kill a natural enemy? It is equally important to define what is meant by "compatibility?" Biorational insecticides and miticides are considered to be more selective to natural enemies and potentially more compatible than most conventional insecticides and miticides because they are active on a broad range of target sites or systems (Croft 1990). In fact, several commercially available biorational insecticides/miticides state that their products are not disruptive to beneficial insects and mites. However, research conducted worldwide has shown that biorational insecticides/miticides may in fact be harmful to certain natural enemies. Although biorational insecticides/miticides may not be directly toxic to a particular natural enemy there may be indirect effects such as delayed development of the host and natural enemy inside, delayed adult emergence, and/or decreased natural enemy survivorship (Croft 1990). In general, the harmful effects of biorational insecticides and miticides may be due to direct contact, host elimination, residual activity, or sublethal effects (Parrella *et al.* 1999):

Direct contact: directed sprays of biorational insecticides/miticides may kill natural enemies or in the case of parasitoids they are killed while in developing hosts.

Host elimination: biorational insecticides/miticides may kill hosts, which may lead to natural enemies dying or leaving because they are unable to locate additional hosts.

Residual activity: although spray applications of biorational insecticides/miticides may not directly kill natural enemies, any residues may have repellent activity thus influencing the ability of parasitoids or predators to locate a food source.

Sub-lethal effects: biorational insecticides/miticides may not directly kill a natural enemy, but may affect reproduction such as sterilizing females, reducing the ability of females to lay eggs or impact the sex ratio (number of females vs. males). Additionally, foraging behavior may be modified thus influencing the ability of a parasitoid or predator to find a host (Elzen 1989). Also, those parasitoids that host feed such as the greenhouse whitefly parasitoid, *Encarsia formosa* may inadvertently consume residues on hosts after a spray application. Residues on a potential host may make them unacceptable to a parasitoid or predator.

Differences in natural enemy susceptibility to biorational insecticides/miticides may be due to a number of factors including 1) whether the natural enemy is a parasitoid or predator, 2) species of natural enemy, 3) life stage (i.e., egg, larva, pupa, and adult) sensitivity, 4) developmental stage of host, 5) rate of application, 6) timing of application, and 7) type or mode of action of biorational insecticide or miticide used. All these differences are complex primarily

due to the interactions that may occur among the factors mentioned above and the variability in natural enemy sensitivity. Further complicating the "picture," the harmful effects from biorational insecticides/miticides may not be associated with the active ingredient but due to inert ingredients such as carriers or solvents (Cowles *et al.* 2000).

Biorational insecticides/miticides are generally more specific in pest activity and more physiologically sensitive to natural enemies than conventional insecticides/miticides (Croft 1990). A number of biorational insecticides/miticides used in greenhouses have been evaluated for both their direct and indirect effects on natural enemies. Below are descriptive examples, based on studies, on the compatibility of biorational insecticides and miticides with various natural enemies.

EFFECTS OF PESTICIDES ON NATURAL ENEMIES

INSECT GROWTH REGULATORS

The insect growth regulators that have been evaluated for both their direct and indirect effects on natural enemies include the juvenile hormone mimics pyriproxyfen, and kinoprene; the chitin synthesis inhibitors diflubenzuron and buprofezin; and the ecdysone antagonists tebufenozide and azadirachtin.

Pyriproxyfen. Pyriproxyfen, in laboratory studies, is non-toxic or harmless to the larval and adult stages of the green lacewing, *Chrysoperla carnea* (Medina *et al.* 2003) and predatory bugs, *Orius* spp. with no harmful effects on adult female oviposition and egg viability (Nagai 1990). Pyriproxyfen is also non-toxic to the predatory bug, *Orius laevigatus* via ingestion and residual contact (Delbeke *et al.* 1997). Although harmless to certain predatory insects, pyriproxyfen is toxic to immature parasitoids developing inside the silverleaf whitefly, *Bemisia argentifolii* nymphs (Hoddle *et al.* 2001). Natural enemy species may influence compatibility as demonstrated with pyriproxyfen, which appears to be harmless to *Eretmocerus eremicus* (Hoddle *et al.* 2001) and *Encarsia pergandiella*, but is highly toxic to *Encarsia formosa* (Liu and Stansly 1997).

Kinoprene. This insect growth regulator is consistently harmful to certain natural enemies, especially parasitoids. As mentioned above, the rate used may influence natural enemy susceptibility. For example, kinoprene reduces adult emergence of the leafminer parasitoid, *Opius dimidiatus* (Lemma and Poe 1978) and the aphid parasitoid, *Aphidius nigripes* (McNeil 1975) at all rates tested. Applications of kinoprene may inhibit adult emergence when applied to hosts containing the larval and pupal stages of certain parasitoids (McNeil 1975). It has been shown that kinoprene is extremely toxic to the aphid parasitoid, *Aphidius colemanii* when exposed to directed sprays and one-day old residues (Olson and Oetting 1996). Furthermore, kinoprene-treated poinsettia (*Euphorbia pulcherrima*) leaves are harmful to the silverleaf whitefly parasitoid, *Eretmocerus eremicus* six and 96 hours after treatment (Hoddle *et al.* 2001). Although harmful to parasitoids, kinoprene is less toxic to certain predators and different life stages. For example, applications of kinoprene did not negatively affect ladybird beetle eggs (Kismali and Erkin 1984).

Diflubenzuron. Diflubenzuron has minimal impact on natural enemies when applied either directly or indirectly under laboratory conditions. However, the life stage (egg, larvae, pupae, and adult) treated influences the effects of this chitin synthesis inhibitor. For example, diflubenzuron is harmful to the early larval stages of green lacewing (*Chrysoperla carnea*) whereas later larval stages are not affected (Medina *et al.* 2003; Niemczyk *et al.* 1985). It has been demonstrated that the young larvae of the mealybug destroyer, *Cryptolaemus montrouzieri* when treated with diflubenzuron fail to develop into adults whereas diflubenzuron has minimal impact on the citrus mealybug parasitoid, *Leptomastix dactylopii* (Mazzone and Viggiani 1980).

Buprofezin. Buprofezin is toxic to the larval stage of predatory ladybird beetles whereas it is less toxic to adult ladybird beetles (Smith and Papacek 1990), although it may have a sterilizing effect on some species (Hattingh and Tate 1995). Buprofezin is less harmful to other predators as demonstrated in a laboratory study where applications of buprofezin did not negatively effect the development (nymph to adult) of the predatory bug, *Orius tristicolor* (James 2004). In general, buprofezin is less toxic to parasitoids (Jones *et al.* 1998). For example, buprofezin does not effect oviposition of the two whitefly parasitoids, *Eretmocerus* sp., and *Encarsia luteola* when the young or adults are exposed to spray residues. Additionally, buprofezin has no effect on the foraging behavior of adult *Eretmocerus* sp. (Gerling and Sinai 1994).

Tebufenozide. In laboratory studies, tebufenazide is harmless to the green lacewing, *Chrysoperla carnea* (Medina *et al.* 2003). This insect growth regulator, which is primarily used against caterpillar larvae, does not affect adult green lacewing female reproduction (Medina *et al.* 2003).

Azadirachtin. Azadirachtin applications have been shown to negatively affect green lacewing, *Chrysoperla carnea* females by inhibiting oviposition (Medina et al. 2003). However, in a large-scale laboratory study, applications of azadirachtin were not toxic to the egg and adult stages of the predatory mites *Phytoseiulus persimilis* and *Amblyseius cucumeris* when exposed to treated bean leaves (Spollen and Isman 1996). Studies have also shown that the number of eggs laid by the aphid predator, *Aphidoletes aphidimyza* are not negatively affected by azadirachtin (Spollen and Isman 1996).

INSECTICIDAL SOAP AND HORTICULTURAL OIL

Direct spray applications (wet sprays) and short-term residues of insecticidal soap and horticultural oil are toxic to most natural enemies, especially parasitoids. However, once the residues have dissipated they are less harmful. Studies with the western flower thrips predatory mite, *Neoseiulus* (=*Amblyseius*) *cucumeris* have indicated that this mite is more sensitive to horticultural oil than insecticidal soap (Oetting and Latimer 1995). Direct applications of horticultural oil are harmful to the predatory mite, however, 1 to 2% concentrations have been shown to be less toxic. Although insecticidal soap appears to be minimally harmful to the predatory mite, sprays of a 4% insecticidal soap have been shown to be very toxic (90% mortality after 48 hours) (Oetting and Latimer 1995). Direct spray applications of insecticidal soap are extremely toxic to the twospotted spider mite predatory mite, *Phytoseiulus persimilis* (100% mortality), whereas there are no harmful effects 3 days after release (Osborne and Petitt 1985).

BACTERIA

In general, sprays of *Bacillus thuringiensis* (*Bt*) are safe to most predators including ladybird beetles, green lacewing, and certain predatory bugs. However, initial sprays may delay the development of certain natural enemies. The effects of *Bt* on the different life stages of natural enemies have been shown to be highly variable (Croft 1990). Additionally, the effects of *Bt* may take longer to impact natural enemies compared to other biorational insecticides. It appears that the larval stage of certain natural enemies such as green lacewing (*Chrysoperla* sp.) and ladybird beetles are more susceptible to *Bt* sprays than adults (Kiselek 1975). It is important to note that any lethal or sub-lethal effects may not be directly caused by the bacteria, but indirectly by altering the available food source or killing hosts before they complete development (Marchal-Segault 1975).

FUNGI

Entomopathogenic fungi vary in how they impact natural enemies depending on whether natural enemies consume spores or they are directly affected by sprays. Natural enemies may ingest fungal spores when either grooming (cleaning themselves) or when feeding on a contaminated host or food source. The fungi *Metarhizium anisopliae* and *Beauveria bassiana* can infect and harm ladybird beetles, depending on the concentration. Direct sprays of *M. anisopliae* and *B. bassiana* results in 97% and 95% mortality, respectively of adult ladybird beetles. However, the severity of the effect is very much dependent on the concentration of spores applied (James and Lighthart 1994). Applications of entomopathgenic fungi may indirectly affect predators that feed on hosts that have been sprayed. For example, 50% of mealybug destroyer (*Cryptolaemus montrouzieri*) larvae died when they consumed mealybugs that were sprayed with a *B. bassiana* product. However, the product was harmless to the adult (Kiselek 1975). Direct applications of the fungus, *Cephalosporium lecanii* had no impact on the longevity of the leafminer parasitoid, *Diglyphus begini* (Bethke and Parrella 1989). In contrast, direct sprays of this same fungus were shown to be harmful to the aphid parasitoid, *Aphidius matricariae* (Scopes 1970) and the greenhouse whitefly parasitoid, *E. formosa* (Ekbom 1979).

SPINOSAD

The impact of spinosad on natural enemies has been extensively studied since its introduction. It has been demonstrated that direct applications (wet sprays) of spinosad are extremely harmful to parasitoids including *Aphidius colemani* and *E. formosa*, however, any toxic effects generally decrease as the spray residues age (Miles *et al.* unpublished). Spinosad applications have been shown to be toxic to the eggs of *Trichogramma* spp. parasitoids and the larval stage (Consoli *et al.* 2001). Applications of spinosad have exhibited toxic effects to *E. formosa* and *Orius laevigatus* shortly after treatment—but populations of both were not seriously affected after 2 to 3 weeks. Spinosad has been shown to not harm the larval stage of the aphid predatory midge, *Aphidoletes aphidimyza* (Miles *et al.* unpublished).

Spinosad appears to be very compatible with many predatory insects and mites. Studies have demonstrated that spinosad has no direct or indirect negative affects to green lacewing (*Chrysoperla carnea*) (Medina *et al.* 2001), ladybird beetle (*Hippodamia convergens*), minute pirate bug (*Orius laevigatus*), big-eyed bug (*Geocoris punctipes*), and damsel bug (*Nabis* sp.) (Thompson *et al.* 2000). Spinosad has also been shown to not directly harm predatory mites

including *Amblyseius californicus*, *P. persimilis*, *A. cucumeris*, and *Hypoaspis miles* at the rates tested (Miles *et al.* unpublished).

UNIVERSITY OF ILLINOIS RESEARCH

A major part of our research effort at the University of Illinois is to assess the compatibility of commercially available insecticides and miticides with natural enemies. For example, we have conducted several studies to test the direct and indirect effects of insect growth regulators on the natural enemies of fungus gnats and mealybugs. In our research, we found that foliar and drench applications of the insect growth regulators novaluron and pyriproxyfen were not directly or indirectly harmful to the soil-predatory mite, *Stratiolaelaps scimitus* (Cabrera *et al.* 2004; Cabrera *et al.* 2005). We have also demonstrated that azadirachtin is safe to use with the citrus mealybug parasitoid, *Leptomastix dactylopii*. Pyriproxyfen was found to be slightly toxic whereas both direct and indirect applications of kinoprene were extremely toxic to this parasitoid (Rothwangl *et al.* 2004). We have also demonstrated that applications of the insecticides buprofezin, pyriproxyfen, and flonicamid are not harmful to the adult stage of the mealybug destroyer, *C. montrouzieri* (Cloyd and Dickinson, unpublished data)

CONCLUSIONS

It is important to note that many studies are conducted under laboratory conditions, which represents a "worse-case scenario" and that if there are no harmful effects under these conditions then it is likely that the biorational insecticide or miticide will not be harmful when used in the greenhouse. In addition, the concentration or rate also influence whether biorational insecticide/miticide will negatively impact natural enemies. In order to avoid any harmful effects to natural enemies it is recommended to make releases several days after an application although applying biorational insecticides or miticides may still decrease host quality thus increasing parasitoid or predator mortality. For example, parasitoid females may not lay eggs in un-suitable hosts and predators may not consume hosts that are an inadequate food source (=poor quality). Applications of biorational insecticides/miticides may also kill a majority of the hosts thus reducing the amount available for natural enemies. Finally, the fact that many biorational insecticides and miticides may need to be applied frequently (depending on the pest population) in order to obtain sufficient control of insect or mite pests increases the likelihood that natural enemies will be exposed to sprays or spray residues, which may have a deleterious effect on foraging behavior or reproduction.

It is apparent that there is variability in the compatibility of natural enemies to biorational insecticides/miticides based on the type of biorational insecticide or miticide, whether the natural enemy is a parasitoid or predator, and stage of development. Biorational insecticides/miticides are effective for controlling many different types of greenhouse pests and are generally less harmful to natural enemies than conventional insecticides/miticides, which suggest that they are more likely to be compatible with natural enemies. However, it is important to know which biorational insecticide/miticide is compatible or not compatible with natural enemies in order to avoid disrupting successful biological control programs.

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BIOLOGICAL CONTROL OF WHITEFLIES AND WESTERN FLOWER THRIPS IN GREENHOUSE SWEET PEPPERS WITH THE PHYTOSEIID PREDATORY MITE *AMBLYSEIUS SWIRSKII* ATHIAS-HENRIOT (ACARI: PHYTOSEIIDAE)

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ABSTRACT

Currently, western flower thrips (*Frankliniella occidentalis*) is controlled in greenhouse sweet peppers with the phytoseiid predatory mite *Amblyseius cucumeris*, the anthocorid flower bug *Orius laevigatus* and the phytoseiid mite *Iphiseius degenerans*. Whiteflies (*Trialeurodes vaporariorum* and *Bemisia tabaci* are controlled by releasing parasitoids and mirid bugs (Miridae).

Cage trials and trials in commercial greenhouse crops with the phytoseiid predatory mite *Amblyseius swirskii* (Athias-Henriot, 1962) have shown a high efficacy against *Frankliniella occidentalis* and against *Bemisia tabaci* in sweet peppers. When the predatory mites were released preventively on flowering sweet pepper plants in a greenhouse in the Netherlands the establishment of *Amblyseius swirskii* was successful. In all trials *Amblyseius swirskii* has shown a very high numerical response to the presence of prey. Biological control of whiteflies with phytoseiid predatory mites, which can be economically reared in large quantities, might be a major step forwards for biological control in greenhouse crops, especially in areas with high whitefly and thrips populations such as Southern Europe.

INTRODUCTION

The greenhouse whitefly, *Trialeurodes vaporariorum*, and the tobacco whitefly, *Bemisia tabaci*, are major pests in greenhouse crops. In commercial greenhouses whiteflies are mainly controlled by releases of the parasitoids *Encarsia formosa* and *Eretmocerus eremicus* against *T. vaporariorum* and *Eretmocerus mundus* against *B. tabaci*. Whitefly parasitoids are not able to establish in a greenhouse when released preventively. Mirid bugs (Miridae) such as *Macrolophus caliginosus* Wagner are expensive and their use is limited to greenhouse tomatoes. Therefore, a biological control agent which is able to establish in a crop before whiteflies enter the greenhouse would be a supplement to the system.

Nomikou *et al.* (2003) showed that the phytoseiid mite *Amblyseius swirskii* (Athias-Henriot), predates on eggs and crawlers of *B. tabaci* and develops well on this prey. Since the late 1980's the predatory mites *Amblyseius cucumeris* is successfully used for control of Western Flower Thrips (*Frankliniella occidentalis*) in greenhouse cucumbers, sweet pepper, eggplants and a large range of greenhouse ornamentals. Although very effective in winter crops in greenhouses in Southern Europe *A. cucumeris* appears not very effective in summer crops. This might be caused by the high temperatures in combination with low humidity conditions during summer. *Iphiseius degenerans* is more adapted to the conditions of the Mediterranean and has proven to be an effective thrips predator in greenhouses in Northern Europe, but this predator is difficult to rear in large quantities. Messelink and Steenpaal (2003) and Messelink *et al.* (2005) showed that *A. swirskii* is a very effective predator of Western Flower Thrips in greenhouse cucumbers. Also in greenhouse trials against greenhouse whiteflies on cucumbers, excellent control was achieved (Messelink, pers. comm.). *A. swirskii* is a common predatory mite in the eastern part of the Mediterranean. The mites used in the following studies have been collected in Israel.

PREDATION AND OVIPOSITION RATE

Rates of predation and oviposition on a diet of thrips larvae were determined according to the method described by van Houten *et al.* 1995. Leave discs of cucumbers (4.5 cm^2) were placed upside down on pads of moist cotton wool, in a climate room at L16:D8, 25° C and 70% relative humidity. Single gravid female mites were placed on each leaf disc. The mites originated from cohorts of young nymphs of the same age which were reared on a diet of cattail pollen (*Typha latifolia*). At the start of the experiment the mites had been laying eggs for 2 days.All leave discs were infested with 12 first instar *F. occidentalis*. During four days the predators were transferred each day to fresh leave discs with 12 newly emerged thrips larvae. It was ascertained that the number of live prey never dropped below 6 per disc. Number mite eggs and killed thrips were assessed daily. Data of the first day were omitted from calculations of predation and oviposition rates. A total of eleven female predatory mites were assessed.

Using the same protocol, 10 gravid female predatory mites were assessed for there oviposition rate when fed with eggs of greenhouse whiteflies (*Trialeurodes vaporariorum*). Each day the predatory mites were transferred to fresh cucumber leaves with eggs of *T. vaporariorum*.

Table 1. Rates of predation and oviposition of *Amblyseius swirskii* on a diet of first instar *F. occidentalis* larvae and *T. vaporariorum* eggs, on cucumber leaf discs (4.5 cm^2 at 25°C and 70% r.h. Predation rate: mean number of larvae killed per female, per day. Ovipostion rate: mean number of eggs laid per female per day. N= number of predatory females; s.e= standard error.

Prey species	N	Predation rate (mean ± s.e.)	Oviposition rate (mean ± s.e.)
F. occidentalis	11	4.9 ± 0.3	2.1 ± 0.2
T. vaporariorum	10	-	2.3 ± 0.1

DIAPAUSE

Diapause experiments were performed according to the method described by van Houten *et al.* 1995. Predatory mites were reared on small plastic arena's (8 x 10 cm) placed on pads of moist cotton. A small roof (2 x 2 cm) made from a piece of transparent plastic was placed on the arena to provide shelter and as an oviposition site. The arena's were provided every second day with fresh cattail pollen and with purple pollen of the iceplant (*Mesembryanthemum* sp.). Iceplant pollen contains ²-carotene. In the absence of ²-carotene in their diet, some mite species do not respond to photoperiod or thermoperiod. Another advantage of the purple iceplant pollen is that egg production by individual non-diapausing females can easily be determined, as the white egg stands out clearly against the surrounding purple intestines. Pollen was provided by dusting it on the arena with a small brush. The colonies were kept in a climate room at 25°C, 70% relative humidity and L16:D8

A cohort of eggs, from 0-16 h after deposition was transferred to a new rearing unit in a climate cabinet under diapause inducing conditions of 19°C, 70% relative humidity and L10:D14. Once the eggs have hatched, 30 young females were carefully transferred to a unit identical to the rearing units and placed in a climate cabinet under diapause inducing conditions of 19°C, 70% relative humidity and L10:D14. It was ensured that ample males were present for insemination of the females. When no egg was seen in a female it was concluded that this female would not lay eggs and, hence, was in a state of reproductive diapause. The female mites with a visible egg were removed. If no egg was seen in a female within 3 days, the conclusion was that it had entered a reproductive diapause.

All 30 female mites were ovipositing. This proves that under the conditions of 19°C, 70% r.h. and L10:D14 this strain of *Amblyseius swirskii* is non-diapausing.

DROUGHT TOLERANCE

The influence of relative humidity on egg-hatching was examined in closed plastic boxes (18 x 14 x 9 cm) at 25°. Eggs from 0-16 h after deposition were transferred to small plastic arena's and floated on different supersaturated salt solutions. Three different relative humidities were obtained by using supersaturated salt solutions of Ca(NO3)2 (50.5% r.h.), KI (69% r.h.) and NaCl (75% r.h.) (Winston and Bates 1960).

Table 2. Egg survival of *Amblyseius swirskii* at different relative humidities at 25°C. N= number of eggs.

Salt solutions	Relative humidity	N	Eggs hatched
Ca(NO3)2	50.5%	154	3%
KI	69%	251	45%
NaCl	75%	160	84%

ESTABLISHMENT OF *AMBLYSEIUS SWIRSKII* IN SWEET PEPPERS

A field trial was conducted in a 7,000 m² commercial sweet pepper crop (var. Derby) in the Netherlands. The goal of this trial was to verify if *A. swirskii* is able to establish in a sweet pepper crop in the absence of prey with only plant pollen as food. When the trial started the plants were flowering, 80cm high and free from pests. *A. swirskii* was released in a plot of 1,500 m². The predatory mites were released in weeks 7 and 10 at a rate of 25 individuals per m² per release. Other natural enemies which were released in the entire greenhouse are: *Orius laevigatus, E. mundus, Phytoseiulus persimilis* and *Aphidius ervi.* Observations were done every other week. Per observation 50 leaves from the higher part of the plants and 25 flowers were chosen randomly. The number of *A. swirskii, B. tabaci* and *O. laevigatus* was assessed.

A. swirskii established well. On the leaves a population of 4 to 5 predatory mites (all stages together) per leaf was reached within 4 weeks and remained at that level until the end of the trial (Fig. 1). In the flowers the *A. swirskii* population reached a peak of 3 predatory mites per flower 10 weeks after the last introduction, but afterwards the population decreased, probably due to the presence of *O. laevigatus* in the flowers (Fig. 2).

The pest level remained low throughout the entire trial period. *F. occidentalis* was not observed at all and *B. tabaci* was found at a level of 1 or 2 individuals per 50 leaves. The only pest which was found frequently was *Tetranychus urticae* Koch at an incidence between 0 – 12% of the leaves.

Despite low pest levels, *A. swirskii* remained present on the plants throughout the season which indicates that *A. swirskii* can be released preventively in a sweet pepper crop. The establishment, speed of population development and persistence in the crop are much better than for *Amblyseius cucumeris.*

BIOLOGICAL CONTROL OF *BEMISIA TABACI* WITH *AMBLYSEIUS SWIRSKII*

A semi field trial was conducted in an 400 m2 experimental plastic tunnel in Aguilas, Spain starting at the end of May until the end of July. The plastic tunnel was divided by 50 mesh screens in 6 compartments of 8 m². 10 poorly flowering sweet pepper plants of 50cm height were planted in each compartment at the start of the trial. *A. swirskii* was released in 3 compartments while the other 3 remain untreated (3 replicates per treatment). *B. tabaci* was released in all compartments. The release schedule is shown in table 3.

To assess the *A. swirskii* and *B. tabaci* population, 3 leaves (top, middle and bottom) from 5 plants per compartment were randomly chosen and observed weekly. All stages of *A. swirskii* and *B. tabaci* were counted separately.

A. swirskii managed to keep the *B. tabaci* population low in all compartments where this predatory mite was released, while in the untreated compartments the *B. tabaci* population increased rapidly. (Fig. 3)

A. swirskii established in all 3 compartments where it was released. After some weeks the first *A. swirskii* was also found in the untreated control cages and the population increased very rapidly. (Fig. 4)

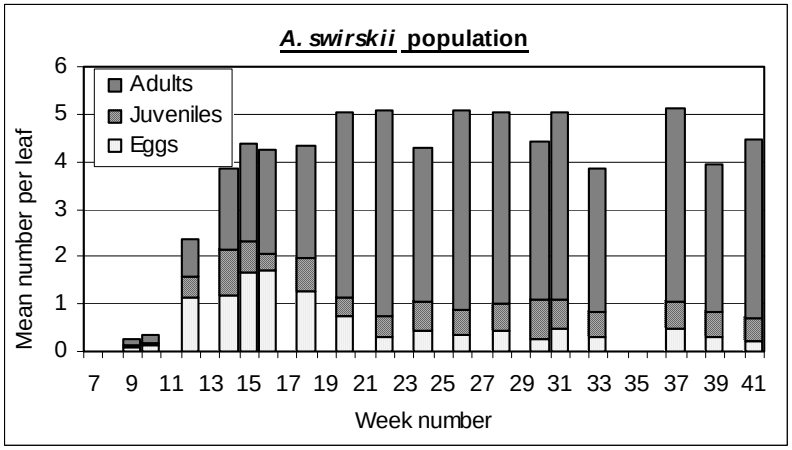

Figure 1. Mean number of *A. swirskii* per leaf. (n = 50).

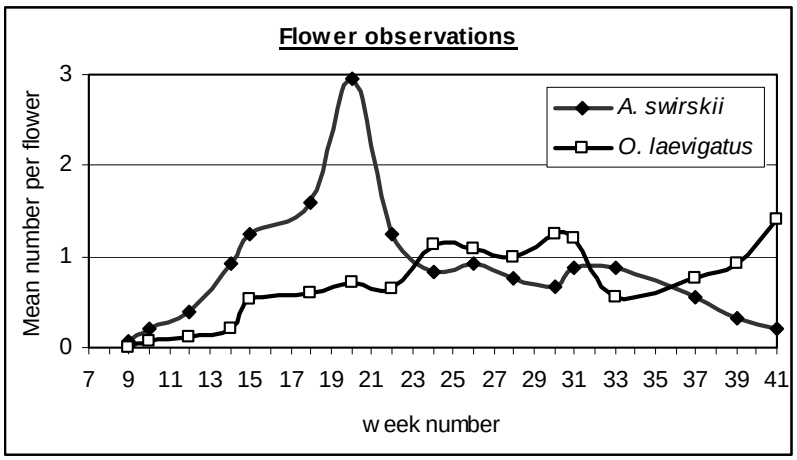

Figure 2. Mean number of *A. swirskii* and *O. laevigatus* per flower. (n = 25).

Table 3. Release schedule (number of adults released per plant) per treatment.

	Day 0		Day 6		Day 7		Day 14	
Treatment	*B.tab.**	*A.swi.**	*B. tab.*	*A. swi.*	*B. tab.*	*A. swi.*	*B. tab.*	*A. swi.*
A. swirskii	2	-	-	80	2	-	4	-
Untreated control	2	-	-	-	2	-	4	-

**B. tab.* = *B. tabaci* and *A. swi.* = *A. swirskii*.

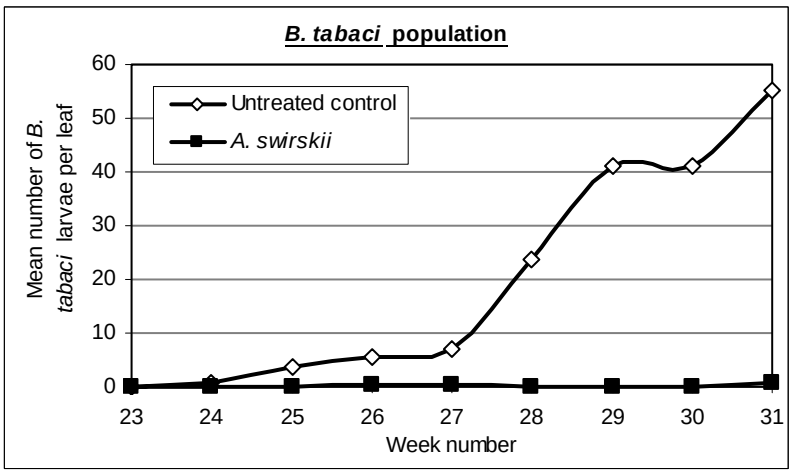

Figure 3. Mean number of *B. tabaci* larvae per leaf. Average of 15 leaves per compartment and 3 compartments per treatment.

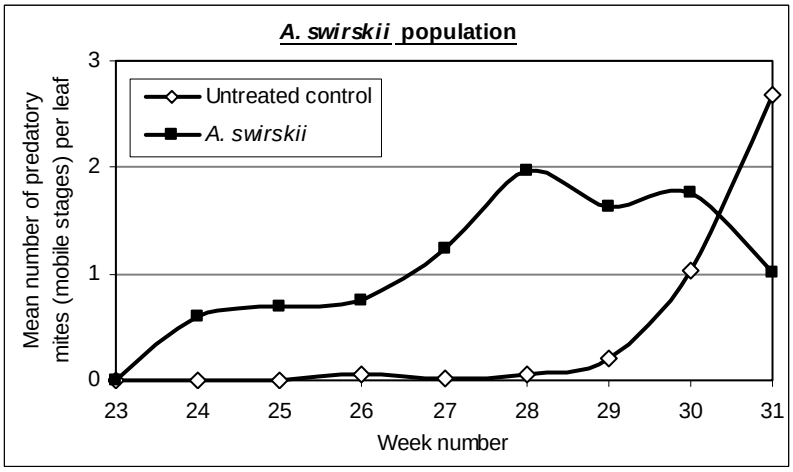

Figure 4. Mean number of *A. swirskii* (mobile stages) per leaf. Average of 15 leaves per compartment and 3 compartments per treatment.

COMPARISON OF FOUR PREDATORY MITE SPECIES AGAINST WESTERN FLOWER THRIPS

This experiment was carried out in 23 walk-in cages of 100 m^2, each cage having 1 row of 13 sweet pepper plants. When the plants had started to flower the western flower thrips and predatory mites were released in the numbers as shown in table 4. The trial was done in the summer period. The maximum day temperature was between 28-30°C with peaks up to 40°C. To monitor thrips and predator populations, samples of 30 leaves and 10 flowers were taken every week.

Iphiseius degenerans and *A. swirskii* established more successfully than *A. cucumeris* and *A. andersoni* (Fig. 5). *Iphiseius degenerans* performed best: the predator population increased rapidly and reached higher densities than *A. swirskii*, particularly in the flowers but also on the leaves,.

The thrips population in the flowers at the last counting is presented in figure 6. *Amblyseius swirskii* was most succesfull in thrips control, followed by *A. cucumeris* released by means of a slow-release breeding sachet, *I. degenerans*, *A, andersoni* and *A. cucumeris*, in descending order.

Table 4. Release rates of predatory mites and thrips in 23 different cages.

Predatory Mite Species	Release rate of predatory mites per plant in wk 24	Release rate of *F. occidentalis* per plant in wk 23, 24, 25, and 26 per cage	Number of Replicates
A. swirskii	30 females	(4 x) 2 females	4
A. andersoni	30 females	,,	4
A. cucumeris	30 females	,,	3
A. cucumeris	1 sachet	,,	4
I. degenerans	30 females	,,	4
Control	-	,,	4

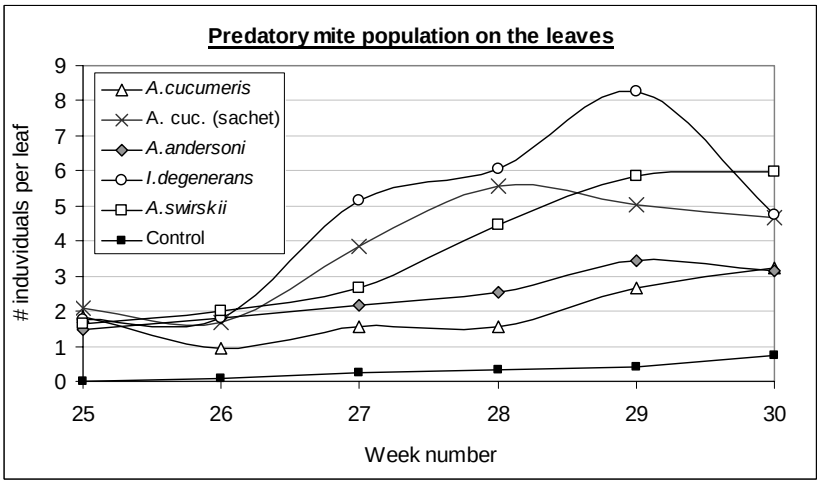

Figure 5. Population fluctuations of 4 predatory mite species on leaves of sweet pepper plants in 23 greenhouses.

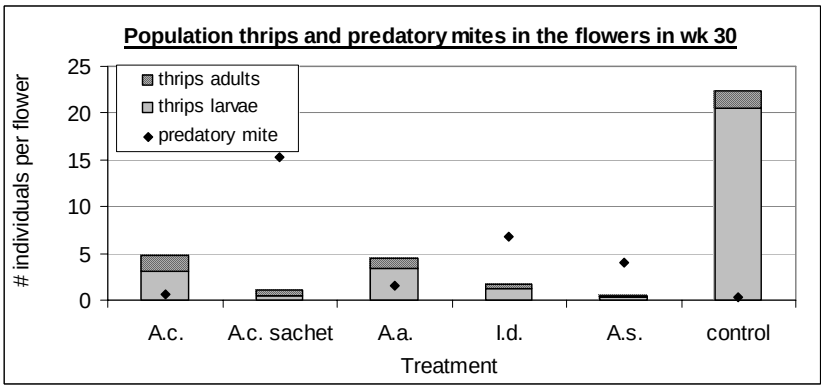

Figure 6. Mean numbers of *F. occidentalis* and 5 predatory mite species in flowers of sweet pepper plants in 23 greenhouses. Ac. = *A. cucumeris,* Aa. = *A. andersoni,* Id. = *I. degenerans,* As. = *A. swirskii* and Ac. sachet = a slow release sachet with *A. cucumeris*.

COMPARSION OF *A. SWIRSKII* AND *A. CUCUMERIS* FOR THRIPS CONTROL

This experiment was performed in 4 cages (3x1x2 m) in an experimental greenhouse. 5 flowering sweet pepper plants of 60 cm height were placed in each cage. Releases of 1 *A. swirskii* per leaf were compared with releasing either 1 *A. cucumeris* per leaf or 3 releases of 10 *A. cucumeris* per leaf at weekly interval. The latter treatment simulates the effect of using slow-release breeding sachets, which is standard practice when releasing *A. cucumeris.* To monitor western flower thrips and predator populations, 5 leaves and 1 flower per plant (25 leaves and 5 flowers per cage) were monitored every week from day 13 onwards.

The cage experiment showed that even when *A. swirskii* was released in dosage 30 times lower than *A. cucumeris*, the establishment of *A. swirskii* was better (Fig. 7). The impact of both predators on the thrips population at these release rates was comparable. Based on these results, *A. swirskii* can be regarded as a promising candidate for thrips control in sweet pepper.

Table 5. Release rates of predatory mites and western flower thrips in 4 different cages.

Predatory mite species	Release rate of predatory mites per cage (number/ leaf)	Release rate of *F. occidentalis* per cage
cage 1: *A. swirskii*	150 adults (1/leaf) on day 6	(3 x) 10 females (day 0, 7, 14)
cage 2: *A. cucumeris*	150 adults (1/ leaf) on day 6	,,
cage 3: *A. cucumeris*	3x 1500 adults (3x10/leaf) day 6, 13, 20	,,
cage 4: control	-	,,

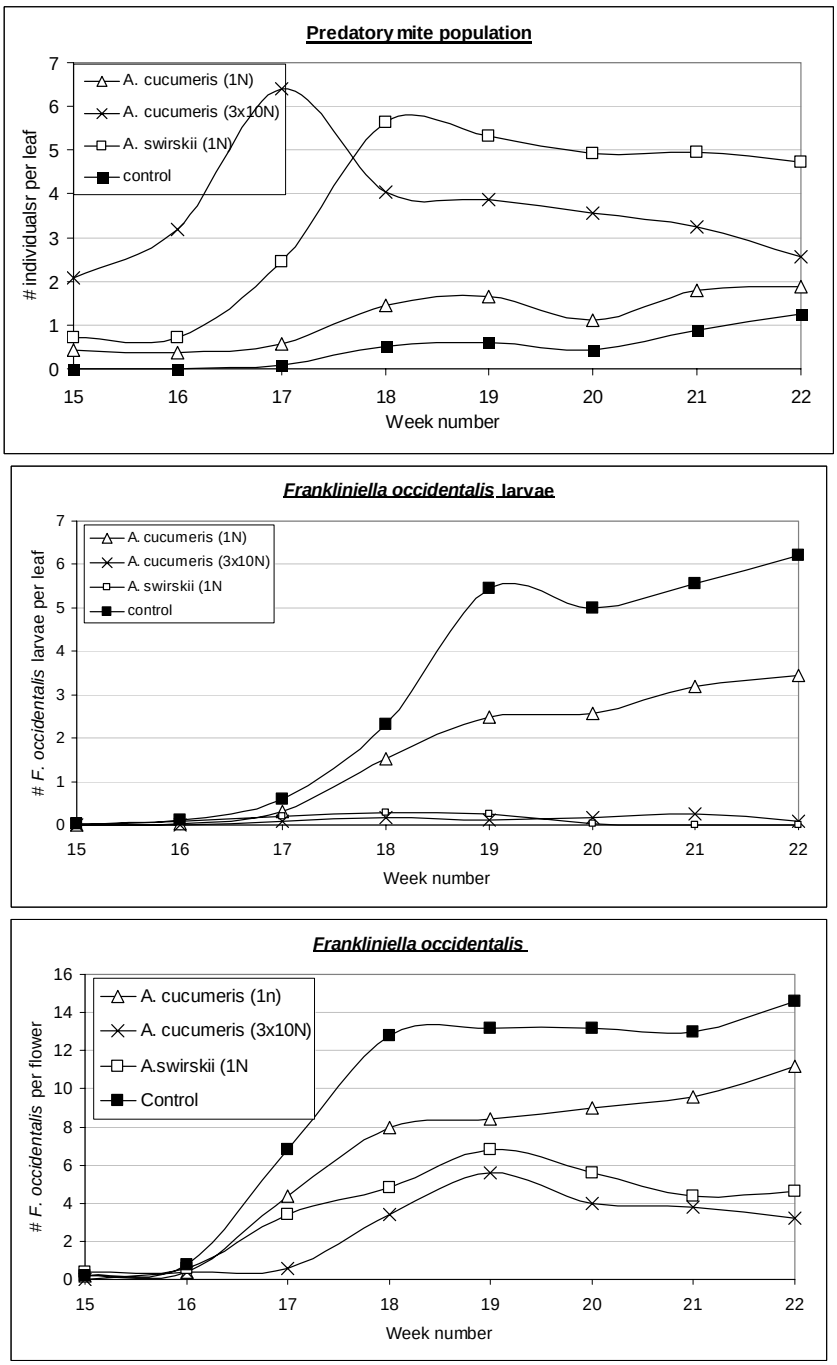

Figure 7. Population fluctuations of *Frankliniella occidentalis* and the phytoseiid mites, *Amblyseius cucumeris* and *A. swirskii,* on leaves and flowers of sweet pepper plants in 4 cages.

CONCLUSIONS

Amblyseius swirskii predates, reproduces and develops well on western flower thrips, greenhouse whiteflies and tobacco whiteflies. Under short day conditions of 19°C and L10:D14 this predatory mite is not sensitive to diapause. Draught tolerance of its eggs is similar to the draught tolerance of eggs of A. cucumeris with an RH_{50} around 70%.

A. swirskii is a promising control agent of whiteflies and western flower thrips on sweet pepper. Moreover, *A. swirskii* can be released preventively when the crop is flowering and remains present in the crop throughout the entire growing season, even while pests levels are very low. The establishment, speed of population development and persistence in the crop are much better than for *A. cucumeris*. Therefore *A. swirskii* may be a new solution for biological control of western flower thrips and of tobacco whitefly in sweet pepper in Northern and Southern Europe. *A. swirskii* is expected to replace *Iphiseius degenerans* and *A. cucumeris* in the future.

Because the biological control system for sweet peppers can be simplified and its robustness greatly enhanced by using this highly efficient predatory mite, *A. swirskii* is expected to become one of the keys to successful development of biological control in sweet peppers in areas with high pest pressures of thrips and whiteflies.

A. swirskii may be a new solution for biological control of both pests in sweet pepper in Northern and Southern Europe. A mass rearing technique for *A. swirskii* has already been developed.

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SELECTION OF NON-TARGET SPECIES FOR HOST SPECIFICITY TESTING OF ENTOMOPHAGOUS BIOLOGICAL CONTROL AGENTS

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ABSTRACT

We present comprehensive recommendations for setting up test species lists for arthropod biological control programs that are scientifically based and ensure that all aspects of potential impacts are considered. It is proposed that a set of categories, including ecological similarities, phylogenetic/taxonomic affinities, and safeguard considerations are applied to ecological host range information to develop an initial test list. This list is then filtered to reduce the number of species to be tested by eliminating those with different spatial, temporal and morphological attributes and those species that are not readily obtained, thus unlikely to yield scientifically relevant data. The reduced test list is used for the actual testing but can (and should) be revised if new information obtained indicates that additional or more appropriate species should be included.

INTRODUCTION

The potential for non-target effects following the release of exotic species has raised concerns ever since biological control programmes were first set up. However, Howarth (1983; 1991) and Louda (1997) highlighted this issue of unwanted non-target effects in biological control and stimulated with these articles intense discussion even beyond the scientific community. Subsequently, a number of papers on non-target effects have been published within the last ten years (e.g., Follett *et al*. 2000a,b; Lockwood *et al*. 2001; Louda *et al*. 2003a; Lynch and Thomas 2000; Lynch *et al*. 2001; Simberloff and Stiling 1996; Stiling and Simberloff 2000;Thomas and Willis 1998). As host-specificity testing of entomophagous biological control agents has lagged behind that of phytophagous biological control agents, recent international efforts have been initiated. These efforts have been aimed at developing guidelines to provide a regulatory framework for the introduction of invertebrates for classical and inundative biological control of arthropods (e.g., OECD 2003). Generally, all these initiatives, research reviews

and guidelines, highlighted *what* should be done or what knowledge is required, but did not provide detailed methods on *how* tests should be conducted to assess potential non-target effects. As an exception, van Lenteren *et al.* (2003) recommended a risk assessment methodology for the evaluation of agents to be used in inundative biological control. Recently, Van Driesche and Reardon (2004) provided guidance to the best practice for assessing host ranges of parasitoids and predators used in classical biological control. Despite these valuable initiatives it is still important to provide standardized methods that can be universally applied for the assessment of potential non-target effects in arthropod biological control. Such methods are particularly relevant for parts of the guidelines where appropriate techniques are lacking to evaluate non-target effects (e.g., indirect impacts, interbreeding, establishment, dispersal and contaminants in agents). Selection of appropriate species for testing potential impacts of candidate biological control agents is the first critical step and although several independent arthropod biological control projects applied different approaches aiming the development of a test species list (e.g., Barratt 1997) a standardized method needs to be developed.

In this paper we review the approaches taken in some recent arthropod biological control programmes. Then we propose recommendations for setting up test species lists for arthropod biological control programmes that are scientifically based and ensure that all aspects of potential direct impacts are considered. Finally, we review the usefulness of selection criteria for setting up test species lists which will depend on the type of results that are generated by host-specificity tests, and the ease of their interpretation.

SELECTION OF NON-TARGET SPECIES FOR TEST LIST: A REVIEW

A review of some recent studies suggests that a variety of strategies have been used to select species for non-target host tests. As a general rule, test lists are based on knowledge from host records extracted from the literature (De Nardo and Hopper 2004; Sands and Van Driesche 2004). We concluded that although phylogenetic considerations were an underlying criterion (i.e., that a particular parasitoid group attacks certain host groups), ecological, biological and socio-economic information was very important for selecting non-target species for study. In addition, availability of test material was also critical for selection of non-target test species in most studies. Phylogenetic considerations were in reality based on taxonomic relatedness (e.g., same genus, same family, etc.) of test species to target host. Ecological features included overlap of geographic range, habitat preference, and feeding niche of species representing different components of the community. Biological characteristics included known host range, phenological overlap of the target and non-target species, dispersal capability of the candidate biological control agent (and parasitized host), morphological similarity, behavioural factors (e.g., feeding, oviposition, host location, etc.), and overlap of the physiological host range of biological control agents. Socio-economic factors included whether a potential test species was commercially important (e.g., a pollinator), beneficial (e.g., predator, weed biological control agent) or of conservation importance (e.g., rare or endangered). The availability of non-target material was considered, and sources included commercial or laboratory cultures, field collections, and progeny of field collected individuals. Many studies state the reasons behind selection of the test species, and all but three studies used at least two of the categories

in their selection. The numbers of non-target species tested in the laboratory ranged from one to 23. In Table 1, studies reviewed are compiled providing information about the selection criteria applied and the number of non-target species selected.

RECOMMENDATIONS FOR COMPILING A NON-TARGET SPECIES TEST LIST FOR ARTHROPOD BIOLOGICAL CONTROL USING INVERTEBRATES

It is widely believed that the criteria used to compile a suitable non-target test list in weed biological control projects are unlikely to provide such a reliable test list for entomophagous biological control agents. There are a number of arguments that support this claim; i) arthropods often outnumber plant species in communities by an order of magnitude (e.g., Kuhlmann *et al.* 2000; Messing 2001), ii) there is a significant lack of knowledge of arthropod phylogeny (e.g., Messing 2001; Sands and Van Driesche 2000), iii) natural enemies of arthropod pests respond to two trophic levels, i.e. the host and its host-plant(s) (e.g., Godfray 1994), iv) disjunct host-ranges appear to be the rule with parasitoids, rather than the exception as in herbivores (Messing 2001), and v) it is much more difficult and time-consuming to rear a large number of test arthropod species than test plant species (Kuhlmann *et al.* 1998; Sands and Van Driesche 2000).

