POSTER PRESENTATIONS

IMPROVEMENT OF RELEASE METHOD FOR *APHIDOLETES APHIDIMYZA* (DIPTERA: CECIDOMYIIDAE) BASED ON ECOLOGICAL AND BEHAVIORAL STUDIES

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ABSTRACT. In many countries, *Aphidoletes aphidimyza* (Rondani) has been used effectively as a biological control agent against aphids, particularly in greenhouses. In Japan, *A. aphidimyza* was registered as a biological control agent in April 1999, and mass-produced cocoons have been imported from The Netherlands and United Kingdom since mass-rearing methods have not yet been established. In recent years, the effect of imported *A. aphidimyza* on aphid populations was evaluated in greenhouses at some Agricultural Experiment Stations in Japan. However, no striking effect has been reported yet from Japan.

The failure of its use in Japan seems to be caused chiefly by the lack of detailed ecological or behavioral information of *A. aphidimyza*. Therefore, we investigated its ecological and behavioral attributes as follows: (1) the survival of pupae in relation to the depth of pupation sites; (2) the time of adult emergence in response to photoperiod during the pupal stage; (3) the importance of a hanging substrate for successful mating; and (4) the influence of adult size and nutrient status on adult longevity and fecundity.

(1) A commercial natural enemy importer in Japan suggests that users divide cocoons into groups and put each group into a plastic container filled with vermiculite to a depth of 100 mm. However, we believe this is too deep for *A. aphidimyza* pupae, since under natural conditions mature larvae spin their cocoons in the top few millimeters to a maxmum depth of 30 mm. Our experiments indicated that the deeper the pupation site in the vermiculite, the higher the pupal and adult mortality.

(2) In nature emergence of *A. aphidimyza* adults peaks soon after sunset. From the imported cocoons, however, most adults emerged near midnight. This means that the imported individuals were apparently no longer in synch with local time after transportation to Japan.

(3) Unlike many other cecidomyiids, *A. aphidimyza* mate by hanging from spider webs and facing each other. Our experiment showed that *A. aphidimyza* could not copulate without a hanging substrate.

(4) The females of *A. aphidimyza* reared with honey solution lived significantly longer and had significantly more eggs than those reared with water alone.

Based on these results, we suggest the following ideas to improve the release methods of use of imported cocoons: (1) keep the cocoons in shallow containers to increase pupal survival; (2) keep cocoons under local photoperiod immediately after arrival in Japan to adjust synchrony to local time; (3) provide hanging substrate sites for adult mating near release locations using spider-web-like substrates such as fishing lines; and (4) provide females with artificial nutrient diet near the release locations.

DEVELOPMENT OF MOLECULAR MARKERS TO STUDY THE ROLE OF COLLEMBOLA AS AN ALTERNATIVE PREY SUSTAINING SPIDERS IN CEREAL CROPS

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ABSTRACT. The conservation and fostering of larger populations of native predator species can be effectively used to regulate crop pests. In order to manipulate generalist predators and use them for this purpose in a safe, environmentally friendly and sustainable manner, food webs in farmland ecosystems must be well understood.

We focused on developing novel molecular techniques to analyze the gut contents of polyphagous predators to investigate how prey species diversity and the availability of nonpest prey help to maintain predator populations within crops. The model system chosen was linyphiid spider predators and Collembola, a major nonpest prey. These spiders have been shown to be potentially important predators of aphids and other pest species in winter wheat crops, while Collembola are a numerically dominant alternative prey of linyphiids. Understanding the role of such nonpest prey in the spider-aphid system provides a model with much wider application to other predator-prey systems. Recent studies, based on the development of molecular markers as tools for the analysis of predator-prey interactions, have shown DNA techniques to be an exciting new approach with enormous potential for the study of complex food webs in farmland and other ecosystems.

Here we report how relevant parts of the mitochondrial gene *Cytochrome Oxidase* I (COI) were amplified by PCR from the three most common species of Collembola in our area of study: *Isotoma anglicana* (Lubbock) (Collembola: Isotomidae), *Lepidocyrtus cyaneus* Tullberg, and *Entomobrya multifasciata* (Tullberg) (Collembola: Entomobrydae). The DNA from these Collembola was sequenced and aligned with sequences for other potential prey species present in the same crop and several species of spider. Specific primers were then designed for the target Collembola species. We used three pairs of these primers, one for each of the three different species of Collembola, all of which amplified short sequences (~200 bp). Specificity analyses were performed using these primers, in which all the species previously sequenced plus many others were tested.

The primers proved to be species-specific, amplifying exclusively the target prey species and no other Collembola, other prey, or spiders, effectively eliminating the possibility that predation might be overestimated though the detection of false positives. Experiments are in progress to measure the detection period for Collembola DNA in the guts of these spiders following ingestion. Preliminary results have already shown that detection of Collembola ingested by the spiders is possible using our primers. Application of these methods will allow us in future to determine the prey range (pest and nonpest) of linyphiid spiders in the field, will help us to quantify the role of predation (by both individual species of predator and predator guilds) in prey population dynamics, and will help us to analyze the effects of biodiversity on the natural regulation of pests.

USING MOLECULAR MARKERS TO DETECT *CACOPSYLLA PYRICOLA* REMAINS IN ARTHROPOD PREDATOR GUTS

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ABSTRACT. The pear psylla, *Cacopsylla pyricola* (Förster) (Homoptera: Psyllidae), one of the main pear pests in the United States, can severely affect the productivity of pear trees in three different ways: by acting as a vector for the pear decline disease, by producing honeydew blackening the foliage and provoking skin russets on the fruits, and by injecting a toxin into the tree causing death. To solve this problem, predators have been studied for use in Integrated Pest Management (IPM) programs against this species. In recent years, the main approach to studying predation in the field by predators that suck liquefied prey has been the use of protein-based techniques, particularly, the development of Monoclonal Antibodies (MAbs). An attractive alternative to the expensive and complex processes involved with the production of MAbs is the development of molecular markers using PCR-based techniques for detection and identification of prey DNA in predator guts.

We developed DNA markers to detect pear psylla remains in arthropod predator guts using specific primers designed from mitochondrial *Cytochrome Oxidase* I (COI) gene sequences. COI gene segments 400 bp long from *C. pyricola*, other psyllid species, other potential prey species present in the same crop, and several predator species included within the Heteroptera, Neuroptera, Coleoptera, and Arachnida were sequenced. Alignment and comparison of these sequences allowed the design of 12 pairs of primers potentially specific for *C. pyricola*.

Here we show the results of one of these pairs of primers, which allow the amplification of a 271 bp fragment. When this pair of primers was tested for species specificity against DNA from other prey and predator species, the 271 bp fragment was detected exclusively in species within the *Cacopsylla* genus. These results indicate that primers with high specificity were designed from the COI gene to detect prey remains in predator guts. In order to study the ability of the primers to detect the psyllid sequence over time following ingestion, *Anthocoris tomentosus* Pericart (Heteroptera: Anthocoridae) adults were fed *C. pyricola* nymphs and were then either immediately frozen or were held for 2, 4, 6, 8, 16, 24 or 32 h at 22 ºC. Predators were then assayed for the presence of pear psylla DNA in their gut. These primers demonstrated successful detection of psyllid DNA in the predator gut at all digestion periods tested.

DEVELOPMENT OF THE PARASITOID *ALLOTROPA* SP. AND ITS EFFECT ON THE SUPPRESSION OF THE MEALYBUG *PSEUDOCOCCUS CRYPTUS*)

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ABSTRACT. *Allotropa* sp. (Hymenoptera: Platygasteridae) is one of the most important native parasitoids of *Pseudococcus cryptus* Hempel (Homoptera: Pseudococcidae) in Japan. It could parasitize all the nymphal stages of the mealybug. Percentage parasitism of *Allotropa* sp. on the first and second instars did not differ, although percentage parasitism on the third instar was lower than that on the first and second instars. Development of this parasitoid was faster in the older stadium of the nymph, although the developmental duration did not differ in the first and second instars. Therefore, the first and second instars were considered as suitable host stages for the parasitoid. Lower developmental threshold temperature and the thermal constant of *Allotropa* sp. were 10.1‡and 518.1 degree-days. *Allotropa* sp. has five generations per year in Kuchinotsu, compared with four for its host

When *Allotropa* sp. females were released onto the first instar of *P. cryptus* on potted citrus trees in a room at 25 ! under a photoperiod of 16:8 L:D, the parasitoid suppressed the first mealybug generation. However, the parasitoid did not reproduce well, and mealybug density increased about 80 days after parasitoid release. I conclude that a single release of *Allotropa* sp. cannot suppress *P. cryptus* for more than two months.

NON-TARGET HOST ACCEPTANCE AND PARASITISM BY *TRICHOGRAMMA BRASSICAE* UNDER LABORATORY AND FIELD CONDITIONS

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ABSTRACT. The egg parasitoid *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) is released in European maize fields against the European corn borer (*Ostrinia nubilalis* [Hübner]) in large numbers. This practice has raised concerns that parasitoid females might attack eggs of non-target hosts outside release fields, especially those of butterflies. We carried out no-choice tests on acceptance and suitability of 23 non-target butterfly species in the laboratory, including species named on the red list of endangered species in Switzerland. From direct observations we conclude that many nontarget hosts were accepted at a high level, and several even showed a higher acceptance rate than the target pest. With a subset of six non-target species we evaluated the impact of *T. brassicae* in field cages (2 x 2 x 2 m). Eggs were glued on the host plant along with *Ephestia kuehniella* Zeller eggs which served as a control. Parasitism rates were low under these semi-field conditions. Finally, we exposed the eggs of two non-target butterflies in meadows at 2 and 20 m distance from the edge of maize field where we released 120,000 *T. brassicae* females per ha. Nontarget parasitism rates reached up to 11% at the 2-m distance, while no parasitism was observed at the 20-m distance from the edge of the maize field. These results are discussed with respect to potential risks for butterfly populations due to mass released *T. brassicae* in Switzerland.

SELECTIVE BREEDING FOR HIGHER FECUNDITY IN *CATOLACCUS GRANDIS* (HYMENOPTERA: PTEROMALIDAE)

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INTRODUCTION

The potential of *Catolaccus grandis* (Burks) as a biological control agent against the boll weevil, *Anthonomus grandis* Boheman, a key pest in most cotton producing areas of North America, has been established (Summy *et al*., 1995). In the Lower Rio Grande of Texas, boll weevil densities and cotton fruit damage were significantly lower in fields treated with the parasitoid than in fields treated with insecticides. Up to 90% mortality of boll weevil third instars and pupae was recorded in three parasitoid-release fields, and mortality was substantially less in three insecticide-treated fields (King *et al.*, 1995). Lint yield was significantly higher in the parasitoid release fields (mean = 1047 lb/acre) than in insecticide-treated control (mean 929 lb/acre), yet chemical insecticide applications were reduced from 11 to 5. King *et al.* (1993) outlined a strategy for integrating augmentation into short-season cotton production as a regional approach. Such strategy requires the mass production of millions of parasites. The technical feasibility of mass producing *C. grandis in vitro* and *in vivo* (the latter in either its natural host *A. grandis* or in an artificial host, *Callosobruchus maculata* [Fabricius]) has been demonstrated (Rojas *et al.*, 1996; Bárcenas *et al.* 1997a). A *C. grandis* strain with higher fecundity could improve not only mass production efficiency but also field effectiveness. This quantitative trait may be improved by artificial selection. The response of *C. grandis* to four selection cycles of selective breeding for higher fecundity is documented.

MATERIALS AND METHODS

The CHS1-CP laboratory strain from the *C. grandis* Germoplasm Bank at the Colegio de Postgraduados (Bárcenas *et al.* 1997b), was used as the baseline strain. This colony was founded with 90 females and 36 males from Chiapas, Mexico, and the parasitoid had been reared for three years using *C. maculata* as the host.

The original strain was characterized by analyzing fertility table parameters in 50 individual females. Each freshly emerged virgin female was confined with two 6- to 12-day old males in petri dishes (10 x 10 x 1.5 cm) and maintained at 27 ± 1 °C, 60 ± 10% RH, and a 14:10 L:D photoperiod. Water, honey, and 12 cells of parafilm-encapsulated fourth instar host larvae were exposed for 24 h and replaced daily from the first day of female emergence to death. The number of *C. grandis* eggs were recorded daily. The number and sex of the resulting adult offspring was also recorded. Life table parameters were estimated according to Ravinovich (1982). Preoviposition period, fecundity, and sex ratio were also estimated.

A 20-day maximum fecundity period was estimated (Table 1), so the useful life of the colony is around 30 days. The accumulated fecundity during the first 12 days after emergence correlates with that accumulated at 20-30 days (r = 0.92 and 0.79, P<0.05), so the former was used as selection parameter.

The offspring of 30% of the most fecund females during the first 12 days after emergence were selected in each of four cycles. The number of females evaluated was 150 in the first selection cycle and 100 in the rest. The selected colony was increased during three to four generations before the following selection cycle. The resulting colony after four selection cycles was characterized during 30 days after emergence and compared with the original unselected colony.

RESULTS AND DISCUSSION

Although fecundity was the only selection parameter, other related parameters were indirectly selected. Pupal weight increased significantly, by 33% (2.7 to 3.7 mg). Greenberg *et al.* (1995) documented a significant correlation of 0.94 between pupal weight and fecundity in *C. grandis*. According to their equation (Y = -83.58 + 66.35X), the expected number of eggs from a 3.68 mg female reared on boll weevil larvae is 160.6. However, a female of the same weight reared on *C. maculatus* (a smaller host) oviposited 283.1 eggs, i.e., 76% higher, which supports the hypothesis of adaptation to a factitious (nonnatural) host.

The preoviposition period decreased 42.6% (5.4 to 3.1 days). The number of precocious females (those that start oviposition within the first three days after emergence) increased 61%. Accumulated fecundity increased significantly by 38.8% at day 12 and 25.8% at day 20, but the 17.1% increase by day 30 was not significant. Given that the useful life of a laboratory colony is 22-30 days, the fecundity increases are important for *C. grandis* mass rearing.

The maximum fecundity period during the first 30 days after emergence was 14 days for the selected colony and 16 days for the original colony. The original colony oviposited a maximum of 12 eggs per day, while the selected colony reached 18 eggs per day. This is a desirable field behavior because the selected females would oviposit most of their eggs immediately after release. Fertility increased 27.8% (72-92% fertile females), a desirable character in laboratory and field efficiency.

In the original colony, sex ratio was 1.5:1 females: male at 12 and 20 days, and decreased to 1:1 by day 30. In contrast, the selected colony maintained a 1.5:1 sex ratio throughout the 30-day period. The net reproductive rate (R_o) (number of females produced by each female) increased from 35.0 to 65.3 at day 30, a significant and desirable increase for mass rearing. The generation time had a tendency to increase, but the daily intrinsic rate of increase (r_m) did not change.

A heritability of 0.21 was estimated for accumulated fecundity during the first 12 days after emergence. Further genetic improvement might be obtained by continuing selective breeding.

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IMPACT OF AN INTRODUCED PARASITOID (*PTEROMALUS PUPARUM*) ON THE ABUNDANCE AND DYNAMICS OF THE RED ADMIRAL BUTTERFLY (*BASSARIS GONERILLA*), A PRELIMINARY REPORT

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ABSTRACT. *Bassaris gonerilla* Fab. is an endemic butterfly in New Zealand. Its distribution is determined by the availability of the larval food plant, *Urtica ferox* Forst. There is anecdotal evidence that populations of the butterfly have declined since the early 1900s, perhaps due to the introduction of the generalist pupal parasitoids *Pteromalus puparum* L. (Hymenoptera: Pteromalidae) and *Echthromorpha intricatoria* Fab. (Hymenoptera: Ichneumonidae). *Pteromalus puparum* was introduced to New Zealand in 1933 for the biological control of the cabbage white butterfly (*Pieris rapae* [L.]); however, it also attacked non-target species such as *B. gonerilla*. The ichneumonid *E. intricatoria* attacks a wide range of Lepidoptera native to New Zealand and is thought to have invaded from Australia around 1915.

There are no data on red admiral abundance before versus shortly after the introduction of these parasitoids, so the quantification of parasitoid impacts demands a novel approach. A population model is proposed as a means of separating parasitoid impacts from other potentially regulatory mechanisms. This occurs in two steps. Initially, a model is built to describe total mortality of red admiral populations. Second, the mortality due to parasitism (as determined by laboratory and field manipulations) is removed, assuming no compensatory changes occur in rates of other subsequently acting factors. The modified model, with parasitoid mortality removed, is then used to predict the red admiral density that would have occurRed before the introduction of parasitoids.

Preliminary data have been collected to estimate parameter values for the model. Butterflies emerged from only 9% of red admiral pupae collected from three regions (Wellington, Canterbury, and Dunedin); 68% of pupae were parasitized by *E. intricatoria*, 8% by *P. puparum*, and 15% were dead (N=261). However, this percent parasitism is likely to be an over-estimate, because many of the bushes where the pupae were collected had very visible, high densities of pupa on them and it is probable that parasitoids were also attracted to these populations. Furthermore, intensive monitoring of sample plants in the Canterbury region showed much lower overall rates of parasitism (26% by *E. intricatoria*, 14% by *P. puparum*), although this could be a reflection of temporal and spatial variation in parasitism. The parasitoids' response to host density will be measured in the future by placing trap pupae out in the field at varying densities. Data from Canterbury field sites revealed low hatching rates of red admiral eggs (5%), mainly due to high parasitism rates (57%, N=374) by an unidentified *Telenomus* spp. The stage specific survival rates for the five larval instars, in order, were 59, 50, 40, 31, and 25%.

CLASSICAL BIOLOGICAL CONTROL OF COFFEE BERRY BORER, *HYPOTHENEMUS HAMPEI* (COLEOPTERA: SCOLYTIDAE) IN COLOMBIA WITH AFRICAN PARASITOIDS

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INTRODUCTION

Coffee (*Coffea arabica* L.) is the most extensively consumed beverage in the world. It is grown throughout many regions of the world, including Central Africa, Asia, and Central and South America. Brazil, Vietnam, and Colombia produce about 45% of the world's coffee. Colombia grows coffee on 2.3 million acres and supplies 10% of the world's coffee demand. Colombian coffee is also recognized as high quality because of its unique acidity, body, and aroma.

Coffee berry borer, *Hypothenemus hampei* (Ferrari), is the most important insect pest on coffee crops worldwide (Baker, 1999). The female leaves an infested berry and colonizes new beans in order to provide food and protection for its progeny (Ticheler, 1963). The life cycle of this insect occurs exclusively inside the bean, so its control is very difficult in such a protected habitat. This insect is already present in most of the coffee producing countries, directly damaging the beans and decreasing the quality of the beverage, thus making its damage of great importance (Bustillo *et al*., 1998).

Coffee berry borer was found in 1988 in Colombia, when it was detected on the border with Ecuador (Bustillo *et al.*, 1998). Currently, coffee berry borer is present in all coffee fields in Colombia, and the infestation has reached levels of up to 13% of the coffee produced by the entire country.

After the appearance of coffee berry borer in Colombia, the National Coffee Research Center (Cenicafé), a branch of the Colombian Coffee Grower's Federation (Federacafé) developed strategies to control coffee berry borer under an Integrated Pest Management (IPM) scheme. The basis of the IPM program was manual control by picking all the beans in the field during the harvest periods, since coffee berry borer has a high reproductive rate and can continue its life cycle inside coffee beans that fall to the ground. A great number of insecticides were tested, including both high and low toxicity materials. Coffee berry borer infected with *Beauveria bassiana* Bals. (Vuillemin) was found, and a strain of this entomopathogen was isolated, tested and then used as a spray to control populations in the field (Bustillo *et al*., 1998).

African parasitoids were a component of this IPM program that captured great public attention. Since coffee berry borer completes its life cycle inside the berry, organisms that could reach the pest in its microhabitat are highly desirable. *Cephalonomia stephanoderis* Betrem and *Prorops nasuta* Waterston (both Hymenoptera: Bethylidae) were introduced first, followed later by *Phymastichus coffea* La Salle (Hymenoptera: Eulophidae). *Heterospilus coffeicola* Schneideknecht (Hymenoptera: Braconidae) is the next prospective candidate likely to be introduced in the future.

We address in this paper the results obtained through the research on African parasitoids and their impact on coffee berry borer carried out in Colombia since 1989. Most of this information is available in cited literature.

Cephalonomia stephanoderis (Betrem) *and Prorops nasuta* Waterson

Prorops nasuta was the first African parasitoid used for biological control of coffee berry borer in the Americas. This wasp was introduced to Brazil from Uganda in 1929 and released in 1930 (Hempel, 1934). Biological control efforts, however, were later suspended with the development of chemical insecticides.

Cephalonomia stephanoderis was discovered by Ticheler in Ivory Coast in 1960 (Ticheler, 1963), but it was used only as a local form of natural control until 1988 when it was introduced into Mexico and Ecuador (Moore and Prior, 1988).

These parasitoids were introduced in Colombia in 1989 and 1990, both from a colony maintained in Ecuador and from new material collected in Africa (with quarantine services provided in England). These two parasitoids show very similar life cycles (Benavides and Portilla, 1990). Female wasps contact the coffee berry borer female inside the berry. After paralyzing the coffee berry borer female, the parasitoids consume any coffee berry borer eggs and small larvae. They then lay their own eggs on the bigger coffee berry borer larvae, pre-pupae and pupae (Bustillo *et al*., 1997). Attempts to rear these parasitoids on infested ripe beans were previously made in all countries where they were introduced, but the results were not satisfactory (Baker, 1999).

The coffee berry borer's life cycle was first studied on different materials such as ripe beans, processed beans, humidified coffee, and coffee grains with 45% water content. The last of these proved to be the best material in which to rear coffee berry borer and its parasitoids (Benavides and Portilla, 1990).

In the laboratory, the life cycle of coffee berry borer requires only 28 days, even though 45–60 days are needed under field conditions. Coffee berry borer parasitoids (*C. stephanoderis* and *P. nasuta*) were successfully reared in the laboratory, although *P. nasuta* production was lower than that of *C. stephanoderis* (Portilla and Bustillo, 1995).

Field studies with these parasitoids have been carried out since 1991. *Cephalonomia stephanoderis*, the first coffee berry borer parasitoid to be successfully reared, was released in eight experimental plots located at four different altitudes (Benavides *et al.*, 1994). Three releases of parasitoids, in proportions of 1:1, 1:2, and 1:3 wasps per infested bean, were made during the first months of the experiment, and observations on the pest infestation level and rate of parasitism were made for three consecutive years. There was no harvesting of the coffee beans, thus the plots were left to resemble the parasitoids' natural habitat. The results showed this parasitoid successfully established in coffee crops in Colombia, being more efficient at lower altitudes and causing levels of parasitism as high as 60%.

Prorops nasuta was released, in small numbers, as long as they were being reared in the laboratory, but no evaluations were conducted after the releases. The first field study was carried out five to six years after the first releases. *Prorops nasuta* was found in 73% of randomly chosen coffee farms where parasitoids had previously been released, and in 53% of those farms where no attempts to release the parasitoid had ever been made. Also, *C. stephanoderis* was found in 27% of farms that had previous releases of this parasitoid and 10% where it had not been released. The level of the parasitism of coffee berry borer-infested berries was up to 8.3% by *P. nasuta* and 1.3% by *C. stephanoderis.* These results suggested that *P. nasuta* had a more active host searching behavior in the field and perhaps was able to exert higher control at lower coffee berry borer infestation levels (Quintero *et al*., 1998).

Amplified Fragment Length Polymorphism (AFLP) analysis was performed on bulk DNA from *P. nasuta* and *C. stephanoderis* in order to establish genetic differences. DNA fingerprints were obtained showing distinguishable and very well differentiated bands between these two populations (Fig. 1).

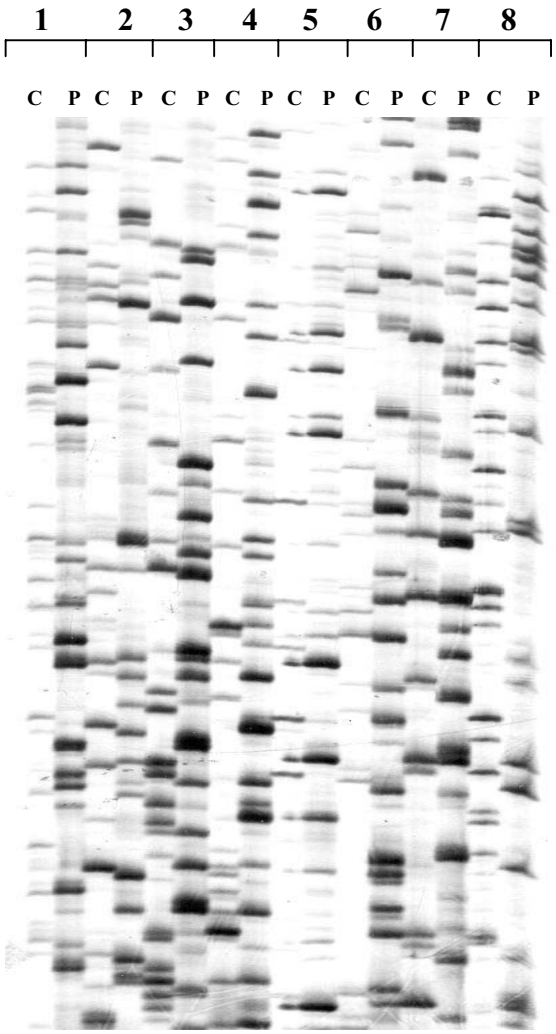

Figure 1. DNA fingerprints of *Cephalonomia stephanoderis* Betrem (C) and *Prorops nasuta* Waterston (P) generated with eight primer combinations (1 to 8) through AFLPs.

Phymastichus coffea La Salle

Phymatichus coffea is a parasitoid of coffee berry borer adults that was discovered in 1987 in Togo (Borbon, 1990). The wasp paralyzes the coffee berry borer female when it begins to bore into the bean, then the parasitoid lays two eggs inside the abdomen. After the eggs hatch, larvae feed internally on the host and then pupate. The male pupates in the pronotum of the host, and the female in the abdomen. New adults of *P. coffea* emerge from the host in about 38 days.

Phymatichus coffea was introduced into Colombia through quarantine in England in 1995, mass reared under laboratory conditions, and released in the field in 1997. In 1998 *P. coffea* was recovered in field plots, and levels of parasitism of 41% and 67% were observed (Baker, 1999).

Two million adults of this parasitoid have been released over 33 farms in Colombia, causing 2 to 6% parasitism. Releases are still taking place. Although these parasitism levels are not as high as desired, the parasitoid does appear to be adapted to the environmental conditions found in coffee crops in Colombia.

Heterospilus coffeicola Schneideknecht

Adults of H. coffeicola are predators of the immature stages of coffee berry borer. This insect was discovered in Uganda in 1923 (Hargreaves, 1926). The adult female lays one egg inside an infested coffee berry; the egg hatches and the larva feeds on eggs and larvae of coffee berry borer. An exploratory trip to Uganda by Jaime Orozco in 1997 showed that this predator causes about 16% of coffee berry borer mortality in the field (Baker, 1999). Attempts to rear the parasitoid in the laboratory in Uganda were unsuccessful. Nevertheless, it was possible to rear the wasp under field conditions once the female had laid her eggs on infested berries (Baker, 1999). Work to develop an artificial diet for this species is continuing.

Artificial Diets

Research on artificial diets has been performed in order to mass produce coffee berry borer and its parasitoids. Early artificial diets (Villacorta–140 diet and Ecobrovil-160) served as a basis for development of the currently used diet, called CENIBROCA (Portilla, 1999). This artificial diet uses more common ingredients; therefore, diet cost was reduced by about 90%. Studies in the laboratory found the optimal humidity and temperature for coffee berry borer rearing to be 60% RH and 27 ºC. Under those conditions, five generations of coffee berry borer can be reared without loss of fertility (Portilla, 1999). *Cephalonomia stephanoderis* can be reared on artificially produced coffee berry borer, yielding a sex ratio of 1:6 females to males. Artificially produced parasitoids were compared in a field trial against a wild parasitoid population and one reared in coffee parchment. The parasitism in these three treatments ranged between 58% and 61%, and no statistical differences were found (Baker, 1999).

In an initial trial to mass rear coffee berry borer and its parasitoid *C. stephanoderis*, 20,000 coffee berry borers in various life stages were harvested per 750 ml of diet. These hosts allowed the production of 10,000 parasitoids from 480 founders (Baker, 1999). Research on artificial diets is still in progress.

DISCUSSION

Controversy has arisen about which parasitoid might be the best species for control of coffee berry borer. *Cephalonomia stephanoderis* was successfully mass reared, established after release and reached high levels of parasitism; but the level of parasitism decreased faster than expected in the field. Nevertheless, the predatory effect of this parasitoid on the host is very significant, and it is easier to rear than the other parasitoids.

Prorops nasuta has a more aggressive searching behavior when released, but it is more difficult to rear. This wasp has been recovered in higher numbers than *C. stephanoderis* from the field, even when released in lower numbers. It is perhaps better adapted to the environment in the coffee plantations in Colombia since it has been recovered in higher proportions than *C. stephanoderis* from plots where no releases were conducted. However, the greater difficulty in rearing *P. nasuta* favors use of *C. stephanoderis,* at least in augmentative programs.

Phymastichus coffea appears to be easy to rear and effective when in contact with its host. However, this parasitoid has not yet been produced in as large numbers as *C. stephanoderis. Heterospilus coffeicola* seems a likely new agent, but research is still needed before predictions can be made about its performance in the field.

Many authors have shown that these four parasitoids provide considerable control of coffee berry borer in natural conditions and in experimental plots. The unpredictable weather conditions in tropical coffee-producing areas have complex effects on coffee berry borer parasitoids. Some species may be favored during certain periods, while others are suppressed. We believe that the more parasitoids that are introduced, the better the chances will be to keep coffee berry borer populations under natural equilibrium on a permanent basis. In addition, development of an inexpensive artificial diet appears to offer promise for augmentative releases of coffee berry borer parasitoids. We look forward to continue production of coffee in Colombia with the highest quality in harmony with the environment. To this end, we hope to continue developing clean techniques such as biological control and manual control rather than encouraging chemical control as the only alternative to control coffee berry borer.

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THE EFFECTS OF HABITAT DIVERSIFICATION ON A GENERALIST INSECT HERBIVORE POPULATION AND IMPLICATIONS FOR BIOLOGICAL CONTROL BY A PARASITOID WASP

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ABSTRACT. Preference for one host plant over another can be used to manage generalist insect herbivores in some agricultural crops. However, this approach has not always been found to be effective in diverting insect herbivore populations away from target agricultural crops. Hence preference is not the only factor to be considered when diversifying crop habitat for insect management. Another factor of importance to overall population growth is performance of the insect herbivore, since growth and survivorship vary with host plant species. We investigated the effect of habitat diversification on the generalist insect herbivore, *Lygus hesperus* Knight (Hemiptera: Miridae), and its performance as influenced by mortality due to a natural enemy, *Anaphes iole* Girault (Hymenoptera: Mymaridae). The results indicated that preference alone was insufficient for reducing the insect herbivore population and subsequent damage to a target agricultural crop. In addition, *A. iole* abundance and performance differed between host plants. Collectively, these results suggest that, if habitat is to be diversified toward the goal of generalist insect herbivore management, the host plant species used for diversification must be selected carefully. Ideally, a host plant species used would be one that the insect herbivore prefers, and one on which it suffers high mortality due to natural enemies. Research is ongoing to investigate this approach as a simple biological control strategy.

A HISTORICAL AND GEOGRAPHICAL ANALYSIS OF WORK ON HABITAT MANIPULATION FOR ARTHROPOD BIOLOGICAL CONTROL

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ABSTRACT. Habitat manipulation, along with reducing pesticide-induced mortality of natural enemies, offers scope to conserve biological control agents. This study, the first stage of a comprehensive analysis of the literature, investigated historical and geographical trends in such work.

The CAB Abstracts computer database was searched to identify relevant publications in the years 1973 to 1999. Seven or fewer habitat manipulation papers were published each year between the early 1970s and mid 1980s. There was an increasing rate of habitat manipulation publication from the mid 1980s to mid 1990s and fluctuating numbers of papers (15-25 per year) in the second half of the 1990s. The United States and Europe (especially the United Kingdom, Switzerland, and Germany)

together accounted for around half of habitat manipulation publications. General reviews and laboratory or modeling studies accounted for 14% of publications. China, Oceania (especially Australia and New Zealand), Russia, and Africa each accounted for 4-9% of all publications.

To gauge the amount of habitat manipulation activity in relation to overall biological control activity, a second search identified publication activity in all fields of biological control. This showed that the percentage of biological control publications concerned with habitat manipulation was low–less than 1%. There was a modest but erratic increase in percentage of habitat manipulation publications since the 1970s.

Further analysis will consider agent and target taxa, agricultural system, level of success, and degree of adoption.

