ALTERNATIVES

Accomplishments of the University of Arkansas Alternative Pest Control Center 1989-1995

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FOREWORD

The Research Center for Alternative Pest Control (RCAPC) was established in 1989 as a federal-state partnership to address the critical national priority to find effective, economical alternatives to chemical pesticides in crop production systems in order to minimize chemical inputs into agricultural ecosystems. The stated goal of the Center was to "establish and maintain a multidisciplinary research center for the collection, evaluation and development of biological alternatives for control of agricultural and urban pests including weeds, insects, plant diseases and nematodes." The Center was intended to build on the existing strengths in biological control, plant breeding and related crop management areas at the University of Arkansas that focused on developing crop production systems with minimal chemical inputs. The initial objectives of the Center were to 1) explore natural and managed ecosystems for the collection, isolation and identification of candidate biological control agents, 2) conduct biological and ecological evaluations of promising native, exotic and genetically improved biological control agents and crop plants, 3) develop procedures for the production, formulation and application of promising biological control agents and provide technology transfer to industry, 4) evaluate field performance and integrate effective biological control agents in agricultural production systems, and 5) elucidate the pesticide load in the environment and assess the environmental impact resulting from the integration of biological control agents into agricultural production systems.

Arkansas Senator Dale Bumpers was instrumental in helping to obtain federal funding to match state resources to establish and maintain the Center during its formative years. The Center was structured so that all resources were devoted to funding research projects that applied to the Center objectives and were competitively awarded to Division of Agriculture faculty in the University of Arkansas system and cooperating USDA scientists.

In 1990, the University developed a strategic plan for alternative pest control for FY 92-97. The strategic plan identified limitations in greenhouse, growth chamber and laboratory space as a major constraint for Center projects. Therefore, an additional federal, state, private partnership was created to overcome this limitation. The Rosen Center for Alternative Pest Control was completed in 1995 and now serves the University of Arkansas as a state-of-the-art research and teaching resource devoted to alternative pest control.

The Center was reviewed by USDA, CSREES and a team of research scientists from around the nation in 1992. Based on the input from the review team, several changes were instituted to improve the coordination of the Center and Center projects. The Center objectives were modified slightly to accurately reflect the research being conducted by Center scientists. In particular, research to determine the pesticide load in the environment was discontinued and the objective was directed towards assessing the environmental impact resulting from the integration of biological alternatives into agricultural production systems. The Center projects were divided into seven broad program areas (Biological Control of Insects, Biological Control of Diseases, Biological Control of Weeds, Pest Management, Host Plant Resistance, Allelopathy and Environmental Fate and Risk Assessment). Each program area was represented by a faculty member who served on a coordinating committee

charged with coordinating Center projects and assisting Division of Agriculture administrators with the oversight of the Center.

With the completion of the Rosen Center for Alternative Pest Control, sufficient research capacity had been built at the University of Arkansas so that the Center became self-sustaining. At the request of the University of Arkansas, federal funding for the Center was discontinued in 1995, so that federal resources could be used for capacity building in other areas. Such an action serves to illustrate the value of the federal-state partnership in agricultural research where combined resources can be used to target priority needs and can be used to increase research capacity to serve the citizens of this nation. The Center continues to serve Arkansas and the nation as a resource in alternative pest control, and the Center will continue to be a strategic priority for the Arkansas Division of Agriculture in the future.

This publication was developed to highlight some of the research conducted by both University of Arkansas and cooperating USDA agricultural scientists from the inception of the Center through 1995. It by no means covers the full extent of the Center research over this time period, but rather provides examples of some of the research conducted in each of the seven program areas. Publications published to date from Center funded research projects have been included in an appendix.

Over the life of the Center, a number of new biological control agents and pest resistance genes were discovered that will reduce our need for chemical pesticides; new crop management systems were investigated that will require fewer chemical treatments; new methods were developed to reduce the impact of agricultural chemicals; and new procedures were developed to ensure the safe use of biological control agents.

Some highlights detailed in this publication include:

- The discovery of a new soil fungus that destroys the eggs and larvae of the soybean cyst nematode, a significant pest problem of soybeans in the southern United States.
- The development of new biological control strategies for bollworms and budworms, important insect pests of cotton and other crops.
- The discovery of rice plants that suppress important rice weeds naturally.
- The development of a cotton management computer program that increases profitability and reduces insecticide inputs.
- The identification of natural insect-deterring compounds in plants that can be used to increase natural plant resistance to insect pests.
- The development of new procedures for assessing the potential risks of introduced biological control agents.
- The discovery of fungi that attack morning-glory, pigweed, crabgrass and other weeds of economic importance.
- The development of reduced rate technology for herbicides to minimize the amount of chemical required for effective weed control.
- The discovery of a new biological control agent for cotton aphids.
- The discovery of fescue endophytes that promote drought tolerance and deter insects but do not cause cattle toxicity.

Contents

Chapter 1

Biological Control of Insects

Biological Control of Insects

D.C. Steinkraus, T.J. Kring, S.Y. Young and G.W. Felton

entral to the development and justification of the Alternative Pest Control Center was the quality of the existing entomology programs within the University. Center research efforts focused on both strengthening and expanding these programs, particularly in the pursuit of pertinent biological information, evaluation of efficacy of biological control organisms and implementation of biological control tactics into various agricultural and pest management systems.

## BIOLOGY OF BIOLOGICAL CONTROL ORGANISMS

Control of the Cotton Aphid by an Entomopathogenic Fungus

The cotton aphid, *Aphis gossypii* Glover, has long been a pest of cotton (Isely, 1946; Slosser et al., 1989). Heavy infestations distort leaves, stunt plants, cause sticky cotton and support sooty molds, lowering yield and lint quality. Cotton aphid problems have intensified in recent years, possibly due to insecticide resistance (O'Brien et al., 1992). Fortunately, fungal epizootics rapidly reduce high aphid populations in mid-south cotton. The causal agent, *Neozygites fresenii* (Nowakowski) Batko, was first identified in 1990 (Steinkraus et al., 1991). Epizootics occur from early July through August and often reduce aphid populations in five to 10 days (Steinkraus et al., 1992b, 1995; Boys et al., 1996).

Research on prevalence of the fungus showed that it was present in 87.5% and 77.8% of commercial cotton fields in 1992 and 1993, respectively. There was a significant trend in both years for a decrease in prevalence rates from south to north, suggesting that epizootics may progress from the south (Steinkraus et al., 1995). This information has value in predicting when and where epizootics will occur in Arkansas. An additional 10 cotton fields were intensively sampled during 1992 and 1993. In all 10 fields, epizootics occurred that rapidly reduced aphid populations.

Several factors were found to be responsible for the rapid and widespread aphid declines seen in the cotton fields. First, the incubation period, i.e., the time from infection until death of an aphid, is three to four days at 25 C (Steinkraus et al., 1993b). This rapid fungal life cycle means that two to three generations of N. fresenii can occur in about nine days, with each generation resulting in a tremendous increase in fungal inoculum. Primary conidia of N. fresenii are actively discharged from an aphid cadaver into the air and onto leaf surfaces surrounding the dead aphid. Laboratory studies showed a mean of 3,052 primary conidia discharged from each infected aphid, with 76% entering the air (Steinkraus et al., 1993b). During an epizootic, millions of primary conidia enter the air from infected aphids on each plant. The aerial spore flora in cotton fields during epizootics was monitored using Rotorod and Burkard aerial spore samplers (Steinkraus et al., 1996). Results showed a remarkable periodicity to spore discharge by the fungus (Fig. 1). At 12:00 a.m. primary conidia began entering the air, reached a peak at 3:00 a.m., declined towards 9:00 a.m. and were scarce during daylight hours. Spore discharge during the early morning hours of relatively cooler temperatures, darkness and high relative humidity insures that the spores have the greatest likelihood of surviving to infect aphids throughout the fields. Experiments with sentinel aphids placed in cotton fields at night during an epizootic showed that aerial spores were able to infect 48% of the aphids after 8 hours exposure (D.C. Steinkraus, unpublished data). This demonstrates that the extremely high numbers of aerial conidia present in cotton fields along the Mississippi River and in other cotton growing areas of Arkansas play an important role in the rapidity with which epizootics spread within fields and between fields.

In 1993, 1994 and 1995, a pilot study was conducted in conjunction with scientists at the Cooperative Extension Plant Disease Diagnostic Clinic in Lonoke, Arkansas. Cotton aphid collecting kits were distributed to co-
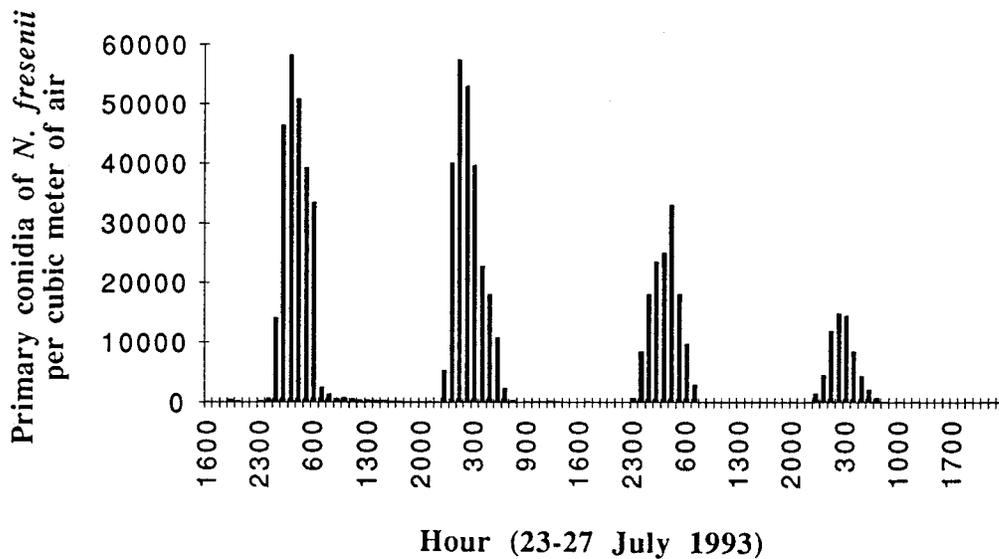
Fig. 1. Spores of the fungus were collected during an epizootic in aphids using a Burkard sampler in a commercial cotton field in Arkansas. The data show the periodicity to spore discharge and the enormous numbers of spores present per cubic meter of air during an epizootic.

operating extension agents and consultants in cotton producing areas of Arkansas. When aphids reached the economic threshold, cooperators collected samples of aphids and mailed them via overnight delivery to the Diagnostic Clinic. Samples were analyzed, and within 24 hours cooperators were informed of the fungal levels in the sample. This information was used in making IPM decisions on cotton aphid management, and surveys indicated that participants found the information useful (Steinkraus et al., 1993d; Steinkraus and Hollingsworth, 1994; Hollingsworth et al., 1994).

Information gained from studies on N. fresenii is widely used by Extension agents and cotton growers in Arkansas, Louisiana, Alabama, Florida, North Carolina, Georgia and Mississippi (Hollingsworth et al., 1994; 1995). Growers frequently rely on the fungus to reduce the aphid populations. Thus, N. fresenii provides valuable natural control, resulting in fewer applications of insecticides on cotton, preservation of natural enemies and a lessening of insecticide residues in soil, air and water.

EVALUATION AND ENHANCEMENT OF THE GENERALIST PREDATOR *ORIUS INSIDIOSUS*

Overwintering biology of Orius insidiosus (Say), an important predator of many pest insects in Arkansas

(Elkassabany et al., 1996), was described. The predator passes the winter as an adult in a state of reproductive diapause (reduced activity) in a variety of habitats. Fall-planted wheat was found to be a major overwintering site, one that affords considerable protection from the environment and has a ready supply of free water and prey (especially thrips).

Orius insidiosus is commercially produced by several insectaries and is distributed worldwide. Although production techniques vary among companies, common concerns are 1) storage of individuals prior to sale and 2) sale of individuals during the winter months in greenhouses. A critical day length of 12-13 hours was discovered, below which more than half of the O. insidiosus population enters diapause (Ruberson et al., 1991). Also it was found that a storage condition (10:14 L:D, 15 C) that resulted in low mortality and little impact on reproductive capacity or longevity of individuals after eight weeks of storage (eight weeks is more than twice the expected lifespan of adults under field conditions) (Fig. 2). A population of O. insidiosus was genetically selected that does not undergo a reproductive diapause even when held under conditions that induce diapause in 95% of feral O. insidiosus (Ruberson et al., 1991). The selected population performs similarly to wild populations, with no significant reduction in the number of days in which

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females lay eggs or the number of eggs laid per day or per female lifespan (Fig. 3). The selected strain lives up to 40% longer than an unselected wild population and could be sold for use in greenhouses in the winter without fear of the population entering diapause. These rearing technologies were provided to three commercial insectaries during 1994 and 1995.

These research efforts have provided commercial insectaries with techniques to enhance rearing technology of O. insidiosus, thus promoting increased use of biological control. Completed biological studies, including description of O. insidiosus overwintering behavior, aid in evaluating opportunities when the predator may be relied upon to provide adequate pest control.

Role of Host Plants in Bollworm-Budworm Susceptibility to Diseases

A common assumption among pest managers is that biorational tactics, such as the use of insect pathogens and HPR, are compatible when used together in an IPM system. This assumption was tested with Bacillus thuringiensis (Bt) and the H. zea nuclear polyhedrosis virus (HzNPV).

In one test, the combined effects of the cotton allelochemical gossypol and Bt on neonate bollworm and budworm growth were examined. Larvae reared on artificial diets containing 0.5% (f. wt.) gossypol grew significantly more slowly than larvae reared on diets without gossypol (control) or with 0.1% gossypol. A consis-

tent but generally nonsignificant increase in growth rates for larvae reared on diets containing a 0.1% gossypol was observed compared to larvae reared on control diets. Larval growth rates decreased with increasing Bt concentration. The combined effects of gossypol and Bt were independent (i.e., additive) in all cases. In a related test, the combined effects of cotton cultivars differing in gossypol (and other terpene aldehydes) content and Bt on bollworm and budworm growth was examined. Larval growth was highest on the two cultivars with relatively low gossypol content ('Stoneville 213' glandless and Stoneville 213 glanded) and significantly lower on the cultivar with relatively high gossypol content ('DH216'). Again, larval growth rates decreased with increasing Bt concentration, and the combined effects of cotton cultivar and Bt on larval growth were independent.

The effects of diet on the susceptibility of the bollworm to HzNPV using laboratory bioassays were also examined. In one test, larvae fed cotton after administration of HzNPV suffered significantly less mortality (36%) than those fed either tomato or artificial diet (75% and 65% mortality, respectively). If larvae were reared on artificial diet, dosed on plant foliage disks and returned to artificial diet, differences in virus-induced mortality were observed (Forschler and Young, 1992). In another test, the addition of condensed tannin and tan-
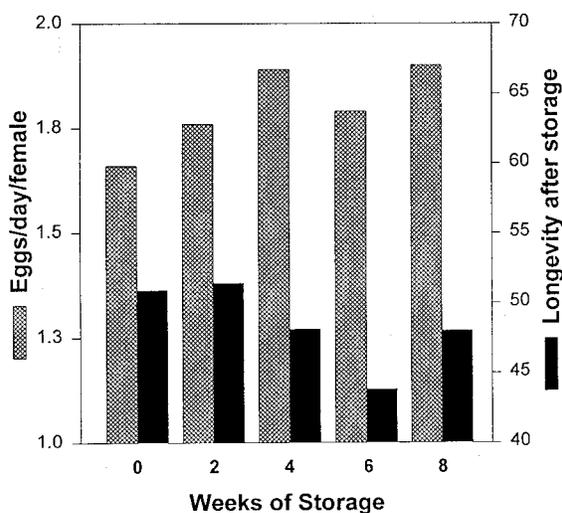

Fig. 2. Daily reproduction and longevity of *Orius insidiosus* after removal from a selected storage regimen (10:14, L:D photoperiod, 15 C).
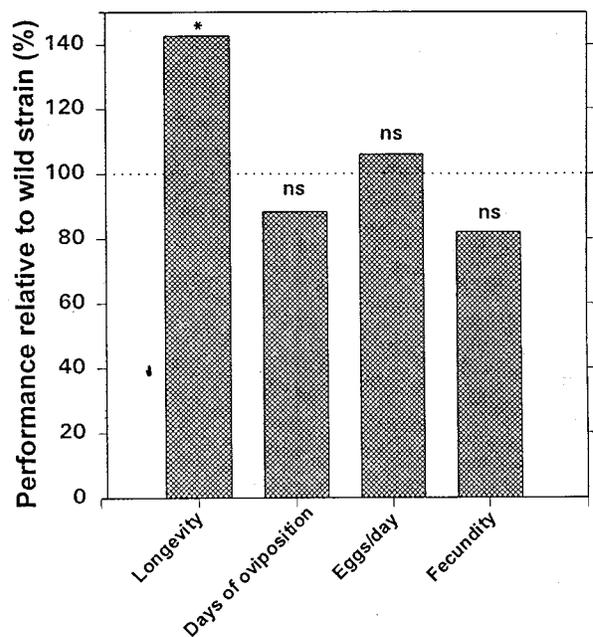

Fig. 3. Longevity and reproductive parameters of a genetically selected non-diapausing strain of *Orius insidiosus* relative to a wild-type field-collected strain.

nic acid to synthetic diet at concentrations of 0.025, 0.05 and 0.1% (w/v) resulted in reduced mortality of bollworm larvae treated with HzNPV on these diets (Young et al., 1995; Meade et al., 1995).

These results suggest that HzNPV and HPR may be incompatible pest management tactics while Bt and HPR may be compatible. If these results can be applied to the field, pest managers might expect better control of larvae with NPV on crops or cotton varieties low in tannin than on high-tannin cotton varieties. In the case of Bt and gossypol, however, our results indicate that greater degree of pest suppression could be obtained when these two tactics are used in combination than when each is used alone and suggest that pyramiding resistance factors such as terpene aldehyde content and Bt toxins in transgenic cotton could be a useful pest management strategy.

INVESTIGATIONS OF *COTESIA MARGINIVENTRIS* DEVELOPMENT IN TOBACCO BUDWORM LARVAE

Cotesia marginiventris (Cresson) is one of the most valuable lepidopteran parasitoids found in Arkansas cotton fields. With increased use of Bt with ovicides to control early-season pest populations of tobacco budworm and bollworm as a means to manage pyrethroid resistance, it is important to understand the impact of this biological insecticide on naturally occurring parasitoid populations. Laboratory and field studies were conducted to evaluate the impact of Bt and thiodicarb on tobacco budworm mortality and C. marginiventris survival.

In laboratory studies, survival of C. marginiventris was inversely related to Bt concentration and directly related to timing of exposure. As expected, survival to adult C. marginiventris was inversely related to thiodicarb concentration. Parasitoid survival from larvae exposed to a 50-ppm thiodicarb concentration, however, did not significantly differ from control survival. Parasitized and non-parasitized tobacco budworm larvae were also exposed for two days to cotton squares taken from fields treated at recommended rates of Bt and thiodicarb and then transferred to a standard laboratory rearing medium. Overall, results indicate poor control of late-second instar tobacco budworm in the absence of parasitization (<33%) regardless of treatment. Emergence of C. marginiventris from tobacco budworm did not differ with respect to insecticide treatment (>90%). Results of these tests indicate that thiodicarb is more effective than Bt in controlling late second to third instar tobacco budworm larvae. Furthermore, results suggest that larvae

parasitized prior to insecticide application should survive exposure, thus having negligible direct impact on the field parasitoid population within host larvae (Atwood et al., 1995).

BACILLUS THURINGIENSIS-INDUCED MOVEMENT OF LARVAE ON COTTON

Javelin[R] WG was evaluated two days after application to cotton leaves and terminals for effects on location, mortality and leaf area consumption of exposed bollworm and tobacco budworm larvae. Results indicated that Bt rates and sampling times did not generally alter location of larvae that remained on leaves. Bt application to cotton terminals demonstrated that first instars of both species tended to move from meristems and squares to other sites on terminals with increasing Bt rates or sampling times. An increase in Bt rate resulted in greater larval movement off cotton leaves and terminals and higher larval mortality in both species. In general, increasing Bt rate decreased leaf area consumption in both species, but this decrease was significantly and inversely correlated with larval mortality only in bollworm. Pupal and adult stages in both species were also affected by Bt exposure. These behavioral changes indicate that such effects need to be considered when evaluating Bt applications in pest management systems on cotton (Jyoti et al., 1996).

EFFICACY EVALUATIONS

Bacillus thuringiensis

Bacillus thuringiensis products were evaluated on crops other than cotton. In one test on heading grain sorghum, most Bt products (at recommended rates) did not significantly reduce bollworm numbers over the control at three and six days after treatment. In a second test, all Bt products tested reduced bollworm larval numbers over the control at four and seven days. However, only Javelin WG at 2.2 gm/ha was as effective as Sevin XLR Plus at 3.5 L/ha after four days (Young and Ali, 1992). Sorghum webworm, Nola sorghiella Riley, larval numbers on heading grain sorghum were reduced after four and seven days by all Bt products tested (ABG-6237F, Javelin WG, Dipel 4 LF and MVP-F) at most rates, except for MVPF at four days. None of the products tested were as effective as Sevin XLR Plus (Young, 1992a).

In a test on soybean, low rates of all Bt products tested provided good control of populations of velvetbean caterpillars, Anticarsia gemmatalis Hubner, and green cloverworm, Plathypena scabra (F.), reducing numbers of both pests by 65 to 90% at four and seven days (Young

and Forschler, 1992). A series of tests have also shown Bt products to be effective against soybean looper, *Pseudoplusia includens* Walker, on soybean. Soybean looper numbers were reduced by greater than 50% by recommended rates of the products at four and seven days after application. Generally, Design WP, Condor F, Sandoz 415 and Sandoz 420 (Costar) resulted in the lowest numbers of surviving larvae (Young, 1992b, 1994; 1995).

Results of tests with Bt products against small larvae of the armyworm, *Pseudaletia unipuncta* (Howarth), on heading winter wheat showed larval numbers were not reduced over the untreated control (Steinkraus and Young, 1994; unpublished results, 1994). Since laboratory bioassays on semisynthetic diet revealed that some products at low concentrations killed small larvae (S.Y. Young, unpublished data), tests were conducted to determine if coverage was adequate in field tests. Results of a spray table test showed that mortality was high in small larvae fed wheat leaflets sprayed with the equivalent of 1.0 lb/acre of Javelin WG. However, when larvae were fed leaflets collected from plants in plots sprayed with Javelin WG, mortality was much less when leaves were collected from the lower portion of plants. These results suggest that, under field conditions, little spray was reaching the lowest leaves of plants where small larvae feed.

Maximum Infestation Level

Microbial insecticides may have a role to play in control of bollworm and tobacco budworm in row crops in Arkansas. However, due to their mode of action, they do not kill target pests as rapidly as chemical insecticides and are not equally infectious to small and large larvae. Therefore, knowledge of the age structure of the pest population and the effects of microbials on the different age classes is essential for satisfactory results with microbial insecticides. Two microbial insecticides, Bt (Javelin) and HzNPV (Elcar), were tested in 1990 and 1991 on cotton, soybean and grain sorghum in field tests to determine natural mortality levels in populations of natural larvae of different instars. Larvae in the grain sorghum and soybean tests were 100% bollworms. On cotton the species composition was 59% and 41% bollworms and tobacco budworms in the first test, respectively, and 9% and 91% bollworms and tobacco budworms, respectively, in the second test. Both Bt and HzNPV caused greater larval mortality on soybean and grain sorghum than on cotton. Greater than 80% of bollworms were killed by either Bt or HzNPV on soybean at the high rates. On cotton, neither microbial insecticide caused larval mor-

tality higher than 58% even at rates of 4 lb of Javelin or 160 larval equivalents of HzNPV per acre. There was a lack of a pronounced increase in larval mortality with increasing rates of the microbials that most likely is due to the difficulty of applying these stomach poisons to larval feeding sites. Larvae feeding on grain sorghum and soybean feed on more exposed sites than on cotton, as indicated by higher mortality on these crops (Steinkraus et al., 1992a).

Viruses

Baculoviruses, NPV and granulosis (GV) viruses have been evaluated against lepidopterous larvae on crops. The persistence and efficacy of HzNPV on heading grain sorghum was compared with three multiple-enveloped NPV's from other hosts: *Autographa californica* (Speyer), *Heliothis armigera* Hubner and *A. gemmatalis*, to which the bollworm is susceptible. Bioassay of NPV by feeding florets from sprayed heads to second instar showed that the viruses were rapidly inactivated and only low levels of activity remained on any treatment four days after application. In a small-plot test, the viruses were applied at 1.5, 3.0 and 6×10^{11} polyhedral inclusion bodies (PIB)/ha. Mortality of collected corn earworm larvae and larval population reduction were greater in the HzNPV than in the three multiple-enveloped viral treatments. The AgNPV was least effective. All virus preparations required one week or longer to significantly reduce larval populations at all rates (Young and McNew, 1994).

Results of tests on the effects of temperature and larval age of the soybean looper on PiNPV-induced mortality on soybean showed that mortality in the laboratory was significantly higher when PiNPV-treated larvae were held at 30 C (75.8%) than at 25 C (69%) or 35 C (65.2%). In the field study, larval mortality across rates of 6×10^{11} and 1.5×10^{11} PIB/ml was higher in late instars than in early instars, ranging from 20% in second instars to 38% in fourth instars (Ali and Young, 1992a).

The development and use of baculoviruses as biological insecticides have been limited by their failure to provide control comparable to recommended chemical insecticides. Stilbene fluorescent brighteners have shown potential as synergists when fed with these viruses. In laboratory bioassays on semisynthetic diet, a brightener, Calofluor white M2R, enhanced activity of NPV of soybean looper, beet armyworm, bollworm and tobacco budworm against their respective hosts up to 1000 times at rates of 0.14 $\mu g/mm^2$ of diet surface (Zou and Young, 1994). In a spray table test, the percentage mortality from PiNPV of second instar soybean loopers fed cotton and

soybean leaves sprayed with 1×10^6 PIB/ml of PiNPV alone was significantly increased with the addition of 0.1% (soybean leaves only) and 1% brightener. In a field test on artificially infested soybean, mortality from PiNPV at 6×10^{11} PIB/ha of second to fourth instars was significantly increased by the addition of 0.1% and 1.0% brightener. A field test on a natural population of soybean looper larvae of mixed age showed that 0.3% and 1.0% brightener significantly enhanced mortality from virus of PiNPV-treated (3-15×10^{11} PIB/ha) larvae. Mortality in 1.5×10^{11} PIB/ha PiNPV treatments was increased from 24.4% to 46.4% and 49.5% by the addition of 0.3% and 1.0% brightener, respectively (Zou and Young, 1996).

Results of tests with three baculoviruses—P. unipuncta NPV, P. unipuncta GV and Anagrapha falcifera (Kirby) NPV—against small armyworm on heading winter wheat showed that none of the viruses reduced larval numbers below that in the control in any treatment. Laboratory bioassay on semisynthetic diet had shown the viruses to be virulent against small larvae of armyworm (Steinkraus and Young, 1994; unpublished data. 1995). Research is continuing on natural mortality factors in P. unipuncta populations, including entomopathogenic fungi (Steinkraus et al., 1993c), viruses, nematodes and parasitoids (D.C. Steinkraus unpublished data).