One question that remains paramount with regards to the selection of non-target test species in arthropod biological control programmes is whether the host range of the parasitoid considered for use is restricted to one of a few closely related groups of herbivorous insects, or whether other factors such as phylogenetic disjunction in host range (a host range that includes phylogenetically unrelated species) are apparent. While it is commonly viewed by biological control scientists that initial predictions and assessments of parasitoid host range may be based on phylogeny, it is agreed that other highly relevant criteria , such as, ecological similarities shared between the target pest and other species in the field, should also be addressed as well as consideration of safeguard species selection. Thus, a more reductionist approach may be appropriate and selection of non-target test species is best carried out on a case-by-case basis.

At present, there is no standard protocol to refer to when compiling a species test list for assessment of an entomophagous biological control agent's host range. Numerous studies carried out in recent years illustrate that an array of criteria have been used to compile test species lists (Table 1).

In light of this, recommendations are proposed for developing a species list for host specificity testing of entomophagous arthropods (Fig. 1). The first step involves the collation of all recorded information on field hosts of not only the candidate biological control agent, but also of closely related species (see De Nardo and Hopper 2004). Literature reports and museum collections can provide valuable information relating to this but confirmation of the quality of the data must first be sought from a taxonomic expert as a precautionary measure. It must also be recognized that host records tend to be compiled using data from agricultural and forest habitats and often focus on more economically important species.

Table 1. Summary of selection criteria used in recent studies assessing host-specificity of entomophagous biological control agents.

Agent and Target	Selection Criteria Used	# Non-target Selected	Reference
Agent: *Cotesia erionotae* Wilkinson [Hymenoptera: Braconidae] Target: *Erionota thrax* (L.) [Lepidoptera: Hesperiidae]	Phylogenetic: 1 sp. in the same family Socioeconomic: commercially important spp.	4 Lepidoptera spp.: 1 Hesperiidae 3 Papilionidae	Sands *et al.* (1993)
Agent: *Trichogramma nubilale* Ertle and Davis [Hymenoptera: Trichogrammatidae] Target: *Ostrinia nubilalis* Hübner [Lepidoptera: Crambidae]	Socioeconomic: rare and endangered species Biological: wide host range of Trichogramma spp.; phonological overlap of target and non-target spp.; dispersal of agent and mortality during dispersal	1 Lepidoptera sp.: 1 Lycaenidae	Andow *et al.* (1995)
Agents: *Ageniaspis citricola* (Logvinovskaya) [Hymenoptera: Encyrtidae] *Citrostichus phyllocnistoides* (Narayanan) *Cirrospilus quadristriatus* Subba [Hymenoptera: Eulophidae] Target: *Phyllocnistis citrella* Stainton [Lepidoptera: Gracillariidae]	Ecological: leaf mining and gall forming flies; unrelated leafminers Phylogenetic: 1 sp. in same genus as target Socioeconomic: beneficial species (weed biocontrol agents)	4 Diptera spp.: 1 Agromyzidae 1 Cecidomyidae 2 Tephritidae 1 Coleoptera sp.: Chrysomelidae 12 Lepidoptera spp.: 2 Bucculatricidae 1 Gelechiidae 5 Gracillariidae 1 Lyonetiidae 1 Pterophoridae 1 Pyralidae 1 Tortricidae	Neale *et al.* (1995)
Agents: *Diachasmimiorpha longicaudata* (Ashmead) *Psyttalia fletcheri* (Silvestri) [Hymenoptera: Braconidae] Targets: *Ceratitis capitata* (Wiedemann) *Bactrocera dorsalis* (Hendel) *Bactrocera curbitae* (Coquillett) [Diptera : Tephritidae]	Ecological: plant tissue of similar size and shape to that of target hosts; feeding niche Socioeconomic: weed biocontrol agent Biological: Morphology of parasitoid ovipositor, searching behaviour Availability: obtained from culture; field collected	2 Diptera spp.: 2 Tephritidae	Duan and Messing (1996; 1997) Duan *et al.* (1997)
Agents: *Cotesia rubecula* (Marshall) *Cotesia plutellae* Kurdjumov [Hymenoptera: Braconidae] Targets: *Pieris rapae* L. [Lepidoptera : Pieridae] *Plutella xylostella* (L.) [Lepidoptera : Plutellidae]	Ecological: taxa in geographic region and habitats where agent is abundant Biological: behaviour, attractiveness to host plant volatiles Availability: field collected material	14 Lepidoptera spp.: 1 Plutellidae 1 Tortricidae 1 Pyralidae 2 Nymphalidae 1 Arctiidae 8 Noctuidae	Cameron and Walker (1997)

Table 1. Summary of selection criteria used in recent studies assessing host-specificity of entomophagous biological control agents (continued).

Agent and Target	Selection Criteria Used	# Non-target Selected	Reference
Agent: *Microctonus aethiopoides* Loan [Hymenoptera: Braconidae] Targets: *Sitona discoideus* Gyllenhal *Listronotus bonariensis* (Kuschel) [Coleoptera: Curculionidae]	Ecological: feeding niche; habitat overlap Phylogenetic: taxa from subfamilies and tribes related to target Socioeconomic: weed biological control agents Biological: Phenology; diurnal activity, feeding and oviposition behaviour Availability: field collections	11 Coleoptera spp.: 11 Curculionidae	Barratt *et al.* (1997; 1998; 2000; 2004)
Agent: *Aphidius rosae* Haliday [Hymenoptera: Braconidae] Target: *Macrosiphum rosae* (L.) [Hemiptera: Aphidae]	Ecological: habitat where target occurred Biological: behaviour, attractiveness host plant volatiles Availability: species from glass house and field collections	7 Hemiptera spp.: 7 Aphidae	Kitt and Keller (1998)
Agents: *Cotesia flavipes* Cameron *Cotesia sesamiae* (Cameron) *Cotesia chilonis* (Matsumura) [Hymenoptera: Braconidae] Target: *Diatraea saccharalis* (F.) [Lepidoptera: Pyralidae]	Ecological: habitat preference of agents Biological: physiological host range overlap of agents	None	Rutledge and Wiedenmann (1999)
Agent: *Comsilura concinnata* (Meigen) [Diptera: Tachinidae] Target: *Lymantria dispar* (L.) [Lepidoptera: Lymantriidae]	Ecological: habitat overlap Biological: temporal overlap Socioeconomic: threatened species	3 Lepidoptera spp.: 3 Saturniidae	Boettner *et al.* (2000)
Agent: *Trichogramma brassicae* Bezenko [Hymentoptera: Trichogrammatidae] Target: *Ostrinia nubilalis* Hübner [Lepidoptera: Crambidae]	Biological: temporal overlap Availability: collected by light trap, economically important pest	23 Lepidoptera spp.: 3 Arctiidae 2 Geometridae 1 Hesperiidae 1 Lycaenidae 9 Noctuidae 2 Pieridae 1 Pyralidae 1 Satyridae 1 Sphingidae 1 Tortricidae 1 Yponomeutidae	Orr *et al.* (2000)
Agent: *Pseudacteon curvatus* Borgmeier [Diptera: Phoridae] Targets: *Solenopsis invicta* Buren *Solenopsis richteri* Forei [Hymenoptera: Formicidae]	Phylogenetic: taxonomically unrelated spp. Biological: ovipositor morphology; similarity of non-targets to target species	19 Hymenoptera spp.: 19 Formicidae spp. (12 different genera)	Porter (2000)

Table 1. Summary of selection criteria used in recent studies assessing host-specificity of entomophagous biological control agents (continued).

Agent and Target	Selection Criteria Used	# Non-target Selected	Reference
Agent: *Aphantorhaphopsis samarensis* (Villeneuve) [Diptera: Tachinidae] Target: *Lymantria dispar* (L.) [Lepidoptera: Lymantriidae]	Ecological: European spp. collected in wild in areas of target occurrence; NA species collected from field and reared	56 Lepidoptera spp.: 45 European spp.: 5 Arctiidae 1 Drepanidae 8 Geometridae 2 Lasiocampidae 1 Lycaenidae 5 Lymantriidae 1 Nemeobiidae 10 Noctuidae 2 Notodontidae 6 Nymphalidae 2 Saturniidae 1 Sphingidae 1 Thaumetopoeidae 11 North American spp.: 4 Arctiidae 1 Danaidae 1 Lymantriidae 2 Noctuidae 3 Saturniidae	Fuester *et al.* (2001)
Agent: *Trichogramma platneri* Nagarkatti [Hymenoptera: Trichogrammatidae] Target: *Cydia pomonella* (L.) [Lepidoptera: Tortricidae]	Phylogenetic: Lepidoptera (known hosts) and non-Lepidoptera Biological: host egg characteristics Availability: 9 spp. from commercial cultures; 7 spp. from field-collected specimens reared in laboratory	2 Coleoptera spp.: 1 Cerambycidae 1 Chrysomelidae 1 Diptera sp.: 1 Muscidae 2 Hemiptera spp.: 1 Lygaeidae 1 Pentatomidae 11 Lepidoptera spp.: 1 Bombycidae 1 Danaidae 1 Gelechiidae 2 Noctuidae 1 Pyralidae 2 Saturniidae 1 Sphingidae 2 Tortricidae 1 Neuroptera sp.	Mansfield and Mills (2002)
Agent: *Trigonospila brevifacies* (Hardy) [Diptera: Tachinidae] Target: *Epiphyas postvittana* Walker [Lepidoptera: Tortricidae]	Ecological: community interactions Availability: field collections	14 Lepidoptera spp.: 12 Tortricidae 2 Oecophoridae	Munro and Henderson (2002)

Table 1. Summary of selection criteria used in recent studies assessing host-specificity of entomophagous biological control agents (continued).

Agent and Target	Selection Criteria Used	# Non-target Selected	Reference
Agent: *Laricobius nigrinus* Fender [Coleoptera : Derontidae] Target: *Adelges tsugae* Annand [Hemiptera: Adelgidae]	Ecological: Habitat similarity/dissimilarity and vulnerable host stage occurs at same time as target Phylogenetic: Same genus, same family unrelated families Availability: Field collected in nearby ornamental trees or forest or from greenhouse colony	6 Hemiptera spp.: 3 Adelgidae 2 Aphididae 1 Diaspididae	Zilahi-Balogh *et al*. 2002
Agent: *Trichogramma brassicae* Bezenko [Hymenoptera: Trichogrammatidae] Target: *Ostrinia nubilalis* Hübner [Lepidoptera: Crambidae]	Ecological: habitat and temporal overlap of hosts and released agent Socioeconomic: species at risk	23 Lepidoptera spp.: 1 Hesperiidae 3 Lycaenidae 8 Nymphalidae 1 Papilionidae 1 Pieridae 6 Satyridae 2 Sphingidae 1 Zygaenidae	Babendreier *et al*. (2003a) Babendreier *et al*. (2003b)
Agent: *Trichogramma brassicae* Bezenko [Hymenoptera: Trichogrammatidae] Target: *Ostrinia nubilalis* Hübner [Lepidoptera: Crambidae]	Phylogenetic: representative Lepidopteran spp. Availability: laboratory culture, 2 Noctuidae collected from field	6 Lepidoptera spp.: 3 Noctuidae 1 Plutellidae 2 Tortricidae	Babendreier *et al*. (2003c)
Agent: *Trichogramma brassicae* Bezenko [Hymenoptera: Trichogrammatidae] Target: *Ostrinia nubilalis* Hübner [Lepidoptera: Crambidae]	Ecological: predator groups represented in target (corn) ecosystem Availability: Coleoptera and Diptera spp. commercially available, Neuroptera collected from field and reared	2 Coleoptera spp.: (1 family) 1 Diptera sp. 1 Neuroptera sp.	Babendreier *et al*. (2003d)
Agent: *Cotesia glomerata* (L.) [Hymenoptera: Braconidae] Target: *Pieris rapae* L. [Lepidoptera: Pieridae]	Socioeconomic: endangered status	2 Lepidoptera spp.: 2 Pieridae [in same genus (Pieris) as target]	Benson *et al*. (2003)
Agent: *Trichogramma minutum* Riley [Hymenoptera: Trichogrammatidae] Target: *Choristoneura fumiferana* (Clemens) [Lepidoptera: Tortricidae]	Ecological: geographic distribution Biological: oviposition phenology, voltinism, overwintering stage, host-plant preferences, egg mass type and location	2 Lepidoptera spp.: 1 Lycaenidae 1 Nymphalidae 23 Lepidoptera spp.: in 4 families 14 Hesperiidae 5 Lycaenidae 7 Nymphalidae 1 Papilionidae	Bourchier (2003)

Table 1. Summary of selection criteria used in recent studies assessing host-specificity of entomophagous biological control agents (continued).

Agent and Target	Selection Criteria Used	# Non-target Selected	Reference
Agent: *Peristenus digoneutis* Loan [Hymenoptera: Braconidae] Target: *Lygus lineolaris* (Palisot de Beauvois) [Hemiptera: Miridae]	Ecological: Habitat and temporal overlap of hosts and released agent Phylogenetic: According to maximum fit cladogram of Lygus and its outgroup Biological: Geographical distribution; temporal pattern of occurrence Availability: Set up of culture and field collected	9 Hemiptera spp: 9 Miridae (different tribes)	Haye (2004)
Agent: *Celatoria compressa* Wulp [Diptera: Tachinidae] Target: *Diabrotica v. virgifera* LeConte [Coleoptera: Chrysomelidae]	Ecological: Habitat and temporal overlap of hosts and released agent Phylogenetic: One representative species from the sister genus of Diabrotica in the Old World; One representative not closely related Coleopteran Socioeconomic: Beneficial species including weed biocontrol agent Biological: Geographical distribution; temporal pattern of occurrence; similarity in host size Availability: obtained from culture and field collected	9 Coleoptera spp.: 7 Chrysomelidae 1 Curculionidae 1 Coccinellidae	Kuhlmann *et al*. (2005)

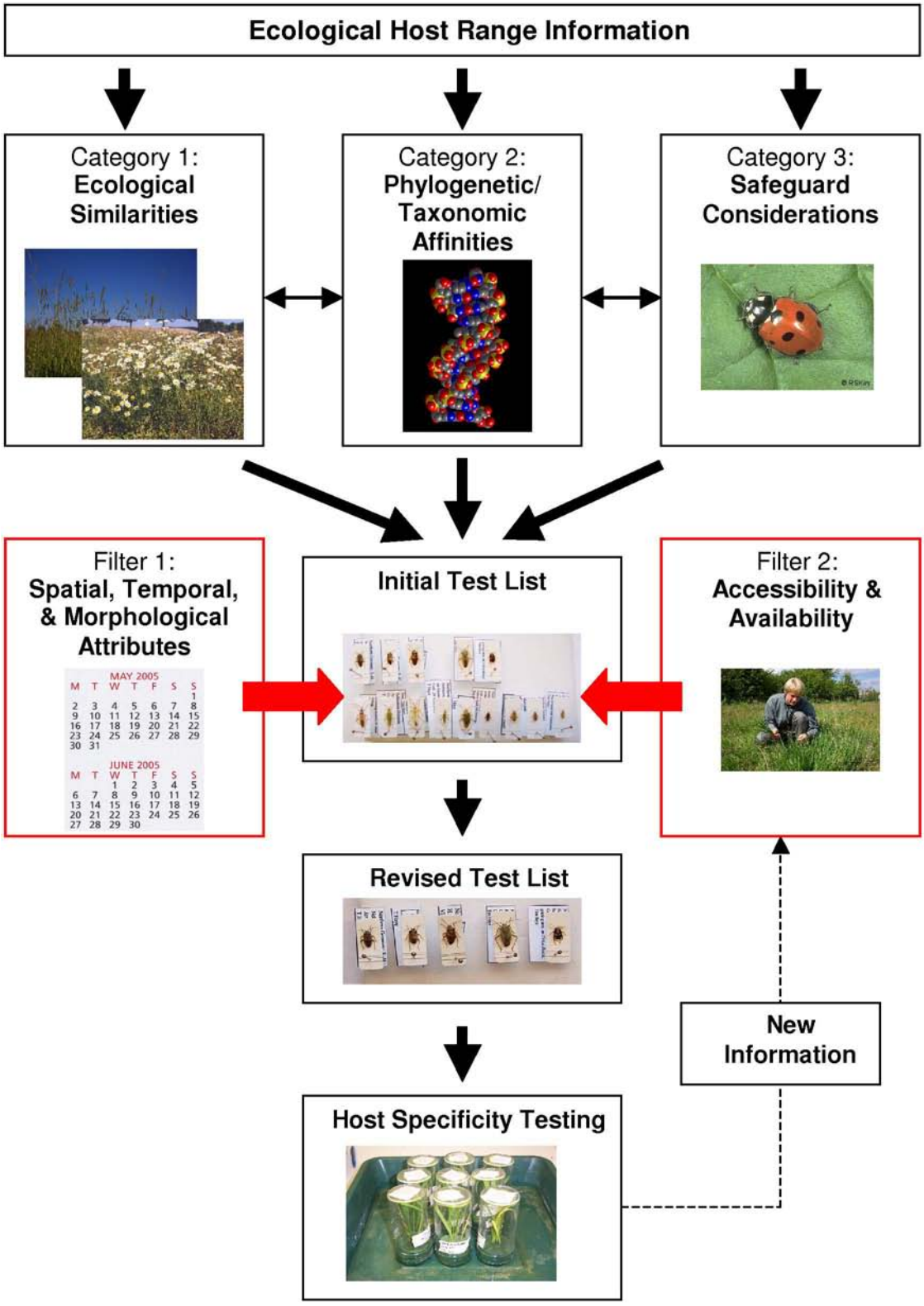

Figure 1. Recommendations for selecting non-target species for host specificity testing of invertebrates for biological control of arthropods.

The general consensus is that experiments must be performed in order to thoroughly determine the ecological (realized) host range of a potential biological control agent (Hopper 2001). This can be achieved through carefully planned field studies to determine parasitoid-host complexes in the area of origin of the candidate biological control agent. Knowledge of the host species attacked by the candidate agent and its close relatives in its native range will facilitate the selection of appropriate test species for host range testing in the proposed area of introduction (Kuhlmann and Mason 2003; Kuhlmann *et al.* 2000). It is also recommended that comparable field studies be conducted in the area of introduction to provide insight into which herbivore species would be exposed to the candidate biological control agent, both 'in space and time'. If little is known about the target pest (see Barratt 2004), these initial studies are especially necessary in order to generate the information required for selection of appropriate non-target test species.

An initial test species list can then be established based on this knowledge of ecological host range of the candidate biological control agent in its native habitat. We propose three different categories from which test species should be selected (the category order holds no relevance):

Category 1: *Ecological Similarities*: Species, which live in the same/ adjacent habitat (e.g., on arable land and adjacent field margins) or feed in the same micro-habitat (e.g., on same plant species, or in galls) as the target species;

Category 2: *Phylogenetic/ Taxonomic Affinities*: Species which are taxonomically/ phylogenetically related to the candidate biological control agent (according to modern weed biological control programmes);

Category 3: *Safeguard Considerations*: 'Safeguard' species, which are either beneficial insects (e.g., pollinators, other biological control agents) or rare and endangered species that belong to the same family or order. Additionally, host species of congeneric species of the candidate biological control agent could be selected when appropriate.

Available information may be limited such that it becomes necessary to focus on selecting species that fit into one category more than another category. However, the selection of species that are associated with more than one category should be a priority.

It is likely that the initial non-target test list will consist of at least 50 species, as is often the case for the final plant test list in weed biological control programmes. The rearing of such a number of insect species is unrealistic, however, being far more laborious and time-consuming than growing the equivalent number of plant species. Field collection of suitable stages for testing would provide an alternative to laboratory rearing, although confirmation that the collected species are not already parasitized or diseased would be required.

It has been suggested by Sands (1997) that testing more than 10 species of non-target arthropods may be impractical, and in those cases where the non-target species test list is long, often the number of species could be reduced to a more manageable size. In addition, carefully designed tests on a few species related to the target will provide adequate informa-

tion relating to the host specificity of candidate agents (Sands 1998). We therefore propose that the test species list can be reduced by filtering out those species with certain attributes (listed below) that do not overlap with those of the target species and are thus not suitable hosts. Attributes that can lead to the elimination of certain species from the list include; non-overlapping geographical distribution, different climate requirements, phenological asynchronization and host size which is outside of the range that is accepted by the candidate biological control agent (*Filter 1* in Fig. 1). The latter attribute can be tested by offering target species or other host species of different size classes to the candidate biological control agent. Phenological asynchronization of the potential non-target test species can be determined by studying the herbivore complex that inhabits the potential area of introduction of the biological control agent. Species that are neither available nor accessible in large enough numbers for adequate experimental replicates to be conducted should also not be considered for host specificity testing (*Filter 2* in Fig. 1). For rare and endangered species, it is acceptable to test congeners as surrogates.

Following this filtering process, the host-specificity test list might focus on approximately 10 to 20 non-target species. However this should not necessarily be considered as a final test list. Results from on-going host specificity testing and parallel studies to assess the chemical, visual and tactile cues emitted by the host or its host-plant(s) and involved in the agent's host-selection behaviour may shed new light on which non-target species may be at risk of being attacked by the candidate biological control agent. As is the case for weed biological programmes we propose that the revised test species list should be periodically revisited during the pre-release studies of arthropod biological control programmes (indicated by the feedback loop in Fig. 1). In North American weed biological control programmes, test plant lists that have been submitted to and approved by the Technical Advisory Group at the beginning of a programme may be subject to revision during later stages of the pre-release studies. New information gathered during the pre-release studies may lead to scientifically based justification for removal or addition of test species.

It is our belief that this reiterative process is of greater relevance in arthropod biological control programs because of the requirement to keep the test list as short as possible while still providing a reliable host range profile for the candidate biological control agent.

RESULTS AND INTERPRETATION OF HOST-SPECIFICITY TESTS WITH PARASITOID BIOLOGICAL CONTROL CANDIDATES

The usefulness of selection criteria for setting up test species lists depends on the type of results that are generated by host-specificity tests, and the ease of their interpretation. The goal of host-range testing should be to carefully select test species and choose host-selection bioassays so that the biological control agents will reject at least some of the tested species. The interpretation of results from host-specificity tests is notoriously difficult when a large number of test species are accepted. This is also true for those cases where significant differences in attack rates among the test species were found, because spatial and temporal distribution of preferred and less-preferred hosts in the area of introduction is usually highly variable (e.g., Schaffner 2001).

Based on the experience from pre-release studies in weed biological control projects, one might expect that a discriminating host-selection behavior under confined conditions can be plausible in host-specificity studies with more or less specialised parasitoid species that are considered as classical biological control agents. However, general concern has been expressed about the interpretability of results from laboratory host-specificity tests with parasitoid species, since parasitoids may display a more indiscriminant host-selection behaviour in containment than herbivorous insects (e.g., Sands 1997).

The limited number of published host-specificity studies available to date suggests, though, that tests on the basis of a carefully selected test species list can indeed provide reliable data on the fundamental host-range of parasitoid biological control candidates with a supposedly narrow host-range. The selection criteria used in these studies for setting up the test species list are reviewed in an additional paper (Kuhlmann *et al.* submitted); here we focus on the interpretability of the results obtained in the host-specificity tests.

Using multiple-choice cage experiments, Neale *et al.* (1997) exposed 17 non-target leafmining species on their respective host-plants to three parasitoids of the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracilariidae). The test species were selected on the basis of taxonomic and ecological criteria. No adult parasitoids were recovered from any of the non-target species exposed to the three biological control candidates.

Barratt *et al.* (1997) tested the laboratory host specificity of two classical biological control agents, *Microctonus aethiopoides* Loan and *Microctonus hyperodae* Loan (Hymenoptera: Braconidae), which had already been released into New Zealand. Two of the twelve weevil species exposed to *M. aethiopoides* and 7 of the 11 weevil species exposed to *M. hyperodae* were not accepted or not suitable for larval development. The narrower host-range of *M. hyperodae* displayed in the no-choice cage experiments was corroborated by data from a field study assessing the realized host-range of the two species in the area of introduction. A single record for each of two non-target species were reported for *M. hyperodae*, while *M aethiopoides* was recovered from 13 different non-target species.

The host-specificity of the supposedly specialist parasitoid *Cotesia rubecula* (Marshall) and of *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae), which had previously been recorded from several Lepidoptera species, were experimentally assessed by Cameron and Walker (1997). In the laboratory no-choice host-specificity tests, *C. rubecula* readily accepted the target host, *Pieris rapae* L. (Lepidoptera: Pieridae), for oviposition, but none of the other nine Lepidoptera species offered. In contrast, *C. plutellae* oviposited in all species tested, and completed its development in 8 out of the 13 test species. The authors concluded that laboratory tests based on suitability of hosts for parasitoid development are appropriate for demonstrating high degrees of specificity such as found in *C. rubecula*.

Kitt and Keller (1998) studied the host-specificity of *Aphidius rosae* Haliday (Hymenoptera: Braconidae), a parasitoid of the rose aphid *Macrosiphum rosae* (L.) (Hemiptera: Aphidae). In no-choice and choice experiments, only *M. rosae* and *Macrosiphum euphorbiae* (Thomas) were frequently attacked; single attacks were observed on each of two additional aphid species, while three aphid species were not attacked at all. Host suitability tests revealed that *M. euphorbiae* is not a suitable host for *A. rosae*. In wind-tunnel experiments females

were strongly attracted to roses, but not to the odours of various other plant species. The results of these laboratory studies provide strong evidence for a very narrow host-range of *A. rosae*.

A series of no-choice and choice tests with 21 different ant species were carried out by Porter (2000) to study the host-specificity of the decapitating fly *Pseudacteon curvatus* Borgmeier (Diptera: Phoridae), a biological control agent against the invasive fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). In these tests, which were conducted in small plastic trays, no *P. curvatus* larvae or pupae resulted from any of the 19 ant species from 12 non-host genera. Two congeneric, native fire ants were successfully parasitized by *P. curvatus*, indicating that the host-range of this parasitoid is likely to be restricted to fire ants of the genus *Solenopsis*.

Fuester *et al*. (2001) carried out field and laboratory studies to assess the host specificity of the tachinid fly *Aphantorhaphopsis samarensis* (Villeneuve), a biological control agent against gypsy moth. In choice oviposition tests, one out of eleven North American non-target species was attacked and supported larval development. The susceptible non-target species belongs to the same family as the target species, the Lymantriidae. In tests where nine European non-target Lepidoptera were artificially inoculated with maggots of *A. samariensis*, no puparia were obtained. These findings were in agreement with extensive field studies in Europe, during which no verifiable recoveries of *A. samariensis* from non-target species resulted. One questionable recovery each was made from two lymantriid species.

Kuhlmann *et al.* (2005) applied the recommendations outlined above for host specificity testing of *Celatoria compressa* Wulp (Diptera: Tachinidae), a candidate biological control agent of the western corn rootworm, *Diabrotica virgifera virgifera*. The final test list comprised nine Coleoptera species. Naïve and experienced *C. compressa* females did not parasitize eight non-target species but they did accept the red pumpkin beetle, *Aulacophora foveicollis* Lucas was attacked regardless of the presence or absence of *D. v. virgifera*. These studies showed *that C. compressa* has a high degree of host specificity and is restricted to a few genera in the tribe Luperini of the subfamily Galerucinae within the family Chrysomelidae.

In contrast, Haye (2004) selected seven non-target species to define the fundamental host range of *Peristenus digoneutis* Loan (Hymenoptera: Braconidae), a parasitoid of *Lygus* plant bug species in Europe. Laboratory choice and no-choice tests demonstrated that all selected non-target species were attacked and were largely suitable for parasitoid development. Haye (2004) also studied the ecological host range in the European area of origin to compare laboratory and field results. It was shown that *P. digoneutis* was reared from ten hosts in the field, including three *Lygus* species and seven non-target hosts from the subfamily Mirinae. However, the proportions of *P. digoneutis* in the larval parasitoid guild of non-target hosts were less than 5%.

In general, the published studies that report laboratory assessment of the host-specificity of supposedly specific entomophagous agents provide evidence that a careful selection of non-target test species and host-specificity tests based on host-selection behavior and host suitability allow a thorough assessment of the fundamental host-range of parasitoid biological control candidates. However, it is too early to draw any general conclusions from such a limited set of published host-specificity studies as shown by Haye (2004). Several of the para-

sitoid species which have been thoroughly tested up to date may have been selected because they were likely to display a very discriminating host-selection behavior in containment. As in weed biological control projects, it appears to be much more challenging to predict the ecological host-range when parasitoid biological control candidates do not display a discriminating host-selection behavior in containment, or when they have a relatively broad fundamental host-range. A relatively broad host-range may be particularly common in parasitoids aimed for use in inundative biological control projects. In these cases, laboratory host-range studies may be of limited value, and a thorough risk assessment will need to consider additional aspects, such as dispersal as well as long-distance and short-distance host-searching behaviour of the biological control candidate (Babendreier *et al*. 2005; Orr *et al*. 2000).

CONCLUSIONS

Selection of non-target species for inclusion in host range testing for exotic entomophagous biological control agents must be done carefully to ensure that appropriate species are chosen. While phylogenetic relationship (taxonomic relatedness) is a useful starting point, other attributes such as ecological similarities, biological habits, socio-economic considerations, and test species availability are of primary importance and have been used in the limited number of studies conducted to date. Because the number of plant species screened in weed biological control (typically 40-100) would be prohibitive for testing entomophagous biological control agents one of the key aspects in host specificity testing in arthropod biological control programmes lies in setting up a test species list that is both scientifically sound and manageable. This is a challenging task, particularly since host-selection by parasitoids is often triggered by an additional trophic level (host and host-plant) than that by herbivores.

The recommendations proposed will help improve the host specificity testing of entomophagous biological control agents. Compilation of a test species list is in itself a valuable step in the pre-release assessment because it provides a mechanism for assembling and synthesising relevant information and knowledge. Hopefully, new evidence from thorough host specificity tests will accumulate relatively quickly so that the proposed recommendations for the non-target selection procedure, which are based on a relatively small data set of experimental parasitoid host range assessments, can be thoroughly tested and refined as necessary.

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HOST RANGES OF NATURAL ENEMIES AS AN INDICATOR OF NON-TARGET RISK

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ABSTRACT

Potentially, the introduction of exotic natural enemies or mass release of biological control agents may lead to unwanted non-target effects. Whether or not such effects occur will mainly depend upon the host range of the biological control agent and the presence of non-target species in the area of release. Host-specificity testing is an important aspect of host-range assessment – perhaps the most important, and the easiest conceptually for regulators. Usually, laboratory based manipulative experiments will form the core of host-range assessments, but there is little information on how to determine host ranges. Here, we present a framework for step-wise host-range testing with levels of increasing complexity that should allow to avoid over- and underestimation of the host range of a biological control agent. Next, the interpretation of data obtained with host-range testing is discussed and conclusions are drawn about the importance of host-range testing in future biological control projects.

INTRODUCTION

Contrary to the thorough host-range evaluations applied in the search for natural enemies of weeds (Wapshere 1974), host ranges of biological control agents for insect or mite control were usually not extensively studied until recently. The earlier lack of concern for non-target effects combined with the fact that very few non-target effects were ever found in insect biological control resulted in hardly any host-range assessment or screening studies before the 1990s with the exception of Australia, which started in the 1980s.

Several publications have appeared in which ideas or methods for host-range testing are presented; they are reviewed in van Lenteren *et al.* (2006a). Aspects of risk assessments have been developed and applied during the past two decades, though often in a preliminary way and not always satisfactorily (van Lenteren *et al.* 2006b). Decisions about release of exotic natural enemies are still often based on short term decisions strongly influenced by financial and social benefits reflecting national priorities, and tend to ignore environmental ethics especially where risks are difficult to quantify. However, there are several positive developments taking place currently, which commenced with the design of a Code of Conduct for the import and release of exotic biological control agents (IPPC 1996). A recent review in which the implementation and use of this Code of Conduct is evaluated (Kairo *et al*. 2003) led to the following conclusions: (1) the CoC is widely used currently, (2) with the CoC several requests for importation could be rejected based on good reasons, (3) the CoC made evaluation procedures generally more rigorous and lengthy, but did not lead to fewer introductions, (4) most users were positive about the implementation of the CoC, but also that (5) the CoC lacks procedures for, among others, host-range assessment schemes and host-range testing methods that need to be developed with high priority.

Although there is still much debate on how to test host specificity, several protocols for host-range determination have been designed and used during the past decade (Barratt *et al*. 1997; Sands 1998; van Lenteren *et al*. 2003). An important conclusion from recent papers on risks of releasing exotic biological control agents is that host-range assessment should form the focus of every natural enemy risk assessment, because the width of the host range will, together with the numbers of natural enemies that are released and the dispersal capacity of the natural enemy, determine the probability that non-target effects will occur. Several sources of information may be incorporated into a host-range assessment, including literature records, field observations in the area of origin, and physiological, behavioral and ecological observations and experiments; all these aspects are reviewed in van Lenteren *et al.* (2006a). Usually though, laboratory based manipulative experiments to test host ranges will be performed.

Developing a list of appropriate nontarget species is a difficult task and is discussed in detail by Kuhlmann *et al.* (2006). In addition to what one would logically select as potential indigenous non-target species, species should be included that are of *conservation concern* or important biological control agents, i.e. any non-target species considered to be at risk from introduced biological control agents, causing declines in distribution or density, or local and regional extinctions. Species of conservation concern may not necessarily be taxonomically closely related to the target species but their ecological, cultural or conservation significance are considered sufficient to justify an expansion of the host-range testing schedule.

In this paper we attempt to answer the question of how to test the host specificity of arthropod biological control agents, and we present a framework for host-range testing. Next we will discuss the interpretation of data obtained with host-range testing, and finally some conclusions are drawn.

DEVELOPING HOST-RANGE TESTS

Hypotheses about host ranges of natural enemies generated from the literature and field surveys can be tested in formal laboratory host-range tests (Sands 1998). Host-range tests aim to demonstrate if a natural enemy can feed, develop or reproduce on a nontarget species. Laboratory testing can become quite complicated as a result of multitrophic chemical communication, learning and wide host ranges, involving many host plant species. Host preferences are determined not only by the choice of species offered, but also by the physiological condition and experience of the natural enemy under investigation. Host-range testing is relevant only if proper controls are included. Hence, before a specific testing scheme is designed, knowledge needs to be obtained about the multitrophic system in which the natural enemy forages in order to make the tests meaningful. Particularly, behavioral variation including learning, intraspecific variation and genetic changes occurring during laboratory rearing of natural enemies, may complicate host-range testing and these are reviewed in van Lenteren *et al.* (2006a).

FRAMEWORK FOR HOST-RANGE TESTING

The above-mentioned considerations may lead to the conclusion that host-range testing is too complicated and produces unreliable results. But based on the very limited number of negative non-target effects known in biological control, we may conclude that biological control workers have generally done an excellent job in making predictions about such effects in the past. However, with an increasing number of non-specialists involved in biological control work, there is great need for a basic methodology to perform host-range testing.

Below we present a design for a testing scheme to determine host ranges of insect natural enemies. Because of the large variation in natural enemy – host relationships, this testing sequence should be considered as a basic approach, which will need to be adapted for specific situations. The test sequence we present may be simplified if this can be based on the biology of the natural enemy. Depending on the multitrophic system under consideration, one does not necessarily have to start with step 1, but can start with approaches in e.g. large arenas that allow a much more precise estimate of the host range. So, the tests described below are examples. There are a great many potential designs, and these will be determined by the nature of the interaction between the natural enemy (parasitism, predation) and the habitat occupied by the organism.

Step 1: Small arena no-choice black-box test. The aim of this test is to answer the question: does the biological control agent attack the non-target organism in the appropriate stage on the relevant part (e.g., the leaf or a root) of its natural host plant? A positive control is performed with the target species; a negative control is done with the target and non-target species without the natural enemy to check survival of target species under test

conditions. Consider that extensive stinging and superparasitism can lead to host mortality and prevent parasitoid development, and thus potentially underestimate the host range. For predators, consider the effect of cannibalism on prey range.

If none of the non-targets is attacked and the target species (=positive control = pest species) is attacked at a rate approaching that in the field, one can stop testing, because no direct effects on the tested non-target species in field are expected. If non-target hosts are attacked, even at very low rates, further testing is mandatory.

Step 2: Small arena no-choice behavioral test. The aim of this test is to answer the question: does the biological control agent consistently attack the non-target organism on the appropriate substrate of its natural host plant? A positive control is performed with the target species; a negative control is done with the target and non-target species without the natural enemy. Superparasitism in the confines of a small arena may lead to unnatural mortality of the host. Therefore, special precautions may be necessary to deprive individual hosts from repeated oviposition after first oviposition to avoid host mortality. With predators, the occurrence of cannibalism in small arenas need to be taken into account. This no-choice test can overestimate the risk of including the non-target species in the host range of the natural enemy.

If the target host (= positive control = pest species) is attacked at a rate approaching that in the field, and the non-target host is not attacked at all, one can stop testing, because no direct effects on non-target species in the field are expected. If attack rates are above zero for target and non-target host, but the attack on non-target hosts is significantly lower than on target hosts, the hazard to non-target hosts under field conditions might be low to acceptable, and further testing should be considered. If non-targets are only attacked at the end of the observation period (long latency time), then the risk of direct effects on these species is small. If non-target species are consistently attacked, with a latency time similar to the target, and attack rates on target and non-target hosts do not differ significantly, non-target effects might be considerable and further testing is mandatory.

Step 3: Large arena choice test. The aim of this test is to answer the question: does the biological control agent attack non-targets when target and non-target species are present in a semi-natural situation on their natural host plants? Present multiple host plants each with their own non-target species and the target species in a large arena. Offer target and non-target hosts in as natural a situation as possible and on their natural host plants. Positive controls are done in the same type of cage with the natural enemy and the target host only and the natural enemy and the non-target host only; a negative control is done with the target species and non-target species, but without the natural enemy. Care should be taken that the same number of total hosts is present at the start of each treatment. The experiments should be terminated before the target host is eliminated, or in case of parasitoids before most target hosts are parasitized. Consider that extensive stinging and superparasitism can lead to host mortality and prevent parasitoid development, and thus potentially underestimate the host range. With predators, consider the occurrence of cannibalism.

Non-target species that are easily attacked on their natural host plants, i.e. with similar latency times as target hosts and with similar attack rates, pose a high risk for non-target effects. If latency times of attack on a non-target species are much higher and attack rates are much lower than in the target control, the natural enemy displays a strong preference for the target species, but may be prone to attack the non-target species under situations where the target species is not present. If latency times in the choice test and the non-target control are much higher than in the target control and the attack rates are much lower in the choice test and non-target control than in the target control, the risk of direct effects on the non-target species under field conditions is small.

Step 4: Field test. The aim of this test is to answer the question: does the biological control agent attack the non-target when the non-target and the target species are present in their respective habitats? This test can only safely be done in the area of release if the biological control agent cannot establish in this area (e.g., agents from tropical areas to be used in greenhouses in temperate climates). The test can be done in the native area of the natural enemy if the non-target species also occur in this area. Release the natural enemy in the non-target habitat, and determine if there is attack of non-target species. Control: put target species on target host plant in the non-target habitat. Replicate the approach in a number of plots.

If the target species is easily attacked, and no or low attack of non-target species occurs, a low risk for direct effects on non-target species is expected. If the biological control agent easily attacks non-target species on their host plants in their natural habitat, it poses a very high risk for non-target effects.

INTERPRETATION OF HOST-RANGE DATA

Interpretation of host-range data is difficult, among others because of the confusing effect of test conditions. Regularly observed confusing effects of test design are: (1) overestimated host ranges, in which non-hosts are used by agents when deprived for long periods from their normal hosts, (2) overestimated host ranges in which non-hosts are used when in close proximity to the normal host due to transference of stimuli, and (3) underestimated host ranges in which valid, but less preferred, hosts are ignored in the presence of a more preferred host. The disruption of insect behavior when they are held in confinement, or outdoors in cages, is well known for biological control agents generally (Sands and Papacek 1993). Sometimes a particular host will be accepted in laboratory trials but when released into the field, the agent will ignore it. This anomaly commonly leads to overestimated host-range predictions for an agent and may lead to discontinuation of evaluation studies that, if continued, may have shown high degrees of host specificity. Not all potential agents are affected by confinement during tests for host preference or specificity but it is important to be wary of this problem arising and, depending on the suspected nature of the problem, adjust the design of experiments to minimize or prevent overestimated host ranges in agents. If laboratory host-range tests remain inconclusive decisions whether or not to release an agent may depend on information from its native range or countries where it has already been introduced (van Lenteren *et al.* 2006).

For mono- or slightly oligophagous and for clearly polyphagous biological control agents, the above host-range testing framework will usually lead to clear answers about risks for non-target species. Indeed, in a number of cases, host-specificity data from mono- or slightly oligophagous species found in the literature were confirmed when exposed to new non-target host species (e.g., Cameron and Walker 1997). But exceptions do occur. For example, natural enemy species that were considered to be monophagous or that had a rather restricted host range, were found to attack a number of other host species in the area of release (e.g., Barratt *et al.* 1997; Brower 1991). Conclusions about host specificity can, therefore, seldom be made alone on data collected in the area of origin of the biological control agent, although this is an important first step.

The most difficult group for interpretation of host-range data will be the more pronounced oligophagous and slightly polyphagous biological control agents. These agents might first of all not be the most efficient natural enemies and result in intermediate or partial control, and may also show more severe non-target effects when compared to strongly monophagous species. This group of natural enemies needs to be studied with high priority.

Host-range data have earlier been used to reject introductions. For example, Sands and Van Driesche (2000) reported that certain egg parasitoids were not released in the United States for control of pest Hemiptera because they were shown to attack at least 20 species of unrelated native Hemiptera. The decision not to release them was based on their wide host ranges and lack of evidence that they were effective in suppressing the target pest in their native ranges (Jones 1988).

Frustratingly little information is available about potential changes in host preference over time. While to our knowledge no recent example is available for insect parasitoids, some herbivorous insects like tephritid fruit flies that attack fruits of their host plants provide a well known example for an evolutionary host race formation in ecological time dimensions (Berlocher and Feder 2002). Apple maggot flies seem to have switched to cherries within the last century (Jones *et al*. 1989). Nevertheless, such host-range expansions, host shifts, or host race formations seem not to occur so often that they represent a major concern for the release of otherwise host specific insect natural enemies.

CONCLUSIONS

Determination of host specificity, particularly of generalist natural enemies, will always be a complicated and time-consuming affair. First there is the problem of the selection of appropriate non-target species to be tested and which set of tests to use. We propose to use the sequential test that is summarized above for the determination of host ranges of new exotic natural enemies. We already indicated that, depending on the type of natural enemy and the ecosystem where it will be released, the testing sequence might need to be adapted. We also realize that this sequential design will undergo changes with growing experience.

After host-range testing, there is the issue of interpretation of data obtained with the various tests. For all these phases, arthropod biological control workers have just started to develop a theoretical and methodological background. Finally, the risk posed by and the benefits resulting from the release of the exotic biological control agent should be weighted

against the risks and benefits of any other control method under consideration (van Lenteren *et al*. 2003; van Lenteren *et al*. 2006b; van Lenteren and Loomans 2006).

An exhaustive data search of Lynch *et al.* (2001) in which more than 5000 recorded biological control cases were analyzed and 30 international biological control experts were contacted for additional information, and information provided in van Lenteren *et al.* (2006b), has underlined our ignorance of the degree to which non-target effects occur. Host-range testing combined with pre- and post release studies need to become standard procedures in each biological control project (Coombs 2003). That this does not necessarily result in fewer introductions of exotic biological control agents has been shown by the recent evaluation of the IPPC Code of Conduct (Kairo *et al*. 2003). But it does lead to higher costs and delay of introduction. However, if higher costs and later introduction do result in fewer serious mistakes, the investments are certainly justified.

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EFFECTS OF TEMPERATURE ON THE ESTABLISHMENT OF NON-NATIVE BIOCONTROL AGENTS: THE PREDICTIVE POWER OF LABORATORY DATA

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ABSTRACT

The European Union (EU) and its constituent governments are committed to increasing the success of biocontrol in Europe, and are currently seeking a pan-European balanced regulatory system to aid this objective. The concept of 'balance' recognizes that (a) the complexity of any licensing system must be proportionate to risk, (b) industrial producers of biocontrol agents have limited RandD budgets, but (c) there can be no compromise on environmental safety. Whilst there is common agreement between agencies responsible for environmental protection, regulators and industrial producers, about the ecological information required to assess the establishment potential of non-native species, the research methods by which such data can be generated (if not available in the literature), have not been fully developed or tested. The inappropriate use of 'climate matching' between native and introduced ranges as a 'proxy' for cold tolerance and overwintering ability is one example of this problem.

Most predatory insects and mites used in glasshouse biocontrol in the UK originate from tropical and semi-tropical climates. For this reason, the licensing system for the introduction of non-native species has operated under the assumption that winter would act as a natural barrier to the establishment of such species outside of glasshouse environments. This view has been challenged by the establishment in the wild of the predatory mite *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) and the discovery of the predatory mirid *Macrolophus caliginosus* Wagner (Hemiptera: Miridae) outside of glasshouses in winter. Whilst the impact of these species on native ecosystems is unknown, their establishment is considered undesirable. This paper describes a series of experiments used to determine a range of thermal characteristics (developmental threshold, day-degree requirement per generation, supercooling point, lethal times and temperatures, field survival) of five non-native biocontrol agents. A strong correlative relationship was found between the time at which 50% of populations die in the laboratory at 5°C ($LTime_{50}$) and duration of winter survival in the field. The comparative data provide a retrospective ecophysiological explanation for the establishment of *N. californicus* and occurrence of *M. caliginosus* outside of glasshouses, and also indicate that *Delphastus catalinae* (Gordon) (Coleoptera: Coccinellidae), *Eretmocerus eremicus* (Rose and Zolnerowich) (Hymenoptera: Aphelinidae) and *Typhlodromips montdorensis* (Schicha) (Acari: Phytoseiidae) would not survive outdoors in the UK under current climatic condi-

tions, and would therefore be 'environmentally safe' introductions. The experimental protocol applied to these species could be used as part of a routine, stepwise testing procedure for 'establishment potential' in the licensing system of non-native biocontrol agents in the UK and other parts of the world.