THE EFFECTS OF ALYSSUM FLOWERS ON THE FITNESS OF THE LEAFROLLER PARASITOID *DOLICHOGENIDEA TASMANICA* (HYMENOPTERA: BRACONIDAE)

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ABSTRACT. Many parasitoid wasps feed on nectar or pollen, and the presence of these resources in agricultural ecosystems may enhance the effectiveness of biological control. To investigate how important floral resources are to the fitness of parasitoids, a laboratory cage experiment was conducted comparing longevity, realized fecundity and offspring sex ratio of *Dolichogenidea tasmanica* (Cameron) with and without alyssum flowers (*Lobularia maritima* [L.]). The experiment was set up in a randomized block design with six treatment and six control cages. Cage temperature averaged 18 °C. Each cage held one female and two male wasps, along with a potted alyssum plant with flowers (treatment) or without flower (control). All cages were also provided with water on cotton wool. Each female wasp was confined with an excess of second instar leafroller larvae (*Epiphyas postvittana* [Walker]) in a box with artificial leafroller diet for four hours each morning throughout the life of the female. The same box of leafrollers was presented to a female for three consecutive days before a fresh box was used. Leafrollers were reared at an average temperature of 20 °C, and the number of parasitoid cocoons and the sex of the wasps produced every three days were recorded.

Female wasps caged with flowers lived seven times as long ($P < 0.001$) and males three times as long ($P < 0.01$) as those caged without flowers. Significantly more leafrollers were parasitised per day by wasps with access to flowers than by those without ($P < 0.01$). The offspring of wasps in control cages were almost exclusively male, compared with a sex ratio approaching 50:50 produced in treatment cages ($P < 0.05$). These results show that the fitness of *D. tasmanica*, as represented by longevity, realized fecundity, and sex ratio, is strongly affected by the presence of food in the form of flowers. This study demonstrates the importance of providing biological control agents with easily accessible sources of food.

BIOLOGICAL CONTROL OF THE MANGO MEALYBUG, *RASTROCOCCUS INVADENS* (HOMOPTERA: PSEUDOCOCCIDAE) IN AFRICA

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INTRODUCTION

Mango (*Mangifera indica* L., Anacardiaceae) is an ancient fruit of Indian origin (Butani, 1975) under cultivation for over 6000 years (Hill, 1952). Today, mango is a fruit of great importance throughout the tropics (Laroussihle, 1980). It is sold at local markets in Africa and constitutes an important source of energy and nutrients. Mango is also a valuable ornamental shade tree and contributes to the protection of soil against erosion. Until recently, damage to mango trees by insect pests and diseases in Africa was insignificant (Butani, 1975; Laroussihle, 1980). In 1986, however, a mealybug–later described as *Rastrococcus invadens* Williams (Homoptera: Pseudococcidae) of Southeast Asian origin–was reported to cause serious damage to various fruit trees, especially mango (Williams, 1986; Agounké *et al.*, 1988). Colonies of *R. invadens* are generally located on the lower side of the leaves, where the insects suck the sap of infested plants. Mealybug infestations, together with sooty mold, seriously affect plant growth, flowering, and fruiting of attacked trees. Chemical and mechanical (i.e., trimming) control measures were adopted, but failed to control the pest (Agounké *et al.*, 1988). Because a perennial plant providing shade and fruit was threatened, the whole community including decisionmakers in towns became concerned. As chemical and mechanical control, together with local natural enemies, appeared ineffective to control the pest, introduction of specific natural enemies from the origin of the pest was considered to achieve a long-term control.

This paper presents results of a successful biological control project in which the International Institute for Tropical Agriculture (IITA), CABI Bioscience, The Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) in Togo, the Food and Agriculture Organization (FAO), and several African biological control programs were involved. Results summarized below demonstrate the success of the program and show that a properly conducted biological control remains an excellent pest management option.

METHODS

Rearing and Releases of Introduced Hymenopterous Parasitoids

Gyranusoidea tebygi Noyes and *Anagyrus mangicola* Noyes were reared in the insectary at the IITA Station in Benin on *R. invadens* as described by Neuenschwander *et al.* (1994). Releases were made from the ground by placing insects on infested trees (Neuenschwander *et al.*, 1994; Bokonon-Ganta and Neuenschwander, 1995).

Laboratory Studies

Host stage selection and sex allocation by *G. tebygi* and *A. mangicola* were studied in no-choice and choice experiments (Boavida *et al.*, 1995; Bokonon-Ganta *et al.*, 1995). Studies on life histories of the two parasitoids were conducted to compare their biological potentials for a better understanding of the outcome of their releases in the same environment. (Boavida *et al.*, 1995 ; Bokonon-Ganta *et al.*, 1995). Host discrimination and larval competition by *G. tebygi* were studied to determine by direct observation a parasitoid's response to parasitized and unparasitized mango mealybugs offered at the same time. (Bokonon-Ganta *et al.*, 1996). Competition between *G. tebygi* and *A. mangicola* was studied in multiparasitized second instar hosts, for two different time intervals between the first and second attack (Bokonon-Ganta *et al.*, 1996).

FIELD STUDIES

The distribution of the mango mealybug and the establishment, spread, and dispersal of natural enemies were studied by surveys (Neuenschwander *et al.*, 1994; Bokonon-Ganta and Neuenschwander, 1995). Impact of the mango mealybug on infested trees and results of the biological control program implemented was assessed in exclusion experiments and population dynamic studies (Boavida *et al.*, 1995) and countrywide entomological and socioeconomic surveys (Bokonon-Ganta *et al.*, 1995; Bokonon-Ganta *et al.*, 2002).

RESULTS AND DISCUSSION

Introduction of the Mango Mealybug and its Hymenopterous Parasitoids

Rastrococcus invadens was first reported in Africa in the early 1980s. The pest was accidentally introduced into Africa from Southeast Asia. *Rastrococcus invadens* was found to be highly polyphagous shortly after its introduction, with mango being the most affected plant species (Löhr, 1985; Agounké *et al.*, 1988). Indigenous predators adapted to *R. invadens* were very scarce and their relative inefficiency to control its populations was confirmed in subsequent surveys (Bokonon-Ganta and Neuenschwander, 1995). Similar observations were made for local natural enemies on the exotic cassava mealybug, *Phenacoccus manihoti* Matile Ferrero (Neuenschwander *et al.*, 1989, 1990, 1991). Mechanical and chemical measures were adopted to control the pest, but appeared ineffective. It was then necessary, in a classical biological control approach, to locate natural enemies offering the best chance to control the pest, and introduce them.

After quarantine by the CAB International, the parasitic wasps *G. tebygi* and *A. mangicola* were introduced and mass reared at the International Institute of Tropical Agriculture (IITA) in Cotonou, Benin, on *R. invadens* maintained on *Ficus polita* Vahl. (Moraceae), a fast growing alternate host plant. Releases were made with the agreement of the Inter-African Phytosanitary Council of the Organization of African Unity (OAU) in collaboration with several African biological control programs. The natural enemies adapted easily to their host and new environment and were found to disperse rapidly. *Gyranusoidea tebygi* was recovered not only in the large mealybug populations sometimes observed in towns, but also on isolated mango trees in farmers' fields (Bokonon-Ganta and Neuenschwander, 1995). After the establishment of *G. tebygi* and the concomitant decline in mealybug populations, the range of host plants infested by the mealybug was drastically reduced and mango remained the only important host (Bokonon-Ganta and Neuenschwander, 1995). Similar observations were made in Gabon (Boussienguet and Mouloungou, 1993).

Laboratory Studies

Host stage selection and sex allocation by *G. tebygi* and *A. mangicola*. Boavida *et al*. (1995) studied the host stage selection, sex ratio, and survival of *G. tebygi i*n hosts of different ages and sizes and found that the parasitoid reproduced on first and second instars of the mango mealybug as reported by Narasimham and Chacko (1988), but also on third instars. In this study, sex ratios were highly female biased. Females had longer developmental times than males, developed faster in larger mealybugs than in smaller ones, and were always larger than males from the same host instar.

Bokonon-Ganta *et al.* (1996) studied the host stage selection, sex ratio, and survival of *A. mangicola* in hosts of different ages and sizes and revealed that the wasp parasitized all host instars of the mango mealybug. First instars were less often encountered and were seldom parasitized. First instars were, however, preferred for host feeding. Handling time per host decreased with increasing host size. Female wasps recognized previously parasitized hosts and, in cases where they attacked them, did not oviposit into them. The sex ratio of emerging parasitoids, expressed as proportion of males, was lowest when mango mealybug was parasitized as mature adult females and increased with decreasing host size, from young adult females to first instars. Female wasps emerging from any size of host were always larger than the corresponding males. Male size increased with that of the host, while female size was independent of host instar at oviposition.

Competition between *G. tebygi* and *A. mangicola*. Introduction of the second parasitoid was justified by reports of persistent "hot spots" of infestations by *R. invadens*, despite the presence of *G. tebygi.* Studies of competition between *G. tebygi* and *A. mangicola* showed that no significant differences were found in the way each parasitoid species examined, attacked, stung, and oviposited into hosts, unparasitized, or previously parasitized by the other species. This suggests that neither species discriminates against each other. The total number of parasitoids of either species emerging did not significantly differ between competition experiments. When *A. mangicola* was the first parasitoid to attack a host, it had no significant advantage over *G. tebygi.* However, when *A. mangicola* followed *G. tebygi* by either 4 or 24 hours, *A. mangicola* clearly won. Overall *A. mangicola* won the competition in 70.9% of all cases.

The competitive interaction between these two parasitoid species contrasts with the one between *Apoanagyrus* (*Epidinocarsis*) *lopezi* De Santis and *Apoanagyrus diversicornis* Howard (both Hymenoptera: Encyrtidae) introduced against the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Homoptera: Pseudococcidae) (Herren and Neuenschwander, 1991). The later-attacking *A. diversicornis* proved to be competitively inferior (Pijls *et al.*, 1990; Gutierrez *et al.*, 1993) and could not be established as a biological control agent against cassava mealybug in Africa (Neuenschwander, 1996; Neuenschwander and Markham, 2001).

Differences in host selection by *G. tebygi* and *A. mangicola*, the demonstrated competitive superiority of *A. mangicola* in multiparasitized hosts together with results of further comparative studies on life history characteristics of *G. tebygi* and *A. mangicola*–most particularly the higher lifetime fecundity and longevity of *G. tebygi*–explain why *A. mangicola* could be established in Africa on *R. invadens* already under control by *G. tebygi* (Neuenschwander *et al.*, 1994; Boavida *et al.*, 1995; Bokonon-Ganta *et al.*, 1995, 1996).

By contrast to Godfray and Waage (1991), who predicted that *A. mangicola* was not a useful addition to the parasitoid complex of the mango mealybug, an effective reduction of mealybug populations was demonstrated in the present study and seems in fact attributable to the establishment of *A. mangicola*.

Field Studies for Measuring Impacts of *R. invadens* on Mango Production and Natural Enemies on Biological Control

From physical exclusion experiments, Boavida *et al.* (1995) concluded that *G. tebygi* was an effective biological control agent against *R. invadens*. This result was confirmed in population dynamic studies over five years that recorded a marked and stable reduction of pest levels (Boavida *et al.*, 1992, 1995), demonstrating that *G. tebygi* was responsible for successful biological control. The same conclusion was drawn from similar population dynamic studies in Togo (Agricola *et al.*, 1989) and Congo (Matokot *et al.*, 1992). Repeated detailed countrywide surveys in Benin (Bokonon-Ganta and Neuenschwander, 1995) based on multiple regression analyses of several interacting abiotic and biotic factors affecting *R. invadens* populations, their damage, and their influence on the growth of mango trees, confirmed that effective control occurred over large areas. These studies were based on 2,067 trees in three surveys across different ecological zones of Benin. The overall yield loss due to infestations by mango mealybug was assessed at 72%. From the first survey year to the third, the percentage of infested mango trees declined from 31.0% in 1989 to 17.5%. During the same period, the mean percentage of infested mango trees having indigenous predators declined from 42.3% to 20.9%. Average mealybug densities declined steadily from 9.7 females per sampling unit in 1989, with 3.2% of all mango trees having densities above 100 mealybugs, to 6.4 in 1991, with 1.3% of all trees having densities above 100 mealybugs. In multiple regression analyses, based on 23 meteorological, agronomic, and plant variables, the duration of the parasitoid's presence proved to be a major factor. It influenced mealybug population densities and sooty mold incidence, which in turn affected the production of new leaves (Table 1) (Bokonon-Ganta and Neuenschwander, 1995). Similar multiple regression analyses have been used in studies on rice pests (Baumgärtner *et al.*, 1990), cassava mealybug (Neuenschwander *et al.*, 1989, 1990, 1991; Chakupurakal *et al.*, 1994), and stem and ear borers of maize (Gounou *et al.*, 1994).

A socioeconomic study for assessing the impact of *R. invadens* on mango production and the results of its biological control by the exotic parasitoids was carried out (Bokonon-Ganta *et al.*, 2002). Information obtained in interviews concerned the host plant, the pest and its social effects, the control efforts and damage by the pest to fruit production.

Most producers attributed the observed improvement to the success of biological control. Similar observations on the awareness amongst the local population of the value and practice of biological control of *R. invadens* were made by Vögele *et al.* (1991) in Togo. Based on production estimates by producers, the negative impact of the pest on plant production and the positive impact of the introduced natural enemy were demonstrated. Socioeconomic studies extend the already quantified effect of *G. tebygi* on the mealybug populations and the leaf loss reduction (Bokonon-Ganta and Neuenschwander, 1995) to fruit production. Each mango farmer gained on average $328 per year through this biological control program. Including operational costs, the present value of IITA's involvement was estimated at $U.S. 1.75 million. Other organizations provided additional support (GTZ, FAO, CAB International, and the Benin Plant Protection Service). The total depreciated cost of biological control of mango mealybug in Benin, taking into account the initial activities in Togo, amounted to $U.S. 3.66 million. Compared with the benefit of $U.S. 531 million, the benefit-cost ratio was calculated at 145:1.

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Table 1. Multiple regression analysis for assessing the importance of factors influencing mango mealybug, *Rastrococcus invadens* Williams, populations. (data obtained in Bénin, from 1989 to 1991).

Variables		Regression statistics	
		All trees (N=2067)	
		B	t_b
Dependent variable			
Y	Mealybugs[a] in log (x+1)		
Independent variables			
X1	Rainfall x 104	2.872	5.56*
X2	Human population density	0.195	11.66*
X3	Young leaves	0.003	5.00*
X4	Medium aged leaves	0.003	3.84*
X5	*A. tubercularis*[b]	0.047	1.97*
X6	Duration *G. tebygi*	0.009	7.57*
Intercept		-0.020	
Explained variance, R^2		0.141	

[a] Total number of adult female mango mealybugs, estimated on 48 leaves per tree.
[b] The scale, *Aulacaspis tubercularis* Newstead, played an important role but was never seen to interfere in any way with the mealybug.

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SPECIES-SPECIFICITY AND SENSITIVITY OF A PCR-BASED ASSAY FOR *PERISTENUS STYGICUS* LOAN (BRACONIDAE), A PARASITOID OF *LYGUS* SPP. (MIRIDAE)

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INTRODUCTION

Lygus spp. (Heteroptera: Miridae: Mirini), which are serious crop pests in North America, are attacked by numerous predators, parasitoids, and pathogens in North America and Europe (Day, 1999). Among the parasitoids of congeneric mirids occurring in Europe that exhibit good host specificity, the southern Mediterranean species *Peristenus stygicus* Loan (Hymenoptera: Braconidae: Euphorinae) appears to be a promising agent for reducing populations of southwestern U.S. *Lygus* species, including *Lygus hesperus* Knight (Drea *et al*., 1973). Because natural populations of *Lygus* species and of their parasitoids tend to be very patchy in southern Europe, and because several different *Peristenus* species and hyperparasitoids co-occur at many locations, it has been necessary to devote considerable time and effort to field collections in order to obtain large numbers of parasitoids for research in the native range of this species and for shipment to cooperators in the United States (Coutinot and Hoelmer, 2000).

Traditional methods for monitoring the presence and identity of *Lygus* parasitoids in host populations have required either dissections of nymphs, which provides estimates of parasitism levels but not identification of parasitoid species, or rearing parasitoids from parasitized nymphs, which is not complete until the following spring. Both procedures are time consuming and labor intensive. This hinders the development of studies relevant to understanding field patterns of parasitism, the evolution of parasitoid populations, and the interaction of native and exotic *Peristenus* species with overlapping geographic and host ranges.

Such studies and mass collections could benefit from the recent development of molecular approaches–such as PCR–as a diagnostic and quantitative technique in veterinary parasitology (Robledo *et al*., 2000; Zarlenga *et al*., 2001). The sensitivity of PCR amplification for detection of trace quantities of target DNA in heterogeneous samples has made this technology an ideal choice for identifying parasitoids and has already been used to detect *Peristenus digoneutis* Loan, an introduced parasitoid of European origin of *Lygus lineolaris* (Palisot de Beauvois) (Tilmon *et al*., 2000). Here we describe a method for efficient and rapid DNA isolation of *P. stygicus* from *Lygus* sp. nymphs that facilitates sensitive, species-specific PCR-based diagnosis of parasitism. The study reported herein was designed to assess the specificity and the sensitivity of the PCR-based assay.

MATERIALS AND METHODS

Insects

Adult *P. stygicus* used in the laboratory experiments were obtained from laboratory colonies reared on *Lygus pratensis* (L.) and *Lygus rugulipennis* Poppius. Hosts and parasitoids in laboratory cultures were originally collected from various locations in southern Europe. *Lygus* nymphs (developmental stage N2 + N3) were exposed to female adult parasitoids until stinging was observed. Each parasitized nymph was held individually in a vial at 22 °C, 14:10 L:D and given fresh green beans for food. At 6-

month intervals, two groups of *Lygus* nymphs were parasitized by *P. stygicus* females to determine the accuracy of the assay in detecting parasitoid eggs and first through fourth larval instars within hosts. Parasitoid development was monitored by dissecting samples each day, so as to ensure the right stage of the parasitization. At the conclusion of each developmental stage within the experiments, the nymphs were immediately frozen at –20 °C.

PCR-Based Assay

Total nucleic acid isolation from individual specimens followed the method of using DNAzol reagent according to the manufacturer's instructions (Life Technologies, San Diego, U.S.A.). DNA concentration and quality were estimated by optical density at 260 and 280 nm, respectively, as well as by ethidium bromide staining after running on agarose gel 1%.

We selected the mitochondrial protein-coding gene cytochrome oxidase I (COI), because this gene exhibits interspecific variability within genus and a high number of copies per cell (Simon *et al*., 1994). Initial amplification of this target region was performed for *P. stygicus* individuals from the Drôme Department in France and the Andalucia region in Spain using primers C1-J-2183 and TL2-N-3014 (Simon *et al*., 1994). PCR reaction mixtures contained Qiagen reaction buffer, 0.5 ¼M of each primer, 250 ¼M each dATP, dCTP, dGTP and dTTP, 2 units of Taq DNA polymerase (Qiagen), and DNA template in a total volume of 25 ¼l. Samples were heated to 92 °C for 2 min and then the reaction mixtures were cycled in a DNA thermal Cycler (Hybaid) 35 times at 92 °C for 30s, 52 °C for 1 min, 67 °C for 1 min with a final extension at 67 °C for 7 min. PCR products were run out on agarose 1% gels in 1x TAE buffer and stained with ethidium bromide. All amplifications were sequenced by Genome Express (Grenoble, France) in an Applied Biosystems 377 DNA sequencer.

COI sequences of *L. lineolaris, P. digoneutis, Peristenus pallipes* (Curtis), *and Peristenus conradi* (Marsh) obtained from Genbank (respective accession numbers AF 189240, AF 189241, AF 189242, AF 189243) were aligned with *P. stygicus* sequence using Clustal X program (Thompson *et al*., 1997). Optimal nucleotide primers used for diagnosis PCR-assay were designed for a 636-base-pair target region within the *P. stygicus* COI gene using the Primer 3 program (Rozen and Skaletsky, 1998; Whitehead Institute for Biomedical Research, MIT Center for Genome Research, Cambridge, Massachusetts, U.S.A.).

Assessment of Specificity and Sensitivity for the PCR Assay

To test the specificity of the PCR-based assay for *P. stygicus*, we performed the diagnostic test on DNA from *Lygus pratensis* (L.) and *Lygus rugulipennis* Poppius, *P. digoneutis, Peristenus rubricollis* Thomson, *P. conradi, Adelphocoris* sp., and the hyperparasitoid *Mesochorus* sp. Assay sensitivity was determined directly by PCR in a spike and recovery format in the presence of a constant amount of host nymph DNA. Decreasing quantities (5 ng to 5 pg) of *P. stygicus* DNA were mixed with a constant amount (50 or 150 ng) of *Lygus* spp. DNA. PCR reactions, using primers designed for the diagnosis assay, were performed under the same conditions as described above, except that the number of PCR cycles was increased to 40. For all steps of the PCR reactions, either positive controls containing *P. stygicus* DNA or negative controls containing no template were included. PCR products were run out on agarose 1% gels in 1x TAE buffer and stained with ethidium bromide.

RESULTS

A sequence of *P. stygicus* was deposited in Genbank (Accession number AF414390). Based on the sequence obtained and compared with those of *Peristenus* spp. and *L. lineolaris* (Fig. 1), the rational design of primers allowed specific detection of the parasitoid in host nymph with an amplicon of 636 base pairs (bp).

Figure 1. Alignment of the partial sequence of the COI gene from *Peristenus stygicus* (S), *Peristenus digoneutis* (D), Genbank AF189241, *Persitenus pallipes* (P), Genbank AF189242, *Peristenus conradi* (C), Genbank AF189243, and *Lygus lineolaris* (L), Genbank AF189240. PCR primers for specific diagnosis of *P. stygicus* (IF and IR) are underscored.

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S    AGTGTATATT TTAATTTTGC CTGGGTTTGG AATTATTTCT CATATAATTT ATAATGAAAG GGGTAAAAAA GAAACATTTG GATGTTTAGG TATAATTTAT 100
                      IF
D    .A.A...... ........A. .A..A..... T......... .......... .......... .......... .......... .T........ ..........
P    .A.T..-... ........A. .......... ... ..... ..... ... .......... A..G...... .......... .T.....G.. C.........
C    ...A...... ........A. .A..A..... G........C .......... .......... ...G...... ..G....... .......... A.........
L    .A.A...... ........A. .A..A..... .C.A..C..A .....TG..A GAC.A..... A..A.....C ...G...... ...CA..... A.........
S    GCTATAATAA CAATTGGAAT TTTAGGGTTT ATTGTTTGGG CTCACCATAT ATTTACTGTG GGGATAGATG TTGATACTCG GGCATATTTT ACCTCTGCAA 200
D    .......... .T.....T.. ......A... ........A. .A..T..... ......A..T .........A .......... ...T...... ..T.....T.
P    .......... .......... .......... ........A. ....T..... ......A..T .....G...A .......A.. A..T...... ..T.....T.
C    .......... .......... C.....A... ........A. ....T..C.. .........A .........A .......A.. A..T...... ..T.....T.
L    ..C....... ........C. AC....A... ..C..A..A. .A..T..... ......A..A ..T....... .A..C..C.. A.....C..C ..A..A..T.
S    CAATGATTAT TGCTGTACCA ACGGGAATTA AAATTTTTAG GTGATTAGCG ACTTTTAGAG GAGTTAAGAT AAAATATAAT TTAAGAATTT TGTGATCTAT 300
D    ....A..... ......T..T ..A..... .......... T........T ..A..... .T..A..A.. .......... .......... .A...G.A..
P    .T..A..... ......T..T .....T.... .......... T..G.....T ..A.....T. ....A..A.. .......... .......... .......G..
C    ....A..... ...G.....T ..T..... .......... T......... ..A.....G. .TA.A..A.. .......... .......... .A.....G..
L    ....A..... .......... ..A..T..C. ....C..C.. T........A ...A..CAT. ..AA...T.. T..C...TCA G..CCT..A. .A...AGAT.
S    GGGGTTTATT TTTTTATTTA CTATAGGTGG TTTAACAGGG GTAGTTTTAT CTAATTCTTC GGTGGATTTA TTGTTACATG ATACATATTA TGTAGTTGCT 400
D    A..A...... .......... .......G.. .........A ..T..... .A........ T..A...... C.T....... ....T..... ...T..C...
P    A..T...... .......... .C....... .......... .....A.... .A........ T..T..C... C.T....... .......... ...T......
C    A..A...... .......... .......A.. .C.......A .......... .......... T..T..C..G C.T....... ....T..... ...T......
L    A..A..C... ..CC.T.... .A.....A.. .C.T..T..A ...A.....G .......A.. .A.T...A.T A.CC.T..C. .......... ......A..A
S    CATTTTCATT ATGTTTTATC TATGGGGGCT GTATTTTCTA TTATTGGTGG GGTAATTTTT TGATATTCGT TATTTATAGG TATTTCAATA AATGAAAAAT 500
D    .......... .....C.T.. ...A..A... ........A. .......... AT........ ......C.CC ......C... .T.A...T.. .....T....
P    .......... .....C.T.. ...A..... ........A. .......... AT........ ......C.C. ......C... GG.A..TT.. ..........
C    .......... .......... .....T..A .......... .......G.. CT........ .......... .....C... GG.A...T.. ...A......
L    ..C..C.... ....AC.... A..A..A... ......G.C. ....C..AA. AT.T..CCAA ......C.AC ....C.C... N..AA.C... ..CCC.....
S    GACTAAAAAT TCATTTTTAT TTGATTTTTA TTGGGGTTAA TATGACTTTT TTCCCTCAAC ATTTTTTAGG ATTGGGAGGT ATACCTCGTC GTTATAGTGA 600
D    ..T....... .......... ..A..... ....T..A.. ...A..... .......... .......... ...AA....G ........G. .G.....G..
P    ..T....... .......... ..A..... ....T..A.. ...A..... .....C.... .......... A..AA....G ........A. .A.....A..
C    ..T.....G. .......... ..A..... ......A.. ...A..... .....A.... .......... G...A....G .......... .G.....A..
L    ..T....G.. C..A..CATA ACC..A.... ....A..A.. ...A..C... ..T..A.... .......... G..AA....N .....A..A. .G...TCA..
S    TTATCCTGAT ATATATATAA CTTGGAATGT GTTATCTTCT TTGGGATCAA TTATTTCTAT AGTAGGAATA TTATATTTTA TTTACATTTT TTGAGAAAGA 700
                                    IR
D    .......... .......... A...A...T. A......... A.A..... .......... .A.TA..... .......... ....T...A. ......G..G
P    .......... .......... A...A...A. T......... A.T..G.... .......... ...T..T... ..G....... ....T...A. .........G
C    ......G... ..G....... TA..A...A. T.....A..A A.A..T.... .......... .A.T..T... .......... ....T...A. .........T
L    .......... .G..TC.C.. .A..A..C.. AA.C..A..A A.T.....T. GA.....AT. .A...C.G.. A.CATA..A. .CAT....A. A..G......
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Species-Specificity of the PCR-Based Assay for *P. stygicus*

The primers did not anneal with the sequence of the host *Lygus* spp. resulting in the absence of the PCR product. None of the other parasitoid species that attack *Lygus* spp. and *Adelphocoris lineolatus* Goeze in France, *P. rubricollis*, *P. conradi*, *P. digoneutis*, or the hyperparasitoid *Mesochorus* sp. nor the European alfalfa bug *A. lineolatus,* yielded the expected 636 bp amplicon. All specimens of species tested produced the approximately 850 bp COI amplicon using primers CI-J-2183 and TL2-N-3014 (Simon *et al*., 1994), indicating that the template DNA preparations were of amplifiable quality.

Sensitivity of the PCR-Based Assay for *P. stygicus*: Spike and Recovery Studies

Assessment of the sensitivity of the PCR-based assay by the spike-recovery experiments in the *Peristenus* and *Lygus* DNA indicated that the lowest limit of detection was less than 15 pg of *P. stygicus* DNA in the presence of 150 ng of *Lygus* DNA. The diagnosis assay is independent of the life stage of both parasitoids and host nymphs. As suspected, the use of this technique led to the detection of ovipositional events that had gone undetected by simple dissection, possibly because the eggs of *P. stygicus* tend to stick to host fat body tissue, from which they cannot easily be separated.

DISCUSSION

Although considerable progress has been made in recent years with regard to detection of parasitization (Zarlenga *et al*., 2001), there is a critical need in biological control programs in particular for species-specific and sensitive diagnostic methodologies that will also facilitate the evaluation of the effects of parasitoids on target populations. Although several methods have been used to identify and to differentiate immature parasitoids within hosts, including random amplified polymorphic DNA

(Kazmer *et al*., 1995) and DNA hybridization probe (Greenstone and Edwards, 1998), specific attention is given now to the development of PCR methodologies and their applications to diagnosis of parasitism.

The PCR method described here for the detection of the parasitoid *P. stygicus* within its host *Lygus* nymph is species-specific, appears simple and reliable, and can certainly be adapted to other similar host-parasitoid systems. PCR amplification using our specific primers allows accurate and early detection of parasitization as well as processing of large numbers of samples. The entire PCR detection process can be completed within six hours. Due to their specificity and sensitivity, the use of PCR-based diagnostic assays for routine diagnosis of parasitism could become the state-of-the art methodology in quarantine laboratories. Despite substantial improvements in recent years in the monitoring of biological control strategies, and the knowledge of ecological interactions between the host and its parasitoids in particular, there is a clear need for the application of innovative approaches to address problems related to diagnosis, quality control and prevention of culture contamination. The application of PCR assays will play a major role in this process and will enable a more customized biological control strategy.

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ENSURING COMPATIBILITY OF BIOLOGICAL CONTROL OF *ICERYA PURCHASI* MASKELL WITH CONSERVATION IN THE GALÁPAGOS: DEVELOPMENT OF A PROCEDURE TO EVALUATE RISK

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INTRODUCTION

From a conservation viewpoint, the introduction of a biological control agent for a plant pest should be considered only if strong evidence is available to demonstrate that high infestations of the pest species are altering the composition of native plant communities or contributing to the mortality of threatened species or their habitats. Biological control becomes a viable option in a conservation context only if the potential detrimental effects of the biological control agent on the environment and nontarget organisms are shown to be small in relation to the damage inflicted on native species by the target pest. This is more likely when the biological control agents have a very narrow feeding range. Risk assessments are particularly important for natural enemies that will be introduced into island ecosystems. Small populations of native species, many with restricted ranges that have already been reduced by human-related impacts may be exposed to an increased risk of extinction or a decrease in their geographical range with the introduction of alien organisms (Samways, 1988; Howarth and Ramsay, 1991; Samways, 1997).

In the Galápagos Archipelago, the cottony cushion scale, *Icerya purchasi* Maskell, was first reported in 1982. Dispersed by wind currents and humans between the islands, this cosmopolitan and polyphagous pest has now colonized 15 islands in the Galápagos (Causton, 2001). In 1996, the Charles Darwin Research Station (CDRS) at the request of the Galápagos National Park Service (GNPS) initiated studies to determine whether *I. purchasi* was having sufficient detrimental impact on the native flora and fauna to merit the introduction of a biological control agent. This is the first time that classical biological control has been considered for the Galápagos.

In March 1999, host range testing of *Rodolia cardinalis* Mulsant was begun in order to identify potential negative effects that this natural enemy of *I. purchasi* might have on Galápagos biodiversity, particularly on the Galápagos scale insect fauna and their associated predators. Tests were considered necessary as earlier introductions of *R. cardinalis* preceded host testing methodologies. Although the lack of negative reports elsewhere provides support for the safety of introducing *R. cardinalis*, the vulnerability of island biota requires special attention. Few studies have been conducted on the feeding range and impact of predators used as biological control agents, in part due to the past disregard of the potential nontarget impacts of such predators when used in agricultural or other anthropogenic

landscapes (Van Driesche and Hoddle, 1997; Strong and Pemberton, 2000). A risk analysis was presented to a technical advisory committee in 2001 and it was concluded that sufficient evidence existed to justify liberating *R. cardinalis* into the Archipelago.

The principal objective of this paper is to explain the procedures used to evaluate the risks to ecosystem conservation of introducing this biological control agent. The risk assessment methodology is presented in a schematic format. Whether this methodology is likely to succeed in correctly assessing the potential risks of biological control to the conservation of areas such as the Galápagos is discussed, and recommendations for improvement are made.

RISK ASSESSMENT METHODOLOGY

The initial outline of the methodology was based on the guidelines of the Code of Conduct for the Import and Release of Exotic Biological Control Agents (FAO, 1996). Procedures were developed to address three key questions: (1) Does *I. purchasi* weaken plant growth and contribute to the mortality of native flora and fauna in the Galápagos? (2) Are there any natural enemies of *I. purchasi* already present in the Galápagos? (3) Will *R. cardinalis* have any detrimental effects on nontarget organisms or the environment in general? These steps are presented in flow charts and are summarized below.

Question 1. Impact of *Icerya purchasi* on Galápagos ecosystems and justification for classical biological control

Activities carried out were a literature review, field surveys, and experimental trials (Fig. 1).