Beauveria bassiana for Control of the Tarnished Plant Bug

The tarnished plant bug (TPB), Lygus lineolaris (Palisot), is an important pest of cotton, causing terminal abortions, shedding of squares and bolls and malformed seeds and lint (Pack and Tugwell, 1976; Hanny et al., 1977). Due to the development of insecticide resistance, the TPB is increasingly hard to control with insecticides. After boll weevil eradication and introduction of Bt cottons, TPB may become a major pest of cotton because it will no longer be controlled concurrently with insecticide applications made for boll weevils and heliothines. Sucking insects such as the TPB are generally not susceptible to microbial agents such as viruses, Bt's or protozoa; therefore, studies were initiated using the fungus Beauveria bassiana (Balsamo) Vuillemin for control of TPB (Steinkraus, 1996). The two B. bassiana isolates used were ARSEF 3769, isolated from TPB in Arkansas, and Mycotrol WP (Mycotech, Butte, Montana), a recently registered Beauveria product. ARSEF #3769 was highly effective versus TPB in laboratory assays and elicited a significant dose response (Table 1). Field tests on cotton and canola were promising. In 1993 tests, 92.6% and 88.5% of control TPB were alive

five and seven days posttreatment, respectively. In contrast, only 11.2% (n=143) and 25.3% (n=142) of the TPB in the cages sprayed before or after bugs were added were alive at five days posttreatment. By seven days posttreatment, 100% of both B. bassiana-treated groups had died of mycoses. Field tests in 1994 showed that B. bassiana spores persisted on the plants for up to four days on the plant, under ambient conditions of sunlight, temperature and relative humidity.

In a 1995 field test, significant numbers of bugs were killed in the imidacloprid, imidacloprid plus Mycotrol and Mycotrol alone treatments (Table 2). Most interesting was the result that combining imidacloprid and a low rate of Mycotrol resulted in significantly more mortality (97.9%) at five days posttreatment than any other treatment. Dead TPB held in moist conditions revealed that a high percentage of the bugs exposed to B. bassiana died with mycoses.

The results indicate that B. bassiana by itself could be an effective control agent for TPB in cotton if time until death of the pest is not a major factor. There may be situations, such as in alternate or trap crops that are breeding areas for TPB, or in wind strips, conservation areas and weedy field borders, in which killing the TPB in six to seven days is satisfactory. The data show that combining imidacloprid with B. bassiana significantly increased the mortality in exposed bugs above that of either material alone, although the reasons for this are unclear. Future studies directed at formulation, mass production and optimum delivery methods could result in a useful material for TPB control.

IMPLEMENTATION
Releases of Biological Control Organisms
Neozygites fresenii

The cotton aphid fungus, N. fresenii, has played a beneficial role in controlling aphid populations in the midsouth. In 1994 and 1995, N. fresenii was used in a classical biological control release in the San Joaquin Valley of California because the fungus does not appear to be present in the cotton growing areas of California and Arizona. Methods were developed to produce, store and release the fungus (Steinkraus et al., 1993b; Steinkraus and Slaymaker, 1992b). Aphids were infected in vivo and then, shortly before aphid death, placed at 75% RH. This results in inert, dry, infected aphids that can be stored for up to one year in a freezer. Dried infected aphids were brought to California and placed on leaves among A. gossypii on cotton at Shafter (1994) and Chowchilla

(1995). The releases were successful in initiating infections in the field aphid populations. Aphids collected from some replicates had 84% infection rates in 1994 and up to 50% in 1995 (D.C. Steinkraus, unpublished data). If *N. fresenii* becomes established, it could be a useful addition to IPM in California cotton.

Ichneumon promissorius

Release and establishment of exotic natural enemies to reduce overall densities of selected insect pests has had significant success in the U.S. over the past 100 years (DeBach and Rosen, 1991). The bollworm/tobacco budworm (heliothine) complex is attacked by a large number of beneficial insect species in most agricultural and natural habitats. However, there are no known parasites of the pupal stage of this pest complex. Heliothines burrow in the soil and form pupal chambers approximately 10 cm below the soil surface (Kring et al., 1993). A parasite from Australia, *Ichneumon prommissorius* (Wilkinson) (Hymenoptera), was identified that burrows into heliothine pupal chambers and oviposits a single egg within a pupa. In cooperation with scientists in Georgia, Oklahoma and Texas, a large-scale rearing and release program was implemented in an attempt to establish this beneficial species in the U.S. Center scientists developed and refined rearing protocols and coordinated release programs in the four states. The release program was initiated in 1993, with releases of more than 75,000 parasites in Arkansas alone. Although recoveries of both adult and immature (inside heliothine pupae) parasites have been made in Arkansas during each year of release, establishment cannot be verified until recoveries are made several generations after the last releases.

Entomopathogenic Nematodes for Control of Bollworm

Few natural enemies in Arkansas greatly impact *H. zea* once it enters the soil to pupate (Kring et al., 1993). In studies on pupal mortality in an Arkansas field corn, a small percentage of *H. zea* pupae were killed by a facultatively pathogenic nematode, *Chroniodiplogaster aerivora* (Cobb) (Steinkraus et al., 1993a). The nematode was evaluated in the laboratory as a natural enemy of *H. zea* and found to be unsatisfactory. Recently a new nematode species, *Steinernema riobravis* Cabanillas, Poinar and Raulston, was discovered in Texas, causing regular and significant mortality in *H. zea* pupae in irrigated corn. In Arkansas field corn tests in 1994, significant numbers of *H. zea* larvae pupating in soil were killed and infected with *S. riobravis* (Table 3). *Steinernema riobravis* was capable of infecting *H. zea* in both irrigated and unirrigated soil (Feaster and Steinkraus, 1996). It kills *H. zea* quickly, preventing moths from emerging and infesting cotton or other crops. This nematode deserves further study in Arkansas on persistence and spread of *S. riobravis* and the possibility of area-wide introduction and establishment.

Table 1. LC_{50} and LC_{90} values for *Lygus lineolaris* 5th instar nymphs and adults immersed in *Beauveria bassiana* spore suspensions.

Day Post Treatment	LC_{50}		LC_{90}	
	nymph	adult	nymph	adult
4	1.5×10^8	-	9.0×10^{11}	
5	2.2×10^6	3.7×10^6	8.2×10^8	4.0×10^8
6	2.0×10^5	-	3.2×10^7	
7	9.0×10^4	8.4×10^4	1.4×10^7	1.2×10^7
8	2.0×10^4	-	2.5×10^6	-

Table 2. Mortality in *Lygus lineolaris* (tarnished plant bug) in a 1995 field test in Arkansas.

Treatment	Mean percentage mortality days posttreatment			
	4	5	6	7
Imidacloprid + Mycotrol Low	86.3 a*	97.9 a	98.9 a	98.9 a
Imidacloprid	63.5 a	67.3 b	67.3 b	75.0 ab
Mycotrol high	37.7 b	61.3 b	83.9 ab	91.5 a
Mycotrol low	27.6 bc	52.0 b	70.4 b	84.7 ab
Silwet Control	6.7 c	7.6 c	9.5 d	18.1 c
Water Control	10.7 c	13.6 c	19.4 cd	27.2 c

*Means with the same letter in a column are not significantly different. T-tests (LSD), $\alpha = 0.05$

Incorporation of *Bacillus thuringiensis* in Cotton Pest Management Systems

The tobacco budworm and bollworm are major pests of the cotton crop in the United States, and the budworm has developed widespread resistance to recommended chemical insecticides. An alternative strategy for management of the budworm-bollworm complex often involves the use of Bt products in combination with a chemical ovicide and (or) larvacide.

Effectiveness of Bt (Javelin WG) against first instar tobacco budworm and bollworm was studied in the laboratory on cotton terminals and in the field on cotton. Javelin was more effective against the tobacco budworm than against the bollworm, and most mortality on both species occurred within four days (Ali and Young, 1993a). Application of 0.5 lb of Javelin in the field to first instars resulted in mortality after three days of 69 and 57% for budworm and bollworm, respectively. Survival in both species generally decreased as the Javelin rate increased. In bioassays on semisynthetic diet, Bt products were three to five times less effective against bollworm than tobacco budworm (Ali and Young, unpublished data). In another field test, Bt effectiveness decreased as larval size increased.

Spray volume did not affect the activity of Bt against tobacco budworm in treated cotton terminals. *Bacillus thuringiensis* on cotton in the field was rapidly lost due to sunlight (half life approximately 2.5 days), and persistence of Bt did not differ among rates (Ali and Young, 1992b, 1993b, 1996). Control of tobacco budworm on cotton did not differ with stage of development of the cotton plant (Abbas Ali and S.Y. Young, unpublished data).

The ovicidal and larvacidal activity of several chemical insecticides (Bolstar 6EC, Larvin 3.2 and Ovasyn) alone and in mixtures with Bt were evaluated against tobacco budworm eggs on cotton. The percentage of hatched eggs two days old at application ranged from 21.7% to 41.1% with the ovicides. Mortality of larvae that hatched from these eggs was also high. The chemicals were much less effective against younger eggs, and egg hatch ranged from 44.8% with thiodicarb 3.2 (0.125 lb/acre) to 75.8% with sulprofos 6 EC (0.125 lb/acre). *Bacillus thuringiensis* did not prevent egg hatch but did provide a high level of mortality in larvae from eggs that were close to hatching at application. Chemical insecticide-Bt mixtures significantly reduced survival of hatching larvae over that of the chemical insecticide alone only for Ovasyn-Bt mixtures applied against newly-deposited eggs (Ali and Young, 1993c).

The increased use of Bt in cotton pest management systems (with foliar Bt or genetically engineered Bt-cotton) indicates that an increased reliance on pest control provided by beneficial insects may be possible. Parasites (especially Trichogramma spp.) attacking heliothine eggs account for significant suppression of heliothine populations (40-50%) in the absence of insecticides (Metcalf and Luckman, 1982) but are severely hampered by standard insecticides targeting early-season pests (Jacobs et al., 1984). Recently completed studies found that ovicides (always tank-mixed with Bt in Arkansas) reduced parasitism rates as severely as standard insecticides (Fig. 4) (Kring and Smith, 1995). Center experiments found, however, that ovicides did not kill the majority of developing parasites within the host eggs. Parasites success-

Table 3. Mean percentage (SE) of bollworm larvae or pupae infected with *Steinernema riobravis* and percentage survival to adult stage after collection from soil.

No. nematodes/ m² soil	Post-treatment irrigation	% infected			% *H. zea* survival to adult		
Experiment 1							
0		0	(0)	a*	59.4	(1.3)	a
	+	0	(0)	a	66.9	(4.9)	a
3.7 × 10⁶		72.6	(2.6)	b	17.1	(5.9)	b
	+	79.2	(7.6)	bc	8.5	(4.5)	bc
1.2 × 10⁷		85.9	(2.7)	bc	9.4	(3.0)	bc
	+	91.3	(5.0)	c	1.1	(1.1)	c
Experiment 2							
0		0	(0.0)	a	66.2	(4.9)	a
	+	0	(0.0)	a	60.6	(8.2)	a
5.2 × 10⁵		69.7	(12.6)	b	11.1	(4.9)	b
	+	78.5	(5.5)	b	7.6	(4.6)	bc
5.3 × 10⁶		89.4	(4.7)	b	0	(0.0)	c
	+	94.8	(3.1)	b	0	(0.0)	c

*Within a column, means followed by the same lower case letter are not significantly different, ANOVA, Fisher's Least Significant Difference, $P \leq 0.05$. Data were transformed (arcsin sqrt [%/100]) before analysis.

fully emerged from more than 90% of parasitized eggs treated with ovicides. Although parasites emerged from less than 50% of parasitized eggs treated with a standard insecticide (Fig. 4), this was higher than expected. It is apparent that the host egg affords a considerable degree of protection for the developing parasites. This high degree of survival of developing parasites after treatment identified a need for studies on the impact of insecticide residues (on cotton leaves) on emerging parasites. Surprisingly high mortality (>90%) was observed in adult parasites after emergence even five days after treatment with both the ovicide and the standard insecticide (Fig. 4). It was not until 10 days after treatment that the ovicide residues disappeared, although residues from the standard insecticide were still in high enough concentrations to cause more than 50% mortality of adults. Under field conditions parasites require up to 10 days to complete development (from oviposition to adult emergence) within the host egg. These studies indicate that only the proportion of the parasite population in the earliest stage of larval development within the host egg has a significant chance of surviving an application of the Bt-ovicide mixtures currently recommended in the mid-south. These findings explain the low levels of parasitism observed in treated fields and indicate that unless ovicides can be omitted from the Bt applications, the parasites attacking heliothine eggs should not be relied upon for any significant level of added control.

Beneficial arthropods are also thought to play a role in management of larval tobacco budworms and bollworms when Bt and (or) ovicides are used. Results of large plot tests in 1993, 1994 and 1995 have shown, however, that populations of beneficial arthropods are significantly reduced by ovicidal rates of thiodicarb as well as chemical larvacides. Only Bt alone failed to reduce beneficial arthropods, and Bt used alone did not provide a significant level of heliothine control in the tests. Given the severity of impact of the tested insecticides on populations of beneficial insects, the current cotton pest management systems cannot consistently rely on any significant level of pest suppression by beneficial arthropods. When the population was mostly bollworm (97%), the Bt-thiodicarb mixture was significantly less effective against this pest complex than conventional chemical larvacides.

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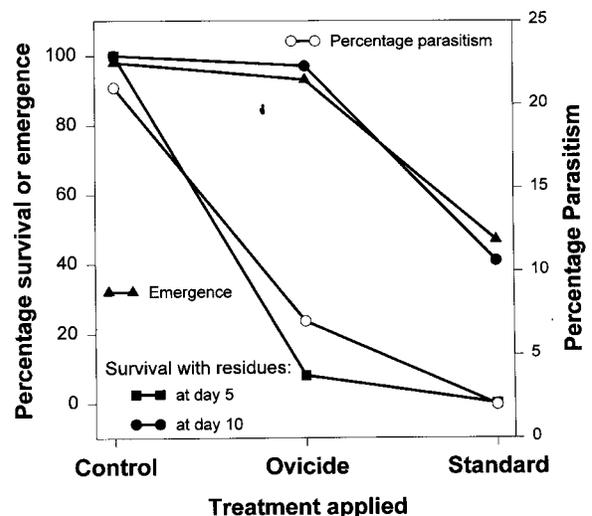Feaster, M.A., and D.C. Steinkraus. 1996. Efficacy of Steinernema

Fig. 4. Impact of chemical ovicide and standard insecticide applications on *Trichogramma pretiosum* percentage parasitism, emergence from treated host eggs and survival in contact with insecticide residuals.

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Chapter 2

Biological Control
of Plant Diseases

Biological Control of Plant Diseases

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INTRODUCTION

lant diseases are important constraints to the economical production of crops. In addition to pesticides, cultural practices such as crop rotation, tillage and soil fertility have long been relied on for the management of diseases. Through research we have realized that many of these cultural practices suppress disease by encouraging the large and diverse population of microorganisms in soil or on plants to suppress plant diseases. Thus, many of our diseases currently are being managed through biological control. The challenge for researchers is to identify the organisms responsible for disease suppression and develop and utilize these organisms as economical control strategies. What follows are a number of research projects designed to isolate, identify, develop and utilize biological organisms for the control of plant diseases.

BIOLOGICAL CONTROL OF SUDDEN DEATH SYNDROME OF SOYBEAN

Sudden death syndrome (SDS), caused by the fungus Fusarium solani, is a mid-season disease of soybean and is associated with intensive soybean production systems. Fungi were isolated from the rhizosphere and rhizoplane, the zone of soil influenced by the presence of the root, of healthy or mildly diseased plants collected in areas of fields severely affected by sudden death syndrome of soybean at the Pine Tree Station, Colt, Arkansas; a field near Stuttgart, Arkansas; and a field near Piggot, Arkansas. Forty-six of the 151 candidate fungi evaluated demonstrated control activity when applied to roots of healthy two-week-old 'Lee 74' soybeans and challenged with conidia of the pathogen four days later based on foliar disease symptoms. Of the 46 isolates selected from the initial screening tests, 19 were F. solani; 13 were F. oxysporum; there was one each of F. avenaceum, F. acuminatum, Pithomyces sp. and Diplodium sp.; two were Gliocladium spp., and nine isolates were not identified. Sixteen of the isolates demonstrated significant control activity in two further tests: F. solani isolates PC8SC, PC5SD, PC6PD, PD16PF, PC5SE and PC15SD; F. oxysporum isolates PD6PB, PC6PB, PD5PD, PC5SC, PD6PD, PC9SE and PD7SD; Pithomyces sp. PD95A; and unknown isolates PC9PA, PC8SD and PC9PA.

Fourteen isolates from the results in initial greenhouse tests were evaluated in the field in 1992. Three additional isolates were included in a 1993 test. Similar to greenhouse evaluations, Lee 74 seedlings were grown in vermiculite in the greenhouse for two weeks and then dipped in a suspension of the biocontrol agent, replanted and incubated in the greenhouse for four days and transplanted in a field naturally infested with the SDS pathogen at the Pine Tree Station. In both years, high levels of variability resulted in no significant differences in disease development among any of the fungi tested and the control. However, some treatments had much less disease than the control, and one isolate of F. oxysporum, PC5PD, had less than half the level of disease of the control both years. The three additional isolates tested in 1993 also had less disease than the control. In 1992, soybean yields with one isolate were significantly greater than the control.

Many of the fungi isolated from the rhizosphere and rhizoplane of soybean plants controlled SDS in greenhouse evaluations. Fungi dem-

onstrating biological control activity were primarily isolates of *F. solani* and *F. oxysporum*. The predominance of isolates of *Fusarium* spp. demonstrating biological control activity may reflect the population level of this genus in the soil, the isolation methods or an inherently higher level of biological control activity by this fungus than by other fungi. Competition for infection sites or nutrients or induced resistance may be the control mechanisms of the isolates in the study reported here, since none demonstrated antibiosis, the production of toxic chemicals, at either high or low nutrient levels. This is the first report of biological control of SDS by any microorganism.

BIOLOGICAL CONTROL OF SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES*

Soybean-cyst nematode (SCN), *Heterodera glycines* Ichinohe, was first detected in the Mississippi River Valley in 1956 in Tennessee and Missouri and soon was found in other soybean-growing areas. Concerns about the toxicity of chemical pesticides used to control nematodes have increased the interest in alternative control strategies. Biological control with nematode-parasitic fungi, particularly parasites of nematode eggs and females, have shown potential.

A sterile hyphomycete fungus, ARF18, was isolated from nematode-suppressive soils in Arkansas in 1987. ARF18 has resisted extensive efforts to induce sporulation, and few characteristics can be used to identify it. Morphological and culture characteristics include clear, septate hyphae (4.0 μm wide) with Woronin bodies and the formation of sclerotium-like structures (SLS) on certain media.

Populations of SCN were extracted from 76 soil samples from 33 counties and parishes in 10 states along the Mississippi and Missouri Rivers. Each population was identified to race by standard procedures. Surface-disinfested eggs were placed on agar in Petri plates, and growth of ARF18 was seen within five days. ARF18 was isolated from eight populations in four states, Kentucky, Louisiana, Mississippi and Tennessee, where ARF18 had not been found previously. All of the new locations were south of 37° N Latitude.

One isolate of ARF18 from Arkansas was grown on 31 media made with extracts from parts of various plants. Growth of the fungus ranged from very poor on wheat straw or radish extract to very good on green pea juice or green pea extract medium. The eight isolates from other states and the two from Arkansas grew well on potato dextrose agar (PDA), corn meal agar (CMA) and nutrient agar (NA), and minor but significant differences in growth were observed. Average radial growth was greatest on CMA followed by PDA then NA. All isolates formed SLS on the bottom and sometimes on the sides of the Petri plates on CMA and PDA but not on NA. SLS averaged 0.20 mm (range 0.05-1.00 mm) in diameter and numbered as many as 350 per cm². Growth of ARF18 was not affected by pHs from pH 5 to pH 8.5.

ARF18 is unique among nematopathogenic fungi in that it penetrates directly through the cyst wall of SCN then kills the eggs. Direct penetration is particularly important for biological control of cyst nematodes because the cyst wall protects the eggs. ARF18 parasitizes eggs, second-stage juveniles, young females and mature females. ARF18 also parasitizes the nematode species *H. schachtii*, *H. leuceilyma*, *H. trifolii*, *H. lespedezae*, *Cactodera betulae* and *Meloidogyne incognita*. *Globodera tabacum virginiae* and *Rotylenchulus reniformis* are not parasitized. The fungus does not affect the growth of soybean, cotton, rice or wheat.

When the 10 isolates of ARF18 were tested for comparative parasitism of eggs in vitro, cysts in control dishes had an average of 259 ± 90 eggs and J2 in two experiments. The J2 numbers represented eggs that hatched during the test. The cysts had an average of 225 ± 93 or 84% healthy eggs, 9 ± 8 (range 0-40) or 4% unhealthy eggs and 25 ± 23 (range 3-89) or 12% J2. Unhealthy eggs had an abnormal appearance but were not necessarily parasitized. In contrast, cysts on fungus-treated plates averaged 6% healthy eggs, 66% parasitized eggs and 24% J2, most of which hatched during the test, for 10 isolates on three media in two experiments. Healthy eggs, parasitized eggs and J2 averaged 5%, 71% and 24% on CMA; 6%, 74% and 20% on PDA; and 8%, 64% and 28% on NA, respectively. When the two experiments were analyzed separately, significant differences (P = 0.01) were found among media,

among isolates and in the interactions among media and isolates.

Formulations of inoculum of the fungus have been evaluated. The efficacy of the pelletized inoculum of the fungus is affected by the size of the pellet and the concentration of the fungus within the pellet. To test the efficacy of different formulations, ARF18 was cultured on seven different organic or inorganic carriers (diatomaceous earth, millet, southern clay, pea, alginate pellets, rice and wheat grain) for one month and tested in the greenhouse. Different inoculum levels were applied and compared with the control without additives or with sterilized pellets 2, 5 and 10 g/10-cm-d pot. After two months, egg counts indicated that all formulations were effective ($P > 0.01$) in controlling SCN with population level reductions of 89-99%. A low inoculum level (2 g/pot) was as effective as a high level (10 g/pot). Diatomaceous earth was the most effective carrier. Field applications do not always reduce SCN populations the year they are applied, but tests involving nonhosts of SCN indicate that ARF18 should stay in the soil and become effective over a period of time. The economics of formulation and application of ARF18 have not been determined.

Nine SCN-susceptible soybean cultivars, 'Davis', 'FFR561', 'Hutcheson', 'Lee74', 'Lee No-Nod', 'P9391', 'P9591', 'Sharkey' and 'Tracy', were grown in soil infested with SCN race 14 and ARF18. Fungus-infested soil had three to five SCN eggs/g soil compared to 111-245 eggs/g soil in the controls, a reduction of 95-99%. Hutcheson soybean was grown in soil infested with ARF18 and SCN race 2, 3, 4, 5, 9, 12 or 14. Fungus-infested soil had five to 12 SCN eggs/g soil compared to 69 to 196 eggs/g soil in the controls, a reduction of 83-97%. This study indicates that the efficacy of the fungus ARF18 is not affected by soybean cultivar or SCN race.

ARF18 was tested in mixtures with each of three predatory fungi, Dactylaria brochopaga, Althrobotrys oligospora and Hirsutella rhossiliensis, and each was tested alone for the control of SCN and root-knot nematode (RKN), M. incognita. Tests were conducted in the greenhouse and microplots. ARF18 reduced SCN population levels more than 90%, but parasitism of RKN was less. There was no evidence of additional effects of predatory fungi for either soybean cyst or root-knot nematode.

DNA restriction fragment length polymorphisms (RFLP) have been used increasingly to characterize fungi. These techniques can be particularly useful for characterizing genotypes of fungi of unknown taxa. The mtDNA RFLP analysis detected no polymorphisms among the 10 geographically diverse isolates of ARF18 examined. All 10 isolates exhibited identical mtDNA fragment patterns with each of three endonucleases, EcoRI, HaeIII and PVUII.

The biological, chemical and physical factors varied greatly among the 76 samples. Logistic regression analyses gave low predicted probabilities values, indicating that none of the soil factors were suitable for predicting the presence of ARF18 in a particular soil.

Microplot tests in the summer of 1995 in which a clay formulation supplied by a commercial manufacturer was used had a 60%+ reduction in total egg numbers at a rate of 28 kg granule/ha. The number of healthy eggs was reduced even further. A rate of 11.2 kg/ha also greatly reduced the number of healthy eggs, but the differences were not significant.

Greenhouse tests during the winter of 1995-96 have indicated that isolates of a fungus that is similar to ARF18 in morphology but different in RFLP studies may be much more effective as a parasite of SCN.

BIOLOGICAL CONTROL OF SHEATH BLIGHT OF RICE WITH INDIGENOUS RICE-FIELD MICROORGANISMS

Microorganisms from rice fields were isolated and evaluated for the control of sheath blight, caused by R. solani AG1-IA, and their integration into commercial rice disease management.

Soil and crop debris samples were collected in 1992 and 1993 from 36 rice fields and two research plots in 10 Arkansas counties featuring conventional rice/soybean culture. Overwintered sclerotia of R. solani AG1-IA were extracted from samples by wet seiving and hand picking. Sclerotia were surface disinfested 3 min in 1% bleach then plated on CMA amended with antibiotics and NA to assess viability and microflora. Plates were incubated at room temperature (RT) or 10 C for one to four weeks. Fungi and bacteria grow-

ing from sclerotia were isolated and stored at 10 C until needed.

Dual cultures of R. solani and the isolated fungi and bacteria on PDA- and CMA-coated cellophane revealed Trichoderma, Pythium, Penicillium, Gliocladium, Aspergillus and Mortierella spp. to be the most antagonistic. Trichoderma, Pythium and Gliocladium spp. were intensely mycoparasitic on R. solani, and certain isolates appeared to enzymatically dissolve the pathogen's hyphae. Other organisms tested were more weakly antagonistic, mycoparasitic or non-interactive than those mentioned.

Field surveys for water-line microflora of rice in several counties in eastern Arkansas were conducted between early July (internode elongation) and mid-August (heading). Tillers (100) were randomly collected from the same area of these fields at weekly intervals, and stem sections (0.5 cm) of the water line area of each tiller were excised and placed on CMA + and NA plates and incubated (RT) under lights for 7-14 days. Fungi and bacteria from the segments were isolated and stored as before, and laboratory assays for antagonism, competition and mycoparasitism to R. solani were conducted.

Trichoderma and Pythium spp. were again the most aggressive mycoparasites of R. solani in culture while several binucleate Rhizoctonia spp., R. oryzae, Sclerotium hydrophilum and R. oryzae-sativae were the most competitive. The last three fungi are epiphytes or minor parasites of the rice plant, with life cycles similar to the sheath blight fungus. Approximately 75% of the bacterial isolates tested were antagonistic to R. solani in culture, with level of antagonism dependent on the nutrient medium. Additional fungal (117) and bacterial (181) isolates were eventually tested in dual culture with R. solani but generally did not show high levels of antagonism.

Promising candidates from the dual culture assays were screened on rice plants in the greenhouse under conditions conducive to sheath blight. Depending on the organism, candidates were inoculated as floating alginate pellets, sclerotia or cell/spore suspensions sprayed onto plants. Two rice cultivars, 'Katy' (considered sheath blight tolerant) and 'Lemont' (highly susceptible), were used in these tests. Candidates were co-inoculated with R. solani or inoculated one week prior to R. solani inoculation to allow

prior establishment depending on the experiment.

Organisms that consistently reduced sheath blight incidence (I) and severity (RLH) included R. oryzae, S. hydrophilum, R. oryzae-sativae, UB106 bacterium and one Gliocladium sp. An unusual Penicillium sp. recovered and tested in 1994 also was effective under greenhouse conditions but was not field tested.