INTRODUCTION

Biological control has a long history of use in pest management, both as a method of control in its own right, and in combination with other techniques as part of IPM programs. In some respects, the importance and success of biological control has been overshadowed, historically by pesticides, and more recently, by the prospect of insect-resistant GM crops, both of which have been viewed as a more generic approach to pest management, capable of being targeted against a range of pests in different climatic zones. However, there is now widespread international agreement on the need to reduce over-reliance on chemical pesticides, at the same time as the future of GM crops looks uncertain, particularly in Europe, not least because of public concern over risks to human health and the environment. By contrast, biological control is regarded as safe and environmentally friendly.

The definition of 'success' in biological control depends in part, on the environment into which an organism is released. In classical biological control, where relatively low numbers of a non-native predator or parasitoid are released into a new country or region of the world, often against an exotic pest, success can usually be defined by the ability of the introduced species to suppress numbers of the target pest below economic levels, and to become permanently established in the new area, thus reducing the need for re-releases. In inundative biological control, where large numbers of non-native natural enemies are released into glasshouses, pest suppression is again a criterion for success, but there is also the expectation that any organisms that escape from the protected environment will die out rapidly, and not cause any disruption to the native ecosystem. This is the 'paradox of establishment': in classical biological control, establishment is a key feature of success, whereas in inundative biological control in glasshouses, establishment outside of the protected environment is considered potentially deleterious.

INTERNATIONAL CONTEXT

Over the last 10-20 years there has been a developing trend toward international regulation for the import and release of non-native biological control agents, including the International Plant Protection Convention and the Convention on Biological Diversity. At the same time, various countries have introduced their own legislation to regulate importation of exotic species (United States, Canada, Australia, New Zealand, United Kingdom). Recently, a number of organizations have developed guidelines for the import and release of non-native biological control agents, in which an environmental risk assessment forms a central component. At the present time, the guidelines previously issued by FAO, EPPO and OECD are being harmonized to provide comprehensive guidance for EU member states and European countries under the auspices of IOBC-WPRS (Bigler *et al.* 2005) whilst the International Standard for

Phytosanitary Measures (ISPM3) will soon provide revised advisory guidelines for all introductions of non-native biological control agents worldwide.

It is evident that biological control practitioners, programs and producers will become subject to greater regulation in the future than hitherto. It is however acknowledged that any new regulatory framework should be 'balanced', whereby the complexity of the licensing system is proportionate to the risk, without compromising environmental safety. However, there is already an identified problem that may hinder the implementation of new regulations: whilst the guidelines provide clear statements about the range of information that should be included in an environmental risk assessment, they do not indicate the methods by which such information should be obtained, especially when it is not available from the published literature.

ENVIRONMENTAL RISK ANALYSIS

It is self evident, but not always recognized, that there can be no long term negative effects on native species and ecosystems unless exotic species become permanently established in new environments; transient 'summer only' survival is unlikely to have any major impact. For this reason, an environmental risk analysis should first focus on the likelihood of successful establishment of non-native species.

The two most important factors affecting the establishment of non-native biological control agents are climate (especially temperature) and availability of prey. This knowledge can be utilized in the design of risk assessment protocols. In a step-wise testing procedure to assess the outdoor establishment potential of non-native species released into glasshouses in cool temperate climates, a case can be made for firstly investigating the effects of temperature on development and winter survival, followed by experiments on host range and non-target effects, in those species that appear to be capable of developing in summer and surviving through winter.

The difficulties of assessing the establishment potential of non-native biological control agents intended for inundative release in glasshouses are well illustrated by recent experience in the UK. Successful biological control has been implemented in glasshouses with the management of the whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) by the parasitoid *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae), and the spider mite *Tetranychus urticae* (Koch) (Acari: Tetranychidae) by the predatory mite *Phytoseiulus persimilis* (Athias-Henriot) (Acarina: Phytoseiidae). These two schemes have operated successfully over decades without any recorded establishment outside of the glasshouse in the cool climates of western Europe, or any deleterious effects on native fauna. Over the last 10-15 years, a number of 'new' species have been licensed for release in UK glasshouses. Although the UK licensing system requires companies to compile an environmental risk assessment dossier containing physiological and ecological information on the subject species, including overwintering ability and host range, this 'critical information' is often unavailable. As a classic example, in the absence of any direct assessment of cold tolerance, it has been assumed on the basis of 'climate matching', that winter would be an effective barrier to establishment in the UK of species originating from warmer climates. This assumption is incorrect, as evidenced by the outdoor establishment of the predatory mite *Neoseiulus californicus* after a first release

in 1991, and occurrence outside of glasshouses in winter of the predatory mirid *Macrolophus caliginosus* following release in 1995.

In the light of the definite establishment of *N. californicus* and the possible establishment of *M. caliginosus*, a series of studies were undertaken to investigate the thermal biology of these two species, and two other species that had been licensed in the UK for the same periods of time, and for which there had been no reports of establishment or outdoor occurrence in winter (*Eretmocerus eremicus* and *Delphastus catalinae*). The same series of experiments were then conducted on a further species (*Typhlodromips montdorensis*) that was currently under study as a candidate for release in the U.K. (see Hart *et al.* 2002a,b; Hatherly *et al.* 2004; 2005; Tullett *et al.* 2004; for full details). It was envisaged that a comparative analysis of the thermal biology of established and non-established species might identify indices with 'predictive power' that could be applied to future candidate species in a step-wise risk analysis protocol.

MATERIALS AND METHODS

DEVELOPMENTAL THRESHOLD AND THERMAL BUDGET

Individuals of *N. californicus, M. caliginosus, E. eremicus, D. catalinae and T. montdorensis* were reared from egg to adult at a range of temperatures (5° to 35°C depending on the species) and the time taken to complete development recorded. The data were analyzed by weighted linear regression and the developmental threshold estimated by extrapolation of the linear relationship between development and temperature to the x (temperature) axis, and the thermal budget (day degree requirement per generation) by taking the reciprocal of the slope (Campbell *et al.* 1974).

Annual voltinism. The developmental threshold temperature and thermal budget values for each species were compared with daily temperature records over a 10 year period to calculate the annual number of available day degrees and hence the number of generations that could be completed each year. The temperature data were further divided into nominal summer (April to September) and winter (October to March) periods to indicate if development could continue throughout the year or was restricted to summer.

COLD TOLERANCE

All experiments were carried out on both immature stages (larvae, nymphs) and adult organisms of the five species, with and without a period of prior acclimation (usually 7 days at 10°C). This regime was known to increase the cold tolerance of other species and was intended to identify any acclimation ability, rather than to produce 'fully acclimated, winter hardy' populations.

Supercooling points. The freezing temperature (supercooling point or SCP) was measured by cooling the organisms (n = 20 to 50 depending on species) at 1°C min^{-1} in a Peltier cooling device, alcohol bath or differential scanning calorimeter, depending on the size of the specimens. The SCP was detected by the release of heat (exotherm) when the organisms froze.

Lethal temperatures. Replicate samples (3-5 x 10-50 specimens, depending on species) for each exposure temperature were cooled at 0.5 or 1°C min^{-1} in a programmable alcohol bath to range of sub-zero temperatures (-5° to -20°C, depending on the species), exposed at the minimum temperature for 1 min, and then warmed back to the rearing temperature at the same rate. Survival was assessed 24h after exposure.

Lethal times. Replicate samples (3-10 x 10-50 specimens, depending on species) were maintained with and without target prey for increasing periods of time (days, weeks or months as appropriate) at -5°, 0° or 5°C, and mortality assessed 24h after return to the culture temperature.

FIELD EXPOSURES

Replicate samples (5 x 40-50 specimens) were placed in the field within sealed 'quarantine boxes', with and without prey, for increasing periods of time (days, weeks or months depending on the species), and returned to the laboratory after different exposure periods. Survival was assessed within 24h.

DIAPAUSE

The occurrence of diapause was investigated by maintaining different life cycle stages of *N. californicus* and *T. montdorensis* in various 'diapause-inducing' regimes (different LD cycles and temperatures), and monitoring reproduction in the emerging adults after return to normal rearing conditions.

RESULTS

The results for the two predatory mites, *N. californicus* and *T. montdorensis* are presented in Table 1 as examples of the types of data obtained in the range of experiments conducted on the five species. Full details on all species are given in Hart *et al.*, 2002a, b; Tullett *et al.*, 2004; Hatherly *et al.*, 2004, 2005.

The developmental threshold is lower in *N. californicus* than *T. montdorensis*, though both species can complete an average of 6 generations under UK summer conditions; a key difference between the species is the ability of a non-diapausing strain of *N. californicus* to both develop and reproduce in winter. The freezing temperatures of adult females of the two species were similar and did not change after a period of acclimation. In both species there was evidence of substantial pre-freeze mortality with $LTemp_{50}$ values considerably above the mean SCP. However, the most striking differences between the species were in the $LTemp_{50}$, $LTime_{50}$ (at 5°C), and maximum survival times in the field in winter. In all these indices, *N. californicus* was clearly the more cold hardy species.

The data in Table 1, together with that for *M. caliginosus*, *E. eremicus* and *D. catalinae* were then analyzed (Pearson product moment correlation with Bonferroni correction for multiple comparisons), to identify any relationship between laboratory indices of development and cold tolerance (developmental threshold, thermal budget, SCP, $LTemp_{50}$ and $LTime_{50}$)

and survival in the field in winter. The only significant correlation was between the $LTime_{50}$ (at 5°C) and maximum survival time in the field ($r = 0.97$, $P < 0.005$, Fig. 1; Hatherly *et al*. in press).

In the laboratory, *N*. *californicus* had the longest $LTime_{50}$ and survived for the longest in the field (over 3 months); the mites also reproduced before dying. By contrast, *T*. *montdorensis* has a short $LTime_{50}$ and died out quickly in the field. Also, provision of prey extended the survival time of *N*. *californicus*, with 10% still alive after about 4 months, when observations ended.

Table 1. Ecophysiological data for *Neoseiulus californicus* and *Typhlodromips montdorensis* as part of a risk assessment protocol.

Index	*N. californicus*	*T. montdorensis*
Development		
Developmental threshold (C)	8.6	10.3
Thermal budget (DD)	142.9	108.7
Mean annual voltinism	7	6
Development in winter	Yes	No
Cold tolerance		
Mean SCP ± SE (C)		
Acclimated female	-22.2 ± 0.4	-22.4 ± 0.5
Non-acclimated female	-21.6 ± 0.3	-24.1± 0.6
LTemp50 ± 95% fiducial limits (C)		
Acclimated female	-17.7 ± 0.3	-11.5 ± 1.0
Non-acclimated female	-13.9 ± 0.3	-6.7 ± 1.1
LTime50 ± 95% fiducial limits (days at 5 C)		
Acclimated female	65.4 ± 2.5	11.6 ± 1.1
Non-acclimated female	38.6 ± 1.9	9.5 ± 1.1
Field survival		
Maximum survival time (days)		
Without prey	100	35
With prey	112*	35
Reproduction in winter	Yes	No
Ability to diapause+	No	No

*10% still alive after 112 days, +Refers to tested strain

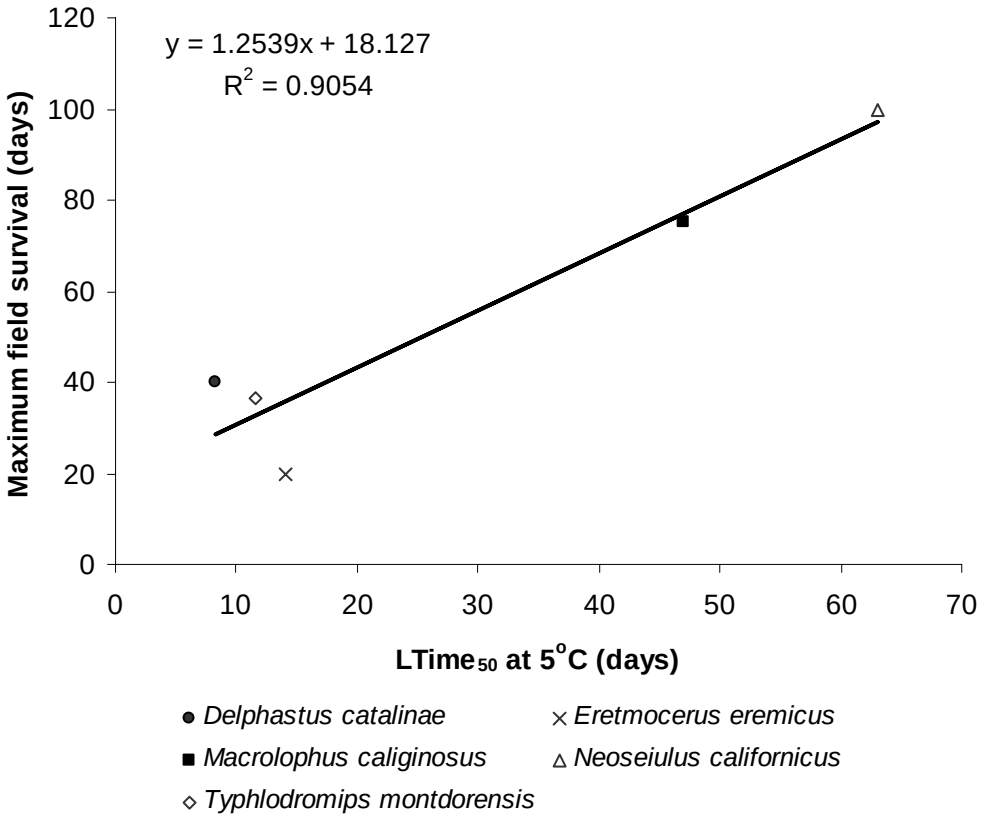

Figure 1. Relationship between maximum field survival (days) and $LTime_{50}$ at 5°C (days) for five non-native biological control agents (data refer to unfed adults of all species except *E. eremicus* that were exposed as unfed larvae).

DISCUSSION

Environmental risk assessment (ERA) of non-native biological control agents, regulated by worldwide, European or country-specific legislation, is an inevitable reality over the next 5-10 years. Irrespective of the proven historical safety of biological control with non-native species, the 'precautionary principle' is now pervasive across all methods of pest management. The common task of scientists, the biological control industry, regulatory bodies, and environmental agencies, is to design and implement a system where the complexity and level of testing in the ERA is proportionate to the risk, without compromising environmental safety.

The recent IOBC-WPRS guidelines (Bigler *et al.* 2005), based on similar documentation from OECD (OECD 2004) and EPPO, together with a comprehensive review of risk assessment of non-native biological control agents (van Lenteren *et al.* in press) have all highlighted an essential requirement for any ERA: the testing should be conducted in a 'step wise' manner, such that species that are either demonstrably safe, or likely to establish and impact on native species or ecosystems, are identified early in the process. The likelihood of establishment is clearly a crucial component in an ERA, especially for inundative releases into glasshouses.

In cool temperate climates, two dominant factors will determine the establishment potential of species escaping from glasshouses: overwintering ability and sources of prey. These two factors must therefore be the central focus for any risk assessment. However, the 'step wise' concept suggests that the first stage of the assessment should focus on overwintering, because if a species is unable to survive through winter, establishment is impossible, and hence, any consideration of effects on non-target prey becomes irrelevant.

The analysis presented in Fig. 1 indicates that the $LTime_{50}$ at 5°C is a reliable predictor of the winter field survival of five non-native biological control agents, representing different taxonomic groups and trophic guilds. It is important to stress that this predictive relationship should not be viewed in isolation; it is one component of an ERA. Also, there is clearly a limit to the sensitivity of the system in terms of estimating maximum survival times in the field. The real value of this approach is that it enables candidate agents be classified into different 'risk categories'. For example, *D. catalinae*, *E. eremicus* and *T. montdorensis* are representative of a 'low risk' group, where 100% field mortality occurs within four weeks and any establishment is highly unlikely. An 'intermediate risk' group would contain *M. caliginosus,* where survival may persist for extended periods outdoors in winter with limited establishment. *Neoseiulus californicus* would fall into a 'high risk' group where some strains are able to overwinter in diapause and non-diapause strains survive long enough to develop and reproduce.

An indication that a non-native species is able to survive through winter in a new environment is not in itself a reason to reject a licence application. Other forms of risk assessment should then be carried out, on host range and dispersal (van Lenteren *et al.* 2003; in press), and in the final analysis, it may be decided that the overall benefits of release outweigh the risks.

In critically reviewing the contribution that studies on thermal biology, cold tolerance and overwintering can make to an ERA, there are a number issues to consider, including: the possibility that the observed relationship may have occurred by chance, that other indices have similar predictive power, and the extent to which the system is applicable to insects and mites with different levels of cold hardiness.

There are sound ecophysiological reasons to believe that the observed relationship is based on a representative index of cold tolerance that links the laboratory to the field and is not a 'chance occurrence'. It is known that the vast majority of insects show some pre-freeze mortality, in some cases, with 100% death above the SCP. For this reason, the SCP temperature in isolation is not a reliable indicator of cold hardiness, and hence, no correlation with field survival would be expected (and was not found). For insects and mites that originate from warm climates, where pre-freeze mortality is extensive, it is intuitive to predict that the duration of survival at low temperatures (0° to 5°C) in the laboratory would be reflected in field survival, and this was shown to be case. A similar relationship has been reported for a range of native and non-native crop pest species in the UK (Bale and Walters 2001).

It is interesting that no other laboratory index of thermal biology was correlated with field survival. In some respects, the most misleading information relates to the estimation of the developmental threshold and annual number of available day degrees. Both *N. californicus*

and *T. montdorensis* can complete an average of 6 generations in UK summers, but their winter survival is markedly different. Estimates of annual voltinism are clearly important, but are not a reliable indicator of winter survival or establishment potential.

The final consideration concerns the applicability of this system to other insects and mites with different levels of cold tolerance. The current analysis includes species in which pre-freeze mortality occurs after exposures of days or a few weeks (*D. catalinae*, *E. eremicus* and *T. montdorensis*) up to several months (*M. caliginosus* and *N. californicus*). These two groups would be classified as 'chill susceptible' and 'chill tolerant' respectively according to Bale (1996). In terms of the world-wide distribution of insects and mites, there are very few 'true' freeze susceptible species (where there is no mortality above the SCP), and only a small number of freeze tolerant species. These species tend to inhabit the coldest regions of the world, and none have ever been used as biological control agents. In summary, it seems reasonable to conclude that the current protocol is applicable to virtually all insects and mites that are likely to be considered as non-native biological control agents, and can make a valuable contribution to a step-wise environmental risk assessment.

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HOW TO ASSESS NON-TARGET EFFECTS OF POLYPHAGOUS BIOLOGICAL CONTROL AGENTS: *TRICHOGRAMMA BRASSICAE* AS A CASE STUDY

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ABSTRACT

We show key elements of the risk assessment conducted for *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae), an egg parasitoid which is successfully used for control of the European corn borer in European countries. The main factors that we addressed in this study were: the potential of establishment; acceptance and parasitism of non-target butterflies under laboratory, field-cage and field conditions; the searching efficiency in non-target habitats; the dispersal capacities; and the potential for effects on other natural enemies in maize.

Although high parasitism of non-target butterflies and other natural enemies were observed under laboratory conditions, very few eggs of the non-target species were attacked in the field. These findings may be explained by a low host searching efficiency and the observation that female *T. brassicae* do disperse only a few meters per day. We conclude that the possibility of using invertebrate agents with a broad host range in inundative biological control should not *a priori* be excluded, however, a thorough environmental risk assessment should be performed prior to release.

INTRODUCTION

Egg parasitoids of the genus *Trichogramma* are used for inundative biological control against a range of agricultural pests. In fact, *Trichogramma* spp. are the most widely used natural enemies in inundative biological control worldwide and both native and exotic species have been mass reared and released The vast majority of *Trichogramma* species are known to be polyphagous attacking a wide range of lepidopterans as well as insects belonging to other orders (e.g., Thomson and Stinner 1989). Due to this wide host range concerns have been expressed already several years ago that mass released *Trichogramma* may threaten non-target species in natural habitats (Andow *et al.* 1995; Orr *et al.* 2000).

Concerns about detrimental effects of introduced species on the native fauna have been increasingly expressed over the last two decades. There is now general agreement that the potential for non-target effects has to be evaluated before releasing biological control agents. During the last 10 years, several guidelines addressing non-target effects have been developed. For instance, the Organisation for Economic Co-operation and Development (OECD) developed guidelines to provide 'light regulation' for invertebrates used in classical and inundative biological control (OECD 2004). Despite these initiatives which basically aim to provide guidance on what data should be considered for environmental risk assessment, there is still a debate on how these data can be obtained. Van Driesche and Reardon (2004) provided a 'guide to best practice' on how to conduct host specificity testing which generally forms an important part of the risk assessment. Babendreier *et al*. (2005) recently published a comprehensive review on the methods used to assess non-target effects in biological control and a book in which questions on environmental risk assessment of arthropod biological control are addressed will be published in the near future (Bigler *et al.* 2006).

In this paper we summarize key elements and results of an environmental risk assessment project conducted for *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) in Switzerland from 1998 to 2002 which was part of the EU funded project 'Evaluating Environmental Risks of Biological Control Introductions into Europe' (ERBIC).

PRIME FACTORS FOR NON-TARGET EFFECTS

Host specificity is one of the bottom lines in the assessment of non-target effects (Van Driesche and Reardon 2004; Van Lenteren *et al.* 2006) and hence, only agents with a narrow host range are considered for release in classical biological control. However, less specific agents are sometimes used in inundative biological control. One example is *T. brassicae* which is used since many years in European countries for control of the European corn borer, and it is known that this species attacks eggs of other lepidopterans and other non-target insects. We tested host acceptance and parasitism of *T. brassicae* on non-target butterflies and predators in maize fields under laboratory, semi-field and field conditions. Further we hypothesized that host searching efficiency in non-target habitats could be another important factor responsible for adverse effects on non-target butterflies.

In contrast to classical biological control, overwintering and establishment are negative properties of non-native agents if used for inundative biological control. If establishment does not occur, the risk for non-target species is limited to the period of release and possibly the following weeks if females can reproduce on target or non-target hosts in the crop or in other habitats. Therefore the risk of non-target impacts is spatially limited and of transient nature (Lynch *et al*. 2002). If *T.brassicae* would be able to survive the winters, reproduce on non-target host eggs in the area of introduction and disperse, there is potential for permanent effects on a large geographical scale.

OVERWINTERING

In order to test for the ability of *T. brassicae* to establish in Switzerland, two experiments were conducted. The first one was designed to study whether *T. brassicae* would survive outdoor winter conditions in Switzerland. Eggs of six non-target host species parasitized in the laboratory by *T. brassicae* were exposed under outdoor conditions (in Zurich) every two weeks between 26 September and 7 November. Control eggs were kept in an environmental chamber at 25 °C, 70% RH (for details see Babendreier *et al.* 2003a).

We found that *T. brassicae* is able to overwinter successfully on eggs of six lepidopteran species in the families Tortricidae, Noctuidae, Plutellidae, Pyralidae and Crambidae. Between 75% and 100% emergence was observed in the following spring for all of the six host species exposed on 26 September. On later exposure dates, spring emergence decreased significantly and no development of *T. brassicae* occurred from host eggs parasitized on 7 November.

In a second experiment, we evaluated at what time of the year diapause induction under field conditions occurs. Eggs of the flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) were offered to *T. brassicae* females at five consecutive dates at weekly intervals from 27 August to 24 September. After parasitization in the laboratory, the eggs were exposed under outdoor conditions until emergence occurred. We found that the period of diapause induction is equal to the dates which allowed successful development and overwintering of *T. brassicae*. In order to evaluate the effect of overwintering on the fitness of females that had spent the winter in diapause inside the eggs of *E. kuehniella* under outdoor conditions from 17 September 1999 to beginning of May 2000, we measured the fecundity of 30 females. Fecundity of females that overwintered outdoors was not significantly different from the fecundity of females that were reared in the laboratory without diapause at 25 °C.

Our results demonstrate that the egg parasitoid *T. brassicae* is able to overwinter successfully in northern Switzerland and that is has the potential to establish if host eggs were available.

PARASITISM OF NON-TARGET BUTTERFLIES

Since *T. brassicae* was known to be polyphagous, we concentrated on butterflies because of the strong environmental concerns for this group of insects. We exposed eggs of 23 non-target lepidopteran species, including nine endangered species of Switzerland, to single *T. brassicae* females under no-choice conditions in the laboratory (Babendreier *et al.* 2003b). Most of the species were well accepted and parasitized at the same level as the target, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae). In addition to oviposition, we also measured the number of times a female rejected a host egg before acceptance as well as the time from first host egg contact to acceptance.

In a next step, we investigated parasitism of six non-target butterfly species by *T. brassicae* in field cages of 2 x 2 x 2 m (Babendreier *et al.* 2003c). Eggs of the non-targets were glued on

host plants together with *E. kuehniella* eggs (multiple choice) and exposed for 24 hours to the females. Parasitism of non-target species in field cages ranged between 2.5% and 18.7%. We found that parasitism was density dependent.

Field trials were then carried out in maize fields and adjacent meadows (Babendreier *et al*. 2003c). We released 30,000 female *T. brassicae* in a plot of 50 x 50 m. This corresponds to the number of females released in commercially treated maize fields. All release plots were situated inside the maize fields but bordering the meadows. We exposed eggs of two non-target hosts together with eggs of *E. kuehniella* as a control. Eggs were exposed for 3 days at 2 m distance inside the maize field and at 2 m and 20 m distance outside the maize field in the meadow. At each distance, we attached 30 single eggs of the non-targets and 30 egg masses of *E. kuehniella* (50 –100 eggs each). As a control for natural occurrence of *Trichogramma* spp., we placed 30 egg masses of *E. kuehniella* on leaves of maize plants in two fields that were 1-2 km away from the treated fields.

Parasitism rates of *E. kuehniella* egg masses inside maize fields averaged 40% compared to significantly lower parasitism rates of 26.2% and 12.6% for eggs of the two non-targets. In the meadow, at 2 m distance from the maize field, parasitism rates decreased to 2.3% and 6.1% for the non-targets and 9.8% for *E. kuehniella* while no single egg was found parasitized in the meadow at 20 m distance from the maize field.

HABITAT SPECIFICITY

In order to evaluate whether the low parasitism in meadows can be generalized and to understand the underlying mechanisms, we studied the searching efficiency of *T. brassicae* in several non-target habitats such as meadows, flower strips and hedgerows. At the same time, *T. brassicae* was released at rates of 120,000 females/ha in plots of maize and one of the selected non-target habitats (plot size 24x24 m). Sentinel egg clusters of *E. kuehniella* were applied to the plants and recollected after 3days. Parasitism of sentinel egg clusters was 1.6 - 3.6% in meadows and 2.0 - 4.0% in flower strips while the respective figures were 57.6% – 66.7% and 19.2% - 46.9% in maize (Babendreier *et al*. 2003d). Subsequent field cage experiments confirmed the higher parasitism rates in maize compared to meadows, flower strips and hedgerows.

To investigate the factors responsible for the low parasitism in non-target habitats, the behavior of individual *T. brassicae* females was observed on common meadow plants. Single females were directly observed on different plants and parameters such as mean walking speed, turning angles and number of wasps leaving the plants were measured (Babendreier *et al*. 2003d). Significant differences in these variables were found between maize and four meadow plants. The most pronounced effects were found between maize and red clover, a very common plant in meadows in Switzerland with very hairy leaf surface. In a laboratory choice experiment, carried out with all five host plant species together in cages, we obtained highest parasitism on maize and lowest on red clover, confirming the behavioral observations.

DISPERSAL

While dispersal is a prerequisite of a successful classical biological control agent, it may be a negative feature in the context of non-target effects of inundatively released agents. The ultimate question is how many released biological control agents will enter a given non-target habitat or, more precisely, what densities of the agent can be found in certain distances from the release fields. To answer this question, experiments were carried out to investigate the dispersal behavior of *T. brassicae* (Babendreier *et al.* 2002; Kuske *et al*. 2003; 2004; Mills *et al*. 2006). The first experiment aimed to establish the degree to which *T. brassicae* will leave maize fields where they were released. Traps consisting of a plastic transparent sheet (30 x 21 cm), sprayed with glue on both sides, were placed at the edge of a maize field. These traps were mounted on wooden sticks at a height of 40-70 cm and positioned inside the field (0.8 m from the edge), at the edge of the field and outside the field (0.8 m from the edge). After one week the numbers of male and female *T. brassicae* on each side of the trap were counted. The results indicated a strong decrease in numbers from inside to outside of the maize field.

Kuske *et al.* (2003) increased the scale of this experiment and placed traps of the same type at distances up to 40 m away from the edge of maize fields. Traps were placed directly above the vegetation and exposed for one week before and during the first and the second commercial release of *T. brassicae* as well as for three weeks following the second release. A strong decrease in numbers with distance was observed and, altogether, it can be concluded from these experiments that a large fraction of *T. brassicae* will not leave the field. Moreover, the experiments have shown that *T. brassicae* will be present in non-target habitats close to the release field only for one or two weeks after releases.

In order to investigate the distance that individual *T. brassicae* travelled in a given time period, about 100,000 wasps were released from parasitized eggs from a central release point in a meadow (Babendreier *et al*. 2002). Sticky traps that had been placed at distances of 2, 4, 8, 16, 32 and 64 m in four directions from this release site were changed daily and all *T. brassicae* that had been collected were counted. This experiment revealed that *T. brassicae* only flies a few meter per day (Mills *et al.* 2006). Finally, sticky traps were used to study whether hedgerows may act as a barrier for dispersing *T. brassicae* (Babendreier *et al*. 2002).

INTRAGUILD PREDATION AND INDIRECT EFFECTS

After demonstrating that non-target effects will most likely be restricted in space and time, we decided to conduct a final experiment on potential effects on populations of other natural enemies in maize. In a tiered approach, experiments were conducted on the host acceptance of *T. brassicae* towards eggs of *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), *Episyrphus balteatus* (De Geer) (Diptera: Syrphidae), *Coccinella septempunctata* L. and *Adalia bipunctata* L. (both Coleoptera: Coccinellidae) under laboratory, greenhouse cages and field conditions (Babendreier *et al*. 2003e). While no offspring emerged from eggs of *A. bipunctata*

and *C. septempunctata,* high parasitism rates were obtained for *C. carnea* and *E. balteatus* eggs in laboratory experiments. However, we observed significantly increased mortality on *A. bipunctata* eggs, compared to the control and also found young instars of *T. brassicae* inside *A. bipunctata* eggs. In a second experiment where the host acceptance behavior of the parasitoid female was directly observed for 10 min, 10% of *T. brassicae* females were found to oviposit in eggs of *A. bipunctata* but development of parasitoid offspring failed.

In greenhouse cages, parasitism rates of *C. carnea* eggs (7%) and *E. balteatus* eggs (0.4%) were significantly lower than parasitism of *E. kuehniella* eggs (21 and 27%, respectively) that were used as a control in the two experiments. In the field, only 3.1% of *C. carnea* eggs were parasitised by *T. brassicae*. This was significantly less than the observed parasitism rate of *E. kuehniella* egg clusters (64%). From direct observations of the parasitoids host acceptance behavior and the low parasitism rates observed in cages and under field conditions we conclude that ecologically relevant adverse effects of mass released *T. brassicae* on natural enemies in maize are unlikely to occur.

Finally, we aimed to assess the potential for negative effects on the native larval parasitoid *Lydella thompsoni* Hert. (Diptera: Tachinidae) (Kuske *et al.* 2004). In Switzerland, this tachinid was found to develop the first generation on the two non-target lepidopteran species *Archanara geminipuncta* Haworth (Lepidoptera: Noctuidae) and *Chilo phragmitellus* Hb. (Lepidoptera: Crambidae) living on common reed plants, *Phragmites australis* (Cav.), while subsequent generations attack the European corn borer in maize. Severe parasitism of the two non-target lepidopterans by *T. brassicae,* immigrating from maize fields into reed habitats could lead to negative effects on the tachinid due to competition. Under laboratory conditions, both non-targets were found to be suitable hosts for *T. brassicae.* However, parasitism rates were low, either because eggs are hidden between leaf sheaths and the stalk of the host plant or because of low attractiveness of the eggs. Field experiments and surveys of the two non-target lepidopteran species were conducted in common reed habitats located amongst maize fields with *T. brassicae* releases. No single egg of the two non-target species was found parasitized, indicating that negative effects on the native tachinid due to mass releases of *T. brassicae* are unlikely.

CONCLUSIONS

We have provided an example on how to conduct a full environmental risk assessment for a polyphagous biological control agent. The study on non-target effects of *T. brassicae* mass releases demonstrates that the final conclusion on environmental risks could be drawn only after investigating host range, establishment, dispersal and competition in laboratory and field experiments. We have evidenced that low dispersal capacities and low host searching efficiency in non-target habitats were the main determinants to explain the relatively low level of risk associated with this egg parasitoid. Our results indicate that the structural complexity of the plants and of the habitat play a role for the low searching efficiency. We conclude that the possibility of using agents with a broad host range in inundative biological control should not *a priori* be excluded, however, a thorough environmental risk assessment should be performed prior to release.

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TOOLS FOR ENVIRONMENTAL RISK ASSESSMENT OF INVERTEBRATE BIOLOGICAL CONTROL AGENTS: A FULL AND QUICK SCAN METHOD

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ABSTRACT

The deliberate or accidental introduction of species from their native ranges to new environments is a major threat to biological diversity. Biological control is both an important management tool for controlling threats to agriculture an the environment as well as - in rare cases - a potential threat to the environment itself. The newly adopted International Standard for Phytosanitary Measures No. 3 (ISPM3) offers a framework for risk assessment and focuses specifically on the shipment, import, export and release of biological control agents. Guidelines for information requirements of exotic natural enemies and methods for risk assessments are currently in development. The major challenge in developing risk assessment methodologies is to develop protocols and guidelines that will prevent serious mistakes through import and release of potentially harmful exotics, while at the same time still allowing safe forms of biological control to proceed. We expect that a risk assessment methodology for biological control agents will integrate information on the potential of an agent to establish, its abilities to disperse, its host range, and its direct and indirect effects on non-targets. In this presentation, we first propose a comprehensive risk evaluation method (full scan) for new natural enemies and, second, a quick scan method for natural enemies already in use. The outcome of our evaluation of150 biological control agents, commercially available in northwest Europe, will be discussed.

INTRODUCTION

Measures to protect the environment, and people in it, have involved a wide variety of approaches and underlying principles (Calow 1998). Risks posed to human and animal health and to ecosystems from chemicals, genetically modified organisms and from biological introductions are widely assessed, based on scientific methods and procedures (Simberloff and Alexander 1998). Risk assessment is a tool that can be used to support exclusion of invasive

species as well as to assess the potential impact of those that have become established. Risk assessment can be used in decision-making to help determine if action should be taken, and, if so, what kind (Wittenberg and Cock 2001). There is, however, still a great need for research on risk assessment procedures and methods to evaluate biological introductions. Although regulations for biological control agents of weeds have been more strict than those of pests, risk assessments have not always been accurate enough to prevent ecological side effects on nontarget hosts (Louda *et al.* 2003). Invertebrate biological control agents (IBCAs) are applied across the world to control pest species in agricultural, urban and natural ecosystems. In the past 100 years many exotic natural enemies have been imported, mass-reared and released as biological control agents for pest control in areas outside their origin. In few cases, negative effects of these releases have been reported, mostly of generalist predators, often vertebrates (Lynch and Thomas 2000; van Lenteren *et al.* 2005). The current popularity of biological control may, however, result in problems: an increasing number of projects will be executed by persons not trained in identification, evaluation and release of biological control agents, an increasing number of agents and products will become available for the control of pest organisms, and the internet increasingly lowers access, sales and demands for public use.

The International Plant Protection Convention (Rome 1951; IPPC 1997) and the Convention of Biological Diversity (CBD 1992) are the two conventions which are most relevant for biological introductions of economical and environmental concern. Obligations on contracting parties include development of scientifically based risk assessment procedures and methods. Whereas for plant pests there is a long history of such procedures and measures, for introductions of organisms of environmental concern these are relatively new (IPPC 2004). Since 1992 more and more countries have put legislation in place concerning biological introductions that threaten species habitats and biological diversity. This has increased the international interest in risk assessment as a legislative tool. The FAO Code of Conduct (FAO 1996) has brought about important changes in the regulation of IBCAs in developed (EPPO 1999; 2000; NAPPO 2001) and developing countries (Kairo *et al.* 2003), but these were still largely non-legislative instruments. The recently revised ISPM3 (IPPC 2005) includes assessment of environmental risks and offers contracting parties a minimal standard when putting regulation in place. In addition, its recognition by the WTO-SPS agreement, provides that ISPM3 will be an international binding instrument that offers a format for trade in and release of biological control agents (WTO 1994). Except that there is a need for generic risk assessment schemes for all types of biological introductions, there is a specific need for schemes tailored for biological control and other beneficial organisms. Here we summarize new tools for assessing environmental risks of biological control agents that have been developed recently (van Lenteren *et al.* 2005; van Lenteren and Loomans 2005), consisting of a full and a quick scan analysis.

ECOLOGICAL DETERMINANTS

Various qualitative methods are used to generate a cumulative risk index for potential quarantine pests by adding qualitative or quantitative scores, such as low, medium, high (APHIS 2000; NRC 2002), assign numerical scores in a questionnaire (EPPO 1997; MacLeod and Baker 2003) or using successive matrices (Biosecurity Australia 2001; Murray 2003). Simi-

larly quantitative risk assessment models have been developed for weed introductions (Pheloung *et al.* 1999; Williams and Newfield 2002) and their biological control agents (Wapshere 1974). Risk assessment procedures for inoculative and inundative biological pest control need to be more tailored to its specific requirements and needs, and support a well-balanced decision making process, properly weighting its principal beneficial and potential detrimental impact (Sheppard *et al.* 2005). Environmental risk assessment should preferably be placed in a general framework for regulation of import and release of biological control agents (OECD 2004), including

- characterization of the agent (taxonomic, biological characteristics),
- risks posed to human and animal health,
- efficacy, quality control and benefits of use, and
- environmental risks.

The latter category, assessment and analysis of environmental risks, demands integration of many aspects of their biology, as well as information on ecological interactions identified above. The risk posed by introduced species, whether invasive and of ecological or of economic concern, including biological control agents (Simberloff and Alexander 1998; van Lenteren *et al.* 2003), is determined by the following ecological factors:

- the potential of an agent to establish in its novel environment,
- its abilities to disperse,
- its host range, and
- its direct and indirect effects on nontarget species.

Any risk-assessment of IBCAs should include information on these factors. The first three factors mainly determine to what extent the intrinsic attributes of a species determine its environmental impact (direct and indirect effects). The intrinsic factors of successful invaders and of successful biological control agents partly have common denominators. It is a critical issue to develop risk assessment schemes that recognize these potential conflicts of interest and distinguish keystone values subsequently.

ENVIRONMENTAL RISK ASSESSMENT TOOLS

The following account is largely summarized from van Lenteren and Loomans (2005) and van Lenteren *et al.* (2005), with additional references. In contrast to most PRAs for pests of phytosanitary importance, pathway analysis for biological control agents is of secondary importance as they are mostly deliberately introduced. Performing an ecological risk assessment prior to first introduction is then essential as addressed in ISPM3, thus avoiding undesired establishment of an IBCA. Nevertheless, potential IBCAs also are entering a country by range expansion or by accident as stowaways on infested plants and hosts and are discovered when they already passed ports of entry. When there is no legal justification for eradication measures, as for most IBCAs which are not of phytosanitary importance, regulation of an exotic IBCA present but still contained in a country, can be covered indirectly by performing a risk assessment prior to its commercial release (IPPC 2005).

Depending on the stage of the regulatory process, either a comprehensive full scan can be used as a tool for risk assessment, or a quick scan, based on the same environmental determinants as indicated above. A quick scan is an initial screening of available information for known nontarget impact to exist or to expect, revealing any invalid, missing or incorrect information. It is supposedly fast, less costly than a full scan, but mostly indicative and qualitative in its results. A full scan, on the other hand, includes all these elements as well, but is more thorough, comprehensive, evaluating and extrapolating potential hazards, including the use of generated data and performing a complete risk-analysis.

FULL SCAN

Any comprehensive environmental risk assessment will first identify the hazards (intended as potential to cause harm), subsequently estimate the risk (intended as the likelihood of that potential being realized) of environmental importance (intended to refer to the routes of exposure for both humans and animals) (Calow 1998). Risk assessment includes a risk identification and evaluation procedure and should be closely tied to risk management, risk-cost-benefit analysis and risk communication. Van Lenteren *et al.* (2003) proposed a first general framework of the first step, a risk assessment methodology for import and release of inundative biological control agents. Their method integrates and indexes the five ecological determinants mentioned above. A numerical value (1-5) is assigned to the likelihood (L) and magnitude (M) of each of the five elements to quantify risks. The overall ecological risk index (ERI) was based on multiplying values for L and M for each element and adding the values of all five elements. The minimum score was thus 5 (5 * 1x1) and the maximum score 125 (5 * 5x5). Thirty-one cases of natural enemy introductions where thus analyzed in retrospect. Although a clear categorization was obtained with an ERI ranging from 7-105, we encountered some practical and intrinsic drawbacks: calculation and evaluation of such a cumulative ERI would require a substantial amount of information and experimentation before any evaluation can be made, and when these are not available (mis)interpretation could lead to manipulation in decision making. In addition, the ecological elements are not independent and not equal in importance, they should not be rated equally and cannot be indexed in a cumulative way as we previously did. To optimize the process and avoid unnecessary research efforts and costs, we suggest a more advanced, stepwise risk assessment procedure (van Lenteren and Loomans 2005).

In contrast to the procedure of the cumulative risk assessment method described above, the decision to release is based on a tiered approach of each of the ecological determinants, using successive individual matrices of L*M matrix as indicated before. Prevention of entry and establishment is the first and most cost-effective line of defense against biological introductions, such as plant pests or other invasive species (Baker *et al.* 2005; Wittenberg and Cock 2001). Establishment is therefore considered as the first factor in line. When establishment (survival, reproduction, over-wintering) in the novel environment is aimed at, like in classical biological control (CBC) programs, host specificity (and host range testing) is considered the most relevant element, etc. The step-wise procedure of environmental risk assessment is shown in Table 1. For some steps (3, 4 and 6) successive ERI levels (L*M) are calculated according to

Table 1. Generic key to procedures of environmental risk assessment of invertebrate biological control agents (after van Lenteren *et al.* 2005).

Step #	Topic/Condition	Go To
Step 1	**Origin**	
	native to area of release	Step 6
	exotic to area of release	Step 2
Step 2	**Biological Control Program**	
	import and release for permanent introduction (CBC)	Step 4
	establishment not intended (ABC)	Step 3
Step 3	**Establishment**	
	certain	Stop
	possible	Step 4
	not possible	Step 6
Step 4	**Host Range Includes**	
	attack of related and non-valued nontargets	Release
	attack of related + unrelated and/or valued species	Stop
Step 5	**Dispersal**	
	local, moderate	Step 6
	extensive	Stop
Step 6	**Ecological Impact (direct and indirect effects)**	
	likely - permanent	Stop
	unlikely - limited - transient	Release

the approach of van Lenteren *et al.* (2003) and depending on its outcome, the procedure stops or continues.

When we applied the proposed stepwise risk assessment procedure (Table 1) to biological control agents commercially available in Europe (EPPO 2002), obviously risky species were eliminated early in the process. Other species that scored - erroneously - a high cumulative index in the first quantitative risk assessment procedure (van Lenteren *et al.* 2003), such as *Trichogramma brassicae*, were not eliminated early in the new procedure. In concordance with recent experimental data these are recommended for further release. See van Lenteren and Loomans (2005) for a full report.

QUICK SCAN

Under certain conditions a more qualitative 'quick scan' method could be used to assess potential adverse environmental effects based on currently available information only:

1. For *newly introduced organisms* a quick scan can act as an initial screening step for governments to initiate the evaluation process and to assess the status and of the species or population. level of containment prior to first import of a new organism into their country for research or production. For the applicant it helps before first introduction of a natural enemy to quickly evaluate the biological and ecological characteristics and to determine the potential research effort he will have to make to get an approval after efficacy testing is resolved. When after a thorough evaluation on efficacy a release is still considered, a comprehensive risk analysis would apply.
2. In countries developing new regulations a quick scan would allow governments to assess the environmental risk for *natural enemies already in use* to distinguish IBCAs with minor effects from those with large effects, based on evidence of ecological impact. Species considered safe for continuation of release can thus be exempted from further regulatory measures.
3. A quick scan can be used to assess the environmental risk of mass-releasing natural enemies originating from areas within the same ecoregion, but not present in the area of release itself (initial step 1 and 6 in Table 1). Thus, the results of a quick scan could help to establish lists of species that can be used in certain, specified regions or (parts of) ecoregions of the world (*ecoregional "white lists"*). This would result in strongly reduced costs for regulation of the major part of biological control agents currently used and continuation of current biological control programs.

We applied the quick scan method, based on the information requirements and ecological determinants as outlined above, to 150 species of natural enemies currently commercially available in The Netherlands (EPPO 2002; Loomans and Sütterlin 2005). About 5 % of the species were considered too risky for (continuation of) release and 80 % of the species were considered safe. For the remaining 15% information initially was either still partly inadequate, inappropriate or lacking to complete the quick scan. However, when no evidence was available on any significant nontarget effects, or not foreseen, it was advised for most species to continue release. In 2005, 134 species were placed on a "white list", which will be exempted from further regulatory measures in The Netherlands. All other species, IBCAs and other beneficial organisms, will need authorization by derogation.

CONCLUSIONS

The intrinsic factors of successful invaders and of successful biological control agents partly have common denominators. An environmental risk assessment (ERA) can help to reveal, and where possible distinguish, potential conflicts of interest in the application for certain taxa, guilds, species or populations of biological control agents and to distinguish keystone values subsequently. Thus, we can increase efficacy and avoid direct and indirect nontarget effects. In order to be of practical use, the risk evaluation method in a full scan should preferably be 1. quantifiable, so that the environmental effects of different biological control agents can be compared and choices can be made, and 2. consist of a tiered or stepwise procedure so that the clearly safest agents or the unequivocally hazardous natural enemies will be identified quickly and with lowest possible costs involved. The applicant needs to provide sufficient

and reliable information to issue a permit or derogation for import and release. For natural enemies already in use (~200 species worldwide), the quick scan risk evaluation method consists of steps and questions which are the same as in the advanced method, but will be based on available data only. The results of a quick scan could help to establish lists of species that can be used in certain, specified areas or (parts of) ecoregions of the world.