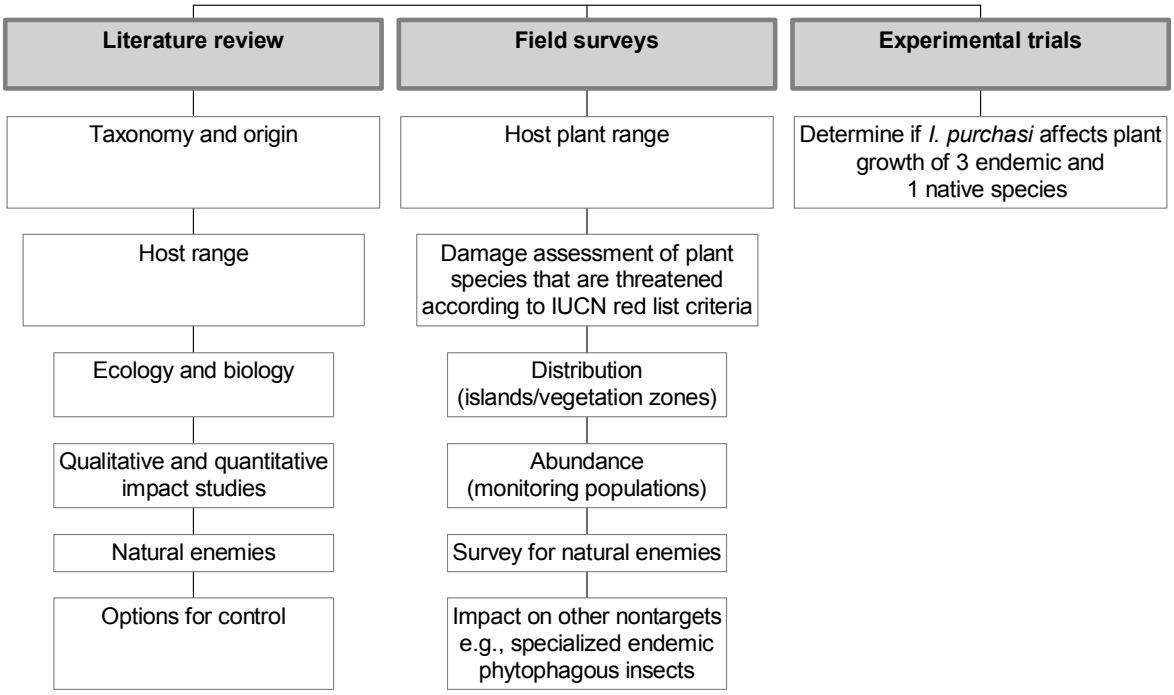

Figure 1. Activities carried out to evaluate the impact of *Icerya purchasi*.

Step 1: Literature review. Abstracts and the internet were searched. Information was compiled on the host range, ecology, and biology of *I. purchasi.* Qualitative and quantitative impact studies carried out on this pest in other parts of the world were reviewed.

Step 2: Field surveys. Monitoring surveys were carried out in the Galápagos from 1996 to 2000 to determine the host range and distribution of *I. purchasi.* Naturalist guides, Galápagos National Park rangers, visiting scientists, and CDRS staff were involved in the monitoring program. Where possible, the degree of damage was recorded and infested plants were checked for the presence of other species of insect that might also be responsible for causing plant damage. For example, damage caused by a less visible stem boring species could be misinterpreted as an effect of the scale insect.

Particular emphasis was placed on carrying out damage assessments of plant species that are threatened according to IUCN criteria. In one case, where enough information was available on the insect guilds associated with an endangered plant species, we were able to study the presence and absence of specialized phytophagous insects before and after colonization of this invasive species. Additionally, a monthly monitoring program was carried out between 1998 and 2000 to study the abundance of *I. purchasi* on three affected native plant species (*Acacia macracantha* Willd., *Piscidia carthagenensis* Jacq., *Parkinsonia aculeata* L.) on Santa Cruz Island**.** This information also provides baseline data for post-release monitoring.

Step 3: Experimental trials. There are many accounts attributing plant damage to *I. purchasi*; however, we were unable to find quantitative data to demonstrate this. Moreover, studies of a closely related species, *Icerya seychellarum* Westwood, suggest that the impact of scales on plant growth is hard to isolate from other variables in the field and can only be demonstrated under controlled conditions (Newberry, 1980a,b,c; Hill et al., 1988). Consequently, experimental trials were carried out to compare growth of infested versus healthy potted plants of those species that have shown high mortality in the field likely to be attributable to *I. purchasi*. These plants were *A. macracantha* (a possible endemic), *Phaseolus mollis* Hook f. (endemic), *Scalesia helleri* B. L. Rob (endemic, threatened), and *Laguncularia racemosa* L. (native mangrove, providing habitat for endangered species).

Question 2: Presence of Natural Enemies of *Icerya purchasi* in the Galápagos

Studies were carried out to determine whether any species already present in the Galápagos feed on *I. purchasi*. This information was necessary to identify (1) resident species that could be used in a biological control program, and (2) species that may have an increased risk of interacting with *R. cardinalis*. A list of potential natural enemies was compiled and the literature was reviewed for their feeding habits. Entomophagous Coccinellidae (10 species) were considered to be the most likely predators of *I. purchasi* in the Galápagos. Feeding trials were carried out on nine of these species by exposing starved adult beetles to all stages of *I. purchasi.* Additionally, all *I. purchasi* encountered during field surveys from 1996-2000 were checked for parasitoids and predators.

Question 3: Potential Impact of *R. cardinalis* on Nontarget Species

Step 1: Review of literature and museum specimens (Fig. 2). A literature search was carried out on the feeding habits and ecology of both *R. cardinalis* and other *Rodolia* spp. for indicators of prey specialization and the likelihood of adaptability to Galápagos ecosystems. Databases and search engines on the Internet were reviewed including "Scalenet" (http://www.sel.barc.usda.gov/SCALENET/SCALENET.HTM) and CAB Abstracts. The feeding habits of other species belonging to the same genus were considered to be a useful indicator of the potential feeding range of *R. cardinalis*, as coccinellid scale insect predators are known to exhibit restricted feeding ranges. Museum curators and coccinellid specialists were contacted for details found on specimen labels and additional information. Nevertheless, caution should be exercised with labels as they often fail to mention whether the prey supports reproduction or development of the control agent. Hodek (1996) in his review of coccinellids found that adult behavior has often been misinterpreted and points out that an adult coccinellid found on top of a scale insect is not necessarily an indication that it is using this species as a food source. The honeydew of scale insects is often used for short-term survival by coccidophagous insects when their

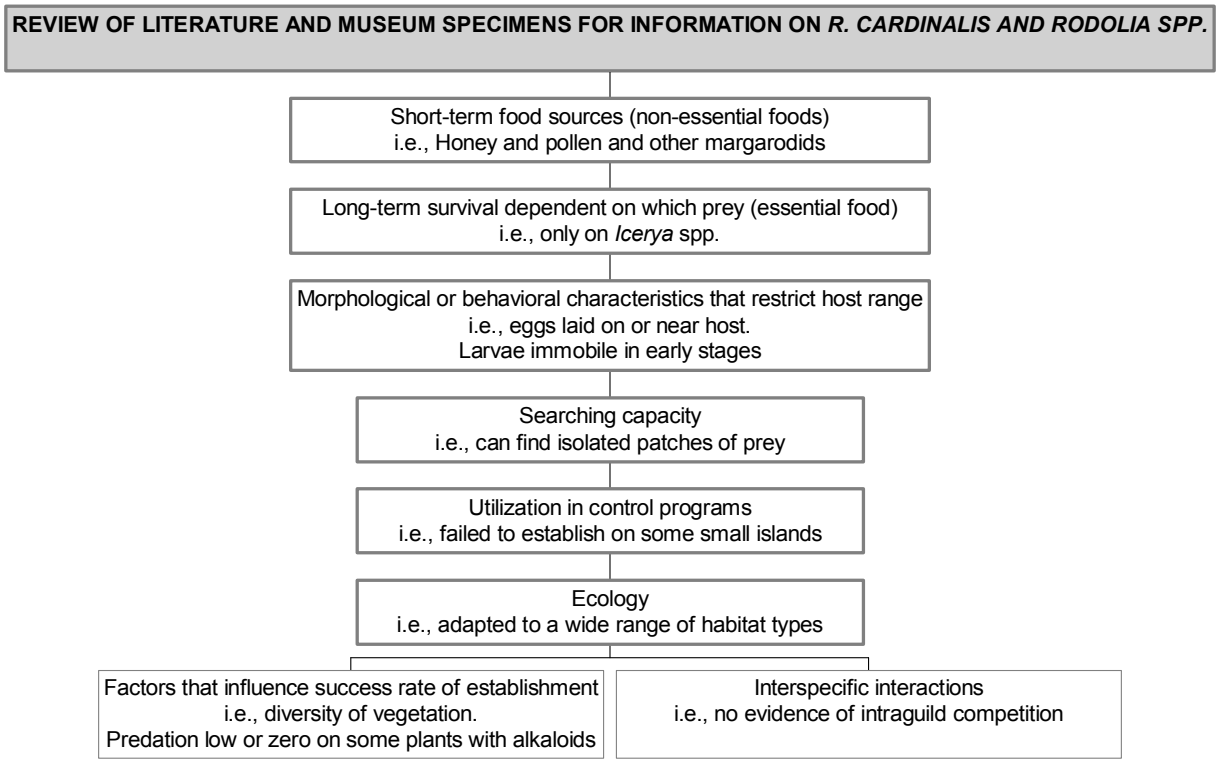

Figure 2. Key areas identified for literature search on *Rodolia cardinalis* and other *Rodolia* spp.

prey is not available, or the insect may have been simply resting on a branch that happens to contain a scale insect.

When considering the feeding habits of a biological control agent it is important to distinguish between short-term food resources that are used for survival and prey that are needed to complete development. Temporary foraging on alternative food sources may, in some cases, be necessary and may not affect the population numbers of the nontarget prey (Sands, 1997). Hodek (1996) calls these "nonessential" and "essential foods."

Other indicators of host specialization considered include morphological and behavioral characteristics that restrict prey range, reports of intraguild competition and the rate of success of the agent in establishing after liberation in various locations (no success may indicate that the agent cannot switch to other species).

Information was also sought on the ecology, geographical range, and dispersal capacity of *R. cardinalis* to determine the likelihood of it adapting to the different vegetation zones in the Galápagos. Climate in the Galápagos was compared with that in the beetle's native range using Climex. The searching capacity of the beetle and the influence of plant defense systems and other biotic factors on predation were also considered because they might influence establishment.

Step 2: Selection of potential nontarget organisms for testing (Table 1). The centrifugal testing methodology designed for use with phytophagous insects (Wapshere, 1974; Harley and Forno, 1992) has also been applied successfully to predators and parasitoids (Sands, 1997, 1998; Kuhlmann et al., 1998; Kirk and Thistlewood, 1999; Lopez and Kairo, 2003) and was used to identify native species potentially at risk to predation by *R. cardinalis*.

Table 1: Criteria used to select potential nontarget organisms of *Rodolia cardinalis*

Selection criteria	Potential nontargets (No. of species)	Possible impact
Native Galápagos species that belong to the Margarodidae	*Margarodes similis* Morrison (subterranean)	Predation
Native representatives of closely related familes	Ortheziidae (1), Eriococcidae (2), Pseudococcidae (7), Diaspididae (3)	Predation
Families of Homoptera that are reportedly attacked by the agent or related species according to literature and museum labels and are represented by native species in the Galápagos	Aphididae (3), Pemphigidae (1)	Predation
Native species that are morphologically similar to *I. purchasi*	Ortheziidae (1), Eriococcidae (2) and Pseudococcidae (10)	Predation
Native species that are found on plants damaged by *I. purchasi*	Ortheziidae (1), Pseudococcidae (4), Eriococcidae (2), Diaspidiidae (1), Aphididae (1)	Predation
Native predators of *I. purchasi* and other scale insects	Neuroptera (4), Lepidoptera (1), Coccinellidae (10)	Competition, Predation
Endemic or endangered insectivorous species that may feed on *R. cardinalis*	finches (13), mocking birds (4), warbler (1), lizards (1)	Toxic reaction produced by feeding?

A list of native scale insects was compiled from the literature and additional field surveys were carried out in 1999 and 2000. Test species were native scales (superfamily Coccoidea) that were closely related to *I. purchasi* or species that belong to Homoptera families that have been reported as prey of *R. cardinalis* in the literature. Additionally, species were tested that are similar to *I. purchasi* in having ovisacs composed of white wax filaments as the presence of wax stimulates foraging and oviposition in some coccinellid species (Merlin *et al.*, 1996; Dixon, 2000) and may influence prey selection by *R. cardinalis*. In some cases, where a desired native test species could not be collected, an alternative, related species was tested even if it was an introduced species. Where possible, before carrying out host tests, the ecology of the potentially vulnerable species was studied to eliminate species that occupied niches that were unlikely to be visited by the agent.

Field surveys and simple feeding trials were also carried out to identify the prey range of arthropod predators of scale insects in Galápagos. Species that were common or that might be displaced by *R. cardinalis* were identified. Furthermore, insectivorous vertebrates, especially species that also feed on *I. purchasi*, were also considered as potential nontarget species as some toxicity experiments have demonstrated that at least one species of coccinellid (*Coccinella septempunctata* L.) is toxic

to vertebrates (Marples *et al.*, 1989). According to the coccinellid specialist, J. L. Hemptinne (pers. comm., 2000) the effects on vertebrates of feeding on *R. cardinalis* or other members of the subfamily Novinii have not been systematically studied. In the event that *R. cardinalis* does produce a toxic alkaloid, Galápagos vertebrates may not have learned to recognize distasteful or poisonous insects that are usually identified by their bright colors unless species found in the Galápagos also use aposematism as a defense mechanism.

Step 3: Experimental trials (Fig. 3). Trials were designed to test the response of a starved larva or adult *R. cardinalis* to a nontarget species. This method is commonly known as "no-choice" testing (Van Driesche and Hoddle, 1997), where the survival of the agent on a test species (treatment) is compared with individuals fed on the target prey (control). Tests with adults included two types of controls (following the methodology of Lopez and Kairo, 2003): one being the target prey (*I. purchasi*) to provide baseline data to compare with responses to nontarget species, and the other control being just water, to estimate mortality under starvation conditions. Neonate *R. cardinalis* larvae, isolated from *I. purchasi* as eggs, were used to determine whether *R. cardinalis* could complete development on other scale insect species in the Galápagos. In addition, late instar larvae and adults of *R. cardinalis* that had previously fed on *I. purchasi* were tested for their ability to switch between prey. "Naive" adults (ones never exposed to *I. purchasi*) were also tested in case prey selection by adults is influenced by previous feeding on *I. purchasi*. Beetles were donated by CSIRO Entomology-Brisbane and had been previously screened for pathogens and parasitoids. Beetles were tested in small containers (9 mm dia.) and all tests were conducted at 24-26°C with a 12-hour photoperiod in the Insect Containment Facility of the Charles Darwin Research Station, Santa Cruz, Galápagos.

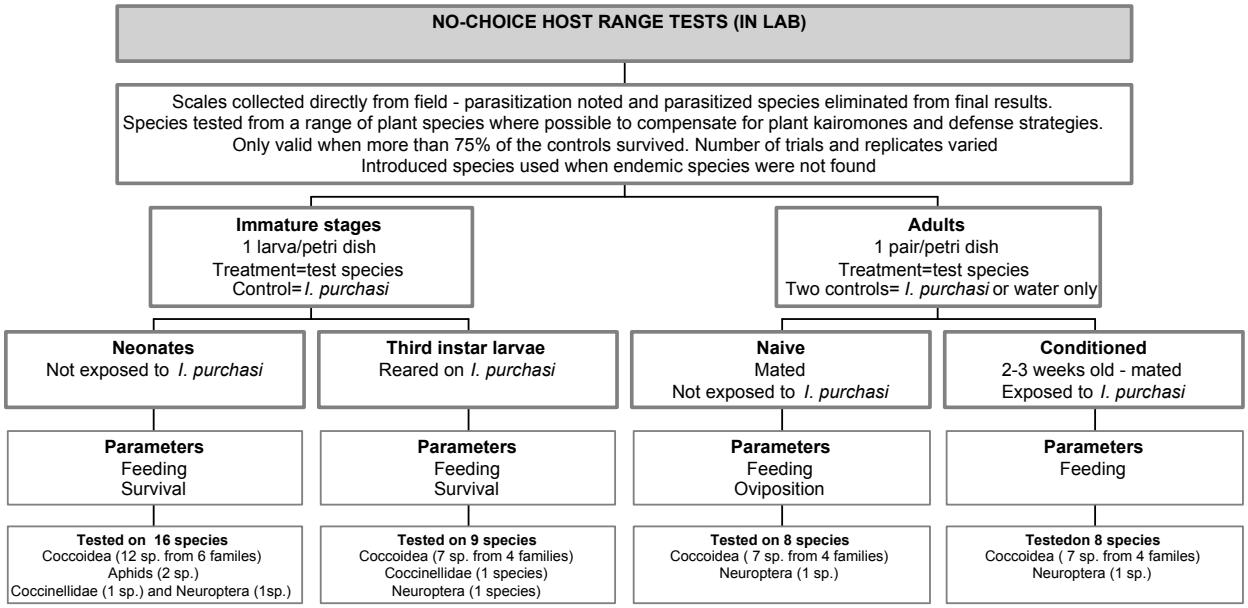

Figure 3. Procedures used for feeding range trials of *Rodolia cardinalis*

The number of trials and species tested depended on the availability of *R. cardinalis* eggs, larvae, and adults, and of nontarget prey (Fig. 3). Field-collected scale insects were used in the tests rather than laboratory-reared individuals, which allowed testing of more species in a shorter time period. Individuals used in the tests, in addition to other field-collected specimens, were reared after the trial was terminated to check for the presence of parasites. All insects that were associated with parasites were eliminated from the trials, as *R. cardinalis* has been shown to avoid eating *I. purchasi*

scales that are parasitized by *Cryptochaetum iceryae* (Williston), although it will eat all parts of the target prey except for the parasite in times of prey scarcity (Quezada, 1969). Where possible, *R. cardinalis* larvae and adults were tested on nontarget prey species without the host plant and with a range of natural plant hosts to allow for the possible influence of plant kairomones and plant defense strategies (i.e., pubescence or trichomes) that might interfere with the foraging capacity of the predator.

Simple trials using individuals of *I. purchasi* buried in soil were also carried out to test the excavation abilities of *R. cardinalis* given that the nontarget species most closely related to *I. purchasi* in the Galápagos is the subterranean *Margarodes similis* Morrison (Margarodidae). In addition, experimental trials were carried out on two species of Galápagos finch, *Camarhynchus parvulus* Gould and *Geospiza fuliginosa* Gould, to determine whether the haemolymph produced by *R. cardinalis* contains an alkaloid that is toxic to birds. The survival and weight of finches that were force-fed larvae and adults of *R. cardinalis* was compared with individuals that were fed on a diet of insects typically consumed by these species.

RESULTS AND DISCUSSION

Field and experimental trials carried out in this study suggest that at high levels of infestation, *I. purchasi* can seriously affect the flora of Galápagos. This invasive species has been able to disperse throughout the archipelago and evidence shows that it can restrict plant growth and in some cases kill plants. At least 62 native species are recorded as host plants, 16 of which are threatened according to the IUCN criteria. Mortality has been recorded on nine endemic and 10 native species so far, including populations of threatened species such as the Critically Endangered *Scalesia atractyloides* Arn. One species, *Darwiniothamnus tenuifolius* Wild. has been reclassified as threatened explicitly as a result of damage caused by *I. purchasi*. Although, the distribution of infested plants is generally patchy within a plant population (with the exception of *D. tenuifolius*), it is likely that some changes in plant species composition have occurred at the level of habitat. Furthermore, indirect effects on three species of endemic Lepidoptera associated with *D. tenuifolius* have been observed (L. Roque-Albelo, pers. comm., 2001) suggesting that scale damage could influence other specialist endemic arthropod fauna dependent on threatened plant species.

Experimental trials effectively demonstrated the impact of *I. purchasi*, but were limited in number by the absence of information on the biology of endemic plant species and the absence of needed quarantine facilities to test species from other islands. Our trials showed that *I. purchasi* seriously damaged three plant species, including the white mangrove, *L. racemosa*. Mangroves are habitat for specialized invertebrate and vertebrate fauna such as the Critically Endangered mangrove finch, *Cactospiza heliobates* (Dvorak *et al*. 2001). The breeding population of this species is restricted to one area in northern Isabela Island, only 4 km from current infestations of *I. purchasi*, and this population could be at risk if the scale continues to spread.

Although our studies indicate that *I. purchasi* is a threat to the Galápagos flora, one of the problems we encountered was collecting concrete evidence from the Galápagos and other parts of the world to prove that the scale reduced plant vigor and increased plant mortality under natural conditions. The paucity of baseline data on these endangered plant species, in addition to factors such as water and nutrient stress, made it difficult to isolate the effects of *I. purchasi* in the field. Obtaining further evidence would only be possible by carrying out experimental trials on plant physiology that were beyond the scope of this study. Similar obstacles were found with assessments carried out on *I. seychellarum* in Aldabra (Newberry, 1980a,b,c; Hill *et al*, 1988). But as in our study, these authors concluded that there was compelling observational evidence to suggest that the scale they studied was influencing plant survival.

No monophagous natural enemies of *I. purchasi* were found in the Galápagos, precluding the possibility of augmentative biological control of this pest. Biological control using *R. cardinalis* has been successfully applied in 50 or more ecosystems, including island ecosystems, but surprisingly, reliable information on its feeding habits was scarce. Results from our feeding range studies and observations of *R. cardinalis* in other countries, including in its native Australia, suggest that the absence of reports of negative ecological side effects are justified. Although some may argue that a small test arena such as the one used in this study may produce erroneous results, previous studies (Matsuka and Watanabe, 1980; Ragab, 1995) showed that *R. cardinalis* will oviposit and mate under these conditions. Where possible, however, field conditions should be simulated and adults tested in large rooms with potted plants with the nontarget in "choice" and "no choice" situations (Sands and Van Driesche, 2000). Furthermore, it would be preferable that all nontargets used for testing should be screened for several generations to ensure that they are free of natural enemies. This was not possible in this study as little is known about the biology of native nontargets and their host plants, but again, this method was deemed acceptable (with the exception of aphids) following observations that few coccid species in Galápagos harbor parasitoids or pathogens. This method would not be acceptable for testing nontarget species on continents.

The checklist of Galápagos scale insects is far from complete and nothing is known about the distribution and status of these species. Nevertheless, we were able to test a wide range of species that included representatives of all families, none of which could be used for long-term development by *R. cardinalis*. Moreover, literature reviews and basic research on the biology of these species permitted us to eliminate many species from the list of risk species on the basis of habitat separation (e.g., root versus aerial feeders). According to observations by Brancatini and Sands (pers. comm., 1999) adult *R. cardinalis* could use nectar and pollen as a temporary and alternative food source before beetle numbers decline in response to low prey numbers. Beetles may therefore interact with native pollinating insects in the Galápagos during times of prey scarcity, but are not expected to have any harmful effect on them–to our knowledge, insect pollinators in the Galápagos do not specialize on particular plant groups, but little information is available. Likewise, insufficient information on the ecology of native invertebrate scale insect predators prevented us from thoroughly evaluating the potential impact of *R. cardinalis* on all species. However, interspecific competition is doubtful for the following reasons: (1) *R. cardinalis* feeds specifically on Margarodidae; (2) resident coccinellids and most other scale insect predators in the Galápagos do not feed on Margarodidae, and (3) there is little habitat overlap between the prey of native coccinellids and *I. purchasi.*

Evaluations carried out on insectivorous vertebrates were also limited by the need to invest considerable effort before the experiments in developing techniques for maintaining individuals in captivity. As a result, only two species of finch were tested, but neither species showed adverse reactions after being fed *R. cardinalis*. Moreover, the post-feeding behavior of both species suggested that the beetles were distasteful. All individuals refused to eat *R. cardinalis* voluntarily.

CONCLUSIONS

The methods described in this risk assessment were considered to be sufficiently rigorous to demonstrate the costs and benefits of introducing a biological control agent into an area of high conservation value. In practice, however, the lack of baseline data on the flora and fauna of the Galápagos and economic constraints limited the research that was carried out. The decision of whether to extend the project was ultimately defined by how long the GNPS was prepared to permit trials to continue at the risk of losing some species endangered by *I. purchasi*. Acquiring additional information on the ecology of invertebrate predators and other nontarget species would have required several years of further research, as would the physiological trials on plants to quantify *I. purchasi* damage in the field. Not-

withstanding, the final conclusion reached by the GNPS was that the research demonstrated that a large number of endangered plant species are at risk from *I. purchasi* and that the use of classical biological control is unlikely to have significant negative impacts on this protected area.

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COMMUNITY BASED PRODUCTION AND UTILIZATION OF EGG PARASITOIDS FOR THE CONTROL OF MAJOR LEPIDOPTEROUS PEST OF CORN

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(TITLE ONLY)

A BIOLOGICAL ASSESSMENT OF A *GLYPTA* SP. (HYMENOPTERA: ICHNEUMONIDAE) AS A PARASITOID OF *CHORISTONEURA ROSACEANA* (LEPIDOPTERA: TORTRICIDAE)

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ABSTRACT. Three species of *Glypta* (Hymenoptera: Ichneumonidae) have been identified as key parasitoids of the obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) in organically managed apple orchards in the southern interior of British Columbia, Canada. The species designated as *Glypta* 2 (the *Glypta* genus requires taxonomic revision) overwinters in early instar leafroller hosts that have themselves overwintered in the orchard. *Glypta* 2 also has been found to parasitize the summer leafroller generation. The objective of this research was to study how this *Glypta* species interacts with the obliquebanded leafroller host under laboratory conditions. This biological assessment is needed to understand how to appropriately manipulate the parasitoid as a biological control agent in the field.

After emergence and mating, two to five days (at 20 °C) are required before the adult female begins oviposition. *Glypta* 2 females are synovigenic. More than one egg is frequently oviposited per host. The *Glypta* 2 egg is large and easily visible through the host's integument, enabling visual confirmation of parasitism. Up to seven parasitoid larvae have been observed within a single host under laboratory conditions, although at most only one survives. Parasitoid mortality due to superparasitism is common in first-instar hosts.

Host instar preference studies were conducted using laboratory colonies of both the parasitoid and host species. Significantly more eggs were oviposited within first and second instar hosts as opposed to fourth instar hosts. No eggs were oviposited in fifth instar hosts. When hosts were dissected seven days after parasitization, most *Glypta* 2 larvae in hosts parasitized as third instars and all *Glypta* 2 in hosts parasitized as fourth instars were dead. When parasitized third instar hosts were allowed to develop to completion, successful adult parasitoid emergence was significantly lower than parasitized first or second instar hosts.

The impact of the developing *Glypta* 2 parasitoid on the host's food consumption was used as a measure of the parasitoid's ability to reduce leafroller feeding damage. This was determined by weighing frass from unparasitized hosts and hosts parasitized as second instars, until host pupation or parasitoid emergence occurred. When the total frass produced per host instar was compared, *Glypta* 2-parasitized obliquebanded leafrollers in the fourth instar produced significantly less frass than female, unparasitized fourth instars. Parasitized hosts in their fifth and sixth instars produced significantly less frass than all unparasitized fifth and sixth instar larvae.

We concluded that the *Glypta* 2 species has good potential as a biological control agent of the obliquebanded leafroller to both reduce the host population and decrease feeding by parasitized late instar larvae. Parasitism would need to be targeted against the first two host larval instars.

DEVELOPMENT AND OPTIMIZATION OF PCR-BASED TECHNIQUES FOR THE GUT ANALYSIS OF CARABID PREDATORS FEEDING ON SLUGS

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ABSTRACT. Millions of pounds worth of damage is caused annually to arable crops in the United Kingdom by *Deroceras reticulatum* Müller, which is the major pest slug in much of Europe, North America, and elsewhere. Previous research has established that carabid beetles are important predators of this pest and can significantly reduce slug numbers and distribution in the field. Currently the primary method for investigating predation is by monitoring numbers of predators and prey in the field, combined with analysis of predator gut samples using prey-specific monoclonal antibodies (MAbs) and ELISA. A novel alternative approach is to amplify the DNA of the target prey from within the predator's gut using specific primers and polymerase chain reaction (PCR). Our previous work has shown that molecular techniques can provide an assay system that is at least as specific and sensitive as MAbs and ELISA by using multiple copy genes and by amplifying small fragments of DNA.

In this study the beetle *Pterostichus melanarius* Illiger was used to investigate predation on the slug *D. reticulatum* using molecular techniques. Specific primers were designed to detect *D. reticulatum* DNA, targeting parts of the mitochondrial 12s rRNA gene, after the secondary structure for predator and prey DNA sequences had been resolved. Following alignment with sequences for other species of slug and the predator, seven primer pairs were designed which amplified fragments ranging in size from 113 bp to 294 bp. The results of cross-reactivity tests confirmed that two primer pairs were species-specific with the remaining five pairs also co-amplifying a closely related species of slug. A laboratory-based DNA decay rate experiment was conducted to determine the detection period of prey DNA following digestion by the beetle for up to 72 h, with and without the provision of alternative prey. The detection periods for different sized fragments of digested DNA were compared. Results indicated that slug DNA can be detected from beetle foreguts for extended periods depending upon the size of the fragment being amplified.

TRANSGENIC BT CROPS IN INTEGRATED PEST MANAGEMENT: IMPACTS ON ARTHROPOD BIOLOGICAL CONTROL

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INTRODUCTION

Transgenic crops modified to produce insecticidal proteins derived from genes of the bacterium *Bacillus thuringiensis* Berliner (Bt) are one of the first practical examples of genetic engineering of crops for insect pest control. Currently, a number of transgenic crops expressing Bt genes that encode crystalline (Cry) proteins have been commercially released and are widely used by farmers. These Bt crops provide control of economically important insect pests in both developed and developing countries such as the United States, Canada, and China (Shelton *et al*., 2002). Some Bt crops, such as Bt cotton and corn, have been widely adopted by farmers worldwide, and are likely to become a widespread pest control tactic in many pest management programs (James, 2001).

Bt crops have a novel mode of plant resistance that involves lethal effects of plant-produced Cry proteins on target insect pests. This mechanism of insect pest control is similar to the antibiosis of conventional plant resistance, which involves adverse effects of a plant on insect survival, development, or reproduction (Herzog and Funderburk, 1985). Conventional plant resistance and biological control by natural enemies (predators and parasitoids) have long been emphasized by agricultural scientists as the core tactics around which integrated pest management in crops and forests should be built (Herzog and Funderburk, 1985; Kogan, 1998).

In this article, we evaluate the potential impact of Bt crops on arthropod biological control and their potential for use in crop IPM programs. First, we assess possible impacts of Bt crops on arthropod natural enemies (predators and parasitoids). Second, we discuss the possible consequences of Bt crops for tritrophic interactions among plants, phytophagous insect pests and their arthropod natural enemies. Finally, we discuss the use of Bt crops with biological control programs for development of Bt crop-based IPM programs.

EFFECTS OF BT CROPS ON ARTHROPOD PREDATORS AND PARASITOIDS

Effects of Bt crops on arthropod natural enemies may be classified into two categories: toxicological effects and ecological effects. While the toxicological effects result from direct toxicological action of the insecticidal traits (i.e., Cry proteins) expressed in Bt plants, the ecological effects result primarily from removal or reduction of populations of the target insect pests, which serve as food sources for their natural enemies. To evaluate impacts of Bt crops on biological control, both types of effects need to be considered.

Toxicological Effects

Purified Cry proteins or transgenic plant tissues have been tested against a battery of non-target beneficial insects, including predators and parasitoids, as part of the ecological risk assessment data requirements for Bt crop registrations (Table 1). Besides the regulatory guideline studies with predators and parasitoids, a number of published laboratory and field studies have also examined potential effects of Bt Cry proteins, transgenic Bt crop tissue, or whole plants on a number of non-

target arthropods, including biological control agents (e.g., Mascarenhas and Luttrell, 1997; Orr and Landis, 1997; Pilcher *et al.*, 1997; Hilbeck *et al.*, 1998 a, b and 1999; Riddick and Barbosa, 1998; Riddick *et al.*, 1998; Lozzia *et al.*, 1998; Manachini *et al.*, 1999; Meier and Hilbeck, 2001). To date, these studies have consistently shown that Bt Cry proteins or Bt plant tissues have minimal or limited toxicity against arthropod predators and parasitoids. These recent findings are also supported by our current knowledge of the mode of action and specificity of Bt Cry proteins (Table 2), as well as by the historical safety records of Bt microbial pesticides (Table 3).

Table 1. Standard regulatory guideline tests for analysis of intrinsic hazard (toxicity) of the insecticidal traits (purified Cry proteins) of Bt crops (US EPA, 1989, 1994)[a].