Various methods of formulation and application were investigated in the laboratory using different candidate organisms, usually fungi. Most experiments focused on incorporation of the organisms into floating alginate pellets as a logical method of application to flooded rice fields. Mineral oil (15% v/v) seemed the best overall floatant tested. Most fungi tested survived at least 12 months in alginate/mineral oil pellets at 10 C. Bacteria did not survive or store well in alginate pellets, even when calcium gluconate solution was used as the gellant instead of the standard calcium chloride solution. Bacteria and certain conidial fungi continued to be applied as spray suspensions in tests for this reason.

The most promising candidate organisms from greenhouse experiments were assayed in the field during 1993 and 1994 using aluminum ring plots. Organisms were co-inoculated with R. solani approximately 10 days prior to the panicle differention stage of the rice. Inoculum was applied as floating alginate pellets or as sprays of cells or conidia as previously mentioned. Two locations (Pine Tree Experiment Station, Colt, Arkansas, and a grower's field in Lawrence County) were used with the same cultivar, 'Lacassine', at both sites. R. oryzae was the most consistent organism in reducing sheath blight under field conditions (Table 1). Some isolates of this fungus cause a minor disease known as bordered sheath spot of rice, although our isolate did not cause any noticeable symptoms on rice. Apparently, R. oryzae can successfully compete for the rice sheath with R. solani in the field and, as demonstrated in culture, may hyperparasitize the pathogen as well. S. hydrophilum also reduced sheath blight in the field tests, although less effectively than R. oryzae. Other candidates were inconsistent at best. Both R. oryzae and S. hydrophilum also reduced yield loss from sheath blight (Table 1) at a level equivalent to the standard fungicide used, propiconazole.

Table 1. Results of field tests on biocontrol of sheath blight of rice, 1993 and 1994.*

	1993			1994		
Location/treatment	DI[†]	Yield[‡]	Loss[§]	DI	Yield	Loss
Pine Tree						
Not Inoculated	0	6281 a[¶]	0	0.17	6003 a	0
R. solani	0.79	4925 c	22	0.71	4967 c	17
Propiconazole[#]	0.64	5501 b	12	0.70	4711 c	22
S. hydrophilum	0.61	5512 b	12	0.46	5843 a	3
S. hydrophilum	0.59	5533 b	12	0.60	5961 a	1
R. oryzae-sativae	0.65	5480 b	13	0.54	5544 b	8
R. oryzae	0.51	5811 ab	7	0.23	6110 a	+ 2
Gliocladium sp. (GL1)	0.72	5149 bc	18	0.51	5726 ab	5
UB106 Bacterium	0.74	5427 b	14	0.68	5341 b	11
Lawrence						
Not Inoculated	0.22	5480 a	0	0	6976 a	0
R. solani	0.86	3685 e	33	0.83	5053 d	28
Propiconazole	0.39	5085 b	7	0.77	5608 c	20
S. hydrophilum	0.54	4925 b	10	0.64	5982 b	14
S. hydrophilum	0.68	4519 c	18	0.69	6100 b	13
R. oryzae-sativae	0.71	4647 c	15	0.66	6100 b	13
R. oryzae	0.44	4850 b	12	0.28	6933 a	1
Gliocladium sp. (GL1)	0.82	4187 d	24	0.75	5459 c	22
UB106 Bacterium	0.61	4316 d	21	0.71	5555 c	20

*R. solani = Rhizoctonia solani AG1-IA applied as floating alginate pellets; All treatments except "Not Inoculated" had Rhizoctonia solani applied as floating alginate pellets in addition to the listed treatment.
[†]DI = mean disease symptom height/tiller height.
[‡]Yield = kg/ha at 12% moisture.
[§]Loss = percent loss as compared to uninoculated control.
[¶]Means followed by the same letter were not significantly different according to Tukey's HSD test (P = 0.05).
[#]Tilt 3.6EC (Ciba-Geigy Corp.) applied at 320 ml ai/ha in 94 l/ha water approximately 10 days after panicle differentiation.

BIOLOGICAL CONTROL OF SOILBORNE WHEAT DISEASES

The ability to colonize the rhizosphere is considered essential for bacteria to function as biological control agents for soilborne plant pathogens. A study was conducted to determine if selected strains in the genera Bacillus (D-60R, D-39Sr. 147R, 58R), Pseudomonas (2-79R), Streptomyces (D185S), Xanthomonas (88SE) and a coryneform bacterium (D-56SR) (with antibiotic-resistance markers) reported to colonize wheat roots, inhibit root pathogens and/or improve wheat growth and yield would colonize wheat roots under environments diverse from those from which they were isolated. Field plots were established on a poorly drained Tunica silty clay soil at the Northeast Research and Extension Center, Keiser, Arkansas, and on a well-drained Roxanna silt loam soil at the Vegetable Substation, Kibler, Arkansas in 1990 and 1991. Winter wheat was the previous crop at both sites. The experimental design was a split-strip plot with the soil treatments (nontreated, fumigated, low and high inoculum of Gaeumannomyces graminis var. tritici (the pathogen causing take-all) as the main plots and seed treatments (strains and checks) planted as strips across the main plots. Plant samples for determining rhizosphere colonization were collected four to six weeks after planting and again in the early spring.

This study demonstrated that the ability of selected bacterial strains to colonize roots of soft red winter wheat from seedborne inoculum was stable over four environments in Arkansas that differed in soil texture, matric potential or oxygen diffusion rate. Strain 2-79R had the highest overall rhizosphere population on seminal roots in the fall and seminal and crown roots in the spring (7.8, 6.6 and 6.2 colony forming units (cfu)/ g dry root weight, respectively), which were similar to rhizosphere populations of another derivative of strain 2-79 on soft white winter and spring wheats in the state of Washington where it was isolated. Strain D-39Sr had significantly

greater relative colonization of crown roots than strains D-56SR or 2-79R. All strains examined had better colonization of crown roots in the spring than strain D-185S. For strains D-39Sr, D-56SR, D-60R, 58R and 88SE, the average rhizosphere population for each strain on seminal roots in the spring was 4.9, 5.3, 4.5, 4.3 and 5.5 cfu/g of root, respectively, comparable to rhizosphere populations on hard red spring wheat in Montana 83 days after planting. The Streptomyces strain D-185S, however, tended to have higher populations in Montana than in Arkansas.

It appears possible to develop bacterial biological control agents that colonize diverse wheat genotypes well under diverse environments. Rhizosphere populations were highly correlated (r = 0.74 - 0.76) with initial population on the seed, and rhizosphere populations in the spring were highly correlated (r = 0.91) with rhizosphere populations in the fall. The ability to colonize crown roots that develop from late fall through early spring appears to be the most stringent test of rhizosphere competence. This study also demonstrated that competition from the native soil microflora is not a severe impediment to establishing certain bacterial strains in the rhizosphere using seedborne inoculum, with strains colonizing seminal roots almost as well in fumigated and nonfumigated soil. Rhizosphere colonization by these strains was not associated with disease suppression or enhanced plant growth or yield.

Pythium root rot of wheat occurs wherever wheat is grown, with Pythium spp. being the most frequently isolated root pathogen from soft red winter wheat in Arkansas. No cultivars are resistant to Pythium root rot, and fungicide seed treatments do not give sufficient disease control. A study was conducted to identify bacterial strains from wheat roots in Arkansas that suppressed Pythium root rot and compare their efficacy to bacterial strains from other locations.

More than 600 bacterial strains from the rhizoplane were obtained from wheat roots at two locations in 1991. Strains were evaluated for control of Pythium root rot in greenhouse assays, and eight strains from Arkansas and three strains provided by D.M. Weller suppressed Pythium root rot in at least two of five assays. Bacterial strains that suppressed Pythium root rot in growth chamber assays were evaluated further for in vitro

antibiosis against three Pythium spp. and for efficacy under field conditions.

Biological seed treatments significantly (P = 0.05) reduced incidence of Pythium infection in two of four location-years from 1991-1993. Compared to the nontreated check, strains 2-79R (Pseudomonas fluorescens), 1-23 (Burkholderia cepacia) and 1-30 (Pseudomonas sp.) reduced incidence of infection at Keiser in 1992, and strain 2-79R reduced incidence of infection at Kibler in 1993 (Table 2). The fungicide metalaxyl reduced the incidence of infection at Kibler in 1993. In field experiments in 1994, metalaxyl (Apron 30 FL) seed treatment followed by a metalaxyl (Ridomil 2E) soil drench at Kibler and Keiser and strain 5-40 (Pseudomonas sp.) plus metalaxyl seed treatment at Kibler reduced the incidence of infection on a root system basis. A greater number of treatments reduced the incidence of infection near the point of seed attachment. In 1994 Metalaxyl and strain 2-79R seed treatments appeared to be responsible for the reduced infection at Keiser and Kibler, respectively. Seed treatment with both strain 2-79R and metalaxyl was the only treatment that reduced infection at both locations. At Kibler, eight treatments had significantly (P = 0.10) greater yield than the nontreated check. These treatments included strains 1-23, 2-79R and 5-40 as seed treatments, 1-23 plus metalaxyl, 1-23 plus in-furrow spray, 5-40 plus metalaxyl, metalaxyl alone and in-furrow phosphorus.

This study demonstrated that several bacterial strains inhibited Pythium spp. in vitro and frequently suppressed Pythium root rot in growth chamber assays. However, in the field, root rot suppression and yield enhancement were incon-

Table 2. Effect of seed treatment on incidence of Pythium infection on wheat four to six weeks after planting, 1991-1993.

Seed treatment	Seedling with infected roots (%)			
	Keiser			Kibler
	1991	1992	1993	1993
1-23	93	54*	70	94
1-30	95	58*	75	88
2-79	93	58*	60	81*
Nontreated	93	75	55	95
Metalaxyl	-	63	47	85*

*Means within each column marked with an asterisk (*) were significantly (P = 0.05) less than the nontreated check.

sistent across experiments and generally small in magnitude.

BIOLOGICAL CONTROL OF THE COTTON SEEDLING DISEASE COMPLEX

Seedling diseases are the most important disease problem of cotton in Arkansas. This disease complex has continued to be important in spite of the widespread use of fungicides effective against these pathogens. Slower developing plants and skips in plant stand may result in difficulty in management of the crop and other pests the remainder of the growing season. Pathogens causing seedling diseases include *Pythium spp., Rhizoctonia solani* and *Thielaviopsis basicola*.

A number of biological agents from the University of Arkansas program, other Universities, the USDA and industry have been examined in a series of field experiments. Data from selected experiments representing the successes and challenges of working with biologicals are presented.

A field experiment in 1989 examined two registered biological products, Quantum and Dagger, for biological control of cotton seedling diseases at Southwest Research and Extension Center, Hope, Arkansas. Seedling diseases were severe in 1989 as a result of an extended cool and wet period after emergence. Isolation from seedlings from the nontreated control plots indicated that *Pythium spp.* were the pathogens most responsible for seedling disease, with *Pythium spp.* being isolated from 84% of the plants and *R. solani*

from less than 1% of the plants.

Stand counts were improved for all treatments over nontreated seed, except for the captan treatment and the Terraclor Super X treatment 11 days after planting (Table 3). It appeared that certain treatments did not extend plant protection over the period stand counts were taken. This was especially noticeable for captan and Dagger, which showed decreases in stand over time similar to nontreated seed. The biological treatments, Quantum (*Bacillus subtilis*) and Dagger (*Pseudomonas fluorescens*), provided protection from damping-off equivalent to that of a standard fungicide treatment under the severe disease pressure that occurred. In most field studies, biological agents did not increase cotton plant stands while chemical fungicides gave significant stand responses. Additional biological control agents tested have included isolates and formulations of *Gliocladium virens, Trichoderma harzianum* and *Arthrobacter globiformis*.

In an effort to expand the use of alternative control strategies, additive biological control strategies were examined to control seedling diseases of cotton using 16 interrelated cotton genotypes and the microbial products Kodiak (*Bacillus subtilis*), Dagger and GL-21 (*Gliocladium virens*) at two locations, the Delta Branch Station, Clarkedale, Arkansas, and the Southwest Research and Extension Center, Hope, Arkansas. Some differences in susceptibility of cultivars were demonstrated in controlled environmental studies. In 1994, differences in stand among cultivars in the field were similar to reaction of the

Table 3. Influence of chemical and biological treatments on cotton plant stand

Treatment*	Plant Stand (%)			
	11 days	21 days	42 days	Mean[†]
Seed treatment				
Metalaxyl+PCNB+Quantum[‡]	64 a[§]	65 a	62 a	64 a
Metalaxyl+PCNB[‡]	63 a	64 a	60 ab	62 a
Quantum[‡]	64 a	61 a	58 ab	61 a
Captan	52 ab	42 bc	40 cd	45 bc
Granular In-furrow				
Dagger G	59 a	52 ab	48 bc	52 ab
Terraclor Super X	56 ab	58 a	53 abc	56 ab
Nontreated	44 b	32 c	26 d	34 cd

*Rates for the treatments: metalaxyl (4.2%) + PCNB (16.7%) + Quantum (12.5%) at 7.8 ml/kg seed; metalaxyl (6.2%) + PCNB (25.0%) at 5.2 ml/kg seed; Quantum at 7.8 ml/kg seed; Dagger granular at 16.8 kg/ha; Terraclor Super X granular at 11.2 kg/ha; Captan 400 at 3.3 ml/kg seed.
[†]Significant time by treatment interaction.
[‡]Treatment applied to captan-treated seed.
[§]Means within a column followed by the same letter are not significantly different, LSD (P = 0.05).

lines in the growth chamber, especially to Rhizoctonia solani, the primary pathogen in both field studies. There was no interaction between microbial products and cultivar, and none of the microbial products increased stand while the fungicide seed treatment gave significant stand increases (Table 4).

Table 4. Plant stand for 16 interrelated cotton genotypes as influenced by chemical and biological treatments.

	Plant stand at 21 days(%)	
Treatment	Clarkedale	Hope
Fungicide*	58 a[†]	74 a
Kodiak	48 b	71 b
GL-21	40 c	
Dagger		72 b
Untreated	48 b	72 b

*RTU Baytan Thiram + Apron (2.0 + 0.5 ml/kg seed).
[†]Means within a column followed by the same letter are not significantly different, LSD (P = 0.05).

One of the critical aspects to successful control with biological agents is determining and solving problems that cause the erratic performance of some of these products, either by improving formulation or by changing the organisms used. Additive control strategies may be one option to increasing the consistency of disease control.

Chapter 3

Biological Control of Weeds

Biological Control of Weeds

D.O. TeBeest and G.J. Weidemann

INTRODUCTION

Since its inception, biological weed control using fungal plant pathogens has been achieved utilizing two major strategies. One, the classical approach, utilizes pathogens introduced from the geographic origin of the weed to suppress the weed population following introduction and release. This strategy is most appropriate in non-cropland situations in which weed suppression rather than outright control is desired. In recent years, several pathogens have been introduced into the continental United States and an additional two have been introduced into Hawaii to control weed infestations in parklands and rangelands using the classical approach.

The other major strategy, commonly called the bioherbicide approach, was developed in Arkansas. In this approach, an endemic pathogen is applied to the weed population in large numbers to achieve maximum control much like a chemical herbicide. This strategy is most appropriate to cropland, turf or other high-management systems in which maximum weed suppression is desired. Work began on this approach in 1969 and resulted in the commercialization of the first bioherbicide for row crops in 1982 when Collego™ was registered as a microbial herbicide to control northern jointvetch in rice and soybean fields of Arkansas, Mississippi, Louisiana, Tennessee and Missouri. Collego was sold commercially from 1982 until 1995 and was composed of the endemic fungus, *Colletotrichum gloeosporioides f.sp. aeschynomene*. Collego is considered the model for successful development and use of a pathogenic fungus as a microbial pesticide. Its development spurred interest in developing other pathogens as microbial pesticides worldwide. To date, over 200 pathogens have been evaluated in either the classical or microbial pesticide strategies. Of these, 15 are considered to be successful biological control agents.

Work on biological control of weeds with plant pathogens has shown that many recently discovered pathogens are not useful as microbial pesticides because of intrinsic biological limitations preventing effective use. Some of these limitations are host ranges that are either too broad or too narrow, environmental restrictions that prevent infection of plants in the field, an inability to sporulate in mass culture and host resistance. These limitations have instigated research on methods and techniques to reduce or eliminate these restrictions.

In addition, the widespread use of biotechnology has raised questions regarding the impact of using microbes in the environment. As additional products composed of fungal plant pathogens are developed and registered with the Environmental Protection Agency, the inadequacy of the research base for addressing potential risks associated with their use has become apparent.

In addition to studies of several new potential weed control agents, funds obtained through the Alternative Pest Control Research Center were utilized to conduct research on methods to enhance the efficacy of potential biocontrol agents and to address potential risk issues associated with the use of biocontrol agents. Both of these research areas have broad application to other research programs worldwide.

GENETIC AND PHYSIOLOGICAL ENHANCEMENT OF BIOLOGICAL WEED CONTROL AGENTS

Pathogen efficacy can be improved by modifying the genetics of the organism or by utilizing methods to enhance the physiological activity of the agent. Genetic enhancement can be achieved through traditional sexual mating or by utilizing biotechnology. Using the Collego agent, C. gloeosporioides f.sp. aeschynomene, as a model system, studies were conducted to investigate the potential of using sexual mating to improve pathogen efficacy and to develop an effective genetic transformation system for this organism. In addition, tank-mix additives were investigated for their ability to enhance the efficacy of this biocontrol agent.

Development of an Effective Transformation System for *Colletotrichum gloeosporioides*

The fungus Colletotrichum gloeosporioides f.sp. aeschynomene (CGA) infects and controls northern jointvetch in rice and soybean fields. However, it is unable to control a related weed species, Indian jointvetch, also found in rice fields. Repeated attempts to find a pathogen of Indian jointvetch or to select a strain of CGA pathogenic to Indian jointvetch were unsuccessful. As a result, genetic transformation was investigated for its potential to produce a genetic variant with the potential to control this additional weed.

An efficient transformation system usually is comprised of two parts, a reliable method of producing transformable protoplasts from cells that can incorporate exogenous DNA and a selectable marker that can be used to isolate transformed cells. The marker consists of a cloned gene on a plasmid vector that permits rapid selection of a transformed cell. Three different selectable markers, B-tubulin, hygromycin and nitrate reductase, were investigated to develop a transformation system for CGA.

Resistance to the antibiotic benomyl is encoded by a B-tubulin gene clone possessed by several fungi resistant to benomyl. For this reason, resistance to benomyl is a common selectable trait utilized in a number of fungal systems. Two plasmids, pBT6 and pSI50, encoding a tubulin gene from a benomyl-resistant strain of Neurospora were tested, and a plasmid, pCG7, containing a tubulin gene from a benomyl-resistant strain of C. graminicola was used in repeated transformation experiments with two strains of CGA. The experiments were conducted to determine if transformation was possible, if transformations were stable, if the vector was integrated into the fungal genome and if pathogenicity was retained (Table 1).

In general, colonies resistant to benomyl were obtained after transformation of CGA with plasmid vectors. However, with each plasmid and strain combination tested, it was apparent that transformation resulted in the loss of virulence to northern jointvetch. Average disease values of transformants were approximately half of that normally obtained for the wild type isolates. In many cases, virulence was nearly lost, while in a few cases virulence was retained at near parental levels.

Molecular analyses of the transformants also were conducted to determine if transformants were the result of integration of vector DNA into the fungal genome. The results of repeated analyses showed that in all cases the vector DNA was initially integrated into genomic DNA but was subsequently lost. However, the phenotype of resistance to benomyl was retained in nearly every case. It was concluded that benomyl resistance with the tubulin gene was not a useful transformation system for this fungus.

The antibiotic hygromycin B has been used as a selectable marker to transform several fungi. Two plasmids, pDH25 and pMSC1, encoding for hygromycin phosphotransferase, a gene that

Table 1. Pathogenicity of putative transformants of the fungus *Colletotrichum gloeosporioides* f.sp. *aeschynomene* with plasmids encoding resistance to benomyl.

Strain	Plasmid	Number of Transformants	Disease Index* (Avg)	Disease Index (Range)
3.1.3	pBT6	13	2.4	0.4 - 4.4
	pSI50	11	3.2	0.6 - 5.0
	pCG7	20	2.1	0.1 - 4.9
CLA5a	pCG7	3	2.1	2.2 - 4.5
	Total	47	Avg.2.8	0.8 - 4.7

*Disease index: 0 equals a healthy plant to 5 equalling death of northern jointvetch seedlings. Seedlings were inoculated at three to five days after emergence and incubated at 28 C.

detoxifies the antibiotic and permits direct selection on hygromycin, were used to transform CGA. Protoplasts were produced from spores, and transformants were selected on plates containing hygromycin. Twenty-seven hygromycin-resistant colonies were obtained after protoplast transformation with plasmid pMSC1. Nearly all of the putative transformants remained virulent on northern jointvetch (NJV) when inoculated to two- to five-day-old seedlings in replicated, controlled environment experiments (Table 2). Hygromycin-resistant colonies were re-isolated from all seedlings in these tests.

DNA analysis of a number of these isolates was conducted to compare integration and hybridization patterns of the HYG B gene in the genome of CGA. Southern blots showed that genomic DNA of all putative transformants hybridized with a 2-kb fragment of pMSC1 that contained the hygromycin phosphotransferase gene. Integration appeared to be random and unstable. After serial transfer on nonselective media, all transformants lost hybridizable fragments within the genome, although most retained resistance to hygromycin.

The results with a hygromycin selection marker were similar to results obtained with benomyl. Although selectable transformants were obtained, a hybridizable marker was not retained within the fungal genome although the resistance phenotype was maintained. These results make the hygromycin and benomyl resistance transformation systems unsuitable for use.

Nitrate reductase genes have been isolated and cloned from several fungi and have been used to selectively transform fungi. Nitrate reductase genes from several fungi were examined for use as selectable markers in CGA. Results of hybridization experiments with these plasmids showed that cloned nitrate reductase genes did not specifically identify a nitrate reductase gene in Colletotrichum. Therefore, polymerase chain reaction amplifications were used to identify a similar gene in Colletotrichum. Degenerate PCR primers were made using conserved amino acid sequences found in the nitrate reductase proteins of several fungi and plants. The products of several PCR reactions were run on a 0.8% agarose gel. Six bands ranging from 1.6 kb to 0.650 kb appeared on the gels. A Southern blot was made of the gel and probed with a 1.9 kb XhoI fragment from pNit3, the nitrate reductase gene from Neurospora. Four bands, designated A, B, C and D, approximately 1.6, 1.3, 1.0 and 0.650 kb, respectively, hybridized to the probe and were cloned.

Probes were made from the four cloned fragments and were hybridized to a Southern blot of HindIII digests of genomic DNA from CGA, C. trifolii, C. gloeosporioides (CRP) from pecan, A. niger and N. crassa. A probe constructed from band A hybridized to single bands in CGA and CRP approximately 4 kb in size. A probe constructed from band B hybridized to a single bands in CGA and CRP approximately 5 kb in size and to a single band in Neurospora approximately 1 kb in size. A probe constructed from band C hybridized to a single band in CGA approximately 2 kb in size and a single band in CRP-2 approximately 6.5 kb in size.

Most interesting, however, a probe constructed from fragment D hybridized to numerous bands (more than 30) in CGA (all sizes) and to only three bands in CRP. The fragment cloned as D appeared to be an unusual repetitive sequence. It has been found in CGA and, in similar form, in C. gloeosporioides f.sp. jussiaeae but is not repetitive in any other C. gloeosporioides. The repeat does not appear to hybridize with DNA from any other fungal species, including other species within the genus Colletotrichum. Approximately half of the repeat has been sequenced. The sequence information has been used to determine homology to other genes currently identified. At this time, no other genes have been identified that have a high degree of homology to this fragment.

Table 2. Pathogenicity of hygromycin-resistant transformants of *Colletotrichum gloeosporioides* f.sp. *aeschynomene* to northern jointvetch.

Experiment	Transformants	Plants	Disease Index*
1	27	54	5
2	27	216	5
3	27	216	4.5
			Avg. 4.6

*Disease Index: 0 equals healthy to 5 equalling plant death of northern jointvetch seedlings. Seedlings were inoculated at three to five days after emergence and incubated at 28 C.

Development of a Sexual Mating System for *Colletotrichum gloeosporioides*

Compatible isolates of *Colletotrichum gloeosporioides* are known to produce a *Glomerella cingulata* sexual stage on plants and in culture (Fig. 1) while others are not known to reproduce sexually. A search for sexually compatible isolates of *C. gloeosporioides* with different host specificities was conducted to determine if gene transfer was possible through sexual mating. Initially 12 *C. gloeosporioides* isolates were chosen based on their host specificities so that a wide range of host plant families were represented.

Sexual compatibility between strains of *C. gloeosporioides* with different host specificities was observed in laboratory experiments utilizing a defined medium and plant substrate (Table 3). However, it was unclear whether these same isolates could reproduce under more natural conditions since a sexual stage has not been observed in nature for many of the isolates. Stems of northern jointvetch plants were first inoculated with spores from either CGA or CRP2 and placed in a growth chamber for one week. These same stems were then inoculated with the other sexually compatible isolate. The plants were placed in a growth chamber for another one to three weeks,
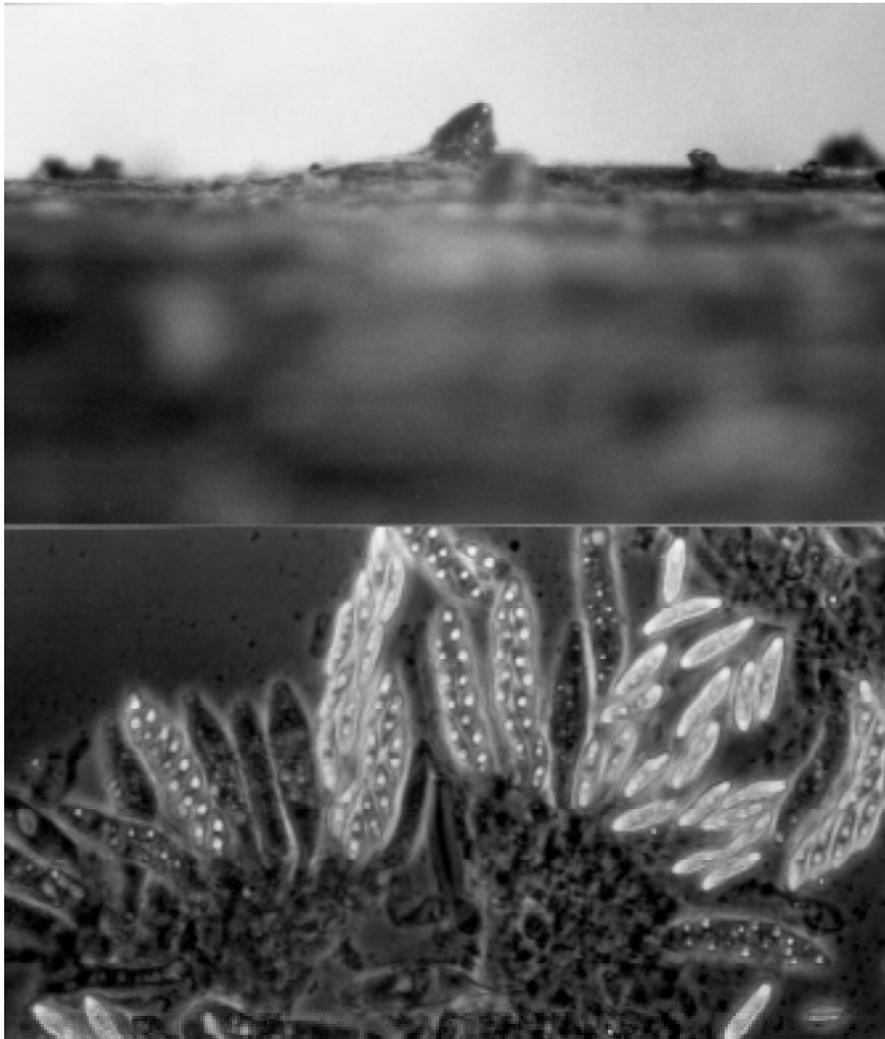

Fig. 1. The sexual stage of *Glomerella cingulata*. Panel A. Perithecia resulting from a cross of *Colletotrichum* isolates from pecan and northern jointvetch. Panel B. Asci and ascospores found in fertile perithecia of the above cross.