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CHOICE OR NO-CHOICE TESTS? EFFECTS OF EXPERIMENTAL DESIGN ON THE EXPRESSION OF HOST RANGE

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ABSTRACT

Estimation of the host range of entomophagous biological control agents (parasitoids and predators) is complex. It is not always possible to inoculate all test organisms with eggs or neonates to determine "physiological suitability". We argue that, for the host range testing of parasitoids, it is important to initially employ test procedures that will maximize the probability that the test species will be accepted for oviposition. This is vital to ensure that our testing methods do not generate data with a false impression of host specificity. No-choice tests are generally thought to maximize the expression of host range. The main reason for this may be increases in readiness to oviposit induced by host deprivation per se and/or associated changes in egg load, which has the potential to counteract any effects of prior experience. Sequential no-choice tests should only be used with caution as they have the potential to produce false negative results if the period of access to the lower ranked host is insufficient to allow time dependent changes in responsiveness of the parasitoid to become apparent, or if insufficient controls are utilized. Choice tests including the target host have the potential to mask the acceptability of lower ranked hosts, thereby producing false negative results. Examples where wider host ranges have been expressed in no-choice tests than in choice tests, and vice versa are presented. Sufficient variation exists that we recommend that researchers routinely use both assay methods for host range testing of parasitoids and predators.

INTRODUCTION

The most common methodologies employed for host range estimation are no-choice and choice tests (Van Driesche and Murray 2004). The way that scientists decide on the appropriate laboratory-based methodologies for the accurate estimation of field host range of proposed biological control agents however is an interesting issue. The accurate assessment of field host range of parasitoids and predators is complex because of the relationships the target and test organisms invariably have with their food plant. It is critical therefore that all potential non-target impacts are elucidated by the methodologies selected.

The assessment of host range in endoparasitoids is complicated as it is usually not possible to inoculate all test organisms with eggs or neonates to determine "suitability" (although

exceptions do exist, Fuester *et al.* 2001; Morehead and Feener 2000;). Such inoculation tests require an experimental separation between the act of oviposition and subsequent larval development. This is commonly achievable for herbivorous insects but is generally impossible for endoparasitoids. Thus, a testing regime to determine the host range of endoparasitoids is usually denied a useful tool: the so-called physiological host range test.

Whether it is parasitoids or predators that are under consideration as potential biological control agents, it is important to employ test procedures that will maximize the probability that the test species will be accepted for feeding or oviposition (Withers and Barton Browne 2004). Unless acceptance of at least one of the offered hosts occurs, there is a danger that a lower ranked but potential host may be left out of further experimental analysis. Without this acceptance being revealed, a realistic risk assessment process cannot proceed. We believe some test designs can definitely produce false negative results, and it is this we want to eliminate in host testing. In this paper, we discuss the potential implications of choice and no-choice test designs on maximizing the expression of host acceptance. This will focus primarily on oviposition in parasitoids, although most of the concepts are also relevant to predators.

Behavioural and physiological factors. In the chapter by Withers and Barton Browne (2004), the potential influences of various factors on the expression of host range in parasitoids and predators was reviewed. In theory, factors such as the physiology of the parasitoid and aspects of the test design such as the proportion of target to non-target species have the potential to impact on the outcomes of host range assays by altering the probability the parasitoid will attack non-target species. Withers and Barton Browne (2004) concluded that prior experience and time-dependent state of the parasitoid could alter the test outcomes, and the impact of these factors on the test outcomes could differ with different test types. We will briefly discuss three of these factors and then examine the test designs in more detail.

EFFECTS OF EXPERIENCE

Thanks to the high quality of the literature (e.g., Turlings *et al.* 1993; Vet *et al.* 1995), we now have a good appreciation of the complexity of experience effects on host-related behaviour in parasitoids (Withers and Barton Browne 2004). Significantly altered behaviour has been demonstrated in relation to experience by the adult parasitoid of the host it was reared in or on (rearing host), the complete plant-host complex and/or some of its components. This behaviour modification can occur with or without oviposition into hosts. There is strong but indirect evidence that any enhancement in responsiveness to a familiar host or plant-host complex is generally greater than any enhancement in responsiveness to an unfamiliar (novel) non-target or its plant-host complex (Fujiwara *et al.* 2000; Petitt *et al.* 1992).

It is commonly expected that an experienced parasitoid will be biased towards the host or plant-host complex that it experienced during rearing or previous laboratory trials. What influence this has on host range tests depends (i) upon the history of the parasitoids used in the tests, (ii) how the target and non-targets are presented in the tests, and (iii) the magnitude and nature of the effects of the previous experience. For example, the experience gained by a parasitoid of the rearing host and its host plant during larval development and subsequent adult emergence is likely to result in enhanced responsiveness to cues from this plant-host complex. Such an effect would be reinforced by continued contact with, and possibly ovipo-

sition experience on, the same plant-host complex, especially if the parasitoids were not removed from the rearing colony before or shortly after eclosion.

There are ways that experience-induced bias towards the target species can be reduced. The most difficult effect to avoid is any enhanced responsiveness towards the rearing host (which is usually the target pest) as a result of experience acquired at eclosion or shortly afterwards. For crucial tests, methods such as dissecting the parasitoid pupae out of the host (for endoparasitoids) or removing it from the host (ectoparasitoids) and washing the exterior of the parasitoid pupal case prior to eclosion can be used. This is probably the only method that can be applied to reduce experience effects in oligophagous parasitoids that have no high quality alternative host for rearing. The presentation of target and non-target species to the parasitoid on a neutral or "inert" substrate such as artificial diet or glass is a valuable means of avoiding a possible bias towards the parasitoid host's plant that was used during rearing. However, this is often impossible wherever test species are inseparable from their plants, such as with internally placed eggs, internally feeding larvae or when test species require the presence of the food plant for the duration of the assay. The most practical solution to minimize bias as a result of prior experience is collecting the parasitoids immediately after they have eclosed from their pupae and storing them in the absence of hosts and plant material (unless this is also food for the parasitoid).

READINESS TO OVIPOSIT WITH HOST DEPRIVATION

Another significant influence on insect behaviour, and hence the outcome of host testing will be the impact of time-dependent changes in responsiveness (Barton Browne and Withers 2002). This has been defined as changes in threshold in relation to elapsed time since an insect last fed or oviposited. The behavioural threshold for the acceptance of hosts can be expected to decrease with increasing periods of deprivation. Therefore female parasitoids that have been deprived of oviposition will show greater responsiveness to cues associated with oviposition sites (Barton Browne and Withers 2002; Papaj and Rausher 1983).

The most important practical result of this is that the probability of a parasitoid attacking a non-target host species that induces a lower stimulation to oviposit (is "lower ranked") increases with the period of time since they last successfully oviposited. Increased acceptance of lower ranked hosts by *Holomelina lamae* Freeman as time elapses since they eclosed may be an example of this (Fig. 1).

Further evidence for this phenomenon in parasitoids comes from experimental work on superparasitism, as superparasitized hosts are known to be lower ranked. Hosts already parasitized by conspecific females are increasingly accepted for oviposition by female parasitoids as they become increasingly deprived (e.g., Hubbard *et al.* 1999; Klomp *et al.* 1980). Similarly parasitoids that have recently suffered from a low encounter frequency with unparasitized hosts (e.g., Babendreier and Hoffmeister 2002) subsequently show increased acceptance of parasitized hosts. So in conclusion, time-dependent increases in responsiveness will act to increase the probability that lower ranked or non-target hosts will be accepted for oviposition in test assays.

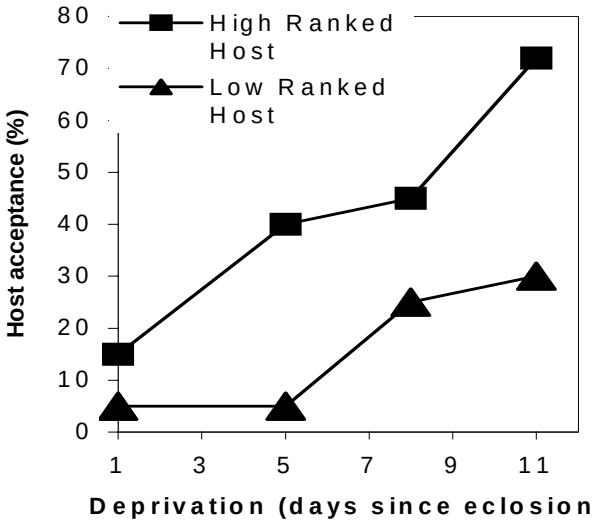

Figure 1. Probability of acceptance of higher ranked host, *Lymantria dispar* (L.) in no-choice tests, compared to the lower ranked host, pupae of *Holomelina lamae* Freeman by host-deprived *Brachymeria intermedia* (Nees). Adapted from Drost and Cardé (1992).

OVIGENY CHARACTERISTICS

Life history theory predicts that stimulation to oviposit is influenced, at least in part, by egg load (Mangel 1989). There is an abundance of empirical data that supports this prediction for parasitoids (Withers and Barton Browne 2004). However the effect of host-deprivation on egg load, and therefore the potential contribution of deprivation to any increased readiness to oviposit, is totally dependent on ovarian physiology. For example, a female of a pro-ovigenic species does not increase its egg load during host-deprivation so any increase in readiness to oviposit in a pro-ovigenic species cannot be attributed to egg load. Conversely, females of synovigenic species may increase their egg load, up to a point, during a period of host deprivation (e.g., Eliopoulos *et al.* 2003). The extent to which this happens is dependent on the nutritional reserves stored within the body and/or the availability of foods during the period of deprivation. This is particularly relevant in parasitoids that also feed on their hosts as host-deprivation will deprive the females of both nutrients for oogenesis as well as depriving them of the opportunity to oviposit. For example, when the host-feeding species, *Aphytis melinus* DeBach is maintained on honey but deprived of hosts, there is a reduction in egg load due to oosorption (Collier 1995). It is therefore vital that the ovarian physiology of the parasitoid is understood prior to the selection of host testing methodology, in order to understand its potential influence on the outcome of host tests.

No-choice tests. No-choice tests present the potential biological control agent with one non-target test species at a time. Thus if 10 non-target species are to be tested, there will be a series of ten cages (with replicates for each), plus appropriate controls (Van Driesche and Murray 2004). It is not usual for all tests to be undertaken at exactly the same time, due to the phenology and seasonality of the non-targets and availability of adult parasitoids, but this is acceptable if tests are sufficiently replicated with appropriate target species controls.

Potentially many factors could influence the outcome of no-choice tests. Time-dependent changes in responsiveness are likely to be significant factors acting upon parasitoids when subjected to tests with hosts that produce a lower stimulation to oviposit (are lower ranked). Encounter rates are likely to be lower if the test hosts are presented on plants/substrates that induce lower or no innate host searching preference. This, and the lower preference for the test host may lead to low oviposition rates. As discussed above both low encounter rates and low oviposition rates during the test have the potential to result in an increase in host-deprivation in the parasitoid (Barton Browne and Withers 2002).

In no-choice tests, if the parasitoid has had any experience of the target or aspects of the target's plant-host complex, this may act to reduce the probability of acceptance of unfamiliar hosts (non-targets). It is not known how long lasting the effects of experience are (Barton Browne and Withers 2002). What is likely however is that time-dependent effects have the potential to override the effects of experience if the duration of the no-choice test is long enough. This is why there are significant benefits in undertaking behavioural observations during host range tests. Only observation will elucidate whether temporal changes in attack behaviour are present that would indicate time-dependent changes in responsiveness are acting upon the parasitoid.

SEQUENTIAL NO-CHOICE TESTS

Although not commonly used, it is important we also consider the method of sequential no-choice tests in which insects are given no-choice access to a sequence of two or more test species, in which the target species is also presented at least once in the sequence. In parasitoid host testing, sequential no-choice tests are almost invariably used to assess host acceptance behaviours for oviposition. The sequence chosen for the presentation of target and non-target species can be varied according to the biology of the parasitoid, as can the duration of presentation and any "rest" durations between presentations.

A theoretical analysis of the potential outcomes of some sequential no-choice experimental designs in phytophagous insects has been undertaken (Barton Browne and Withers 2002). One of the most popular designs is the test sequence A - B - A (where A was the higher ranked host, and B a lower ranked, although acceptable host). Barton Browne and Withers (2002) concluded that the outcome of sequential no-choice tests varied according to the period of time for which the insects were given no-choice access, particularly access to host B. If the parasitoid oviposited during the first access to host A (which was often the aim – to ensure the parasitoid was physiologically and behaviourally ready to oviposit), it may not accept host B when it first entered its no-choice access to the non-target host B. Whether it does accept host B during the test depended on whether the test was run for a sufficient length of time for time-dependent processes to act upon the parasitoid to lower its acceptance threshold to a level whereby host B stimulated attack behaviour. Hence the chance that the lower ranked host was scored as unacceptable was negatively related to the duration of the period of access to this host. To help control for time-dependent effects, a control should be run at the same durations of presentation of the order A – A – A.

Another variation on the sequential no-choice test gives "rest" periods (deprivation) where no hosts are available, in between the periods of access to hosts. This allows time-

dependent effects to increase the stimulation to oviposit during the period of no access to hosts, and in theory should increase the probability that the parasitoids will oviposit in host B. This is, in effect, equivalent to prolonging the period of access to less preferred hosts in a sequential no-choice test (Barton Browne and Withers 2002).

Sequential no-choice tests of the design B – A – B – A – B – A for 2 hours each with no rest period between tests (where A is the target, and B the non-target species) were used to test oviposition responses of *Trichopoda giacomellii* (Blanchard) (Tachinidae) (Coombs 2004). This method was chosen instead of multiple choice tests where the authors were concerned false positive results might occur due to priming (i.e. central excitatory state caused by the presence of target species). The exposure duration was chosen "after observing oviposition patterns of the parasitoid on its target host". It is likely the duration was appropriate to the biology of *T. giacomellii* because the non-target native species *Glaucias amyoti* (White) were attacked during their 2 hour presentation time and the test results have since been supported by post-release field studies showing *G. amyoti* is being parasitized at a comparable low level in the field (1%) (Coombs 2004).

Porter and Alonso (1999) used another variation of sequential no-choice oviposition testing. These experiments used a design of A – B and B – A, with presentation times of 60-90 mins with a variable duration of 30 mins or more between presentations to recapture flies. This method permits the comparison of what effect prior oviposition experience on a target (A) has on the acceptance of the non-target (B). This example is interesting in that it has the appearance of central excitation. The only instances where both parasitic flies *Pseudacteon tricuspis* Borgmeier and *Pseudacteon litoralis* Borgmeier attacked the non-target (B) native fire ant *Solenopsis geminata* Forel were when they were first presented some time after the no-choice test on the target A (imported fire ants). It is not known how long the effects of central excitation last, but they are generally considered to be short lived. The duration between presentations in this case therefore probably excludes central excitation as an explanation. Controls of the design A – A could also have been employed here to elucidate any temporal patterning of oviposition.

Sequential no-choice tests of the design A – B – A were used by Gilbert and colleagues (Porter and Gilbert 2004) with the aim being to screen the motivational status of field-caught flies, which were the only ones available for host specificity testing at the time. Only those individuals that successfully attacked the first presentation of the target host A (imported fire ants, *Solenopsis* spp.) were used in the following B – A tests. Seldom are the effects of oviposition experience effectively understood or controlled for in these sequential tests. But this is always the case when field-caught individual parasitoids are used in host range testing. This level of uncertainty may be taken into account to some extent with the use of non-parametric statistical tests appropriate to sequential, non-independent data sets.

Our conclusion on the use of sequential no-choice oviposition testing of parasitoids are that it should be attempted with caution, and only when the physiology and behaviour of the parasitoid is understood in terms of its temporal patterning of oviposition. This is due to the high risk that a test of the design A – B – A, where the duration of access to the non-target B is too short, will produce a false negative result.

Choice tests. In choice tests, two or more host species are presented to the test insect simultaneously and thus the response is a measure of preference for one species in the presence of another species (Van Driesche and Murray 2004). Tests that offer more than two choices pose several challenges for experimental design as well as for statistical analysis (Hoffmeister 2005; Mansfield and Mills 2004). In the context of non-target risk assessment for biological control, the comparison between the target host and a single non-target host is usually more straight forward than a multiple choice situation.

It is generally expected that host preferences will be more clearly expressed by parasitoids in choice tests compared to in no-choice tests. This is because the impacts of time-dependent changes in responsiveness (that increase host acceptance of lower ranked hosts), as discussed above, will not occur when high ranked hosts are available for oviposition (Van Driesche and Murray 2004). For example, when a parasitoid enters a choice test containing two species of host (one high ranked, the other low ranked in relative acceptability) and each host is offered on its own food plant (Barton Browne and Withers 2002), we assume that the high ranked hosts will be contacted and accepted for oviposition first due to an inherent preference in the parasitoid for searching the food plant of the high ranked host first. Therefore when the lower ranked hosts are eventually located in the cage, they are less likely to be attacked, as they shouldn't stimulate the parasitoid sufficiently to oviposit. The outcome of choice tests therefore are expected to be a greater difference in parasitism (or attack rate, searching time, proportion of parasitoids produced) between the target and lower ranked host than would be expressed in a no-choice tests.

There are a number of other aspects of a choice test that have the potential to alter the outcome of the test (e.g., ratio of host abundance, the duration of the test permitting all target hosts to become parasitized). The effects of experience either with or without access to the plant-host complex may increase responsiveness towards the experienced host species or decrease responsiveness away from the novel non-target species. Any such experience-induced increases in responsiveness towards the target would in effect exaggerate the apparent difference between the rankings of the two hosts. This has implications for the interpretation of results from choice tests, particularly when (as is often the case) the target species and non-target species are presented on different host plants. In choice tests, increased contrast in ranking between the plant-host complexes would, in itself, increase the probability that attack on the non-target species will fail to be revealed.

COMPARING RESULTS OF NO-CHOICE TO CHOICE TESTS

While taking species-specific ovarian physiology into account as was discussed above, we can see that both time-dependent increases in responsiveness as well as effects of experience, many of which are unavoidable, are responsible for why we expect parasitoids to show a wider host range (greater acceptance of non-target species) when tested in no-choice tests than in choice tests that include their target host. This concept of greater acceptance in no-choice situations has also been clearly demonstrated with parasitoids expressing host acceptance behaviour for different developmental stages of the same host species. For instance Neveu *et al.* (2000) showed that in no-choice tests the parasitoid *Trybliographa rapae* Westwood (Figitidae) accepted and reproduced equally in first, second and third instars of the cabbage root fly, *Delia radicum* L

(this was not explained by any superparasitism). However when all larval stages were offered simultaneously to parasitoids in an equivalent choice test, an oviposition preference was clearly expressed towards the third instar (Neveu *et al.* 2000).

If we generally expect to see a wider host range expressed by parasitoids from no-choice tests than from choice tests, then this should be reflected in results from the literature. Some examples that support this conclusion have been summarized in Table 1. Note the majority of these examples are of quantitatively greater acceptance in no-choice than in choice tests.

It would be tempting to generalize that no-choice tests are the most suitable laboratory assay for revealing the maximal physiological host range of parasitoids. It is a well accepted notion in weed biological control that a no-choice test will seldom produce a false negative result (Hill 1999; Marohasy 1998; Van Driesche and Murray 2004). However, as mentioned above, parasitoids and predators bring a whole new level of complexity to laboratory assays. There are just as many examples in the literature where both no-choice and choice tests revealed extremely similar results in terms of the host acceptability (Table 2). This suggests both methods can be equally suitable for revealing attack on non-targets. Of more concern are examples of parasitoids where non-target attack has occurred in a choice test, which was not revealed in a no-choice test.

We are aware of only two unambiguous examples where parasitoids attacked a non-target species in choice tests but did not attack those same species in no-choice tests. The first example is of the parasitoid *Sphecophaga vesparum* Curtis (Ichneumonidae) being investigated as a biocontrol agent for *Vespula germanica* (F.) and *V. vulgaris* (L.) (Field and Darby 1991). *Sphecophaga vesparum* oviposited in (and then successfully developed in) two adjacent larvae within wax cells obtained from a hive of the non-target wasp *Ropalidia plebeiana* Richards. This occurred however, when the larvae had been presented in a choice test alongside cells of the target wasp, and were not guarded by adult wasps as would occur in the field. In the equivalent no-choice tests, no parasitism occurred on the unguarded non-target wasp larvae (Field and Darby 1991). The authors implied that *S. vesparum* may have been stimulated to oviposit in the nearby non-target cells because of the presence of their natural host and/or the preferred food source which is saliva of larval *Vespula* spp. (the target) in the choice test. One possible behavioural explanation for this observation may be that stimulation elicited by kairomones of the target species or the ingestion of the target saliva have generated an excitatory state in the female parasitoids central nervous system leading her to accept non-target species ("central excitation" *sensu* Dethier *et al.* 1965). In the field these species are unlikely to nest in such close proximity (*V. germanica* nests are subterranean and *R. plebeiana* nests are arboreal), leading to the conclusion that the result of the no-choice test is likely to reflect the field situation in this case.

In the second example, *Aphidius rosae* Haliday (Braconidae) showed complete rejection of the non-target *Macrosiphum euphorbiae* (Thomas) when presented on the same host plant (rose) as their target host *Macrosiphum rosae* (L.) but only when the parasitoid had prior oviposition experience acquired after being held with *M. rosae* during the preceding two days (Kitt and Keller 1998). Naïve *A. rosae,* in comparison, showed similar attack rates on the non-target *M. euphorbiae* in no-choice tests but a direct comparison was not available (see Table 3 in Kitt and Keller 1998). One possible behavioural explanation for this outcome is that host

Table 1. Examples of non-target species that received greater attack in no-choice tests than in choice tests that included the target.

Parasitoid Species	Parasitoid Family	Target	Non-target accepted more in no-choice test	Conclusion	Reference
Pelidnoptera nigripennis (F.)	Diptera: Sciomyzidae	*Ommatoiulus moreleti* (Lucas)	Two species of Paradoxosomatidae	Eggs dislodged so no development on non-targets	Bailey (1989)
Thripobius semiluteus Boucek	Hymenoptera: Eulophidae	*Heliothrips haemorrhoidalis* Bouché	*Hercinothrips bicinctus* Bagnall	Species not attacked in the field	Froud and Stevens (2004)
Trichogramma platneri Nagarkatti	Hymenoptera: Trichogrammatidae	*Cydia pomonella* (L.)	*Sitotroga cerealella* (Olivier)	Sitotroga was not parasitized in multiple choice tests	Mansfield and Mills (2004)
Cotesia rubecula (Marshall)	Hymenoptera: Braconidae	*Pieris rapae* (L.)	*Pieris napi olearacea* (L.)	Never yet attacked in field, but is in both test types	Van Driesche *et al.* (2003)

Table 2. Examples of non-target species being attacked at a similar rate in both choice tests with target and in no-choice tests.

Parasitoid Species	Parasitoid Family	Target	Non-target accepted similarly	Conclusion	Reference
Microctonus hyperodae Loan	Hymenoptera: Braconidae	*Listronotus bonariensis* (Kuschel)	*Nicaeana cervina* (Broun)	Pupal parasitism rates consistent	Goldson *et al.*, (1992); Barratt *et al.* (1997b)
Cotesia glomerata (L.)	Hymenoptera: Braconidae	*Pieris napi oleracea* (L.)	*Pieris rapae* (L.)	Parasitism rates consistent in both test types	Van Driesche *et al.* (2003)
Pseudacteon curvatus Borgmeier	Diptera: Phoridae	*Solenopsis invicta* Burden	*S. geminata* (F.), *S. xyloni* (MacCook)	Parasitism rates generally consistent	Porter (2000)
Laricobius nigrinus Fender	Coleoptera: Derodontidae	*Adelges tsugae* Annand	*Adelges piceae* (Ratzeburg), *Adelges abietis* (L.), *Pineus strobi* (Hartig)	Oviposition preferences (mean number of eggs laid in ovisacs) consistent	Zilahi-Balogh *et al.* (2002)
Dichasmimorpha kraussii (Fullaway)	Hymenoptera: Braconidae	*Bactrocera latifrons* (Hendel)	*Eutreta xanthochaeta* Aldrich	Probing responses and parasitism consistent	Duan and Messing (2000)

acceptance behaviour in favour of the target host was only modified in parasitoids with prior oviposition experience of the test host.

The example of attack of the non-target weevil *Sitona lepidus* Gyllenhal by *Microctonus aethiopoides* Loan (Barratt *et al.* 1997a) is sometimes quoted as being an example of a greater level of attack in choice than in no-choice tests (Van Driesche and Murray 2004). However the apparent difference in parasitism on *S. lepidus* (6% in choice c.f. 1% in no-choice) may be partially explained by a rapid host immune response. We cannot exclude the possibility, however, that a heightened excitatory state was induced in the parasitoid through being held in the presence of both target and non-target adult weevils within the choice test cage (Barratt *et al.* 1997a).

To summarize, particularly for polyphagous parasitoids, choice tests may be more suitable than no-choice tests for assessing the order of preference if the hosts are closely ranked (Mansfield and Mills 2004; Van Driesche and Murray 2004). Returning to the example from Kitt and Keller (1998), the use of naïve parasitoids (no prior oviposition experience with the target) produced the more useful data for the estimation of non-target species at risk using no-choice tests, whereas relying on oviposition-experienced parasitoids would have produced a false negative result. In parasitoids, Van Driesche and Murray (2004) suspect that false negative results in a choice test also containing the target species may be less likely than in herbivorous insects, and that the potential for false positives may in fact increase. Barratt (2004) similarly believes that choice tests can contribute different information but are probably less informative for insect rather than weed biological control agents. Our conclusion is that as the host range of parasitoids predicted by both methods differs, both methods should ideally be used in combination. Whether a wider host range is expressed in no-choice or choice tests depends on the species tested and on the relative strengths of any deprivation effects acting on the one hand, and the effects of experience and/or central excitation acting on the other. In many cases behavioural observations during both choice and no-choice tests could be instrumental in allowing us to make accurate interpretations of the data, and their value cannot be underestimated.

CONCLUSIONS

No-choice tests remain the most useful method for assessing host acceptance behaviour of parasitoids. As the duration of no-choice tests increases, the potential for time-dependent effects to act upon the parasitoid will increase. Similarly because of time-dependent effects, sequential no-choice tests should be attempted with caution as false negative results can occur when the period of exposure to non-targets is too short.

In choice tests, host experience may have a significant influence on the expression of host preference. Exposure to the host or plant-host complex at eclosion, even without actual oviposition experience, can bias host preference towards the natal host, obscuring acceptance of lower ranked hosts. This should be minimized by collecting the parasitoids immediately or soon after eclosion. The presentation of the hosts during the test itself (on an inert substrate, on the same host plant, or on different host plants) may overcome the potential effects of experience.

Finally, we believe that as the parasitoid host range predicted by the host range testing methods discussed in this paper have been shown to differ, ideally both no-choice and choice methods should be used in combination. In unusual cases where the results predicted by no-choice and choice tests differ significantly, further research will be required. The biology of the natural enemy involved will need to be examined and ideally the behavioural mechanism responsible for the discrepancy should be elucidated. Undoubtedly as more research is carried out on this topic, our understanding of how to interpret the results of different types of tests will increase.

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PARASITOID CASE HISTORY: AN EVALUATION OF METHODS USED TO ASSESS HOST RANGES OF FIRE ANT DECAPITATING FLIES

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ABSTRACT

The first three papers in this section have discussed factors that affect the efficiency and success of laboratory host range tests. This paper presents an evaluation of how well those factors applied to our investigations of host ranges of fire ant decapitating flies in the genus *Pseudacteon* (Diptera: Phoridae). We initially discuss the nature of the fire ant problem (Hymenoptera: Formicidae: *Solenopsis* spp.) and the need for effective self-sustaining biological control agents. We briefly review the biology of *Pseudacteon* decapitating flies, the overall results of our host range tests, and the current status of field releases of these biological control agents. We conclude by discussing how well the recommendations of the three initial papers about 1) statistical procedures, 2) biotypes and cryptic species, and 3) experimental design, plus a recent book on the subject of host range testing, apply to our experiences with fire ant decapitating flies.

BACKGROUND OF PARASITOID SYSTEM

THE FIRE ANT PROBLEM AND NEED FOR SELF-SUSTAINING BIOLOGICAL CONTROL

The major problem with invasive fire ants (Hymenoptera: Formicidae: *Solenopsis* spp.) is that there are so many of them. In north Florida pastures, fire ant densities average 1,800-3,500 ants per square meter or about 1.5-3.0 metric tons of fire ants per square kilometer (Macom and Porter 1996; converted from dry weight to wet weight). Economic damage to agriculture, electrical equipment, and human health in the United States is estimated at nearly 6 billion dollars per year (Lard *et al.* 2001; Pereira *et al.* 2002), not including environmental damage.

Fire ant populations in their South American homeland are about 1/5 as dense as populations normally found in North America (Porter *et al.* 1997). This intercontinental difference in fire ant densities was not explained by differences in climate, habitat, soil type, land use, plant cover, or sampling protocols (Porter *et al.* 1997). Escape from numerous natural enemies left behind in South America is the most apparent explanation for the intercontinental population differences. Classical or self-sustaining biological control agents are currently the only potential means for achieving permanent regional control of fire ants.

BIOLOGY OF *PSEUDACTEON* DECAPITATING FLIES

Information on the life history, phenology, and biogeography of South American *Pseudacteon species*, is accumulating (Porter 1998a; Folgarait, *et al.* 2002; 2003; 2005a; 2005b; Calcaterra *et al.* 2005). At least 20 species of *Pseudacteon* flies (Diptera: Phoridae) have been found attacking fire ants in South America (Porter & Pesquero 2001; Brown *et al.* 2003). Up to nine species of these flies have been found at a single site (Calcaterra *et al.* 2005). Each species has a distinctively shaped ovipositor that is presumably used in a lock-and-key fashion to lay eggs in a particular part of its host's body. Female flies usually contain a hundred or more eggs (Zacaro & Porter 2003). During oviposition, one egg is rapidly injected into the ant thorax with a short hypodermic shaped ovipositor (Fig 1A). Shortly after hatching, maggots of *Pseudacteon* flies move into the heads of their hosts where they develop slowly for two to three weeks (Porter *et al.* 1995a). Just prior to pupation, the third instar maggot appears to release an enzyme that dissolves the membranes holding the exoskeleton together. The maggot then proceeds to consume the entire contents of the ant's head, a process that usually results in rapid decapitation of the living host. The headless body is usually left with its legs still twitching (Fig. 1B).

Figure 1. A) Female decapitating fly (*Pseudacteon*) preparing to inject an egg into the thorax of a fire ant worker (*Solenopsis*). B) Decapitated fire ant worker with a fly maggot consuming the contents of its head. UGA1390062, UGA1390063

The maggot then uses hydraulic extensions to push the ant's mouth parts aside, after which it pupates within the empty head capsule, positioned so that the anterior three segments harden to form a plate that precisely fills the ant's oral cavity (Porter 1998a). The rest of the puparium remains unsclerotized and is protected by the ant's head capsule, which functions as a pupal case. Pupal development requires two to three weeks depending on temperature.

Adult flies are generally mature and ready to mate and oviposit about three hours after emergence. Based on laboratory observations at 20 °C, adult *Pseudacteon* flies may live up to two weeks (Chen *et al.* 2005); however, higher temperatures and activity associated with oviposition will shorten their lives to one to three days (Porter 1998a). Once phorid attacks commence, fire ant workers become keenly aware of the presence of the flies. A single female fly usually stops or greatly reduces the foraging efforts of hundreds of fire ant workers in only a minute or two (Porter *et al.* 1995b). As soon as a fly appears, most workers rapidly retreat into exit holes or find cover. Other workers curl into a stereotypical c-shaped posture (Porter 1998a). Some fly species inhibit fire ant foraging as long as they are present, often for periods of several hours (Folgarait & Gilbert 1999; Wuellner *et al.* 2002). Reduced foraging activity appears to facilitate competition from ants that might otherwise be excluded from food sources in fire ant territories (Feener 1981; Orr *et al.* 1995; Morrison 1999; Mehdiabadi & Gilbert 2002). The overall impact of these flies on fire ant populations is unknown; however, it is clearly sufficient to have caused the evolution of a number of phorid-specific defense behaviors (Porter 1998a).

HOST SPECIFICITY OF *PSEUDACTEON* DECAPITATING FLIES

Based on the highly specialized behavior and life history of *Pseudacteon* flies, we conclude that they pose no threat to any arthropod except for ants (Porter 1998a). Based on the results of our host range tests (Porter & Gilbert 2004), we conclude that *Pseudacteon* decapitating flies are only a realistic threat to fire ants in the genus *Solenopsis*. None of the flies tested, to date, were attracted to other genera of ants in the field (Porter *et al.* 1995c, Morrison & Porter 2005c, Vazquez & Porter 2005) and the few attacks that occurred in the laboratory did not produce any parasitized workers (Porter & Gilbert 2004). It is theoretically possible for *Pseudacteon* phorids to switch to ant hosts in different genera because several species have done just that during the process of evolution (Disney 1994). However, this is only likely to occur in evolutionary time scales of hundreds of thousands of years. Even then, such switches would be limited to a small subset of ants of similar size (Porter 1998a). A major constraint on the evolution of host shifts and the broadening of host range is that phorids apparently use species-specific alarm pheromones to locate ant hosts (Vander Meer & Porter 2002). In almost eight decades of exposure to an expanding population of *S. invicta*, none of several species of *Pseudacteon* flies which attack native fire ants in North America have made the shift to the more abundant introduced species. All comparative and experimental evidence weighs heavily against the possibility that any of the fire ant decapitating flies from South America would ever become a generalist parasite of ants within ecological or microevolutionary timeframes.

Several of the *Pseudacteon* species proposed for release present a finite but acceptable risk to the native fire ants *Solenopsis geminata* (Forel) and *Solenopsis xyloni* MacCook (Porter & Gilbert 2004). The primary risk suggested by our specificity testing is that occasional attacks on these non-target native ants might occur. Several *Pseudacteon* species can also complete development in native fire ants. However, all of these species are much more successful at attacking imported fire ants than either of the native fire ant species tested. They also have a strong preference for imported fire ants over native fire ants when allowed to choose. These data justify a conclusion that *Pseudacteon* flies present a much greater risk to imported fire

ants than either of the native fire ants tested. This being the case, the likelihood is that these flies will actually benefit native fire ant species rather than harm them because imported fire ants are the primary enemy of native fire ants (Porter 2000). Furthermore, risks to native fire ants must be balanced against the possible benefits of these flies to hundreds of native arthropods and dozens of native vertebrates threatened by high densities of imported fire ants (Wojcik *et al.* 2001). This small risk is justified, in light of the benefit of finding an economic, self-sustaining, and target-specific biological control of imported fire ants.

RELEASE AND ESTABLISHMENT OF DECAPITATING FLIES IN THE UNITED STATES

Field introductions of South American fire ant decapitating flies in the United States began after careful analyses of risks and benefits as elaborated in three Environmental Assessments for field release which the authors separately prepared with and for officials at USDA/APHIS six, eight, and ten years ago. Three species of South American decapitating flies have been released in the United States. The first species was *Pseudacteon tricuspis* Borgmeier in Texas (Gilbert & Patrock 2002) and Florida (Porter *et al.* 1999). This fly attacks medium to medium-large fire ants and is especially abundant in the fall. A biotype of this species from near Campinas, Brazil is well established in eight states in the southeastern United States. Flies released in Florida have spread at least 180 km from their release sites (Porter *et al.* 2004). A second biotype of this species from northern Argentina has been released at several sites in Texas along with the first biotype, but its establishment, while likely, still needs to be confirmed by biochemical markers. Two biotypes of *Pseudacteon curvatus* Borgmeier have also been established in the United States, one on black and hybrid fire ants in Alabama, Mississippi, and Tennessee (Graham *et al.* 2003; Vogt & Streett 2003; Parkman *et al.* 2005) and the other on red fire ants in Florida (Vazquez *et al.* 2005), South Carolina (Davis & Horton 2005), and Texas (L.G. unpublished). This fly only attacks small fire ants and is especially abundant in the late summer. Impacts of this fly have yet to be assessed, but this fly often occurs in higher densities than *P. tricuspis*. A third species of decapitating fly, *Pseudacteon litoralis* Borgmeier, has been released at two sites in north Florida (Summer 2003, Fall 2004). First generation flies were recovered, but establishment has not been confirmed. This fly attacks medium-large to large fire ants and is most active in the morning and late afternoon until dark. A fourth species of decapitating fly, *Pseudacteon obtusus* Borgmeier, is being held in quarantine until permits can be obtained for its field release.

Studies of the impacts of these flies are ongoing, but field studies show that the impacts of a single species of fly (*P. tricuspis*) are not enough to rise above the 10-30% sensitivity of field tests (Morrison & Porter 2005a; 2005b). The introduction of additional species of decapitating flies and other natural enemies will increase the likelihood of permanently reducing imported fire ant populations in the United States.

EVALUATION OF RECOMMENDATIONS

The preceding authors in this section (Hoffmeister 2005; Hopper *et al.* 2005; Withers & Mansfield 2005) and those in a recent book (Van Driesche & Reardon 2004) have made a number of recommendations about procedures for assessing the host ranges of potential self-sustaining biological control agents from foreign countries. For the purposes of discussion,

we will divide these recommendations into six categories: 1) existing knowledge about the taxonomy and host specificity of potential biological control agents; 2) the importance of biotypes and cryptic species in host range tests; 3) selecting appropriate non-target organisms for testing; and 4) choosing the best ways to handle and select biological control agents for specificity tests; 5) experimental design for assessing host specificity; and 6) recommendations for proper statistical analysis of experimental data. We will proceed to discuss how well recommendations in each of these categories applied to our studies of the host ranges of fire ant decapitating flies.

EXISTING KNOWLEDGE

Explore literature. Generally, the first recommendation in assessing host ranges is to explore existing literature about identification and host records of potential biological control agents (Sands & Van Driesche 2004; Hoddle 2004). This is important advice. When we searched the literature, we found that all *Pseudacteon* species with host records had been collected attacking ants. We also found that more than 20 species of *Pseudacteon* flies had been described that attacked *Solenopsis* fire ants (Borgmeier 1925; 1962; 1969; Borgmeier & Prado 1975; Disney 1994). Indeed it appeared that *Pseudacteon* had diversified in a fire ant adaptive zone.

Contact experts. Hoddle (2004) recommended that taxonomists, museum curators, and other experts should be contacted for information. Contacting experts provided us with a wealth of information early in our programs. In particular, phorid specialist, Brian Brown shared his "*Pseudacteon* scrapbook" with us. This resource included references, descriptions, and illustrations for most of the species of flies that attacked fire ants. He also assisted with identifications when existing keys to the genus proved marginal and he provided taxonomic advise on numerous other occasions. David Williams and Don Feener provided additional literature about *Pseudacteon* flies as well as advise about their biology. Harold Fowler introduced SDP to these flies in the field. Roberto Brandão provided access to Thomas Borgmeier's collections at the Museum of Natural history in São Paulo. Roger Williams and Angelo Prado were also consulted about work they had done with these flies. In short, our colleagues provided an important foundation on which we were able to build.

Identification errors. Sands & Van Dreische (2004) warn that care must be taken to evaluate and validate old host records because some are not reliable. Indeed, we found two instances where improper identification of ant host records made it appear that three species of flies were less specific than they really are (Porter & Gilbert 2004). We also found evidence that a fourth species is likely more specific than generally reported (Porter & Gilbert 2004).

BIOTYPES AND CRYPTIC SPECIES

Hopper *et al.* (2005) caution that host range testing needs to be done on each new population of biological control agents being considered for field release. This is because cryptic species or biotypes can have different degrees of host specificity. We found this to be true with at least two species of *Pseudacteon* flies. In particular, we found that *P. tricuspis* appears to be two cryptic species, one of which attacks red fire ants and the other of which attacks black fire ants (Porter and Pesquero 2001). Similarly, we found that a biotype of *P. curvatus* collected from black fire ants in Buenos Aires, Argentina could not be established on red fire ants in the

United States while a biotype of *P. curvatus* originally from red fire ants in Formosa, Argentina was easily established on red fire ants in the United States (Vazquez *et al.* 2005). We also found that the two *P. curvatus* biotypes differed in their abilities to attack and develop in the two non-target native fire ants in North America (Porter 2000; Vazquez *et al.* 2004). These data indicate that each new population of a biological control agent needs to be screened for host specificity before field release, at least until the variability of host specificity is well understood within a particular species or genus. However, we do not think it appropriate to require separate permits for each new biotype of a species unless the new introduction falls outside of the host-specificity envelope already permitted for that species.

SELECTING NON-TARGETS FOR TESTING

Barratt *et al.* (1999) recommend that host range tests begin with closely related species in order to maximize the probability of identifying potential non-target host species. If closely related hosts are not suitable hosts, then additional testing with more distantly related organisms can often be greatly reduced because of the low probability that they would be suitable hosts. We generally agree with this line of reasoning. However, we initially tested more distantly related ant hosts to confirm literature observations that these flies were likely limited to ants in the genus *Solenopsis* (Porter *et al.* 1995c). If this screening test had shown broader than expected host ranges, further work with some or all of the fly species may have been abandoned. However, once we were convinced that *Pseudacteon* flies were likely very host specific, we focused our host range tests on the near native congener *S. geminata* and later on another native congener *S. xyloni* (Porter & Gilbert 2004). Two species of flies (*P. tricuspis* and *P. litoralis*) were not able to attack and develop in the native fire ants. Therefore, they were only tested with an abbreviated number of ants from other genera (Porter & Gilbert 2004). However, two species of flies (*P. curvatus* and *P. obtusus*) were capable of developing in one or more of the native congeners (Porter & Gilbert 2004) and as a result, they were both tested with a full battery of appropriately sized native ants from other genera (Porter 2000; Porter & Gilbert 2004).

HANDLING AND SELECTING BIOLOGICAL CONTROL AGENTS FOR TESTS

Withers & Browne (2004) and Withers & Mansfield (2005) make a number of suggestions for handling and selecting biological control agents for host range tests. Their suggestions are designed to "maximize the probability of attack on non-target species" in laboratory tests. Basically, their suggestions were to: 1) test biological control agents in groups, 2) use both naïve and experienced females, 3) select large females over small ones, 4) rear test agents on alternate hosts when possible, 5) deprive females of food prior to the test to increase motivation to oviposit, 6) use females deprived of oviposition opportunities for an appropriate amount of time, 7) test pro-ovigenic agents when young, and 8) use small test chambers. Several additional suggestions related to plant substrates, diet, and mating were generally not applicable to *Pseudacteon* flies.

1. Test in groups. This is a good recommendation for *Pseudacteon* flies. We have tested flies individually (Gilbert & Morrison 1997) but our preference is to test groups of 6-15 females when availability permits (Porter 2000; Folgarait *et al.* 2002; Vazquez *et al.* 2004; Porter & Gilbert 2004). A major benefit of groups is that a hundred or more flies can

easily be evaluated with only 8-12 test runs whereas individual testing would require a hundred or more test runs. Furthermore, tests with individual flies are often not dependable for many reasons including mating failures, ants killing flies, sick flies, no motivation to oviposit, etc. Finally, group testing is biologically normal because most *Pseudacteon* species attack gregariously in the field.

2. Naïve and experienced females. We used naïve females when using lab-reared flies and experienced females when using field-collected flies. We did not find evidence that prior experience in the field restricted subsequent host acceptability in lab trials. To the contrary, we actually have some evidence suggesting that flies attacking *S. invicta* in the lab are primed to approach non-target ants if exposed to them while they are still motivated. Specifically, tests with two species gave slightly higher rates of oviposition attempts (albeit unsuccessful) on non-target ants after having recently attacked the target species (Porter & Alonso 1999). Similarly, motivation to attack was generally short lived after Gilbert & Morrison (1997) transferred flies from target to non-target ants.

3. Large females. Withers & Browne (2004) recommended the use of large females on the assumption that they would have more eggs to lay and consequently be more motivated to oviposit. The relevance of this recommendation depends on details of an insect's life history. In the case of *Pseudacteon* females it is probably better to use a mixture of all sizes. This is because fire ant workers vary greatly in size and large and small female phorids attack different sizes of host workers (Porter 1998). Furthermore, small females could be more motivated to lay eggs because, under some circumstances, they do not live as long as large flies (Chen *et al.* 2005), thus canceling any benefits of small versus large.

4. Rear on alternate hosts. The suggestion about testing the host range of agents reared on alternate hosts has merit in some systems, but is largely impractical for most *Pseudacteon* species because their production rate is either very low or non-existent on alternate hosts. We know of no instance in which a *Pseudacteon* species from South American fire ants could be successfully cultured on North American fire ants or vise versa. Nevertheless, we were able run a small test to see if *P. curvatus* flies reared on the native fire ant *S. geminata* switched from their normal preference to *S. geminata*. We found that flies reared on the alternate host (*S. geminata*) showed little or no inclination to attack the alternate host indicating that host preferences in this fly were more genetic than facultative (Porter 2000).

5. Deprive food. This recommendation has little relevance for phorid flies that attack fire ants. Although we routinely deprived *Pseudacteon* flies of food in our tests, this is because they show little interest in feeding and the presence of food in oviposition chambers appears not to have much effect on fly health or parasitism rates. Also, most *Pseudacteon* species appear to be pro-ovigenic (Zacaro & Porter 2003) so feeding does not facilitate egg development.

6. Deprive oviposition opportunities. This recommendation applies best to insects with longer life spans. Depriving phorid flies of oviposition opportunities to improve motivation in host range tests is probably not necessary and could be counterproductive.

Indeed, if anything, *Pseudacteon* females are more likely to approach novel hosts immediately after exposure to normal host ants. *Pseudacteon* flies are usually very short lived when ants are available to attack (1-4 days) and oviposit most vigorously when they are young.

7. Test pro-ovigenic agents when young. Withers & Browne (2004) stated that pro-ovigenic agents would likely be best tested when they were young because they are often short-lived while synovigenic agents needed to be tested after eggs have matured and are ready to be laid. This is good advice for *Pseudacteon* flies because they are both pro-ovigenic and short lived. Nevertheless, we prefer tests which run for the full adult life of the flies because it gives them full opportunity to oviposit across all age ranges.

8. Small test chambers. We used small test chambers (Porter & Gilbert 2004) mostly because of limited space in our quarantine facilities; nevertheless, the use of small chambers in our tests rather than large ones probably did improve the likelihood of oviposition because the females could simply use visual or other short-range cues to find their hosts. This was good because it maximized the probability that test flies would oviposit in both target and non-target hosts. The down side of the small chambers is that we were not able to evaluate host specificity associated with long-range host detection.