Test Groups	Surrogate species	Test Substance	Rout and duration of Exposure	Safety Margin of Test Cry Protein Concentrations[a]
Beneficial insects	Honey bee Ladybird beetle Green lace wing Parasitic wasp	Purified Cry Protein or pollen	Continuous feeding for 7 - 30 days	10 - 100 x the protein expression rate in Bt plant
Soil invertebrates	Earthworm Collembola	Purified Cry proteins or Crop plant tissues	Continuous feeding for 14 - 28 days	10 - 100 x the protein concentration in soil containing all Bt crop plants.
Avian species	Quail or chicken	Seed meal	Continuous feeding for 30 days	10 - 100 x protein expression rate in Bt plants
Aquatic animals	Daphnia Fish	Purified Cry protein, Pollen, or seed meal	Continuous feeding: 2 days for Daphina and 14 - 70 days for catfish.	10 -100 x the run-off rate of the protein from Bt crop plants

[a]To date, the existing database from the U.S. EPA containing the standard regulatory guideline studies for Bt crop registration has not showed any significant toxicity of the Cry proteins used in registered Bt crops (cotton, corn, potato) to those non-target organisms including arthropod predators and parasitoids (U.S. EPA, 2001).

In addition, entomophagous arthropods (predators and parasitoids) rarely consume green plant tissues, and the route of exposure to plant-produced toxins for predators and parasitoids is primarily (if not solely) through the consumption of an intermediary herbivore prey species that feeds on the toxin-producing plants. Recent studies by Head *et al.* (2001a) and Raps *et al.* (2001) have shown that levels of Cry proteins in insects that feed on Bt-plants, if any, are generally many fold less than those in the original plant tissues. For some phloem-feeding insects, such as aphids, levels of Cry proteins in insects feeding on Bt corn plants were not detectable by both ELISA or sensitive bioassays (Head *et al.*, 2001). Thus, the exposure risk of plant-produced Cry proteins to predators and parasitoids, if any, would be limited.

In conclusion, Bt crops expressing Cry proteins have both low intrinsic toxicity and offer limited exposure to arthropod natural enemies (predators and parasitoids), and thus are not likely to have severe toxicological effects on arthropod natural enemies occurring in the target crop systems.

Table 2. Comparison of levels of the specificity of Cry protein-based Bt pesticides and Bt crops expressing engineered Cry genes that encode full length or truncated Cry proteins (Federici, 2002).

Levels of Specificity[a]	Bt Microbial Pesticides (Crystal Protein Spores, Cell Bodies, Surfactant, Inactive Carrier):	Bt Crops (Full Length or Truncated Cry Protein in Plant Tissues):
1. Primary Route of Exposure	Ingestion of treated or contaminated substrates	Ingestion of Bt plant tissues
2. Protoxin (protein crystal) solubilization	Occurring following ingestion under appropriate midgut pH	Circumvented
3. Protein toxin activation	Solubilized protein is activated via appropriate cleavage by midgut proteases	Same process as for Bt microbial pesticides
4. Toxin binding to midgut receptor	Active toxin binds to specific receptors on midgut microvilli	Same process as for Bt microbial pesticides
5. Formation changes of the toxin to enter membrane and form pores (i.e., toxicity)	After binding to midgut receptor, toxin requires specific processing to change its formation, so that it can enter the midgut membrane and oligomerize to form pores	Same process as for Bt microbial pesticides

[a]This table illustrates that most of the inherent specificity that accounts for the safety of Cry proteins used in commercial microbial insecticides still applies to these same proteins when they are used to confer Bt crops with resistance to selected target insects. Whether a full length or truncated protoxin, Cry proteins produced by Bt crops must still be properly activated after ingestion, and must successfully meet the criteria for binding and membrane insertion defined by at least four levels (1, 3, 4 and 5) of specificity to be toxic.

Table 3. Toxicity of various Bt microbial formulations to different groups of arthropod predators and parasitoids at test concentrations equal to or higher than the recommended field application rates (Oregon State University, 1999).

Species	Order: Family	Crop type	Response type	Average toxicity rating[a]	SD	Number of Studies
Coccinella septempunctata	Coleoptera: Coccinellidae	not specified	mortality	2.00	1.67	6
Cryptolaemus montrouzier	Coleoptera: Coccinellidae	not specified	mortality	1.00	0.00	8
Chrysoperla carnea	Neuroptera: Chrysopidae	Forest	mortality	1.77	0.68	13
Telenomus alsophilae	Hymenoptera: Scelionidae	Apple	mortality	2.57	0.79	6
Trichogramma cacoeciae	Hymenoptera: Trichogrammatidae	not specified	mortality	1.91	0.30	11

[a]Toxicity rating: 1 = 0% mortality; 2 = <10% mortality; 3 = 10 - 30% mortality; 4 = 30 - 90% mortality; 5 = >90% mortality.

Ecological Effects

Like any insect control technology, Bt crops are designed to reduce populations of selected target insect pests. The reduction of the targeted pest populations will thus inevitably eliminate or reduce food supplies for the associated natural enemies, and are likely to result in "food-chain" effects. Pilcher (1999) showed that the abundance of the specialist parasitoid *Macrocentrus grandii* Goidanich was 30 to 60% lower in Bt corn expressing Cry1Ab for European corn borer (ECB) control than isogenic non-Bt corn. However, such food-chain effects of Bt corn were not seen for other predators and parasitoids that do not rely on ECB as their primary food source (Pilcher *et al.*, 1997; Pilcher 1999; Orr and Landis 1997). From the pest management perspective, this type of "food-chain" effect of Bt crops on the specialist predators and parasitoids is a normal consequence of effective reduction of the selected target pest populations, and should not be considered as unintended adverse effects of Bt crops. In addition, populations of generalist predators and parasitoids will be little affected due to absence or very limited effect of Bt crops on their alternative prey or host species.

Tritrophic interactions among Bt crops, phytophagous arthropods and their natural enemies

Ecologists have long recognized the importance of tritrophic level interactions among plants, herbivores, and their natural enemies in the evolution of plant-insect interactions (e.g., van Emden, 1966; Price *et al.*, 1980). Many published studies have documented that plant traits have a role in modifying interactions between herbivores and their enemies (see review in Price *et al.*, 1980), and thus affect the efficacy of biological control (van Emden, 1986). Depending on the characteristics of the interaction, plant traits may have positive, negative, or no effects on the role of the third trophic level in suppressing the pest populations (see review in Price *et al.*, 1980; van Emden, 1986).

Unlike many conventional insect-resistant traits (e.g., increased production of natural plant defense compound or trichomes), the Cry proteins expressed in currently commercialized Bt crops result in extremely rapid death of the selected target pests. In addition, because of their highly selective insecticidal activities, Bt Cry proteins generally have no or very limited effects on non-target organisms, including the third trophic level (predators and parasitoids). For these reasons, Bt plants are unlikely to have a significant role in mediating the interaction between the second and third trophic levels in the crop ecosystem.

Integration of Bt crops with biological control in IPM programs

Many crops have complexes of pest species that must be controlled simultaneously for effective crop protection. Based on their ecological characteristics, these crop pests may be classified into three categories: key pests, sporadic pests, and secondary pests (Croft and Hoyt,1983). Control measures for key crop pests form the foundation on which IPM programs rest. These measures may involve chemical control, cultural control, biological control, or control through plant resistance (e.g., Bt crops). In general, chemical control-based IPM programs often involve some level of broad-spectrum pesticides, and offer little opportunity for integration with biological control. For this reason, recent IPM researchers have emphasized the development of nonchemical methods that would replace regular use of broad-spectrum insecticides against key crop pests, and thus increase opportunities to achieve biological control of the secondary and sporadic pests.

To date, Bt crops primarily have been used to control target key pests in several crops, such as Colorado potato beetle, *Leptintarsa decemlineata* (Say), in potato and lepidopteran pest complexes in cotton and corn. Prior to the development of transgenic Bt crops, effective control of these key pests was frequently achieved by extensive chemical control programs, which largely excluded biological control programs against the secondary crop pests (such as aphids, spider mites, and white flies) via conservation or augmentation of arthropod predators and parasitoids. Al-

though the high efficacy of Bt crops in reducing the selected key pest populations may operate in their own right, the foundation formed by Bt crops for key pest control has positive effects on control of secondary or sporadic pests by their natural enemies because of this high target specificity.

Recently, large-scale field studies with transgenic Bt cotton have demonstrated that the effective control of key lepidopteran pest complexes by Bt-cotton provided a favorable foundation around which biological control of secondary cotton pests such as whiteflies and aphids by their natural enemies has become possible (Head *et al.*, 2001b). In a multiple-year field study, Reed *et al.* (2001) also demonstrated that transgenic Bt-potato eliminated needs for weekly Bt microbial sprays, biweekly permethrin sprays, or two in-furrow applications of systemic insecticides for *L. decemlineata* control. This allowed the survival and reproduction of naturally occurring generalist predators such as big-eyed bugs (*Geocoris* sp.), damsel bugs (*Nabid* sp.), pirate minute bugs (*Orius* sp.), and spiders (Araneae), which together effectively suppressed the population of green peach aphid (GPA), *Myzus persicae* (Sulzer), a vector of potato viral diseases. In these crop systems, the use of Bt crops to target key pests apparently has an advantage over chemical control to form a favorable foundation around which biological control and other control measures can be integrated into the IPM system.

CONCLUDING REMARKS

It has been nearly a decade since several major transgenic Bt crops, such as cotton, corn, and potato, were first commercialized in the United States. Bt crops have become increasingly accepted by farmers as effective alternatives to chemical insecticide applications for control of key crop pests in both developed and developing countries (James, 2001). Because Bt crops have the characteristics of conventional plant resistance for crop protection, they have great appeal for crop production and management systems. Based on ecological risk analysis of the toxicity and specificity of Bt Cry proteins and possible tritrophic interactions among Bt crops, herbivores and natural enemies, we see great potential for integrating Bt crops with biological control in IPM programs. This is especially true where Bt crops control key crop pests and allow survival and population growth of generalist natural enemies for effective biological control of secondary pests.

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TEST PROCEDURE TO EVALUATE THE RISK THAT INSECT-RESISTANT TRANSGENIC PLANTS POSE TO ENTOMOPHAGOUS ARTHROPODS

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INTRODUCTION

Assessing the ecological risks of insect-resistant transgenic crops is one of the challenges of this new technology. The product of genes for insect-resistance are most often confined to particular plant parts (e.g., stem or leaves) and are usually targeted against specific insect pests. However, the possible risks that these new plants pose to the environment cannot be ignored. Most attention has been paid to transgene dispersal and cross pollination with wild related species (Dale, 1994; Crawley *et al*., 2001) and the possible development of resistance towards the insecticidal protein in the target pest (Gould, 1998). Risks that transgenic plants might pose to non-target arthropods and the consequences for biological control have received less attention. To date, most transgenic crops have been ones expressing Bt toxins that encode for crystalline (Cry) proteins (delta-endotoxins). These Cry proteins confer effective protection to the crop from damage by certain phytophagous insect pests. Cry proteins are also the primary active components of Bt-based microbial insecticides (Bt sprays). Bt sprays are widely used in agriculture and are generally considered safe due to their specificity. However, laboratory studies have shown that Bt plants can affect non-target arthropods such as Lepidoptera (Losey *et al*., 1999), as well as beneficial insects (Hilbeck *et al*., 1998a).

International regulation of plants expressing insecticidal traits is still in its infancy. To protect non-target organisms such as natural enemies (predators and parasitoids), some countries require data from laboratory tests similar to those used for registration of pesticides. Tests for pesticides seek to determine direct (acute) effects on selected beneficial organisms (Candolfi *et al*., 2001). No such standard test methods exist for use with plants that express insecticidal proteins. Natural enemies can contact insecticidal toxins from transgenic plants either by feeding (1) on plant parts or tissues where the insecticidal protein is expressed, (2) on pollen or nectar (in cases where the insecticidal protein is present), or (3) on herbivores that have ingested the insecticidal protein. Thus the routes of exposure by which arthropods contact insecticidal proteins expressed in plants differ from the ways insects contact insecticides sprayed on a crop.

Following commercialization of various newly registered Bt plants, seed companies have been asked to conduct faunistic surveys and field tests to determine the risks transgenic plants pose to non-target arthropods, including natural enemies. This approach requires the collection and identification of a large number of arthropods, which is both very labor intensive and expensive. Such field studies often produce results that are difficult to interpret, as various factors (biotic, abiotic, landscape) make it extremely difficult to link population changes of a species to one specific factor, such as the transgenic crop. Moreover, not all arthropod species collected in the crop are necessarily exposed to the insecticidal toxin. We propose a logical stepwise procedure for assessing the risks of transgenic insect-resistant plants pose to non-target natural enemies. Bt maize is examined as a model system , but the same procedure can also be applied to other insect-resistant transgenic crops.

HERBIVORES AND NATURAL ENEMIES IN THE MAIZE SYSTEM

In Europe, there are over 30 phytophagous species that are considered potential pests in maize (Chiang, 1978). The most important of these pests are the European corn borer (*Ostinia nubilalis* Hübner), aphids, wire worms, pink cut worm (*Sesamia nonagriodes* Léfèbre), leaf beetle (*Oulema melanopa* L.), and frit fly (*Oscinella frit* L.). Other species that are important only regionally are spider mites (*Tetranychus* sp.) found in Spain (Eizaguirre and Albejas, 1987) and the corn weevil (*Tanymecus dilaticollis* Gyllenhal) found in Bulgaria and Hungary (Gerginov, 1987). Although this list of herbivores does not include all the species that feed on maize, these are the species most commonly used as prey or hosts by predators and parasitoids. As such these are the species that should be included in risk assessments of Bt maize in Europe.

The most frequently encountered predator and parasitoid species in maize in Europe include several species of Coccinellidae, Carabidae, Syrphidae, Chrysopidae, and Anthocoridae, as well as various hymenopteran and, to a lesser extent, dipteran parasitoids.

EXPOSURE OF NATURAL ENEMIES TO BT TOXIN EXPRESSED IN MAIZE

To determine which natural enemies might be exposed to insecticidal proteins, it is necessary first to assess which herbivores (prey or host) ingest the Bt toxin. Since some natural enemies also exploit plant materials, such as pollen, nectar, or plant sap, or consume the honeydew produced by various Homoptera, it is important to have information on the expression of the toxin in the different plant parts and on the feeding behavior of the natural enemies.

Herbivorous arthropods primarily are exposed to Bt toxin from maize by ingesting plant tissues containing the toxin. In addition, other herbivorous insects may ingest Bt toxins when they feed on plants surrounding the crop that are dusted with crop pollen. In maize, all the important

chewing pests (stem borers, cut worms, wire worms, leaf beetles, and corn weevil) ingest Bt toxin when feeding on maize plants (Table 1) since the plant parts they eat contain the toxin. In contrast, not all piercing-sucking herbivores ingest the toxin. Aphids, for example, do not ingest the toxin because it is not transported in the phloem (Raps *et al*., 2001). In contrast, spider mites, which feed on parenchyma cells, do ingest the toxin, as has been confirmed through ELISA tests of spider mites reared on Bt maize (Dutton *et al*., 2002). Thrips have also been shown to ingest the toxin. ELISA tests have confirmed this finding and immunohystochemical studies (Dutton *et al*., unpublished) show that the toxin is present in mesophyll cells, which include the cells fed upon by this arthropod.

Table 1. Feeding behaviour and ingestion of *Bt*-toxin by main non-target herbivores in maize.

Herbivores	Feeding site	Mode of feeding	Ingestion of toxin
stem borer	stalk and leaf tissue	chewing	yes
cut worms	stalk and leaf tissue	chewing	yes
aphids	phloem sap	piercing-sucking	no[a]
wire worm	roots, stalk and leaf tissue	chewing	yes
cereal leaf beetle	leaf tissue	chewing	yes
corn weevil	leaf tissue	chewing	yes
frit fly	leaf tissue	rasping-sucking	yes
thrips	epidermis/mesophyll	piercing-sucking	likely
spider mites	mesophyll	piercing-sucking	yes

[a] no Bt detected in phloem (Raps et al., 2001)

Exposure of parasitoids and predators to Bt toxin can be predicted based on the feeding behavior of each species. Table 2 summarizes potential exposure to Bt toxin of the most common natural enemies (larvae and adults) found in maize. The only natural enemies that are neither exposed as larvae or adults to the toxin are parasitoids in the genus *Aphidius* and the predator *Aphidoletes aphidimyza* Rondani. Although as adults these arthropods feed on nectar or honeydew, exposure to the toxin through these routes is unlikely as maize produces no nectar and honeydew from aphids feeding on Bt maize does not contain the toxin (Raps *et al*., 2001). Based on these predictions, sensitivity tests for these two species is unnecessary. For risk assessments of pesticides, *Aphidius rhopalosiphi* (DeStefani Perez) is one of the two indicator species that must be tested (Candolfi *et al*., 2001). For assessing the risks of Bt maize, testing this species would be redundant. In contrast, all other five species that are potentially exposed to the Bt toxin (Table 2) should be screened for their sensitivity to the toxin (first tier laboratory test). Only the developmental stages that are exposed to the toxin should be tested. For example, only the adult stage of *Episyrphus balteatus* De Geer should be assessed, since larvae feed only on aphids, which do not ingest the toxin. Adults, which feed on pollen, are exposed to the toxin. For the rest of these natural enemies, first tier laboratory tests should be conducted with both larvae and adults.

Table 2. Exposure to Bt-toxin by common natural enemies (larva and adult stages) in maize.

		Prey					Plant	
		larvae[a]	eggs[b]	aphids	thrips	mites	pollen	sap
Coleoptera: Coccinelidae								
Coccinella septempunctata	adult	+	-	-	+		+	
	larva	+	-	-	+		+	
Coleoptera: Carabidae								
Bembidion spp.	adult	+	-	-	+	+	+	+
	larva	+	-	-	+	+	+	+
Diptera: Syrphidae								
Episyrphus balteatus	adult			-			+	
	larva							
Diptera: Cecidomyiidae								
Aphidoletes aphidimyza	adult							
	larva			-				
Neuroptera: Chrysopidae								
Chrysoperla carnea	adult						+	
	larva	+	-	-	+	+		
Heteroptera: Anthocoridae								
Orius spp.	adult	+	-	-	+	+	+	+
	larva	+	-	-	+	+	+	+
Hymenoptera: Aphidiidae								
Aphidius spp.	adult							
	larva			-				

[a] all lepidopteran and coleopteran larvae
[b] from all arthropods
+ = food source containing *Bt*-toxin, - = food source not containing *B*t-toxin

PRINCIPLES OF A TIERED TEST PROCEDURE: FROM LABORATORY TO FIELD TESTS

The tiered procedure starts by testing species in the laboratory (first tier) with so called "worst case" conditions. Testing then moves sequentially to semifield (second tier) and field (third tier) experiments (Fig. 1), according to threshold levels reached in the initial tiers. Given the lack of experience with transgenic plants, conservative threshold values have been assigned. These thresholds are the same as those that were used during the first years of pesticide testing. For pesticide testing a 30% threshold (recommended application rate causing 30% mortality of the test organism) has now been replaced with hazard quotient (HQ) value (Candolfi *et al*., 2001). The HQ is calculated by dividing the crop-specific application rates, by the LR_{50} (lethal rate 50, the application

rate causing 50% mortality of the test organism) derived from worst-case laboratory studies generated using two sensitive indicator species, *A. rhopalosiphi* and *Typhlodromus pyri* (Scheuten).

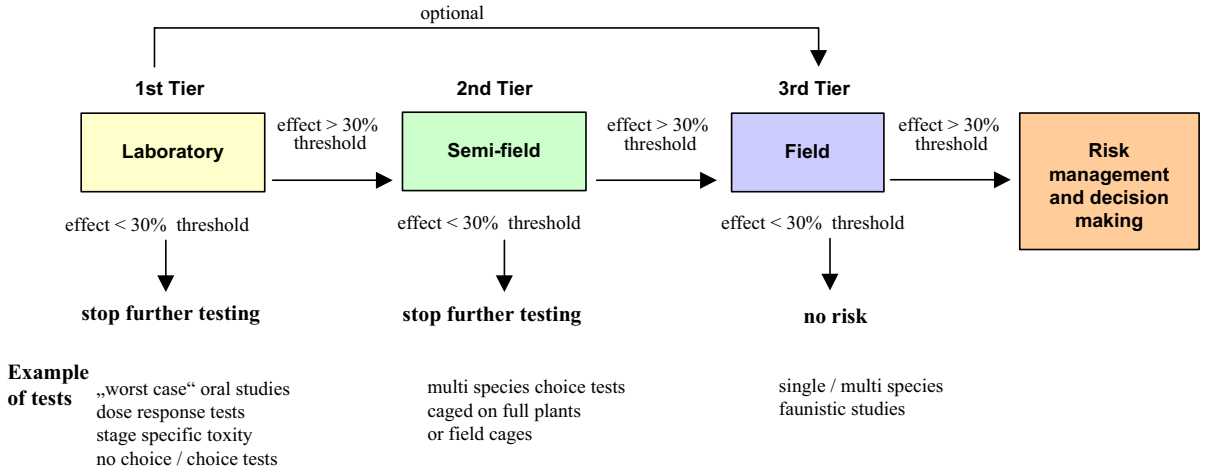

Figure 1. Sequential test procedure for assessing ecological risks of insect resistant transgenic plants on nontarget natural enemies.

Laboratory First Tier Tests

Laboratory tests are ideally single factor bioassays where cause and effect relationships are straightforward and interpretation of results is relatively unbiased. Experimental conditions are well defined, and arthropods are kept under constant control. Biological parameters of a specific stage of a single species are measured. Endpoints (assessment parameters) are well known from ecotoxicological research where mortality (e.g., LD_{50}, LC_{50}), reproduction, lifespan or other life parameters are standard variables. Since the purpose is to determine sensitivity, it is reasonable to start such experiments by adopting worst case conditions that aim to ensure maximum exposure of the organism. Tests must be conducted at the developmental stage in which the organism is exposed to the toxin. If worst case tests in the first tier give no indication of lethal or sublethal effects (threshold levels < 30%) on life cycle parameters, it is very unlikely that unacceptable effects would occur in the field and further testing is not necessary.

Depending on the host-prey spectrum of each natural enemy, second tier tests may have to be performed accordingly. For monophagous natural enemies, in which no effects were observed in the first tier, further tests in the seond tier will not be needed. An option, in the case of specialist natural enemies is to go straight to third tier field tests. For polyphagous natural enemies, second tier tests will be required with either single or multiple prey choice or no-choice tests. Since polyphagous natural enemies in the field feed on a wide range of contaminated and noncontaminated prey, the selection of prey used in the second tier tests must take this into account. It is important that suitable prey are selected in order to minimize indirect effects due to low prey quality.

Semi-field Second Tier Tests

Semi-field tests should simulate to some extent known field conditions. For such tests, single or multiple species should be offered to the natural enemy. The test system should consist of whole plants in greenhouses or outdoors. Single or groups of plants are caged and prey together with the species

tested are introduced. In these tests, free movement of the natural enemy tested and prey offered should be allowed. Natural enemies should be given the choice of prey that they would normally feed upon in the field. The type of endpoint (lethal or sublethal effects) to be assessed will depend on findings of the first tier test.

Field Third Tier Test

Field trials for regulatory purposes are required only if significant effects (above the defined threshold) are observed in the second tier tests. Field tests should be applied to those key species for which effects in lower tier testing were observed. Field tests should go beyond the mere collection, counting, and identification of non-target species. Field tests should provide ecological information and answer questions related to the effects observed in laboratory and semi-field tests.

Defining the effects on populations of the selected key natural enemies should be the goal of field experiments. However, it should be noted that effects identified in field trials can be due to many biotic and abiotic, direct and indirect, biological and landscape factors that can make it extremely difficult to assign population changes of a particular species or of a functional group of organisms to a single factor. Valuable information on interactions and ecosystem responses may be gained, but data can be difficult to analyze and interpret due to the system's high complexity and spatial and temporal variation. Field studies that employ the tiered system described here will be more efficient and less costly than full faunistic studies.

CHRYSOPERLA CARNEA (STEPHENS): A CASE STUDY

Given that both larvae and adults of *Chrysoperla carnea* (Stephens), an important predatory insect in row crops, are exposed to the Bt toxin in a maize field (Table 2), first tier tests are recommended for this species. Worst-case experiments in which *C. carnae* larvae were fed high concentrations of Bt toxin (Cry1Ab) incorporated in an artificial diet showed harmful effects on this predator (Hilbeck *et al*., 1998a). These findings received much attention as they were the first evidence that a non-lepidopteran insect was sensitive to the Cry1Ab Bt toxin expressed in maize. To evaluate the ecological relevance of this finding, second tier semi-field tests were necessary.

As a first step it was important to determine the exposure of the predator to the Bt toxin in the field. This can be defined according to the feeding behavior of the predator in the field. *Chrysoperla carnea* larvae feed primarily on aphids. Aphids do not ingest the toxin (Raps *et al*., 2001). In the field, spider mites can also be an important prey herbivore for *C. carnea.* Spider mites ingest the toxin when feeding on Bt-maize. However, when *C. carnea* larvae were fed only contaminated spider mites, no harmful effects on *C. carnea* were observed (Dutton *et al*., 2002). Lepidoptera larvae are fed on relatively seldom by *C. carnea* in the field and are known to be of suboptimal quality for this predator (Klingen *et al*., 1996). Negative effects were only observed when poor quality contaminated prey, lepidopteran larvae (*Spodoptera littoralis* Boisduval or *O. nubilalis*) were provided to the predator (Hilbeck *et al*., 1998b; Dutton *et al*., 2002).

Given that *C. carnea* larvae in the field seldom come into contact with the Bt toxin since they feed mostly on aphids (Meier and Hilbeck, 2001) and that negative effects are only observed when the predator ingests continuous and high concentrations of the toxin, no further third tier field tests should be required. Our scheme predicts that *C. carnea* is not at risk in the field and indicates that field tests are not necessary. Indeed a number of faunistic field studies confirm our prediction that Bt maize does not pose a risk to *C. carnea* (Orr and Landis, 1997; Pilcher *et al*., 1997; Candolfi *et al*., 2000).

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LOW COST MASS PRODUCTION OF PREDATORY BENEFICIAL INSECTS

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ABSTRACT. Beneficial insects are widely accepted as effective control agents against arthropod pests and are an integral part of many successful IPM systems. However, for beneficial insects to be cost-effective and readily available treatments, mass-production systems are required to provide a continuous supply of insects at the optimum life-stage. To lower the cost of beneficial insect production, Entomos concentrated on (1) developing artificial diets based on components readily available in large, lot-tested batches, (2) eliminating use of fresh meat or insect material, (3) relying on generalist predators, and (4) automating diet and insect handling. Many of these objectives now have been met.

U.S. Patent 6,129,935 has been granted as a utility patent for rearing predatory beneficial arthropods on an artificial diet with carbohydrates, fats, and a 5:1 ratio of intact protein to protein hydrolysate. An additional 24 claims have been granted in a composition patent on these same diets. Oviposition of the pink spotted lady beetle, *Coleomegilla maculata* (De Geer), fed a 95:5 mixture of this artificial diet to *Ephestia kuehniella* Zeller eggs is essentially equivalent to that on the moth eggs alone. Several systems for presentation of the artificial diet to the predators have been examined, with the most efficient being a polymer encapsulation of the diet. Encapsulated diet is stable for over two weeks at room temperature without antibiotics, and the capsules can be prepared with a high-throughput machine. The artificial diet and diet encapsulation technology, along with several handling improvements, have resulted in cost reductions of approximately 100-fold for production of *C. maculata*. Further cost reducing and handling improvements have been identified. Concentration on generalist predators also has allowed development of a field evaluation program aimed at reducing the complexity of augmentative biological control, since generalists have the potential to control more than one pest. Entomos produces *C. maculata*, *Geocoris punctipes* (Say), and *Orius insidiosus* (Say), and they are among the best known efficacious predators for a range of pests including mites, aphids, whiteflies, thrips, chinch bugs, arthropod eggs, and small lepidopteran instars.

Additional research is required to elucidate the dynamics of these predators in pest control. Entomos is seeking collaborators to perform field trials for an IPM systems approach using the generalist predators we rear to determine application rates at different pest populations to deliver consistent biological control.

BIOLOGICAL CONTROL OF CUBAN LAUREL THRIPS (THYSANOPTERA: PHLAEOTHRIPIDAE) IN CALIFORNIA

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INTRODUCTION

The Cuban laurel thrips, *Gynaikothrips ficorum* Marchal, is a cosmopolitan pest of *Ficus microcarpa* L., var. *nitida* and *retusa*, throughout the tropical and temperate world and was introduced into California sometime before 1959 (Brown and Eads, 1979). Because the thrips feeding causes the *Ficus* leaves to fold or roll into a gall in which the thrips primarily live (Rivnay, 1947), chemical control is difficult to achieve. Successful control of the insect has been achieved in some areas through the introduction of an anthocorid predator, *Montandoniola moraguesi* Puton (Lewis, 1973). This species and two other unidentified anthocorid predators were released in southern California in the mid 1960s, but no establishment was reported (Clausen, 1978). Although the Cuban laurel thrips does not cause significant damage to the health of infested trees, feeding causes the leaves to curl into unsightly, misshapen, and discolored leaf rolls that can greatly diminish the aesthetic quality of the plants (Paine *et al.*, 1991).

METHODS

Samples were taken from two inland and two coastal sites in southern California over a period of one to two years. Each site contained 8 to 10 *F. microcarpa*. From each tree selected, ten 30-cm branch tips were removed with a pole pruner and returned to the laboratory for processing. For each branch, we recorded the number of leaf galls, the total number of leaves, the number of thrips (as adults or immatures), and number and types of predators seen.

To obtain *M. moraguesi* for release in California, sites in Hawaii were examined. Four locations on Oahu were sampled once in November of 1995 to also estimate the field predator-prey ratio at Hawaiian sites. Individual leaf galls were examined and numbers of Cuban laurel thrips and *M. moraguesi* were recorded. The anthocorids were retained for release in California. The predators collected in Hawaii were processed in quarantine, and adults were removed for use in laboratory rearing. A greenhouse colony was established to support field release of the predators. At two sites, one inland and one coastal, 100 adult anthocorids per tree were released.

RESULTS

Although we did find immature and adult *M. moraguesi* some months after release, none were found the following year. Comparison of data from 1988 with our data in the 1990s showed an increase in the number of anthocorids (Fig. 1-4) and the number of anthocorid species. In 1988 sampling, only the anthocorid *Macrotracheliella nigra* Parshley was found associated with thrips in the leaf galls. In our 1996 and 1997 samplings, the anthocorid *Macrotrachelia nigonitens* Stål (first United States record for the genus—Dr. David Horton, U.S. Department of Agriculture, pers. comm.) had largely supplanted *M. nigra* in coastal southern California, while *M. nigra* remained dominant inland. In addition, *Orius* spp. were occasionally found as the thrips population peaked during the late summer. Even with all these predators in the environment, the ratio of predators-to-prey only occasionally approached the levels seen in our Oahu samples (Fig. 5), although thrips-related complaints have all but disappeared over the last few years.

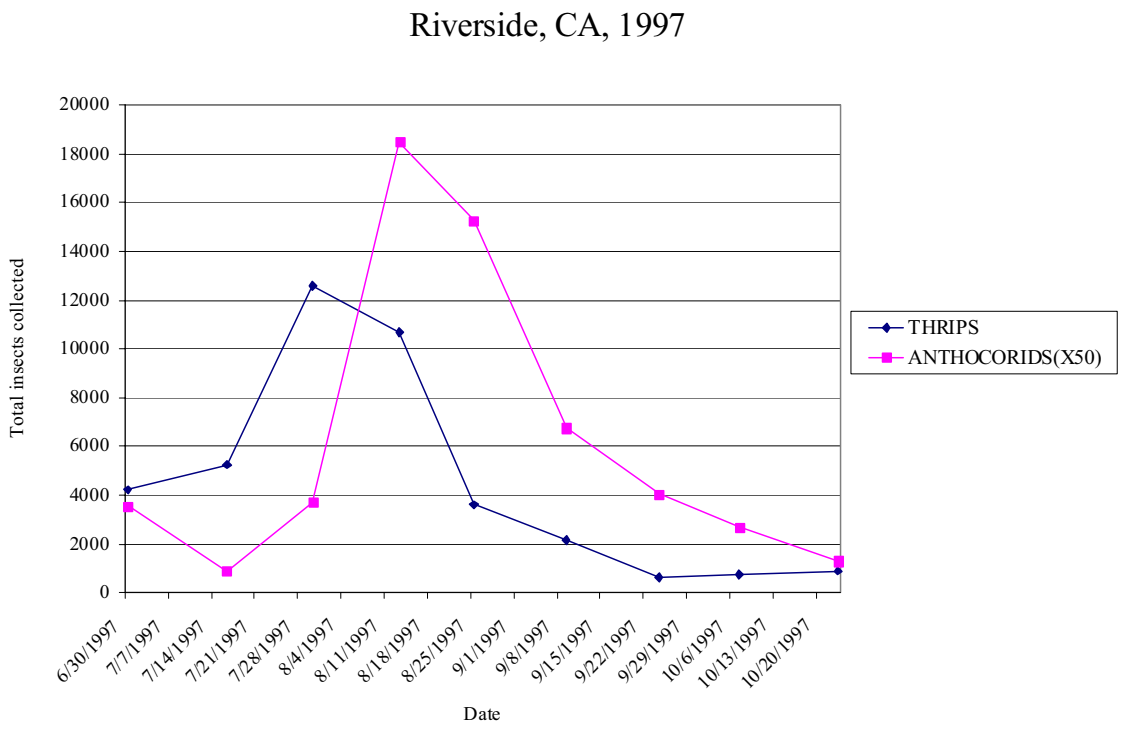

Figure 1. Total number of insects collected from *Ficus nitida* leaf galls during weekly sampling during summer-fall 1997 at a location in downtown Riverside, California, U.S.A.