Table 3. Sexually compatible isolates of *C. Gloeosporioides/G. cingulata* from distantly related plant hosts.

Isolate*	Aeschynomene	Carya	Ludwigia	Malue	Mangifera	Medicago	Persea
Aeschynomene	-†	+	+	+	+	+	+
Carya		-	+	+	+	+	+
Ludwigia			-	+	+	+	+
Malus				_	+	+	+
Mangifera					-	-	+
Medicago						-	+
Persea							-

*Each isolate is identified by the genus of the plant from which it was isolated. (Common names: *Aeschynomene* - northern jointvetch; *Carya* - pecan; *Ludwigia* - winged water primrose; *Malus* - apple; *Mangifera* - mango; *Medicago* - alfalfa; and *Persea* - avocado.

†All of the isolates are self-sterile. A + indicates a successful cross (defined as *G. singulata perithecia* with asci and ascospores), and a - indicates an unsuccessful cross.

and stem portions were excised and placed on mating media. Following incubation, the stem pieces were examined for the presence of perithecia with ascospores under a microscope. Perithecia with ascospores were found on the northern jointvetch stems whether the plants were inoculated with CGA (northern jointvetch pathogen) or CRP2 (pecan pathogen) first.

Progeny were isolated from several *C. gloeosporioides* crosses to facilitate analysis of the mating system in *C. gloeosporioides*. Several new RFLP markers were developed for use in analyzing progeny from these crosses. Southern blots containing genomic DNA digested with restriction enzymes were probed with a cutinase gene from *C. gloeosporioides*, an rRNA gene repeat from *Neurospora crassa*, a glutamate dehydrogenase gene, a glyceraldehyde 3-phosphate dehydrogenase gene or the repeat sequence from *C. gloeosporioides f.sp. aeschynomene*. A mitochondrial DNA probe was used in the analysis of progeny from these crosses to determine the maternal parent. Results from these experiments showed that sexual recombination had occurred and that normal Mendelian inheritance of the nuclear markers was observed in the crosses (Table 4). On the other hand, mitochondrial DNA was uniparentally inherited by the progeny in a single perithecium, as expected, indicating that one isolate serves as the maternal parent.

Progeny from crosses were analyzed for pathogenicity to northern jointvetch and apple. None of the ascospore progeny were pathogenic to northern jointvetch and exhibited varying degrees of pathogenicity to apple, ranging from almost non-pathogenic (similar to CGA) to highly pathogenic (similar to CRP2). Progeny from CRP1 X CRP2 and CGA X CRP2 crosses were also analyzed for sexual compatibility with parents and siblings. Results of these backcrosses indicate that this fungus appears to have a bipolar mating system with a number of alleles at each locus.

Use of Adjuvants to Enhance the Pathogenicity of *Colletotrichum gloeosporioides*

The need for a free moisture period remains a significant constraint to assuring the consistent performance of most biocontrol agents. Previous studies demonstrated that crop oils and other surfactants could be utilized to reduce the free moisture requirement of bioherbicides without the use of specialized application equipment. Seven crop oils and 15 commercial surfactants were tested to determine their effect on spore germination and infection of northern jointvetch by CGA. Surfactants that showed a stimulatory effect on spore germination in plate studies were examined for their influence on infection of the weed host under disease-limiting conditions.

Table 4. Segregation of nuclear RFLP markers among progeny from crosses between *Glomerella cingulata* isolates Cla-5A and CRP-2.

RFLP Marker	Cla-5A Genotype	CRP-2 Genotype	Chi^2
GDH*	28	23	0.31
cutinase	23	29	0.49
rDNA	16	18	0.03
Repeat 1	21	31	1.56
Repeat 2	23	29	0.48
Repeat 3	30	22	0.94

*GDH = glutamate dehydrogenase, cutinase = cutinase probe, rDNA = ribosomal DNA, Repeat 1, 2 and 3 = bands 1, 2 and 3 from *Colletotrichum*. Chi^2 values are the statistical test for significance of the data presented.

To determine spore germination, surfactants were added to spore suspensions at 0, 1, 5 and 10% (v:v). The suspensions were placed on water agar and incubated for 24 hours at room temperature. Both percent germination and germ tube length were determined microscopically. To determine the influence on infection structure formation, drops of each suspension were placed on sterile glass microscope slides in sterile petri plates. After 24 hours incubation, each suspension droplet was covered with a cover slip and examined microscopically to determine the number of spores producing appressoria.

Sunflower, soybean and olive oil significantly increased spore germination and germ tube length when added to the spore suspension at the highest rate. The other crop oils and commercial surfactants either had no effect or reduced germination or germ tube length. Five crop oils (sunflower, canola, soybean, safflower and peanut) significantly increased appressorial formation at one or more of the treatment rates.

Sunflower, soybean and olive oil were examined for their influence on infection and disease development on northern jointvetch under disease-limiting conditions. Each crop oil was added to the spore suspension at 0%, 5% and 10% (v:v) just prior to plant inoculation. Following inoculation, plants were placed into a 28 C dew chamber, except for one three-pot replicate, which was placed directly into a 28 C growth chamber. The remaining pots were removed from the dew chamber 4, 8 and 12 hours after inoculation and placed into the growth chamber.

Application of the fungus in combination with 10% sunflower or soybean oil resulted in significantly greater disease than in plants inoculated with the fungus alone under moisture-limiting conditions of less than 12 hours. In particular, the addition of 10% soybean oil reduced the free moisture requirement for significant disease development to eight hours from the 12 hours normally needed. Results suggest that the free moisture requirement, a major limiting factor to the consistent performance of many biocontrol agents, can be minimized by the addition of crop oils or other tank-mix additives.

THE DEVELOPMENT OF RISK ASSESSMENT METHODOLOGIES FOR BIOLOGICAL WEED CONTROL AGENTS

As biological control agents continue to reach the marketplace, the public remains concerned about potential environmental risks associated with the use of these agents. This is particularly true if the organism has been genetically modified using the tools of biotechnology. However, scientists now possess powerful new tools for genetically characterizing biocontrol agents, examining the potential for genetic exchange, examining their behavior in the environment and developing risk assessment models. Collego has proved to be an excellent model for the development of risk assessment methodologies with broad application to other organisms.

Genetic Characterization of Microbial Biocontrol Agents

A critical component of risk assessment is the capability to genetically characterize the biocontrol agent such that it can be differentiated from other related organisms in the environment. Protein isozymes and DNA RFLP's were investigated as means to better identify and differentiate fungal pathogens of weeds from related pathogens of crops.

Thirty-three isolates of Colletotrichum gloeosporioides representing host-specific populations obtained from weed hosts (northern jointvetch and round-leaved mallow), crop hosts (citrus and pecan) and a pasture legume (Stylosanthes) were characterized using isozyme electrophoresis. Protein extracts were electrophoretically separated using discontinuous polyacryamide gel electrophoresis. Following electrophoresis, gels were stained for 11 different enzymes. A cluster analysis of the similarity values resolved distinct clusters that corresponded to source host.

Isolates of Colletotrichum representing eight species including C. coccodes, C. graminicola, C. lindemuthianum, C. malvarum, C. orbiculare, C. trifolii, C. truncatum, several host specific forms of C. gloeosporioides and an isolate of Glomerella cingulata were examined for relatedness and diversity using molecular markers. Probes, constructed from genes encoding for ribosomal DNA, glutamate dehydrogenase and glyceraldehyde-3-

phosphate dehydrogenase, were used to identify band patterns in genomic DNA digested with two restriction endonucleases. RFLP banding patterns were obtained for all of the above isolates for each probe/enzyme combination. Bands were measured according to molecular size and the data compiled into a data matrix on the basis of the presence or absence of a band at each locus. A total of 83 characters were scored for each taxa. An heuristic search was done using PAUP with Neurospora crassa used as an outgroup to root the tree. A dendrogram obtained from the RFLP data suggests that there are three major groups within the genus Colletotrichum. One group contains the "gloeosporioides" isolates. A second group contains five species all taxonomically related to C. orbiculare and an isolate of C. gloeosporioides from Malva. The third group consists of all other species included.

The results of the isozyme and RFLP studies demonstrated that both provide useful tools for determining genetic relatedness within C. gloeosporioides and other species and for differentiating host-specific populations of weed biocontrol agents from crop pathogens for risk assessment purposes.

Population Dynamics of Microbial Weed Control Agents

The potential for gene exchange between any two populations depends upon the size of each population, the ability of the two populations to compete and disperse and the ability of the two populations to reproduce. Therefore, experiments determining the role of disease increase and dispersal of pathogens in weed control and the role of strain competition and reproduction were investigated in field and controlled environments using CGA.

The interaction of inoculum concentration and plant age was tested in small plots containing plants from 15 to 65 days old. Inoculum consisting of one, two or three plants with 10 sporulating lesions of CGA per plant was introduced into all plots 15 days after transplanting. The number of lesions and plant mortality was determined for all plants every five to seven days after inoculation. Analysis of the data indicated that disease spread rapidly in all plots. Mortality was greatest and first recorded among the youngest plants with plants dying within three weeks.

By the last counting date, nearly 95% of the 15- and 30-day-old plants were dead in each plot while only 25% to 50% of older plants were killed.

The effect of inoculum concentration levels on control of northern jointvetch was also tested in the field in large plots. In these tests, four inoculum levels consisting of one or 10 lesions per plant, an aerial application made by spraying plants and a noninoculated control were used to determine how many lesions were required to control northern jointvetch. The number of lesions used to initiate disease in the first two levels (one or 10 lesions per northern jointvetch plant) was established by mechanical inoculation with spores of CGA. For the spray treatment, northern jointvetch plants were sprayed with spore suspensions of the fungus (1×10^6 spores/ml). The results showed that the fungus increased rapidly on plants within one week after inoculation. Disease severity reached the maximum level in three weeks. Development of disease was similar for both the one lesion and sprayed treatments while it was greater on plants inoculated with 10 lesions.

The results of these experiments confirm for the first time that control of northern jointvetch is the result of an epidemic caused by inoculation of plants with viable spores of the fungus. Control of the weed is successful if the initial number of lesions resulting from aerial inoculation is approximately five lesions per plant or greater. Natural increase of the disease apparently results from dispersal of spores from lesions to new infection courts.

Dispersal of the pathogen appears to be a key factor in development of epidemics on northern jointvetch and by other biological control agents on their hosts, although it is more commonly associated with the classical strategy. Dispersal also is an important factor in assessing the risk associated with using biocontrol agents.

Dispersal of Colletotrichum species is thought to occur by wind-driven rain and by insects. These dispersal mechanisms were investigated in field plots and in greenhouse and laboratory tests. Surveys conducted in Arkansas in the early 1990s showed that northern jointvetch is distributed in rice fields in patches, with plant densities ranging from 0.5 to 8.4 plants/m². Similarly, disease in northern jointvetch is unevenly distributed

among patches with incidence of disease ranging from 0% to 100%. These findings suggest that disease development in one patch is independent of development within another patch. High mortality of northern jointvetch was observed in patches with the highest levels of disease. Grasshoppers were often observed feeding on or around lesions, and treefrogs were observed resting on infected plants.

In subsequent experiments, 20% of the grasshoppers collected from fields infested with diseased northern jointvetch were found to be infested with the fungus when grasshoppers collected from these fields were placed on healthy northern jointvetch plants. In controlled experiments in which pathogen-free grasshoppers were placed on anthracnose lesions then transferred to healthy plants, about 65% to 75% of the grasshoppers transmitted the fungus to healthy plants. Thus, the grasshoppers Conocephalus fasciatus and Melanoplus differentialis appear to be important vectors for the dispersal of the fungus in rice fields.

Green treefrogs, Hyla cinerea, also were captured in rice fields from northern jointvetch plants infected by the fungus. Captured frogs were placed in containers with healthy northern jointvetch seedlings, and an average of 90% of the seedlings became infected by the fungus with as many as 10 lesions/plant. Dispersal by treefrogs was further quantified in greenhouse experiments by monitoring disease development from a point source in simulated rice-weed patches. The treefrogs transmitted the fungus to 95% of the northern jointvetch plants in these tests. Transmission of the fungus by the treefrog occurred by mechanical means and resulted from treefrogs using northern jointvetch plants as shelters in the rice ecosystem.

Controlled dispersal tests were conducted in replicated field plots arranged in a "Y" formation where each arm of the "Y" contained three plots surrounding a central plot to determine the extent to which the fungus can be dispersed on northern jointvetch. Plots were separated from each other by 0.5 to 1.0 m, and each replication was separated by grass borders. Two weeks after transplanting 12 northern jointvetch plants into each plot, a single plant with 10 sporulating lesions of CGA was introduced into the central plot. Analysis of the data indicated that the fungus spread throughout all 10 plots of each replicate within two weeks after inoculation. The number of lesions per plant generally was highest on plants in the central plot and diminished rapidly on plants in plots along each arm. Northern jointvetch plants were killed in all plots by the end of the summer, although mortality was greatest in the central plots and was correlated with lesion numbers. In related experiments conducted in rain towers, dispersal of the fungus from sporulating lesions to healthy northern jointvetch was limited to approximately 1 m/rain episode. In experiments in which rice and northern jointvetch seedlings were intermixed when placed in test chambers in mixed populations, dispersal was limited to less than 0.5 m/episode.

Results from these studies demonstrated that dispersal of the fungus causing northern jointvetch anthracnose is a significant factor in the control of northern jointvetch by this disease. Wind-driven rain, insects and treefrogs all contribute to the dispersal of this fungus on its host. Natural dispersal from rain, insects and treefrogs is not sufficient to control weed infestations without augmentation of the pathogen. However, control of individual plants results from disease increase resulting from wind-driven rain and insect and treefrog dispersal of the pathogen.

Environmental risk of a biological pesticide often can be related to the persistence of the agent in the environment and the population dynamics of these strains in comparison to wild type populations in natural ecosystems. This is particularly true for genetically engineered organisms, where regulatory guidelines often require estimates of persistence, dispersal and competitiveness in the field. These questions typically are addressed by using genetically marked strains.

Greenhouse and field experiments were conducted with CGA to determine the competitiveness of mutant (benomyl-resistant and nitrate non-utilizing) strains in mixed populations with a wild type strain.

In greenhouse competition experiments, mutant strains were allowed to compete with a wild type strain for a limited host base. Experiments were initiated with the introduction of diseased northern jointvetch seedlings into a predetermined population of healthy plants in such a way that strains were introduced in equal pro-

portions. Disease was permitted to progress for 10 generations, with each generation concluding with the removal of dead or severely infected seedlings and replacement with an equal number of healthy seedlings. Thus, strains competed for available infection sites, and healthy plants were inoculated with inoculum produced on previously infected plants.

Results of these experiments indicated that a benomyl-resistant mutant strain was rapidly replaced by the wild type isolate. By the third to fifth generation, the benomyl-resistant strain was reduced to less than 10% of the total population, and after 10 generations, the benomyl strain comprised less than 5% of the population. On the other hand, a nitrate non-utilizing (nit) mutant strain remained in equilibrium with the wild type strain during all 10 generations.

The same three strains were used in field experiments. In these tests, treatments consisted of the wild type plus the benomyl mutant, the wild type plus the nit mutant and all three strains. Epidemics were initiated by introducing plants mechanically inoculated with each of the strains. Disease was permitted to develop unchecked, and new lesions were sampled repeatedly during the next few months. At each sample time, approximately 150 to 200 lesions were collected by cutting small pieces of tissue from the lesion margins. Selective media were used to identify each strain.

Within the first month, the wild type strain achieved a higher proportion of the population than the benomyl mutant. The proportion of the benomyl mutant was reduced to approximately 40% of the final population by the end of the season in both years (Fig. 2). On the other hand, the nit mutant strain was much less competitive than the wild type (Fig. 3). The final proportion of the nitrate non-utilizing strain at the end of season was only 10% of all sampled lesions.

In experiments in which all three strains were used, neither mutant strain was competitive with the wild type strain (Fig. 4). In both years of the study, the proportion of the mutant strains decreased from the initial levels of 33% to less than 10% of all lesions sampled.

Based on these studies, it is clear that genetically marked strains may not always be effective substitutes for predicting the behavior of a wild

strain in field experiments, and the predictive qualities of such strains must be evaluated carefully. A careful study of individual infection components may be required to explain differences in the competitiveness of individual strains.

Risk assessment models can be developed by incorporating infection components into standardized disease models to study strain differences. Analysis of infection components of 10 mutant and wild type strains of CGA was conducted in growth chambers under optimal conditions for infection. Five infection components, including infection structure formation, infectiv-
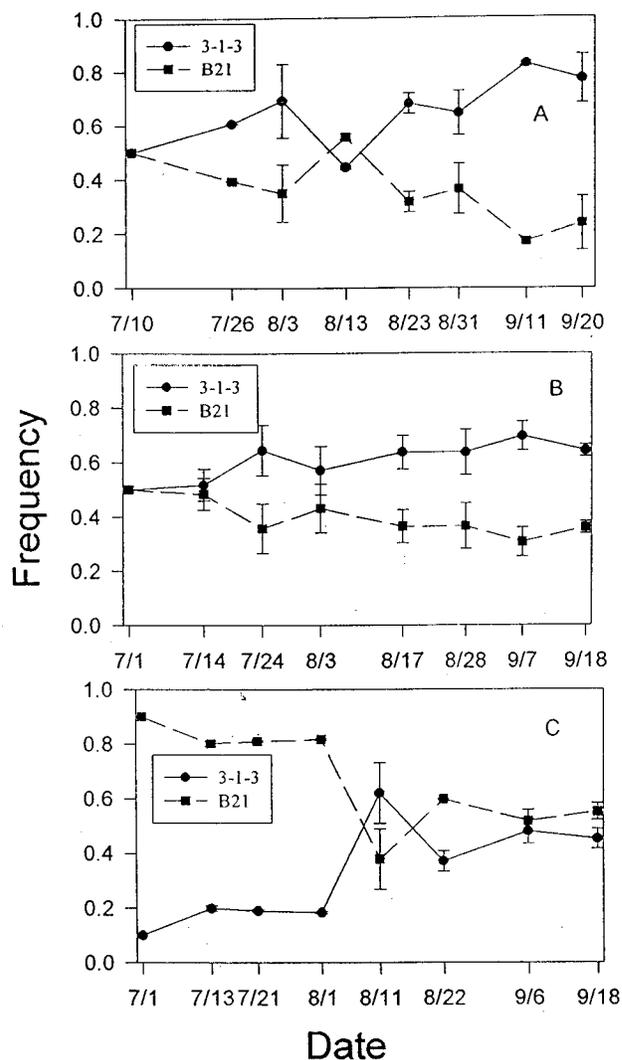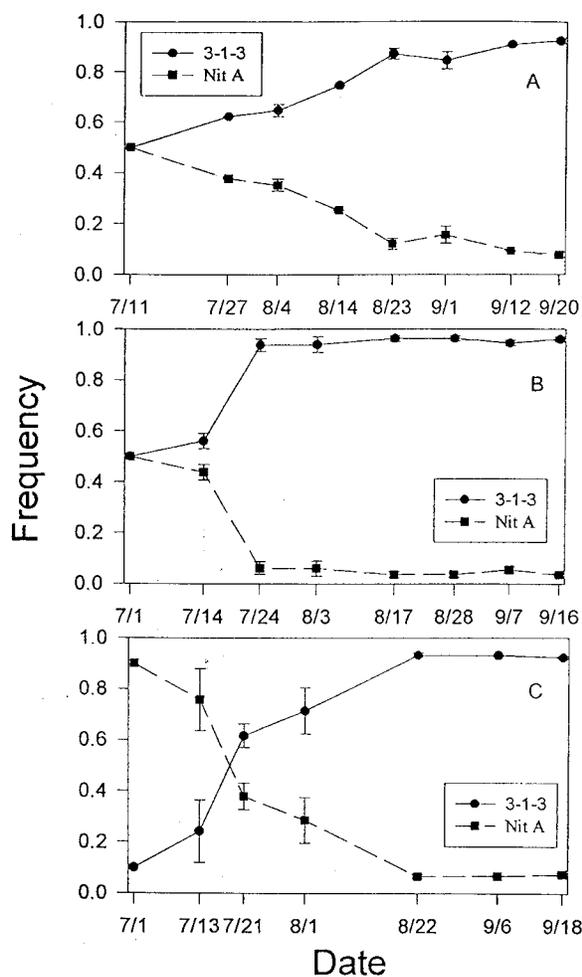

Fig. 2. Competition between a wild-type strain and a benlate-resistant strain of *Colletotrichum gloeosporioides* on northern jointvetch. Frequency is the proportion of each isolate in lesion samples for each isolate at each sample date.

Figure 3. Competition between a wild-type strain and a nitrate nonutilizing strain of *Colletotrichum gloeosporioides* on northern jointvetch. Frequency is the proportion of each isolate in lesion samples for each isolate at each sample date.

ity, latent period, lesion expansion and sporulation, were measured. Differences among strains for each infection component were tested by incorporating the component parameters for each strain into the model. Results indicated that three of the four benomyl-resistant strains were less competitive than wild type strains. On simulation day 100, the number of lesions produced by the wild type strains was about 100 times greater than that of three of the four benomyl-resistant strains.

Simulations showed that the nitrate non-utilizing strains were more competitive than benomyl-resistant strains. With validation, simulations may be able to be substituted for time-consuming field studies for risk assessment.

SUMMARY

Using the successful commercial biological control agent, Collego, as a model, a number of research questions could be answered about how successful biocontrol agents work to achieve control, how field performance can be increased, the possible impact of genetic modification on an agent and potential risks associated with using these agents in the environment. Information generated from in-depth studies of successful agents can be used to identify and overcome possible constraints uncovered during the development of other organisms for biological control. Because Collego has been used successfully and safely for so many years, it makes an ideal organism to address possible risks associated with the release of a biological agent into the environment. Such information can be used to refine regulatory procedures designed to ensure the safe use of these organisms.
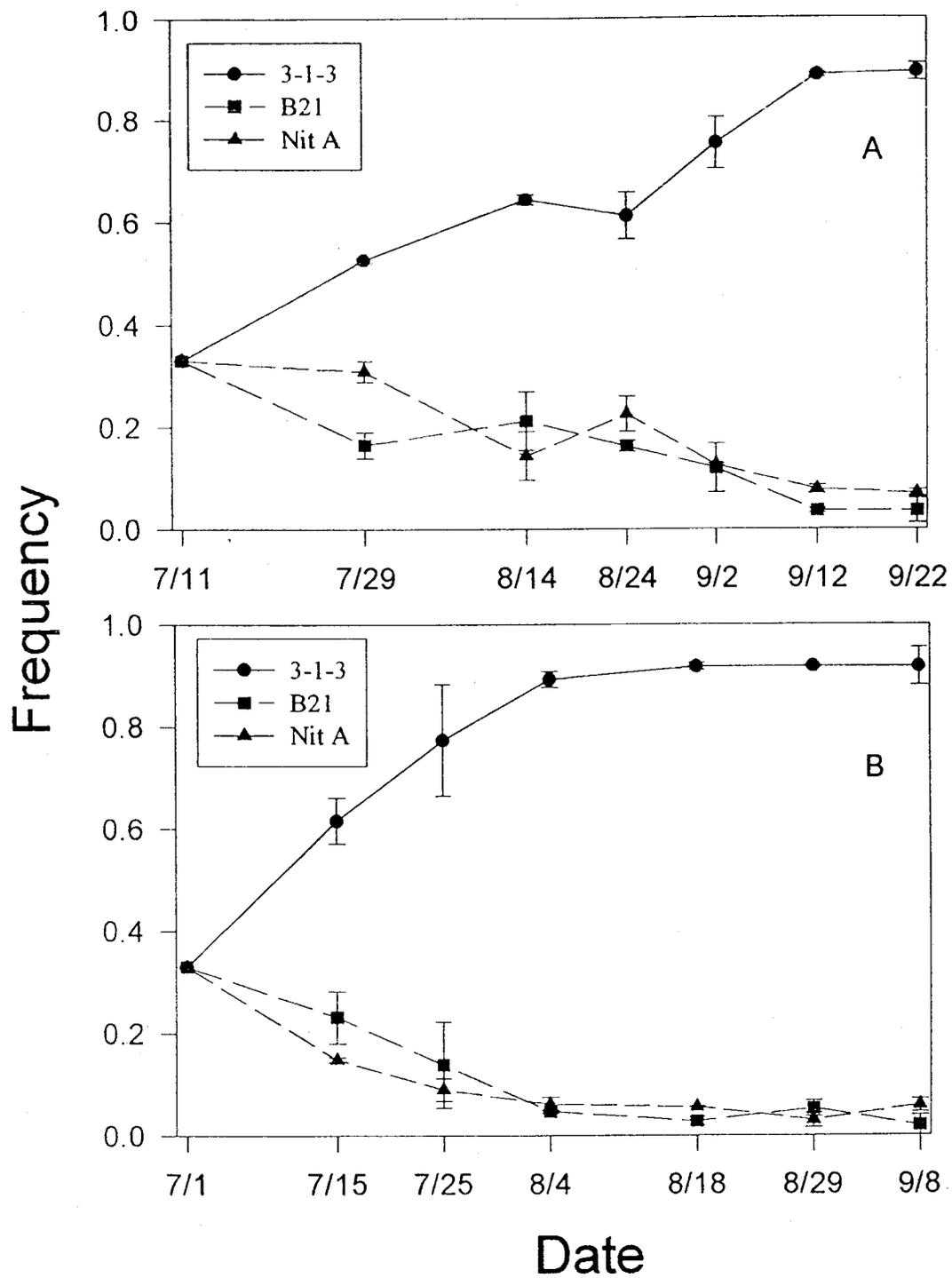
Figure 4. Competition between a wild-type strain and benlate-resistant and nitrate nonutilizing strains of *Colletotrichum gloeosporioides* on northern jointvetch. Frequency is the proportion of each isolate in lesion samples for each isolate at each sample date.

Chapter 4

Pest Management

Pest Management

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COTTON MANAGEMENT BASED UPON FRUITING DYNAMICS

otton can be affected by multiple pests, environment and cultural factors throughout the season. Management decisions often ignore effects and interactions of similar and contrasting factors with decision rules frequently based on addition of single factors. For example, economic levels (Stern et al., 1959) for management of a pest are typically based upon density of the pest species with no provision for adjusting rules for multiple pests or unanticipated interactions. Management in cotton might truly become integrated if decision rules were based on a single focal parameter that would serve as a standard/target of the composite of all plant responses. Due to the perennial nature and indeterminate growth habit of cotton, such a focal parameter must encompass the fruiting dynamics of the plant and provide a means to attain the optimum combination of high yields and early maturation.