EXPERIMENTAL DESIGNS

Van Driesche & Murray (2004) discuss the strengths and weaknesses of a number of experimental designs that have been used with host range testing including no-choice tests, choice tests, sequential tests, open field tests, preference ranking tests, and post-release tests. Withers & Mansfield (2005) evaluate choice and no-choice tests and recommend the use of either no-choice tests or a combination of no-choice and choice tests. During the course of our host range studies, we have used almost all of the experimental designs just mentioned.

No-choice tests. As recommended, we agree that no-choice tests are the best design for determining host ranges of *Pseudacteon* flies in the laboratory, at least when test flies are available in sufficient numbers either from the field or from a laboratory colony. No-choice tests were run with groups of flies (Porter 2000; Vazquez *et al.* 2004; Folgarait *et al.* 2002) for the entire life of the test flies. This allowed us to measure attraction rates, oviposition rates and most importantly parasitization rates.

Choice tests. We conducted binary choice tests when female flies in no-choice tests had demonstrated some abilities to attack and develop in non-target native fire ants (Porter 2000; Porter & Gilbert 2004). The objective was to determine whether females had a preference for the target species over the non-target native species. Our results showed strong preferences for imported fire ants over native fire ants. This preference data together with poor rates of development on native fire ants strengthened the argument that release of these flies would most likely benefit the native ants because of their impacts on imported fire ants (see specificity discussion under Background section).

We also used binary choice tests to screen ants in non-*Solenopsis* genera (Porter & Alonso 1999; Porter 2000; Porter & Gilbert 2004). However, these tests functioned like no-

choice tests since test flies always showed little or no attraction to ants from other genera and no test flies were ever reared from ants in other genera. Testing 3-4 species of non-target ants simultaneously would have increased testing efficiency. The drawback is that if flies had been attracted to any of the species of ants, we would have needed to repeat the tests to make sure that attraction to one ant species was not masking attraction to another (Withers & Mansfield 2005).

Sequential no-choice tests. Sequential no-choice tests were used to investigate the host specificity of several groups of flies transported into U.S. quarantine facilities from South America. Because of the short lifespan of field collected flies (2-5 days) and the time and expense required to hand carry these flies up from South America (1-2 days) we had very few flies and a very short time to conduct as many tests as possible. Gilbert & Morrison (1997) and Morrison & Gilbert (1999) chose to use an A-B-A pattern where the motivation of individual flies was tested against target ants (A) for five minutes and then against non-target ants (B) for 20 min, and finally against target ants (A) again to reconfirm motivation. In these tests, attacking flies moved from trays of target *S. invicta* (A*)* to trays of non-target *S. geminata* (B) initially approached, and sometimes attempted to oviposit in *S. geminata* workers. Typically however, motivation to attack carrying over from exposure to *S. invicta* was short lived and waned quickly after exposure to *S. geminata*. Porter & Alonso (1999) chose to test small groups of three flies in an A-B and a B-A pattern where some flies were first exposed to the target host while others were exposed first to the non-target host (each for periods of 60-90 minutes). This pattern controlled for any effects of recent exposure to the target host.

These sequential tests had two weaknesses: first all of the flies had been collected after they had prior experience with the target host and secondly test times (20 min. or 60-90 min.) could have been too short to overcome the effects of prior experience. Nevertheless, these limitations were largely unavoidable because of transport times, short life spans, and the fact that, at the time, the flies could not be cultured in the laboratory. Fortunately, results from these tests were equivalent to larger no-choice tests run later indicating that prior experience as wild flies is not a major factor affecting host range tests with *Pseudacteon*.

Withers & Mansfield (2005) recommend that Gilbert & Morrison (1997) could have used an A-A-A pattern to control for time dependant effects and similarly that Porter & Alonso (1999) could have used an A-A pattern. We agree that this suggestion could have provided some useful information. However, since the numbers of flies were very limited and many of them only survived one test cycle, we do not feel that the value of this information would have justified using 1/3 of the available flies. In the case of Gilbert & Morrison (1997), the second exposure to the target host in the A-B-A cycle provided most of the information that would have been provided by an A-A-A cycle. In our opinion, activity in an A-A-A cycle would not have been directly comparable to activity in an A-B-A cycle because the presence of the target host caused greatly increased activity that generally sapped the vigor and longevity of test flies. Our challenge was to keep flies alive and vigorous through even a short A-B-A cycle. In the case of Porter & Alonso (1999), an A-A test would have proved that flies exposed first to the target host (A) retained sufficient vigor to attack the non-target host (B). However, in keeping with the behavioral observations noted above for the A-B-A tests, the data showed that test flies were actually slightly more likely to attack the non-target

ant after being exposed to the target ant than vise versa (3/36 versus 0/79 attacking flies, P=0.029, Fisher's exact test, data for two species of flies combined). Thus, for *Pseudacteon*, we consider the sequential no-choice test to be conservative in that it tends to over-estimate the tendency of these flies to attack non-targets.

Open field pre-release and post-release tests. We conducted several pre-release and post-release open field tests with *Pseudacteon* flies. The major advantage of open field tests is that they take into account the long-range search and discovery abilities of test organisms. The major disadvantages of open-field tests are that the selection of potential hosts in pre-release tests are limited to what is available in the country of origin while in post-release tests, the biological control agent has already been released and can rarely be recalled. For the first open field test Porter *et al.* (1995c) used an $AB_1B_2B_3B_n$ design where target ants (A) were presented simultaneously with a menu of non-target ants (B). In subsequent papers (Porter 1998b; Morrison & Porter 2005c; Vazquez & Porter 2005), authors used a sequential B-A-B design where non-target ants (B) were presented for 30 minutes followed by target ants (A) and finally by non-target ants again (B). The advantage of this sequential design is that it allowed us to first determine if flies were attracted to non-target ants when no target ants were present and then it allowed us to determine if the flies would attack non-target ants after large numbers of flies had been attracted to the immediate area by the target ants. Van Driesche & Murray (2004) call post-release tests a "necessary step" in evaluating the accuracy of pre-release predictions. Results from our post-release tests confirmed that our pre-release predictions of host specificity were accurate for both species of flies that are currently established in the United States (Morrison & Porter 2005c; Vazquez & Porter 2005).

Statistical analyses. Hoffmeister (2005) discusses a number of important aspects of statistical design that apply to host range testing including proper controls, randomization, and pseudoreplication. He also discusses the potential importance of using power analyses to describe the power of statistical procedures to resolve differences between effects of interest.

Controls. Proper controls are vital to most kinds of statistical tests, but they are especially important to simple no-choice tests because the failure of a parasitoid to attack a potential non-target host could be due to poor test conditions or unhealthy parasitoids. To control for these possibilities, we randomly assigned test flies to simultaneous controls and treatments. On several occasions, we had to discard a run because the controls failed due to improper handling of the flies. Zilahi-Balogh *et al.* (2005) mention that the use of negative controls (tests without both a parasite and a host together) could have helped with interpretation of their oviposition tests. We did not use negative controls in any of our tests. Negative controls using ants that were not exposed to flies might have been useful in identifying ant mortality caused by parasitism prior to pupation of the parasite. However, based on random dissections of dead workers, we felt that pre-pupation mortality of host ants was not sufficiently large to justify the extra effort needed to quantify it.

Pseudoreplication and randomization. We attempted to avoid pseudoreplication in our tests by randomly assigning subjects to treatments and using experimental units that were independent of one another. However, in practice, flies were usually assigned to test groups using "haphazard randomization" and the locations of test trays were usually rotated sequentially among test groups so that whatever effect tray location might have would be uniformly

distributed across treatments. Finally, our host range data are from specific populations of flies; consequently, our results can only be safely applied to those specific populations. Extrapolating host range results from a single population to all populations of a species is a form of pseudoreplication that can lead to failures in host range predictions (Hopper *et al.* 2005)

Power analyses. We did not use power analyses as discussed by Hoffmeister (2005) in our host range tests. An *a priori* power analysis is useful for predicting the necessary sample size for a test if variability is known (Zilahi-Balogh *et al.* 2005). However, since we rarely knew variability beforehand, we simply continued to increase sample sizes in our tests until standard errors of the means dropped to reasonable levels.

Hoffmeister's (2005) recommendations concerning the use of power analyses to assess the probability of falsely accepting the null hypothesis of "no effect" were not particularly applicable to the kinds of host range tests we did with phorid flies– this was because rates of attraction and parasitism were always very different between target and non-target hosts. Furthermore, if critical aspects of host specificity had been similar enough that they could not be easily resolved statistically, then we would have simply accepted the null hypothesis that no difference existed. We would not have worried whether parasitization rates may have actually been slightly different because they would still have been similar enough to have caused serious concern about the safety of releasing a particular biological control agent in the field.

Hoffmeister's (2005) recommendations concerning power analyses, however, are highly applicable to the assessment of impacts of biological control agents on field populations of target and non-target organisms. In the case of field impacts, it is important to know what power the statistical tests had to resolve differences when no statistical difference was found. This is exactly the problem faced when evaluating the field impacts of *P. tricuspis* on imported fire ants and other ant competitors (Morrison & Porter 2005a). Morrison & Porter (2005a) dealt with the problem by reporting what percent of the mean that two standard errors were. This was done on the assumption that means two standard errors apart would normally be statistically detectable. Power analyses probably provide a more effective way of providing this information.

CONCLUDING REMARKS

The model systems around which many of the general ideas about biological control are framed depart substantially from the phorid–fire ant system in terms both of the enemy and the victim. Conceptually, the decapitating fly – fire ant system resembles host-specific leaf miners and a woody plant host. However, *Pseudacteon* flies are likely to be more host specific than their herbivorous counterparts because the chemical cues they use for host discovery are under selection to be highly distinct among ants for reasons of close physical competition. Ants are mobile, dangerous targets for an attacking fly and the behavior and mechanics of inserting an egg into an armored predaceous host surrounded by aggressive sisters adds additional potential causes for specialized behaviors and morphology in these phorids. Add to these features the likelihood of internal defenses against phorid larvae and it is not surprising that *Pseudacteon* flies exhibit striking host specificity. By contrast the parasitoids of the eggs, larvae and pupae of Lepidoptera, for example, face many fewer challenges that might be solved

by evolving increased specialization. Many practical and theoretical similarities and distinctions of this system and other systems need to be further explored.

CONCLUSIONS

Host range testing is essential because it allows scientists to predict the potential target and non-target impacts of new biological control agents prior to their release in the field. Information about potential impacts, both positive and negative, permits a reasoned decision about whether the likely benefits of releasing a particular agent clearly outweigh the potential problems. The papers in this session and recent books on the subject have set out a number of important procedures and principles that applied to our work with fire ant decapitating flies and to host range testing generally. We would like to emphasize how important it is to do a thorough review of the literature concerning the biology of a prospective agent, the target host, and organisms related to the agent and hosts. We found that biotypes and cryptic species can have different host ranges both as related to target and non-target species; consequently, it is important that biological control practitioners consider this when conducting their tests. We agree that host range tests should be conducted using methods that initially maximize the probability of attack on non-target species. These methods will vary depending on the agent being tested. We attempted to maximize this probability by testing congeners, using small test chambers, using no-choice tests, testing flies of all ages, testing flies in groups, and using both experienced and naïve flies. Good experimental design that uses appropriate controls, randomization, and replication allows valid interpretations to be drawn. Finally, we want to emphasize the need for post-release host range monitoring. Post-release monitoring is important because it verifies the validity of the prerelease testing procedures and provides data that facilitate the release of future biological control agents.

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A PREDATOR CASE HISTORY: *LARICOBIUS NIGRINUS*, A DERODONTID BEETLE INTRODUCED AGAINST THE HEMLOCK WOOLLY ADELGID

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ABSTRACT

The hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae) is an invasive alien pest of eastern North American hemlocks (*Tsuga* spp.) and is the target of a classical biological control program in the eastern United States. Host range testing conducted under quarantine in Blacksburg, Virginia determined the suitability of *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) a predatory beetle, as a biological control agent of this pest. Members of the genus *Laricobius* are known to feed on adelgids. *Laricobius nigrinus*, native to western North America, was tested on three other adelgid and three non-adelgid species of Homoptera in three families. Host acceptance and host suitability tests were conducted on test prey. In paired-choice and no-choice oviposition tests, *L. nigrinus* females preferred to oviposit in HWA ovisacs over the other test species. Feeding tests showed that *L. nigrinus* consumed more eggs of HWA than eggs of *Adelges piceae* (Ratzeburg) and *Pineus strobi* (Hartig), but not of *Adelges abietis* (L.). In larval development tests, *L. nigrinus* only completed development on HWA. These results suggest that *L. nigrinus* has a narrow host range and that it has potential for biological control of HWA. *Laricobius nigrinus* was cleared for field release by USDA APHIS in 2000 based on these findings and NAPPO Guidelines for 'Petition for Release of Exotic Entomophagous Agents for the Biological Control of Pests'. Test design will be discussed in a retrospective analysis in relation to the practical realities of host range testing in this system and compared with what might be the ideal.

INTRODUCTION

HEMLOCK WOOLLY ADELGID

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand is an invasive alien pest of native hemlocks (*Tsuga* sp.) in eastern North America (McClure 1996). This insect was first observed in North America in the Pacific Northwest in the early 1920's where it was described from specimens collected on western hemlock, *T. heterophylla* (Raf.) Sargent (Annand 1924). Since its introduction into the eastern United States in the early 1950's (Souto *et al.*

1996), HWA has spread along the eastern seaboard in parts of 13 states on the eastern sea board (USDA FS 2004).

Eastern hemlock is an important ornamental and forest tree that is very susceptible to HWA attack. Infested trees exhibit poor crown condition, reduced terminal branch growth and needle loss, and have been reported to die within four years after initial attack (McClure 1991). HWA populations in the eastern United States are not regulated by effective natural enemies (McClure 1987; Montgomery and Lyon 1996; Wallace and Hain 2000). In contrast, HWA has little impact on Asian and western North American species of hemlock. Tree resistance and natural enemies have been reported as playing a role in maintaining HWA below injurious levels in these regions (Cheah and McClure 1996; Montgomery and Lyon 1996).

LARICOBIUS NIGRINUS

Members of the genus *Laricobius* are predacious on woolly adelgids (Homoptera: Adelgidae) (Lawrence and Hlavac 1979; Lawrence 1989). *Laricobius nigrinus* Fender is native to western North America (Fender 1945; Hatch 1962; Lawrence 1989). It was found in close association with HWA on western hemlock in British Columbia, Canada (Zilahi-Balogh *et al.* 2003) where HWA is not considered a forest pest. We hypothesized that *L. nigrinus* may play a role in regulating HWA abundance in the Pacific Northwest and therefore warranted investigation as a candidate biological control agent of HWA in the eastern United States.

We studied the life history of *L. nigrinus* over two years in British Columbia (Zilahi-Balogh *et al.* 2003). This beetle is univoltine. Females lay eggs singly within the woolly ovisacs of HWA from January to May. Onset of oviposition by *L. nigrinus* coincides with oviposition by the over-wintering (sistens) generation of HWA. After hatching, larvae feed preferentially on the eggs of HWA. On completion of feeding, mature larvae migrate to the soil to pupate. After eclosion, adults remain in the soil in an aestival diapause resuming activity in late September to early October at about the same time that aestivating first instar HWA sistens resume development (Zilahi-Balogh *et al.* 2003). Adult feeding by *L. nigrinus* in the winter contributes significantly to adelgid mortality (Lamb *et al.* 2005a). The phenology of *L. nigrinus* in Virginia (Lamb *et al.* 2005a) is similar to that in British Columbia (Zilahi-Balogh *et al.* 2003).

A summary of host specificity tests on *L. nigrinus* followed by a retrospective analysis of host range testing procedures addressing issues presented in this symposium are discussed. The issues are: 1) test design (Withers and Mansfield 2005), 2) statistical design (Hoffmeister 2005), and 3) genetics: relation of local populations to whole species (Hopper *et al.* 2005).

MATERIALS AND METHODS

Laricobius nigrinus adults used in this study were field collected from HWA infested western hemlock from coastal British Columbia, and imported to Virginia for quarantine evaluation (Zilahi-Balogh *et al.* 2002). Field collection and testing of adults coincided with the ovipositional period (peak oviposition is early to mid-March) of *L. nigrinus* (Zilahi-Balogh *et al.* 2003). Immature stages tested were progeny of field collected adults. Insects were main-

tained on field collected HWA infested eastern hemlock twig cuttings in environmental chambers at 15°C, 12:12 (L:D) h, and 75-87% RH.

Six species of test prey in the order Homoptera in three families (Adelgidae, Aphididae, Diaspididae) were used in host specificity tests. They were selected based on taxonomic or ecological similarity to HWA as well as availability. Test prey species are listed in Table 1. With the exception of *M. persicae*, all test prey could be encountered by *L. nigrinus* in the natural forest setting in southeast United States.

Table 1. Test prey on associated host plants used in host range tests conducted between February and April 2000 (from Zilahi-Balogh *et al.* 2002).

Test Prey	Distribution	Host Plant
	Family Adelgidae	
Adelges tsugae Annand (HWA)	Asia, North America[a] (Target insect)	*Tsuga canadensis* (L.) Carrière
Adelges piceae (Ratzeburg)	Europe, North Americaa	*Abies fraseri* (Pursh) Poir
Adelges abietis (L.)	Europe, North America, North Africa, India[a]	*Picea abies* (L.) Karst.
Pineus strobi (Hartig)	North America, Europe[a]	*Pinus strobus* L.
	Family Aphididae	
Cinara pilicornis (Hartig)	Europe, Australia, New Zealand, North and South America[a]	*Picea abies* (L.)Karst.
Myzus persicae (Sulzer)	World wide[b]	*Capsicum frutescens* L. var. *grossum* Bailey
	Family Diaspidae	
Chionaspis pinifoliae (Fitch)	North America[c]	*Pinus cembra* L.

[a]Blackman and Eastop 1994; [b]Blackman and Eastop 1984; [c]Kosztarab 1996

The egg stage was used in all tests for members in the family Adelgidae and Diaspididae. Eggs of adelgids are typically laid in a mass by a sessile female and surrounded by flocculence (waxy/woolly filaments). This stage was selected because we found *L. nigrinus* females laying eggs in the woolly ovisacs of HWA (Zilahi-Balogh *et al.* 2003). *Chionaspis pinifoliae* (Diaspididae) over-winters in the egg stage underneath the female scale. In May, these hatch into crawlers which move over the needles for a few days and then settle down to feed (Kosztarab 1996). Host plant material infested with *C. pinifoliae* were field collected in the early spring and held at 4°C until used in tests. HWA differs from the other adelgids tested in that it breaks aestival diapause in late September/October, develops throughout the winter and begins to lay progrediens and sexuparae eggs in February (McClure 1987). In contrast, *A. piceae*, *A. abietis* and *P. strobi* over-winter as early instar nymphs and begin to lay eggs in the spring when buds begin to break (April or May) (Arthur and Hain 1984; Craighead 1950; Friend and Wilford 1933; Gambrell 1931; Johnson and Lyon 1991; USDA 1985). The challenge was synchronizing development of the various adelgid species with that of HWA. This was achieved by moving adelgid infested potted saplings (Table 1) from an outdoor nursery

into a greenhouse (~ 24°C) beginning in January to accelerate development before being used in tests. Test prey in the family Adelgidae and Diaspididae remain attached to their host plant once crawlers settle. Excess individuals were removed from the host plant with fine forceps when numbers exceeded those required for a particular test. Test prey in the family Aphididae were tested at the early instar nymphal stage as adult females exhibited vivipary. Individuals within the family Aphididae were transferred onto or removed from their respective host plant with a fine brush to attain the appropriate number on the host plant cutting.

Host specificity tests (Zilahi-Balogh *et al.* 2002) were of two types – host acceptance and host suitability. Host acceptance tests determine whether a candidate biological control agent will feed and/or oviposit on a host. Host suitability tests determine whether the agent is able to complete development to the adult stage and produce viable offspring on a particular host (Browne and Withers 2002; Kok *et al.* 1992). Host suitability tests therefore are more crucial in determining potential host range.

HOST ACCEPTANCE

Oviposition tests. Both no-choice (single-prey) and paired-choice oviposition tests were conducted to evaluate the effect of prey type on acceptance and preference by *L. nigrinus* females for oviposition. All tests were conducted in 14 x 2.5 cm plastic petri dishes. One male-female pair was placed in a petri dish with either one bouquet of associated host plant twigs housing test prey (no-choice test) or two adjacent bouquets of host plant with associated prey (paired-choice test). A bouquet was made up of two to four terminal tip branches (10-12 cm length) of prey infested host plant held together by wrapping the cut end with parafilm to prevent the twigs from drying out. In the paired-choice tests, HWA was paired with each of the six test prey. The same numbers of prey (~60 individuals per bouquet) were used in each test. Duration of each test was three days. The number of *L. nigrinus* eggs deposited on each plant bouquet was counted at the end of each test (Zilahi-Balogh *et al.* 2002). A 3-day test was selected based on preliminary trials that showed that three days was a long enough interval to get a treatment effect without resulting in host plant desiccation or having to add additional prey.

Adult feeding test. Prey acceptance by adult *L. nigrinus* was examined in a single-prey feeding experiment using eggs of the four adelgid species, HWA, *A. abietis*, *A. piceae*, and *Pineus strobi*. Even though *L. nigrinus* adults preferentially feed on nymphs and adult stages of adelgids, eggs were selected to test because they are uniform in size within and between adelgid species. Adult *L. nigrinus* starved for 12 h, were placed individually in 50 x 9 mm petri dishes containing one of four prey types attached to sections (< 5 cm) of host plant. Egg numbers of test prey were estimated before introduction of the predator. After 3 d, adult beetles were removed and the number of eggs that remained were counted (see Zilahi-Balogh *et al.* 2002 for details).

Host Suitability. Development and survivorship of *L. nigrinus* were followed from the egg to adult stage on all test prey except *M. persicae*. We did not evaluate *M. persicae* because it was the only test prey that *L. nigrinus* females did not oviposit on during the oviposition tests. *Laricobius nigrinus* eggs (d•24 h old) were transferred individually onto test prey in petri dishes as described above in the adult single-prey feeding test. The stage of test prey

used was similar to that described for the oviposition tests. Egg hatch was followed daily. Other stages were examined daily or every other day for survivorship until adult emergence. Fresh prey was added each time an individual larva was examined. Larval molt was determined by recording the presence of an exuvium. Once the pre-pupal stage was reached, moistened sterilized peat was placed at the base of each petri dish and acted as a pupation medium. The pre-pupal stage was determined to be the stage that mature larvae left the twig with abundant prey and appeared to be actively searching for a suitable pupation site (Zilahi-Balogh *et al.* 2002).

RESULTS AND DISCUSSION

HOST ACCEPTANCE

Oviposition tests. In both the no-choice and paired-choice oviposition tests, *L. nigrinus* females laid significantly more eggs in HWA ovisacs ($P < 0.0001$ to 0.02) over the other test prey (Zilahi-Balogh *et al.* 2002). In the paired-choice test, no eggs were laid on host plants housing non-adelgid prey (*C. pilicornis, C. pinifoliae,* and *M. persicae*). Oviposition was more than five times greater on HWA than on adelgid test prey (*A. piceae, A. abietis, Pineus strobi*) in the paired-choice tests. These differences indicate an ovipositional preference for HWA over these other adelgids (Zilahi-Balogh *et al.* 2002). In no-choice tests, no eggs were laid on sweet pepper housing *M. persicae,* and very few eggs (mean: $d \leq 0.2$ eggs) were laid on host plants housing the other non-adelgid Homoptera (*C. pilicornis* and *C. pinifoliae*). In no-choice tests, *L. nigrinus* laid ~ 2 to 12 times more eggs in HWA ovisacs over the other adelgid non-target prey.

Adult feeding test. In this no-choice feeding test, eggs of all the test adelgids were fed on by adult *L. nigrinus.* Significantly more eggs of HWA were consumed than eggs of the *A. piceae* and *Pineus strobi,* but not *A. abietis.* Though not statistically significant, *L. nigrinus* adults consumed on average 2x more eggs of HWA (48.4) than *A. abietis* (24.7) (Zilahi-Balogh *et al.* 2002).

HOST SUITABILITY

Laricobius nigrinus only completed development to the adult stage on a diet of HWA. *Adelges piceae* and *P. strobi* supported larval development to the fourth instar, providing evidence of larval feeding, but did not support further development. Larvae provided with *A. abietis, C. pilicornis* or *C. pinifoliae* did not survive beyond the first instar (see Zilahi-Balogh *et al.* 2002 for details).

RETROSPECTIVE ANALYSIS

TEST DESIGN

Host specificity tests are designed to determine host acceptance and host suitability (defined earlier) (Kok *et al.* 1992). No-choice and choice tests have been used widely to evaluate host

ranges for both weed and arthropod biological control (Sands and Van Driesche 2003; Van Driesche and Hoddle 1997; Van Driesche and Murray 2004a).

No-choice tests combine the biological control agent with a single test species for a set period of time (Van Driesche and Murray 2004a; Withers and Mansfield 2005). Sequential no-choice tests involve the presentation of target and non-target hosts in a sequence. Choice tests utilize two or more test species with the biological control agent simultaneously (Withers and Mansfield 2005). The paired-choice test includes two treatments (i.e., hosts or prey) being offered simultaneously to the biological control agent. In our tests, the target prey (HWA) was always paired with a non-target prey. We used both no-choice and paired-choice for ovipositional preference and no-choice tests for adult feeding and larval development. Both no-choice and choice tests contribute to information on possible ecological host range of the biological control agent and ideally both should be used in combination (Withers and Mansfield 2005).

Estimation of physiological host range examines the suitability of a candidate biological control agent to survive and complete development on a test host/prey. No-choice larval development tests are able to determine physiological host range and may be more restrictive than no-choice oviposition tests. Physiological host range testing can be challenging when assessing endoparasitoids as it requires observing whether the parasitoid develops and emerges from a test species that has been previously accepted by a female in an oviposition test (Van Driesche and Murray 2004a; Withers and Mansfield 2005). However with a predator, eggs can be transferred easily onto test prey and assessed for feeding and development (Zilahi-Balogh *et al.* 2003). We were able to assess host suitability for larval development to the adult stage. In our case, even though *L. nigrinus* developed to the fourth instar on several non-target hosts, it was only on HWA that this predator developed to the adult stage.

No-choice tests are important in host range testing because negative results can provide good evidence that a test species is not likely to be a field host. Host acceptance in a no-choice test can identify low ranked hosts missed in choice tests. Choice tests are useful in ranking order of preference within a list of possible hosts (Van Driesche and Murray 2004a). With choice tests, we expect a bigger difference in predation or oviposition between target and non-target (lower ranked hosts) (Withers and Mansfield 2005). In our oviposition tests, *L. nigrinus* accepted more non-target hosts than in the paired-choice tests. In the paired choice tests, none of the non-adelgid test prey were accepted as hosts for oviposition. This is consistent with what we expect.

Physiological and behavioral factors can influence the outcome of host range lab assays whether they are choice or no-choice (Withers and Mansfield 2005). Several relevant to our study system are discussed.

Prior experience. A confounding factor in interpretation of results from no-choice and choice tests is prior experience to host or prey (Withers and Mansfield 2005). Studies on both parasitoids and predators have shown there is an enhanced responsiveness in foraging behavior with prior experience to that host (prey) or volatile (Van Driesche and Murray 2004a; Withers *et al.* 2000; Withers and Browne 2004; Withers and Mansfield 2005).

A weakness in our test design is prior experience of adult *L. nigrinus* to HWA prior to tests. *Laricobius nigrinus* adults used in host specificity tests were field collected and therefore were preconditioned to the target prey. This has introduced bias in favor of the target prey (HWA). Though not ideal because of preconditioning of *L. nigrinus* to HWA, it was a practical reality in our system. *Laricobius nigrinus* is a difficult species to rear in the laboratory because of the obligatory aestival diapause exhibited by adults. We initially experienced high mortality in aestivating adults in laboratory culture. A mass rearing protocol has subsequently been developed for *L. nigrinus* (Lamb *et al.* 2005b), but it can only be kept in culture if reared on HWA. No artificial diet has been developed for this species yet. Withers and Browne (2004) suggested that predators and parasitoids should be reared and maintained on species other than the target host (prey) or on artificial diet if possible in order to minimize any experience-induced bias in favor of the target species, especially in the context of choice tests. The use of artificial diet to rear insects can creates some inherent problems because such diets are seldom optimal for development.

Time dependent effects. The period of food or oviposition site deprivation can have major effects on the acceptance threshold of a biological control agent to host cues (Browne and Withers 2002). The consequence of host deprivation is that deprived insects may accept a wider range of hosts than non-deprived individuals (Browne and Withers 2002; Withers and Mansfield 2005). In our studies, beetles were deprived of prey for 12 h prior to feeding tests, but were not deprived prior to oviposition tests. Had females been deprived of host prior to oviposition, would the outcome of the tests be different? We do not think so because of the longevity of *L. nigrinus.* Long-lived species are more likely to resorb eggs in the absence of suitable oviposition sites, as is typical of synovigenic species.

Physiological state of test insects. An important consideration in all bioassays with insects is ensuring that all test insects are of a similar physiological age and have been exposed to the same conditions. When doing oviposition bioassays, it is important to have an understanding of the life history and reproductive biology of the biological control agent. In our case, we were dealing with a predator that is univoltine, and undergoes an obligatory aestival diapause for ~ 4 months of the year (Zilahi-Balogh *et al.* 2003). As mentioned earlier, practical considerations necessitated the use of field collected beetles. Beetles were collected in February, within the ovipositional period of *L. nigrinus* (Zilahi-Balogh *et al.* 2003).

Negative controls. Though not discussed by Withers and Mansfield (2005), the use of negative controls (arenas with no predators) in no-choice feeding tests and controls (with no prey) in oviposition tests are useful for interpretation of results (Van Driesche and Murray 2004b). Negative controls in a feeding test account for any mortality in prey not attributed by the biological control agent, while a no-prey control in an oviposition test can account for the potential of prey dumping in the absence of prey-related cues (Van Driesche and Murray 2004b). We did not include negative controls in our feeding tests or a no-prey control in our oviposition tests. In retrospect, we should have considered these controls, but do not think that it would change our findings. Had we used a no-prey control, and oviposition in this treatment was not significantly different from non-adelgid homopteran hosts, we might have been able to conclude that oviposition on these non-target hosts may be due to egg dumping

rather than host acceptance. In our feeding tests, we were not assessing mortality. We assessed the difference between the number of test prey eggs present before predator introduction and number of test prey eggs present after the predator was removed three days later.

STATISTICAL DESIGN

Hoffmeister (2005) argued that the problem in host range testing is assigning a probability of accepting the null hypothesis of no effect, i.e. that the biological control agent does not include a given non-target host into its host range. This may be impossible to prove with certainty, but what is required is utilizing an experimental design that aims at achieving accuracy and precision from the sample population that is tested. This requires a robust experimental design and decision by researchers on the magnitude of an effect that is desirable to be detected, appropriate sample size to use, and knowledge of the power of the statistics used (Hoffmeister 2005).

Statistical power. The power of a statistical test, defined as 1-β is the probability of rejecting the null hypothesis when the null hypothesis is false and should be rejected (Zar 1984). Power is dependent on the α-level, variance, sample size (n) and effect size (Quinn and Keough 2002). Power analysis can be done *a priori*, for a given level of variability, sample size and power (0.80 is common) to determine how big the change (i.e., effect size) is needed before it would be detected as significant (Hoffmeister 2005; Quinn and Keough 2002).

In our study, preliminary no-choice and paired-choice oviposition tests were done to determine an appropriate length of time to use for a bioassay that allowed for adequate oviposition to occur without host plant material desiccating or having to add additional host material. The number of replicates used for these preliminary tests were n=12 and n=20. Using the variance from the preliminary tests, we could have conducted power analysis to calculate the minimum detectible effect size for a given level of power, or calculate sample size to decide on how much replication is necessary given a level of power, variability, effect size and α (α=0.05 is standard) (Quinn and Keough 2002). Instead, after appropriate analyses of the preliminary tests, we determined that n=12 was a reasonable sample size to get a significant treatment effect. Sample sizes in oviposition tests ranged between n=11 and n=20. We used n=7 in the no-choice feeding test. The limited sample size is this case was due to the limited availability of test predators. Even with this limited number of replications, when we compared number of eggs eaten by *L. nigrinus* when adult predators were presented with eggs of either target prey (HWA) or non-target prey, the predator consumed significantly more HWA eggs than two of the three non-target prey. Though not statistically significant, *L. nigrinus* adults consumed ~ 51% fewer non-target *A. abietis* eggs than target HWA eggs (Zilahi-Balogh *et al.* 2002). A larger sample size might have shown a significant difference in predator consumption between HWA and *A. abietis* eggs.

Statistical analysis. Both paired-choice and no-choice tests were used in our study. The response variable in these tests is quantitative (i.e., number of eggs laid, number of prey consumed). Therefore ANOVA and paired-t tests are an appropriate choice as long as data are normally distributed and there is homogeneity of variance (Horton 1995; Zar 1984). Prior to analysis, data were examined for normality using the Shapiro-Wilk W Test and for homogeneity of variance using Levene's test for Equality of Variance (SAS 1989). The Shapiro-Wilk

test was done on the difference between paired observations in the paired-choice tests. Transformation of data using log (x+1) prior to analysis was done as necessary to correct for heterogeneity of variance and/or non-normal sample distributions. Parametric tests on transformed data were selected over non-parametric tests as they are more powerful than non-parametric tests.

Pseudoreplication. Pseudoreplication is defined as the use of inferential statistics to test for treatment effects with data from experiments where either treatments are not replicated (though samples may be) or replicates are not statistically independent (Hurlbert 1984). If treatments are spatially or temporally segregated, if replicates of a treatment are interconnected somehow, or if replicates are only samples from a single experimental unit, then replicates are not independent (Hurlbert 1984). It is important to determine the experimental unit. Steel and Torrie (1980) define the experimental unit as the unit to which one application of a treatment is applied. The treatment is the procedure whose effect is to be measured and compared with other treatments (Steel and Torrie 1980). For all experiments in this study, the experimental unit was an individual *L. nigrinus* adult, male-female pair, or egg (larva) in a petri dish. The treatment was the host/prey material (host plant with associated homopteran prey) in which the predator was exposed. Pseudoreplication did not apply to our study.

GENETICS: RELATION OF LOCAL POPULATIONS TO WHOLE SPECIES – IMPLICATIONS FOR HOST RANGE TESTS

In classical biological control it has been common practice to introduce natural enemies from many geographic locations (Unruh and Woolley 1999). However, it has been well documented that different populations have shown differences in host affinities and behavior (Hopper *et al.* 2005, and references within). The term biotype has commonly been used for populations that display differences in some biological attributes (Unruh and Woolley 1999). Diehl and Bush (1984) categorized insect biotypes by their genetic polymorphisms, non-genetic polyphenisms, geographic variation and host races. Molecular genetics provides tools to unraveling this variation. Hopper *et al.* (2005) discussed the implications of using distinct populations in host range testing.

All collections of *L. nigrinus* evaluated under quarantine were collected from the same site in a HWA infested western hemlock seed orchard near Victoria, British Columbia, Canada and thus would be considered the same 'local' population. Although this may not represent all existing populations of the species, it allowed for the elimination of inter-population variations.

CONCLUSIONS

A summary and interpretation of our test results is shown in Table 2. Although adult feeding tests indicated feeding acceptance on other adelgid species in addition to HWA, no-choice larval development tests showed that *L. nigrinus* only completed development to the adult stage on HWA. Based on the larval development tests, we concluded that these adelgid species are not suitable hosts for completion of larval development of HWA. If we solely based our conclusions on the paired-choice and no-choice oviposition and no-choice adult feeding

test, our interpretation would be that the other test adelgids would be inside the host range of *L. nigrinus* (see Table 4, Sands and Van Driesche 2000). Oviposition and feeding tests are concordant with larval development tests. We consistently see HWA ranked as the most preferred host. Non-host adelgids rank second, while non-adelgid hosts rank at the bottom.

Table 2. Summary of results of acceptance and suitability tests of Homoptera prey screened as hosts of *Laricobius nigrinus* (from Zilahi-Balogh *et al.* 2002).

	Acceptance[a]		Suitability[a]	
Test species	**Oviposition**	**Adult feeding**	**Larval development**	**Final host status[b]**
Adelgidae				
Adelges tsugae Annand	+	+	+	Yes
Adelges piceae (Ratzeburg)	+	+	-	No
Adelges abietis (L.)	+	+	-	No
Pineus strobe (Hartig)	+	+	-	No
Aphididae				
Cinara pilicornis (Hartig)	+	x	-	No
Myzus persicae (Sulzer)	-	x	x	No
Diaspididae				
Chionaspis pinifoliae (Fitch)	+	x	-	No

[a] +, positive response on test prey; -, negative response on test prey; x, test not conducted;
[b] Whether the species could serve as a host to L. nigrinus.

Laboratory host range tests are further strengthened by the synchrony between ovipositional period of *L. nigrinus* and presence of suitable oviposition sites (i.e., HWA ovisacs) in the field (Zilahi-Balogh *et al.* 2003). There is poor synchrony between ovipositional period of *L. nigrinus* and availability of suitable oviposition sites with the non-target adelgids tested (Arthur and Hain 1984; Craighead 1950; Friend and Wilford 1933; Gambrell 1931; Johnson and Lyon 1991; USDA 1985). When this information is combined with the larval development tests, we predict that these adelgids are outside of the ecological host range of *L. nigrinus*. We conclude that adult feeding by *L. nigrinus* may occur under natural field conditions on the other test adelgids, but that these hosts are phenologically and/or physiologically unsuitable for larval development.

Though not without flaws, we believe our host specificity tests provide a consistent pattern in regards to the predicted ecological host range of *L. nigrinus*.

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GENETICS: RELATION OF LOCAL POPULATIONS TO THE WHOLE "SPECIES" – IMPLICATIONS FOR HOST RANGE TESTS

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ABSTRACT

Populations of parasitoids collected from different host species or geographical regions can differ in host specificity. Where the necessary research has been done, such populations have usually been found to represent various stages of speciation. Here, we review the literature on variation in host specificity among populations and sibling species of parasitoids. We then summarize our results on the evolution and genetics of host specificity in *Aphelinus varipes* Foerster and *Aphelinus albipodus* Hayat and Fatima (Hymenoptera: Aphelinidae). Populations of *A. varipes/albipodus* from *Diuraphis noxia* (Mordvilko), *Ropalosiphum padi* (L.), and *Aphis glycines* Matsumura (Homoptera: Aphididae) collected in France, Georgia, Israel, China, Korea, and Japan differed in parasitism of seven aphid species in five genera and two tribes on four host plant species in no-choice laboratory experiments. Some populations showed narrow to monospecific host use, others attacked most or all host species tested. Most populations were reproductively isolated by pre-zygotic, behavioral barriers involving female choice. However, some allopatric populations where partially or completely reproductively compatible in laboratory crosses, although they differed in host specificity. A molecular phylogeny based on three nuclear and two mitochondrial genes indicated that these compatible, allopatric populations are distinct lineages, and morphometric analyses showed subtle differences

between them. Our conclusion is that *Aphelinus varipes/albipodus* is a rich complex, with populations in various stages of speciation. Although there was some concordance between phylogenetic affinities of host species and parasitoid species, other cases showed flips in host use between closely related taxa in the complex. We have been able to introgress genes for use of a novel aphid species from one parasitoid species to another in laboratory crosses, and we are using these crosses to map genes involved in host specificity. The take-home lessons for biological control are: (1) parasitoids in what appears to be a single species, but collected from widely different geographical regions or from different host species, may differ greatly in host specificity and thus should be tested separately, and (2) allopatric sibling species with different patterns of host use may introgress if placed in sympatry, which could lead to evolutionary changes in host use.

INTRODUCTION

Populations of parasitoids collected from different host species or geographical regions can differ in host specificity. Parasitoid species may consist of distinct host races that switch little between host species in the field (Cameron *et al.* 1984; Henter *et al.* 1996; Hufbauer 2002; Nemec and Stary 1983; Powell and Wright 1988; Stary 1983). Differences in host use among populations may often be explained by unrecognized sibling species. Evidence accumulated during the last decade suggests that sibling species of parasitoids may be far more common than previously realized (Campbell *et al.* 1993; Clarke and Walter 1995; Gauld and Janzen 2004; Kazmer *et al.* 1996; Pinto *et al.* 2003). Here, we review some of the literature on variation in host specificity among populations and sibling species of parasitoids, summarize our results on this issue, and draw conclusions concerning biological control introductions.

LITERATURE REVIEW

Microctonus aethiopoides (Hymenoptera: Braconidae) from different regions and host species differ in parasitism of *Hypera postica* versus *Sitona* spp. (Coleoptera: Curculionidae) (Sundaralingam *et al.* 2001) and also in parasitism of different *Sitona* spp. (Loan and Holdaway 1961; Phillips *et al.* 2002; Sundaralingam *et al.* 2001). Some of the differences parasitism result from differences in encapsulation by the host (Phillips *et al.* 2002). *Microctonus aethiopoides* from different sources differ in nuclear and mitochondrial DNA sequences (Vink *et al.* 2003). Although Vink *et al.* (2003) found no morphological differences among sources, Sundaralingam (1986) was able to discriminate between parasitoids from *H. postica* in France and those from *Sitona discoideus* in Morocco using eight quantitative traits. Furthermore, parasitoids from *H. positica* in France and *S. discoideus* in Morocco were partially reproductively isolated, with much lower frequences of males courting and females accepting insects from the other source (Sundaralingam *et al.* 2001). These results suggest that some of the differences in host use among populations of *Microctonus aethiopoides* can be explained by confounding of cryptic, sibling species.

Aphidius ervi (Hymenoptera: Braconidae) comprises a complex of populations, some of which have been recognized as host races or sibling species based on patterns in parasitism of

their aphid hosts, reproductive compatibility, morphology, and molecular markers (Atanassova *et al.* 1998; Pennacchio *et al.* 1994). Stary (1975) synonomized many species in a morphology-based revision of *Aphidius colemani* (Hymenoptera: Braconidae), another major parasitoid of aphids. But subsequent research has shown that *A. colemani* is a complex of reproductively isolated sibling species with different patterns in host use (Messing and Rabasse 1995; Ode and Hopper, unpublished data).

Populations of *Apocephalus paraponerae* (Diptera: Phoridae), a parasitoid ants in Central and South America, show differences in morphology, molecular markers, and host specificity sufficient to consider them cryptic species (Morehead *et al.* 2001). Populations of *Pseudacteon tricuspis* (Diptera : Phoridae) appear to be cryptic species with different host ranges (Porter and Gilbert 2005). Populations of *Pseudacteon curvatus* (Diptera : Phoridae), which are being introduced to control imported fire ants in North America, also show differences in host specificity which may affect their potential for impact on non-target native ants (Porter and Gilbert 2005; Vazquez *et al.* 2004;).

Leptopilina boulardi (Hymenoptera: Figitidae), a parasitoid of *Drosophila* spp., shows geographical variation with a genetic basis in responses to different host-associated odors (Campan *et al.* 2002) and ability to avoid encapsulation by its hosts (Dupas *et al.* 2003). *Asobara tabida* and its sibling species *Asobara rufescens* (Hymenoptera: Braconidae) also show geographical variation in ability to overcome encapsulation by their hosts (Kraaijeveld and Godfray 1999; Kraaijeveld *et al.* 1994).

HOST USE IN *APHELINUS VARIPES* COMPLEX

Although *Aphelinus varipes* has been reported from 40 host species across several genera of aphids (Kalina and Stary 1976), we found distinct patterns of host use among *A. varipes* from different hosts and regions (Fig. 1) as well as different populations within a region (Fig. 2). We measured host use in single-host-species laboratory experiments, where female parasitoids had the choice of whether to oviposit or not in a particular host species. This is frequently the choice parasitoids make in the field. "Choice" tests in the laboratory provide different species in close spatial and temporal proximity, but the behavior on encountering a particular host is still whether to parasitize in or not. Our goal was to determine host acceptance/suitability in an environment that appears to harbor only one aphid species on only one plant species and where parasitoid females re-encounter this combination repeatedly with a full egg complement after a relatively long period without encountering other host species. In these experiments, we exposed 100 aphids (mixed stages) on host plant to individual, naïve, mated female wasps for 1 day, with 10-20 replicates per host-species/parasitoid-source combination. We measured parasitism as the number of mummified aphids produced during this exposure.

Most of these populations in the *A. varipes* complex had fixed differences in DNA sequences, subtle but highly significant differences in morphology, and were reproductively incompatible. It appears that *Aphelinus varipes/albipodus* is a rich complex, with populations in various stages of speciation. Thus, the host range reported in the literature for *A. varipes* is incorrect because sibling species have been confounded.

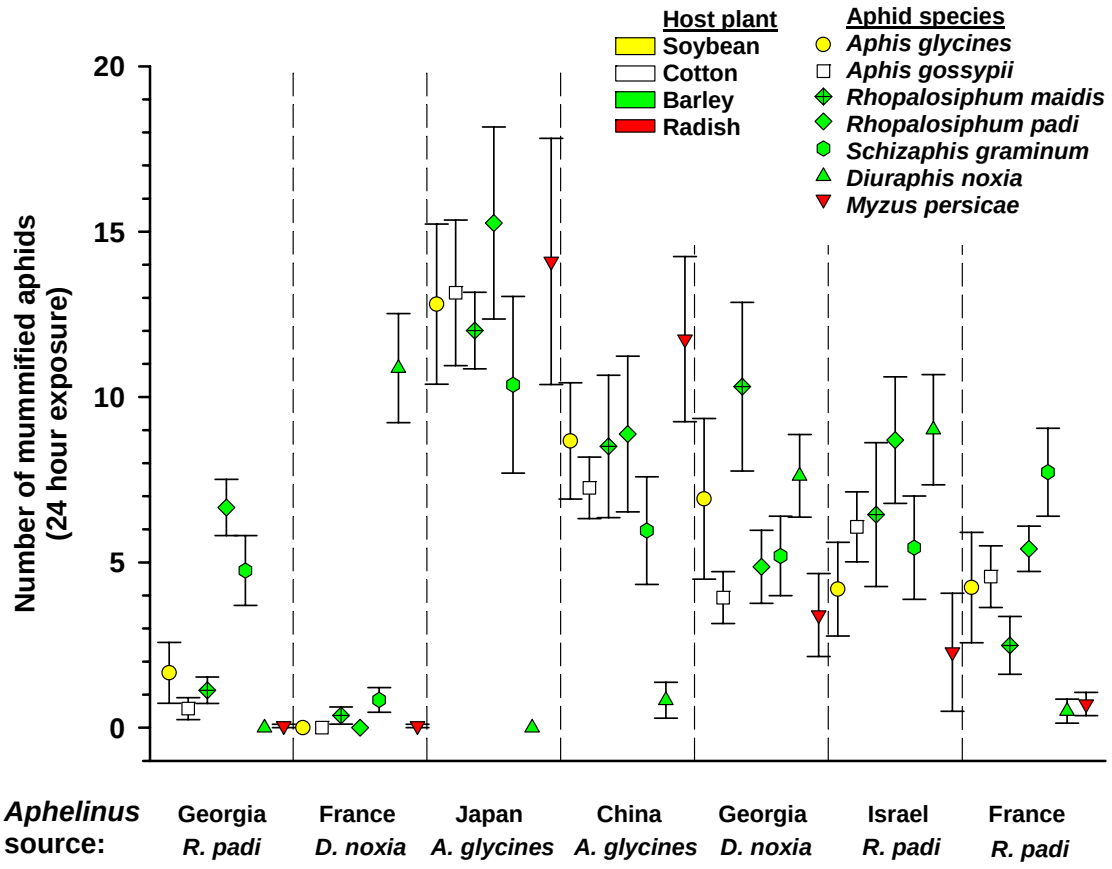

Figure 1. Host specificity in *Aphelinus varipes* complex: differences among host and regional sources.