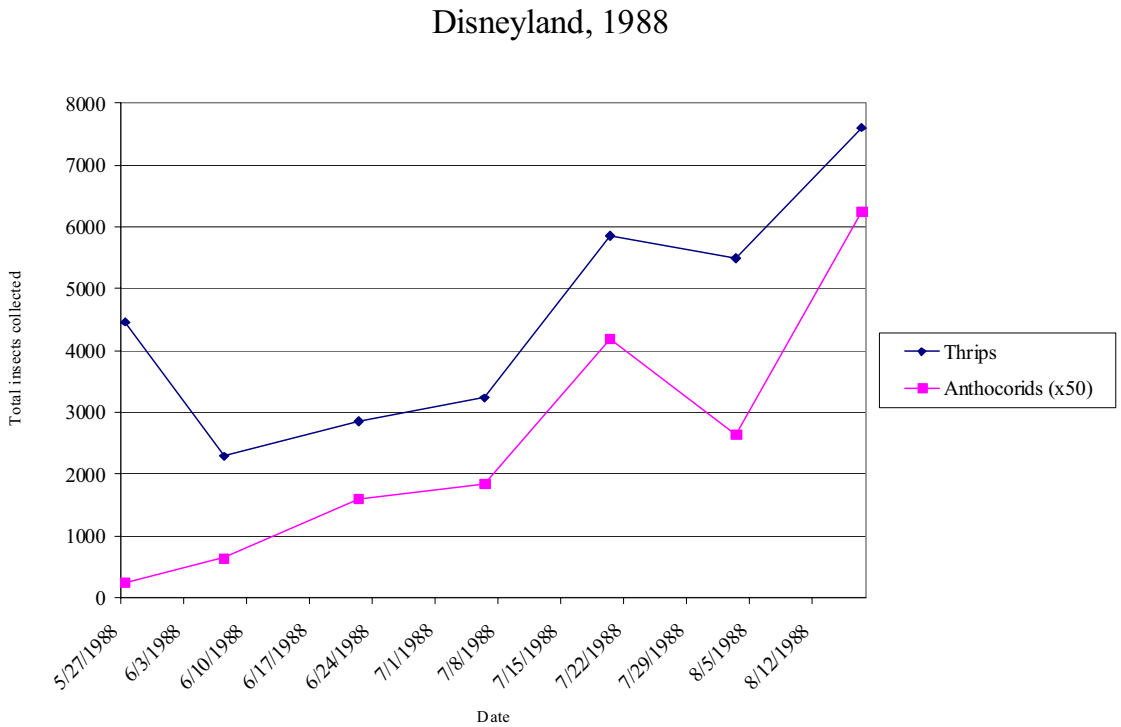

Figure 2. Total number of insects collected from *Ficus nitida* leaf galls during weekly sampling during spring-summer 1998 in the Disneyland parking lot.

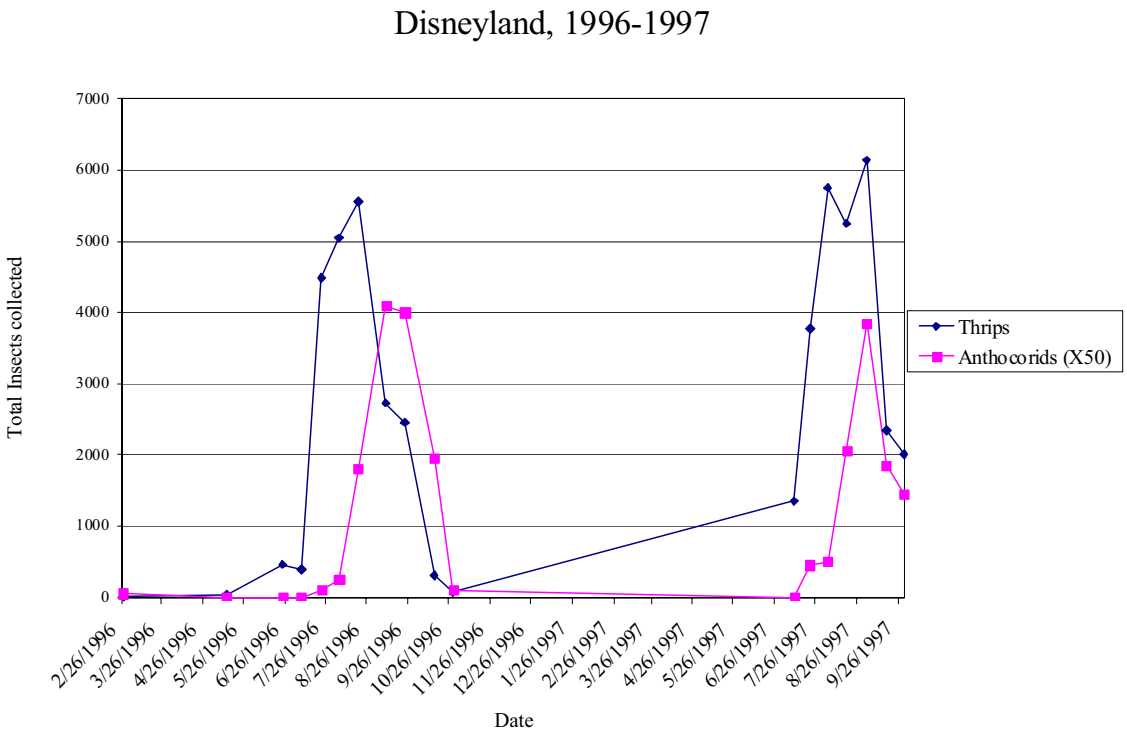

Figure 3. Total number of insects collected from *Ficus nitida* leaf galls during weekly sampling during 1996-1997 at a location near Disneyland.

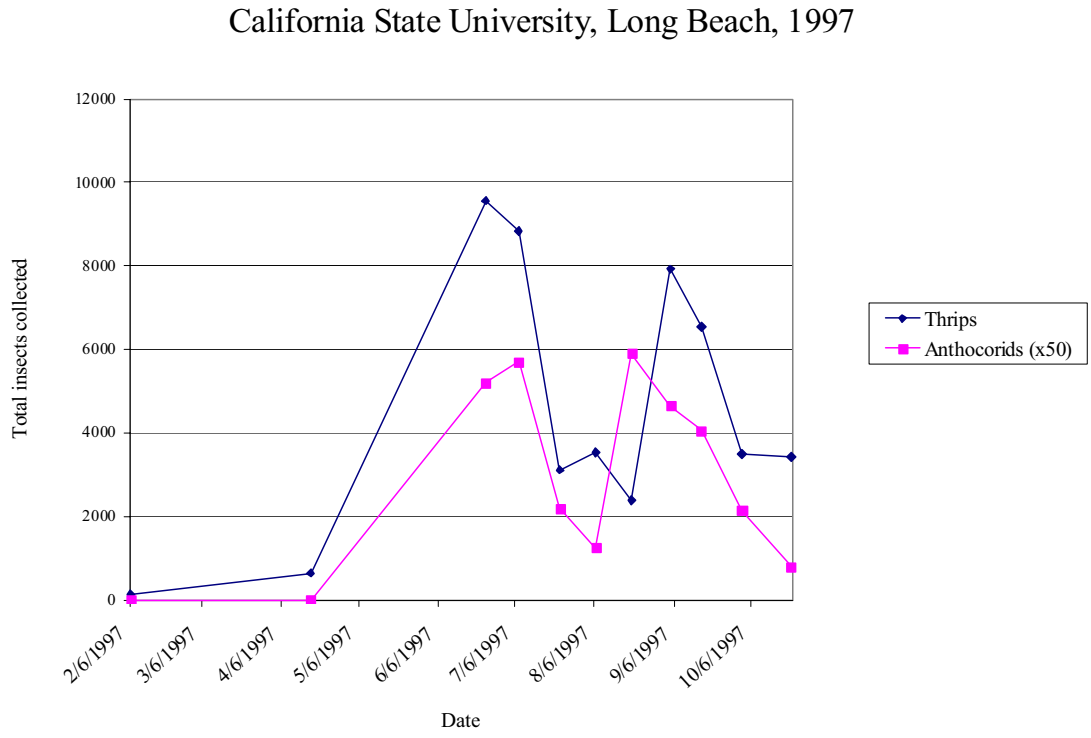

Figure 4. Total number of insects collected from *Ficus nitida* leaf galls during weekly sampling from February through October 1997 at a location on the campus of California State University, Long Beach.

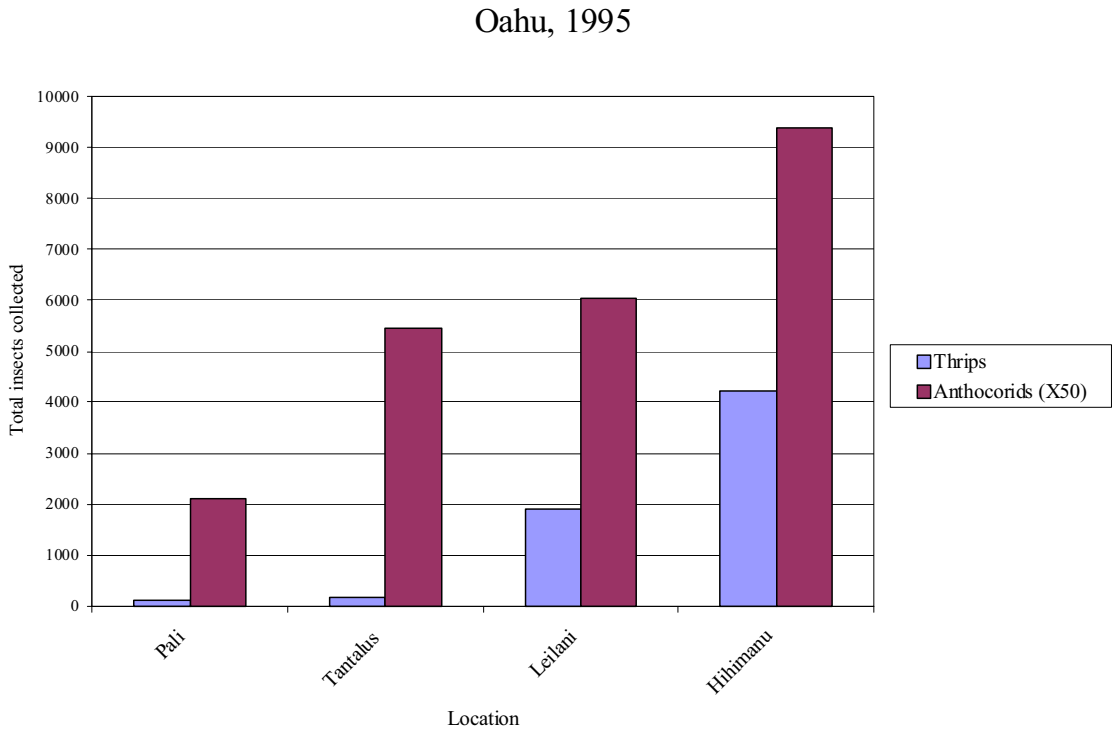

Figure 5. Total number of insects collected from *Ficus nitida* leaf galls at four locations on Oahu, Hawaii, November 1995.

DISCUSSION

The release of *M. moraguesi* in Hawaii successfully reduced the population of the Cuban laurel thrips to a level such that the aesthetic damage to *Ficus* spp. was reduced to acceptable levels (Lewis, 1973). It was thought that introductions of this predator into California might produce comparable control of the thrips. Our most recent releases of this anthocorid may have been a case of too little, too late. Although there is no certainty as to the fate of the earlier predator releases, two local species of anthocorids have occupied the niche we sought to fill with *M. moraguesi*. Several months after our releases of adults of this predator, we captured a few immature *M. moraguesi*. The following year we found no *M. moraguesi* in our samples, although we did continue to collect many other predators, primarily two non-native anthocorids, *M. nigra* and *M. nigronitens*. Competition or abiotic factors are the most likely causes of the failure of *M. moraguesi* to become established. Although coastal southern California has a mild Mediterranean climate, it does have cool winters that may be unacceptable for many tropical organisms.

ACKNOWLEDGMENTS

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USE OF MONOCLONAL ANTIBODIES TO MONITOR SPIDER PREDATION ON CEREAL APHIDS AND THE EFFECTS OF ALTERNATIVE PREY

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ABSTRACT. Low-input production systems, minimal tillage, the under-sowing of crops, and diversification of agricultural environments can all result in an increased quantity and diversity of invertebrates within arable ecosystems. This enhancement in the availability of alternative prey could have a profound effect upon the role of generalist predators in biological control. A dense and diverse prey fauna may stimulate a rapid growth in the spider population prior to the arrival of pests such as aphids, enabling the spiders to prevent growth in pest numbers by establishing a favorable predator-pest ratio. Conversely, alternative prey may reduce the ability of the predators to control the pests, especially if the pest species is non-preferred, as suggested by laboratory studies using spiders and aphids. For these reasons it is necessary to ascertain the extent to which non-pest prey populations influence spider predation in the field and the effect this has on regulating pest density.

Linyphiid spiders, which are the dominant family of spiders within agroecosystems in northern Europe, were shown to locate their webs nonrandomly with respect to the availability of their potential prey. There was a significantly greater number of prey at sites with webs than at sites without webs, even where microhabitats were matched. Within these prey-rich patches, nonpest food resources such as Collembola, Diptera, Hymenoptera, Coleoptera, and Thysanoptera were abundant.

An antiaphid monoclonal antibody was created and shown to be aphid-specific, cross-reacting with no other invertebrates. Aphid remains were detectable in spiders using this antibody for more than seven days. Spiders were collected from a crop of winter wheat and tested by ELISA.

Of 2,531 linyphiid spiders tested, 26% contained significant quantities of aphid protein. The concentration and quantity of aphid protein present within these spiders was found to be disproportionately high when pest density was low early in the season. Such feeding when prey are scarce could regulate pest densities, possibly until the arrival of specialist natural enemies. When aphid

availability increased 13-fold, the concentration of aphid protein present within female and male spiders increased by 30% and 84%, respectively. When availability of the dominant alternative prey (Collembola) was high, the consumption of aphids per spider was significantly reduced. This negative relationship suggests that enhancement of alternative prey numbers later in the season, when aphid densities are high, could reduce the impact of linyphiid spiders on aphid populations. However, if agricultural practices are implemented that enhance the density and diversity of nonpest prey early in the season, prior to and during the early establishment phase of the aphids, the spider population could be increased, improving the potential of a large population of spiders and an assemblage of other generalist predators to control aphids.

UNDERSTANDING AND ASSESSING THE ECOLOGICAL IMPACTS OF DIFFERENT PEST CONTROL STRATEGIES

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ABSTRACT. This paper is an analysis of the relative agro-ecological impacts of different pest control strategies. Synthetic insecticides and transgenic insect-protected crops are contrasted with biological and cultural controls, and host plant resistance. Examples are drawn from two systems: potatoes in the northwestern United States where the predominant pest insect is the Colorado potato beetle (*Leptinotarsa decemlineata* [Say]), and corn in the midwestern United States where the corn rootworm (*Diabrotica* spp.) causes substantial damage. The approach we take is risk-assessment based, showing the value that this framework can bring and highlighting gaps in our knowledge, as well as inconsistencies in the standards that are applied in different cases. It is apparent that some of the imposed requirements are more reflective of precedents already set rather than consistent standards of oversight being applied, and that judgments concerning the value of different pest control strategies are often made in isolation and do not consider the risks and benefits of all possible alternatives. Furthermore, whether a particular strategy will be successful and sustainable depends as much on how that strategy is implemented as on the nature of the strategy.

DISCRIMINATION BY FEMALES OF THE PARASITOID *MICROPLITIS RUFIVENTRIS* BETWEEN HEALTHY AND VIRUS-INFECTED COTTON LEAFWORM LARVAE

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ABSTRACT. Efforts were devoted to determining if *Microplitis rufiventris* Kok females can discriminate between non-infected and nuclear polyhedrosis virus-infected *Spodoptera littoralis* (Boisduval) larvae (SlNPV), using biological methods for re-isolating the control "non-infected" and "experimental hosts" (virus-infected) after removing the female parasitoid. The distribution of eggs by mated *M. rufiventris* females among NPV-infected and non-infected hosts showed a clear ($P<0.01$) oviposition preference for NPV-infected larvae. When a *M. rufiventris* female was given a choice between a NPV-

infected larva and a virus-free but previously parasitized larva (with one previous oviposition in the host), the wasp female showed a more marked preference for NPV-infected larva. This preference was almost absolute.

These results suggest that the two biological agents, SlNPV and *M. rufiventris* wasp compete for the same resource (e.g., *S. littoralis* larvae) and thus are incompatible agents. The results suggest a different strategy will needed when this virus' use is combined with *M. rufiventris* wasps if a successful integrated pest management program is to be achieved.

AUGMENTATIVE RELEASE OF *TRICHOGRAMMA* SPP. FOR BORERS IN SWEET CORN–RESULTS OF A FIELD STUDY AND CONSUMER SURVEY

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ABSTRACT. In a 1,200 m^2 experimental field of 2,300 sweet corn plants, we compared the use of pheromone traps and plant development as means to time releases of *Trichogramma* spp. for control of Asian corn borer, *Ostrinia furnacalis* Guenée. The first moth trap captures were observed on April 28, 1998. Egg masses were found in the experimental field on May 19, and egg hatch began on May 25.

In the first test plot, parasitoid releases started in early June, two weeks after the peak of moth trap catch. In the second plot, parasitoid releases were started in late June, one week before male flower emergence (tasseling). Weekly releases continued until mid July. A local strain of wasp reared in our laboratory was used. A weekly release rate of 40 females per m^2 resulted in a significant reduction in numbers of damaged ears (81% lower than in the control plot), but not in numbers of damaged stalks. The parasitism rate of egg masses was significantly higher in the pheromone plot. Between the two release plots, the number of plants with host egg masses and parasitized egg mass were almost the same, but in the plant development plot, ears on plants with parasitized egg massES were damaged more. Parasitism rates within individual egg masses in the plant development plot were lower than that in pheromone plot, and there was a negative correlation between parasitism and the number of eggs per mass.

In addition to the field study, a survey was conducted of members of a cooperative for analysis of customer attitude to produce grown using less pesticide. Respondents ranged in age from 10 to 70. Of 84 respondents, 98% saw themselves as health-conscious and they reported that they would pay more for pesticide-free produce. However, 20% rejected pesticide-free produce even if slightly damaged by insects and 60% didn't persist in purchasing such ecofriendly produce if not readily available.

This system meets the requirements for success in practical use, namely, high effectiveness, self-determination of the timing by producers, fixed release number, and easy handling. Technical advances aimed at cutting cost by reduction of the number or duration of releases or numbers of releasing points are unlikely to completely eliminate loss in yield and quality. Adoption of this biological control method for preventing environmental pollution will require increased consumer understanding.

EFFECT OF HOST SIZE ON THE SEX RATIO OF *SYNASTER LEPIDUS*, A PARASITOID OF EUCALYPTUS LONGHORNED BORERS (*PHORACANTHA* SPP.)

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(TITLE ONLY)

ASSESSING RISKS OF ARTHROPOD BIOLOGICAL CONTROL: EXOTIC ENEMIES AND NONTARGET EFFECTS

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ABSTRACT. The 2001 National Invasive Species Management Plan calls for better screening methods for nonindigenous biological control agents for animal pests. To address this challenge, we used historical data to develop risk assessment models for introducing nonindigenous arthropod biological control agents into the continental United States. The research was limited to entomophagous biological control agents that were established between 1900 and 1981. We investigated 85 species for which reasonable documentation of establishment and success or failure in a biological control program could be found. Species were characterized using 13 life history traits and eight descriptive variables. Nontarget effects on native species were recorded from published literature. We used proportions tests to develop predictions for success and likelihood of nontarget effects based on histories of established species.

In the first set of tests we examined the proportion of successes versus failures. Predators had a lower proportion of success than parasitoids ($P < 0.05$), and polyphagous species (those that attack more than one family of hosts or prey) had a lower proportion of success than monophagous or oligophagous species ($P < 0.01$). Biological control species had a higher proportion of successes when the sex ratio was female dominated ($P < 0.06$); when there were multiple generations per year ($P < 0.01$); when the oviposition site was within or on the host ($P < 0.01$); and when the feeding was internal to the host ($P < 0.05$). Analysis showed evidence that biological control agents had a lower proportion of successes in forests than in crops or orchards ($P < 0.01$) and on target hosts in the order Lepidoptera ($P < 0.01$). Documented presence of native enemies and nontarget hosts or prey significantly decreased the probabilities for success ($P < 0.05$, $P < 0.01$, respectively).

There was no information about nontarget effects in 48 of the 85 cases. Lowest incidence of nontarget effects was associated with species having a female-dominated sex ratio ($P < 0.05$), lower host mortality (< 100 hosts attacked per individual parasitoid or predator) ($P < 0.01$), monophagous host range ($P < 0.01$), and moderate dispersal ability ($P < 0.01$). Nontarget effects were significantly higher in forest systems than in agricultural crop or orchard systems ($P < 0.01$). In addition, the proportion of nontarget effects was lowest in biological control programs against the target family

Diaspididae (armored scales) ($P < 0.05$) and when native enemies and nontarget hosts or prey were not present in areas of introduction ($P < 0.05$, $P < 0.01$).

This study, based on historical evidence, quantifies the associations between life history and descriptive traits and outcomes in biological control programs. The results are based on available published literature. The approach can be useful in developing guidelines for intentional introductions in arthropod biological control programs. More complete data on life history traits of biological control agents, pre-release surveys for native natural enemies, and post-release monitoring data for nontarget effects will significantly improve the models.

DISPLACEMENT OF AN INDIGENOUS NATURAL ENEMY BY AN INTRODUCED EXOTIC PARASITOID?

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(TITLE ONLY)

A SURVEY OF HORSEFLY EGG PARASITOIDS IN CHIANG MAI PROVINCE, THAILAND

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(TITLE ONLY)

CAN WE SELECT SPECIALIST PREDATORS FROM GENERALISTS?

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(TITLE ONLY)

BULB MITE BIOLOGICAL CONTROL

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(TITLE ONLY)

EFFECT OF OVERWINTERING CONDITIONS ON THE EMERGENCE OF *DIACHASMA ALLOEUM* REARED FROM THE PUPARIA OF BLUEBERRY MAGGOT

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ABSTRACT. The Opiine parasitoid, *Daichasma alloeum* Muesebeck, frequently parasitizes apple maggot, *Rhagoletis pomonella* Walsh. Percentage parasitism is usually low, with variations from year to year and from one region to the next. In 1999, we successfully reared *D. alloeum* from *Rhagoletis mendax* Curran, blueberry maggot, puparia. Previous records indicated *R. mendax* as a potential larval host, but confusion regarding the species status of *R. mendax* identified *D. alloeum* as a parasitoid of apple maggot and not blueberry maggot.

In 2000, blueberries were collected from an unsprayed abandoned plot in southwestern Michigan. Blueberries were suspended over screened trays for 21 d to allow maggots to exit the fruit and pupariate. Eight replicates of 50 puparia were placed in 59 mL soufflé cups containing moist vermiculite. Four of the eight replicates were incubated at 5 °C for 5 months, while the remaining four cups were left at room temperature (22 °C) on a 16:8 light/dark (L/D) cycle with 70% relative humidity. When puparia were kept at room temperature for 48 d and subjected to 16:8 L/D regime with 70% RH, an average of 3.5 *D. alloeum* per replicate emerged and puparia parasitism rates ranged from 2 to 12 %. However, when puparia were subjected to overwintering temperatures (5 °C), and then exposed to the same conditions (16:8 L/D regime with 70% RH) the mean number of *D. alloeum* that emerged was significantly higher, averaging 7 per replicate with parasitism rates ranging from 10 to 16%. We recorded no significant differences in the behavior of the adults from puparia exposed or unexposed to overwintering conditions. The results indicate that low temperature may be a requirement for optimum performance of *D. alloeum* with respect to parasitism of puparia from blueberry maggot.

IMPACT OF NATURAL ENEMIES ON POPULATIONS OF THE GLASSY-WINGED SHARPSHOOTER, *HOMALODISCA COAGULATA* (SAY), IN NORTHERN FLORIDA

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INTRODUCTION

Nearly five decades of sampling of *Homalodisca coagulata* (Say) populations in Florida and Georgia have shown that the insect is highly mobile and feeds upon more than 200 native plant species (Turner and Pollard, 1959; Adlerz, 1980; Ball, 1982; Mizell and French, 1987). However, populations of this insect at the North Florida Research and Education Center (Monticello, Florida) appear not to reach the levels observed at nurseries nearby in Florida or in localities where it has been found in California. We hypothesized that the abundance and diversity of natural enemies at the Monticello Research Station, possibly associated with ecologically oriented management practices, might account for this leafhopper's low densities there.

Our objective was to assess the potential impact of natural enemies on the GWSS through identification and quantification of its parasitoids and predators in conjunction with monitoring of sharpshooter populations.

MATERIALS AND METHODS

Nine survey plots, each including eight species of potted plants 2-3 m tall, were established on an open grassland at the Monticello Research Station, Florida. Plot replicates were set in a 3 x 3 grid pattern with 75 m between adjacent plots. The plant species were chosen based on the high frequencies at which GWSS had been recorded feeding or laying eggs on these plants during extensive prior observations of both wild and planted vegetation in Georgia and Florida (authors' unpublished data). These plants were crape myrtle (*Lagerstroemia indica* L.), holly (*Ilex* sp.), *Pyracantha sp*., *Euonymus japonica* L., redbud (*Cercis canadensis* L.), peach (*Prunus persica* [L.] Basch), orange (*Citrus* sp.), and grape (*Vitis* sp.).

Plots were established the first week of June in 2000 and 2001, and all plants in the plots were visually inspected each succeeding week through the last week of October for GWSS adults, nymphs, and egg masses. Egg masses were left in place and each was dated and then checked every following week until parasitoid exit holes, signs of predation, or evidence of glassy-wing sharpshooter hatching was observed and recorded. A single large circular hole on top of each egg has been determined to be caused by *Gonatocerus* spp. parasitoids (Sahad, 1982); multiple smaller holes per egg are attributable to *Zagella* sp. parasitoids; and total or partial removal of the egg mass cover and empty egg compartments are the result of predation by tree crickets or earwigs.

In 2001, the presence and number of post-egg-stage predators on the crape myrtle trees on each plot were also recorded every week. Crape myrtle trees were chosen for this purpose because GWSS adults and nymphs had been found to feed preferentially on this species. Predators of adults and nymphs were captured in the act of predation under natural conditions and were identified. Spiders, tree crickets, and lizards were the most common predators found on the crape myrtle trees. Individuals of each spider and lizard species were captured and provided continuously with sharpshooter prey in laboratory conditions to determine their maximal predation rate, to approximate their potential field impact on glassy-wing sharpshooter populations. Individual ventilated Plexiglas cages were stocked with single, three-branched potted plants of *Baccharis halimifolia* L. This plant were selected because in the last 10 years of field observation of *H. coagulata* it has been determined that *B. halimifolia* is one of the native plants that it is in the top five preferred hosts in northern Florida (Turner and Pollard, 1959; authors unpublished data). Within each of three cages, 1, 2 and 4 predators of each species were successively exposed for 24 h to 5, 10 and 20 adult glassy-winged sharpshooters.

RESULTS AND DISCUSSION

Seasonal rates of parasitism plus predation of eggs in masses were 80.7% ± 13.4 (± 95% CI) for 2000 and 89.7% ± 12.15 (± 95% CI) for 2001 (Fig. 1). The predators most frequently found in the plots were *Lycosa punctulata* Hentz (Lycosidae); *Peucetia viridans* (Hentz) (Oxyopidae); *Salticus* sp., *Sitticus* sp., and *Phidippus* sp. (Salticidae); *Sinea spinipes* Fabricius (Reduviidae); *Anolis* sp. (Iguanidae); and *Cyrtoxipha* sp. (Gryllidae). Mean number of spiders per crape myrtle tree across the season during 2001 were 2.8 ± 0.8, 1.2 ± 0.5 and 2.1 ± 1.1 per week for Oxyopidae, Lycosidae, and Salticidae, respectively, and this combined number of spiders was found capable of consuming seven adult and six nymphal glassy-winged sharpshooters daily. These levels of predation closely approximate the average numbers of sharpshooters found per crape myrtle tree per sampling occasion: 10 ± 3 for adults and 1.9 ± 0.7 for nymphs. Although under field conditions these predators would not necessarily concentrate on GWSS alone, the fact that the GWSS was the most abundant prey on the trees where the predators were found makes it likely that glassy-winged sharpshooters constitute a large proportion of their diet.

CONCLUSIONS AND MANAGEMENT IMPLICATIONS

Our results indicate that at the Northern Florida Research Station, mymarid and trichogrammatid parasitoids together with tree crickets and earwigs leave only about 10% of the population of glassy-winged sharpshooter eggs to develop further. Predators of nymphs and adults that include several species of spiders–*L. punctulata*, *P. viridans,* and *Phidippus* sp., as well as *S. spinipes* and green anoles lizards. Management practices at the North Florida Research and Education Center differ from those of commercial nurseries in several ways that might encourage populations of these natural enemies. First, there has been no spraying of any pesticides in the meadow area for several decades. Second, clover seeds were spread in an earlier year to provide a cover crop to favor beneficial insects early in the season. Clover attracts aphids, which provide food for ladybugs and honeydew for many species of natural enemies, as well as bearing flowers with abundant nectar. Third, the meadow is mowed only a few times per season, permitting a diversity of herbaceous species to grow among the grass. Floral and especially extrafloral nectaries on these species provide regular sources of nutrition that may help to maintain beneficial insect populations in the area.

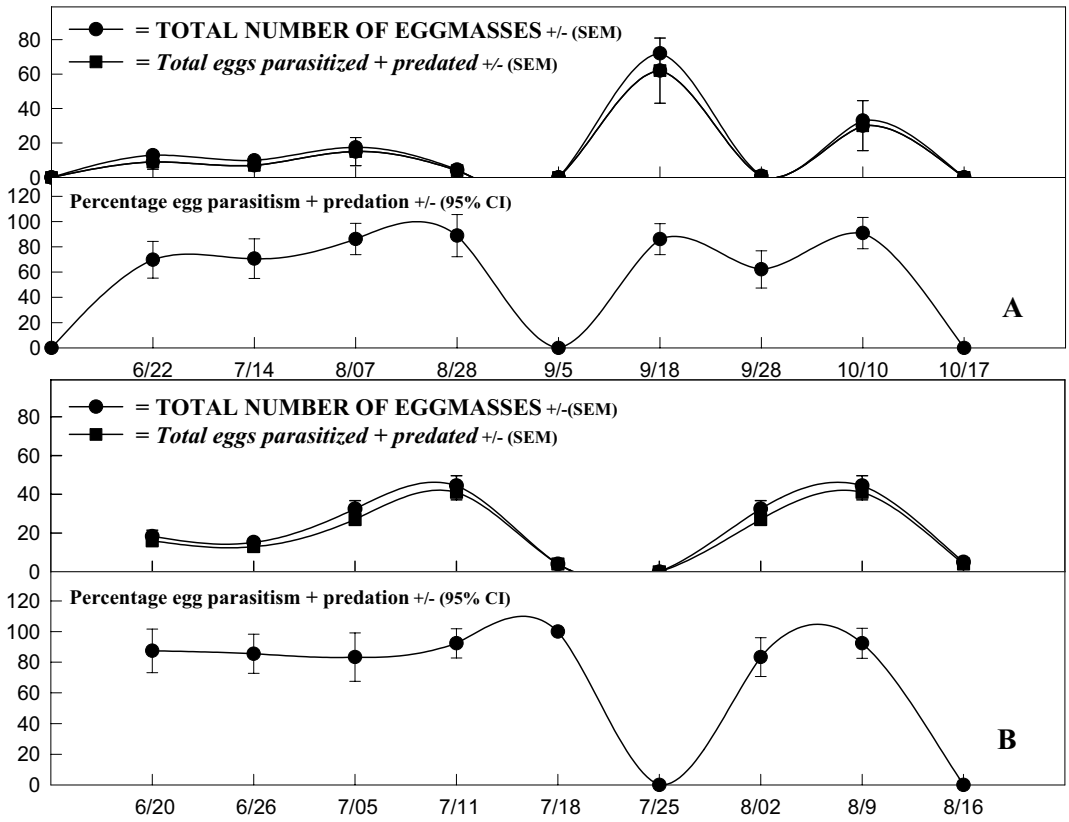

Figure 1. Total number of egg masses parasitized and predated in the year 2000 (A) and 2001 (B).