In addition to measuring the fruiting dynamics, a focal plant monitoring parameter should 1) be sensitive to discrete changes, 2) be accurate to provide confidence in data, 3) require relatively little time to measure to be cost effective, 4) be easily measured to avoid physical and mental fatigue precipitating errors and 5) be easily understood and taught. With these criteria in mind, the COTMAN system is primarily based upon one plant measurement, the number of "squaring nodes," measured sequentially throughout the effective fruiting period (Bourland et al., 1992ab; Oosterhuis et al., 1994). Prior to flowering, the number of squaring nodes is equal to the num-

ber of sympodial branches. After initiation of first flowers, number of squaring nodes is the number of sympodia above a first-position white flower (nodes-above-white-flower or NAWF). Thus, number of squaring nodes provides a direct measure of potential fruiting capacity of the plant throughout the square development period. The physiological basis of squaring nodes was established by relating NAWF to measurements of crop growth and yield, including whole canopy photosynthesis, boll retention, boll size and number of seed in first-position bolls (Oosterhuis et al., 1992).

The COTMAN system consists of two expert systems, SQUAREMAN and BOLLMAN, with plant monitoring data provided by the SQUAREMAP and NAWF techniques, respectively.

SQUAREMAN utilizes square retention and plant height data collected by the SQUAREMAP technique (Slaymaker et al., 1995; Danforth et al., 1995), which commences with initiation of fruiting (first square). Early versions of SQUAREMAP were tested and reported using the name TOPMAP (Bourland et al., 1994b). SQUAREMAP data are entered into the SQUAREMAN computer program, which determines total nodes, height, vigor indices, number of squaring nodes and square retention by mainstem node and groups of nodes, as well as whether square retention differs significantly from the previous sampling date. The square retention variables give a direct measure of plant response to incipient pest and environmental stress. Plant growth pattern is graphically displayed by the number of squaring nodes over sampling dates and can be compared to a pre-deter-

mined target curve. The vigor index measurements are similar to those previously developed and have similar applications (Kerby and Goodell, 1990; Hake et al., 1990). SQUAREMAN addresses two critical questions: 1) Is square retention acceptable?, and 2) Are plants progressing at an acceptable rate?

Once flowering is initiated, attention switches to NAWF. NAWF monitors the growth and development of cotton plants by determining the number of main-stem squaring nodes on a plant after it begins to flower (Bourland et al., 1992b). Waddle (1982) recognized that the occurrence of a white flower within 3 in. of the plant terminal indicated that the cotton plant had ceased producing new bolls. In a subsequent 1983 unpublished report, N.P. Tugwell and C.W. Smith used number of main-stem nodes instead of distance to measure this phenomenon. Bernhardt et al. (1986) suggested the use of this measure to sequence termination of insecticide application. BOLLMAN provides guidelines on end-of-season decisions, e.g., when to terminate insecticides, irrigation, fertilization and plant growth (apply defoliants) and how to sequence harvest by plant maturity (Zhang, 1994).

A goal of in-season management is to optimize the probability of developing plant structure with high fruit retention, so as to produce optimum yield with early maturity. SQUAREMAN assists in achieving this goal by providing a mechanism to assess growth pattern and square retention. By comparison to a target growth pattern, deviant patterns can be manipulated when detected early. Square retention can be monitored in relation to plant development, insect infestations, water, diseases, nutrients and other factors affecting the crop.

The number of squaring nodes measured by both SQUAREMAP and NAWF provides a sequential, dynamic evaluation of growth pattern through most of the season. We postulate that the optimum combination of early maturation and high yield can be achieved if plant development tracks a pre-determined "target growth curve" (Oosterhuis et al., 1994). To meet the target growth curve, a crop should commence squaring and initiate first flowers and then cutout by 35, 60 and 80 days after planting, respectively. Number of squaring nodes at first flower should

be 9.25 (25 days from square to flower divided by 2.7 vertical fruiting interval) and decline linearly thereafter.

NAWF has been used to measure variation in maturity associated with several factors, including cultivars, irrigation, fertility, plant density, insecticides and insect infestations (Bagwell et al., 1992; Benson et al., 1995; Bourland et al., 1991, 1992b; Holman, 1996). Typically, we found that fruiting patterns were already established by the time plants had initiated flowering. Demands of fruit load relative to vegetative growth after flowering inhibited remediation of growth patterns provoked by earlier plant stress, i.e., NAWF below target curve. This experience with NAWF demonstrated the need to monitor the plant prior to flowering and led to the development of SQUAREMAP. Fields in which plants are not following the target pattern can be detected early in the season by SQUAREMAP. Our ability to remediate deviant patterns should improve as deviation patterns are detected earlier in the season. For example, the plant growth regulator PGR-IV, applied two weeks after defruiting (to simulate an insect attack), increased fruit set and development and gave the same yield as the control in 1994 and 1995 tests.

To understand the effects of deviant patterns, Holman (1996) made weekly releases of tarnished plant bug, Lygus lineolaris (Palisot de Beauvois) nymphs at the base of cotton main stems, allowing them to distribute and feed normally. Bollworm, Helicoverpa zea (Boddie), larvae also were released on the same plants so that the composite square shedding could be attributed to both insect species. The insects were released as treatments to provide a range of square shedding, which was assessed weekly with SQUAREMAP. At the time of first flower, first-position square abscission percentage ranged from 1 to 10, 23 to 60 and 7 to 77 in 1992, 1993 and 1994, respectively.

Cotton plants compensated for abscission of at least 19% of first-position squares (Yield, kg/ha, $Y = 1026.71 + 1.72X$; $R^2 = 0.06$). Undercompensation for abscission was evident when it exceeded 26% ($Y = 1201.51 - 8.29X$, $R^2 = 0.87$). However, even low abscission caused some crop delay, i.e., a delay of one day for each 5-6% square shed (days to cutout, $Y = 93.47 + 0.18X$, $R^2 =$

0.79). Therefore, the effects of deviant pattern depended largely upon whether overcompensation or undercompensation occurred.

The point of economic damage for overcompensation was defined with plant-based decision rule one, damage level, DL:

$$DL = C / MP,$$

but for the undercompensation zone, decision rule two was used:

$$DL = [(C-KMP) / (VD + MP)] + K,$$

where C is equal to cost of control, M is crop delay associated with 1% first-position shed, P is insect control cost per day in late-season, V is value of cotton per unit weight, D is the weight of the reduced yield in the undercompensation zone from abscission prior to first flower.

As cost and price varied, damage levels changed but tended to remain near 20%, which is similar to levels used to supplement conventional Extension recommendations based upon insect numbers.

Use of plant-based decision rules will reduce the emphasis on inspection for insect numbers/population densities, but determination of insect species, life and seasonal history will remain important. Primary decisions would rest with the plant-based rules with rapid detection of changes being facilitated with SQUAREMAN. The behavior tendency of plant bugs and bollworms to feed on tiny squares insures easy detection. The fruiting dynamics of the cotton plant, its patterns of square development and its compensatory capacity provide time for adequate decisions. For example, consider early plant development when only three sympodia have developed. As much as 60% of the squares could be lost on three sympodia and not exceed a DP = 20% at first flower, if control were promptly achieved.

Thresholds could be used prior to first flower for decision point (DP) for initiating control to prevent abscission from exceeding 20% at first flower:

$$DP = 9.3 DL / S,$$

where 9.3 is the desired number of sympodial nodes on vigorous plants at first flower, DL is the damage level as calculated above, and S is the number of sympodia at time of sampling.

END-OF-SEASON MANAGEMENT USING BOLLMAN

Using BOLLMAN, end-of-season decisions are based upon the maturation of the "last effective boll population" (Oosterhuis et al., 1994). To be "effective," a flower population must have a high rate of retention and potentially have the chance to develop an acceptable size and fiber quality. Flowers become less effective if they occur either in the plant apex or very late in the season. "Cutout" then is defined as the flowering date of the last effective boll population. BOLLMAN uses crop-oriented rules when cutout is determined by position of flowers on plants and weather-oriented rules when cutout is determined by a critical date in the season. Once cutout is established, end-of-season decisions can be sequenced by the subsequent heat unit (HU) accumulation, i.e., maturation of these developing bolls.

Observation of mature cotton plants indicates few harvestable bolls near the plant terminals. In early work, a critical value of five NAWF was considered the optimum base for initiating HU accumulation for timing defoliation. To confirm this critical value of cutout, date and NAWF for first-position white flowers were labeled (Bourland et al., 1992b). As NAWF decreased, the probability of first-position white flowers successfully developing into a boll decreased, and bolls that were produced were smaller. Thus, NAWF = 5.0 was chosen as a conservative indicator of the last flowers that will effectively contribute to yield. Although some flowers will produce harvestable bolls at NAWF less than five, their relative retention and boll size is low. Recent research on cotton grown in different environments and under different management conditions has further validated the value NAWF = 5 as defining cutout. With cutout defined, plant maturity can be measured by the number of days from planting to cutout (Danforth et al., 1994). Days from planting to cutout (NAWF = 5) is easily determined by regressing NAWF by days from planting, then solving for days to NAWF = 5 (Bourland et al., 1991).

In BOLLMAN, the last possible date that a flower population will likely mature into an effective boll population is defined by long-term weather data and a user-supplied risk factor. The

latest possible cutout dates that would allow the last boll population to accumulate 850 HUs in 95% of years in Arkansas ranged from late July to early August (Zhang, 1994). If cutout (NAWF = 5) is attained before the latest possible cutout date, BOLLMAN indicates that the field has a "Type I" growth pattern and initiates crop-oriented rules. Otherwise, the field is defined as a "Type II" field and uses weather-oriented rules.

Currently, termination of late-season insecticide application is often based on the number, size and firmness of top bolls. At the time of defoliation, attention is directed to the number of open bolls in the bottom half of the plant. There is a temptation to protect the top bolls too long and to defoliate too soon relative to the maturity of the last potentially effective bolls. Boll susceptibility to attack by adult boll weevil, Anthonomus grandis (Boheman), and by the larval stage of the bollworm relative to physiological age of the boll should provide an objective criteria for terminating insect control. Individual insects were caged on single bolls of a known physiological age and removed after three days (Bagwell and Tugwell, 1992). Boll age was negatively correlated with the number of bolls successfully penetrated by the insects. The lignified endocarp appeared to be the site of resistance to insect penetration. At 350 HU, only 5 and 12% of the bolls were successfully penetrated by the boll weevil and bollworm, respectively.

Timing defoliation based upon HU accumulation of the last effective boll population requires timely crop cutout. Failure to reach cutout (NAWF = 5) by late July or early August necessitates defoliation prior to maturation of the last potentially effective bolls, so that weather risks during harvest may be minimized. The percentage of lint and gross revenue loss from premature defoliation were determined from replicated plots where defoliants were applied when the last effective boll population had reached varying degrees of maturity as defined by HUs from cutout (Bourland et al., 1994a; Wells, 1991). When defoliated at less than about 750 to 950 HUs, lint yield and gross revenue declined linearly. The intermediate value of 850 HUs appeared to define the optimum defoliation time, with open boll weathering losses estimated at about 2% per day.

Target dates for harvest completion in areas representative of four sites in the Delta region of Arkansas and Mississippi were based on the assumption that field work would be delayed if daily rainfall exceeded 0.5 in. on clay soil or 1.0 in. on loam soil (Zhang, 1994). Weather events in a year indicated clear spatial and temporal patterns in the Delta region. The weather risk, associated with declining solar radiation, temperature and increasing amounts of rainfall, increased as the harvest season progressed. Rainfall events consistently increased to an obvious peak in November. Specific target dates for harvest completion were defined as November 10 in the north (Keiser) and November 1 in the central regions (Marianna and Stuttgart) and the southern region (Stoneville).

BOLLMAN was designed to be a computer-aided management system that integrates expert system, data base and computer graphics technologies. The inputs to BOLLMAN include plant monitoring, long-term and current weather and farm and field information. Output includes a diagnosis of plant growth status and recommendations for termination of insecticide applications, time of defoliation and harvest scheduling.

Benefits of COTMAN

COTMAN's focus on key plant cues allows one to measure effects of a single factor or combinations of factors that may be integrated in any defined way. For example, questions about effects of two insect populations, each below the economic threshold level, are easily answered when the focus is on plant response cues. In a truly expansionist perspective, one could compare different agroecosystems.

A series of field experiments was conducted to further document the economic benefits accruing from the insecticide termination rules in the COTMAN program. In many cases the highest yields were associated with termination timed at the recommended NAWF = 5 + 350HU. In addition, a number of large-plot experiments were pursued in actual grower's fields. These large plots were located in fields that were known to have significant late-season insect infestations and compared terminations at NAWF = 5 +350HU with terminations at NAWF = 5 + 500 to 600HU. Plots from Arkansas, Texas, Mississippi and Louisiana were included in the study. In only one case was the net revenue lower for

the recommended termination rule than for those that continued insecticide treatments later into the season, and this occurred in a late field that had considerable replanted cotton. When all the large plots are examined as a group, net revenues for the recommended termination rule are $52/acre higher than the those of the later termination rules.

TOBACCO BUDWORM
MANAGEMENT IN COTTON

The tobacco budworm, *Heliothes virescens* (Boddie), completes three to four generations per year in central and southern Arkansas. Following spring emergence, overwintering populations develop on wild hosts through May. Succeeding generations develop on cotton beginning in late June and continue through the fall. Management practices target the high field populations that occur during this period. Several management practices can be combined into a plan to better control tobacco budworm. These practices include earliness, population monitoring with pheromone traps and the use of *Bacillus thuringiensis* plus ovicides on both individual fields and large acreages.

Crop earliness is a practice that includes the use of insecticides and tolerant cotton cultivars for control of early-season insects such as thrips. This practice allows for a strong, healthy crop that matures early so the highest late-season tobacco budworm populations are avoided. Cotton cultivars exhibit varying degrees of tolerance to thrips and/or their injury. Cultivars such as 'Deltapine 5415', 'Deltapine 20' and 'Chembred 1135' maintain low thrips populations early season; other varieties such as 'Hartz H1215', 'Deltapine 50' and 'DES 119' maintain high populations early season. The cultivar differences in thrips populations also appear in the number of days to cutout. In 1994, those cultivars maintaining low thrips populations reached cutout in 91-92 days while cultivars with high thrips populations reached cutout in 96-98 days.

Another important practice is monitoring the moth populations through pheromone traps. Peak moth populations can be identified so that appropriate control actions can be taken. Moth trap catches for 1993-1995 showed that second-generation moth populations peaked from late June to early July (Fig. 1). Third-generation populations peaked from late July to early August in 1993 and 1994. A fourth-generation peak was observed in late August 1995. Pheromone trap data can also be used to estimate species composition. Data collected in 1993 and 1994 showed that
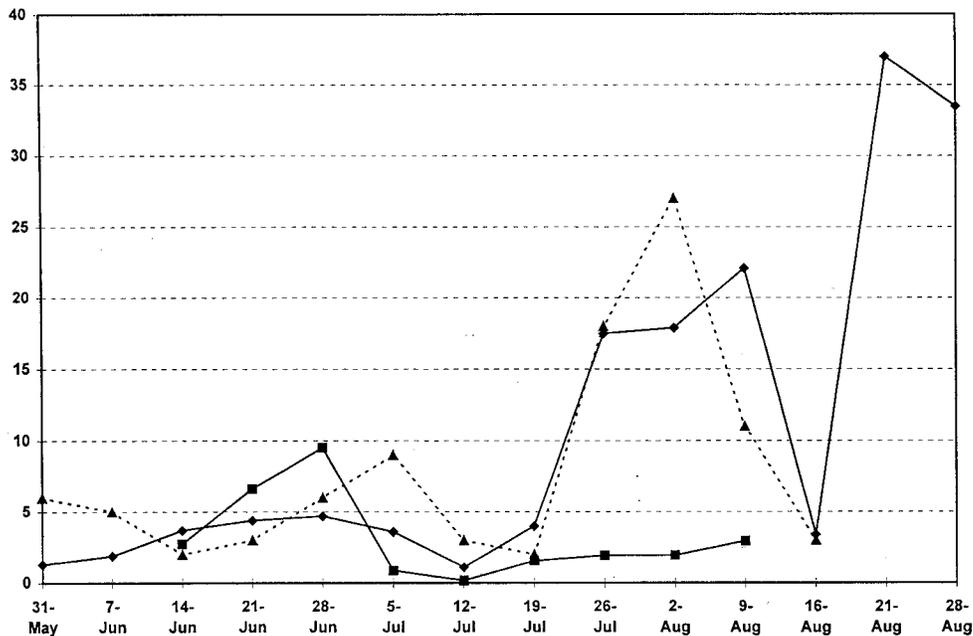
Fig. 1. Average daily tobacco budworm pheromone trap catches, Jefferson County, 1993-1995.

there was a close correlation between tobacco budworm/bollworm moth trap catches and field populations of larvae. This information is important in deciding the class of insecticide to apply.

Tobacco budworm resistance to pyrethroid and organophosphate insecticides is increasing in most cotton growing areas. Due to this increase, research is focusing on alternative insecticides such as *Bacillus thuringiensis* (Bt). One method of using Bt is in combination with low to moderate rates of an ovicide. In 1994, several Bt/ovicide combinations were evaluated for efficacy against tobacco budworm and bollworm larvae in small plots. All treatments except for Bt alone significantly reduced the total number of larvae on the first evaluation date (Table 1). On 7 July, Bt in combination with Larvin®, Curacron® or Lannate® and the ovicides alone significantly reduced the number of total larvae. No differences were observed on the last two evaluation dates.

Area-wide applications of Bt/ovicides were evaluated in 1994. The purpose of these applications was to reduce the second-generation moth population, resulting in a reduced third generation. The first insecticide application was made within two days after the second-generation peak, and the second application was made five to seven days later. Most of the cotton acreage in southern and central Arkansas was treated (Fig. 1). Tobacco budworm trap catches averaged less than five moths per night through the end of the trapping period on August 9. These data suggest that area-wide Bt/ovicide combinations may pro-vide control of early tobacco budworm/bollworm populations, resulting in reduced populations later in the season. Another benefit of this management technique was a minimal increase in insecticide resistance to pyrethroids unlike previous years when large increases were observed.

WEED MANAGEMENT IN SOYBEAN AND COTTON

Reduced rate herbicide technology developed by the University of Arkansas is currently being used by producers on 1 to 1.5 million acres of soybean, resulting in an annual cost savings of $7 to 10 million in the state annually. As a result, current USDA/ERS information shows that Arkansas soybean producers have the least variable cash expenses per acre and per bushel of those in any soybean-producing state. In addition to reducing grower input costs, the reduced rate program is resulting in less herbicide placed in the environment.

In this research project, soybean herbicide rates as low as one-fourth those recommended by the manufacturer continue to be as effective as labeled rates. Herbicide inputs have been further reduced by taking the one-fourth to one-half labeled rates and reducing them another two to three times by applying on a band. A precision cultivator and guidance system was used to accomplish this. Soybean yields were statistically equivalent in treatments with herbicide costs under $7/acre compared to the $27/acre standard program.

Table 1. Efficacy of ovicides in combination with *Bacillus thuringiensis* for control of Heliothine complex in cotton, Jefferson County, Arkansas, 1994.

Treatment/formulation	Rate	Total bollworm/tobacco budworm/10 row ft			
		July 4	July 7	July 27	Aug 15
UTC		22.5 a*	12.25a	4.0 a	5.75a
Design	0.75	14.0 ab	8.0 ab	2.5 a	4.25a
Design + Ovasyn 1.5 EC	0.75 + 0.25	10.5 b	7.75abc	3.75a	6.25a
Design + Larvin 3.2 EC	0.75 + 0.25	9.75b	2.75cd	2.75a	4.25a
Design + Bolstar 6 EC	0.75 + 0.25	8.5 b	7.75abc	4.0 a	4.75a
Design + Curacron 8 E	0.75 + 0.25	11.25b	5.5 bcd	3.5 a	4.5 a
Design + Lannate 2.4 LV	0.75 + 0.25	15.25ab	2.25d	2.25a	5.5 a
Curacron 8 E	1.0	9.75b	1.5 d	2.25a	5.25a
Larvin 3.2 EC	0.25	13.75ab	6.25bcd	3.5 a	4.75a

*Means within a column followed by the same letter are not significantly different (*P* = 0.05)

In 1994 and 1995, the reduced rate concept in soybean was expanded to include Roundup®-tolerant cultivars. Low rates of Roundup, when further reduced to a narrow band, have been effective in controlling a broad spectrum of weeds, including sicklepod. Programs with $4/acre in Roundup costs were more effective than conventional programs with over $25/acre herbicide costs and placed no residual herbicide in the environment.

In cotton, the new herbicides Staple® and Buctril® continue to show excellent potential for greatly reducing herbicide inputs in a crop where those inputs have historically been very high. Both products have received federal registrations for 1996. The labeled rate of Staple is 0.063 lb ai/acre. This alone can substitute for 1 to 2 lb ai/acre of currently used herbicides. Research conducted in this project showed that both Staple and Buctril rates of one-fourth to one-half of the labeled rates can be effective on certain weed species and can be further reduced two to four times on a band. Combination programs using reduced rates of Staple and Buctril have demonstrated the potential to reduce cotton herbicide inputs by eliminating 1 to 3 lb ai/acre of long residual herbicides from the program.

Reduced rate herbicide programs were integrated with allelopathic cover crops, narrow rows and ridge tillage in six experiments. Rye and red clover cover crops had excellent early-season weed control on certain species. Equivalent weed control and soybean yields have been produced by soybean seeded into the cover crop versus conventional methods. However, the expense of establishing the cover crop and killing it later has made the conventional herbicide programs more economical to date. Ridge tillage appears promising for reducing burndown herbicide costs and allowing herbicides to be banded in a cover crop system. Research in these areas has shown enough promise to be continued beyond the close of this project.

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Chapter 5

Pest Control by Host Plant Resistance

Pest Control by Host Plant Resistance

James McD. Stewart*

INTRODUCTION

esticides are applied to food and fiber crops that do not possess adequate natural genetic resistance to specific pests that reduce yield or quality or both. Biologicals are used effectively as alternatives in the management of some but not all pests. On the other hand, host plant resistance (HPR) encompasses a broad range of exceptionally effective strategies elaborated in nature for the co-existence of plants and potential pests. Problems arise only when a particular organism develops mechanisms that defeat the prevailing HPR system of a plant genotype (species) and allow parasitism on the plant. If the plant genotype is to survive, it must evolve a counter strategy that defeats the pest or allows for accommodation that maintains a balance for both organisms. At one extreme, the plant, by virtue of its HPR systems, is a non-host for the preying organism. At the extreme of accommodation, a symbiotic relationship is established between the plant and invading organism that is beneficial to both. A variety of different adaptations and strategies, both unique and generalized, are found between these two extremes of HPR. In addition, with the development of biotechnology, the potential exists for the HPR genetics of one species to be transferred to another not possessing adequate HPR for a particular pest. In this sense genetic engineering can be utilized to enhance HPR by improving the effectiveness of a plant's existing mechanisms or by giving a plant new genetic material not previously available to it (Stewart and Felton, 1992).

Several faculty in the Alternative Pest Control Center (APCC) are pursuing projects to identify and enhance the genetics of naturally occurring HPR or to understand the interactions between plant and pest that can be used to effectively select for improved resistance. These projects include empirical identification of resistance to specific pests, investigation into the mechanisms and constituents of resistance and directed modifications of HPR.

DISEASE RESISTANCE IN FRUIT CROPS

Resistance of Strawberry to Fruit Rotting Fungi

Strawberry (Fragaria x ananassa Duch.) fruit is beset by three major rot organisms, Botrytis cinerea (gray mold), Colletotrichum gloeosporoides (anthracnose) and Phytophthora cactorum (leather rot). Effective screening methods for early detection and genetic evaluation of resistance to these organisms could lead to more rapid development of cultivars with host plant resistance to fruit rots.

A screening technique, based on the growth of conidiophores (fungal fruiting structures) on killed leaf disks, was developed (Olcott-Reid, 1993; Olcott-Reid et al., 1993a) to compare degree of Botrytis cinerea infection on symptomless leaves (versus fruit) of various strawberry genotypes. Densities of conidiophores differed significantly among cultivars and correlated to field ratings of susceptibility to gray mold fruit rot in two of three tests. This method could be used on seedlings in a breeding program before they are transplanted to the field and before they bear fruit.

In an inheritance study of gray mold resistance (Olcott-Reid et al., 1993b), progeny families from two full diallel crosses between resistant and susceptible parents were significantly different in resistance, but heritability was only 0.16. This underscored the need for an early screening technique. Additive gene action ac-

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counted for the majority (55 to 89%) of total genetic variance for gray mold reaction with epistasis accounting for the remainder.

Natural infections of anthracnose and leather rot organisms in the field allowed inheritance of resistance to these two rots to be determined also (Olcott-Reid and Moore, 1995a,b). Heritability of reaction to leather rot was low (0.16 to 0.27) with mainly additive and epistatic gene action. Anthracnose reaction exhibited moderately high heritability (0.55 to 0.64), with strong dominance for resistance and epistatic gene action. Twelve clones with apparent resistance to all three rots were propagated for use in further breeding work.

Table Grape Resistance to Downy Mildew

Downy mildew is a major disease of grapes incited by the fungus Plasmopara viticola. HPR to this disease organism in popular cultivars would reduce the need for fungicides while increasing yield. Because grapes are perennial, early screening techniques could be used to increase the efficiency of selecting resistant breeding material. Most early screening procedures are conducted in greenhouses on grape seedlings or rooted cuttings.

Screening of breeding materials for downy mildew resistance was begun. An objective was to develop a leaf disk technique for early screening of genotypes. Nine Arkansas selections and 10 cultivars were chosen for evaluation. Disks (18-mm diameter) from young, fully expanded leaves from greenhouse plants were placed on moist filter paper and inoculated with approximately 2×10^4, 5×10^4 and 1×10^5 sporangia/ml. At five and 10 days after inoculation, each disk was rated for sporulation and necrosis on a scale of 0 to 5 based on percentage of the disk area infected. Inoculum concentrations were not significantly different in terms of sporulation or necrosis. Resistant genotypes had no symptoms or minor infection. Most of the susceptible material had increased sporulation and necrosis from day five to day 10. Some cultivars (e.g., 'Bacchus', 'Aurelia' and 'Clinton') had no sporulation but did have small flecks of necrosis suggestive of a hypersensitive reaction. Highly susceptible selections (based on previous field observations) had high levels of sporulation and some necrosis on day five, and by day 10 nearly the entire disks were necrotic. Initial correlations of the leaf disk results with field evaluations of the genotypes showed that the disk technique has potential to screen for downy mildew resistance (Brown et al., 1993).

In excess of 2000 parents and progeny seedlings from crosses between downy mildew-resistant and -susceptible parents have been evaluated using the leaf disk procedure and a greenhouse screening procedure. The plants also were screened in the vineyard for resistance. In general, the results of the leaf disk screening correlated with those of the greenhouse screening. However, the susceptible rating by the leaf disk technique is occasionally higher than by the greenhouse leaf technique. Data are being collected to determine the mode of inheritance of downy mildew resistance. Also, to obtain new sources of resistant germplasm, over 110 cultivars and Vitis species are being evaluated (Brown et al., 1995a).

Epifluorescence is another technique being examined as a tool for early evaluation of resistance parameters. It is possible that epifluorescent microscopy can be used to determine if certain leaf characters (i.e., trichomes, number of stomates) influence resistance in grape plants to P. viticola. Preliminary results revealed that the fluorescent stains berberine (leaf trichomes) and calcofluor (fungal structures) could be used to distinguish between various leaf and fungal structures (Brown et al., 1995b).