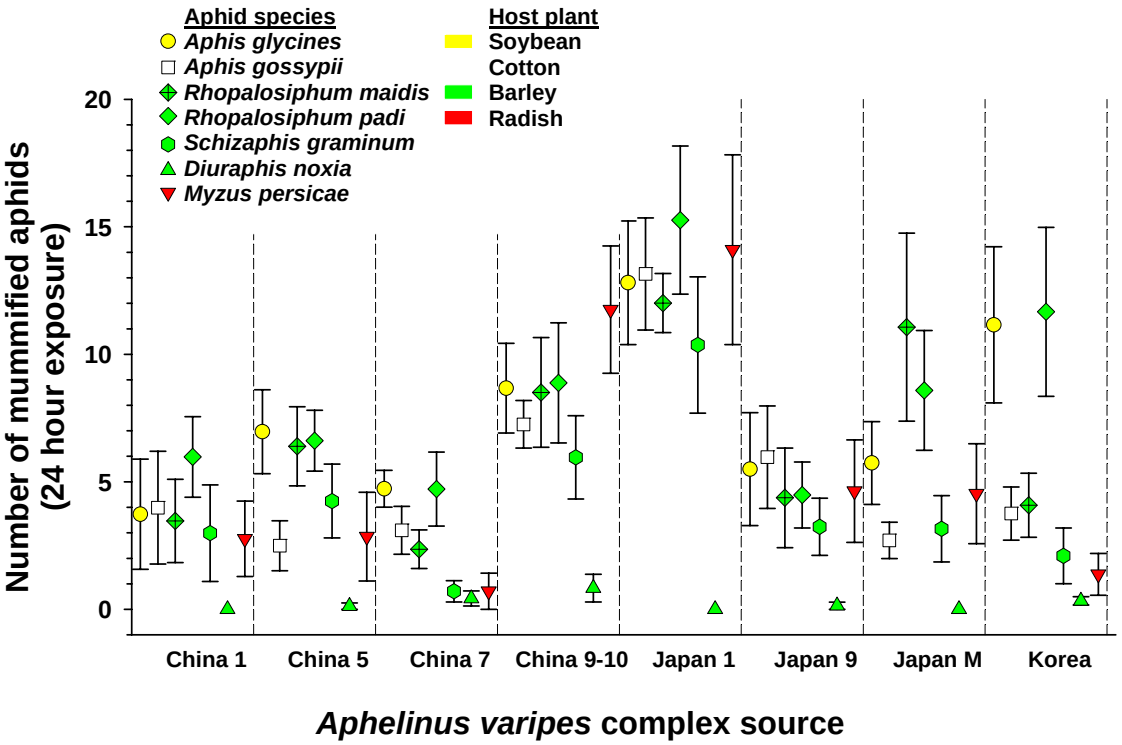

Figure 2. Host specificity in *Aphelinus varipes* complex: differences among populations from *Aphis glycines* in the Far East.

Although closely related species sometimes show similar patterns of host specificity, phylogenetic affinity was not a reliable indicator of host specificity. Even among the rather closely related species and populations in the *A. varipes* complex, use of some host species roughly maps onto the parasitoid phylogeny, but use of other species does not.

Therefore, we need to examine the genetic basis of host switches if we are to predict when they will occur. Two populations in the *A.varipes* complex, one from *D. noxia* in Georgia ('Georgia-*D. noxia*') and the other from *A. glycines* in Japan ('Japan-*A. glycines*') were reproductively compatible, despite differences in DNA sequences, morphology, and host use. 'Japan-*A. glycines*' parasitoids do not parasitize *D. noxia*, whereas 'Georgia-*D. noxia*' parasitoids readily parasitize this host (Fig. 1). By crossing and backcrossing, we have introgressed genes from 'Georgia-*D. noxia*' into the 'Japan-*A. glycines*' background and produced hybrids segregating for parasitism of *D. noxia*.

CONCLUSIONS

The take-home lessons for biological control are: (1) parasitoids in what appears to be a single species, but collected from widely different geographical regions or from different host species, may differ greatly in host specificity and thus should be tested separately, and (2) allopatric sibling species with different patterns of host use may introgress if placed in sympatry, which could lead to evolutionary changes in host use.

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FROM DESIGN TO ANALYSIS: EFFECTIVE STATISTICAL APPROACHES FOR HOST RANGE TESTING

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ABSTRACT

The major goal of host range testing in biological control is to minimize the probability that released biological control agents have unwanted effects on populations of non-target hosts. This leads to a non-trivial problem in statistical hypothesis testing, since the standard approach in statistical tests is to ask whether or not an effect – in this case acceptance of a non-target host – exists and to attribute a precise probability to err only with rejecting the null hypothesis that assumes no effect. The problem is that it is difficult to assign a probability with accepting the null hypothesis of no effect, i.e., that the biological control agent does not include a given non-target insect into its host range. Yet, this piece of information is exactly what we need for high precision and confidence. Confidence in this respect increases with sample size and the statistical effect size, i.e., the difference from the null hypothesis that is considered biologically meaningful. However, sample size is often limited due to limitations in test subjects, research money, and space for testing arenas. Consequently, there is a high premium on using a very good experimental design and employing the most powerful statistical approach available. This paper discusses common problems with experimental designs, emphasizes the necessity to decide on the statistical effect size that is biologically meaningful, points towards the need to determine the statistical power of the host range test employed, and provides an overview about powerful statistical approaches for analyzing experiments on the host range of potential biological control agents.

INTRODUCTION

Over the last two decades ecologists have become increasingly aware of novel and powerful statistical approaches. This trend can be witnessed by a number of recent textbooks on design and statistical approaches in the life sciences (e.g., Crawley 1993; Crawley 2002; Grafen and Hails 2002; Hilborn and Mangel 1997; Quinn and Keough 2002; Ruxton and Colegrave 2003) and changes in approaches used in more recent publications. This reflects both the increased awareness that conclusions in ecological studies need to be drawn in a quantitative manner with high precision and confidence, and that, for a number of reasons, large sample sizes are often difficult to obtain. This is especially so for studies on the host range of agents for bio-

logical control, since these animals have to be tested on a number of non-target hosts. Thus, the need for powerful statistical tools that allow precise analysis from limited sample sizes is especially evident in this field of research. Formerly, the statistical analysis of data in ecological investigations has been fraught with the difficulty that many if not most of the data sampled in these cases are not normally distributed and are thus not suitable for the parametric 'standard' approaches of Analysis of Variance (ANOVA) and Student *t*-tests. Instead, non-parametric statistics like, e.g. Kruskal-Wallis and Mann-Whitney U-Tests have been used that are known to be less powerful. In theory, the lack of power of non-parametric statistics may be compensated by larger sample sizes. However, an increase in sample size is often not feasible for agricultural entomologists who are usually limited by the time that can be invested, the money that can be spent on experiments, and/or the number of replicates that can be obtained through a shortage of either experimental fields or insects to work with.

In this paper, I want to make 4 points: Firstly, that in many experiments of host range testing it becomes most interesting when we do not find a statistical effect, e.g., no effect on non-target hosts, a situation that is inherently difficult to interpret in statistical testing. Secondly, and following from the first point, that it is generally important to determine and to report on the precision with which we can conclude that no effect exists when no statistically significant effect has been found, i.e., the Power of the statistical test. Thirdly, that it is usually advisable to carefully consider the distribution of the data and find the most powerful means of analyzing them. And fourthly, that as yet, not all research questions in insect host range testing can be analyzed with easily accessible powerful statistical methods and that further progress in this field is clearly needed.

Throughout, I will use verbal examples or computer generated (fake) data sets to elucidate my arguments.

β-ERRORS AND THEIR IMPORTANCE FOR INSECT HOST RANGE TESTING

The very basis of statistical testing is that, by performing an experiment, it remains impossible to prove, for example, that a natural enemy will never attack a non-target host or prey. Using a sound experimental design, we can only aim at achieving high accuracy and precision in what we conclude from the sample that we tested. Yet, using standard statistical procedures, there is always some possibility that our interpretation of the data is wrong. This is due to the fact that all the measurement variables we are interested in are usually subject to random variation (i.e., variation between sample units that cannot account for a treatment factor considered) and that our conclusion is based on a sample rather than the entire population. In general there are two ways to err: 1) based on test results we may either conclude that there is an effect when in fact there is none, or 2) we may conclude that there is no effect when in fact there is an effect (Fig. 1). Standard statistical testing is much concerned with the first kind of error, the so called α-error or Type I error, which is returned as *P*-value in test results. However, in insect host range testing, it is often much more important to know the probability of committing a β-error: let us assume that we have tested the mortality of non-target hosts in field cages with and without the presence of a biological control agent, have found 10 and 17 % mortality in control and treatment cages, and have not found a statistically significant deviation from the null hypothesis that states in our case that no difference exists in mortality

of the non-target prey in control cages without and treatment cages with the biological control agent present. Assume further that our statistical test returns a *P*-value of $P = 0.167$. Is it safe to conclude that we cannot reject the null-hypothesis? In this case we would usually state – using words rather than statistical jargon – that in our test the biological control agent did not cause significant mortality of the non-target prey. However, we do not know the β-error (that an effect exists that we did not detect). If we decide to release an exotic natural enemy for biological control based on such results, and if in fact we committed a β-error, i.e. the natural enemy in fact causes mortality of the non-target prey, unwanted non-target effects may be the consequence. This seems much more problematic than committing an α-error, i.e. rejecting a natural enemy for biological control based on tests that falsely led to the conclusion that the biological control agent would cause mortality of non-target prey. Therefore, in non-target testing, it seems fundamental to obtain information about the β-error. This is where power analysis comes into play.

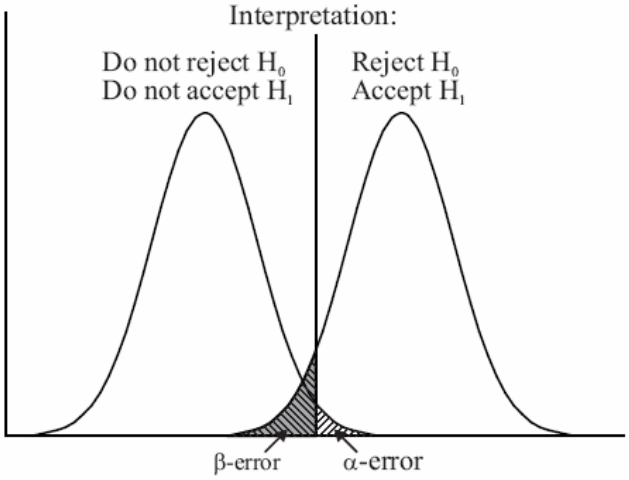

Figure 1. Graphical representation of α-error (area hatched in white and black) and β-error (area hatched in grey and black) probabilities, using a one-sided *t*-test, comparing, e.g., encounter rates of biological control agents with non-target hosts. The curves on the left (for the null hypothesis) and right (for a specified alternative hypothesis) represent the probability sampling distribution of the statistical test done. Note that usually, the alternative hypothesis is not specified, i.e. H_1 is just different from H_0, and the probability distribution of the statistical test done for H_1 is unknown (modified from Quinn and Keough 2002).

REPLICATE NUMBER, EFFECT SIZE, AND POWER OF STATISTICAL TESTS

While the β-error is defined by the probability of not finding an effect when in fact there is an effect, statistical power is the probability of a given statistical test finding an effect (rejecting the null hypothesis) when in fact there is an effect. Hence, power = 1 - β. For any particular test, power is dependent on the α-level, the sample size, the sampling variance and the so called effect size. The effect size can be regarded as the magnitude of the departure from the null hypothesis (observed effect size) or the difference between the values considered in the null and the alternative hypothesis (Fig. 1). Sample size is positively related to power, i.e., with increasing sample size does the power of a statistical test increase. However, this rela-

tionship is not linear, thus a twofold increase in power requires more than a twofold increase in sample size. Power analysis can follow three different routes, it might: 1) be used *a-priori* to define the sample size necessary to detect an effect with a predefined precision, 2) be used *a-posteriori* to calculate the Power of a test that has not detected a significant effect, or 3) to find compromise levels for α- and β-errors when sample size is fixed. The latter is a consequence of the fact that α- and β-errors are closely related. As can be seen in Fig. 1 a decrease in the α-error leads to an increase in the β-error and vice versa (e.g., imagine to shift the interpretation borderline in Fig. 1 between not rejecting H_0 and accepting H_1 to the left; the shaded areas for α- and β-errors would increase and decrease, respectively). Thus, if sample size cannot be increased, and β-errors are of concern one may compromise the α-error in the interpretation of test results, e.g., stating that a significant effect exists up to a *P*-value of 0.2, to use a somewhat extreme example. If sample size can be increased, i.e. before an experiment is carried out, *a-priori* power analysis can be used to define the necessary sample size. However, the effect of size needs to be determined in advance. While there are conventions for small, medium, or large effect size for different tests (Cohen 1998), in non-target tests, one may simply use the deviance from the null hypothesis of no effect as being biologically meaningful.

Let us use the above mentioned example of a field cage test on non-target effects of a biological control agent. If we would consider a mortality of 5 % induced by the biological control agent as the maximum that is acceptable and we know that in such experiments we have a background mortality rate of 10 % with a known standard deviation, we can use the arcsine-transformed proportional values (to allow for parametric tests like *t*-tests) to calculate the effect size. With transformed means of 0.322 and 0.398 and a standard deviation of 0.22, the effect size is 0.341 and thus falls between the values of 0.2 for small and 0.5 for medium effects that are conventionally considered. An *a-priori* power analysis for a one-tailed t-test (we are not interested whether mortality in the treatment is lower than in the control) for an α-error of 0.05 and power of 0.8 (note that this allows a β-error of 20 %) suggests a required sample size of 216, a replicate number that is often unachievable in host range testing. Allowing for 10 % mortality induced by the biological control agent would increase the effect size to 0.645 and reduce the total sample size needed to 78.

While it is usually advisable to conduct *a-priori* power analyses before conducting experiments, often some needed values like the variation around means or, in our example background mortality rates are unknown. Thus, in many cases, power analysis only comes into play, after researchers have not found a statistically significant effect and need to know the confidence with which they can decide not to reject the null hypothesis of no effect. For those *a-posteriori* power analyses, a critical parameter is the effect size assumed. Generally there are two possibilities to determine the effect size. First, the effect size may be computed from the data. However, this does not add new information about the data (see Thomas 1997 for a valuable discussion why this is so). Rather, the effect size should be either determined by using conventions or should – and I would consider this more sensible – be calculated from a biological meaningful effect that we wish to detect.

If, for example, one would have carried out the above mentioned experiments with 10 field cages each for control and treatment and would have found on average 10 % mortality in the control cages and 17 % mortality in the cages with biological control agent and non-target prey, and we would have found no significant effect of the biological control agent on the

mortality of non-target prey (P = 0.167), the power would be 0.277 if the effect size would reflect that we accept a maximum of 10 % mortality induced by the biological control agent. This value is unacceptably low.

Programmes to conduct power analyses are either available for free in the internet or are increasingly often included as modules in current statistical software packages (see Thomas and Krebs 1997 for a list of programs and comprehensive review on this topic and Hoffmeister *et al.* 2006 for a recent discussion and alternative ways of achieving power estimates). However, not all tests are covered yet. For example, to my knowledge, no power analysis is as yet available for Generalized Linear Models (see below).

PSEUDOREPLICATION AND DATA INTERDEPENDENCE, A CLASSICAL ISSUE, UNFORTUNATELY

One of the central assumptions of almost all statistical tests is that data points are independent from each other (one exception is planned dependencies in paired data designs). This said, we might wonder why this assumption is so often violated in experiments (see e.g., Hurlbert 1984). One of the most frequent reasons for data interdependence is pseudoreplication. It occurs whenever inferential statistics are used to test for treatment effects with data from experiments where either treatments are not replicated (though samples may be) or replicates are not statistically independent (Hurlbert 1984). Statistical independence means that each individual data point might positively or negatively deviate from the population average due to random variation not related to the deviation of another point. Although the awareness of researchers to avoid pseudoreplication has increased and fewer studies contain analyses with pseudoreplicated samples (Heffner *et al*. 1996), an alarmingly 46% of 105 studies were found to be pseudoreplicated in a recent study on pseudoreplication in experiments on the olfactory response of insects (Ramirez *et al*. 2000) Thus, pseudoreplication still is an issue in the design of experiments, and much care has to be taken to avoid any spatial or temporal segregation of samples from different treatments. For example, when testing the host range of biological control agents, it is essential that insects for the tests on non-target hosts do not come from one rearing container or incubator and control animals (for the test on target hosts) come from another, or that non-target hosts are always tested in the same container or field cage or on the same plant and target hosts are tested in another cage or on another plant. Equally, positions of experimental units within an experimental chamber or on a field plot need to be switched between treatments to avoid confounding effects of differences in temperature and light conditions etc. In the same manner, the full set of trials on non-target hosts should not be conducted before tests with target hosts are carried out. Randomization of testing order or random assignment to plants or test cages assures that pseudoreplication can be avoided. For further reading, I encourage the reader to take a look at the section on pseudoreplication in Ruxton and Colegrave (2003).

GENERALIZED LINEAR MODELS, POWERFUL STATISTICAL APPROACHES FOR INSECT HOST-RANGE TESTING

Many of the traits to be analysed in biological investigations do not follow a Gaussian (also called "Normal") distribution, and thus standard *t*-tests, analyses of variance (ANOVA) or regression analyses cannot be used to statistically test the effect of a treatment. All these different "classical" methods assume that the distribution of residuals around the fitted model (i.e., the error distribution) is normal (Gaussian). Thus data need to be transformed to achieve a Gaussian distribution or different approaches have to be used. While transformation is often possible, it changes the relationships between parameters in the model. For example, log-transformation of data would make the relationship between parameters in the statistical model multiplicative that has been additive for untransformed values. Thus approaches should be favoured that do not make it necessary to transform values to achieve a Gaussian distribution of data. While non-parametric tests like Mann-Whitney U tests or Kruskal-Wallis tests lack statistical power, Generalized Linear Models can be used to predict responses both for dependent variables that are not normally distributed and for dependent variables which are nonlinearly related to the predictors. They are a generalization of general linear models that underlie classical statistical tests like ANOVA and regression. While in general linear models, the data distribution is Gaussian and the link function is identity, various types of data distribution and link functions (see McCullagh and Nelder 1989) can be chosen, depending on the assumed distribution of the *y* variable values. Table 1 gives the list for the four main generalized linear models that can be used in experiments done to estimate host range of biological control agents.

To give an example, imagine a large arena choice test as suggested in van Lenteren *et al*. 2006. Three different treatments are used, with 10 field cages each: (1) with the target prey (or host which is used synonymously here) and non-target prey present in the same field cage together with the natural enemy, (2) with only the non-target prey and the natural enemy in the same field cage, and (3) with only the target prey and the natural enemy in the same field cage. We are interested in whether the target prey is killed at a higher rate than the non-target prey and whether the mortality of the non-target prey depends upon the fact whether the target prey is available to the natural enemy or not. To achieve independent data, one should not compare whether mortality rates of target and non-target prey are equal within a single treatment. Rather, one should test whether the mortality of non-target prey in treatment (1) is equal to the mortality of non-target prey in treatments (2) and equal to the target prey in treatment (3) (this is our null hypothesis). Again, I use computer-generated data. Given the mortality rates found were 4.1 %, 10.6 % and 50.5 % in (1), (2) and (3), respectively, a Generalized Linear Model with binomial distribution and logit link finds a significant effect overall and also between treatments (Table 2). Thus, in this example, the non-target prey is attacked at relatively low rate and even less so, when target prey are available. This result is visible from the estimates in Table 2, where the estimate for mortality is positive and thus higher in treatment (2) than in treatment (1), and much higher (more than 3 times higher) in treatment (3) than in treatment (1).

Table 1. List of the main generalized linear models that can be used in experiments done to estimate the host range of biological control agents. Link functions indicated are the most frequently used ones. Other can be used in particular cases (see McCullagh and Nelder 1989, for an exhaustive description).

Distribution	Model description	Appropriate link function	Example for data type
Gaussian	General linear model	identity: $f(y) = y$	Morphological data
Binomial	Logistic regression	logit: $f(y) = \log\{y/(1-y)\}$	Proportions like parasitism
Poisson	Log-linear model	log: $f(y) = \log(y)$	Counts like egg load or number of prey consumed
Gamma	Gamma model	inverse: $f(y) = 1/y$	Time durations like survivorship

Table 2. Results of a Generalized Linear Model on computer-generated data for the mortality rates of target and non-target prey in large arena choice tests (for details, see text).

Parameter	Treatment	Estimate	DF	χ^2	Pr > ChiSq
Intercept		-3.2591	1	378.47	<.0001
Target host	(3)	3.2511	1	329.63	<.0001
Non-target prey in no-choice test	(2)	1.0839	1	30.14	<.0001
Non-target prey in choice test *	(1)	0	0	0.0000	

* In the SAS statistics package, which was used here, the last treatment [in this case (1)] is set to zero by convention and the difference between the last and all other treatments [(2) and (3)] is tested.

A special case of Generalized Linear Models exists if measurements are taken repeatedly. If, for example one plans to monitor the mortality induced by the natural enemy on the target and non-target host across a time period after the release of the natural enemy, several data points from the same treatments will be taken. In this case, A GEE model can be specified with the Generalized Linear Model (see e.g., Quinn and Keough 2002) that adequately deals with such data.

TIME DURATIONS AND CENSORED DATA

Time duration data like survival times or latency until attack usually follow an exponential distribution, because the probability λ to die or to become attacked in each time unit is constant. While generally such data can be analysed with Generalized Linear Models with gamma distribution and inverse link function, they cannot if data points are censored, i.e., when we were unable to measure a quantifiable value. Right-censored data origin, for example, from host range experiments in which we measure the latency until attack of target and non-target prey in small arenas with behavioural observation, when a predator did not attacked the prey until the end of the observation (in this case we just know that the latency is larger than the

time of observation, but cannot quantify it properly). If we just ignore those censored values, the interpretation of the test might be wrong. A Cox regression model (= proportional hazards model) can adequately deal with censored time duration data (Cox 1972). Recently, a plethora of different studies have used such an statistical analysis for ecological investigations on insects (e.g., van Alphen *et al*. 2003). Besides using this sort of analysis to study changes in survival time, a Cox survival analysis can also be used when it comes, for example, to testing residence times or giving up times of natural enemies on patches with target and non-target prey, or when testing the latency until a natural enemy attacks a host or prey.

A POWERFUL STATISTIC FOR EVERY PROBLEM? – UNFORTUNATELY NOT

Recent advancements in statistical methods may give the impression that almost every biological problem imaginable in insect host range testing could be analysed with one of the powerful methods described above. Unfortunately this is not so. Besides the banality that good statistics cannot cure poor experimental designs, some of the research questions one will often address in insect host range testing cannot be easily analyzed with powerful statistical methods. For example imagine a no choice test with a natural enemy on target and non-target host. It is statistically not problematic to test the null hypothesis that acceptance of target and non-target prey does not differ. However, this test is not the most interesting research question we might have in mind. If we are to decide whether or not to introduce an exotic natural enemy, we need to know whether the natural enemy will accept the non-target host at all. One approach would be to assume that host acceptance does not vary and, given that we have found in say, 10 replicates on non-target hosts, that they are not accepted while the target host has invariably been accepted. No statistical test would be needed in this case. However, host acceptance usually is variable. Host acceptance experiments with biological control agents of different degrees of host deprivation clearly show increasing acceptance rates with increasing host deprivation (Withers and Mansfield 2005, this issue).

One possibility to solve the problem using statistical methods would be to decide on a threshold of acceptance that can be tolerated, and given one has found no acceptance of non-target hosts in *n* replicate trials, one can compute the probability to obtain a series of *n* host rejections given the threshold level (see Porter *et al*. 1995 for a published example). Alternatively, we might use an exact test based on a binomial distribution. Here, we need to define a null hypothesis (H_0) about the likelihood that a biological control agent accepts the non-target host and an alternative hypothesis (H_A) about a threshold level of this probability that we believe would be crucial to detect. For example, let us assume the H_0 that the biological control agent would have an inherent probability of $\lambda_0 = 0.01$ to accept the non-target host (thus on average, 1 out of 1000 parasitoids would accept the non-target host). Let us further assume that we wish to detect if the true acceptance rate of the parasitoid, our H_A, is $\lambda_A = 0.05$ (the dotted line in Fig. 2). In this case, we would need 32 replicates to obtain a power of > 80 % (Fig. 2). Critical values to detect a significant deviation ($P < 0.05$) from the null hypothesis of 0.1 % acceptance rate of non-target hosts are detected if at least *r* non-target hosts are accepted ($r = 1$ for sample sizes of $1 \leq n \leq 51$ and $r = 2$ for $52 \leq n \leq 100$).

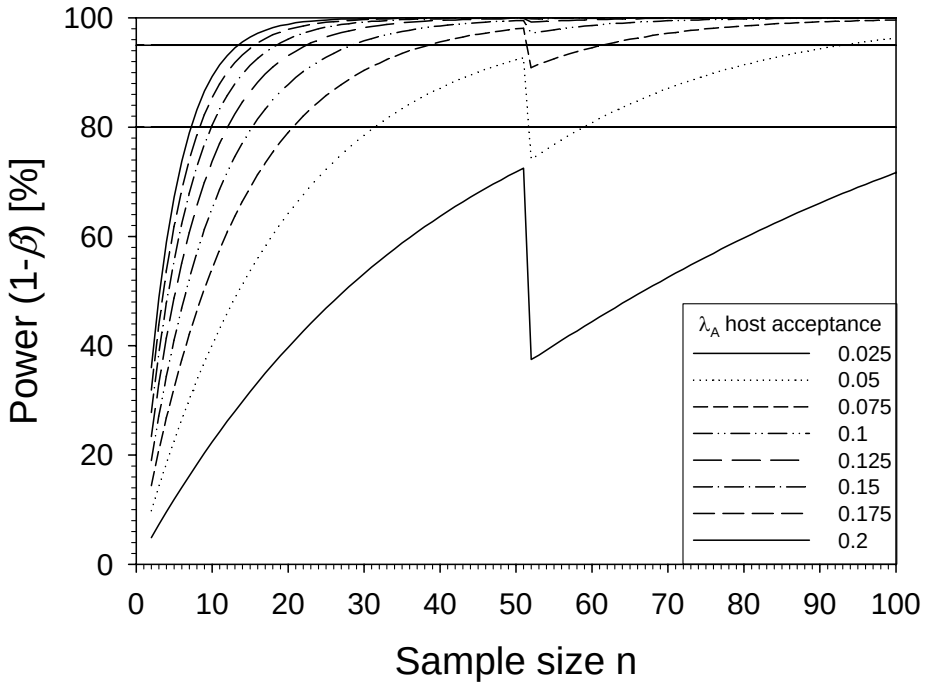

Figure 2. Statistical power for a non-target test based upon an exact binomial test under the null hypothesis H_0 of an acceptance rate of non-target hosts of $\lambda_0 = 0.001$. The tests specifies the Power, given one does not accept (P 0.05) the alternative hypothesis H_A that assumes an acceptance rate of λ_A given in the figure legend, and given fewer than r host were accepted by the parasitoid, with $r = 1$ for $n <$ 52 and $r = 2$ for $n \leq 52$. Horizontal lines mark 80 and 95 % power. See text for details.

A second issue that cannot be solved statistically are optimal designs for choice or no-choice tests. As Withers and Mansfield (2005) point out, there are different benefits associated with no-choice and choice tests. If we think about the statistical analysis of such tests, choice tests can be problematic. Since the same animal will be confronted with target and non-target hosts, we usually wish to obtain more than a single data point for each animal, i.e. for example acceptance rates of target *and* non-target hosts. Thus, some sort of repeated measurement design has to be used in this case (alternatively, only the acceptance rate of non-target hosts is analyzed; see the above example). While analysis of such dependent data is generally possible (see, e.g., GEE models in Generalized Linear Models), an additional problem exists, if target and non-target hosts or prey are exposed to the natural enemy simultaneously. The acceptance of non-target hosts or prey may well depend upon the frequency of target and non-target hosts within the experimental arena. If this is so, every target prey that is removed or every target host that is accepted and that is not replaced alters the experimental conditions of the experiment, and the acceptance of any given host or prey may depend on the current availability of alternative hosts or prey. If exploited hosts or prey cannot be replaced immediately, simultaneous choice test may become almost impossible to interpret. Thus, from a statistical point of view, sequential no-choice tests may be favourable (see Singer 1986 for a discussion), where all effects like the sequence of species presented, the motivational status of the tested insect can be statistically controlled for. Yet, these two tests may lead to very different outcomes biologically (Withers and Mansfield 2005) and thus both tests have their merit, despite the problems associates with simultaneous choice tests.

CONCLUSIONS

In the past, decisions to use or reject a species as biological control agent were more often based on gut feeling than exact scientific methods. Today, sound host range tests are a prerequisite in the evaluation of biological control agents. However, despite great advances in the field (Van Driesche and Murray 2004; van Lenteren *et al*. 2006; Withers and Mansfield 2005), some issues on the interpretation of data are still unsolved. This paper advocates for a rigorous use of Power analyses to obtain a measure of confidence if one does not find significant deviations from the null hypothesis of no effect. Further, the most powerful statistical methods should be used when sample sizes are a limiting factor in insect host range studies. Despite the introduction of a number of new statistical tools, some of the basic statistical problems in host range testing are still unresolved. For example, no standard test is available to calculate a measure of confidence for an experiment where one has not found acceptance of the non-target host in *n* replicates (but see above for a possible method). Until now, researchers working in biological control are largely dependent on educated guesses with respect to how many replicates would be necessary to decide that an insect does not accept a given non-target host (D. Sands, J. van Lenteren, pers. comm.). Thus, further advances in statistical techniques are clearly needed.

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SESSION 14 INTRODUCTION: LEGISLATION AND BIOLOGICAL CONTROL OF ARTHROPODS: CHALLENGES AND OPPORTUNITIES

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SESSION 14 INTRODUCTION

Regulation of biological control agent introduction in most countries is achieved by legislation. Provisions within such legislation vary considerably between countries. Recent global concerns about globalization, and adverse environmental and economic impacts from biosecurity incursions, has in some cases, resulted in reviews of existing, or the enactment of new legislation. In this session we will see how some countries, particularly those who are key users of biological control technology, have developed regulatory frameworks for biological control. These include Europe, the United States (including details of the regulatory process in Hawaii), Mexico, Australia and New Zealand.

We are fortunate to have David Nowell to introduce the recent review of the International Standard for Phytosanitary Measures No. 3 (ISPM No. 3) which provides guidelines for risk management relating to biological control agents. This review of the 1996 'Code of Conduct for the Import and Release of Biological control Agents' has only recently been completed in April this year, and David Nowell as a member of the IPPC Secretariat was directly involved with the review. He will explain the background and process of the review, and outline the aspects of the standard which have received most emphasis during the review. This standard will almost certainly continue to provide guidance for countries who are developing their own legislative systems for biological control regulation, and as pointed out by Franz Bigler and co-authors in this session, the Code may be seen as a first attempt to harmonize regulation of biological control agents globally.

Harmonization of biological control regulation in Europe is the topic of the contribution from Bigler and co-authors. The revised Code is perhaps now the opportunity for Europe at least to harmonize its regulation of biological control, given their shared borders, the biological control requirements that they have in common, and the similar biological control safety concerns of many European countries. So while achievement of harmonization is certainly a political challenge, it is also an opportunity for some countries to review their, in

some cases, inappropriate legislation of biological control. The OECD initiative to harmonize and simplify regulation of commercially produced biological control agents has been a very timely first step in this process. The OECD guidance document is intended to reduce the need for each country to repeat biosafety testing procedures that have already been completed in other countries. Furthermore, it will open up opportunities for commercial producers to expand the use of their products more easily, and facilitate opportunities for use of biological control options.

Continuing with the theme of harmonization across shared borders, Peter Mason from Canada and his co-authors from the U.S.A. and Mexico address the question of whether legislation can facilitate biological control opportunities in North America. To some extent there has been some harmonization in data requirement for entomophagous biological control agent proposals in that the three countries have agreed to conform to NAPPO guidelines. As in Europe, this would achieve gains for biological control by more readily allowing information sharing. Furthermore the authors point out that a scientific approach to the approval process is likely to ensure that only safe and effective biological control agents are introduced. However, currently the regulatory system with the U.S.A. is cumbersome with a mixture of Federal and inconsistent State jurisdiction. Russel Messing provides an overview of the system for biological control regulation in Hawaii, the State where the most rigorous review procedure has been adopted. While the system appears to be exhaustive in ensuring environmental safety of biological control, and allows for a degree of public consultation, it is steeped in bureaucracy that results in frustration and lengthy delays for biological control practitioners. The case is made for the best of the Hawaiian system to be adopted generally in the U.S.A., but improvements made in efficiency and transparency.

Like Hawaii, two island nations where shared borders are not an issue, and complete control over imported biological control agents can be achieved are Australia and New Zealand. Harrison and co-authors describe and compare the regulatory legislation in these countries. The HSNO Act in New Zealand has attracted considerable attention internationally as very environmentally focussed legislation, and the implementation of it by ERMA NZ has been observed with interest. In Australia, biological control agents are regulated by two agencies under three separate Acts, and has been similarly heralded as a thorough and biosafety-conscious approach. The authors provide a useful analysis of the two systems illustrating very clearly some key differences in approach, and the implications of these, particularly in the areas of scope of the regulatory process, opportunity for public participation, and degree of risk-aversion of the regulatory agencies.

In this session we asked the authors to address challenges and opportunities presented by biological control legislation, and several themes have emerged from both perspectives. We take the approach that each challenge in turn presents an opportunity. One of the major challenges for regulators that most authors have acknowledged is the need to manage the uncertainty inherent in risk assessment for biological control agents, specifically host-specificity determination and prediction of post-release impacts based on quarantine laboratory testing. The opportunity here is for researchers to continue to address this issue and to extract maximum value from post-release validation studies. In Europe and North America, the political and/or bureaucratic challenges are to develop regulatory frameworks that recognise

shared borders and the advantages of a coordinated, harmonized approach across sovereign or regional state boundaries. The respective authors have emphasised the opportunities that can be realised from harmonization, and the benefits for biological control that can potentially accrue from such an approach. The authors commenting on the U.S.A. regulatory system have highlighted the bureaucratic complexity and the challenge to legislators to improved efficiency, consistency and public participation in biological control regulation. The opportunity will then be there for biological control practitioners to work within a time-bound and simplified process where they can interact with the public. Finally in Australia and New Zealand, one of the challenges identified (which almost certainly applies generally) is to convince biological control practitioners that the regulatory process should be seen not as an obstacle, but an opportunity for constructive peer review, improvement of the public profile of science as well as the opportunity to conduct high quality research for good of people, the economy and the environment.

We hope that in bringing together this mix of authors from regulatory and science perspectives, we can benefit from the exchange of ideas and an improved understanding of how a range of regulatory systems operate. The biggest challenge and opportunity of all is to capitalise on the best aspects of each and the collective wisdom that has been presented, so that globally we can maximise the opportunity for safe, cost-effective biological control.

HAWAII AS A ROLE MODEL FOR COMPREHENSIVE U.S. BIOCONTROL LEGISLATION: THE BEST AND THE WORST OF IT

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ABSTRACT

The United States currently has no comprehensive, integrated legislative or regulatory framework to manage the permitting of imported biological control agents. There are unresolved questions of whether the USDA - Animal and Plant Health Inspection Service (APHIS) has jurisdiction over parasitoids that are not plant pests; there are differences in protocol between weed and arthropod biocontrol agents; there are unresolved issues of State vs. Federal authority; and there are overlapping and ever-changing requirements from a wide assortment of Federal and State agencies and a diverse array of laws that were designed for other purposes. In contrast, the State of Hawaii has specific, detailed, and exhaustive rules for obtaining import and release permits for natural enemies. In some respects the Hawaii system could serve as a useful model for national protocols - with coordinated scientific evaluation at several levels of specialization, and input from a wide range of concerned parties. However, some aspects of this system lead to bureaucratic entanglements and unconscionable delays that hinder the practice of biological control in the islands. If we could capture the best parts of the Hawaii system and mitigate the legalistic and bureaucratic redundancy, then a thorough, streamlined, efficient, transparent, accountable, and enabling regulatory framework could be put in place that would safeguard non-target species while facilitating biological control and environmentally sound pest management at the national level.

INTRODUCTION

Classical biological control is a powerful tool for pest management that has been used successfully for over a hundred years in the United States to combat invasive arthropod and weed species. For the greater part of the past century, regulations governing the importation of exotic beneficial species were either non-existent or were cobbled together from a diverse array of tangential legislation that was designed for other purposes, often only marginally related to the most important issues of biocontrol. Within the past decade, however, a consensus has emerged, both among conservation biologists and applied (primarily agricultural) entomologists, that some form of regulation specific to the importation of biological control agents should be established. However, the devil is in the details, and the few attempts that the United States Department of Agriculture, Animal and Plant Health Inspection Service

(USDA-APHIS) has made to establish regulations have been (and continue to be) chaotic, poorly understood, and difficult to implement. The increased scrutiny of both hand-carried and shipped packages following the terrorist attacks in the U.S. in 2001, and the bureaucratic re-organization of APHIS (with segregation of a separate Department of Homeland Security) has further complicated and confused efforts to put a manageable regulatory framework in place.

This short paper will first give an overview of the regulatory process in Hawaii, the most stringent system for oversight of biological control in the United States. I'll then briefly compare the State system to the existing U.S. Federal system, and point out the strengths and the weaknesses in Hawaii's rules that can provide valuable guideposts to those charged with establishing a much-needed national policy.

THE REGULATORY PROCESS IN HAWAII

At its core, the system in Hawaii for regulating newly imported biological control agents is a logical and thorough process with some admirable features that were no doubt designed with the best intentions in mind. The applicant is required to file a dossier with the State Dept. of Agriculture Plant Quarantine Branch (PQ) containing information about the taxonomy, bionomics, ecology, and host range of the proposed species introduction, as well as a justification for its importation, person responsible for the insects, description of safeguard facilities, method of disposal, and relevant supporting literature.

PQ then submits the application to two different committees for review. The first advisory committee is composed of disciplinary specialists (for example, a proposed arthropod introduction would be reviewed by the Entomology Committee). This committee is comprised of individuals representing a wide spectrum of opinion and expertise within the state, from agricultural pest management to insect conservation, from University professors to State agricultural entomologists to Museum specialists.

The Entomology Committee's comments are then forwarded for secondary review to another advisory committee with a broader range of expertise. For example, a botanist, a fish and wildlife specialist, a zookeeper, and a public health specialist sit on this Plants and Animals Advisory Committee. This second level of review considers the comments of the entomological experts, as well as the broader ecological and economic context of all new species introductions. Their decision, which is non-binding but highly influential, is passed on to the State Board of Agriculture, which reaches a final decision, that still must, however, by signed by the Governor. In addition, a concurrent Federal permit must be in place before any organism may be removed from quarantine.

The State process has two distinct components: the first requires placing a proposed species introduction on a specific list; the second requires establishing all of the conditions under which an organism on that list can actually be imported and released. As part of the listing process, public hearings are held throughout the state, during which concerned citizens can provide their input regarding the proposed introduction. These public comments are part of the final dossier used by the Board of Agriculture to make its decision.

In theory, the overall system provides for a fair and thorough review with input from all concerned parties, but in practice its implementation becomes bogged down in a bureaucracy that even the administrators of the process have difficulty in understanding and controlling (for details, see Messing and Purcell 2001). The listing process is subject to repeated and long-delayed reviews by the state Attorney General's office to ensure compliance with legal technicalities. The fact that two different steps are required (first the listing, and then the establishment of conditions for release) means that the same dossier is sent to the various committees and attorneys and Board twice (in succession), rather than considering both steps simultaneously. There is no established time schedule for any of the steps, and no accountability for any person or committee that fails to complete a step in a reasonable time frame. Committees and the Board sometimes do not meet for lack of a quorum. Because of the cost of holding public hearings on different islands, applications are held until a sufficient batch accumulates to justify the expense of organizing and holding the meetings. If there is a problem with a single application in a batch, the entire listing process is delayed – including those applications that are problem-free. There is a lack of communication between State and Federal offices, each of which requires the consent of the other. There is no process for online tracking, nor of reporting the status of a submission to the applicant. It is not unusual for an application to take *years*, rather than months, to make it through the listing process, even in cases where no additional biological data are requested. The process has become so onerous that it is significantly hindering the practice of biological control in the state (Messing 2000).

THE UNITED STATES FEDERAL (APHIS) REGULATORY PROCESS

The United States Department of Agriculture Animal and Plant Health Inspection Service has the statutory authority to regulate plant pests entering the U.S. While the agency traditionally has also issued permits for the introduction of entomophagous biological control agents, there are legal questions of whether, for example, a host-specific insect parasitoid can be considered a plant pest. Despite the fact that the Plant Protection Act of 2000 [PUBLIC LAW 106–224; section 412.a] specifies biological control organisms as subject to regulation, APHIS has been reluctant to take on this responsibility overtly, yet at the same time unwilling to relinquish all control given the lack of any other regulatory authority.

Some applicants for biocontrol permits are obliged to write and submit an Environmental Assessment (EA), a legal document that describes the expected impact of a non-indigenous organism on the environment. This document addresses both positive and negative environmental impacts; those deemed to have a higher risk are then required to prepare a more detailed environmental impact statement (EIS); those of lower risk are issued a finding of no significant impact (FONSI). The EA is a requirement of the National Environmental Policy Act (NEPA), but it is *only* required for employees of Federal agencies (or for projects conducted with Federal funds), not for State projects. NEPA also requires consultation with the U.S. Fish and Wildlife Service and other Federal agencies, but again, only for those projects with Federal backing.

APHIS has a fairly well established system for regulating the introduction of weed biocontrol agents (overseen by a Technical Advisory Group, TAG) – since herbivorous

arthropods obviously have the potential to become plant pests. Imported plant pathogens, on the other hand, are considered similar to pesticides and are regulated by the U.S. Environmental Protection Agency (EPA). For entomophagous arthropods, however, the situation becomes murky: APHIS requires a permit to import species into U.S. quarantine facilities, but then generally leaves it to State Departments of agriculture to make final determinations on field release of organisms from quarantine. For several years the agency has issued "letters of no-jurisdiction" for release – in essence turning responsibility over to the states.

In practice, however, and particularly recently, APHIS has instituted new rules, without significant public comment, regulating both the importation of organisms into quarantine and their removal from quarantine. For example, no hand-carrying of biological control agents by foreign explorers is allowed, only licensed, bonded carriers are to carry packages across national borders; shipments are to be routed through APHIS facilities in Beltsville, Maryland prior to their final destination in State quarantine facilities. Removal from quarantine requires consultation and thorough review of dossiers by Canada and Mexico under the auspices of NAPPO, the North American Plant Protection Organization. NAPPO has its own template of data requirements, including detailed plans for post-release monitoring and evaluation.

GUIDELINES FOR A NEW SYSTEM

It is admittedly difficult (though no less necessary) to institute a comprehensive Federal system for regulating imported biological control agents. Efforts are complicated by legitimate concerns for agro-terrorism; by the complex, diverse, and idiosyncratic nature of arthropod biologies; by geographic anomalies inherent in having some states contiguous with international borders while other states are geographically isolated. Furthermore, inter-agency bureaucratic squabbling, inadequate funding, and the short-sighted dissolution by APHIS of the National Biological Control Institute, leave federal, state, private, and university biocontrol practitioners with no adequate channel for communicating needs and concerns to the agency.

Other countries, particularly Australia (McFayden 1997) and New Zealand (Fowler *et al.* 2000), have overcome these obstacles and established effective regulatory policies for biological control. Hawaii, as we have seen, has regulations that are effective in safeguarding the environment (Funasaki *et al.*, 1988, Henneman and Memmot 2001), though not, to understate the case, particularly efficient. Rather than re-inventing the wheel, APHIS should incorporate the best of Hawaii's system while avoiding its bureaucratic pitfalls.

The strengths of the Hawaii system are that there are several layers of review with different perspectives (narrow disciplinary specialists and broader ecological and economic perspectives); that within each committee there is a deliberate choice of individuals with a wide range of opinion and expertise; and that there is a formal public notification and input process, whereby the concerns of all interested citizens are taken into account.