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NO-CHOICE OR MULTIPLE-CHOICE? HOST PREFERENCE ASSESSMENT OF THE GREGARIOUS EGG PARASITOID *TRICHOGRAMMA PLATNERI*

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ABSTRACT. The first step in the risk assessment of a proposed biological control agent is definition of the physiological host range or the complete group of species on which the agent can survive and develop under controlled conditions. If the agent is not monophagous when tested in a no-choice situation it is useful to assess host preference through choice tests with more than one host species present. Choice tests indicate if the target host is preferred over other physiologically acceptable hosts or if all acceptable hosts are equally suitable.

Here we present a comparison of different methods for estimating host preference using the egg parasitoid *Trichogramma platneri* Nagarkatti as a model for risk analysis in biological control. Egg parasitoids of the genus *Trichogramma* are generally polyphagous, but host preferences have been demonstrated for several species. These parasitoids are widely used for inundative biological control of lepidopteran crop pests and concern over non-target effects from large-scale releases have been raised. *Trichogramma platneri* was chosen for these experiments because it is used as an inundative biological control agent against codling moth, *Cydia pomonella* (L.) in California, and the host preferences of this species have not been examined before.

Selective exploitation of six host species (*Chrysoperla carnea* [Stephens], *Cydia pomonella* [L.], *Ephestia kuehniella* Zeller, *Helicoverpa zea* [Boddie], *Manduca sexta* [L.] and *Sitotroga cerealella* [Olivier]) by *T. platneri* was evaluated in no-choice, paired-choice and multiple-choice tests, using number of parasitoids emerged and proportion of female offspring as different measures of preference. *Helicoverpa zea*, *M. sexta*, and *C. carnea* were the most preferred hosts and *S. cerealella* the least preferred, in the no-choice and choice tests. More *T. platneri* emerged from *C. carnea*, *H. zea*, and *M. sexta* than from *S. cerealella*, and a greater proportion of female offspring emerged from *M. sexta* than from *C. pomonella*, *H. zea*, and *S. cerealella* in the no-choice tests. A greater percentage of progeny and a greater proportion of females emerged from *H. zea* and *M. sexta* when paired with any of the other four host species. In the multiple-choice tests *H. zea* produced the greatest percentage of parasitoids and the highest proportion of females, whereas *S. cerealella* was not parasitized at all.

The most appropriate variable to measure selective host exploitation will depend on the type of hosts offered and the type of parasitoid (solitary or gregarious). Percent parasitism is a suitable measure for solitary parasitoids, but should be used for gregarious parasitoids only if the host species tested are of similar size and quality for the parasitoid. If the host species differ in size or quality, the quantity of progeny emerged (measured as absolute numbers or proportions) is more appropriate for gregarious parasitoids. The sex ratio of progeny emerged from different host species although an indicator of host quality was not as effective for determining relative preference of *T. platneri* for the six host species. The number of progeny emerged is the most suitable variable for estimating host preference among gregarious parasitoids. Although no-choice and multiple-choice tests provided the same preference ranking for *T. platneri*, a polyphagous species, the latter tests are likely to be more realistic for more specialized control agents.

ESTABLISHING *PSEUDOSCYMNUS TSUGAE* (COLEOPTERA: COCCINELLIDAE) FOR BIOLOGICAL CONTROL OF HEMLOCK WOOLLY ADELGID, *ADELGES TSUGAE* (HOMOPTERA: ADELGIDAE) IN THE EASTERN UNITED STATES

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ABSTRACT. The hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae), is native to Asia, where it is a harmless inhabitant of several hemlock (*Tsuga*) species. Hemlock woolly adelgid was first observed in the eastern United States around 1950 in Richmond, Virginia. Since then, it has spread into 12 states on the eastern seaboard, from North Carolina to New England, where it is a serious pest of eastern hemlock, *Tsuga canadensis* Carriere, and Carolina hemlock, *Tsuga caroliniana* Engelmann.

In 1992, Mark McClure discovered *Pseudoscymnus tsugae* Sasaji and McClure (Coleoptera: Coccinellidae), a predator of hemlock woolly adelgid in Japan. In 1994, the Connecticut Agricultural Experiment Station and the U.S. Department of Agriculture, Forest Service State and Private Forestry initiated a cooperative agreement to explore the potential of *P. tsugae* as a biological control agent for hemlock woolly adelgid. We found that *P. tsugae* is amenable to mass culturing and possesses other qualities that make it an excellent biological control candidate. *Psuedoscymnus tsugae* produces three or more generations each year in the laboratory under controlled temperature conditions; it is adapted to a wide range of climate conditions; it strongly prefers to feed on adelgids; its life cycle is synchronized with that of hemlock woolly adelgid; and it has a high searching efficiency and dispersal ability. The encouraging results from this project spawned another cooperative effort with the Phillip Alampi Beneficial Insect Laboratory in Trenton, New Jersey to mass-rear *P. tsugae*.

Since 1995, 627,000 beetles have been released at 100 sites in 11 eastern states. *Pseudoscymnus tsugae* has overwintered, established, reproduced, and spread at many of these release sites. In forests where *P. tsugae* has been present for at least three years and where at least 10,000 adults were initially released, beetles are now abundant and can be collected from infested trees. Sampling from a bucket truck documented vertical dispersal, overwintering survival, and reproduction of *P. tsugae* up to 65 feet in the canopy. Horizontal dispersal of 0.58 miles has also been documented. Results have been encouraging when hemlock woolly adelgid densities on hemlock trees in release areas were compared with those in similar control areas (beetles absent in the area) at least half a mile away. Hemlock woolly adelgid densities on monitored branches in release areas were reduced by 43-87% in just five months by a starting population of only 2,400 adult beetles, indicating a remarkable short-term impact of *P. tsugae* on hemlock woolly adelgid. Weather conditions from 1995 to 1999 hampered the biological control effort. A string of mild winters enhanced the survival and growth of hemlock woolly adelgid populations, and a severe drought in 1999 in the eastern United States, significantly reduced hemlock health. However, in the northeastern United States, a two-week period with subzero (F) temperatures in January, 2000 reduced adelgid populations by more than 90%, while *P. tsugae* survived this cold period. With greatly reduced adelgid numbers, hemlocks flourished during the cool, moist spring and summer of 2000 and abundant new growth was evident in spring 2001. Even though milder temperatures during winter 2000-2001 allowed 50 to 60% survival of hemlock woolly adelgid, pest densities have remained low in our release areas.

TOXICITY OF SEVERAL MODERN PESTICIDES TO THE PARASITOID *HYPOSOTER DIDYMATOR* AND THE PREDATOR *CHRYSOPERLA CARNEA*: SIGNIFICANCE OF PENETRATION AND EXCRETION

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INTRODUCTION

Insect growth regulator (IGR) pesticides are selective to many beneficial insects, safeguard the environment and food quality, and do not pose a high risk of stimulating resistance in treated populations. Consequently, they are used worldwide in IPM (Integrated Pest Management) and IP (Integrated Production) systems (Viñuela *et al*., 2000). Among the IGRs most widely used are diflubenzuron, pyriproxyfen, and tebufenozide. Diflubenzuron disrupts insect molting by inhibiting chitin synthesis (Retnakaran and Wright, 1987; Ishaaya and Horowitz, 1998); the juvenile hormone mimic pyriproxyfen suppresses embryogenesis, metamorphosis, and adult formation (Ishaaya *et al*., 1994); and the nonsteroidal ecdysone agonist tebufenozide interacts with the insect molting hormone receptor, especially in Lepidoptera, inducing a premature and lethal molting (Chandler *et al.*, 1992; Smagghe and Degheele, 1994; Dhadialla *et al*., 1998; Carlson, 2000).

In this study, we report on the activity and pharmacokinetics of these IGRs, topically applied, in two enemies of interest: *Hyposoter didymator* (Thunberg) (Hymenoptera: Ichneumonidae) and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). The former is an endoparasitoid of noctuid larvae (Schneider *et al*., 2000); the latter is a cosmopolitan generalist predator (Greeve, 1984; Tauber *et al.,* 2000) and both are commonly found in Spain. We focus on the impact of penetration through the female cuticle and the pattern of excretion in the case of *C. carnea*, and on insecticide penetration through the silken pupal cocoon and its accumulation in the parasitoid´s body in the case of *H. didymator*, discussing the relationships of these processes with the observed toxicity.

MATERIALS AND METHODS

Insects

Insects were maintained in controlled environmental chambers (25 ± 2 °C, 75 ± 5% RH, photoperiod of 16:8 L:D). The parasitoid was reared on third instars of *Spodoptera littoralis* (Boisduval). Adults were provided with pure honey and water. Larvae of *C. carnea* were fed on *Sitotroga cerealella* (Oliver) eggs and adults were provided an artificial diet (Vogt *et al*., 2000).

Insecticides

Dimilin (25% diflubenzuron, WP, AgrEvo, Valencia, Spain), Juvinal (10% pyriproxyfen, EC, Kenogard, Barcelona, Spain), and Mimic (24% tebufenozide, SC, Rohm and Haas, Barcelona, Spain) were used to evaluate lethal and sublethal effects. A C^{14}-radiolabeled isotope of diflubenzuron (specific activity 15 mCi/g), pyriproxyfen (specific activity 181 mCi/g), and tebufenozide (specific activity 23 mCi/g)

were provided by Duphar B. V. (Weesp, The Netherlands), Sumitomo (Osaka, Japan), and Rohm and Haas (Spring House, Pennsylvania, U.S.A.), respectively, to determine rates of penetration, excretion, and distribution in the natural enemies tested.

Toxicity Assays

Two of the most protected developmental stages of the enemies were chosen: < 24 h old *H. didymator* pupae and *C. carnea* adults. Insects were collected from stock cultures and an acetone solution of a given IGR was applied to either the coccon (for parasitoids, total of 1 ml) or the pronotum (for adult predators, total of 0.5 ml), using a hand microapplicator (Burkard, United Kingdom). Every experiment consisted of five replicates of five pupae per dose level and insecticide and at least five replicates of three pairs of *C. carnea* adults. Weights of test insects averaged 19.8 ± 0.005 mg for young pupae of *H. didymator* and 7.5 ± 0.9 mg for adults of *C. carnea*.

For every insecticide, concentrations close to those registered for use in Spain were chosen to evaluate lethal (direct mortality) and sublethal effects (i.e., adult emergence and beneficial capacity [attacked hosts, progeny size, and life span of both sexes] for the parasitoid; fecundity and fertility for the predator).

Six to seven days after treatment, *H. didymator* adult emergence was scored, and adults were transferred to ventilated plastic round boxes (12 cm dia. x 5 cm height) and provided with food and water. Adult survival 15 days after emergence (parasitoid half-life under our environmental conditions) was recorded, and parasitism capacity was evaluated for six 3-d-old females per insecticide, in accord with Schneider *et al.* (2000).

After treatment, three pairs of *C. carnea* per replicate were transferred to oviposition boxes as described by Medina *et al*. (2001). Adult survival was recorded on a daily basis. The average number of eggs per female was counted every 1-2 days, and the cumulative number of eggs in a 10-d-period was used to compare doses. Egg fertility was determined for eggs collected five days after the first oviposition, and the number of hatched larvae were counted five to six days later.

Aplication of C^{14}-Isotopes

Hyposoter didymator pupae (£ 24 h old) and *C. carnea* females (8 d old, once the oviposition had started) were topically treated with 1 ml of an acetonic solution of the corresponding isotope. The rate of penetration, distribution of C^{14}-pesticides into the body, and the pattern of excretion were measured with a Packard Biological Material Sample Oxidizer and a Kontron liquid scintillation counter using the techniques described by Smagghe and Degheele (1993).

Statistics

Data, presented as means ± SD, were analyzed by one-way ANOVA using Statgraphics (STSC, 1987). Means were separated by a LSD multiple range test ($P<0.05$) and in those cases where the F value from ANOVA was not significant, a Bonferroni test was applied. The non-parametric test of Kruskal-Wallis was used to establish differences only when data violated the premises of ANOVA (Milliken and Johnson, 1984). For penetration and excretion, a curve-fitting option of Excel (Microsoft) was performed and the quality of fit was evaluated based on the curve´s correlation coefficient (R^2). T_{50} were calculated from assay data by extrapolation from regression curves.

RESULTS

Diflubenzuron, topically applied to pupae of *H. didymator*, did not modify adult emergence but some delayed effects were observed on the survival rate of adults. At 100 mg a.i./l the number of dead adults 15 days after emergence was increased by 65%. Pyriproxyfen was very harmful to the parasitoid, decreasing adult emergence by 56% and completely inhibiting the development of progeny because all insects died within 15 days. Tebufenozide, in contrast, was totally harmless (Table 1).

Table 1. Effects of diflubenzuron, pyriproxyfen and tebufenozide, topically applied on <24-h-old *Hyposoter didymator* (Thunberg) pupae[a].

Insecticide	Concentration (mg a.i./l)	AdultEmergence (%)	Adultmortality, 15	Attacked hosts (%)	Progeny size (%)
Control	0	92.5±5.0a	39.0±4.0a	90.0±1.0a	98.0±1.0a
Diflubenzuron	100	92.5±5.0a	65.0±5.0b	95.0±1.0a	95.0±1.0a
Pyriproxyfen	75	56.4±4.0b	100c	-	-
Tebufenozide	144	84.0±4.0a	47.0±6.0a	99.0±1.0a	99.0±1.0a

One day after topical application to the silken pupal cocoon of *Hyposoter*, 67% of tebufenozide had penetrated, in comparison to 60% of diflubenzuron and 37% of pyriproxyfen (Fig. 1). When studying the distribution of radioactivity, it was clear that, for the applied insecticides, most material did not reach the parasitoid´s body. The amounts recovered from the silken cocoon, three days after application of the insecticides were 88% for diflubenzuron, 93% for pyriproxyfen, and 98% for tebufenozide.

Half-life values (T_{50}) representing the time that 50% of absorption in the insect body is reached were less than 8 hours for diflubenzuron and tebufenozide and close to one month for pyriproxyfen (Table 2).

Table 2. Regression curves and half-life values (T_{50}) for the penetration of the different insecticides applied topically to pupae of *Hyposoter didymator* (Thunberg).

Insecticide	Equations	R^2	T_{50} (h)[a]
Diflubenzuron	y=5.85Ln(x)+40.59	0.99	5.00
Pyriproxyfen	y=3.71Ln(x)+25.40	0.99	759
Tebufenozide	y=6.54Ln(x)+46.11	0.95	1.81

Topical application of the three IGRs on < 24-h-old *C. carnea* adults, did not reduce survival or fecundity. However, diflubenzuron at the highest dose tested caused 100% inhibition of eggs hatch, in agreement with field results of Vogt and Viñuela (2001). Pyriproxyfen and tebufenozide were harmless (Table 3).

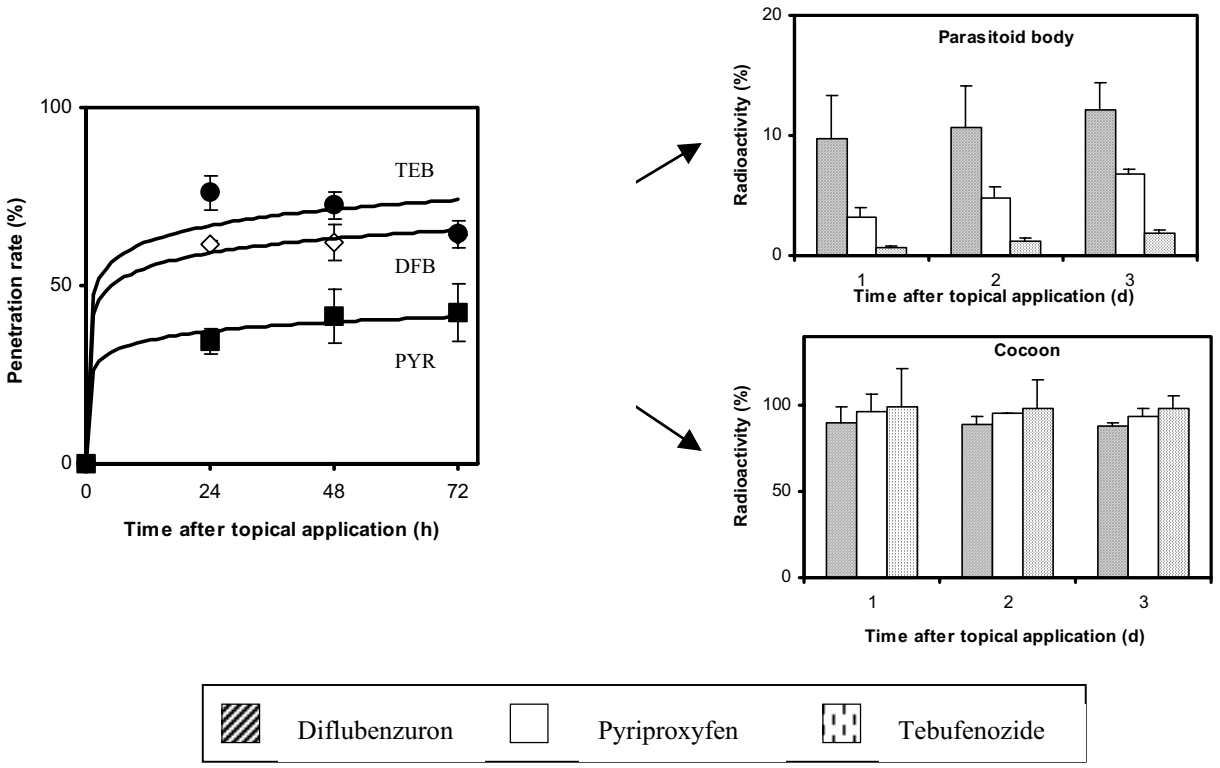

Figure 1. Percentage of penetration of [^{14}C]-isotopes of diflubenzuron (DFB), pyriproxyfen (PYR), and tebufenozide (TEB) on *Hyposoter didymator* (Thunberg) pupae and distribution into cocoon and parasitoid body. (Each data point is the mean ± SD based on three replicates of two pupae.)

Table 3. Effects of diflubenzuron (DFB), pyriproxyfen (PYR), and tebufenozide (TEB) on fecundity and fertility of < 24-h-old *Chrysoperla carnea* (Stephens) adults, when topically applied.

Concentration (mg a.i./l)	Eggs/female/day[c]			Egg hatch (%)		
	DFB	PYR	TEB	DFB	PYR	TEB
Control	28.9±1.8a	35.4±1.2a	24.4±3.5a	86.8±2.2a	82.3±1.6a	84.8±6.8a
1/5 CT	—[b]	35.3±1.6a	27.6±3.6a	—	80.3±3.2a	89.6±6.2a
CT[a]	—	39.1±3.7a	28.3±1.4a	—	83.0±3.5a	90.2±9.8a
2 x CT	26.6±1.8a	41.1±1.5a	29.2±2.8a	0.0±0.0b	82.8±4.3a	76.3±6.6a

[a]CT =Concentration tested (DFB:150 mg a.i./l; PYR: 75 mg a.i./l; TEB: 180 mg a.i./l).
[b] Not tested.
[c]First 10 days of oviposition.

For the three IGRs, penetration curves in *C. carnea* females followed a different profile with respect to the rate of penetration and amounts absorbed (Fig. 2). One day after topical treatment, about 88% of pyriproxyfen was absorbed through the cuticle of *C. carnea* females whereas only about 12% of diflubenzuron and 10% of tebufenozide had been absorbed. Excretion via the feces was different for the three IGRs (Fig. 2; Table 4).

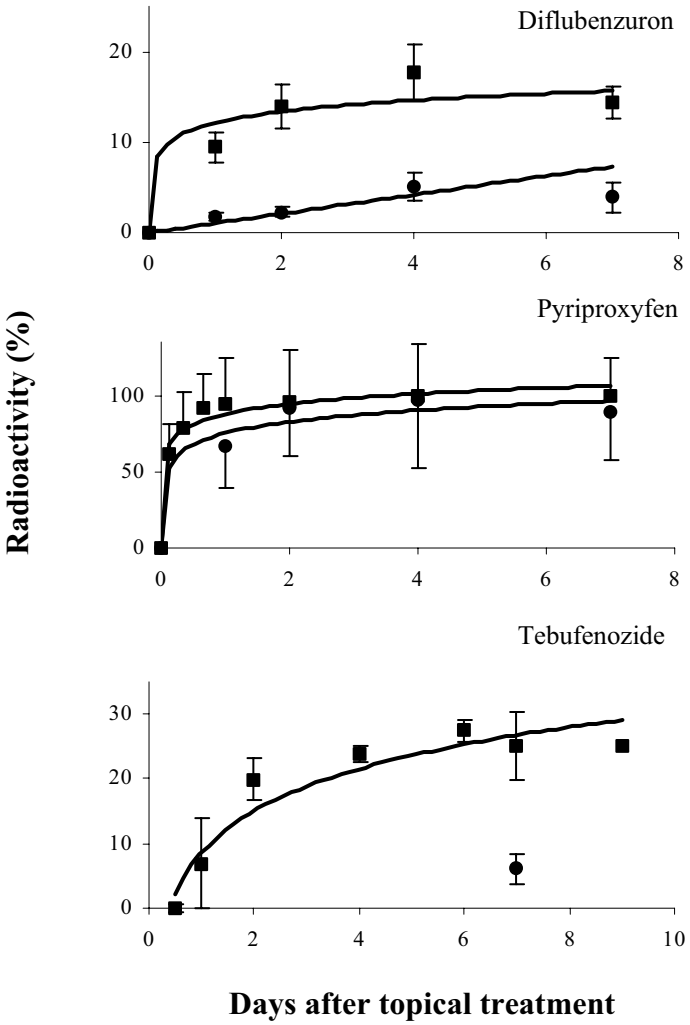

Figure 2. Percentage of penetration (g) and excretion (n) of C^{14}-insecticides in *Chrysoperla carnea* (Stephens). (Each data point is the mean ± SD based on four replicates of four females).

Table 4. Regression Curves and Half-life Values (T_{50}) for the Penetration and Excretion of the Different Insecticides Applied Topically to Females of *Chrysoperla carnea* (Stephens).

Insecticide	Penetration			Excretion		
	Equations	R^2	T_{50} (d)[a]	Equations	R^2	T_{50} (d)[a]
Diflubenzuron	y = 1.80Ln(x) + 12.13	0.90	$1.33x10^9$	$Y = 1.50X^{0.99}$	0.99	48.3
Pyriproxyfen	y = 9.50Ln(x) + 87.97	0.97	0.018	Y = 10.92Ln(X) + 75.18	0.96	0.1
Tebufenozide	y = 8.13Ln(x) + 10.56	0.83	127.6	-	-	

[a]Half-life values T50 (d) are calculated based on regression analysis y=aLn(x)+b, with y=% absorption or excretion and x= time-period after uptake(d).

Only very small amounts of diflubenzuron were excreted, as could be expected when taking into account the low rate of penetration. Seven days after application, nearly 47% of the insecticide applied had been excreted. In sharp contrast, excretion of pyriproxyfen was logarithmic. Fifty percent excretion was reached after 0.1 day, and more than 82% after 2 days. For tebufenozide, about half of the amount applied had been excreted seven days after application.

DISCUSSION AND CONCLUSIONS

Young *H. didymator* pupae were more sensitive to diflubenzuron, pyriproxyfen, and tebufenozide (topically applied) than were adults of *C. carnea*. Insecticide penetration followed a completely different pattern, depending not only on the insect but also on the compound. Diflubenzuron and tebufenozide quickly penetrated the silk cocoon of *H. didymator*, so the T_{50} values were reached in hours. In contrast, pyriproxyfen penetrated quite slowly. In contrast with the rates of penetration observed in *H. didymator*, in *C. carnea* adults, only very low amounts of diflubenzuron and tebufenozide penetrated, and this happened rather slowly whereas more than 80% of pyriproxyfen was absorbed in one day through the female cuticle.

In our study, we have shown the protective role of the silk cocoon of pupae of *H. didymator.* The amount of radioactivity recovered from the body of this parasitoid never surpassed the 12% of the total amount applied. More than 88% of all the insecticides remained in the cocoon.

Chitin synthesis inhibitors such as diflubenzuron are considered more active by ingestion than by contact or topical application because their absorption is relatively low, and this phenomenon has often been claimed as the reason for their selectivity against beneficial insects (Retnakaran and Wright, 1987). However, in *H. didymator* pupae, 60% of all diflubenzuron applied was absorbed within three days after topical application, and only 12% of the compound applied was accumulated into parasitoid tissues. These values are much higher than those recorded for pyriproxyfen and tebufenozide. Both sets of data explain the toxicity observed. In adults of *C. carnea*, penetration of diflubenzuron was extremely low compared with *H. didymator* or pests such as the cotton leafworm *S. littoralis* (Smagghe *et al*., 1997), and the only harmful effect observed was a large reduction in egg hatch in treated females. Fully developed larvae died within the egg shell. Because of its exceptionally low level of excretion, diflubenzuron was retained for a longer period into the female body, including ovaries, where a small percentage was accumulated in developing eggs leading to a total inhibition of egg hatch. This result is in accord with results obtained by Ivie and Wright (1978) in *Musca domestica* L. and *Stomoxys calcitrans* L.

Of the tested compounds, the juvenile hormone pyriproxyfen exhibited the lowest penetration rate through *H. didymator* cocoons (35%). Only 7% of the applied material reached the parasitoid's tissues. However, in spite of this low absorption, high toxicity was observed in the parasitoid, and both adult development and reproduction were totally inhibited. Based on these results, we hypothesize that the low amount of pyriproxyfen retained was present as parent compound or active metabolites. In the case of *C. carnea* adults, penetration through the cuticle was very fast (less than two days after topical application), but so also was the elimination from the female body, thus preventing the accumulation of the compound into the insect's body. Consequently, there were no toxic effects at the concentrations tested.

Similar to results obtained with last-instar larvae of *S. littoralis,* another host of *H. didymator* (Smagghe *et al.,* 2001), there was rapid penetration of the molting hormone agonist tebufenozide through the parasitoid cocoon. More than 80% of the applied amount penetrated the cocoon, but only about 2% or less actually reached the body of the parasitoid. In contrast, the *C. carnea* patterns of penetration and excretion were both slow and low. The low penetration and absorption of

tebufenozide may explain the low toxicity of tebufenozide to these two natural enemies. However, differences in the molting hormone receptors between the species could also be an important factor (Bidmon and Sliter, 1990; Carlson, 2000).

In conclusion, there were no a clear relationships between the pattern of penetration and the toxicity of the compounds. Currently used IGRs have been reported to exhibit good selectivity towards several beneficial insects. However, our results demonstrate that diflubenzuron reduced the life span of *H. didymator* and totally inhibited egg hatch of *C. carnea;* pyriproxyfen was very harmful to the *H. didymator* but harmless to *C. carnea*. Only tebufenozide was completely harmless to both species. Therefore, the use of diflubenzuron and pyriproxyfen in IPM programs involving the use of this parasitoid or predator should be carefully considered.

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PHORID FLIES AS CLASSICAL BIOLOGICAL CONTROL AGENTS OF IMPORTED FIRE ANTS

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ABSTRACT. Phorid flies in the genus *Pseudacteon* are solitary parasitoids of ants. Development occurs inside the head capsule of the host worker, killing the ant in the process. *Pseudacteon* flies may also have relatively large indirect effects on colony-level foraging and interspecific competition, as the presence of a single phorid can modify the behavior of hundreds of workers. Two *Solenopsis* fire ant species from South America, *Solenopsis invicta* Buren and *Solenopsis richteri* Forel, are serious pests in their introduced range in the United States. The high population densities of these two exotic ants may be due in part to an escape from natural enemies. *Pseudacteon* phorid flies from South America are being evaluated as classical biological control agents of imported fire ants in North America. Laboratory studies of one

Pseudacteon species, *Pseudacteon tricuspis* Borgmeier, and its interactions with *S. invicta*, revealed that the presence of this phorid decreased food retrieval by as much as 50%. We have imported select *Pseudacteon* species from Brazil and Argentina to the United States for laboratory evaluation, and are currently mass-rearing and releasing two species, *P. tricuspis* and *Pseudacteon curvatus* Borgmeier at multiple sites in the southeastern United States. In northern Florida, we established the first field population of *P. tricuspis* in October 1997. This species now covers approximately 8,100 square kilometers in Florida, and is continuing to expand its range and increase in abundance. Long-term monitoring of sites with and without flies will elucidate the magnitude of the effect of these parasitoids on target fire ant and associated arthropod populations. *Pseudacteon* flies and other natural enemies will not eradicate imported fire ants in North America, but may reduce fire ant densities.

INTEGRATION OF NATURAL ENEMIES INTO SWEET CORN PEST CONTROL

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ABSTRACT. Sweet corn is attacked by a variety of insect pests that can cause severe losses to the producer. Current control practices are largely limited to the application of broad-spectrum insecticides that can have a substantial deleterious impact on the natural enemy complex. Natural enemies have been shown to provide partial control of sweet corn pests when not killed by broad-spectrum insecticides. New products that specifically target the pest species, while being relatively benign to other insects, could enable growers to have the benefits of natural enemies and still use insecticides as needed.

In field trials in 2000, we found that Avaunt (indoxacarb) and SpinTor (spinosad) are both less toxic to some of New York's major natural enemies in sweet corn (*Coleomegilla maculata* [De Geer], *Harmonia axyridis*, [Pallas] and *Orius insidiosus* [Say]) than the pyrethroid Warrior (lambda-cyhalothrin). Avaunt, however, was highly toxic to coccinellids and SpinTor was slightly toxic to *O. insidiosus* at labeled field rates. Both of these new products were able to provide control of the lepidopteran pests equal to Warrior. Transgenic Bt sweet corn varieties provided excellent control of lepidopteran pests and showed no toxicity to the natural enemies monitored, while Dipel–a foliar spray formulation of *Bacillus thuringiensis* Berliner–provided no significant pest control. The choice of insecticide material had a major impact on survival of both the pests and natural enemies in sweet corn, but the rate and frequency of application had only minor impacts.

BIOLOGY AND ATTEMPTED ESTABLISHMENT OF *LINNAEMYA LONGIROSTRIS* (DIPTERA: TACHINIDAE), A PARASITOID OF *HELICOVERPA ARMIGERA* (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT. *Helicoverpa armigera* (Hübner), a major pest on a variety of food and cash crops, is attacked by a large complex of natural enemies. However, there are a few reports investigating those natural enemies in Africa, even though surveys and reports of mortality factors and rates of parasitism by natural enemies are relatively abundant. *Linnaemya longirostris*, a common tachinid fly is one of the natural enemies of *H. armigera* larvae in Kenya.

We reared natural enemies from *H. armigera* larvae collected from the field, and tried to establish the colony of *L. longirostris* in the laboratory in order to study its ecology and behavior in detail. Samples consisted of the third and fourth instars of *H. armigera* collected from pigeon pea fields at the Kabete campus of Nairobi University in Kenya, in April 2001. Collected larvae were reared singly in petri dishes on artificial diet. Some 80.8% of *H. armigera* larvae were found to be parasitized by *L. longirostris.* Adult tachinids emerging from the samples were easily paired and mated in the laboratory. Mated females (day 11-16) were dissected, and maggots from 10 fertile females were inoculated on last instar host larvae using a fine hairbrush. *Chilo partellus* Swinhoe and *Sesamia calamistis* Hampson were also inoculated to determine their host suitability. However, *H. armigera* was the best host based on percentage parasitism and emerging adult size of the fly among inoculated host species. We introduced newly emerged females to 2- to 6-day-old males. However, none of the emerged adults mated, unlike their parents, which had mated easily under the same conditions. The parent generation did not show any difficulties in mating and produced many offspring in the laboratory.

Culturing tachinid flies is generally considered very difficult because of the difficulty in obtaining matings in captivity. The reasons for this difficulty are unknown and should be investigated further. Larvae of *H. armigera* in the field were highly parasitized in this study.

GIANT WHITEFLY, *ALEURODICUS DUGESII* COCKERELL, AND ITS BIOLOGICAL CONTROL IN FLORIDA

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ABSTRACT. The giant whitefly, *Aleurodicus dugesii* Cockerell, is a tropical species native to Mexico. It occurs in Costa Rica, Guatemala, and Mexico. In the United States it is found in Arizona, California, Florida, Louisiana, and Texas. In Florida, it was first discovered in Red Hill, Volusia County, in December 1996. The population has increased dramatically and spread to at least 13 other counties including Brevard, Flagler, Highlands, Hillsborough, Indian River, Lake, Manatee, Martin, Osceola, Orange, Polk, Seminole, and St. Lucie counties.