In another approach, procedures are being developed to determine if somaclonal variation can be induced in grape plants to alter resistance to P. viticola (Brown et al., 1994). For the table grape cultivars 'Mars', 'Reliance' and 'Saturn', genotypic differences in callusing response to phytohormones were noted. In general, NAA was superior to 2,4-D for callus induction from leaf disks when combined with BAP at 0.2 mg/L. The callus produced on NAA media was amorphous and friable and varied in color. Morphogenesis occurred in 8.5% of calli from the three cultivars grown on NAA media. Media for plantlet regeneration are currently being developed.

Resistance of Apple Germplasm to Summer Rots

Fruit growers face serious biological obstacles to economical production of high quantity and quality of produce. In the southern U.S., the summer rot complex is one of the major obstacles to apple production. Traditionally these organisms are controlled by broad-spectrum fungicides applied for other diseases, but these pesticides are becoming less available. Newer, specific fungicides will not control rots that occur at harvest. A survey of the variation in the causative fungi and evaluation of genotypes for resistance to the rot diseases was begun.

A broad survey determined that the pathogens causing summer rots belong to either *Colletotrichum* or *Botryosphaeria*. *Colletotrichum spp.*, the causative agents in bitter rot, were responsible for 79% of all rot infections in a dozen orchards in several southeastern states (Shi et al., 1995a). Approximately 1000 isolates were obtained from diseased fruit and the causative taxon identified and scored for virulence. Three unique taxa, *C. gloeosporoides*, its telemorphic form *Glomerella cingulata* and *C. acutatum*, were identified as causing bitter rot, with the last being most prevalent. Interaction of apple cultivar and disease reaction for each fungal taxon was such that effective evaluation and screening procedures for resistance to bitter rots must evaluate isolates from each of the three taxa.

Accurate evaluations for resistance require effective and repeatable screening methods. Because these were generally not available for summer rots, they were developed in the initial stages of the project. The following criteria were established. 1) Fruit should be disinfected before inoculation with the test organism. 2) Inoculum should have a spore concentration of $1x10^6$ spores/ml. 3) Isolate suspensions should not be more than seven days old. 4) Inoculated fruit should be maintained between 25 C and 30 C at high humidity for approximately seven days. 5) Disease reaction to epidermal and cortical infections differ, so wound and non-wound spray inoculations must be performed for each fruit genotype. 6) Most reliable results are obtained when apparently mature fruit are tested (Shi et al., 1995b). 7) Disease reactions among wounded genotypes can be differentiated by lesion diameter expressed in linear units or as a percentage of the fruit diameter. Non-wounded fruit infection can be rated as percentage of surface covered with lesions.

To assess the level of resistance within the germplasm pool, 77 genotypes of *Malus domestica* and 17 genotypes of other *Malus* species were evaluated for susceptibility to the three summer rot fungi (Shi and Rom, 1995). A wide range in disease reaction was observed among the domestic genotypes and the *Malus* species. The results indicated that both epidermal and cortical factors contribute to disease resistance and that they function independently. Initial observations on hybrid progeny suggest that level of disease reaction is heritable; hence, breeding for resistance to summer rot in apples should be possible. The response appears to be polygenic with a few major genes active.

Both field and laboratory environments were used to evaluate variation in resistance to *Colletotrichum spp.* among apple cultivars and *Malus spp.* Approximately 25 cultivars were inoculated under field conditions, although fruits were wrapped in plastic for 24 hours following inoculation. Additionally, fruit of 50 cultivars and 25 species of apple were both wound and spray inoculated, sealed in plastic containers and placed in controlled environments. Evaluations were made four weeks after inoculation. Some cultivars, as expected, had no resistance or were highly susceptible to all populations of *Colletotrichum spp.* Complete resistance was not observed in any of the genotypes examined; however, some cultivars and *Malus spp.* had very low susceptibility or partial resistance, with lesions being low in number and very confined.

EVALUATION AND ENHANCEMENT OF COTTON GERMPLASM

More pesticides are applied to cotton than to any other crop. Thus, any increase in HPR of cotton to specific economic pests not only will reduce the cost of production, but should have a positive impact in reducing environmental pesticide load. Two projects under the auspices of the APCC are directed toward the identification and enhancement of genetic resistance in cotton germplasm.

Nematodes

The root-knot nematode (RKN), *Meloidogyne incognita*, causes approximately 2% yield loss per year in cotton (Veech, 1984). In addition, disease complexes between fungal pathogens and *M. incognita* further decrease yields (Starr and Martyn, 1991). The reniform nematode (RFN), *Rotylenchulus reniformis*, is a significant cotton pest in the lower Mississippi Delta and is now recognized as a major problem to cotton production in east-central Arkansas. Early-season nematode populations are suppressed by aldecarb; however, RFN populations are reestablished to high levels by harvest, so treatment must be repeated every year. Host plant resistance is, by far, the best management strategy for nematodes.

Ninety-five accessions of Asiatic cotton were screened for resistance to root-knot nematode (RKN) race 3, collected from an Arkansas cotton field. Week-old seedlings were inoculated with ca. 10,000 RKN eggs, and after 50 days eggs were extracted from the roots with NaOCl and counted. Two *G. hirsutum* genotypes, 'Auburn 634' (resis-

tant) and 'M-8' (susceptible), were included as standards in each test. Most accessions were moderately to highly susceptible; however, several accessions were identified with nematode reproduction less than 10% of M-8. Table 1 lists accessions with significant resistance.

Over half of the accessions in the NPGS Asiatic Cotton Collection and several wild Gossypium species were evaluated for resistance to the reniform nematode (RFN). Significant resistance to this nematode is not known in G. hirsutum cultivars, so only susceptible cultivars 'DPL-50', Auburn 634 and M-8 were included as checks. Replicates of each accession were inoculated with 1800 RFN vermiform isolated from cotton roots. All nematode eggs and juveniles were recovered from the roots and soil of each plant after seven to eight weeks and counted. Approximately 90% of the Asiatic cotton accessions supported less RFN reproduction than the G. hirsutum checks. Ten percent were rated as very resistant (Stewart and Robbins, 1994). Thus, the Asiatic cottons provide a very good source of resistance to RFN. On the other hand, Gossypium longicalyx (F_1 genome) was confirmed to be immune to RFN. The genotypes most resistant to RFN identified in the screen are listed in Table 1.

Seedling diseases

Approximately 200 accessions were evaluated for resistance to the seedling disease pathogens Rhizoctonia solani and Pythium ultimum. Plugs of pure cultures of each organism grown on potato dextrose agar were placed adjacent to the hypocotyls of seedlings maintained at 20 C (Pythium) or 28 C (Rhizoctonia). Susceptibility rating was based on a scale of 1 to 6 where 1 = no symptoms and 6 = dead. Variation was found among A-genome accessions in response to the pathogens, but none was highly resistant. As a group, the Asiatic cottons were more susceptible to the pathogens than upland cotton varieties (Stanton et al., 1994).

Insects

Thrips are early-season pests that delay seedling development. Since they damage cotton immediately following germination and no adequate method is available to predetermine their presence, most producers apply a prophylactic treatment of pesticide as insurance against these pest. Approximately 100 accessions of Asiatic cottons were evaluated for resistance to thrips under field conditions (Stanton et al., 1992). As a group the Asiatic cottons are more tolerant of thrips than upland cotton. When damaged by thrips, many of the Asiatic cotton accessions would recover more rapidly than upland cultivars. Thrips-tolerant accessions are listed in Table 2.

Resistance to Heliothis virescens was determined in a square feeding system. In replicated tests 1-cm flower buds from each of 157 accessions of Asiatic cotton were placed in small cups with two neonate larvae. After six days larval survival and weights were determined and compared to those of larvae growing on artificial diet and to those of larvae fed flower buds of 'Stoneville 506'. Forty-seven of the lines supported lower larval weight than the control cultivar. Ten of the best lines were field tested for resistance by inoculating the plants weekly with budworm eggs. For four of the accessions (Table 2), plants with no chemical control yielded from 75%-90% of the plants in which budworms were stringently controlled with chemicals.

Twenty-four accessions of Asiatic cotton were tested for resistance to the cotton aphid in a no-choice test that measured population development. Five aphid nymphs were placed on the youngest, fully expanded leaf of six plants, and each leaf was enclosed in a mesh screen to exclude natural predators. The infested leaves including aphids were harvested after two weeks and were preserved in fixative and all aphids on each leaf counted. The results were variable within and between accessions; however, one accession (A2-183) gave relatively low population growth on average (68 aphids) and across replications compared to the most susceptible accession (A1-040 with 795 aphids) or the upland checks (255 aphids) (Table 2). Another accession ($A_2$020) had apparently moderate resistance with an average of 122 aphids per leaf. The accessions with apparent resistance to aphids will be retested for confirmation.

Tarnished plant bug, Lygus lineolaris (Palisot de Beauvois), nymphs and adults injure cotton plants by feeding upon terminal buds of seedlings and the squares and young bolls of flowering plants. Tarnished plant bug

Table 1. List of cotton and *Gossypium* genotypes most resistant to nematodes.

Root-knot Nematode		Reniform Nematode	
Accession	Eggs/g root (% of M-8)	Accession	Vermiform (% of DPL 50)
$A_1$045	1.0	$A_1$024	0.8
$A_2$076BN	3.2	$A_2$190	1.5
$A_2$094	3.7	$A_1$015	1.7
$A_2$190	5.1	$A_2$194	1.9
$A_2$033	9.8	$A_2$113	1.9
Auburn 634	<1.0	Auburn 634	109

Table 2. Asiatic cotton accessions with resistance to *Heliothis*, thrips and aphids. All values were significantly different from the respective control.

Insect	Accession	% of check
*Heliothis**	A_1051	61.5
	A_2080	70.5
	A_1028	73.7
Thrips[†]	A_1049	50.0
	A_2154	50.0
Aphids[‡]	A_2183	27.0
	A_2020	48.0

*Evaluated as weight of neonate worms fed flower buds for six days.

[†]Evaluated as damage to field seedlings on a 0 to 7 scale.

[‡]Evaluated as number of aphids on a caged leaf two weeks after infesting with five aphids.

(TPB) damage delays cotton fruiting and crop maturity and can affect yield. Initial work validated a screening protocol for TPB resistance. The protocol consisted of 1) interplanting mustard to insure TPB infestations (Laster and Meredith, 1974); 2) rating TPB injury by percent anther damage (Maredia et al., 1994); and 3) establishing plant maturity based on the nodal development of the plant (Bourland et al., 1992). Subsequently, germplasm evaluations were made 1) to determine if ratings for resistance are influenced by genotype maturity; 2) to determine if public and private cotton breeding programs have made progress in incorporating TPB resistance into commercial lines; and 3) to evaluate available cultivars, breeding lines and exotic lines for TPB resistance.

Two very early maturing cultivars, 'Paymaster HS 200' and 'Tamcot HQ 95', tended to have a higher percent TPB damage than the early ('DES 119' and 'Deltapine 51') and full-season ('Stoneville LA887' and 'Deltapine 90') cultivars, even when planted later in the season. Differences in anther damage ratings were related to genotype rather than maturity group. To evaluate commercial breeding progress in TPB resistance, a set of 14 interrelated and four non-related cotton lines were evaluated for response to TPB at two locations. The time of development of the lines within the respective programs had no effect on responses to TPB. The absence of trends among related lines indicated that improvement in resistance to TPB has not been achieved within the breeding programs represented.

In efforts to identify resistance to TPB, a total of 60 genotypes were screened. Cultivars and breeding lines had limited genetic variability with percent damaged squares ranging from 18 to 36% and from 17 to 40%, respectively. Twenty Asiatic lines ranged between 6 and 31% damaged squares. $A_1$79 had the fewest damaged squares. This, and additional germplasm to be screened, should provide new sources of resistance to TPB.

Enhancement of Exotic Germplasm

An important component in enhancement of germplasm is the development of strategies to efficiently transfer traits to the cultivated species. Upland cotton is a tetraploid species ($2n=4x=56$) that possesses two subgenome chromosome groups designated A and D. Most other *Gossypium* species in the germplasm pool, including the Asiatic cottons (A genome), are diploid ($2n=2x=26$). Direct hybridization between upland cotton and Asiatic cotton is inhibited by a physiological barrier. When hybrids are obtained between these two types of cotton, the resulting 3X plant has an unbalanced set of chromosomes and is sterile. These must be treated with a mitotic poison such as colchicine to double the chromosome number (to 6X) and obtain fertility before trait introgression into 4X cotton can be initiated. Stewart (1995) developed a strategy that will facilitate the transfer of traits from 2X Asiatic cotton to 4X upland cotton. Synthetic hexaploid hybrids were made between diploid D-genome *Gossypium* species and AD-genome cotton to give a 2(DAD) genomic genetic stock. Using ovule culture techniques, the 6X genetic stock can be crossed with A-genome Asiatic cotton to give a 4X hybrid with a genomic constituency like cotton [2(AD)]. Plants with this combination are fertile and compatible with cotton and can be crossed with it by conventional techniques. The problems of hybrid sterility and aneuploidy are avoided with this strategy.

The Asiatic genotypes that were identified as having resistance to one or more of the cotton pests are currently being crossed with the hexaploid genetic stocks and the fertilized ovules cultured to allow the hybrid embryos to develop. The resulting trispecies hybrids will be grown and backcrossed to cotton and progeny screened for expression of the resistance.

To capture the immunity of *G. longicalyx* (F_1) to the reniform nematode, this species was crossed to *G. armourianum* (D_{2-1}). The chromosome number of this hybrid was doubled with colchicine to make a tetraploid plant that should be sexually compatible with cotton because the F_1 genome is closely related to the A genome of cotton. The hybrid is photoperiodic and requires short

daylength to bloom. At such time, it will be crossed with cotton to begin transfer of the resistance genes. Additional strategies for transferring the RFN resistance from G. longicalyx are also being pursued.

ENHANCEMENT OF BIOTIC RESISTANCE IN FESCUE WITH *ACREMONIUM* ENDOPHYTES

The presence of the endophytic fungus Acremonium coenophialum Morgan-Jones & Gams in tall fescue (Festuca arundinacea Schreb.) allows beef producers to use a perennial, cool-season grass for pasture that resists pests and drought (West et al., 1993), thrives on marginal soils, competes effectively against weeds and is easy to manage with minimal use of pesticides. Livestock producers, however, pay a high price for the benefits of the endophyte. Numerous alkaloid endo-toxins are produced by A. coenophialum, of which ergopeptines are primarily responsible for causing fescue toxicosis in cattle. Studies were undertaken to identify strains of Acremonium in Festuca germplasm that lack the ability to produce ergovaline and then transfer such strains into a tall fescue population that will form a new synthetic cultivar. Additional technologies include the development of molecular markers to allow differentiation of endophyte strains.

Five tall fescue accessions from the USDA Plant introduction collection were identified that exhibited no ergovaline production but did contain lolines and peramine (desirable alkaloids for insect resistance) (Holder et al., 1994). Four of five Moroccan accessions obtained through the University of Missouri also fit the desirable alkaloid profile. Five additional accessions collected in North Africa were added to the pool of potential biocontrol agents. Some of the significant accessions are listed in Table 3.

Bioassays of endophyte-infected (E+) and endophyte-free (E-) genotypes representing 10 of the ergovaline-deficient populations showed complete mortality of bird-cherry oat aphids or greenbugs on all the E+ plants Table 4. Insect survival on E- plants varied with genotype, indicating variable host resistance. These bioassay results did not identify any genotypes whose endophytes appeared to be poor candidates for use as biocontrol agents. This was expected since all entries were selected for ability to produce lolines and peramine. Lack of ergovaline production was not necessary to improve resistance to these insects.

Twelve strains of ergovaline-deficient endophytes were introduced into tall fescue, cultivar 'HiMag', via seedling stab inoculation. Syn-1 seed were harvested in 1995 and 1996. Preliminary analysis of seeds and seedling leaf material from the Syn-1 generation indicate virtually 100% transfer of endophytes from the inoculated mother plants and very low to nonexistent ergovaline production, except for one strain, which produced large amounts of ergovaline.

Ten Acremonium isolates from ergovaline-deficient plants and five isolates from high-ergovaline plants indicate genetically distinct taxa in that four mitochondrial DNA RFLP haplotypes were identified among the isolates. Several minor polymorphisms were identified among mtDNA haplotype "A" isolates. Mitochondrial DNA RFLP analysis is useful for identifying genetically distinct taxa; however, substantial genetic diversity can occur among isolates within a mtDNA haplotype. Analysis of RAPDs using different primers allowed finer distinction of isolates within the taxa. There were no obvious banding patterns associated with ergovaline production potential. Interestingly, the "A" haplotype was common across a wide diversity of Festuca whose origins ranged from northern Europe to Morocco.

Genetic variability for ergovaline production potential is present among Acremonium endophytes in Festuca germplasm collected from its native habitat. Morocco had the greatest frequency of ergovaline-deficient E+ Festuca germplasm of all countries sampled. The Atlas mountain range of Morocco is home to diverse communities of Festuca mairei and Festuca arundinacea. High genetic variability in Festuca is apparently associated with high phenotypic variability in Acremonium with respect to the production of ergopeptine alkaloids. Insect bioassays indicated a high level of host resistance to aphids in some Festuca genotypes, thereby masking the ability of their endemic endophytes to enhance insect resistance. Further bioassays will be conducted with artificially inoculated tall fescue populations that show a range in host resistance to aphids. Progress has been made in identifying Acremonium endophytes that do not produce ergopeptine toxins when introduced into improved cultivars of tall fescue, but that will retain biocontrol activity.

IDENTIFICATION, ISOLATION AND UTILIZATION OF GENES FOR PEST

RESISTANCE

With the advent of molecular genetic techniques to insert foreign genes into plants, the potential to increase HPR through genetic engineering has become a reality. With the reality has come the realization that our knowledge concerning the diversity of genetic mechanisms by which plants and other organisms defend themselves is limited.

Induced Resistance Against *Helicoverpa zea*

The biochemical mechanisms of induced resistance to the bollworm Helicoverpa zea in soybean, cotton and tomato were investigated. Induced resistance is activated by a pest feeding on the plant or by some other cue of impending damage from the pest. Under conditions of low pest pressure, plants can fully utilize valuable resources for growth and reproduction rather than defense. Induced resistance is genetically based and can be manipulated for crop improvement.

H. zea was found to be particularly susceptible to plant defenses that impose nutritional and oxidative stress upon the insect (Bi and Felton, 1995; Bi et al., 1994; Felton, 1995a,b; Felton and Gatehouse, 1996; Summers and Felton, 1994, 1996). This multicomponent system is composed of several plant oxidative enzymes, including lipoxygenases, peroxidases, polyphenol oxidases, ascorbate oxidases and diamine oxidases (Bi and Felton, 1995; Felton et al., 1994a,b; Felton and Summers, 1993). The digestive system of the insect is strongly alkaline and oxidative, an environment that favors the oxidative degradation of key nutrients (e.g., ascorbic acid, amino acids, lipids) by these enzymatic systems (Johnson and Felton, 1996a,b). Consequently, the project is focused on

Table 3. List of ergovaline-deficient and ergovaline-high accessions of fescue.

Code	Species	Ergovaline level	Source
32	*F. arundinacea*	Deficient	USDA PI
60	*F. arundinacea*	Deficient	Collection
84	*F. pratensis*	Deficient	USDA PI
85	*F. arundinacea*	Deficient	Collection
245	*F. arundinacea*	Deficient	USDA PI
273	*F. arundinacea*	Deficient	USDA PI
281	*F. arundinacea*	Deficient	USDA PI
310	*F. mairei*	Deficient	Univ. Missouri
330	*F. arundinacea* var. *cirtensis*	Deficient	Univ. Missouri
340	*F. arundinacea* var. *atlantigena*	Deficient	Univ. Missouri
350	*F. arundinacea* var. *atlantigena*	Deficient	Univ. Missouri
360	*F. arundinacea*	Deficient	USDA PI
62	*F. arundinacea*	High	USDA PI
152	*F. arundinacea*	High	USDA PI
193	*F. arundinacea*	High	USDA PI
223	*F. arundinacea*	High	USDA PI
233	*F. arundinacea*	High	USDA PI

Table 4. Classification of fescue genotypes according to mortality reaction of greenbug and bird cherry oat aphid to non-choice tests of E+ and E- isolines.

Insect Pest	Mortality only in E+ plants*	Mortality in E+ plants and some survival in E- plants[†]	Mortality in both E+ and E- plants[‡]
	---------------------------------- Genotype code no. ----------------------------------		
Bird cherry oat aphid	233[§], 340	152[§], 193, 273	32, 62, 84, 223[§] 245, 281, 310 330, 350
Greenbug	32, 84, 152[§] 223[§], 330	281, 340	62[§], 193[§] 233[§] 245, 273, 310, 350

*Category 1, high degree of endophyte-enhanced aphid resistance.
[†]Category 2, moderate host resistance, some endophyte-enhanced aphid resistance.
[‡]Category 3, high degree of host resistance, endophyte contribution to resistance unknown.
[§]High ergovaline-producing genotypes when E+. All others are ergovaline-deficient types.

identifying and characterizing the inducibility of these gene products. Specific objectives are 1) to assess the toxicity of the plant oxidative enzymes, 2) to identify crop accessions possessing high levels of the enzymes and 3) to evaluate the effectiveness of the induced enzymes in resistance.

Induced resistance in each of the three crop species was effective against all larval stages of H. zea. Cotton and soybean field plants, wounded for three days by feeding 4th instar larvae, supported less weight gain (over four days) of both neonates and 5th instars subsequently added to the plants, compared to unwounded control plants (Table 5). In all cases, larvae feeding on wounded plants had significantly smaller weight gains ($P <$ 0.01). Herbivory elicits a cascade of biochemical responses within minutes that continue for days. However, each crop species differs in the specific oxidative enzymes that are induced (Table 6). In addition to the induction of oxidative enzymes, the amount of the antioxidant ascorbic acid is significantly decreased in wounded tissues. This is particularly important because ascorbate is an essential nutrient for the insect, and it also serves as an important antioxidant for the insect in protection against oxidative stress imposed by the induced enzymes (Felton and Summers, 1993).

Four cotton cultivars, 18 soybean accessions and 29 tomato germplasm accessions have been examined for insect resistance. Two accessions of soybean had more potent induced resistance than commercial varieties examined. These accessions had highly inducible lipoxygenase and peroxidase and very high levels of polyphenol oxidase. These accessions appear to possess valuable genes for resistance and may be important as breeding lines. The screen for resistance in tomato germplasm involved Lycopersicon peruvianum and one accession of L. glandulosum. Lines were tested for leaf feeding resistance to H. zea, measured as weight change of third instars after 48 hours feeding, with 'Ozark Pink' as the susceptible L. esculentum control. Susceptibility was defined as weight gain > 30 mg. Three lines, PI 128663, PI 251307 and PI 390669, were as susceptible as Ozark Pink, and one, PI 128649, was consistently resistant with larval weight loss averaging -5 mg. Other accessions ranged between these extremes. Characterization of the basis of resistance is in progress.

Methods to increase the naturally induced response of plants to herbivory are being investigated. Jasmonic acid, a plant product, is able to induce the defensive response in soybean and cotton against neonate and later instar larvae (Table 7). The most pronounced effect was in soybean. These studies aid in understanding plant defense signaling and could provide a novel means to enhance natural resistance in plants.

As a result of this project, new sources of insect resistance in plants are recognized in the gene products lipoxygenase, ascorbate oxidase, diamine oxidase, polyphenol oxidase and peroxidases. These genes are strongly induced in crop plants by insect feeding. Knowledge of this system of resistance provides an additional trait for selection in the development of genotypes with superior HPR.

IDENTIFICATION AND UTILIZATION OF GENES FOR HPR

A project to identify endogenous and foreign bioactive proteins and peptides that may have potential to improve host plant resistance to cotton through genetic engineering was initiated.

Lectins

Lectins are glycoproteins that cause agglutination of red blood cells based on their binding affinity to specific polysaccharide groups that occur in the cell walls. Plant lectins often have affinity for the polysaccharides, such as chitin, found in fungal cell walls and in the gut walls of many insects. These lectins are thought to disrupt membrane function. Cotton was found to produce no lectins detectable by affinity chromatography. As an alternative, a lectin was isolated from rice bran and used in feeding tests to determine its effect on the growth of Heliothis virescens larvae. At 0.1% of the diet by weight, rice lectin inhibited the rate of growth of the larvae and decreased pupal weight. Pupation was delayed two to three days compared to larvae on diet without lectin. Gene clones of barley lectin, which is very similar to rice lectin, nettle lectin and hevein, were obtained by Dr. Thea Wilkins from a third laboratory. A member of Dr. Wilkin's group genetically engineered these into cotton, and seeds have been obtained from the transformed plants that were regenerated. A program is underway at the University of Arkansas to grow these seeds and test for any enhanced insect resistance associated with expression of the lectin genes.

Chitosanase

Plants respond to pathogen invasion by producing a number of proteins, called pathogenesis-related (PR) proteins, that inhibit the growth of many microorganisms. Some of these are hydrolytic enzymes such as glucase and chitinase that attack the cell walls of the pathogens.

Chitin occurs in the cell walls of many fungi, but they also contain chitosan, which is not hydrolysed by chitinase. It was determined that cotton possesses both chitinase and chitosanase enzymatic activity, but the level of expression was not particularly high. A small quantity of the chitosanase enzyme was purified to near homogeneity and an attempt made to get a partial microsequence of the amino acids that could be used to develop a degenerate DNA probe for the gene. The protein appears to possess a glyco-moiety that blocks the microsequencing reaction. In an alternative approach, numerous indigenous microorganisms were examined for chitosanase activity as a potential source of a foreign gene to put into cotton. Five organisms were isolated with this activity (three fungi and two bacteria), and, after preliminary investigation, one bacterium was selected for further work. The bacterium was identified as a Bacillus species by rDNA sequence, and the genome of the organism was cloned into an expression library. A clone of an 8-kb fragment of DNA was isolated that possessed the activity, and with exonuclease digestion this was reduced to a 2-kb fragment that still retained the chitosanase activity. The nucleotide sequence of the fragment is currently being obtained to establish the coding region of the gene. The identified region will be fused with a plant gene promoter and terminal sequence, then genetically engineered into cotton.

Magainin

Magainin is a 23-amino acid peptide produced by the skin of the African clawed frog that has wide antimicrobial activity against bacteria. The peptide was tested for its effects against the major fungal disease organisms of cotton, including Pythium, Rhizoctonia, Thielaviopsis, Fusarium and Verticillium. Mycelial growth of all but Pythium was strongly inhibited at 0.05 g/l of the peptide. A synthetic gene with select changes in the nucleotide sequence of the region coding for the peptide is currently being synthesized for insertion into cotton.

SUMMARY

Ultimately, the most cost-efficient and environmentally benign strategy to control pests on an economic crop is for the crop to possess genetic resistance to the pathogen or insect that is causing damage. The genetic resistance can occur naturally within the germplasm pool available to the crop, or, with the techniques of biotechnology, it can be genetically engineered into the crop. Several projects within the APCC have the objective of enhancing host plant resistance. Common features of research concerned with HPR in different crops are 1) the development of methodologies to measure the effects of pathogens and insects and the varying degrees of HPR and 2) surveys to identify sources of resistance among the available germplasm pools for that crop. Once genetic resistance is identified, strategies to transfer the resistance into elite genotypes must be developed. Much of the research under the HPR umbrella has been in these phases of the projects, and the results have been notably successful. As these projects continue, the identified genetic resistances will be transferred to elite germplasm using the screening techniques and strategies developed under APCC auspices.