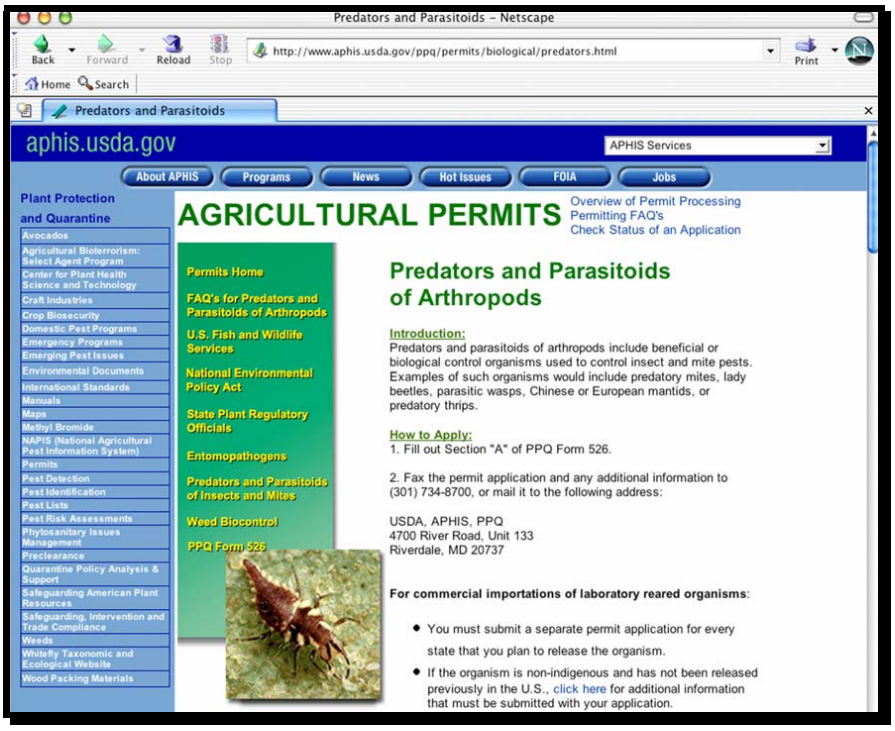
The fact that Departments of Agriculture oversee the biocontrol permitting process (both in Hawaii and at the Federal level) is an historical accident; targets for biological control and environmental impacts of greatest concern have traditionally been in agricultural settings. It may be argued, however, that this a case of the fox guarding the henhouse, since the Depart-

ments' mandate is by nature agro-centric. Now that biological control is becoming more commonly accepted as a tool for pest management in non-agricultural settings (Hoddle 2004), and as environmental impacts are increasingly viewed in non-economic terms, it is more logical to have an independent environmental agency supervise the process, as is done in Australia and New Zealand.

The weaknesses in Hawaii's permitting system, alluded to earlier, are partially the result of specific state laws specifying the need for continuous revising and updating of complete lists of imported non-indigenous species. The listing process is frought with legal technicalities that necessitate repeated review by attorneys who have little knowledge of biology; with no equivalent Federal listing requirement, much of this bureaucracy could be reduced. The use of strictly formatted templates for data entry and evaluation could also help eliminate recurring legal reviews.

APHIS' own TAG system, with a Technical Advisory Group evaluating proposed weed biocontrol agents, could be readily adopted for arthropod biocontrol agents, and made even stronger by adopting Hawaii's system of a two-tiered review process (i.e., specialists and "generalists"). To the extent that NAPPO consultation requires additional review by neighbor countries, the key to a fair and timely response to the applicant is to have as many of these reviews as possible conducted simultaneously.

Online forms and electronic input of all dossier information, comments, and concerns can make the entire system more efficient, responsive, and transparent. APHIS has recently started to gather input on the best way to take this step, but at present the only information available on their web site is a downloadable form (PPQ 526), a list of NAPPO dossier guidelines, and some answers to Frequently Asked Questions (see figure showing home page below).



Timeliness is of utmost concern when biocontrol practitioners are rearing living colonies of arthropod predators or parasitoids at great expense and possible irreplacable loss of genetic diversity. Timelines in the permit process should be strictly specified and firmly adhered to, so that a lack of response during a specified comment period does not stall an application, but rather is interpreted as "no objection". Input from the general public can be obtained by electronic notification of a broad suite of interested parties, and by appropriate public advertising and a transparent web site.

Chemical pesticides continue to become less available to land managers due to insect development of genetic resistance and loss of product registration due to public health concerns. However, invasive species continue to plague our farms, cities, and natural ecosystems at an increasing rate; thus biological control is becoming more important than ever as a valuable tool for safe and cost effective pest management. For the benefit of the nation's agriculture as well as its natural environments, APHIS (or another Federal agency) should adopt regulations that are thorough, streamlined, efficient, transparent, accountable, and that facilitate rather than hinder the practice of biological pest control.

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HARMONIZATION OF THE REGULATION OF INVERTEBRATE BIOLOGICAL CONTROL AGENTS IN EUROPE

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ABSTRACT

The regulation of import and release of invertebrate biological control agents is not harmonized yet in Europe. Each country has its own regulatory system in place that is legally based on either the nature protection and/or the Plant Protection Act. The publication of the FAO Code of Conduct in 1996 for import and release of exotic biological control agents was the turning-point for the activities related to the import and release of biological control agents in Europe. An EPPO expert panel developed from 1998 to 2002 two guidelines on the safe use of biological control and established a list of biological control agents widely used in the EPPO region. An EU funded project with the goal to develop scientific methods for evaluating environmental risks of biological control introductions into Europe (ERBIC) was conducted from 1998 to 2002. In 1999, the OECD initiated a working group with the aim to develop a guidance document on appropriate regulation of invertebrate biological control agents. Biological control industry was very concerned about these developments and proposed to the International Organization for Biological Control (IOBC/WPRS) to co-ordinate harmonization among European countries. A commission of the IOBC/WPRS was put in place in 2003 with the aim to facilitate and harmonize regulation in Europe. In 2004, the EU released a call for project proposals with the aim to develop a balanced system for harmonized registration of biological control agents.

INTRODUCTION

In the past, Europe has generally been a source rather than a recipient of invertebrate biological control agents in comparison to other countries with extensive experience in classical biological control, such as Australia, Canada, New Zealand, South Africa and the U.S.A. These countries had legislation and procedures in place relatively early to regulate imports and to analyze risks of exotic biological control agents (Sheppard *et al.* 2003). Most classical biological control programs in Europe have focused mainly on controlling exotic pests in the Mediterranean region. Today, there is a growing interest in classical biological control of invasive weeds throughout Europe, especially in conservation areas (Waage 1997). Increasing international trade in agricultural products and growing accidental introductions of organisms related to tourism and global trade are nowadays important sources of new imports of exotic pest species into Europe, as demonstrated by Bin and Bruni (1997) for Italy. Many of these introduced organisms are candidates for classical biological control if they establish in conservation reserves where they may threaten native species and communities. Most if not all European countries are signatories of the Convention on Biological Diversity and, thus, have the obligation to prevent the introduction and, as far as possible, to control those alien species that threaten indigenous ecosystems and habitats. Because chemical and mechanical control of such organisms may have negative effects on ecosystems greater than those of the introduced alien species itself, classical biological control may offer adequate solutions.

Protected crops grown in glasshouses have developed rapidly in many European and Mediterranean countries, and the protected environment has favored the temporary or permanent establishment of imported pests. On the other hand, customers in most European countries are increasingly concerned about pesticide residues in food, and food quality regulations are becoming more stringent in Europe where most of the glasshouse crops are marketed. This situation offers new avenues for non-chemical pest control. Biological control by augmentation or inundation has developed during the last 35 years and is now a major component of pest control in protected crops. About 90 species of invertebrate biological control agents are presently on the EPPO list of widely used and commercialized biological control agents in the EPPO region (EPPO 2002), and many more are under investigation for future release. Some agents on this list may have disappeared from the market, but new ones have come up in the meantime. Europe leads the world in this activity, and national regulatory agencies have therefore an obligation to rule and facilitate international trade in an efficient and appropriate way.

Regulations for introductions of invertebrate biological control agents differ between European countries and some have yet to establish these (Bigler 1997; 2001). Obligations in international laws and agreements, and an increasing interest in the import and release of exotic biological control agents calls for harmonized and better regulation between European countries. In many cases introductions of invertebrate biological agents are administered under regulations which were established for other purposes, such as plant quarantine, wildlife conservation and genetically modified plants. The application of appropriate regulatory procedures is important in order to maintain public confidence in biological control and to facilitate introductions and the commercial use of exotic biological control organisms.

ACTIVITIES AND DOCUMENTS TO FACILITATE HARMONIZED REGULATION IN EUROPE SINCE 1995

THE FAO CODE OF CONDUCT

The FAO Code of Conduct for the Import and release of Exotic Biological Control Agents was adopted in 1995 by the FAO Conference and published in 1996 as the International Standard for Phytosanitary Measures No. 3 (IPPC 1996). One objective of the Code was to provide a standard for those countries that are lacking adequate legislation and procedures to regulate import and to analyze risks related to biological control agents. The document lists in a generic way the responsibilities of the authorities and importers and exporters of biological control agents. Furthermore, it recommends that governments already fulfilling the objectives of the Code may adapt their existing regulatory systems in the light of this guidance. This objective of the Code may be interpreted as being the first attempt to harmonize regulation of biological control agents worldwide. The revised version of this Code of Conduct has extended its range from classical biological control to inundative biological control, native natural enemies, microorganisms and other beneficial organisms, and also includes evaluation of environmental impacts (IPPC 2005). The publication of the FAO Code can be considered as the turning-point for a number of activities related to import and release of invertebrate biological control agents in Europe.

EPPO GUIDELINES AND 'POSITIVE LIST'

Shortly after the Code's publication, the European and Mediterranean Plant Protection Organization (EPPO) together with CABI Bioscience organized a workshop on safety and efficacy of biological control in Europe (EPPO 1997). In its recommendations, the scientific committee of the workshop noted that "…practices for the import of macrobiological agents at present vary greatly between European countries. These practices should be harmonized, with appropriate conditions recommended for importation for different purposes, e.g. research, classical, commercial biocontrol." The workshop suggested that an EPPO Panel should be established, and promote the adoption of harmonized practice for the import of invertebrate biological control agents. The workshop broadly endorsed the FAO Code and recommended that guidelines be drawn up to meet European needs with respect to the different legislations and regulations. It was stressed that the guidance on harmonized regulation should not slow the process of import of biological control agents, be it for first introduction for research or for release later on. The workshop concluded that a certification system should be put in place for Europe instead of a registration procedure, to ensure a 'light' regulatory system with efficient and rapid mechanisms.

The registration system for microbial biological control agents in place in the EU under Directive 91/414/EEC was given as a negative example of regulating biological control agents. This Directive accommodates the registration of microbiological control agents since 1992, and experience over the years has shown that the Directive and its implementation is so stringent that it is basically impossible to register a new microorganism in the EU countries. The workshop decided to establish an expert panel with the aim of drawing up more specific guidelines and to prepare a 'positive list' of invertebrate biological control agents that are

widely used in the EPPO region without any reports on adverse effects. The EPPO panel met a number of times between 1998 and 2002 and the results were published in two guidance documents and in a 'positive list' of organisms for safe use in EPPO countries. The first guideline recommends a system for the first import of exotic biological control agents for research under contained conditions (EPPO 1999). The second document gives guidelines for the import and release of exotic biological control agents, including information on how to prepare a dossier by the applicant for the national authority and on how the authority should examine the dossier (EPPO 2001). The two guidelines stress the importance of a two-step system for import and release, i.e., EU countries should first establish a regulatory process for import of exotic organisms for research under containment. The results of these investigations will provide the necessary data to make decisions on whether the organism can later be imported for release. The approval for release will be granted if further studies show that the organism is safe for the environment and humans. To facilitate and speed-up the use of invertebrate biological control agents in the EPPO region, a list of commercially available organisms was first published (EPPO 2002) with the idea to regularly adapt the list depending on new information.

THE ERBIC RESEARCH PROJECT

In parallel with the EPPO panel activities, the EU-funded research project ERBIC (Evaluating Environmental Risks of Biological Control Introductions into Europe) was executed from 1998 to 2002. Among the objectives, major aims were: 1) to ensure that the introduction and use of biological control agents is done in a way which does not put at risk non-target organisms, 2) to develop rapid and reliable methods to assess the potential risk of import and release of biological control agents in Europe, and 3) to design specific European guidelines to ensure that biological control agents are environmentally safe. One of the main outcomes of the project was the proposal for the environmental risk assessment of exotic natural enemies in inundative biological control (van Lenteren *et al.* 2003). This paper presents for the first time detailed criteria for risk assessment and a ranking system that is based on the quantitative evaluation of more than 30 invertebrate biological control agents used in inundative control in Europe.

THE OECD GUIDANCE DOCUMENT

An initiative starting from a meeting held in Canada in 1999 resulted in an activity of OECD (Organization for Economic Co-operation and Development) countries with the aim to develop a harmonized approach for regulation of invertebrate biological control agents. It was agreed that a harmonized regulatory system in the OECD member countries would be beneficial for biological control and that a 'light' form of regulation would be appropriate. The development of harmonized guidance for regulation requirements would enable companies to submit the same applications to many countries, and would allow regulatory agencies to benefit from each other's reviews. The document (OECD 2004) proposes guidance to member countries on information requirements for a) the characterization and identification of the organism, b) the assessment of safety and effects on human health, c) the assessment of environmental risks and d) the assessment of efficacy of the organism. It is however, the decision of member countries whether and how these organisms are regulated, and countries

may require additional information to meet national or international requirements. With native or established organisms and with those long in use in a country, substantially reduced information requirements may be appropriate.

A BOOK ON METHODS OF RISK ASSESSMENT FOR BIOCONTROL AGENTS

Attempts to implement the EPPO, ERBIC and OECD documents into national regulatory systems in a number of European countries have shown that the required information and data are in many cases not available and have to be produced prior to submission of a dossier to the national authority. It became also evident that the framework of environmental risk assessment that should be used for the preparation of the dossiers by the applicants and the evaluation by national authorities was not yet established in Europe. The lack of methodology for risk assessment of invertebrate biological control agents was recognized by the European biological control community, and consequently, 25 experts from across the world gathered for a workshop in Switzerland in 2004 to put together a synthesis of current knowledge, and to provide recommendations for further regulatory guidance in this area. The emphasis was on providing science-based guidance for those assessing and evaluating environmental risks, and on providing up-to-date information on existing methods and their application for evaluating non-target effects. The starting point was to address all the information requirements for environmental risk assessment laid out in the recent OECD publication (OECD 2004). A further aim was to compile all this information for a book, which is to be published by CABI Publishing (Bigler *et al.* 2006).

THE IOBC/WPRS COMMISSION FOR HARMONIZATION OF REGULATION

The European biological control industry was very concerned when the OECD guidance document was published as the information requirements were considered to be too stringent, and manufacturers feared that each national authority in Europe would establish their own regulatory system. As a consequence, the International Biocontrol Manufacturer Association (IBMA) proposed to the International Organization for Biological Control (IOBC/WPRS) to co-ordinate harmonization among the European regulatory authorities. A Commission of the IOBC/WPRS was put in place in 2003 with the objectives to 1) collect information on regulation in European countries and compile an overview, 2) organize a workshop with countries that have participated in the data compilation together with the biocontrol industry and regulators, 3) produce a document that gives detailed guidance on regulation procedures for exotic and indigenous biocontrol agents, 4) up-date EPPO's list of safe organisms, 5) propose a consultation procedure that will allow exchange and use of information and data on biological control agents between European countries and 6) propose a European expert group for invertebrate biological control agents. The first meeting of the Commission was held in 2004 with the participation of scientists, regulators and industry representatives from 15 European countries and resulted in fulfillment of objectives 1 to 3. The document on information requirements for import and release of invertebrate biological control agents in European countries (Bigler *et al.* 2005) gives more specific advice to applicants and national authorities on information required for risk assessment compared to the EPPO and OECD documents cited above, and it reduces data requirements for facilitating regulation, but still respecting concerns of human and environmental safety. Proposals for objectives 4 to 6 will be elaborated in a future workshop.

THE EU CALL FOR A BALANCED SYSTEM OF REGULATION

In October 2004, the Directorate-General for Research of the European Commission released a call for project applications with the aim to develop a balanced system for regulation of biological control agents (micro- and macro-organisms), semiochemicals and botanicals. The call specifies that, despite considerable research efforts on biological control, the number of microbiological products on the market in Europe is currently still low, compared to other countries, e.g., the U.S.A. and Canada. The aim of the task is to review current legislation, guidelines and guidance documents and to compare this with similar legislation in other countries where the introduction of new biopesticides has proven to be more successful. New appropriate and balanced regulatory systems should be designed, provided that no compromises are made to the level of safety. This is the first time that the EU has become involved in regulatory affairs of invertebrate biological control agents with the intent to harmonize national systems of EU countries and hence, it can be expected that in few years from now, the EU members and other European countries may regulate invertebrate biological control agents under uniform principles.

REGULATION REQUIREMENTS IN EUROPEAN COUNTRIES IN 2004

For the preparation of the workshop organized by the IOBC/WPRS Commission on the harmonization of regulation of invertebrate biological control organisms held in 2004, and in fulfillment of objective 1 of the Commission (see above), we have sent out questionnaires to regulatory authorities and biological control scientists in 19 European countries. Replies were returned by all countries, although the quality of information provided differed greatly between countries. Nevertheless, the questionnaires yielded interesting information and data which are presently being compiled and prepared for publication. All countries addressed by the questionnaires have national legislations in place. However, large differences exist in the degree of implementation of regulatory measures of invertebrate biological control agents in these countries as demonstrated in Figure 1. The present status of regulation has been assigned to three categories: a) regulation is implemented to some degree in eight countries (Austria, Czech Republic, Denmark, Hungary, Norway, Sweden, Switzerland, U.K.), b) five countries are working on the design and implementation of a regulation system (Finland, Germany, Netherlands, Slovenia, Spain) and c) six countries have no regulation implemented yet and will not have a regulatory system in place in the foreseeable future (Belgium, France, Greece, Italy, Poland, Portugal).

The results of the survey further demonstrated that the requirements differ largely between European countries. Examples of differences are:

- the assignment of a competent national authority (plant health, pesticide registration or environmental authority),
- the information requirements for evaluation of a dossier and subsequent level of risk assessment,
- whether native species have to be regulated as well; when regulation is required, native species usually follow a “short track” risk assessment, whereas exotic species are assessed more thoroughly,

- the system of regulation: by authorization, by permit, etc.
- listing of biological control species based on commercial availability or on risk based evaluation.

The survey also showed that there is a need for harmonization on a European level. Initiatives with respect to information requirements for import and release have already been taken (Bigler *et al*. 2005).

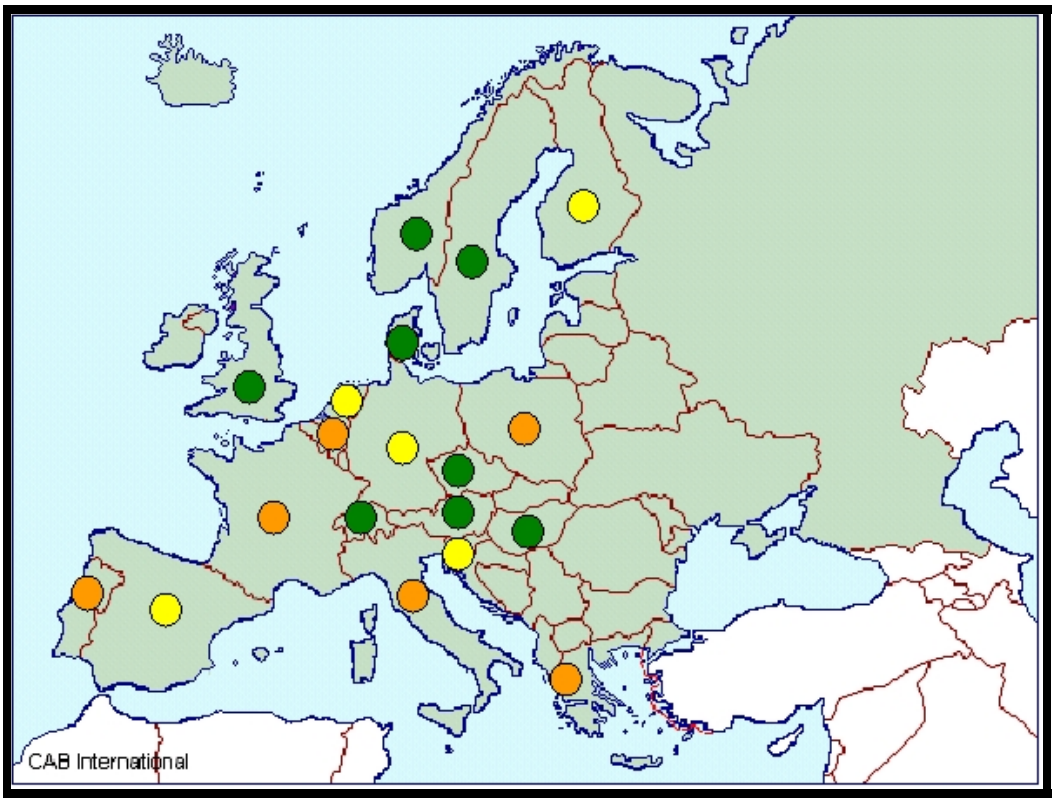

Figure 1. Present status of regulation of invertebrate biological control agents in 19 European countries. Countries where regulation has been implemented are indicated with green dots, countries where regulation is in preparation with yellow dots and those without regulation in place with orange dots.

CONCLUSIONS

Attempts to harmonize regulation of invertebrate biological control agents in Europe have been undertaken since the publication of the FAO Code of Conduct in 1996, and regulatory guidelines developed by international organizations, such as the EPPO and OECD during the last ten years, have been adopted and implemented by national authorities in a few European countries. Given that legislation for the regulation of invertebrate biological control agents differs among European countries and that laws are not yet in place in some countries, responsibilities are often not yet clearly assigned to ministries or government agencies on national levels. Different regulations among European countries may cause serious problems to the biocontrol industry as dossiers must respect national requirements and criteria in those

countries where regulation is in place. This makes applications more time consuming and costly, and can be a factor for a company to decide not to develop the organism to a product if the market potential is estimated low in comparison to the development costs. Past experience has shown that overregulation, i.e., rigid legislation with stringent data requirements may keep such products off the market for a long time or even prevent industry from submitting applications in some countries. This situation has been experienced in the EU since 1992 with the registration of microbial biocontrol agents that are regulated under the Directive 91/414/EEC which largely follows requirements developed for synthetic pesticides. Costly risk assessment studies and long term evaluation of dossiers has kept most products off the market and resulted for the few registered micro-organisms in an average evaluation period per product of over 70 months (Blum *et al.* 2003). Uncoordinated regulation of biological control organisms bear the risk that approval for release in one country may have impacts for others if the organism crosses borders and establishes in other countries. A recent example is the establishment of *Harmonia axyridis,* the Multicolored Asian Lady beetle, in European countries like Switzerland, where the application for release of this coccinellid was rejected in the nineties based on documented non-target effects. Releases in other European countries has resulted in the establishment of the lady beetle and in crossing borders and invading other countries. This and other examples demonstrate that Europe urgently needs a harmonized regulation of biological control agents which will prevent import and release of unsafe organisms, but which will not put an unnecessary burden on biological control.

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HOW CAN LEGISLATION FACILITATE THE USE OF BIOLOGICAL CONTROL OF ARTHROPODS IN NORTH AMERICA?

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ABSTRACT

The use of biological control agents is an integral component of biologically-based pest management strategies. Although there have been many success stories and biological control became synonymous with environmentally friendly pest management, during the last 20 years an increased awareness of biodiversity interactions resulted in concerns being raised about potential negative effects. The outcome has been pressure to improve regulatory oversight of biological control and make the process transparent. In North America, oversight of biological control agents has fallen primarily under federal law and provincial/state laws have occasionally influenced release of biological control agents. Federal laws used are associated with Plant Protection Acts because these regulate plant pests and biological control agents have been viewed as indirect plants pests. In Canada and Mexico this has worked well for regulating entomophagous biological control agents whereas, in the United States there were legal concerns that have now been addressed by including a definition of a "Biological Control Organism" in the U.S. Plant Protection Act. Plant protection laws are appropriate for regulating biological control agents because they are designed to address movement of living organisms associated with plants. Canada, Mexico and the United States are intricately linked both geographically and economically, and efforts have been made to harmonize the data requirements for submissions. The North American Plant Protection Organization (NAPPO) document, "Guidelines for Petition for Release of Exotic Entomophagous Agents for the

Biological Control of Pests" was implemented as a North American standard. The guidelines act as a framework within which there is flexibility for reporting information based on continually improving scientific methods. Judgement of a petition is carried out through an international scientific peer-review process that includes experts in the areas under each heading. Comments are collated and a recommendation is made to the responsible agency in the country where release is intended. To date the process has been effective and this approach continues to provide opportunities for improving oversight based on science and ensuring that only effective agents are used. The future challenge is implementing a process that includes a wider stakeholder community while maintaining objective and scientifically sound assessment of entomophagous biological control agents.

INTRODUCTION

Biological control is a cornerstone of pest management in many parts of the world. Use of entomophagous biological control agents has resulted in important successes in reducing damage from pest species in a variety of manipulated systems and biological control has great value in sustaining environmental health, particularly through reductions in pesticide use. These attributes indicate that use of entomophagous biological control agents will continue and even grow. However, debate is increasing on the need for greater regulatory oversight of biological control agents, including entomophagous species.

Factors that contribute to the need for greater regulation of biological control agents include trade globalization and awareness of the importance of biodiversity. Expanded global trade has resulted in an astounding increase in the numbers of non-native species establishing in new habitats. Estimates suggest that invasive alien species are responsible for annual losses of US$55-248 billion to worldwide agriculture (Bright 1999). More difficult to assess are environmental costs due to habitat loss or species extirpation or extinction caused by invasive alien species (Parker and Gill 2002). Biological control is an important strategy for combating invasive alien species and it has been viewed as being 'environmentally friendly' for more than 100 years. However, during the last decade as science and society have become increasingly aware of the importance of biodiversity to human well-being, a less positive view of biological control, particularly in island environments, has emerged especially with the introduction of generalist predators and non-specific herbivores (Howarth 1991; Simberloff 1992). This perspective is based on non-target/unintended impacts and has stimulated much debate (e.g., Follett and Duan, 2000; Louda *et al.* 2003; Schick *et al.* 1996; Wajnberg *et al*. 2001). Some have concluded that biological control regulation is archaic and Strong and Pemberton (2001) stated that in the United States "In the absence of reform, rational as well as irrational opposition to biological control will grow. Only sensible reform will maintain public support for this powerful tool." There is now a growing consensus that all deliberate introductions of non-indigenous species should be subject to impact risk assessment (Wittenberg and Cock 2001). Furthermore, regulations for biological control agents "... are needed to provide clear guidance as to what introduction can be made legally and to define procedures to resolve any conflicts of interest that may arise." (Van Driesche and Bellows 1996). As Mason and Kuhlmann (2002) concluded, it is clear that regulations for biological control agents are nec-

essary not only for the preservation of biodiversity but for the protection of biological control as a pest management strategy. Messing (2000) suggested that regulations would also help allay some of the concerns about introductions of exotic species that result in exaggerated estimation of the risks in doing so. The challenge is how legislation can facilitate rather than impede entomophagous biological control.

EXISTING REGULATIONS

Regulation of entomophagous biological control agents varies greatly around the world from jurisdictions where there is no regulation to those where specific laws have been enacted and are strictly enforced. Others (e.g., Barratt *et al*. 2003; Hoddle 2003) summarized the status of biological control regulations up to 2002. Since then, new developments have taken place and these will be outlined as they pertain to entomophagous biological control activities in North America. Of particular note are the combined efforts to harmonize the information requirements for submissions to regulatory agencies for approval to release biological control agents.

INTERNATIONAL

Globally, the International Plant Protection Convention (IPPC) provides guidance for "securing common and effective action to prevent the spread and introduction of pests of plants and plant products and to promote appropriate measures for their control" (FAO 1999). A 'Code of Conduct for the Import and Release of Exotic Biological Control Agents' (FAO 1996) and recently updated (Nowell 2005) serves as a framework for regional and national plant protection organizations to develop guidelines/regulations that are appropriate for their jurisdiction. Under this International Standard for Phytosanitary Measures (ISPM No. 3) regional plant protection organizations, such as the North American Plant Protection Organization (NAPPO), are charged with ensuring that appropriate measures are implemented and that proper documentation of movement of biological control agents is made.

Recently, an OECD initiative resulted in the document "Guidance for Information Requirements for Regulation of Invertebrates as Biological Control Agents (IBCAs)" (OECD 2004). This document purports to harmonize data requirements to enable the use of the same data in the approval process among member countries. The OECD document is intended primarily for commercial biological control agents. Such harmonized regulations, by lessening registration requirements amongst members, would minimize costs for developing new agents. While the detailed information requirements set out in the document are helpful, there is concern that in some areas the requirements may be impossible to meet. This is especially the case for risk assessment where the methodologies are largely experimental. It is clearly stated in both the FAO and OECD documents that individual jurisdictions (i.e., countries and their states) may require more detailed information than outlined in the Codes, to meet their own regulations.

North America, Canada, Mexico and the United States do not regulate entomophagous biological control agents under specific biological control acts. Rather, each country regulates these agents under one or more legislative acts, the primary one being a plant protection act.

CANADA

Biological control agents in Canada have been regulated through the Plant Protection Act (PPA) of 1990 (Department of Justice Canada 2005) which is administered by the Canadian Food Inspection Agency (CFIA). In accordance with this Act, an import permit is required for importations of all exotic arthropods into Canada. Conditions attached to the permit may include such restrictions as 'for experimental use in a containment facility only'. Permits are generally valid for a 3-year period and are renewable. The permitting process is based on the provision of information relating to the source, the organism and the end-use (destination). Entomophagous biological control agents are regulated under the PPA with respect to their potential to be indirectly injurious to plants, because plant pests are loosely defined under the Act (Parker and Gill 2002). Furthermore, commercial entomophagous agents are regulated in a similar manner to classical agents and those species with a history of importation without negative effects are generally admitted under permit.

For release of a classical biological control agent or a first release of a commercial biological control agent submission of a petition (based on the NAPPO standard) justifying the release is required. The petition is reviewed by experts and representatives of other agencies, including Environment Canada (EC) and the Pest Management Regulatory Agency (PMRA) and where feasible, provincial government representatives. The review is carried out through a Biological Control Review Committee (BCRC) and depending on the comments, a recommendation is made for or against release to the regulatory entomologists of the CFIA who review all the comments and make a recommendation to the Director of the Plant Health Division (Fig. 1). The process generally takes about 6 months from submission to notification that release is approved or not approved.

The process has worked very well because recommendations are based on the scientific merit of the petition submitted, and although reviews are done mostly on a volunteer basis, these have been completed in a timely manner. A weakness of the Canadian regulatory process is the lack of public participation. Such participation may be warranted and would make the process truly transparent, but the way to accomplish this is not clear.

MEXICO

In Mexico, the importations of biological control agents are regulated through the Plant Health Act of the Mexican States (SARH 1980). In these regulations the Sanidad Vegetal (Ministry of Agriculture) is mandated to authorize the introduction of exotic arthropod species or the mass production of arthropods in insectaries, for use in the biological control of pests, according to requirements set out in Articles 101 and 102. As part of the importation requirements, the organisms must be accompanied by a certificate of biological purity and a certificate of origin provided by the phytosanitary authorities of the exporting country. The permit is granted for one year, and as in Canada, it is renewable.

The importer must submit an application to the General Director of Plant Health of the Ministry of Agriculture. A copy of the application is sent to the Nacional de Referencia de Control Biológico (National Center of Biological Control Reference [NCBCR]), where it is reviewed taking into account phytosanitary and environmental risks. After the review the

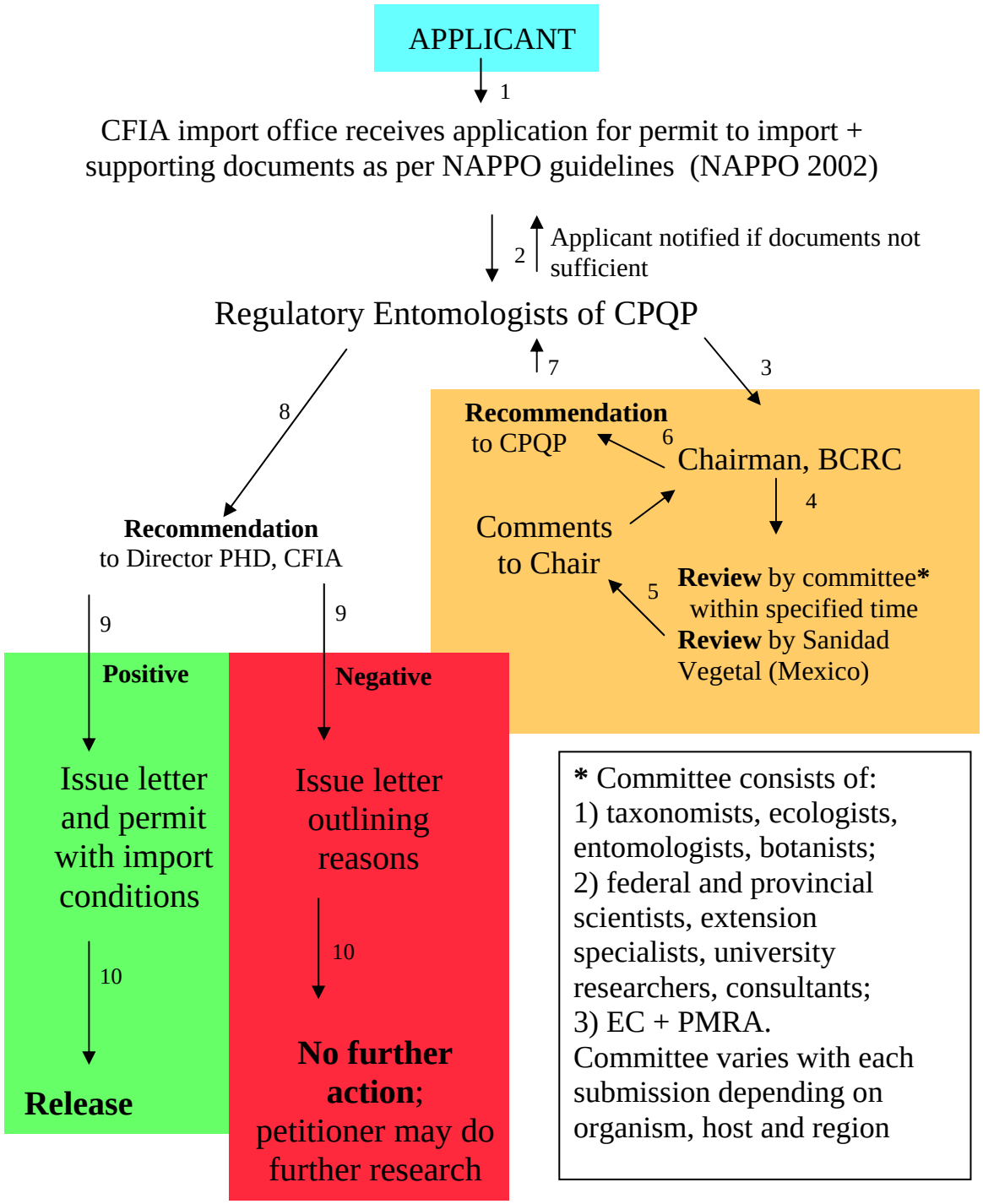

Figure 1. Canadian review process (9 steps) for import and release of new entomophagous biological control organisms. BCRC = Biological Control Review Committee; CFIA = Canadian Food Inspection Agency; CPQP = Centre for Plant Quarantine Pests (CFIA); EC = Environment Canada; NAPPO = North American Plant Protection Organization; PHD = Plant Health Division (CFIA); PMRA = Pest Management Regulatory Agency (adapted from and courtesy of CFIA).

NCBCR issues the authorization or denial through an official letter from the General Director of Plant Health to the applicant (Fig. 2).

In the case of exotic agents (for classic biological control), it is essential to justify the introduction. This includes providing information according to the NAPPO standard on the specificity, biology and behavior of the agent, natural enemies of the biological control agent, results from other countries on the biology and implementation of the agent. For commercial biological control agents information must be provided on the behavior, geographical distribution and any phytosanitary problems associated with the prey or hosts utilized for the rearing; if there are any doubts, an opinion is requested from the Consejo Nacional Consultivo Fitosanitario (National Consultative Phytosanitary Advisory Group) that consists of professionals from academic institutions, research and the government. The processing time is three months for applications for exotic biological control agents and 10 days for beneficial organisms, naturally present or previously introduced and established in Mexico that are mass reared in insectaries.

UNITED STATES

In the United States, biological control agents of plant pests and noxious weeds are regulated by Plant Protection and Quarantine (PPQ), Animal and Plant Health Inspection Service (APHIS) of the USDA (USDA) under the Plant Protection Act of 2000 (APHIS 2005a). This recently enacted legislation provides APHIS the authority to regulate organisms that may directly or indirectly harm plants or plant products. Unlike the previous Federal Plant Pest Act of 1957, the Plant Protection Act also broadly defines biological control agents and recognizes their potential to control plant pests. APHIS is authorized to regulate the importation, interstate movement and environmental release of biological control agents, but may deregulate the interstate movement and environmental release of those agents that APHIS has determined not to be plant pests. APHIS is now in the process of revising its regulations to fully implement this new Act and the following discussion only describes the current regulatory processes for the movement and release of entomophagous biological control agents that were developed under the older Federal Plant Pest Act.

For classical biological control research endeavors involving entomophagous agents, PPQ requires separate permits for importation to containment facilities, domestic movement to other containment facilities, and release to the environment (APHIS 2005b). In general, all movements of entomophagous agents originating from outside the United States are assumed to actually or potentially pose some risk to plants (e.g., pest host contaminants, hyperparasites, unevaluated impacts on plant communities, etc.) or to nontarget species, including endangered or threatened species. Permits for all movements are consequently restricted to Federally inspected containment facilities to prevent the irretrievable release of the organisms to the environment. The permits for containment facilities are issued to facilitate the removal of contaminants from foreign sources, to confirm the identity and purity of the agents, and to develop documentation that can be used to support future applications for release to the environment (i.e., release from containment). We do not anticipate changes to this approach when new regulations are proposed under the Plant Protection Act.

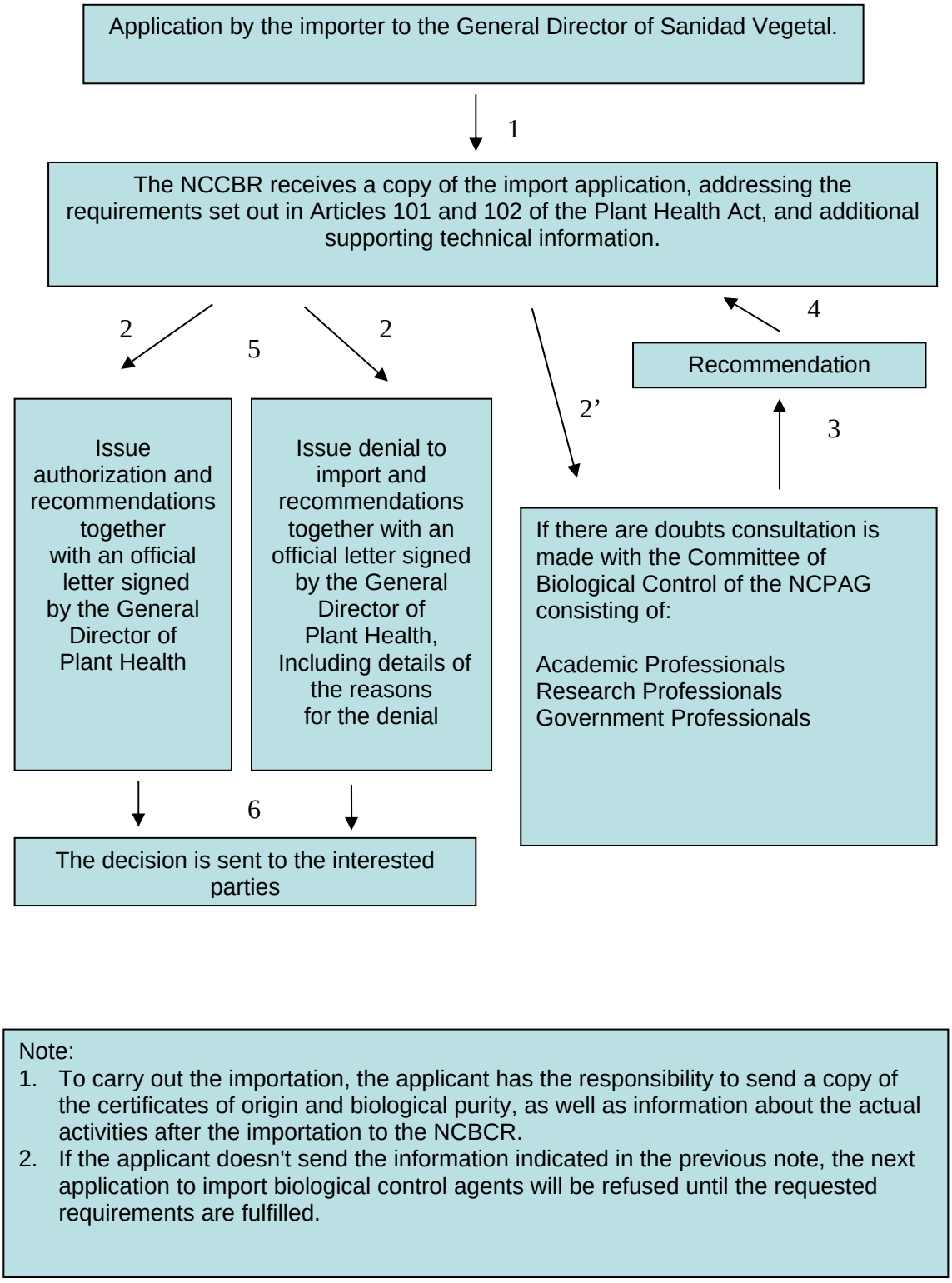

Figure 2. Steps for import and release biological control organisms in Mexico. NCBCR = National Center of Biological Control Reference. NCCPG= National Consultative Phytosanitary Advisory Group.

Following processing of agents and conducting basic biological studies (including host specificity evaluations) in containment, researchers may submit an application to PPQ for environmental release. A supporting document must accompany the application with information equivalent to the NAPPO petition discussed in the Canadian process. PPQ will review the supporting documentation and may request additional reviews with input by Canadian and Mexican counterparts to make a decision on whether or not the agent can be safely released to the environment. Decisions are made based on anticipated indirect or direct plant pest risks including potential impacts on nontarget species, especially endangered and threatened species. Any potential impacts on endangered and threatened species would trigger the Endangered Species Act of 1973 and would require consultation with the United States Fish and Wildlife Service in the Department of Interior. If PPQ determines that the release of the agent will not likely result in adverse impacts to plants and/or nontarget species, a determination of no further regulatory jurisdiction is documented on the permit application and sent back to the applicant. Otherwise the application is denied. When a determination of no jurisdiction is made, the agent may be moved and released throughout the contiguous United States without PPQ permits. Federal permits are still required for movements to and releases in Hawaii, Alaska, Guam, Puerto Rico, American Samoa, and the U.S. Virgin Islands. When PPQ makes a determination of no jurisdiction, individual States may require their own permits under State laws and regulations. This current regulatory process for environmental release of entomophagous agents does not trigger the National Environmental Policy Act of 1972 (NEPA), and no formal environmental assessments are produced to document these determinations of no jurisdiction (technically no Federal permit is issued). However, all subsequent Federal actions, including releases by Federal employees, on Federal lands, or under Federal funding may require compliance with NEPA. We anticipate that PPQ will begin issuing permits for release of entomophagous agents with new regulations under the Plant Protection Act. Such a change will require PPQ to develop formal environmental assessments to document for the public record the information used to make the Federal decision. However, the information currently provided as part of the NAPPO decision is largely what is required to develop a more formal environmental assessment. In addition, we anticipate that PPQ will begin requiring permits for the domestic movement of all entomophagous biological control agents except those formally deregulated by an official listing in the Federal Register. Listing will require an environmental assessment as well as a continuing safety record following establishment in broad areas of the United States.

PPQ permits are required for the importation of entomophagous biological control agents commercially produced outside the United States, including in Canada and Mexico. Commercial import permits restrict the species allowed entry to those agents that are indigenous to and widely distributed in the contiguous United States. All such imports are received and inspected at PPQ inspection stations where identity and purity are evaluated. The inspection process confirms the absence of plant pest risk and Federal permits are not required for subsequent movements within the contiguous United States. As with research releases, State permits may be required for releases in individual states. Equivalently, commercial movements and releases of domestically produced entomophagous biological control agents within the contiguous United States do not require PPQ permits as long as the shipments contain only approved indigenous species and are clear of plant pest host materials and other con-

taminants (e.g., hyperparasites). As with research releases, we anticipate that PPQ will begin requiring permits for the domestic movement of all entomophagous biological control agents except those that are formally listed as deregulated.

It is apparent that in the U.S., several levels of regulations apply to entomophagous biological control agents. As Messing (2000; 2005) has stated, establishment of clear, coherent, and streamlined regulations at the national level will be important to ensuring objective assessment of the risks and benefits of biological control in the U.S.

HARMONIZATION

In North America, there has been important progress in harmonizing the data required for release of entomophagous biological control agents. Petitions submitted to the regulatory agencies (CFIA, APHIS and Sanidad Vegetal) must conform to the standards set out in the NAPPO guidelines (NAPPO 2002). These guidelines were developed by representatives of Canada, Mexico and the United States and are a first attempt to harmonize the data requirements for the three countries. In the case of entomophagous biological control agents the NAPPO guidelines are dynamic and can be changed with the advent of new knowledge.

ARE THESE REGULATIONS AND THEIR OVERSIGHT APPROPRIATE FOR BIOLOGICAL CONTROL AGENTS?

The key to ensuring that arthropod biological control agents are appropriately assessed will be the expertise of the agency (or agencies) in each country that oversees regulation. Depending on the agency mandated with this responsibility, requirements and risk assessments could be based on models used for pesticides (as is the case for microbial agents) or even human pathogens. For entomophagous biological control agents, the most appropriate regulatory models are those already in place for regulating classical biological control agents of weeds. In North America these models are based on ecological theory and assessments are done mainly by scientific experts reporting to regulatory agencies. In addition, they are linked to IPPC standards and thus are in step with regulation of biological control agents in other jurisdictions.

In the context of plant protection, biological control agents are either direct (phytophagous) or indirect (entomophagous) plant pests depending on trophic relationships and the pest status of the associated plant (Fig. 3). Herbivores that feed on weeds are considered to be beneficial plant pests as are natural enemies of herbivores that feed on native endangered and/or important plant species. Similar patterns are apparent for pollinators and decomposers. The biological relationships at each trophic level remain the same regardless of whether the plant is a weed, native species or crop. Because of these complex relationships regulation of entomophagous biological control agents would thus be most appropriate under plant protection acts.