Giant whitefly's life cycle consists of egg, four nymphal instars, and adult stages. Female giant whiteflies lay eggs in a spiral pattern on the underside of host leaves, and at the same time deposit white wax with each egg. The eggs hatch into crawlers after about three days. The fourth nymphal stage, is the so-called pupa. The third and fourth nymphal stages secrete long glassy filaments of wax. Wax filaments are only 1-2 inches long in the field due to wind breakage, but can reach up to 10-18 inches long in the rearing laboratory. The wax filaments become matted and cover the entire underside of the host leaf. Adult giant whiteflies emerge through the T-shaped sutures on the dorsum of the pupal case (fourth stage). Males and females are similar. They congregate in large numbers on the underside of the natal leaf where they feed and oviposit until these leaves fall onto the ground. A complete life cycle from egg to adult lasts about 25-30 days under Florida's warm and humid summers.

Immature and adult giant whiteflies remove large amounts of sap from the plant. The third and fourth nymphal stages secrete wax filaments that are contaminated by excreted honeydew during the feeding process. This leads to the development of sooty mold fungus. During heavy infestation the plant becomes weak, its leaves turn yellow, dry up, fall, and the plant may die. At least 70 plant species are infested by giant whitefly. Among these, hibiscus is the most preferred hosts.

In 1997, a parasitic wasp, *Entedononecremnus krauteri* Zolnerowich and Rose, was obtained from California with the assistance of M. Rose, C. Pickett, and D. Kellum. In May-June 1997 about 500 *E. krauteri* were released in the following counties: Seminole, Indian River, St. Lucie, and Volusia. During a survey in late June, 1997, *E. krauteri* pupae were detected at most of the release sites.

Another parasitic wasp, *Encarsiella noyesi* Hayat, was received in Florida from Tom Bellows (Department of Entomology, University of California at Riverside), in June 1998. About 100 *E. noyesi* were released in Volusia and Indian River Counties on June 12, 1998. On July 30, 1998, at the Volusia county location, where *E. noyesi* was released, *E. krauteri*, *E. noyesi*, and *Encarsia* sp., a native parasite in Florida, were detected. The parasite complex population consisted of *E. krauteri* at 66%, *E. noyesi* at 30% and *Encarsia* sp. at 4%.

During June 2001, a survey was conducted at two newly detected infections of giant whitefly in Winter Haven (Polk county). At one location, of 233 parasites collected, *E. noyesi* was dominant (86%) and the remaining 14% were *E. krauteri*. At another location about 10 miles away, of the 177 parasites collected, *E. krauteri* (88.7%) was dominant, and the remaining parasitoids were *E. noyesi*. Thus *E. krauteri* and *E. noyesi* have established and are providing adequate biological control of giant whitefly in Florida.

COMPARISONS OF NATURAL ENEMY AND PHYTOPHAGOUS ARTHROPOD POPULATIONS, DAMAGE, AND YIELD BETWEEN COMMERCIAL PLANTINGS OF STANDARD AND BT-ENHANCED SWEET CORN

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(TITLE ONLY)

A SEARCH FOR BIOLOGICAL CONTROL AGENTS OF *SITONA LEPIDUS* (SYN. *FLAVESCENS*) GYLLENHAL (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT. New Zealand's low-input, pastoral agricultural system is dependent on the nitrogen-fixing capability of white clover (*Trifolium repens* L.), which is worth about NZ$1.5 billion per year. Unfortunately, this system has been jeopardized by the recent colonisation of New Zealand by *Sitona lepidus* (syn. *flavescens*) Gyllenhal (Coleoptera: Curculionidae). This weevil, which occurs throughout North America and Europe, was discovered in two northern New Zealand localities in 1996 and is now distributed throughout the northern half of the North Island. The larvae feed on the rhizobial nodules and roots of clovers, while adults feed on the foliage.

Sitona lepidus appears not to have any important natural enemies in New Zealand, and efforts to find biological control agents in the Northern Hemisphere began in 1998 through collaboration with CABI Bioscience in Switzerland and the University of California, Berkeley, in the United States. The area of exploration was further increased during 1999 and 2001 by collaboration with the U. S. Department of Agriculture, Agricultural Research Service, European Biological Control Laboratory (EBCL) in France, the Institute of Grassland and Environment Research (IGER) in England, and with scientists from several European countries.

Most effort has been devoted to finding natural enemies of the adult stage of *S. lepidus*, and a combination of two search strategies has been used. One has involved collecting adult weevils in the Northern Hemisphere, sending preserved specimens to New Zealand, then dissecting them to ascertain the occurrence of immature parasitoids. The other has been to maintain live weevils in laboratories in Europe (either EBCL, IGER or CABI Bioscience) to rear parasitoids from them. This latter approach has allowed parasitoids to be tested against New Zealand and European *S. lepidus*.

The *S. lepidus* natural enemies found to date include (1) *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae), which appears widely distributed in Europe; (2) *Microsoma exiguum* Meigen (Diptera; Tachinidae), which appears relatively common in Europe, particularly in France and Swit-

zerland; (3) *Allurus* sp. 1 (Hymenoptera: Braconidae) and a *Perilitus* sp. (Hymenoptera: Braconidae), both of which are found in France; (4) *Allurus* sp. 2, of which a single specimen was recovered from *S. lepidus* collected in England; (5) *Beauveria bassiana* (Balsamo) (Deuteromycetes: Hyphomycetidae), a fungal pathogen that appears to be widely distributed in Europe; and (6) an unidentified pathogen of eggs in the calyces of *S. lepidus* females, which was observed in California, Switzerland, and France.

The strain of *M. aethiopoides* already present in New Zealand (introduced in 1982 for biological control of the alfalfa pest *Sitona discoideus* Gyllenhal) seems unable to overcome the immune system of *S. lepidus*. Accordingly, European parasitoids that can parasitize *S. lepidus* have recently been imported into quarantine laboratories in New Zealand for host range testing. Similarly, the European strain of *B. bassiana* is more virulent against *S. lepidus* than the New Zealand strain, and has been imported for possible development as a biopesticide.

EFFECTS OF SNOWDROP LECTIN ON ADULT PARASITIC WASPS

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(TITLE ONLY)

TRACING SHORT-TERM BENEFICIAL INSECT MOVEMENT USING INSECT-BORNE POLLEN

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INTRODUCTION

Australian cotton producers are attempting to reduce their dependence on insecticides through integrated pest management (IPM), including greater use of naturally occurring predatory arthropods. The full benefits of such a system will not be realized until we have more knowledge of the origins of beneficial, predatory insects colonizing cotton.

Knowing the distances beneficial insects and their prey move, the role of noncrop vegetation (weeds and native plants) and the importance of alternative crops, alternative hosts and prey may allow predictions of the occurrence and abundance of generalist predators within cotton fields. Growers will need to know how farming practices (e.g., crop rotations and management options) may change local beneficial insect populations. Here we investigate whether generalist insect predators move

between cotton and surrounding vegetation through a novel application of pollen as a form of predator marking. Pollen has been successfully used as a marker in insect migration studies (reviewed in Hagler and Jackson, 2001), but has not yet been used to assess short-term movement of generalist insect predators.

MATERIALS AND METHODS

Field Survey of Generalist Insect Predators in Cotton

We sampled from vegetation near Boggabri in northern New South Wales, Australia. Sampling was done in and around one cotton field on each of three farms within a 4 km radius of each other. A standardized sampling technique of running the suction sampler across 20 m of vegetation was repeated five times at each site. Each site was sampled approximately every three weeks between November 1998 and March 1999, and between October 1999 and March 2000. Samples were taken from cotton (*Gossypium hirsutum* L.), wheat (*Triticum aestivum* L.), lucerne (*Medicago sativa* L.), sorghum (*Sorghum bicolor* [L.] Moench), lippia (*Phyla nodiflora* [L.] E. Greene), bishop's weed (*Ammi majus* L.), pasture, eucalyptus trees (*Eucalyptus* spp.), sunflowers (*Helianthus annus* L.), and Paterson's Curse (*Echium plantagineum* L.). Sampling stopped each season when the cotton was within a week of defoliation.

The insects that were collected were frozen in the field and transported to the University of New England, where they were sorted to remove the most abundant insects known to prey on pests of cotton. These insects were labelled and refrozen for later scanning electron microscope (SEM) analysis. Six generalist insect predator species were counted: transverse ladybird, *Coccinella transversalis* (Fabricus); minute two-spotted ladybird, *Diomus notescens* (Blackburn); the nabid/damsel bug *Nabis* (*Tropiconabis*) *kinbergii* Reuter; the red and blue beetle, *Dicranolaius bellulus* (Guerin-Meneville); the green lacewing *Mallada signatus* (Schneider); and the brown lacewing *Micromus tasmaniae* (Walker). We censused only adult populations of the target species.

Scanning Electron Microscope Survey of Pollen

Specimens were prepared for SEM by breaking them into 4 or 5 pieces which were fixed to SEM stubs using double-sided poster tape. SEM stubs were placed in a low-temperature oven (40-60 °C) for 12 hours prior to sputter coating. They were then sputter-coated with gold and the external surface of each piece of insect was examined under at least 500x magnification.

Any pollen found was examined under at least 1,000x magnification and identified using Peter Gregg's pollen SEM photographic library (unpublished) and Jones *et al.* (1995). All pollen species found were photographed. As many pollen species as possible were identified to plant family and species, but because of the poor state of knowledge of Australia's pollen flora, many could not be identified and were given voucher numbers and photographed.

Analysis

The number of pollen types per insect was checked for normal variance using Chi Square goodness of fit test ($c^2 = 513.242$, $p = 0.000$) and Shapiro-Wilks test ($W = 2.2848$, $p = 0.0223$). Both tests suggest that the distribution was non-normal, so nonparametric analyses were used. Comparison of the number of pollen species carried per insect was performed by comparing the group using a Kruskal-Wallis multiple comparison test and between pairs of insect species using Wilcoxon Mann-Whitney tests.

RESULTS

A total of 199 insects were examined for pollen under the SEM. Of these, 170 individuals (85%) carried pollen. Of those with pollen, 151 individuals (89%) carried more than one type ("species") of pollen.

Each insect species carried a significantly different mean number of pollen species (Kruskal-Wallis multiple comparison test, $H = 19.27$, $P < 0.005$; Fig. 1). There were considerable overlaps between species, as reflected in Figure 1. In general, the smaller (e.g. minute two-spotted ladybirds), more glabrous insects (e.g., transverse ladybirds) carried fewer species of pollen than the larger, hairier species (e.g., green lacewings).

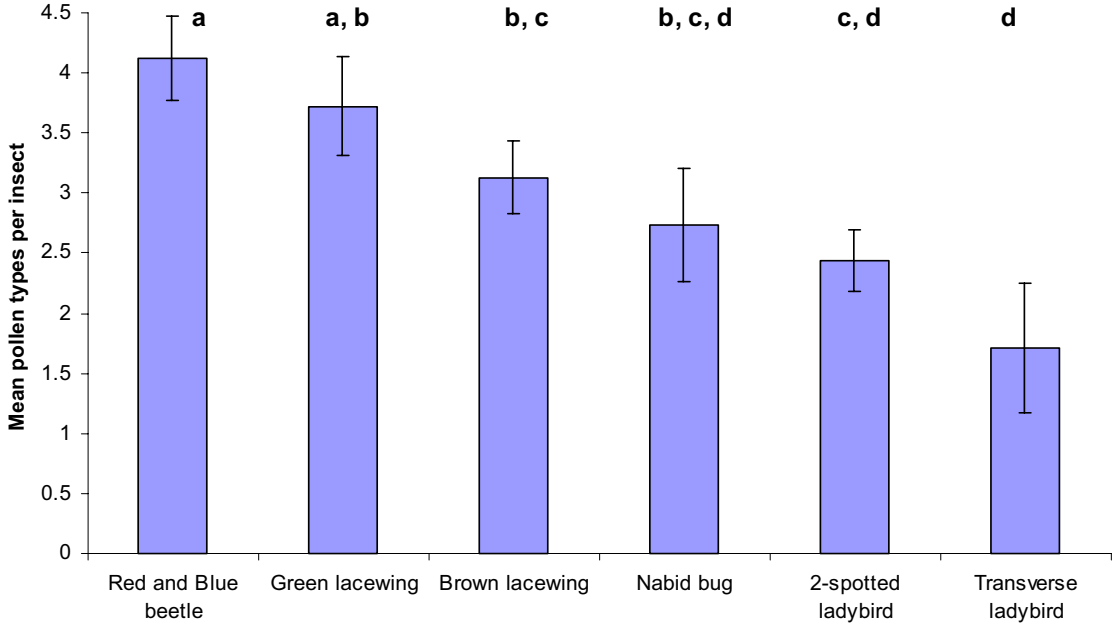

Figure 1. Mean ± standard error number of pollen species found on the exoskeletons of each insect species. The letters above each bar reflect the results of a Kruskal-Wallis comparison. Bars with the same letter are not significantly different.

Some insects also carried spores of a fungus, *Alternaria* sp. We were unable to determine the vegetation type that supported this fungus, but these spores may also be useful markers of movement. The number of pollen species found on insects depended also on the time of year the insects were caught (Figure 2). The presence and variety of flowering species is seasonal, with more species flowering in spring and autumn and fewer flowering in summer.

Pollen on Insects Caught Inside Cotton Fields

We examined 127 insects that were trapped within the cotton fields. Of those, 17 had no pollen on their exoskeleton and most of these were from samples prior to cotton flowering. Of the remaining insects, 110 were carrying pollen. Of those, 78 individuals were caught during the period while cotton was flowering and 46 individuals (59%) carried cotton pollen. During the period that cotton was flowering, 77 individuals (99%) were carrying pollen from sources outside the cotton field. The types of pollen found on those 77 individuals included Bishop's weed (49%), lucerne (9%), sunflower (6%), *Eucalyptus* spp. (28%), Brassicaceae (15%), other Malvaceae (14%), and Paterson's Curse (6%).

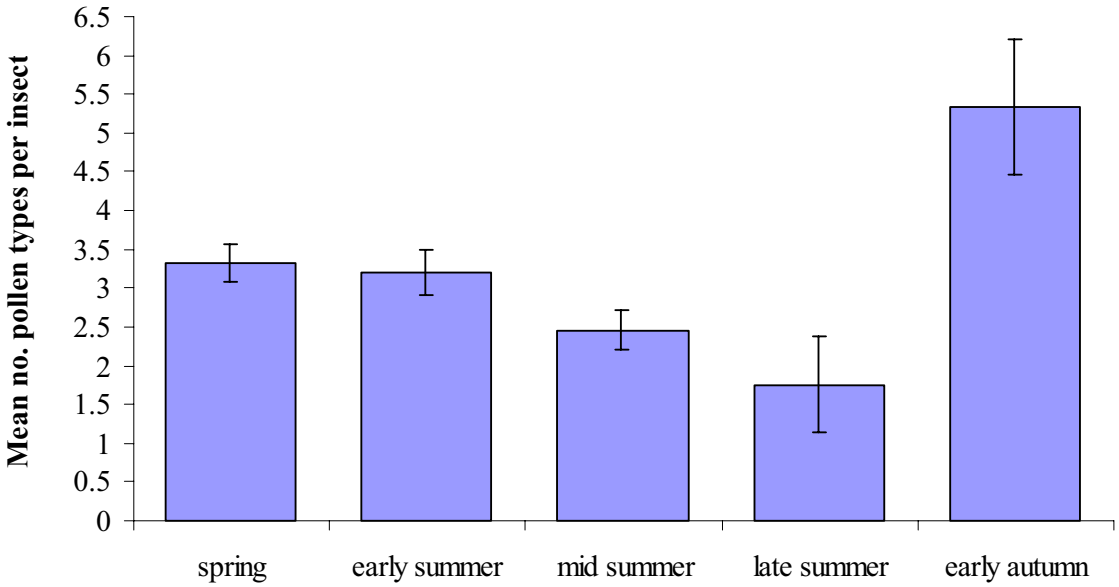

Figure 2. Mean ± standard error number of pollen species per insect caught in different seasons.

Pollen on Insects Caught Outside Cotton Fields

We examined 72 insects caught outside cotton fields. Of those, 60 individuals (83%) carried pollen. Forty one individuals were caught outside cotton fields when cotton was flowering. Of those, 27 individuals (66% of 41) carried cotton pollen. The insects all carried pollen from outside cotton fields including Bishop's weed (54%), lucerne (20%), sunflower (10%), *Eucalyptus* spp. (27%), and Paterson's Curse (7%). With the exception of lucerne, the makeup of pollen on the insects from inside and outside the cotton fields was remarkably similar.

DISCUSSION

In this project we examined the spatial movement of generalist insect predators in a cotton system and surrounding landscape by using pollen to trace short-term insect movement. Examination of the pollen borne on the exoskeletons of generalist insect predators subsampled from field surveys demonstrated that beneficial insects are moving among several types of vegetation. During flowering of cotton, almost all the generalist insect predators caught within cotton fields were carrying pollen from vegetation growing outside the cotton fields. Conversely, more than two thirds of the insects caught outside cotton fields were carrying cotton pollen. All the insect species we examined carried pollen, even those considered not to be regular flower visitors such as nabid bugs, although not all individuals had pollen.

The data from this project suggest that generalist predators, such as ladybirds, lacewings, nabid bugs, and red and blue beetles, visit and move among numerous types of vegetation surrounding cotton fields as well as in the cotton fields. This suggests that any measures to conserve and enhance local populations of beneficial insects must be done on a whole-farm or even an area-wide basis, taking into account crop rotations and land use near cotton fields.

Nabid (damsel) bugs, despite not being considered flower visitors, carried the same mean number of pollen types as flower-visiting species (such as lacewings or ladybirds). Body size, exoskeleton sculpturing and vestiture are probably factors in determining the amount of pollen that adheres to an insect's exoskeleton and, therefore, the number of pollen species retained. For example, transverse ladybird beetles are glabrous compared with other species examined and carried fewer types of pollen.

Pollen on the exoskeletons of beneficial insects is a powerful tool by which to trace short-term insect movement. Pollen marking has several advantages over other marking techniques. Insects are self-marking; pollen is easy to identify to plant family (often to genus or species, particularly in agricultural areas); and pollen is robust enough to withstand most dry collecting techniques. The main disadvantage of the technique is the time and expense required to search insects exoskeletons using SEM.

The findings from this study also have implications for the causes and management of the typical late-summer decline in numbers of beneficial predators (Schellhorn and Silberbauer, 2002) that is commonly associated with the introduction of insecticides damaging to insect predators, such as pyrethroids. Our data suggest that insecticide drift out of the cotton fields may not be the only factor causing the decline in beneficial insect populations during mid-summer (and may not even be the major cause), because beneficial species are likely to eventually encounter a treated field as they move around a district, into and out of insecticide-treated cotton fields. This adds further emphasis to current recommendations on the importance of an area-wide approach to insecticide choice and application method. Learning more about the ways in which beneficial insects use the environment around the cotton fields, particularly other annual crops, contributes a great deal to our ability to manage beneficial insects *in situ* – rather than relying on unpredictable population migrations each season.

ACKNOWLEDGMENTS

We especially thank John and Robyn Watson, Rob Evans and Keith Harris, and Wayne Halder for access to their properties. We also thank Dave Britton, Liza Costa, and Bronwyn Haller for voluntary assistance with field work, Peter Garlick for help with the scanning electron microscopy, Richard Tennant for help sorting specimens in the laboratory, and Nancy Schellhorn for comments on this manuscript.

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POPULATION SYNCHRONIZATION OF *OSTRINIA NUBILALIS* (LEPIDOPTERA: CARAMBIDAE) AND ITS NATURAL ENEMY, *MACROCENTRUS CINGULUM* (HYMENOPTERA: BRACONIDAE)

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INTRODUCTION

Pupation and adult emergence of *Macrocentrus cingulum* Reinhardt are highly synchronized with that of its host, *Ostrinia nubilalis* Hübner (Bruck and Lewis, 1998; Udayagiri *et al*., 1997). However, *M. cingulum* parasitizes late instars, preferably thirds or fourths (Parker, 1931). These host stages are not present until 461 to 767 degree-days after oviposition (Tollefson and Calvin, 1994). Consequently, *M. cingulum* adults must wait 200 to 500 degree days from their emergence before hosts are available for oviposition. Thus, this specialized parasitoid exhibits asynchrony between adult emergence and preferred host availability.

The purpose of this study was to predict the phenology of *M. cingulum* relative to that of third and fourth instars of *O. nubilalis* using host and parasitoid developmental models and known parasitoid adult longevity. As a general rule, parasitoids either have to emerge as adults when preferred hosts are present or live until hosts are available.

MATERIALS AND METHODS

Late instars of *O. nubilalis* were collected from corn in Centre County, Pennsylvania in 1997, 1998, and 2001. Larvae were brought back and kept under laboratory conditions (16:8 L:D and 25 °C) and monitored daily for *O. nubilalis* pupation, eclosion, and death, as well as *M. cingulum* ectoparasitic feeding, pupation, eclosion, brood size and sex. Degree-days were calculated and graphed against percentage completion of a population's entrance into each developmental stage using a base threshold of 12.5 °C. The data were analyzed based on developmental rates established for *O. nubilalis* (Calvin *et al*., 1991). These data were used to construct prediction models for *O. nubilalis* and *M. cingulum* phenological events.

Another model was also constructed using data from adult *M. cingulum* caught on sticky traps and adult *O. nubilalis* caught in flight traps during the 2001 growing season. The timing of *O. nubilalis* entrance into the third and fourth instars was predicted by shifting these curves forward 185 DD and 239 DD, respectively. Fifty percent population death of *M. cingulum* was predicted using a published longevity of 17.6 days (Parker, 1931) and converting to a degree equivalent. This model served as an indicator of the accuracy of the previously mentioned prediction models

RESULTS

Figures 1 through 3 show the results of the prediction models constructed from field-collected *O. nubilalis* larvae that were reared under laboratory conditions in 1997, 1998, and 2001, respectively. The data from these rearing were then pooled to create a fourth model (Fig. 4) displaying phenological events of *O. nubilalis* and *M. cingulum* over the three years that data were collected.

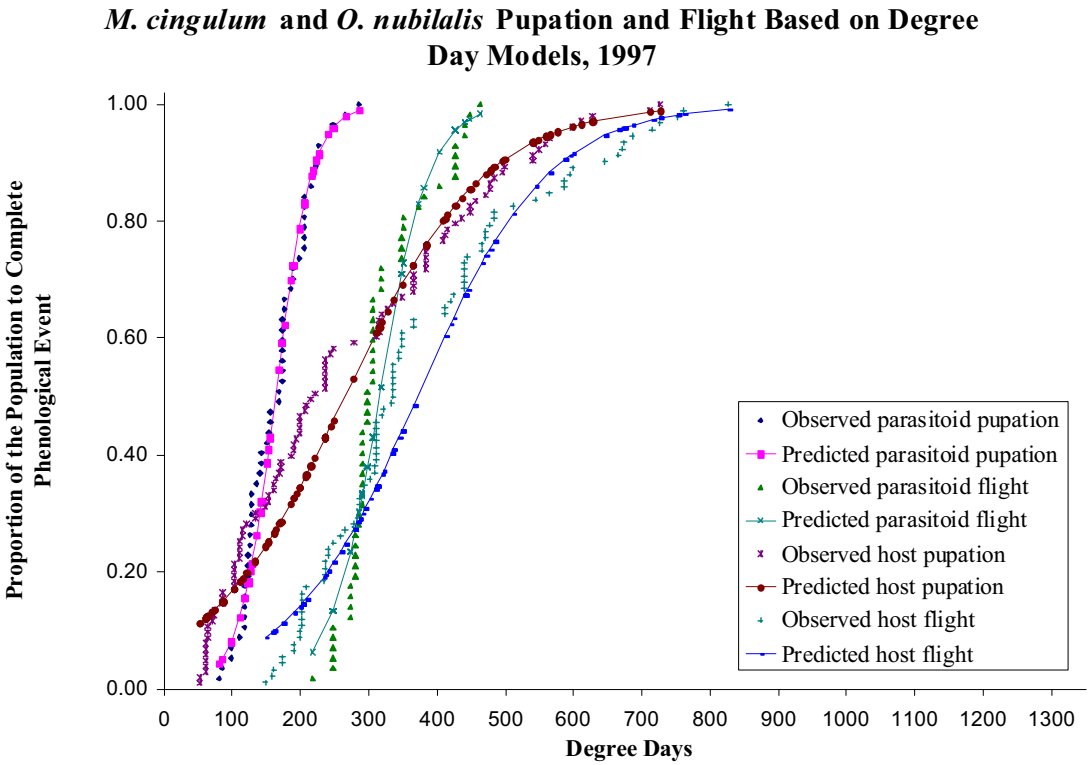

Figure 1. Model predictions of key phenological events of *Ostrinia nubilalis* and *Macrocentrus cingulum*, based on *O. nubilalis* larvae collected from the field in spring 1997 and held under laboratory conditions.

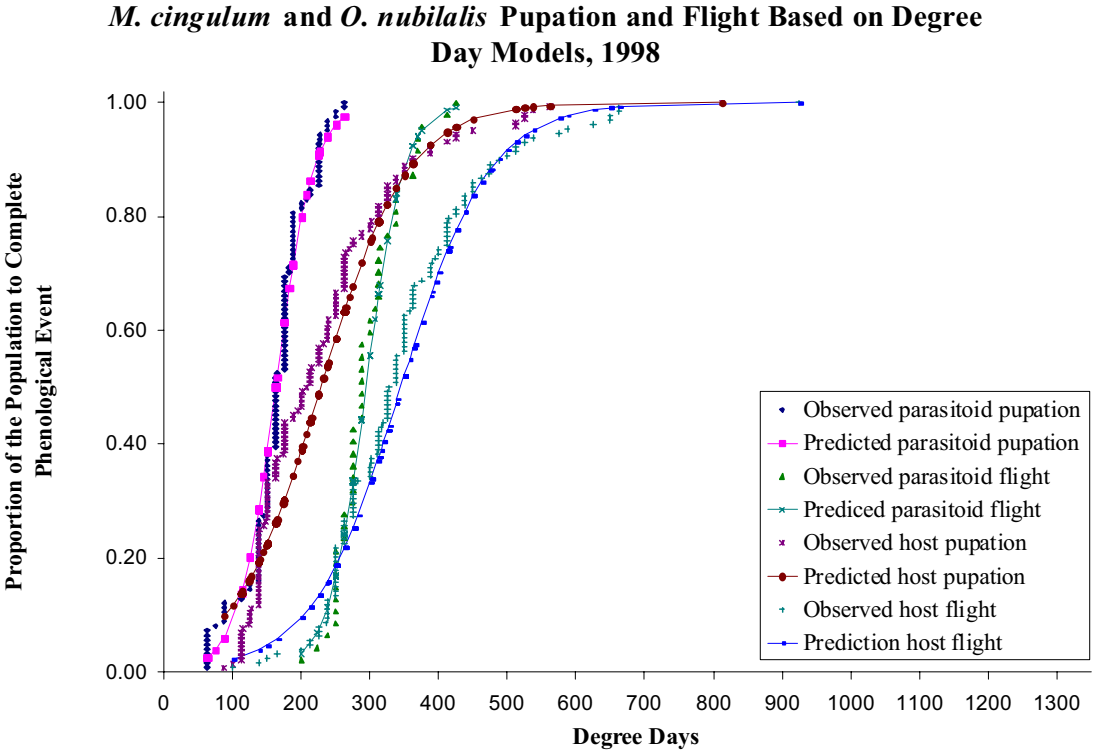

Figure 2. Model predictions of key phenological events of *Ostrinia nubilalis* and *Macrocentrus cingulum*, based on *O. nubilalis* larvae collected from the field in spring 1998 and held under laboratory conditions.

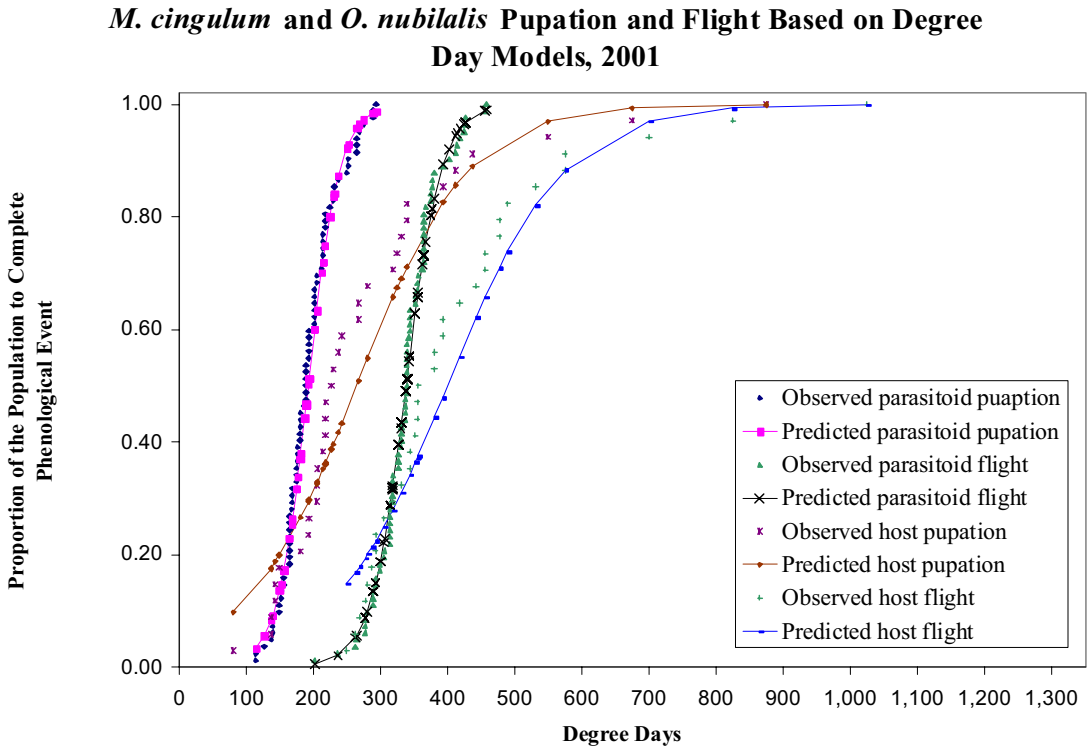

Figure 3. Model predictions of key phenological events of *Ostrinia nubilalis* and *Macrocentrus cingulum,* based on *O. nubilalis* larvae collected from the field in spring 2001 and held under laboratory conditions.

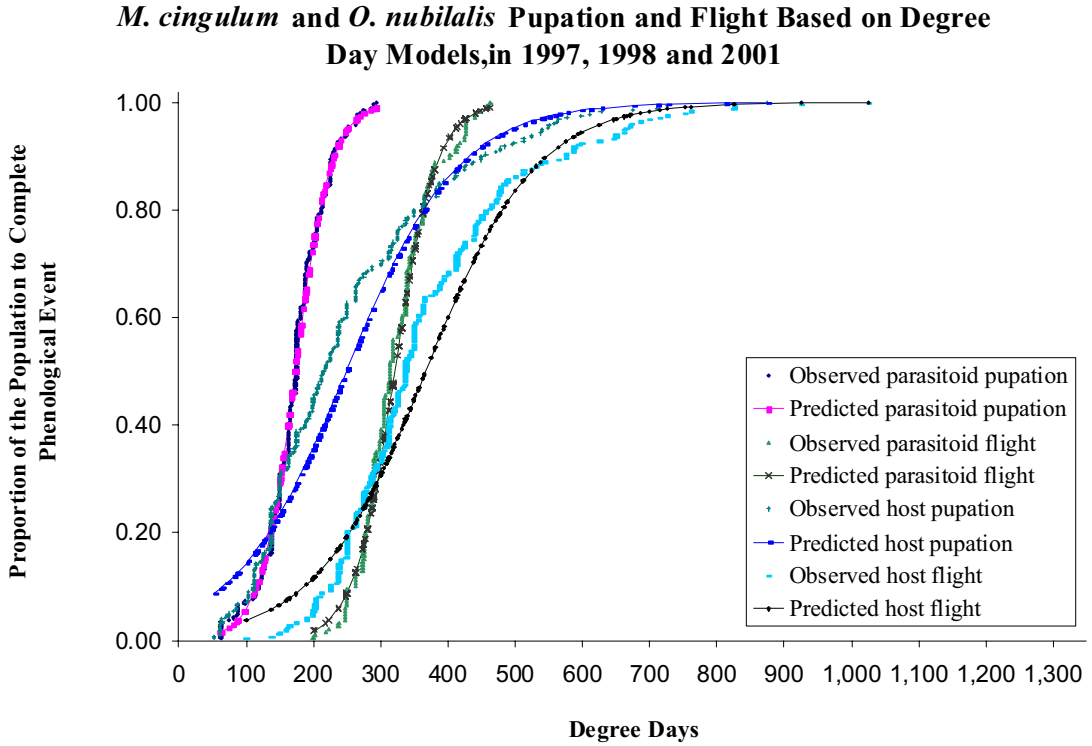

Figure 4. Model predictions of key phenological events of *Ostrinia nubilalis* and *Macrocentrus cingulum,* based on *O. nubilalis* larvae collected from the field in spring 1997, 1998, and 2001 and held under laboratory conditions.