Application of biotechnology to HPR is dependent upon the identification of defensive genetic strategies used by plants against insects and pathogens or upon the identification and isolation of genes for bioactive peptides from other life sources that can be genetically engineered into the economic plant to enhance HPR. While the techniques for genetically engineering many species of crops are

Table 5. Effect of induced resistance on larval growth.

Crop Host	Four Day Larval Wt Gain (mg)	
	Control	Wounded
Soybean		
Neonate-terminal leaves	5.8	4.2
5th instars/leaves & pods	105.1	32.8
Cotton		
Neonate-terminal leaves	9.2	3.6
5th instars-squares	166.3	123.3

Table 6. Effect of herbivory on foliar oxidative enzymes.

Enzyme	Percent of Unwounded Control		
	Tomato	Soybean	Cotton
Lipoxygenase	103	323*	98
Peroxidase	122	161*	261*
Ascorbate Oxidase	105	214*	225*
Diamine Oxidase	105	157*	122*
Polyphenol Oxidase	989*	131	ND

*Significantly different from control at $P < 0.01$. ND= not detectable.

Table 7. Effect of jasmonic acid on induced resistance to *H. zea*.

Larva stage or enzyme	Weight or Activity (% of control)	
	Cotton	Soybean
5th instar	71*	58*
Lipoxygenase	107	136
Peroxidase	127	94
Diamine oxidase	121*	---

*Significantly different from control at $P < 0.01$.

available, much less is known concerning genes that would be useful in HPR. Part of the research within the APCC is involved in this approach to alternative pest control.

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Chapter 6

Weed Control
with Allelopathy

Weed Control with Allelopathy

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INTRODUCTION

Researchers are continuously searching for new weed control strategies that will prevent crop yield loss and reduce production costs in crop production systems. The potential use of allelopathic species may be a solution. Plant communities consist of several species with varying populations that interact with one another. In fact, plants tend to interfere with the growth of one another by interference or a combination of competition and allelopathy (Smith and Martin, 1994). Competition involves the struggle between plants for growth resources as well as space (Munawar et al., 1990; Putman, 1988; Roberts and Dawkins, 1967). Allelopathy, in contrast, is an interaction between plants and/or microorganisms in which a chemical compound is released by a donor species and directly or indirectly affects the growth and development of a recipient species (Muller, 1966; Munawar at al., 1990; Putnam, 1988). These biochemical effects may be either inhibitory or stimulatory to the target plant (Putnam, 1988).

Potential allelochemicals are present in varying concentrations in virtually all plant tissues (Muller, 1966, 1969). The amount of allelochemicals produced by plants is dependent on the type of compound and the plant species (Muller, 1966). Allelochemicals are released into the environment by volatilization, exudation by roots, leaching from plant tissue by rainfall and biotic and abiotic decomposition of plant residues (Johnson and Coble, 1986; Muller, 1966; Putnam, 1988). The method of release influences the effectiveness of the allelopathic compounds. Plant residues placed on the soil surface increase the concentration of allelochemicals in the upper soil layer where most seeds germinate (Munawar et al., 1990). In addition, rate and duration of allelochemical release vary with plant species (Bhowmik and Doll, 1982). The advantages of allelochemicals in a crop cultivar or cover crop or weed to selectively inhibit weeds but not crops are receiving increased attention (Munawar et al., 1990). An allelopathic crop or crop residue may potentially reduce weed growth (Bhowmik and Doll, 1982; Muller, 1966).

Allelopathy has been shown in crops such as cucumber (Cucumis sativus L.) (Putnam and Duke, 1974), oat (Avena sativa L.) (Fay and Duke, 1977), rice (Oryza sativa L.) (Dilday et al., 1989; 1994), rye (Secale cereale) (Barnes and Putnam, 1986; Shilling et al., 1985), sunflower (Helianthus annuus L.) (Leather, 1983) and wheat (Triticum aestivum L.) (Shilling et al., 1985; Steinsiek et al., 1982). These crops release toxic substances into the environment either through root exudation or from the decaying plant material (Punam, 1988; Putnam and Duke, 1974; Shilling et al., 1985).

The USDA/ARS rice world germplasm collection contains 16,476 varieties or accessions from 99 countries. This collection is the primary genetic base for all rice variety development in the U.S. and has been shown to contain accessions with high allelopathic potential (Dilday et al., 1989; 1994). However, of the rice varieties that have been released in the southern rice belt (Arkansas, Louisiana, Mississippi and Texas), all of the genetic diversity in the breeding programs can be traced to only 22 accessions out of the 16,476 (Dilday, 1990).

The use of cover crops is gaining wide acceptance because cover crops improve soil fertility,

minimize soil erosion and exude allelochemicals that suppress weed growth (Leibl and Worsham, 1983; Martin et al., 1990; Smith and Martin, 1994; Shilling et al., 1985). Legume cover crops such as hairy vetch are also used for amelioration of soil N (Brown et al., 1993). Mixing a grass and legume cover crop benefits the companion crop through added N, improved soil cover and weed suppression (Fisher and Davies, 1991). Thus, cover crops are a promising component of reduced or no-till vegetable weed management programs.

In tall fescue (Festuca arundinacea Schreb.) lawns, large crabgrass [Digitaria sanguinalis (L.) Scop.] is often less severe than in common bermudagrass [Cynodon dactylon (L.) Pers.] lawns. Water extracts of 'Missouri-96' tall fescue leaves inhibited germination and root and shoot growth of birdsfoot trefoil (Lotus corniculatus L.) and red clover (Trifolium pratense L.) by 30 to 50% (Peters and Zam, 1981). Extracts from 'Kentucky 31' (KY-31) tall fescue were several times more inhibitory against birdsfoot trefoil during the winter months and the months of peak growth (April-May and October-November) than during the summer months, and allelopathy was also greater with higher nitrogen fertilization rates (Luu et al., 1982). The chemical nature of the inhibitory substances has been partially determined (Luu et al., 1989). However, the connection between tall fescue allelopathy and Acremonium endophytes has not been determined.

One drawback to the use of cover crops is the cost associated with their establishment. Naturally occurring populations of allelopathic weed species may be used instead of cover crops. These weeds also produce potentially allelopathic residue without any establishment costs. In addition to the reduction of production inputs, these natural populations may prove to be more effective. Approximately 90 weed species have been shown to possess allelopathic potential (Putnam, 1988). Weeds are abundant and will naturally dominate a field in the spring and early summer if left undisturbed.

Rice Allelopathy

Field experiments were conducted from 1988 to 1995 to identify rice accessions possessing allelopathic properties to ducksalad [Heteranthera limosa (Sw.) Willd.], purple ammania (Ammannia coccinea) and barnyardgrass [Echinochloa crus-galli

(L.) Beauv.] as part of the USDA/ARS rice germplasm evaluation project at Stuttgart, Arkansas, on a Crowley silt loam (fine montmorillonitic, thermic Typic Albaqualfs). Approximately 14,000 accessions, including checks, were seeded in hills in a 0.75 by 0.75 m grid and evaluated for allelopathy to a natural and uniform infestation of ducksalad, and about 5,000 accessions were evaluated for allelopathy to purple ammania.

Rice allelopathic activity to ducksalad was recorded in July or at panicle initiation and to purple ammania at rice maturity. Allelopathic activity was determined either by measuring the radial area in meters from the base of the rice plant that was affected or by determining the percentage of weed control within the affected area based on the number of ducksalad or purple ammania plants in a check plot.

Field experiments were conducted in 1992 and 1993 to compare the efficacy of dry- and water-seeded rice in row and broadcast patterns on allelopathic activity to ducksalad. Five allelopathic rice germplasm accessions (PI 312777, PI 338046, PI 338065, PI 345920 and PI 373026) and 'Rexmont' (PI 502968), a standard cultivar without allelopathic activity, were tested both years. All water-seeded plots were surrounded with a metal circle to prevent movement of rice seedlings and covered with bird netting immediately after seeding until rice plants were 15 cm tall to prevent bird damage. In dry-seeded rice, aquatic weeds germinated 35 days after seeding or five to seven days after flooding, but in water-seeded rice, the aquatic weeds germinated at the same rate as rice. Unwanted weeds were controlled by propanil, bentazon and fenoxaprop, but in water-seeded rice they were controlled by hand weeding. Visual ratings were taken seven weeks after flooding. At rice maturity, weeds were harvested from the entire plot (2.1 m^2), dried, weighed and expressed as percent reduction compared to Rexmont.

A field experiment was conducted in 1994 to determine the influence of rice allelopathy and N management on aquatic weeds, including ducksalad and purple ammania. Four rice germplasm accessions (PI 312777, PI 338046, PI 366150 and PI 373026) and Rexmont were water seeded. The five N rates were 0, 67, 101, 134 and 202 kg/ha. Three N rates (67, 101 and 134 kg/ha)

were soil incorporated 5 cm deep before flooding. For the 202-kg/ha rate, a split application of 134 kg/ha was soil incorporated, and the other 67 kg/ha was applied at midseason. Grasses were controlled by flooding. The other weeds except ducksalad and purple ammania were controlled by hand weeding. Dry weights of ducksalad and purple ammania were collected at rice maturity.

Derivatization methods for GC-MS analysis of allelochemicals related to rice were established. The methodology was used with previously identified allelochemicals (more than 40) to further chromatographic method development and to establish a GC-MS library for use in the screening for allelochemical content of exudates from rice roots grown in liquid culture.

Initially a Continuous Root Exudate Trapping System (CRETS) was used to determine if allelochemicals released as root exudates into the liquid culture system could be collected (trapped) with XAD-4 resins (Fig. 1). The traps were periodically replaced, the collected resins eluted and evaluated by GC-MS and the Lemna bioassayed for allelochemical content and phytotoxicity, respectively. Other rice liquid culture greenhouse bench systems were also explored, developed and established for the collection and trapping of allelochemicals produced from rice root systems. The first system utilizes a pot that receives nutrient solution from an emitter placed in the root zone and is equipped with a drain tube situated above the sand support to allow for flooded conditions. Several pots of a particular allelopathic or non-allelopathic accession or variety may be connected to a common reservoir and pumping system. Also, alternate pots of the test weed species may be connected to the common reservoir, providing a physical separation while maintaining the potential chemical connection. The nutrient reservoir is maintained in a manner to allow the collection and build-up of allelochemical exudates. Another system is a static-pot system in which each pot is equipped with a bottom drain and a stand-alone reservoir, which can be periodically sampled. The continuous-flow-through system provides for a continuous flushing of the rice root exudates into the common reservoir. Periodic sampling of the reservoirs allows for bioassay and GC-MS analysis. For chemical analysis, culture solutions are collected, extracted by a solid phase disk extraction method (developed in the Pesticide Residue Laboratory)
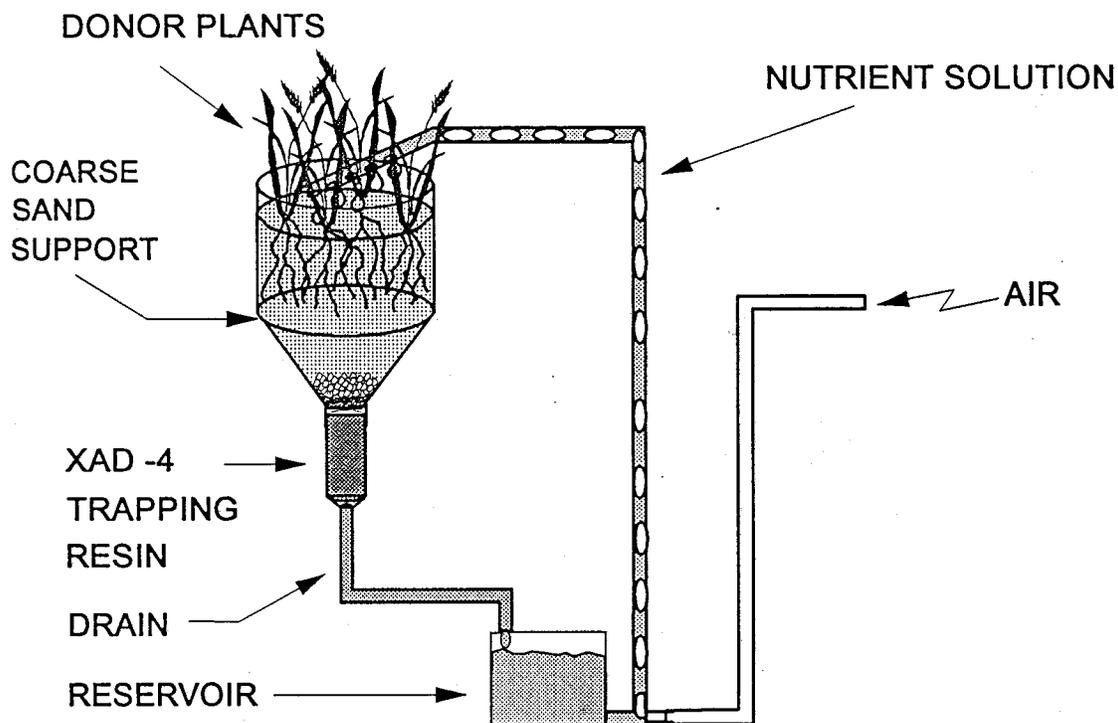

DONOR PLANTS

NUTRIENT SOLUTION

COARSE SAND SUPPORT

AIR

XAD -4 TRAPPING RESIN

DRAIN

RESERVOIR

Fig. 1. CRETS: Continuous Root Exudate Trapping System.

and analyzed by GC-MS to identify the chemical components of rice exudates from allelopathic versus non-allelopathic rice.

Efforts also continue on developing bioassays that utilize the affected weed species to provide data comparable with field observations. This work includes utilizing soil from Stuttgart (Crowley silt loam) that is naturally infested with ducksalad seed in the previously mentioned systems as a replacement for sand support. In order to aid the collection of nutrient solutions for GC-MS analysis and Lemna bioassays, the soil is mixed 1:2 with sand. This allows the flood water to sufficiently permeate through the soil/sand medium and potentially flush exudated allelochemicals out through the bottom drain for collection. Concomitantly, the natural ducksalad infestation provides a bioassay for possible comparison and correlation with both the chemical analysis and Lemna bioassays.

Field data indicated that about 4% or 156 of the rice accessions had a radius of activity greater than 10 cm to ducksalad, and these accessions were significantly different from the check plants that demonstrated no allelopathic activity. Of the 4%, 15 accessions had a radius of activity of 17 to 20 cm and weed control between 65 to 90%, and another eight accessions had a 13- to 15-cm radius of activity and 50 to 85% weed control in the area of activity (data not shown). The accessions that demonstrated allelopathic activity originated in 30 countries. Seven accessions reduced ducksalad dry weight from 91 to 98% of the Rexmont control (Table 1). The accessions that demonstrated allelopathic activity to ducksalad were genetically diverse for other plant characteristics. For example, days from emergence to anthesis ranged from less than 60 days to more

Table 1. Germplasm accession, country of origin and percent control of ducksalad compared to 'Rexmont', a cultivar without allelopathic activity.

Accession	Country	% Control
India AC 2489	India	98
MON X WUAN	China	97
BR41/AKANO/SBLR	U.S.A.	95
India AC 1423	India	95
Afghanistan No. 2	Afghanistan	92
YH 1 (Taiwan)	Taiwan	91
TSAI YUAH CHON	Taiwan	91
Rexmont	U.S.A.	0
LSD $_{(0.05)}$		10

than 140 days, plant height ranged from less than 79 cm to more than 160 cm, grain type included short (<5.50 mm), medium (5.51 to 6.60 mm) and long (6.61 to 7.50 mm) kernels, and most of the genotypes that were less than 110 cm demonstrated no to slight lodging. Agronomic characteristics such as these descriptors are important in selecting parents for varietal development programs. Thus, rice germplasm from two regions, China/Taiwan and Pakistan/India, constitutes a promising genetic base for allelopathy in rice to ducksalad (data not shown). For example, 58% of the most promising accessions that were identified in this study came from either China (Dilday et al., 1989) and Taiwan (Brown et al., 1993) or Pakistan (Einhellig et al., 1985) and India (Brown et al., 1993). Furthermore, these data show that the germplasm from China is a good source of allelopathy to ducksalad for medium-grain or japonica rice, whereas Pakistan is an excellent source for long-grain or indica type germplasm.

Ducksalad produced 246 and 447 g/m² dry weight averaged over row and broadcast seeding methods in dry- and water-seeded Rexmont, respectively (data not shown). In dry-seeded rice, the five allelopathic rice accessions reduced aquatic weed biomass 77 to 88% in broadcast and 62 to 80% in row compared to Rexmont. In water-seeded rice, the five accessions reduced aquatic weed biomass 61 to 83% in broadcast and 27 to 61% in row planting compared to Rexmont. Thus, seeding allelopathic rice in either a broadcast or row pattern, especially broadcast, will improve aquatic weed control.

About 3% or 145 of the rice accessions had a radius of activity greater than 20 cm to purple ammania (data not shown). The accessions that demonstrated allelopathic activity to purple ammania originated in 21 countries. However, 55% of the accessions with allelopathic activity to purple ammania came from the International Rice Research Institute (IRRI) in the Philippines. IRRI knows the pedigrees, and their countries of origin appear to be diverse.

The level of nitrogen management improved the allelopathic activity of the four accessions tested on ducksalad and purple ammania (Table 2). The four accessions were more allelopathic to purple ammania than to ducksalad when compared at the zero nitrogen level. Increasing the

Table 2. Influence of nitrogen (N) management on the allelopathic activity of five rice accessions on ducksalad and purple ammania biomass reduction in water-seeded rice.

| N levels | PI | Reduction of biomass | |
		Ducksalad	Purple ammania
(kg/ha)		----------------%----------------	
	31277	53	72
	338046	53	61
0	366150	53	79
	373026	47	68
	Rexmont	0	0
	312777	69	93
	338046	82	96
67	366150	52	90
	373026	58	88
	Rexmont	0	0
	312777	88	99
	338046	94	98
101	366150	97	99
	373026	92	99
	Rexmont	0	0
	312777	100	100
	338046	100	100
134	366150	100	100
	373026	100	100
	Rexmont	0	0
	312777	100	100
	338046	100	100
202	366150	100	100
	373026	100	100
	Rexmont	0	0
LSD $_{0.05}$		18	9

nitrogen level to 67 kg/ha improved purple ammania control more than ducksalad control; however, at 101 kg/ha, control was comparable. At 134 kg/ha or more, the allelopathic activity or control was 100% regardless of rice accession.

Presently, 5,600 rice germplasm accessions have been evaluated for allelopathy to barnyardgrass (data not shown). Nine accessions have demonstrated an apparent reduction in barnyardgrass growth and development. These accessions originated in Bangladesh, China, Pakistan, Philippines and Vietnam.

Several known allelochemicals have been identified, but it is not yet known if these are the chemicals producing the observed field effects. The presently identified chemicals are 2-hydroxybenzoic acid, 4-hydroxy-3-methoxy-cinnamic acid, 4-hydroxy-3-methoxybenzoic acid, 4-hydroxybenzoic acid, 2-hydroxycinnamic acid and benzoic acid (data not shown). The Lemna bioassay technique is sensitive to most of the potential rice allelochemicals, and detectable levels have been established.

As of January 1996, chromatographic comparisons between rice accessions that exhibit field allelopathy and non-allelopathic activity had not shown obvious quantitative or qualitative differences. Similarly, Lemna bioassays have not demonstrated significant reproducible phytotoxic differences from liquid culture or flood water collected from greenhouse-grown rice. Earlier examinations of rice tissue extracts occasionally demonstrated phytotoxic effects against the Lemna bioassay, but the effect is often non-reproducible.

Fall-Planted Cover Crops in Vegetables

Rye, wheat, hairy vetch (Vicia villosa Roth) and rye plus hairy vetch (2:1 mix v/v) cover crops were seeded into tilled soil 9 and 10 October 1991 and 29 and 25 September 1992 at the Main Agricultural Experiment Station in Fayetteville, Arkansas, and the Vegetable Substation at Kibler, Arkansas, respectively. Cover crops were seeded at 123, 123, 22 and 39 + 15 kg/ha, respectively, for rye, wheat, hairy vetch and rye plus hairy vetch in 6- by 4-m plots, replicated four times. A plot without cover crop, disked twice before planting the vegetable crops but with no cultivation during the growing season, was the standard. Plots were established on a Captina silt loam with 1.5% organic matter and 6.5 pH at Fayetteville and on a Roxana silt loam with 1.2% organic matter and 6.9 pH at Kibler. Each year, cover crops were desiccated with paraquat at 0.84 kg ai/ha plus 0.25% v/v nonionic surfactant in the spring between 17 April and 4 May when rye and wheat were booting and vetch was starting to bloom. 'Dixie' yellow crookneck summer squash and 'Merit' sweet corn were direct seeded with a single-row, no-till planter with a fluted coulter*, double disc openers and double press wheels into the standing cover crops, and four-week-old, greenhouse-grown 'Mt. Pride' tomato plants were transplanted by hand two to three weeks after desiccation.

For sweet corn, herbicide treatments were half and full rates of atrazine plus metolachlor (2.2 + 2.2 kg ai/ha), applied preemergence. Herbicide treatment for summer squash was sethoxydim (0.34 kg ai/ha) for grass weeds augmented at four weeks after planting (WAP) with a post-directed application of paraquat (0.5 kg/ha). Postemergence weed control treatment for tomato was a minimum labeled rate of sethoxydim (0.22 kg/ha) followed by reduced rate of metribuzin (0.28 kg ai/ha). For tomatoes, herbicide treatments were strip-applied across cover crops; each subplot consisted of one crop row. All herbicide applications were made at 187 L/ha spray volume. Each cover crop within the vegetable crop had a no-herbicide plot.

Studies at Fayetteville were replanted four weeks after cover crop desiccation in 1993 because

of hail damage. Fertilizer was applied to the vegetable crops based on soil analysis. Tomato plants were staked and pruned to two stems per plant. Pesticide applications and irrigation were made as needed.

In general, major weeds in 1992 at Fayetteville were yellow nutsedge (Cyperus esculentus L.), large crabgrass and goosegrass (Eleusine indica L. Gaertn.). Major weeds at Kibler were Palmer amaranth (Amaranthus palmeri S. Watts) and goosegrass. Total weed emergence five weeks after cover crop desiccation was lower in all summer squash plots with a cover crop than without a cover crop (Table 3). The rye plus vetch in sweet corn and tomato also reduced weed emergence. The reduced weed emergence is due to cover crop residues physically impeding emergence of weed seeds and reducing light penetration to the soil surface, thereby limiting seedling growth, or due to allelopathic compounds exuded by the cover crop residues. Standing rye residue reduced emergence of large crabgrass, redroot pigweed (Amaranthus retroflexus L.), common lambsquarters (Chenopodium album L.) and common purslane (Portulaca oleracea L.) (Mohler and Calloway, 1992). However, perennial weeds that grow from underground rhizomes or tubers, such as yellow nutsedge, were not affected by the presence of cover crops. In fact, yellow nutsedge density increased by 30 to 60% in the second year in Fayetteville, and yellow nutsedge control with herbicides declined in 1993, regardless of cover crops (Burgos and Talbert, 1996).

All cover crops except vetch alone reduced emergence of sweet corn and summer squash (Table 3). This could be due to planting interference by the residue, improper furrow closure or harbored pests in cover crop residues. However, since cover crops afforded similar impediments to crop emergence and the like, reduced stand in rye plus vetch, rye alone and wheat may also be partly attributed to allelopathy of these residues. Also, in 1992, there was increased feeding on squash seedlings by cucumber beetles in cover crop plots (Burgos and Talbert, 1995). Pest incidence was low in 1993.

Summer squash yields were generally lower in 1992 than in 1993 because of a heavy beetle infestation and escaped Palmer amaranth from a post-directed paraquat application (Table 3). Low pest numbers and better weed control in 1993

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*John Deere 7100, Deere & Co., P. O. Box 663, Moline, Illinois 61266-0663.

Vegetable crop	Cover crop	Total weed density* Fayetteville	Kibler	Crop emergence 2 WAP†	Yield‡ 1992 - herb.	+ herb.§	1993 - herb.	+ herb.
		----- no./m² -----		no./4.2 m	---------------------- mt/ha ----------------------			
Summer squash	Rye + vetch	--¶	63	8	3.2	5.4	2.1	33.0
	Rye	--	99	5	< 1	1.2	4.4	30.5
	Wheat	--	90	5	1.0	1.5	4.4	21.9
	Vetch	--	89	12	4.2	9.6	6.8	28.1
	No cover	--	274	12	1.5	8.0	11.9	37.3
	LSD (0.05)	--	172	6	3		9	
Sweet corn	Rye + vetch	44	26	9	4.8	5.8	7.1	9.3
	Rye	36	58	10	2.8	6.0	5.6	8.6
	Wheat	24	34	13	3.6	8.2	5.8	6.9
	Vetch	98	80	21	6.5	10.5	7.8	8.1
	No cover	112	125	24	5.5	11.4	6.5	8.2
	LSD (0.05)	NS	88	3	2		NS	
Tomato	Rye + vetch	98	91	--	52	45	21	46
	Rye	258	154	--	21	39	15	39
	Wheat	123	152	--	25	33	6	35
	Vetch	222	166	--	33	46	14	33
	No cover	197	193	--	21	42	7	36
	LSD (0.05)	NS	71	--	18		NS	

*Taken five weeks after cover crop desiccation.
†WAP = weeks after planting.
‡Averaged across locations except for sweet corn and tomato in 1993, which was only Fayetteville data.
§Summer squash: sethoxydim (0.34 kg/ha) POST, paraquat (0.50 kg/ha) POSTDIR; sweet corn: atrazine (1.1 kg/ha) + metolachlor
 (1.1 kg/ha) PRE; tomato: sethoxydim (0.22 kg/ha), metribuzin (0.28 kg/ha) PRE.
¶Summer squash was planted only at Kibler; tomato was transplanted.

resulted in higher yield with herbicides. The reduced summer squash yield in 1992 by rye and wheat indicates a possible allelopathic response.

Sweet corn yield in 1992 was reduced by all cover crops except vetch, with or without herbicide (Table 3), as a result of stand reduction and reduced sweet corn growth between four and nine weeks after planting (data not shown). No yield differences were observed in Fayetteville in 1993, probably due to replanting sweet corn at four weeks after cover crop desiccation or when the effects of cover crop residues had dissipated.

Between vegetable crops, growth and yield of tomato were least affected by the presence of cover crop residues (Table 3). Transplanted vegetable crops generally fare better in the presence of residues than direct-seeded crops, probably because of greater tolerance of a larger plant to limiting conditions presented by the cover crops. Without herbicides, in 1992 across locations, tomato plants in rye plus vetch yielded higher than rye, wheat and no cover crop treatments. Highest response to herbicide application was observed in plots without cover crop. Thus, cover crop residues suppressed weed growth. As in sweet corn, no differences in yield were detected in 1993 because of replanting the experiment at Fayetteville four weeks after cover crop desiccation due to hail damage.

Tall Fescue Allelopathy

Allelopathic effects of 'Apache', 'Aquara', 'Arid', 'Crossfire', 'Falcon', 'Hubbard 87', 'Kentucky 31', 'Richmond', 'Safari', 'Shenandoah', 'Silverado' and 'Twilight' turf-type tall fescue cultivars were evaluated in bermudagrass turf for large crabgrass suppression in the fall and following spring. Also, in 1987 and 1992, National Turfgrass Evaluation Program tall fescue tests containing 65 and 92 cultivars, respectively, were overseeded with strips of large crabgrass seed. Density and percent large crabgrass cover were collected.

A three-frond duckweed (Lemna minor L.) bio-assay (Einhellig et al., 1985) was conducted on water extracts of leaves of the 12 tall fescue cultivars. After seven days of growth, frond number, growth rate and dry weight were obtained. Sample preparation is by aqueous blending of weighed tall fescue leaves, centrifuging debris, filtering supernatant and pipetting aliquots into a 24-well tissue culture cluster plate.