The entomophagous biological control agents that have come under the regulations outlined above are beginning to be carefully scrutinized. For example, based on petitions reviewed in Canada, 64% (7/11) of the biological control agents recommended by the BCRC and CFIA regulatory entomologists have been approved for release since 2000. Those submissions that were not approved were for agents for which host specificity could not be dem-

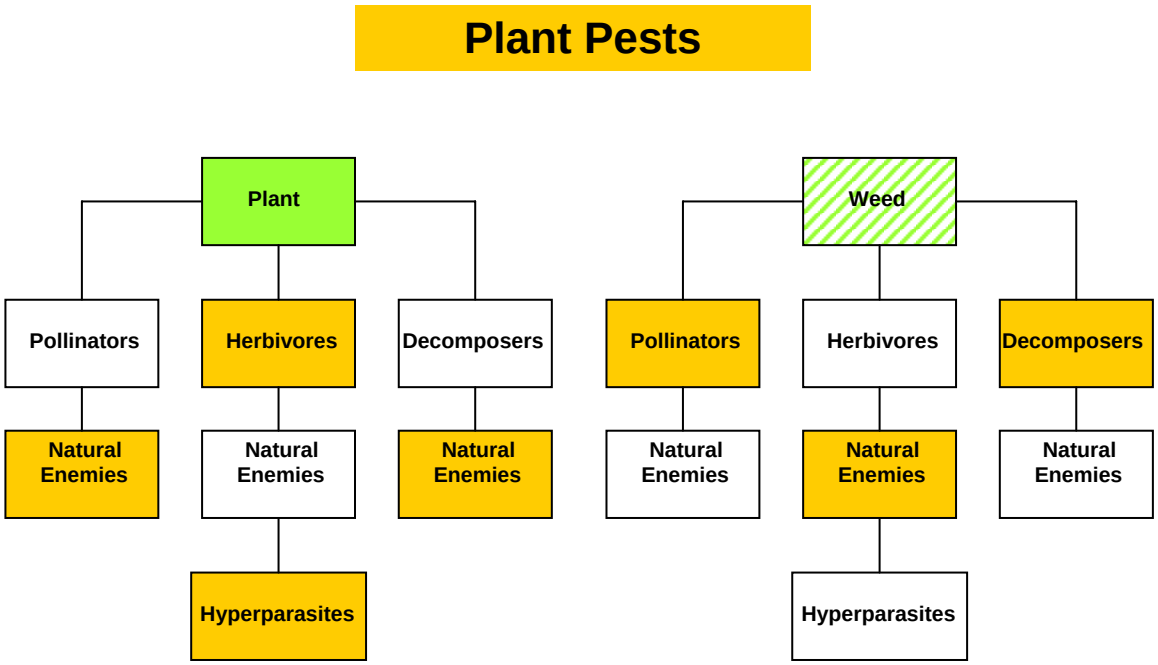

Figure 3. Pest status of trophic groups associated with 'valued' and 'non-valued' (=weed) plants. Shaded boxes indicate 'pest' groups.

onstrated or for targets for which a native North American species might be more suitable for biological control. The arguments in support of release have been based on scientific studies and have been peer reviewed. Turnaround from the time of petition submission until approval or rejection of the agent for release is six months.

There are several limitations to the current system in North America. There is a perceived lack of transparency of the approval process. Public input is not yet incorporated into the review of petitions, nor is it a required part of the justification for initiating biological control projects. Reviews should incorporate comments from all interested parties in all three countries.

Another shortcoming of the current process is the availability of appropriate methodology for assessing impacts of entomophagous biological control agents. Risk assessment is usually interpreted as meaning the greater the specificity of a biological control agent, the less the risk for non-target impacts. However, for arthropod biological control agents, host specificity testing has lagged behind that for weed biological control agents because historically the concerns for non-target impacts on invertebrates has not been as great (Waage 2001). Furthermore, the shear complexity of raising arthropods for testing has created a research bottleneck. Historical published data and collections continue to be an important source of host range determinations. Protocols used for assessing host range of weed biological control agents are well-defined but these are not necessarily appropriate for entomophagous biological control agents (Barratt *et al.* 1999; Kuhlmann *et al.* 2000; Mason *et al.* 1999; Sands 1998). However, biological control researchers are actively developing appropriate protocols (Bigler *et al.* 2006; Van Dreische and Reardon 2004). The NAPPO guidelines used in North America are flexible in terms of the detail of host range data that are required for a petition for release

of an entomophagous biological control agent. This flexibility was intended to facilitate continued release of safe agents while screening methods and interpretation of results are being developed. As this knowledge becomes more sophisticated, the guidelines can be updated.

COMPLIANCE

A major challenge for regulation of entomophagous biological control agents is to ensure compliance on the part of biological control practitioners. The present process relies on an honour system where submissions are made voluntarily by ethical individuals/agencies. Like inspection of international shipments and detection of inappropriate commodities, ensuring that all entomophagous biological control agents released are approved may be impossible. The best strategy to promote compliance will be timely review of submissions and fair assessments.

CONCLUSIONS

Increased regulation of entomophagous biological control agents in North America is inevitable. While no comprehensive legislation such as a 'Biocontrol Act' exists in Canada, Mexico or the United States, exotic invertebrates imported for release as biological control agents are being regulated under existing plant protection and associated acts. As demonstrated by the regulatory processes in Canada and Mexico, review of submissions for release of entomophagous biological control agents is timely and scientifically based. This encourages compliance by practitioners and safety of the agents based on best available knowledge. While the future of using entomophagous biological control agents will be that of greater scrutiny, appropriate legislation and regulation will ensure continuing effectiveness and increased safety.

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REGULATION OF THE RELEASE OF BIOLOGICAL CONTROL AGENTS OF ARTHOPODS IN NEW ZEALAND AND AUSTRALIA

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ABSTRACT

Regulation of biological control agents in New Zealand is legislated by the Hazardous Substances and New Organisms (HSNO) Act 1996 and administered by the Environmental Risk Management Authority (ERMA New Zealand). In Australia the Department of the Environment and Heritage and the Agriculture Fisheries and Forestry Australia - Australian Quarantine Inspection Service jointly regulate the import, testing and release of biological control agents under the Quarantine Act 1908, Wildlife Protection (Regulation of Exports and Imports) Act 1982 and Biological Control Act 1984. A comparison of the two regulatory systems highlights the pivotal role of information from the host-specificity testing in the decision making process and the valuable opportunity for researchers to interact with the public.

INTRODUCTION

Historically, releases of exotic biological control agents and associated regulations were within the framework of quarantine and plant protection legislation managed through agricultural authorities. However, an increasing public understanding and concern for the environment towards the end of the 20th century brought environmental issues associated with such releases to the fore along with an increasing involvement of environmental authorities. Parallel to this, environmental legislation being implemented around the world following the Convention on Biological Diversity (CBD) Decision VI/23 in 1992 on "alien species that threaten ecosystems, habitats or species", designed to protect against such invasions, adopts the 'precautionary approach' within it. This has in turn has led to increasingly precautionary attitudes towards classical biological control releases.

The legislative risk assessment process for biological control agents prior to permissions being granted for release has therefore increased in scope and also complexity in most countries as the regulatory responsibilities for releasing exotic organisms now equally concern both agriculture (the traditional arena) and the natural environment. Similarly proposed re-

leases of genetically modified organisms (GMOs) have also instigated general concerns about releasing novel genotypes into the environment along with increased awareness of critical issues in ecological risk analysis of such introductions recognized internationally through the Cartagena Protocol on Biosafety. Finally international plant protection legislation has also adopted policy in relation to biological control releases. The International Plant Protection Convention (IPPC) Code of Conduct for the Import and Release of Exotic Biological Control Agents and its recent updates are an illustration of this. It is within this context that we review the current regulations for biological control agent releases in New Zealand and Australia comparing attitude to risks as well as procedural differences.

REGULATION OF BIOLOGICAL CONTROL AGENTS IN NEW ZEALAND

The introduction of biological control agents (BCA) into New Zealand is regulated under the HSNO Act by ERMA New Zealand. Practitioners of biological control may apply for 'containment approval' to import a BCA for host-specificity testing followed by a 'full release approval' when they wish to release the agent. Applications are assessed in accordance with the purpose of the Act which "is to protect the environment, and the health and safety of people and communities, by preventing or managing the adverse effects of …new organisms". This is done by taking into account the following matters identified in the Act:

i. Sustainability of native and valued introduced flora and fauna
ii. The intrinsic value of ecosystems
iii. Public health
iv. The culture and traditions of Mâori (indigenous people)
v. Market economy
vi. International obligations

ERMA New Zealand is an 'autonomous' crown entity, partially funded by government that reports to the Minister for the Environment and is overseen by the Ministry for the Environment. Under the Crown entities legislation, ERMA New Zealand must have regard to government policy when directed by the Minister for the Environment but importantly, statute provides that the Minister may not give a direction that relates to the exercise of its core decision making powers to consider or grant approvals. ERMA New Zealand is composed of three parts; the Agency, the Authority and the Mâori Advisory Committee. The Agency works directly with applicants to facilitate submission of, and process applications but the decision making power resides with the Authority. The Authority is a quasi-judicial body[1] of 6-8 people appointed by the Minister for the Environment who are selected to represent a 'balanced mix of knowledge and experience in matters likely to come before the Authority'[2] so may or may not have a scientific background. In making their decision the Authority undertakes a risk, cost, benefit (RCB) analysis using a consistent methodology prescribed by regulation in 1998[3].

[1] Under the HSNO Act they have the same immunities and privileges of High Court judges when undertaking their core decision making powers and the power to operate under 'court-like' procedure ie to permit cross-examinations or questions of clarification.
[2] Section 16 of the HSNO Act.
[3] The Hazardous Substances and New Organisms (Methodology) Order 1998.

In the case of a full release application this RCB is done on information provided by the applicant, submissions (these may be received from members of the public, government departments, industry and community groups), the Agency and, where relevant, external experts and the Mâori Advisory Committee. Figure 1 summarises the application process for a full release application for which the applicant is charged NZ$30,000. It should be noted that in addition to obtaining an ERMA New Zealand approval applicants must also obtain an Import Permit under the Biosecurity Act 1993 from the Ministry of Agriculture and Forestry (MAF). MAF is responsible for New Zealand's Import Health Standards (IHS) designed to prevent accidental or illegal introductions of viable organisms (in this case associated organisms such as pathogens).

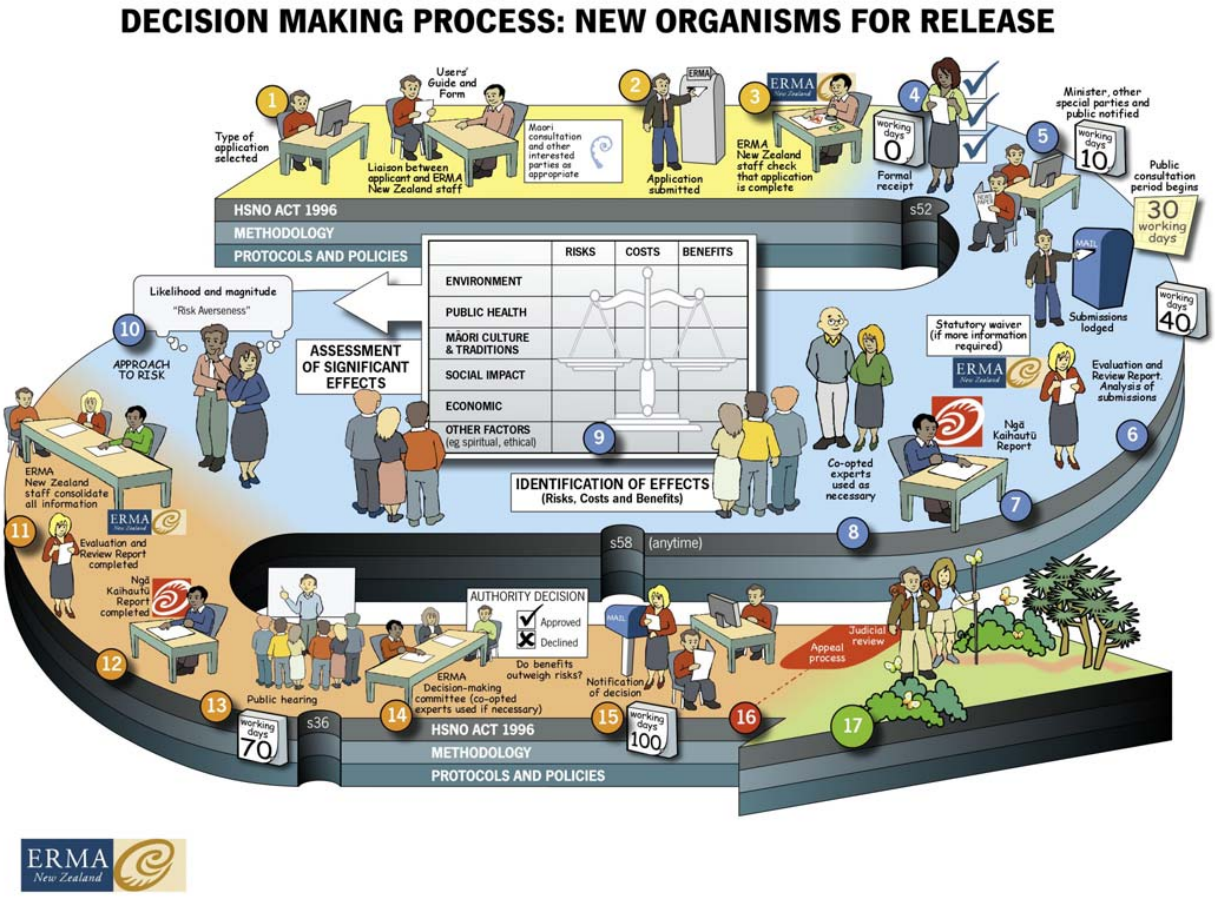


Figure 1. A diagrammatic representation of the application process for the full release of a biological control agent in New Zealand.

REGULATION OF BIOLOGICAL CONTROL AGENTS IN AUSTRALIA

Introduction of BCAs is regulated by two departments the Department of Agriculture, Fisheries and Forestry – Biosecurity Australia (DAFF-BA) and the Department of the Environment and Heritage (DEH) under three pieces of legislation:

i. the Quarantine Act (1908)
ii. Biological Control Act (1984)
iii. Environment Protection and Biodiversity Conservation Act (1992)

DAFF-BA is responsible for managing risks to primary industries and agriculture whilst the DEH is responsible for managing risks to the environment. Approvals are issued and implemented by the Department of Agriculture, Fisheries and Forestry – Australian Quarantine Inspection Service (AQIS).

The Australian process for arthropod targets is similar to that for weed targets and is all encompassing with four major steps to the process as summarised in Figure 2 and listed below:

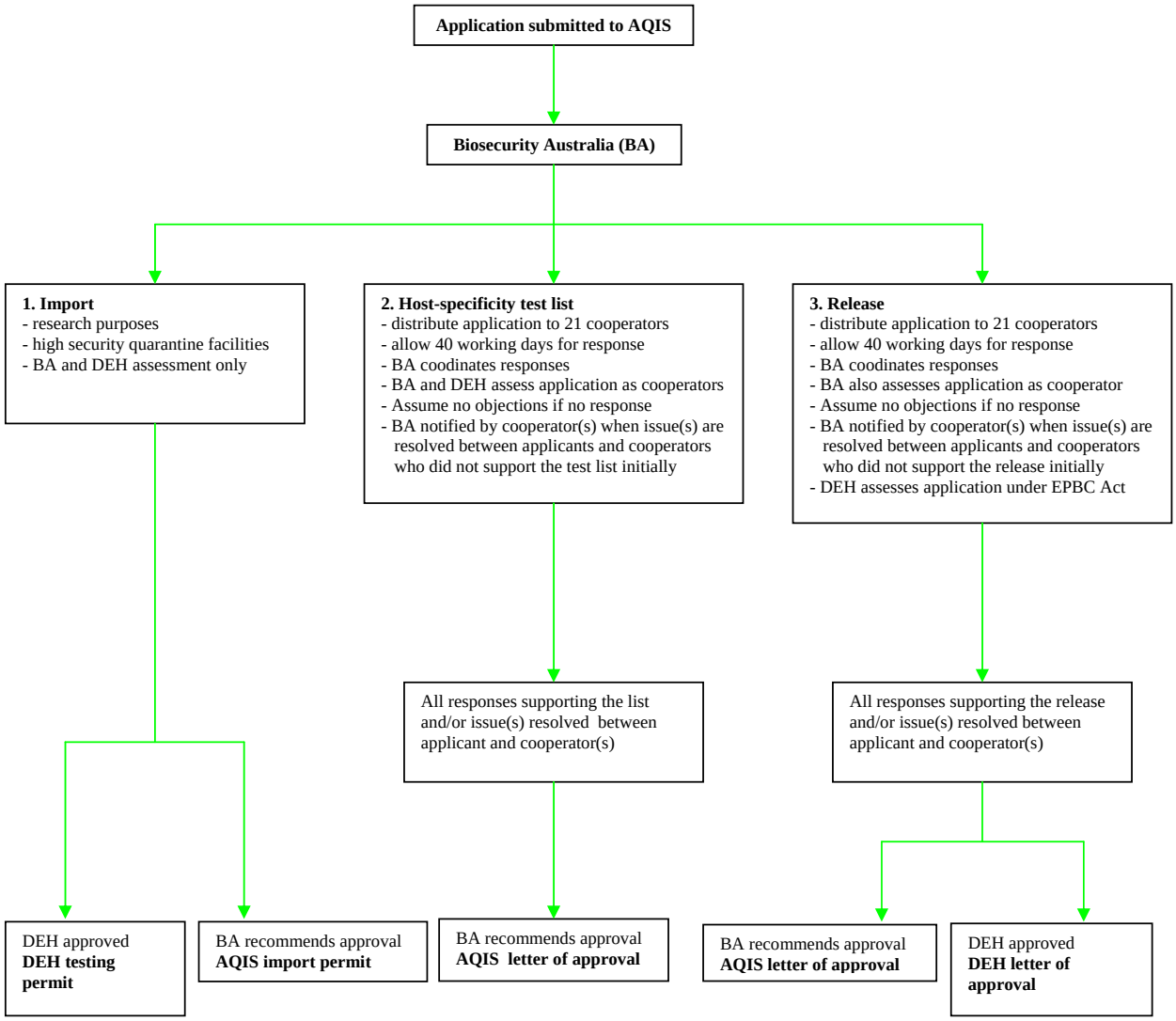
1. A potential BCA is identified and approval is sought to import into containment.
2. Application submitted for acceptance of list of species against which the potential agent will be tested for specificity
3. Application submitted to release biological control agent
4. The applicant reports on BCA establishment, efficacy and any non-target effects.

The Australian process currently allows for two phases of public comment through the DEH; one phase prior to importation when terms of reference for the assessment of likely impacts of the agent on the environment and one phase with respect to the draft release application through the DEH web site (Sheppard *et al*. 2003). Following this the final assessment is tabled in Parliament to allow comment from government departments. The final decision to release is made by the relevant Minister on the advice of the associated department. Approval may be reviewed within 5 years of approval.

Australia operates biological control within a formal legislative acceptance of its benefits under the Biological Control Act (1984) (Cullen and Delfosse 1985). This Act itself was set up to assist in the resolution of conflicts of interest by allowing for public consultations and enquiries, but is rarely used in practice as biological control projects with significant conflicts of interest rarely eventuate (Sheppard *et al*. 2003). No biological control project against an arthropod pest has ever been scrutinized under the Act.

COMPARISON OF THE TWO SYSTEMS

Table 1 summarises some of the key components of the systems. A comparison of the two systems reveal that there are some key differences in the way in which this ‘guidance’ has been implemented (Table 1).



Abbreviations:
AQIS - Australian Quarantine and Inspection Service
BA - Biosecurity Australia
DAFF - Australian Government Department of Agriculture, Fisheries and Forestry
DEH – Australian Government Department of Environment and Heritage

Figure 2. DAFF and DEH protocol for biological control agent applications. Note that application for import and for release will need to be submitted to both AQIS and DEH separately; application for hos specificity test list only needs to be submitted to AQIS. Credit: Australian Government, Department of Agriculture, Fisheries and Forestry, http://www.affa.gove.au; retrieved April 18, 2005.

PROCESS SCOPE

The entire process from importation of potential BCA through to host-specificity testing and eventual release is regulated in Australia. In New Zealand only the import into containment for host-specificity testing and subsequently the release is regulated, with the applicant determining how host-specificity testing is done. While the New Zealand process provides the

Table 1. Comparative analysis of the key components of the New Zealand and Australian regulatory systems.

Component	New Zealand	Australia
Process scope	Regulates import into containment and release but not host-specificity testing.	Regulates import into containment, host-specificity testing and release.
Public participation via a hearing	Occurs if requested (has happened in every case to-date).	Only if the agent is declared under the Biological Control Act (never happened for an agent proposed against an arthropod).
RCB analysis scope	Includes direct and indirect effects.	Limited to direct effects.
Risk averseness	Risk neutral or averse.	Risk neutral or accepting (at present).
Decision-maker	Quasi-judicial body and not necessarily government employees or scientists.	Minister for the Environment and Heritage and the Chief Plant Protection Officer.
Post approval activities	None - organism is no-longer 'new' so is not subject to HSNO regulation.	Post-release monitoring of establishment, efficacy, and non-target effects is required but not enforced.

applicant with more autonomy, the Australian process would seem to avoid the risk errors/ omissions in the host-specificity testing as it is regulated. For example, a decision on a recent application for full release of a weed BCA in New Zealand was delayed over a year as the Authority were concerned that the applicant had failed to include key species in the host-specificity testing. The Agency is attempting to avoid this happening again by making potential applicants more aware of the importance of adequate host-specificity testing. In the past New Zealand applications have relied heavily on host-specificity testing data from overseas and the regulation of host-specificity testing means this would not be an option in the Australian system.

PUBLIC PARTICIPATION

The New Zealand system has a unique feature where any person may make a submission on a publicly notified application[4] and request a public hearing into the application. While hearings may be viewed by the applicant as an obstacle, this is the only opportunity for the applicant to discuss in person their application with the decision-makers. This interaction has in the past provided a valuable forum for clarification of issues that have contributed to positive outcomes for applicants. Submitters also comment favourably on having the opportunity to 'be heard'. In an article discussing regulation of genetically modified organisms in New Zealand, which is also covered under the HSNO Act, Herrera (2005) noted that the public participation "gives New Zealanders more power to participate in the approval process...than any other people in the world." Holding a public hearing remains a practical option in New Zealand due to the comparatively small population and limited geographical area. It is anticipated that

[4] All full release applications must be publicly notified whereas applications to import new organisms into containment are only publicly notified if the Agency considers that there will be significant public interest in the application.

attempting to hold such a hearing in Australia would be a significantly larger and more costly undertaking. However, the Australian public do have an opportunity to comment on applications in a written form.

SCOPE OF EFFECTS CONSIDERED IN THE RCB ANALYSIS

In a review of regulators worldwide Sheppard *et al.* (2003) noted that "currently only the New Zealand approach closely matches a full ecological risk-benefit-cost analysis". This is probably a reflection of the fact that the HSNO Act requires a wider range of effects to be considered beyond the biophysical as demonstrated in the following two case studies. Furthermore, there is also some acceptance of a quantitative approach to risk-benefit analysis conducted by the Authority, for example in economic analyses of potential savings of insecticides.

In Australia the process still reflects a historical bias that biological control releases are largely beneficial, the decision-makers being somewhat risk accepting to risk neutral in attitude. As a result, beyond evaluating the potential risks to non-target species, there is no formal requirement for an extensive evaluation of potential benefits or secondary indirect effects of BCA. That means the Australian system does not follow as clearly a formalised RCB analysis approach as that adopted in New Zealand.

New Zealand Case Study. *Pseudococcus viburni* or obscure mealybug is a pest of pipfruit with its presence resulting in the formation of sooty mould which can result in fruit being unsaleable. In 2000 the release of the parasitoid *Pseudaphycus maculipennis* (Mercet) (Hymenoptera, Encyrtidae) (Fig. 3) was approved as a biological control agent of *Pseudococcus viburni* (Maskell) (Hemiptera, Pseudococcidae).

Figure 3. *Pseudaphycus maculipennis*. Photo: Shaun Forgie, HortResearch. UGA1390027

The Authority considered the most important potential adverse effect associated with approving this application to be parasitism of native mealybugs. This concern was in relation to a particular endemic mealybug but it was also pointed out that because of the incomplete knowledge of the native fauna, there was a potential for effects on as yet undescribed species. If this adverse effect was realised this would have flow-on effects to Mâori culture.

The Authority considered the most significant potential benefit of approving the application to be reducing the application of organophosphates, which would subsequently reduce:

- Insecticide residues in soil
- Impacts on human health through residues on food, spray drift and occupational exposure to insecticides
- A reduction in adverse effects of insecticides to native insects with flow-on cultural benefits to Mâori

- A reduction in adverse effects of insecticides to beneficial insects with flow-on benefits to integrated pest management of apples systems
- A reduction in the development of insecticide resistance.

The Authority also noted the economic gains to the horticultural industry via direct savings in insecticide applications, and improved sustainability.

Australian Case Study. In 2004 the release of *Eretmocerus hayati* (Zolnerowich and Rose) (Hymenoptera, Aphelinidae) (Fig. 4) a parasitoid for the control of *Bemisia tabaci* (silverleaf whitefly) was approved.

Figure 4. *Eretmocerus hayati.* Photo: CSIRO Entomology. UGA1390028

Bemisia tabaci (Gennadius) (Homoptera, Aleyrodidae) is a pest of ornamental nursery crops, vegetables and cotton causing feeding damage and reducing quality through the formation of sooty mould.

A summary of the potential impacts on the Australian environment noted that the results of host-specificity testing "predicts an extremely narrow host range". It also stated that "the risk to non-target whitefly is extremely low", particularly when compared to the risk of the widespread use of pesticides.

The discussion of the benefits in the application was limited to recognising that the amount of insecticide applied against the pest has "reduced the profitability of growers and has threatened the viability of existing low pesticide input management strategies".

RISK AVERSENESS

Inherent in the New Zealand legislation is a need for the decision-maker to consider indirect impacts. Due to the wide scope of the risk assessment (as previously discussed) and because there is no mechanism for compensation to affected parties, the New Zealand decision-makers are likely to be risk averse. In comparison, the Australian system provides for compensation of individuals exposed to adverse effects and so decision-makers are likely to be risk accepting or risk neutral.

DECISION-MAKER

In New Zealand the focus has been to select decision-makers that are experts in a wide range of fields to better represent the opinion of the general New Zealand public:

- Retired Foreign Diplomat
- Professor of Chemistry
- Hazardous Substances Advisor to public sector groups
- Senior Lecturer in Mâori
- Senior Scientist of Insect Ecology
- Associate Professor of Molecular Biology
- Senior Scientist of Molecular Biology
- Partner in a law firm

Advice on scientific, cultural, ethical and economic issues is provided by the Agency or relevant external experts. All documentation has to be produced in a manner that is also accessible to a lay audience. In Australia there is a reliance on scientific experts and staff in the Ministers office to aid the Minister in making a decision. This means that in New Zealand there is a degree of separation from the politics of the day which is in contrast to the Australian system where Ministers may be lobbied by special interest or industry groups. Although the New Zealand Authority is not completely removed from the influence of the political arena as has been previously mentioned, members are appointed by the Minister. It should be noted that in New Zealand there are limited grounds of appeal in relation to the merits of an application, however, given the quasi-judicial nature of the Authority the High court can undertake a judicial review of administrative decision-making. In its decision-making the Authority is required to take into account the need for caution in managing adverse effects where there is scientific and technical uncertainty about those risks.

In Australia the decision can be challenged through the courts. In such a case, however, the agency that made the releases can apply to have the biocontrol agent declared under the Biological Control Act. To achieve this, a public enquiry is required and the outcome must be a clear demonstration that the benefits of releasing the agent clearly out-weigh the risks. Once the agent is declared under the Act the agency responsible for the release is legally protected from indemnity. Not surprisingly certain agencies have requested the Act be simplified so that all agents can be declared under it prior to release. However, this would require a major revision of the Act and so has not occurred. In practise biological control projects with significant conflicts of interest are no longer undertaken.

POST APPROVAL ACTIVITIES

In a continuation of the more holistic approach of the Australian system, applicants are required to submit a report to AQIS 12 months after release of the BCA regarding establishment, efficacy and any non-target effects. As the full release approvals granted in New Zealand have no associated controls post-release monitoring is not regulated, but is often encouraged. Recent changes to the HSNO Act have introduced a new category of approval, 'conditional release', which differs from full release in that controls can be placed on approvals for the purposes of mitigating risk, including but not limited to the following:

- Controlling the extent and purposes for which organisms could be used
- Requiring any monitoring, auditing, reporting, and record-keeping
- Compliance with relevant codes of practice or standards
- Development of contingency plans to manage potential incidents
- Limiting the dissemination or persistence of the organism or its genetic material in the environment
- Requiring the disposal of any organisms or genetic material
- Limiting the proximity of the organism to other organisms
- Setting requirements for any material derived from the organism

- Imposing obligations on the approval user (e.g., training, number of approval users)
- Specifying the duration of the approval

The requirement for controls that 'mitigate risks' associated with an individual approval presents challenges for decision-makers wanting assurances regarding the outcomes of an approval. Conditional release provides an opportunity for decision-makers to limit importations of BCA to the same geographical location from which individuals for testing were collected, hence mitigating the risk of non-target effects due to 'ecotype' differences. While not applicable to the parasitoid scenario, conditional release could allow for pre-release monitoring of effects using sterilised BCA.

FUTURE CHALLENGES AND OPPORTUNITIES

The challenges that the regulation of BCA present to researchers in the field are immediate and obvious. Concerns about the additional costs and time associated with gaining regulatory approval has resulted in an additional obstacle to the scientific community. However, participation in the regulatory system presents many opportunities for researchers beyond the obvious attainment of approval. Key to both the New Zealand and Australian system of regulating BCA is the results of host-specificity testing. Having to provide assurances to regulators that adverse effects are unlikely to occur has challenged researchers to ensure that testing protocols are robust and sound. This has generated opportunities for investigating the principals and practices of host-specificity testing. In Australia this is part of the regulatory system and ERMA New Zealand is also taking a pro-active role in promoting and supporting research in this area by acting as partner in a recent successful bid by experts for government research funding. The regulatory system provides an opportunity for peer review of host-specificity testing to ensure rigour and accuracy of results, particularly in the Australian system. In New Zealand, this process takes place, but only after the application has been received.

Both the New Zealand and Australian systems provide researchers with an invaluable opportunity to interact with members of the public. Applicants can use the process as an avenue to achieve public education of a science the benefits of which are poorly understood. A recent report released in New Zealand has demonstrated the value of this kind of interaction in enhancing a more positive image in the public perception of science. When discussing the issue of human biotechnology (HBT) researchers found that in discussion groups which did not include scientists, the attitudes of members of the public "towards scientists became more negative and they grow more concerned about HBT. On the other hand, when engaged in dialogue with scientists, their attitudes became more positive towards scientists and HBT, they had more empathy with scientists, and they had less concern about HBT" (Roper *et al.* 2004).

In conclusion, while it would initially appear that the regulation of biological control agents present obstacles to researchers, if applied constructively it may have the potential to provide other benefits beyond ensuring the release of efficacious agents that will cause minimal adverse side effects.

DISCLAIMER

The views presented in this publication are those of the authors and not necessarily their employer.

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INTERNATIONAL GUIDELINES FOR THE EXPORT, SHIPMENT, IMPORT, AND RELEASE OF BIOLOGICAL CONTROL AGENTS AND OTHER BENEFICIAL ORGANISMS (INTERNATIONAL STANDARD FOR PHYTOSANITARY MEASURES NO. 3)

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ABSTRACT

This paper describes the development and review of the International Standard for Phytosanitary Measures (ISPM No. 3) which provides guidelines for risk management relating to the export, shipment, import and release of biological control agents and other beneficial organisms. The standard lists the related responsibilities of contracting parties to the International Plant Protection Convention (IPPC) ('contracting parties'), of National Plant Protection Organizations (NPPOs) or of other responsible authorities, importers and exporters. ISPM No. 3 addresses biological control agents capable of self-replication (including parasitoids, predators, parasites, nematodes, phytophagous organisms, and pathogens such as fungi, bacteria and viruses), sterile insects and other beneficial organisms (such as mycorrhizae and pollinators), including those packaged or formulated as commercial products. Provisions are also included for importation of non-indigenous biological control agents and other beneficial organisms for research in quarantine facilities.

INTRODUCTION

Phytosanitary standards (ISPMs) are developed under the auspices of the International Plant Protection Convention (IPPC) and provide a framework within which national plant protection organisations (NPPOs) can develop regulations to provide for plant protection. The level of phytosanitary protection that is considered appropriate for any given country, is for that particular country to decide. The finalization and adoption of the IPPC occurred after the first publication of ISPM No. 3 (FAO 1996b). In the 1980's onwards there was an increasing volume (both number of species and number of individual specimens) of biological control agents moved internationally, particularly classical biological control agents and those

used for inundative release. Prior to 1995, there was no agreed international guidance for the trans-boundary movement of these live organisms, hence FAO developed ISPM No. 3 to address a specific need. It was decided that the most appropriate place for such an international guideline was within the framework of the IPPC. The FAO Conference adopted ISPM No. 3 in 1995, before the revision of the IPPC (which was adopted in 1997) and the finalization of the World Trade Agreement on the Application of Sanitary and Phytosanitary Measures. There have also been many scientific developments in the knowledge of biological control agents since 1995. It is within this context that ISPM No. 3 was developed and now been revised.

The primary support standard to ISPM No. 3 was ISPM No. 2 (FAO 1996a). More detailed guidance on Pest Risk Analysis is provided in other ISPMs, particularly ISPM No. 11 (FAO 2004a) and ISPM No. 21 (FAO 2004c).

At the second session of the ICPM (October 1999) issues wider than agriculture, such as the impact on the environment and other relevant international agreements, were considered in the context of the IPPC (e.g., the Convention on Biological Diversity). The ICPM established an expert working group to consider this and other relevant issues. An output of the working group was that the recommendation that ISPM No. 3 be amended "to include consideration of risk of spread of biological control organisms to other countries".

Prior to revision, the scope of ISPM No. 3 was relatively narrow and primarily applicable to classical biological control agents. Although conceptually it encompassed the principles of the IPPC and SPS Agreement and could in practice be applied more widely, it was not explicit on a number of important phytosanitary issues e.g. pest risk analysis. Therefore, the scope of ISPM No. 3 was broadened to encompass the principles and articles of IPPC, in particular Article VII 2 (g) "*Contracting parties may make provisions, with adequate safeguards, for the importation for purposes of scientific research or education, of plants and plant products and of specimens of plant pests. Adequate safeguards likewise need to be taken when introducing biological control agents and organisms claimed to be beneficial.*" Hence the revised standard has incorporated guidelines that cover other beneficial organisms with particular reference to sterile insects as well as biological control agents.

In addition, ISPM No. 3 was considered by the ICPM for possible review in 2001 (five years after adoption, as is standard for all adopted ISPMs) and issues such as the rapid increase in the use of, and trade in biological control agents, as well as developments in biological control practices meant there was a need to update this standard. The standard also needed to be made consistent with other more recently developed ISPMs and phytosanitary concepts within the framework of the IPPC. The revision of ISPM No. 3 was placed on the IPPC work programme and the revision commenced as soon as funding became available.

REVIEW OF ISPM NO. 3

Given the above context and to ensure that all relevant issues were addressed in this process, the ICPM Standards Committee drafted specifications for the review of ISPM No. 3. According to IPPC Specification No. 4 the review needed to include the consideration of:

- Revision of title and text;
- Pest risk analysis procedures appropriate for biological control agents;
- Regulatory guidance developed by the OECD since publication of the standard;
- Issues relating to the transport and handling of biological control agents;
- Possibilities for clarification and emphasis with regards to invasive species and other impacts on the environment, and
- Issues relating to pre and post release monitoring.

Other matters to be considered and addressed where appropriate were:

- Sterile insect technique (SIT) issues;
- Beneficial organism issues, and
- The use of biological control agents that had been genetically modified using modern biotechnology techniques.

An expert working group (including nine independent experts plus the IPPC Secretariat) met in December 2003 at FAO Headquarters in Rome to revise ISPM No. 3. The outcome was a revised draft ISPM No. 3 that was reviewed by the Standards Committee in May 2004. The draft ISPM No. 3 was released for country consultation in June 2004. Many comments were received and all comments from all interested parties had to channel their comments through the NPPOs. Comments provided by the NPPOs were considered by the Standards Committee and the necessary adjustments made to the draft. The final version of the standard was submitted to the seventh session of the ICPM (in April 2005) for consideration. After minor modifications it was adopted as ISPM No. 3 (FAO 2005a and 2005c).

The Standard states that it is "*intended to facilitate the safe export, shipment, import and release of biological control agents and other beneficial organisms. Responsibilities relating to this are held by contracting parties, NPPOs or other responsible authorities, and by importers and exporters.*" However it does not include reference to living modified organisms, issues related to registration of biopesticides, or microbial agents intended for vertebrate pest control.

> *"Contracting parties, or their designated authorities, should consider and implement appropriate phytosanitary measures related to the export, shipment, import and release of biological control agents and other beneficial organisms and, when necessary, issue related import permits."*

As described in this standard, NPPOs or other responsible authorities should:

- *"Carry out pest risk analysis of biological control agents and other beneficial organisms prior to import or prior to release;*
- *Ensure, when certifying exports, that the phytosanitary import requirements of importing contracting parties are complied with;*

- *Obtain, provide and assess documentation as appropriate, relevant to the export, shipment, import or release of biological control agents and other beneficial organisms;*
- *Ensure that biological control agents and other beneficial organisms are taken either directly to designated quarantine facilities or mass-rearing facilities or, if appropriate, passed directly for release into the environment;*
- *Encourage monitoring of release of biological control agents or beneficial organisms in order to assess impact on target and non target organisms.*

Responsibilities of, and recommendations for, exporters include ensuring that consignments of biological control agents and other beneficial organisms comply with phytosanitary import requirements of importing countries and relevant international agreements, packaging consignments securely, and providing appropriate documentation relating to biological control agents or other beneficial organisms.

Responsibilities of, and recommendations for, importers include providing appropriate documentation relating to the target pest(s) and biological control agent or other beneficial organisms to the NPPO or other responsible authority of the importing country."

DISCUSSION

A primary objective of the revision of ISPM No. 3 was to ensure consistency with the IPPC (FAO 1997) and that it was harmonized with relevant IPPC phytosanitary terms (FAO 2005b).

OBJECTIVES OF THE STANDARD

The objectives of the standard are to:

- *"Facilitate the safe export, shipment, import and release of biological control agents and other beneficial organisms by providing guidelines for all public and private bodies involved, particularly through the development of national legislation where it does not exist;*
- *Describe the need for cooperation between importing and exporting countries so that:*
  - *i. benefits to be derived from using biological control agents or other beneficial organisms are achieved with minimal adverse effects;*
  - *ii.practices which ensure efficient and safe use while minimizing environmental risks due to improper handling or use are promoted."*

Guidelines in support of these objectives are described that:

- *"Encourage responsible trade practices*
- *Assist countries to design regulations to address the safe handling, assessment and use of biological control agents and other beneficial organisms*

- *Provide risk management recommendations for the safe export, shipment, import and release of biological control agents and other beneficial organisms*
- *Promote the safe use of biological control agents and other beneficial organisms."*

SCOPE OF THE IPPC

The International Plant Protection Convention (IPPC) is based on securing common and effective action to prevent the spread and introduction of pests of plants and plant products, and the promotion of appropriate measures for their control. In this context, the provisions of the IPPC extend to any organism capable of harbouring or spreading plant pests, particularly where international transportation is involved (Article I of the IPPC, 1997). A pest is defined as "*any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products*".

The IPPC (1997) contains the following provision in relation to the regulation of biological control agents and other beneficial organisms. Article VII.1 states:

> *"With the aim of preventing the introduction and/or spread of regulated pests into their territories, contracting parties shall have sovereign authority to regulate, in accordance with applicable international agreements, the entry of plants and plant products and other regulated articles and, to this end, may:*
>
> *d) prohibit or restrict the movement of biological control agents and other organisms of phytosanitary concern claimed to be beneficial into their territories."*

Section 4.1 of ISPM No. 20 (FAO 2004b) contains a reference to the regulation of biological control agents; it states:

> "*Imported commodities that may be regulated include articles that may be infested or contaminated with regulated pests. ... The following are examples of regulated articles: ... pests and biological control agents.*"

The revised ISPM No. 3 provides international guidelines relating to phytosanitary measures, as well as recommending guidelines for the safe use of biological control agents and other beneficial organisms claimed to beneficial. Phytosanitary concerns with regards to biological control agents include the possibility that newly introduced biological control agents, or organisms claimed to be beneficial may introduce pests or diseases which affect the agent, hence reduce the effect of a biological control program or may severely disrupt an existing biological control program; or may significantly affect non-target organisms, such that there are harmful effects on plant species or plant health. This standard does not alter in any way the scope or obligations of the IPPC itself as contained in the New Revised Text (1997) or conflict with any of the other ISPMs.

Most of the standard is based on the premise that a biological control agent or other beneficial organism may be a potential pest itself, and in this sense Article VII.1c of the IPPC (1997) applies because contracting parties may prohibit or restrict the movement of regulated pests into their territories. In some situations, biological control agents and other beneficial organisms may act as a carrier or pathway for plant pests, hyperparasitoids, hyperparasites

and entomopathogens. In this sense, biological control agents and other beneficial organisms may be considered to be regulated articles as described in Article VII.1 of the IPPC (1997) and ISPM No. 20 (FAO 2004b).

ISPM No. 3 does not specifically cover genetically modified organisms (GMOs). Although GMOs are specifically excluded, the principles of pest risk analysis for assessment of risk and implementation of an appropriate level of protection are still applicable. In addition, this standard does not cover pesticide registration. Pesticide registration is an independent set of processes that differ between countries. The extent to which organisms covered in ISPM No.3 are involved in these registration processes depends on individual countries. In some instances the processes and information required are coincident with the requirements of ISPM No.3. However, the objectives of pesticide registration are different as a whole from those of the IPPC/ISPM No.3, although there may be similar elements.

STRUCTURE

The structure of this revised standard broadly follows that of the original ISPM No. 3, and its content is based primarily on risk management relating to the use of biological control agents and other beneficial organisms. Based on in-country experience, the previous format of ISPM No. 3 was very easy to understand and popular in the field, and so as much of the content and format as possible was retained.

PEST RISK ANALYSIS

The existing standards on pest risk analysis (ISPM No. 2 (FAO 1996a), ISPM No. 11 (FAO 2004a) and ISPM No. 21 (FAO 2004c)) provide the appropriate fundamental processes for carrying out pest risk assessments for biological control agents and other beneficial organisms. In particular, ISPM No. 11 includes provisions for pest risk assessment in relation to environmental risks, and this aspect covers environmental concerns related to the use of biological control agents. Implicit in the development of the output of a risk analysis is the development of risk management plans for organisms being considered.

The IPPC (1997) takes into account internationally approved principles governing the protection of the environment (Preamble). Its purpose includes promoting appropriate phytosanitary measures (Article I.1). Therefore, in carrying out pest risk analyses in accordance with this and other appropriate ISPMs, and in developing and applying related phytosanitary measures (i.e., pest risk management), contracting parties should consider the potential for broader environmental impacts resulting from releasing biological control agents and other beneficial organisms (e.g., the impact on non-target invertebrates).

ISSUES/CHANGES

The content of ISPM No. 3 was not consistent with that of more recent ISPMs in that it included a significant amount of technical implementation details, as well as having a significantly different functional layout and terminology (e.g., see Table 1 for a summary of terminology changes). The revision removed the technical details and adjusted the layout of the text to align more closely with that of other standards.

Table 1. A summary of ISPM No. 3 terminology changes.

Term	New	Modified	Deleted
Authority		x	
Beneficial Organism	x		
Biological Control		x	
Biological Control Agent		x	
Biological Pesticide (biopesticide)		x	
Classical Biological Control Agent		x	
Contamination	x		
Control (of a pest)	x		
Ecoarea			x
Entry (of a consignment)	x		
Establishment		x	
Exotic			x
Import Permit (of a biological control agent)			x
Host Range	x		
Infestation (of a commodity)	x		
Introduction		x	
Inundative Release		x	
Natural Enemy		x	
Organism		x	
Parasitoid		x	
Pathogen		x	
Phytosanitary Measure	x		
Quarantine		x	
Reference Specimens	x		
Regulated Organism	x		
Specificity		x	
Sterile Insect	x		
Sterile Insect Technique	x		

It is recognized that much of the information removed was useful to various parties involved in the practical processes of import and release of biological control agents and other organisms claimed to be beneficial. It is intended that the technical implementation details will be compiled into a set of technical explanatory documents in support of the standard. These documents will not be obligatory, have no official status under the ICPM, and will not be considered official interpretations of ISPM No. 3. However, they may provide examples

of processes and methodologies that could be followed when implementing the standard. According to the ICPM, such explanatory documents need to be developed under the auspices of the IPPC secretariat (otherwise they do not have ISPM explanatory document status).

The general arrangement of ISPM No. 3 (FAO 2005a) is as follows: "designation of responsible authority and description of general responsibilities; pest risk analysis; responsibilities of contracting parties prior to import, documentary responsibilities of importer prior to import; responsibilities of exporter; responsibilities of NPPO or other responsible authority of the importing contracting party upon import; responsibility of the NPPO or other responsible authority before, upon and following release.

The implementation of the guidelines is the responsibility of the contracting parties (usually the NPPO's) or other responsible authorities. Previously, ISPM No. 3 included details and obligations for organisations (e.g., exporters, researchers and importers) that are beyond the scope of the IPPC.

These guidelines are not legally binding under the IPPC, but are indirectly binding through the WTO/SPS Agreement. Advice for parties other than NPPOs, such as exporters, is provided. This advice is for guidance on appropriate process and is not obligatory. The obligations of non-NPPO parties are those contained in the regulations of countries within which they operate. These regulations should have been developed by the NPPO within the framework of ISPM No.3, hence align with the ISPMs objectives.

Reference is made to other international agreements were appropriate, but such references are intentionally vague to ensure it is not implied the IPPC is infringing or interpreting such agreements.

The revision of ISPM No. 3 should improve the understanding of the processes associated with the import and release of biological control agents and/or beneficial organisms, and facilitate the safe trade in such organisms while protecting the environment. This ISPM continues to provide a framework for countries to establish their own phytosanitary measures for biological control agents and/or beneficial organisms i.e., it is not a prescriptive standard that details phytosanitary measures that should be applied in all countries around the world.

Further information on ISPM No. 3 (or any other ISPM or the IPPC) can be obtained from the IPPC Secretariat (ippc@fao.org) or: IPPC Secretariat, FAO-AGPP, Vialle delle Terme di Caracalla, 00100 Rome, Italy.

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