Only four of 65 tall fescue cultivars had less than 8% large crabgrass cover; three of these were in the four densest cultivars, 'Olympic II', 'Tribute' and Hubbard 87. But 'Austin' had only 6.4% large crabgrass with a density of only 5.5 out of 9 for full ground cover, indicating that Austin might be allelopathic. Nineteen of the 65 cultivars were less dense than Austin, and all seven cultivars that had more than 29% large crabgrass were less dense than Austin. KY-31 was the least dense at 2.8 and had the most large crabgrass. KY-31 was expected to be allelopathic (Luu et al., 1982).

More inhibition of duckweed occurred in 1994 from spring than from summer tall fescue samples. In fact, summer samples generally stimulated Lemna growth. Thus, in 1995 monthly sampling of KY-31, Safari and Shenandoah was initiated. A strong tendency exists for less duckweed inhibition with lower extract concentrations, but no instances of stimulation were found during the spring and summer. The plot fertilization and irrigation during the summer may be the reason for continued allelopathic results in the summer. Luu et al. (1982) found less allelopathy during the summer. The inconsistencies in the results make interpretation of the data difficult.

Weed Allelopathy

Weeds and weed seeds with previously determined or perceived allelopathic activity were collected from throughout Arkansas. Immature (four- to six-week-old) and mature (mature seed produced) whole plants were harvested either from field- or greenhouse-grown plants. To evaluate the allelopathic activity of each species, greenhouse, growth chamber and germination studies were conducted. In greenhouse studies, the fresh plant material was immediately placed on the soil surface or chopped and incorporated into the soil of designated flats. Weed residue levels were determined from the previously obtained naturally occurring biomass samples. Studies were conducted using a factorial experimental design and four replications with the factors being placement of residue (intact on surface, mulched on surface and mulched and incorporated) as well as residue rate (0, 0.5, 1 and 2 times the normal biomass production). Test species to evaluate phytotoxicity were soybean, barnyardgrass, large crabgrass, sicklepod (Senna obtusifolia L.), pitted morningglory (Ipomoea lacunosa L.), prickly sida (Sida spinosa L.), Palmer amaranth (Amaranthus palmeri S. Wats) and common cocklebur (Xanthium strumarium L.). Plant counts and heights were recorded at two and four weeks after emergence (WAE). Fresh and dry weights of each species were also taken at four weeks.

Water-soluble extracts of each potentially allelopathic weed were evaluated in weed seed germination tests. Each germination study was established as a randomized complete block with four replications. The seeds used to evaluate allelopathic effects on germination were the same species as those used in the greenhouse study. The water-soluble extracts were obtained from ground fresh plant material. An extract stock solution was made by grinding 500 g of plant material in 1000 ml of deionized water. This solution was considered to be full strength. From the full-strength stock solution, the following dilutions were made: 1:1, 1:3, 1:5, 1:7, 1:11, 1:15, 1:34, 1:49 and a check using deionized water. Whatman #2 filter paper was placed in the bottom of each petri dish and on top of the seeds with 10 ml of the appropriate extract being added. The petri dishes were incubated at 35 C in the dark, and seed germination determinations were made every four days for 20 days.

Aqueous plant extracts were used in the Lemna bioassay. The same dilutions as in the seed germination studies were then filtered and centrifuged to remove debris and sediment, and Lemna culture nutrients were added. The pH was adjusted to 4.4 and the extract filter sterilized for use in the Lemna assay. The dilutions were added to the appropriate wells in the culture plate, and a single three-fronded duckweed plant was added to each well. The plates were sealed and placed in an incubator for a week. After incubation, the plates were removed, and the number of fronds produced was recorded for each well. The Lemna

studies were conducted using a randomized complete block design with six replications.

Cutleaf eveningprimrose (Oenothera laciniata Hill), wild chamomile (Matricaria chamomilla L.), hemp sesbania [Sesbania exaltata (Ref.) Rydb. ex. A.W. Hill], annual fleabane [Erigeron annuus (L.) Pers], plains coreopsis (Coreopsis tinctoria Nutt.), Palmer amaranth, johnsongrass [Sorghum halepense (L.) Pers.], horseweed [Conyza canadensis (L.) Cronq.] and bulbous buttercup were evaluated. Cutleaf eveningprimrose and bulbous buttercup (Ranunculus bulbosus L.) showed the greatest allelopathy potential. All dilutions of bulbous buttercup extract inhibited germination of soybean, sicklepod and pitted morningglory compared to the check. Germination of common cocklebur was reduced by dilutions of 0.25 or greater. All dilutions of the cutleaf eveningprimrose extract inhibited germination of soybean and sicklepod. In the greenhouse, all residue levels of bulbous buttercup reduced the number of pitted morningglory but increased the fresh weight per plant of soybean and common cocklebur. Bulbous buttercup residue placed on the soil surface increased soybean but reduced sicklepod and common cocklebur fresh weight per plant. The 1X and 2X residue levels of cutleaf eveningprimrose reduced the number of common cocklebur when the residue was placed on the soil surface. Fresh weight per plant of common cocklebur was increased by all residue rates of cutleaf eveningprimrose. In general, bulbous buttercup showed more allelopathic potential than cutleaf eveningprimrose.

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Chapter 7

Environmental Fate/ Risk Assessment

Environmental Fate/ Risk Assessment

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INTRODUCTION

Understanding the fate of pesticides in the environment has been the goal of numerous studies. Benefits of pesticides to agricultural production are easy to document; however, identifying and assessing the significance of risks their use may pose to the environment is a much more difficult task.

Continuing concerns at the national level regarding the possible detrimental effects of pesticides on the environment have led to increased efforts to identify, quantify and confirm any pesticide residues present in soil, surface water and ground water. These questions are of special concern to some living in or around areas such as the Mississippi Delta where pesticides have been used intensively for many years.

Since 1979 several studies at the national level have reported the presence of pesticides in ground water. Cohen et al. (1984) indicated that pesticides most likely to contaminate ground water are those highly soluble in water and those that are neither quickly degraded nor strongly adsorbed. By 1985 17 pesticides had been detected in ground water of 12 states (CAST, 1985). Ground water monitoring efforts within Arkansas in the early and middle 1980s resulted in the analyses of water from over 119 wells, springs and municipal drinking water supplies. The majority of the wells included in these studies were used solely for crop irrigation purposes (Cavalier et al., 1989). In contrast to results from several monitoring studies from the upper midwestern United States, these studies, in which each sample was analyzed for 18 pesticides, identified only one well with a pesticide contaminant. The sample from this well contained trace levels of three commonly used herbicides (alachlor, atrazine and metolachlor); these findings were later confirmed by mass spectrometry (GCMS).

Although several monitoring studies have assessed ground water quality on a regional scale, few researchers have assessed ground water at sites where contamination may originate from a spill or inadequate disposal around the site of pesticide loading or mixing. The greatest concentrations of these compounds occurred at acute spill areas, burn piles, container storage areas and mixing/loading areas (Habecker, 1989). At the initiation of studies reported here, there was no information available on the quality of ground water at similar sites in Arkansas.

There are increasing accounts of surface water contaminated by pesticides. The United States Environmental Protection Agency (USEPA) reported that 76% of lake acreage, 65% of stream mileage and 45% of estuarine square miles were affected by agricultural runoff (USEPA, 1992). Runoff has been defined as water containing dissolved or suspended matter that leaves a plot, field or watershed in surface drainage (Leonard, 1990). Specifically, pesticide runoff includes dissolved, suspended and sediment-adsorbed pesticide transported by water from a treated land surface (Leonard, 1990). Depending on the soil properties deep within the soil profile, contaminated surface water may also affect ground water quality. No wide-scale surface water monitoring study had been conducted previously within the state to provide base-line information regarding the pesticide contaminant status of Arkansas surface waters.

Although there are several sources that could presumably result in a pesticide-contaminated aquifer or soil profile, one source would be the intensive use of soil-applied herbicides. Most effective herbicides must possess water solubility properties that permit them to move through the soil to the imbibing weed seedling; this same property provides them with mobility properties that may allow them to move laterally or vertically within the soil profile. The last section of this paper examines the possibility that some herbicides may pass through the upper portions of the soil profile and the subsoil, resulting in their becoming a threat to ground water in the state.

A major impediment to providing extensive data describing the residual pesticide levels currently present in water samples is the cost associated with sample collection, trace level analysis and confirmation of the findings. This cost is then compounded by the fact that numerous pesticides are in use and the need to take multiple samples. It is not uncommon that over 30 pesticides are being used in an agricultural watershed where rotational cropping is practiced. Another barrier complicating large-sample numbers is the temperature-controlled space required to store and transport the samples prior to analyses due to the instability of pesticides during storage in water. Thus, several impediments point to a vital need to develop methods that could allow us to implement functional time- and cost-effective means for analyzing large numbers of water samples.

Solid-phase extraction (SPE) is a growing technology in the development of environmental sample preparation. Recently, advanced SPE technology has produced SPE membrane filters or disks (Hagen et al., 1990; Markell et al., 1991). If these SPE methods are effective for the pesticides being used in the Delta, this technology could conceivably become a significant tool for facilitating large-scale environmental monitoring efforts in our studies, across the nation and on a world-wide basis.

ASSESSING WATER SAMPLES FOR MULTIPLE PESTICIDES

Twelve row-crop pesticides common to the Mississippi Delta were included in an experiment designed to compare the stability of pesticides when stored in water in glass containers compared to that of pesticides stored on SPE filter disks. These disks had been used to extract the pesticides from specially prepared water samples fortified with the 12 pesticides.

A volume of 250 mL of deionized water was fortified at 20 µg/L concentration of each pesticide in water. A 47-mm Empore™ Extraction Disk* was placed on a sintered glass filter funnel apparatus attached to a vacuum source. The entire fortified sample (250 mL) was then added to the filter funnel containing the disk and drawn through at approximately 25 to 30 mL/min. The disk was removed from the filter holder and placed in a polyethylene Ziploc™ bag (Dow Brands, Indianapolis, Indiana) and stored under four different storage regimes for periods of 0, 30, 90 or 180 days.

After the appropriate storage period, the filter was visibly reoriented back on the filter apparatus so that the originally exposed area was above the sintered glass. Borosilicate glass vials (20-mL capacity) were then placed in the base of the vacuum manifold to catch ethyl acetate, which was used to elute the pesticides from the disk. A 1.5-mL aliquot of the 5-ml sample was placed into a sample vial for GC (electron capture) or HPLC (ultraviolet detector) analysis.

The treatments used in this study represented several different conditions of pesticide storage. The four storage treatments included 1) bottled water stored at 4 C, 2) pesticides stored on SPE disks at 4 C, 3) pesticides stored on SPE disks at -20 C and 4) a combination disk storage treatment (4 C for 1 day, then -20 C for the remainder of the storage period). The five storage periods of 0, 3, 30, 90 and 180 days were included to evaluate stability.

For most pesticides, disk storage was equivalent or superior to storage in water. In general, the highest percentage recovery for these pesticides occurred when the disk was stored at -20 C, and the lowest recovery on the disks occurred at 4 C, although these recoveries did not always differ statistically from the other disk storage treatments (Table 1). Each pesticide stored at -20

Table 1. The effect of storage treatment on percentage recovery of benomyl, simazine, fluometuron and atrazine stored on disks or in water, averaged over all storage periods (Senseman et al., 1993).

	Percent Recovery*			
Treatment	Benomyl	Simazine	Fluometuron	Atrazine
Disk Stored[†] @ 4 C	68	65	74	70
Disk Stored[†] @ -20 C	76	71	79	75
Disk Stored[†] @ 4 C, -20 C	70	69	76	70
Water Stored[‡] @ 4 C	63	61	67	64
LSD $_{(0.05)}$[§]	5.4	6.1	3.8	4.6

*Mean values obtained from 20 observations.
[†]Pesticide/water solutions filtered and vacuum dried through C-18 disks.
[‡]Pesticide stored in bottles containing 250 ml water.
[§]Least Significant Difference. If the difference between the two compared values is greater than the LSD, the values are considered to be statistically similar.

C on the C_{18} material gave better recovery than the same pesticides stored in bottled water. Moreover, the lowest recovery occurred when the pesticides were stored in bottled water, but it was not always statistically different from the next lowest value within a selected disk storage treatment. The combination treatment gave the second highest percentage recovery for each pesticide. Simazine showed no differences among disk storage treatments, indicating that temperature had no effect on the recovery of this compound. Although consistent differences in percentage recovery did not occur between specific disk storage treatments among all pesticides tested, these data show that the pesticides partitioned on the disk are at least as stable as and often more stable than the pesticides stored in water over the same duration.

Since the storage stability of pesticides on SPE media was equivalent or superior to that in water, a comparison among storage stability of these disk-stored pesticides from 3 to 180 days was made (data not shown). The highest recoveries of benomyl, simazine, fluometuron and atrazine occurred after three days of storage.

Two distinct cases of statistical differences between disk storage and water storage were represented in trifluralin and captan data. After 180 days of storage, the recovery for trifluralin stored in bottled water was 32% while recovery from disks ranged from 54 to 64%, representing about a 50% loss of pesticide when stored in water compared with disk storage. Similar trends of pesticide loss were shown for alachlor, metolachlor, methyl parathion, pendimethalin, norflurazon and profenofos; however, during the 180 days of

storage in water, loss was not as great as that for trifluralin. Captan demonstrated the most dramatic results. The recovery from water stored in bottles for three days at 4 C was 28% whereas 114% was recovered from disks stored at 4 C. The differences were more pronounced after 30 days when captan had almost completely dissipated in water while 32 to 54% was recovered from disk storage. This stabilizing effect of the C_{18} material has been observed by Green and Le Pape (1987), who stated that materials bonded to a solid phase were more stable.

Since the stability of selected pesticides has been preserved and, in some cases, enhanced by concentrating the pesticides on C_{18} solid-phase extraction disks, this new methogology now allows water samples that formerly occupied the space necessary for storing 500- to 1000-mL bottles to be reduced to a 0.5-mm thick x 47-mm diameter pliable filter.

MONITORING SURFACE WATERS

This three-year, eight-period monitoring study was initiated in the spring of 1989. Counties were selected based on the amount of agricultural chemical usage, agricultural acreage, soils conducive to agricultural runoff and location near major aquifers. In addition, consultation with the Arkansas Department of Pollution Control and Ecology and county agents provided information concerning counties and potential sampling sites. These sites within each county were based somewhat on accessibility, water resource, personnel and equipment needs and available research funds. The four counties in the intensively row-cropped eastern region of Arkansas chosen as

sampling areas were Jefferson, Phillips, Lawrence and Mississippi Counties. In all, 62 sites were selected from both county highway maps and U.S. Geological Survey maps of each county. These sites represented lakes, streams and rivers.

The eight sampling intervals were selected to coincide with times when pesticides are applied. Preplant incorporated (ppi) and preemergence (pre) are applications typically used for pesticides applied in the spring. In the summer months, fewer ppi or pre pesticides are applied, but postemergence (post) applications increase. The fall sampling represented a period in which infrequent pesticide applications would be expected.

At each location, samples were collected by strapping two 1-L amber glass jars in a metal cage with a rope attached. The jars were lowered below the surface of the water 0.5 to 1 m. The bottles were capped and placed in a cooler filled with ice such that the samples could be transported and temporarily held until stored at 4 C before extraction.

For each sampling period, a sampling site in each county was designated as a fortification site where four extra samples were collected in 1-L amber glass jars and fortified with a solution containing the 17 pesticides. The fortified samples were stored with the other samples to measure any degradation during storage.

The 1-L samples were prefiltered by vacuum filtration through Whatman #5 filter paper to remove particulates before pesticide extraction. The filtered samples were then subjected to the extraction procedure outlined previously in the pesticides-on-disk stability studies.

Analytical standards (>98% purity) were used to prepare fortification and standard solutions for the 17 pesticides. Pesticides were identified, quantified and confirmed using gas chromatography-mass spectroscopy (MS) or co-chromatography. The mean recoveries for the pesticides monitored ranged from 72.2 to 98.2%; the lower limit of quantitation (LLQ) for the pesticide analysis ranged from 0.1 to 1 μg/L.

A total of 485 samples were collected in the three-year period. The LLQ for this monitoring study ranged from 0.1 to 0.4 ppb (μg/l) for 16 of the pesticides included (Table 2). Approximately 3% of the 8,245 possible detections were shown to be positive for a pesticide. Fourteen different pesticides were confirmed by MS or co-chromatography. Metolachlor was detected most often of the pesticides detected and represented 64/256 or 25% of the total detections and was found in 64/485 or 13% of the samples. Atrazine was detected 56 times in surface water samples and represented 22% of the detections. Norflurazon (16% of total detections), cyanazine (14%) and fluometuron (8%) were detected at least 20 times in surface water. Of the pesticides detected most frequently, norflurazon was the only one that had not been previously reported in other monitoring studies (Goolsby et al., 1991).

Fourteen detections, or approximately 5% of the total detections, were quantified at levels above safe drinking water limits. Seven of the fourteen concentrations above the health advisory limit (HAL) were from atrazine. The other pesticides detected above health limits were

Table 2. Summary of the most commonly found pesticides in surface waters from the 485 samples on eight dates from Mississippi, Lawrence, Phillips and Jefferson Counties in Arkansas from 1989 to 1991.

Pesticide detection*	No. of detections	Non-detects[†]	Percentiles[‡]		
			0%	50%	100%
		%	----------------------μg/L----------------------		
fluometuron	20	95.9	0.4	0.9	5.2
cyanazine	36	92.6	0.1	0.9	16.6
norflurazon	40	91.8	0.3	1.8	11.5
atrazine	56	88.5	0.1	0.6	10.5
metolachlor	64	86.8	0.1	0.7	20.0
Total	256	---	---	---	---

*Other pesticides detected ten times or less, in order or decreasing frequencies, were metribuzin, alachlor, pendimethalin, simazine, benomyl, profenofos, trifluralin, methyl parathion and propanil.
[†]Non-detects - Defined as [1-(No. of detections/total samples analyzed)] x 100.
[‡]Percentiles - 0% - minimum concentration detected; 50% - the concentration that is higher than 50% of the concentrations detected or the median concentration; and 100% - maximum concentration detected.

alachlor, cyanazine, methyl parathion and si-mazine. Methyl parathion, norflurazon, pendimethalin and propanil concentrations ranged from 2.7 to 3.5 μg/L. None of the other six pesticides detected exceeded 2.0 μg/L, indi-cating that most of the detections were found at relatively low concentrations and below the HAL.

Since most herbicides are soluble in water, and water runs off to nearby waterways, as attested by muddy waters following rainfall events, it is not surprising that low levels of pesticides are found in surface waters. Although surface wa-ters are not normally consumed as drinking wa-ter within the state for a variety of reasons, it is possible that these materials are transported to downstream locations where human consump-tion may occur after treatment. It is anticipated, however, that dilution, adsorption onto water-borne sediments and degradation of aqueous and sediment-bound materials will decrease the con-centrations of pesticides found in these studies. Although the significance of these trace levels of pesticides in water has not been totally resolved, it behooves producers, researchers and regulators to develop means of limiting pesticides from moving from the point of application.

SAMPLING FOR PESTICIDES IN GROUND WATER AT MIXER-LOADER SITES

Approximately 80 commercially licensed pes-ticide applicators and producers were contacted by mail. An introductory letter was sent giving background information of the planned experi-ment. An accompanying questionnaire was sent, which included questions concerning character-istics of the well site such as soil texture, depth of well and pesticides mixed near the well site. Through contacts from the county agents and questionnaire responses, a sampling scheme was conceived that ultimately included 16 sampling sites located in 11 counties in Arkansas. The sites chosen included four aerial applicators and one commercial ground applicator.

In this study, samples were collected five times in a two-year period. In 1990 collections were made during 13-20 June and 4-20 October, and in 1991 samples were collected 10-23 May, 20-26 July and 5-11 November. These sampling intervals were to depict times of the year repre-

sentative of the different types of pesticide ap-plications as discussed in the surface water study.

At each location, samples were collected by flushing the well for 1 min prior to collecting the sample in a 1-L amber glass jar. Four jars of water were collected at each location. Two samples were fortified immediately in the field with a metha-nol solution containing the pesticide analytes to determine any degradation or dissipation of the pesticides from the time in which a sample was collected to the time of sample analysis. The level of fortification was similar to that described in the surface water study. After the samples were collected, the jars were capped with Teflon lids and placed on ice until they could be transported back to the laboratory for more permanent cold storage at 4 C prior to extraction. Pesticide ex-traction and analyses were similar to the surface water study.

Fourteen total detections of eight different pesticides were confirmed by MS during the two-year survey period (Table 3). The LLQ for the pesticides analyzed ranged from 0.1 to 1 μg/L in water. The concentrations of pesticides found in these ground water samples, corrected for degra-dation and percent recovery, ranged from 0.3 to 27.9 μg/L. Three of the 14 confirmations were detected above the HAL.

The detections of atrazine, cyanazine and metolachlor, representing 50% or seven of 14 of the total confirmations in this survey, are con-sistent with earlier studies at Wisconsin pesti-cide mixing/loading facilities. However, the fre-quency of detections in Arkansas was lower and concentrations were below the HAL (Habecker, 1989). According to critical values reported by Cohen et al. (1984), these pesticides would be the most likely to be detected based solely on chemi-cal characteristics.

Two consecutive methyl parathion confirma-tions and one trifluralin confirmation represented the three detections that exceeded the HAL dur-ing the two-year survey. In other surveys, these pesticides were detected infrequently and typi-cally at concentrations below the HAL (Parsons and Witt, 1988; USEPA, 1992). Trifluralin has been observed in other pesticide surveys but not at concentrations that exceeded 1 μg/L. Pendi-methalin detections have been isolated in Iowa and Missouri at concentrations below 1 μg/L

Table 3. Results of pesticide analysis of ground water samples collected at 16 mixer/loader sites at five sampling periods from 1990 to 1991 (Senseman et al., 1994).

Pesticide Screened	Number of Detections*	Concentration on Range of Detection	HAL§	LLQ¶
		---- µg/L ----		
alachlor	0	NA‡	NA	0.1
atrazine	1	0.6	3	0.1
azinphos methyl	0	NA	NA	0.3
benomyl	0	NA	NA	0.4
captan	0	NA	NA	0.2
cyanazine	4	0.5-1.8	10	0.2
fluometuron	0	NA	NA	0.4
imazaquin	0	NA	NA	1.0
methyl parathion	2†	2.3-2.7	2	0.2
metolachlor	2	2.3-4.5	100	0.1
metribuzin	0	NA	200	0.2
norflurazon	1	2.4	NA	0.3
pendimethalin	1	0.3	NA	0.2
profenofos	0	NA	NA	0.2
propanil	2	1.6-27.9	NA	0.2
simazine	0	NA	4	0.1
trifluralin	1†	2.4	2	0.2

*Number of detections confirmed out of 80 samples analyzed in two-year survey.
†Detections were found at concentrations above the Health Advisory Level (HAL).
‡NA - Data not available.
§HAL - Health Advisory Level. Amounts above this level should not be consumed daily.
¶Lower limit of quantitation - defined as the level pesticides were detected and quantified by gas chromatography-electron capture detection or high performance liquid chromatography-UV detection, then confirmed with gas chromatography-mass spectroscopy (MS).

(USEPA, 1992). At mixing/loading sites in Wisconsin, pendimethalin concentrations have been detected only in soil (Habecker, 1989). Norflurazon had not been detected in any of the ground water studies conducted in the United States in recent years (USEPA, 1992). However, results of leaching studies conducted in Louisiana have determined that norflurazon could be an important disappearance pathway (Southwick et al., 1993). Although the soils of the specific sampling sites typically did not exhibit characteristics of a well-drained soil, some pesticide detections still occurred. According to chemical characteristics and field characteristics conducive to ground water contamination, some of these detections seem unlikely to occur (Cohen et al., 1984). Therefore, the dominating influence on temporary contamination of the ground water at the specified sites may be that these water sources have a higher frequency of exposure to pesticide concentrates. Similar conclusions have been reached with regard to atrazine occurrence in the Mahantango Creek watershed where corn production intensity (CPI) was determined as the dominant factor controlling atrazine concentrations regardless of the soil, geologic and well site characteristics (Pionke et al., 1988). These data suggest that greater precautions are needed when using pesticide concentrates near water supplies.

IN SITU PESTICIDE DEGRADATION IN SUBSOIL PITS

This study was designed to evaluate the dissipation rates of five herbicides commonly used in dryland agriculture that have been found as ground water contaminants in other areas. These compounds were alachlor, atrazine, metolachlor, metribuzin and picloram. They were mixed with moist subsoil and stored at depths of 0, 30, 90 and 150 cm. Evaluating their persistence at lower depths in the soil profile can provide information regarding any threat they may pose to nearby aquifers.

A backhoe was used to excavate the soil from typical, tilled farmland to create pits approximately 4.2 m long by 1.5 m wide by 2.0 m deep.

Horizontal rows of these holes 10 cm in diameter by 30 cm deep were then drilled approximately 30 cm apart at depths of 30, 90 and 150 cm. Moist subsoil was thoroughly mixed with 10 ml of an aqueous herbicide-containing solution and the treated soil placed in a glass storage container (approximately 1 pint size) before being placed into the hole carved in the wall of a pit. Soil stored at each depth had been taken from that same depth. After six months to three years, all of the storage containers in a pit were removed and the soil analyzed by chromatography for residual pesticide content. Use of two kinds of storage containers allowed leaching losses to be compared with the amount of herbicide that degraded.

As shown in Table 4, our studies indicate that dissipation of these five herbicides at the soil surface is considerably faster than when we applied it to subsoils and stored them deep in the lower subsoil horizons. Dissipation from samples stored deeper in the subsoil decreased with depth. This study suggests that if a pesticide were to move deeper in the soil profile via a spill, well casing crack, soil cracking during periods of drought, animal burrowing activity or some other means, its probability of becoming a ground water contaminant is enhanced. These data also indicate that dissipation from the surface horizon is rapid enough that a very large majority of a surface-applied herbicide will be dissipated before it can move deeper in the profile. These data also show that the relative dissipation rate in the subsoil cannot be predicted by evaluating surface dissipation rates.

SUMMARY

These studies have shown that multiple determinations of pesticides in water samples can now be achieved in a much more timely and affordable manner. Although contaminants in irrigation wells at farm sites are not routinely found, the occurrence of surface water contamination is more common. Increased stability of pesticides at subsoil depths makes us more aware of the importance of selecting, where possible, compounds that are less mobile or those that will quickly dissipate at the soil surface.

Although we continue to improve our skills in measuring trace levels of pesticides in the environment, we are also keenly aware that our analytical tools now have far greater sensitivity than those of 20 years ago, but our ability to interpret the potential effects of trace levels of contaminants has made far less progress.

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Table 4. Persistence of five herbicides stored in moist topsoil or subsoil at four depths in pint jars in excavated soil pits. DT_{50} is the time in days for 50% of the pesticide to dissipate.

Compounds	Soil	DT_{50} (Days) Storage Depths (cm)			
		Surface	30	90	150
alachlor	sil	7	598	495	277
	lfs	12	455	545	589
atrazine	sil	30	114	196	199
	lfs	30	124	188	294
metolachlor	sil	17	82	147	129
	lfs	30	132	129	132
metribuzin	sil	13	191	119	143
	lfs	14	158	200	300
picloram	sil	25	--*	117	193
	lfs	50	248	121	193

*Insufficient data points.

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Appendix 1
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