DISCUSSION

The models' predictions (Figs. 1-4) suggest that the majority of the adult *M. cingulum* population is emerging significantly before expected third and fourth instar *O. nubilalis* presence, based on previously documented *O. nubilalis* phenological models (Tollefson and Calvin, 1994). Data collected shows that pupation and adult eclosion are temporally synchronized between host and parasitoid populations. *Macrocentrus cingulum*'s rate of pupation and eclosion completion was faster than that of *O. nubilalis*. However, given that the average life expectancy of an adult is 17.6 days, the majority of *M. cingulum* adults are present during the entire period of preferred host presence (Fig. 5).

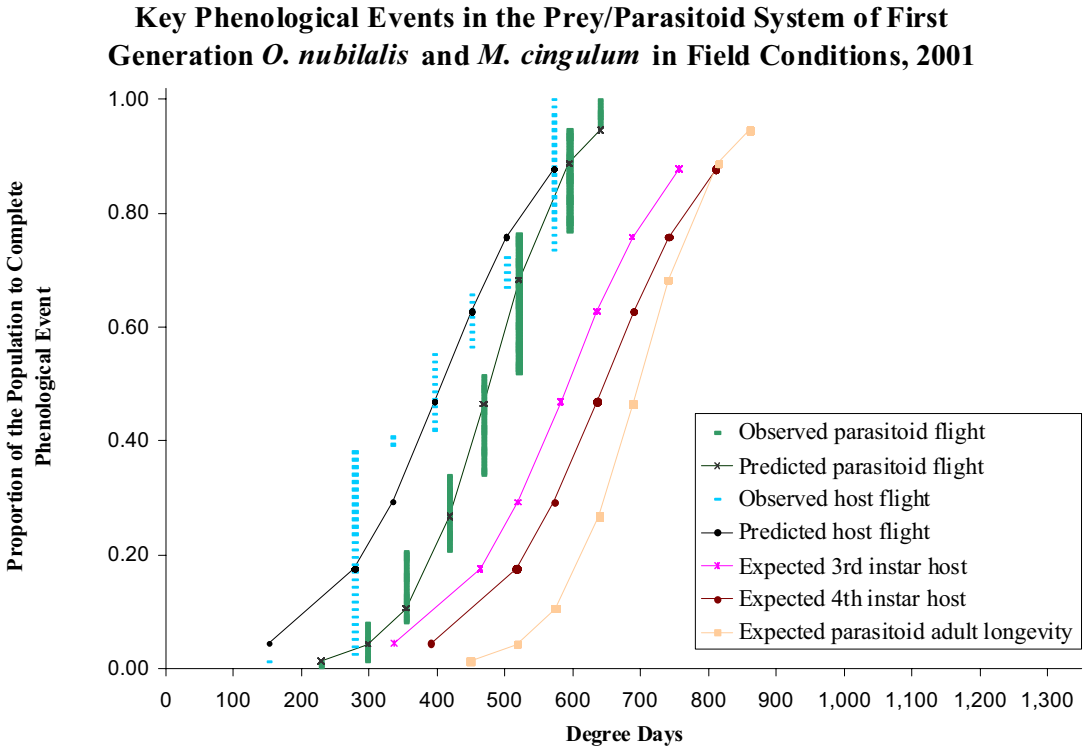

Figure 5. Model for key phenological events of *Ostrinia nubilalis* and *Macrocentrus cingulum,* created from *O. nubilalis* adults caught in flight traps and *M. cingulum* adults caught on Pherecon AM sticky traps in 2001. Third and fourth instar *O. nubilalis* and 50% death curve for *M. cingulum* were extrapolated from flight data based on published expectance of these phenological events.

According to the prediction models and field observations, the preferred hosts of *M. cingulum* are available at the appropriate time. Ongoing field observations confirm the presence of *M. cingulum* during the time that third and fourth instar *O. nubilalis* are present. The viability of *M. cingulum* as an important natural enemy of *O. nubilalis* is dependent on its synchrony with its host's availability. Based on the models' predictions and field observations, the parasitoid is well synchronized with its host.

The data collected from all three years were combined to create a prediction model from several years' data (Fig. 4). In addition, another model was created to show when flight of *O. nubilalis* and *M. cingulum* occurred in the field during the 2001 growing season (Fig. 5). The prediction model created from data collected in 1997, 1998 and 2001 shows that *M. cingulum* adult eclosion occurs between 200 and 440 degree-days above 12.5°C during the growing season. This is comparable to

what was observed in the field during the 2001 growing season. However, a lag time exists between the adult eclosion prediction curves derived from laboratory-reared larvae and the adult eclosion curve derived from field caught adults in flight. Most likely, this lag time can be explained by the methods of collecting data. A period exists between adult eclosion and capture during flight in field conditions. Thus, the model created from field data reflects the prediction models relatively well.

The confirmation of the model generated from field-caught adult hosts and parasitoids allows us to speculate that *M. cingulum* adults wait for their hosts to developed to preferred stages. What the adult parasitoids do during this time is not clear. Females must undergo a three-day preoviposition period after adult eclosion (Parker, 1931). Still, the lag time between parasitoid adult eclosion and preferred host availability is longer than a three-day period. However, the results from this study allow us to understand that *M. cingulum* adults indeed occur while preferred hosts are available. This model can be used for several purposes including scouting and to guide further research.

The use of transgenic Bt-hybrid corn in central Pennsylvania could create adverse effects on this host-parasitoid relationship. Future studies being conducted will address certain issues regarding the direct, indirect and tri-trophic effects of Bt-corn hybrids on the population dynamics of *M. cingulum*. Understanding host-parasitoid synchrony is essential for further studies of direct, indirect and tri-trophic effects that might result from adoption of Bt-corn. For example, if Bt-corn hybrids cause some delay in partially resistant *O. nubilalis*, then sub-lethal effects such as delayed development could hinder the host-parasitoid synchrony documented here. The models produced here will be used in current field studies, which are analyzing these effects. By understanding the above-mentioned interactions between host and parasitoid populations in this tri-trophic system, further analysis of parasitoid population dynamics can be determined. This information can be applied in conservation biological control programs.

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DEVELOPMENT OF A NATIVE PHYTOSEIID, *TYPHLODROMIPS MONTDORENSIS*, AS A COMMERCIAL BIOLOGICAL CONTROL AGENT FOR WESTERN FLOWER THRIPS IN AUSTRALIA

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ABSTRACT. The introduction of western flower thrips, *Frankliniella occidentalis* (Pergande), into Australia in 1993 created the need for an effective biological control agent. The difficulty in importing beneficial species into this country stimulated a search for native natural enemies to develop as commercial biological control agents against this pest.

This process required careful study of biological parameters. For Hemiptera, there can be additional issues to consider, but for others such as phytoseiid mites that are not plant feeders, the following are regarded as important. For the beneficial species to be an effective biological control agent of the target pest, it must be an effective consumer of the target host, it must be able to be reared artificially in large numbers inexpensively, it must have a rate of population increase comparable with or superior to that of the target host, and it must be able to operate effectively in the crop environment for which it is intended. Where export is being considered for the new biological control agent, studies must also be undertaken into factors such as diapause and lower survival and developmental thresholds that are likely to affect its ability to establish in the environment and to survive.

In our project, several phytoseiid mites were selected as having the most potential for success. Investigations were divided into three periods: (1) a survey of diverse, unsprayed habitats in a range of climatic zones throughout Australia to collect and identify phytoseiid mite species associated with thrips; (2) experiments into a range of developmental and reproductive characteristics in relation to temperature, humidity, and day length; and (3) development of crop usage protocols and a mass-rearing system. Throughout these studies we compared the native phytoseiid species against *Neoseiulus cucumeris* (Oudemans), the main phytoseiid species commercially produced overseas for western flower thrips biocontrol in greenhouse crops.

In this poster we briefly describe the process and present some data on the influence of temperature and humidity on development and reproductive activity, and on thrips consumption, for the native phytoseiid species *Typhlodromips montdorensis* (Schicha), known commercially as Montdorensis predatory mite, in Australia.

ANTS AS BIOLOGICAL CONTROL AGENTS OF THE CITRUS ROOT WEEVIL, *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE): DOES THE AGE OF NEONATE WEEVIL LARVAE INFLUENCE PREDATION?

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INTRODUCTION

The citrus root weevil, *Diaprepes abbreviatus* (L.), is an important introduced pest of citrus, ornamentals, and other crops that continues to spread in Florida (Graham *et al*., 1996; McCoy, 1999; McCoy *et al*., 2001). The larvae feed on citrus roots, reduce yield, girdle trees, and facilitate infections by plant pathogens such as *Phytophthora* spp. In citrus, the combination of *Diaprepes* and *Phytophthora* can cause severe tree decline, lead to tree death, and destroy groves within a few years of an initial infestation (Graham *et al*., 1996). In developing an effective IPM program to control *D. abbreviatus*, it would be beneficial to maximize the effectiveness of as many of the natural enemies of this insect as possible. Preliminary research indicates that some of the major mortality agents of *Diaprepes* eggs, larvae, and adults are predators; and that the primary predators are ants (Whitcomb *et al*., 1982; Richman *et al*., 1983; Stuart *et al*., in press).

Florida has a rich and diverse ant fauna (Deyrup and Trager, 1986), and ants can be extremely abundant in Florida citrus groves. Under the proper conditions and with appropriate management, ants could constitute a major source of mortality for *Diaprepes*. A conservation biological control program focusing on appropriate ant species might well be the key to controlling this insect. However, at present, it is unclear which ant species are the most effective predators of *Diaprepes* on the soil surface, in the canopy, and underground, and what strategies might be most effective in promoting and conserving beneficial ant species (Whitcomb *et al*., 1982; Richman *et al*., 1983; Stuart *et al*., in press). Natural variability in the abundance and distribution of ants, combined with the various possible influences of citrus management practices could contribute to considerable variability in predation pressure by ants on *Diaprepes* within and among groves across the state (e.g., see McCoy *et al*., 2001). Our research addresses some of these issues by assessing the role of ants as predators on *Diaprepes* neonate larvae on the soil surface in citrus groves in central Florida. The current study examines one aspect of this predation: the effectiveness of chemical ant repellents that are apparently produced by neonate larvae (Pavis *et al*., 1992).

When *Diaprepes* neonate larvae hatch in the citrus canopy and drop to the soil surface to begin burrowing down to the roots for feeding, they are extremely vulnerable to predation by foraging ants (Whitcomb *et al*., 1982; Richman *et al*., 1983; Stuart *et al*., in press). However, Jaffe *et al*. (1990) observed that first instar *Diaprepes* larvae, although preyed upon, were somewhat repellent to ants of various species, and suggested that they might produce a chemical repellent. Pavis *et al*. (1992) further investigated this chemical repellency with respect to the fire ant *Solenopsis geminata* (F.) and identified two bicyclic sesquiterpene aldehydes that appeared to be responsible for the effect. The concentration of the repellent was highest in newly hatched neonates, decreased quickly with larval age, and was apparently absent from larvae after about four days. Pavis *et al*. (1992) suggested that ant predation on neonate larvae that fell to the soil surface during the first few hours after hatching would

be reduced by about 40% as a result of this chemical repellency. However, field studies indicate that neonates less than 48 h post hatching are highly susceptible to predation by various ant species that are present in the citrus groves of central Florida (Whitcomb *et al*., 1982; Richman *et al*., 1983; Stuart *et al*., in press). The purpose of the present study was to examine the issue of chemical repellency by testing for differential predation by ants on *Diaprepes* neonates of different ages under field conditions.

MATERIALS AND METHODS

Twelve stations were established under the canopy of mature grapefruit trees (one station per tree) at the Citrus Research and Education Center in Lake Alfred, Polk County, Florida. *Diaprepes* egg masses laid between sheets of wax paper were obtained from the U.S. Department of Agriculture rearing facility in Fort Pierce, Florida. Egg masses were maintained at room temperature and allowed to hatch over funnels so that neonates dropping through the funnels could be collected. Neonates were removed from catch containers on a daily basis at 8 AM, and hourly on the day of the experiment. At each station, for each replicate, 20 neonates aged five days post hatching and 20 aged 1-2 h post hatching were placed in separate, paired open plastic dishes (4 mm high x 48 mm diameter), ca. 2-4 cm apart, on the soil surface near the trunk, for 30 min.

A thin layer of sand in the bottom of the dishes effectively discouraged neonates from crawling out of the dish (Richman *et al*., 1983), whereas roughening the outside and inside vertical surfaces of the dishes with sandpaper facilitated the entrance and exit of ants. The number of neonates remaining in the dishes was counted under a microscope at the termination of each replicate. Eight replicates were conducted, with the position of the dishes (i.e., left versus right) being randomized for the first replicate at each station, and then alternated in successive replicates. Observations of predation events, defined as a neonate being removed from an assay dish by a predator, were conducted during the experimental period for all replicates by systematically observing the dishes for 1-2 minute intervals as the test progressed.

A 3-way ANOVA (PROC GLM, SAS Institute Inc., 1990) with two levels for the factor "age" (old and young), two levels for the factor "position" (left and right), and 12 levels for the factor "station" (1-12) was conducted on the percent predation in each dish after arcsin transformation. Means comparisons used the LSMEANS procedure (SAS Institute Inc., 1990). Percent predation on old versus young larvae by various ant species was compared using contingency table analysis and the Chi Square test (PROC FREQ, SAS Institute Inc., 1990).

RESULTS

A total of 2620 of the 3840 larvae (68.2%) were preyed upon, but the predation rate on 5-day old versus 1-2 h old *Diaprepes* neonates showed no significant difference at 68.8% and 67.7%, respectively (ANOVA: $F = 0.44$, $df = 1, 144$, $P = 0.5067$). However, both station and position were significant factors (ANOVA: station, $F = 19.27$, $df = 11, 144$, $P = 0.0001$; position, $F = 5.56$, $df = 1, 144$, $P = 0.0197$), and there was a highly significant interaction between station and position (ANOVA: $F = 6.11$, $df = 11, 144$, $P = 0.0001$). The position of the assay dishes, whether left or right, had a significant impact on the percent predation at five of the 12 stations, and predation was much more intense at some stations than at others (Fig. 1). At one station (No. 11), predation was 100% in both dishes in every replicate.

A total of 368 of the 2,620 predation events (14.0%) were observed directly and involved six species of ants. No other species was observed preying on neonates. *Pheidole moerens* Wheeler and *Solenopsis invicta* Buren were the most active predators and were responsible for 62.5% and 25.3% of

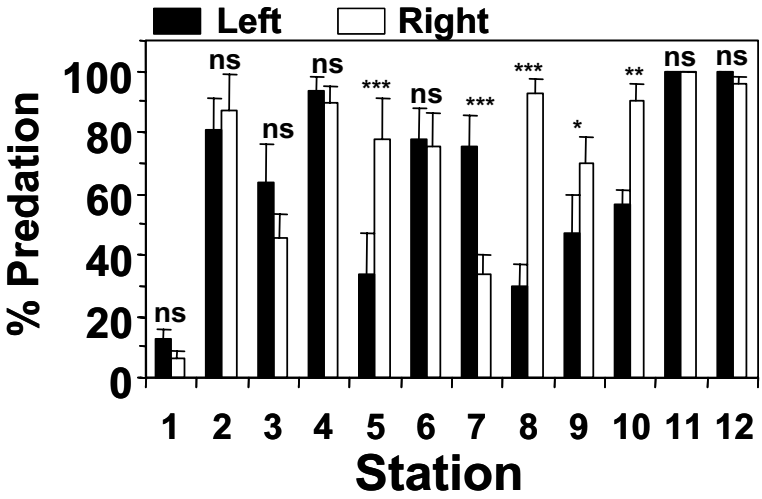

Figure 1. Comparison of percent predation (mean + SE) on *Diaprepes* neonate larvae by ants for different assay-dish positions at different stations. Statistical analysis was by way of ANOVA and LSMEANS: ns, $P > 0.05$; *, $P < 0.05$; ** $P < 0.01$; ***, $P < 0.001$).

these events, respectively. Other ant species and the percent predation observed included *Cardiocondyla emeryi* Forel at 10.1%, *Brachymyrmex obscurior* Forel at 1.4%, *Cardiocondyla wroughtonii* (Forel) at 0.5%, and *Dorymyrmex bureni* (Trager) at 0.3%. A comparison of the number of predation events by *P. moerens*, *S. invicta*, or the other species (pooled) on old versus young larvae revealed no significant differences (2 x 3 contingency table, $X^2 = 4.170$, $df = 2$, $P = 0.124$; Fig. 2). Thus, we found no evidence for species-specific repellency.

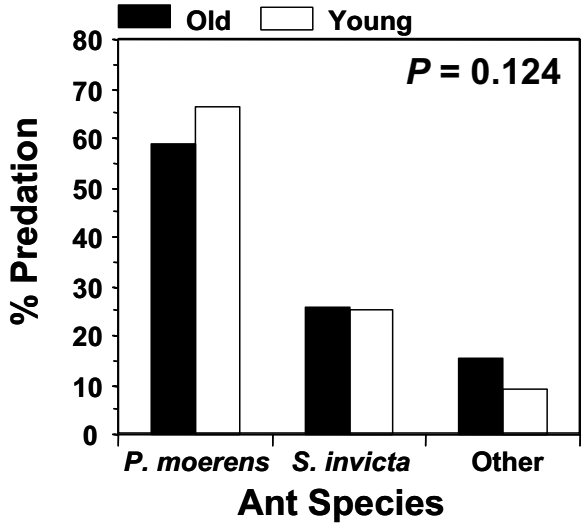

Figure 2. Comparison of percent predation on old versus young *Diaprepes* neonate larvae by *Pheidole moerens* Wheeler, *Solenopsis invicta* Buren, and other ant species (pooled). The *P*-value refers to the results of a 2 x 3 contingency table analysis and the X^2 test.

DISCUSSION

This study indicates that there is no differential predation on 5-day versus 1-2 h old *Diaprepes* neonates by ants in a central Florida citrus grove. Thus, there is no evidence that a quantitative decrease in the chemical ant repellents produced by neonates over this time period as reported by Pavis *et al.* (1992) has any influence on the intensity of predation by this particular ant community. Our results are not necessarily in conflict with those of Pavis *et al.* (1992) since the repellents might be equally effective when present in small or large amounts, or might be totally ineffective against the particular ant species in this study, which differed from the one species in the previous study, *S. geminata*. Furthermore, since Pavis *et al.* (1992) conducted their research on the island of Guadaloupe in the Caribbean, and since the *Diaprepes* population in Florida was introduced, perhaps as a small founder population, it is possible that the weevils in the two studies differ genetically and that the dynamics of the purported chemical ant-repellent system is different as well. Further research is necessary to explore these possibilities.

The results of the present study also show how variable ant predation pressure on *Diaprepes* neonates can be in a Florida citrus grove. Our study detected extensive variation in predation rates among stations placed under a series of grapefruit trees, and detected significant differences in predation rates between paired dishes placed only a few centimeters apart at five of the 12 stations. This kind of variability is probably caused by the distribution of ant nests and foraging activity within the grove. Currently, there is little information on the various factors that influence the distribution, growth, and activity levels of ant colonies of most species in this particular agroecosystem, but such information is necessary if ants are to form the basis for a conservation biological control effort. *S. invicta*, the red imported fire ant, is an exception since many aspects of its biology are well known (Vinson, 1997), but given the many negative impacts that this species has in citrus groves (e.g., causes foliar damage, tends aphids and scales, disrupts harvesting by stinging grove workers; McCoy, 1999), it is unlikely to be a popular candidate for conservation biological control.

As in previous research, this study indicates that ants are very effective and important predators of *Diaprepes* neonates (Whitcomb *et al*., 1982; Richman *et al*., 1983; Stuart *et al*., in press). Thus, ants are likely to be very important factors in the natural biological control of this weevil and have potential as the basis for a conservation biological control program.

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We thank Mark Deyrup (Archbold Biological Station, Lake Placid, Florida) for assistance with ant identification, Karin Crosby (U.S. Department of Agriculture, Agricultural Research Service, Ft. Pierce, Florida) for providing *Diaprepes* egg masses, and the Florida Citrus Research Advisory Council for funding.

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TRICHOGRAMMA CACOECIAE AS A NATURAL OCCURRING EGG PARASITOID OF CHERRY BARK TORTRIX IN THE PACIFIC NORTHWEST (UNITED STATES)

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ABSTRACT. The cherry bark tortrix, *Enarmonia formosana* Scopoli (Lepidoptera: Tortricidae), was first detected in British Columbia, Canada in 1989. Since then it spread southward to Washington in 1991 and was found in Oregon for the first time in 2000. This insect is native to Europe, northern Africa, and western Asia. Since introduction into Canada and the northwestern United States, it has been found on various woody rosaceous plants. Unlike other leaf-rolling tortricids, cherry bark tortrix is a bark feeder. Larvae bore and tunnel under bark. Heavy or repeated infestations can girdle and subsequently kill trees. Some landscape and ornamental cherry trees have been killed as a result of infestations. Because of the importance of woody rosaceous plants to landscape, nursery, and orchard industries, cherry bark tortrix has become a serious threat in the region.

Naturally occurring populations of the egg parasitoid *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae) have been found parasitizing cherry bark tortrix in Bellingham, Anacortes, and Seattle, Washington. In July 2000, 50 parasitized cherry bark tortrix eggs were collected from cherry trees in Anacortes, Washington, and used to establish a colony at Washington State University, Pullman. Mass production of this thelytokous species is feasible. In 2001, field studies were conducted in Seattle to look at the dispersal, parasitism rates, and the overall efficacy of *T. cacoeciae* as a biological control agent for cherry bark tortrix. Our release program was greatly aided by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (APHIS, PPQ) agreement to utilize the APHIS Biological Control Laboratory in Niles, Michigan, to mass-produce *T. cacoeciae*.

In 2001, field experiments in Seattle, Washington, included weekly releases of *T. cacoeciae* to acquire data on their intra- and inter-tree dispersal dynamics. Sentinel traps baited with irradiated eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, were placed at increasing distances from the point of release to measure how far *T. cacoeciae* disperse. Inter-tree dispersal appears to occur less often than intra-tree dispersal, supporting the idea that this parasitoid disperses mostly by walking or hopping and not by flying. Egg parasitism of cherry bark tortrix averaged 55% and reached maximum rates of 95% at certain sites.

ABSENCE OF A SPECIFIC FOOD INTAKE TARGET BY PARASITIZED *MANDUCA SEXTA*: ALTERATION OF A NUTRIENT SPECIALIST TO A NUTRIENT GENERALIST

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ABSTRACT. Last instar *Manduca sexta* (L.) larvae given a choice of two nutritionally extreme defined diets, one with casein but lacking sucrose and a second containing sucrose without casein, selected these diets in a ratio of two parts of the protein diet to one part of the carbohydrate diet. In contrast, parasitized larvae given the same choice consumed the diets in equal amounts. Studies with parasitized larvae reared on individual isocaloric diets with ratios of casein to sucrose varied from 0.25:1.75 to 2.00:0.00, relative to levels of these nutrients in a basal formulation, established that host larvae maintained on the diet with a nutrient ratio of 1:1 supported the greatest parasite burden, in terms of both total parasite biomass as well as numbers of parasites. Nevertheless, choice experiments conducted with isocaloric diets containing both casein and sucrose, but at varying levels, demonstrated the absence of a specific intake target by parasitized larvae. The latter exhibited similar rates of growth on diets varying in ratios of casein to sucrose from 0.125:1.875 to 1.875:0.125, and there was a linear relationship between casein and sucrose intake. In contrast, normal larvae exhibited a nonlinear casein-sucrose intake, and growth was maximal at a casein to sucrose ratio of 1:1. From a nutritional standpoint, normal larvae exhibited the characteristics of specialist feeders, while parasitized larvae behaved like generalist feeders.

AUGMENTATIVE BIOLOGICAL CONTROL OF *THRIPS PALMI* BY THE NONDIAPAUSING ANTHOCORID PREDATOR *WOLLASTONIELLA ROTUNDA* ON GREENHOUSE EGGPLANTS DURING WINTER.

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ABSTRACT. In biological control of greenhouse pests, it is a problem that some species of arthropod natural enemies used for control are inactive during winter because they enter diapause under short-day and low-temperature conditions. This is the case for some anthocorid bugs, predacious mites, and parasitoids in winter greenhouses. Several solutions to this problem have been suggested. One such solution is to use nondiapausing natural enemies from tropical or subtropical regions for the control of greenhouse pests in winter.

Thrips palmi Karny (Thysanoptera: Thripidae) is one of the most serious pests of eggplant both in greenhouses and in open fields in Japan. Populations of *T. palmi* on open-field eggplants have been suppressed by native *Orius* spp., together with selective insecticides. However in winter, *T. palmi* cannot be suppressed by these native predators on greenhouse eggplants, because they enter reproductive diapause under short-day and low-temperature conditions.

Wollastoniella rotunda Yasunaga and Miyamoto (Hemiptera: Anthocoridae), found in Thailand, is a tropical species that preys on thrips, and may be a nondiapausing species. We evaluated the photoperiod effects on diapause induction of *W. rotunda* and the temperature effects on its development and reproduction under conditions similar to those found in winter greenhouses. In laboratory experiments, we found that *W. rotunda* did not enter diapause under 15 ‡and 10:14 L:D photoperiod, and developed and reproduced with high survival when reared on *T. palmi* as prey. Both adults and nymphs of *W. rotunda* were observed attacking *T. palmi* in the field and in the laboratory. Furthermore, *W. rotunda* can be mass-reared with irradiated *Ephestia kuehniella* eggs as prey. Thus, *W. rotunda* has been thought a promising biological control agent against *T. palmi* in winter greenhouses.

We also tested whether *W. rotunda* could control *T. palmi* on caged plants and in a greenhouse crop. The cage experiment showed that *W. rotunda* increased its population on eggplants infested with *T. palmi* in a greenhouse during winter. The cage experiment and the greenhouse release test also showed that augmentation of *W. rotunda* was effective in suppressing *T. palmi* populations in winter greenhouses. From these results, we conclude that *W. rotunda* could be used as a control agent against *T. palmi* on greenhouse eggplants in temperate regions during winter.

ACCEPTANCE OF THRIPS PUPAE AS PREY BY SOIL-LIVING PREDATORY MITES

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(TITLE ONLY)

PACIFIC DAMSEL BUGS–ARE THEY EFFECTIVE PREDATORS IN COTTON?

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ABSTRACT. A thorough understanding of arthropod predator biology and ecology is considered fundamental to evaluate and improve the utilization of these species as biological control agents. For many species, this information is scarce or laboratory based and thus hard to interpret under more realistic field conditions. The effectiveness of the Pacific damsel bug, *Nabis kinbergii* Reuter (Hemiptera: Nabidae), as an arthropod predator in cotton was evaluated in a series of laboratory, greenhouse, and field studies. First, aspects of Pacific damsel bug feeding behavior were studied using cage experiments and direct observations in the greenhouse and field. Second, the temporal and spatial distribution of the Pacific damsel bug and other arthropods was examined in nine commercial cotton fields on the Darling Downs (Queensland, Australia) during the 2000-2001 growing season.

The aim of the study was to provide qualitative and quantitative evidence of (a) Pacific damsel bug prey range and feeding rates under realistic conditions, (b) the influence of varying management strategies such as insecticide application and nursery crops, and (c) the effect of biotic and environmental factors on Pacific damsel bug distribution and abundance.

IMPROVED PRODUCTION AND EFFICACY OF BIOLOGICAL CONTROL AGENTS

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INTRODUCTION

In order to optimize the use of biological control agents in augmentative or inundative releases, it is necessary to have adequate numbers of high quality organisms that are well suited for the environment into which they will be released. For predators and parasites, this requires development of cost-effective rearing methods, knowledge about the induction and termination of diapause, and identification of resistance or susceptibility of these organisms to chemical insecticides that may be present in the agricultural system. For pathogens such as baculoviruses, this requires knowledge of the host range, development of cost-effective propagation systems, protection from ultraviolet light, improvement in the rate of kill of pest insects, and knowledge about the potential for development of resistance to these pathogens. Research at the U.S. Department of Agriculture, Agricultural Research Service, Biological Control of Insects Research Laboratory (BCIRL) in Columbia, Missouri, addresses these issues.

IMPROVED REARING TECHNIQUES FOR BENEFICIAL INSECTS USED IN BIOLOGICAL CONTROL OF INSECT AND WEED PESTS

The use of biological control in biologically based IPM systems often requires augmentation or conservation of natural enemies, particularly when chemical insecticides are also used. Information about the susceptibility of these insects to pesticides and their potential to develop resistance to commonly applied insecticides is necessary in order to optimize the effectiveness of the natural enemies already in the field or those populations increased through augmentative releases. In addition, information about diapause in these insects is needed in order to plan the appropriate timing of their release and of chemical applications. In order to supply the number of insects needed for traditional or augmentative releases, adequate numbers must be available as needed, through mass rearing and long-term storage strategies. Current methods for mass rearing of many insect predators and parasitoids is primarily by *in vivo* methods, which are laborious and inefficient, such that development of *in vitro* techniques is needed for production of economical and consistent populations of natural enemies for biological control or IPM programs.

In order to address these issues, research was undertaken to optimize the production, quality, and effectiveness of mass-reared natural enemies (insects) for the integrated pest management of insect and weed pests by: (1) optimizing artificial diets and other rearing techniques; (2) developing methodologies for induction and termination of diapause; and (3) identifying susceptibility and potential for resistance to insecticides. Diagnostic techniques were developed to monitor quality, fecundity, diapause, insecticide resistance, or stress associated with these methodologies.

Nutrition and Reproduction

The goals of this project were to develop a high-performance diet to support the cost-effective mass rearing of insect predators, to maximize performance on a diet by optimizing developmental and

reproductive parameters, and to develop diagnostic techniques for rapid quality-control analysis of mass-reared insects.

An insect-free artificial diet was developed for the spined soldier bug, *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae), and life history parameters were determined for insects collected from the field and those from a laboratory colony that had been in culture for over 20 years. Insects developed more slowly and laid fewer eggs when reared on this diet, but showed improvement in successive generations. The rearing cost of the diet-fed wild colony was comparable to that of the prey-fed laboratory colony. In contrast, the rearing cost of the diet-fed laboratory colony started out high (F1 generation), then decreased with successive generations. Levels of the egg protein vitellogenin were monitored in hemolymph and ovary samples from adult females using Western blot analysis in order to compare the physiological state of insects reared on prey and diet. Fluctuations in the amounts of vitellogenin in tissues of insects corresponded to changes in the dietary regime and indicated that the quality of the diet during both the nymphal and adult stages affected the fecundity of *P. maculiventris*.

Development and fecundity of *P. maculiventris* reared on the BCIRL artificial diet exceeded the performance of this insect on all other reported diets (Wittmeyer and Coudron, 2001; Wittmeyer *et al.,* 2001). Additionally, the BCIRL diet has been demonstrated to be a cost-effective alternative to natural prey for the rearing of *P. maculiventris*. These results demonstrate the practical value of using an artificial diet when rearing entomophagous insects. The universal application of this diet has been further confirmed by demonstrating its use to rear other beneficial predatory insects, as well as pest insect species. This diet has been used successfully to rear from egg to egg-laying adults, *P. maculiventris*, *Perillus bioculatus* (Fabricius) (Heteroptera: Pentatomidae), the big-eyed bug *Geocoris punctipes* (Say), the lady beetle *Coleomegilla maculata* (DeGeer), and the green lacewing *Chrysopa carnea* Stephens, as well as the green stink bugs *Acrostermum hilare* (Say), and *Thayanat custator accerra* McAtee and the one-spotted stink bug, *Euschistus variolarius* (Palisot de Beauvois). Diagnostic methods that detect early responses of insects to the quality of their diet will assist in the advancement of research in insect nutrition and reproduction.

Diapause

The goals of this research were to identify suitable environmental regimes in order to develop long-term storage of insects based on manipulation of diapause and to identify hormones, proteins, enzymes, and metabolites to monitor progress and aid in developing *in vitro* rearing techniques.

An assay for synthesis of juvenile-hormone-like products by the corpora allata was developed for *P. bioculatus.* Synthesis of these products was approximately equal under diapause-inducing short-day conditions and nondiapause (long-day) conditions. However, the ability of this synthesis to be stimulated by t,t-farnesol, a metabolic precursor of juvenile hormone, was greatly reduced during diapause. In addition, production of viable eggs dropped to zero during diapause, and respiration as measured by CO2 release decreased under SD conditions. This information will be used to further optimize protocols for induction and termination of diapause as a storage method for insects used in biological control and to identify techniques that are diagnostic of their metabolic state.

Pesticide Resistance

The goals of this research were to determine the susceptibility of *P. maculiventris* to commonly applied insecticides, to assess the effects of the newer biorational and broad spectrum insecticides in order to guide IPM decisions, and to determine the probability of emergence of resistance and the mechanism of resistance to these insecticides.

Second instar *P. maculiventris* nymphs were treated with several commonly used pesticides, and mortality was determined over multiple generations. Field collected *P. maculiventris* were highly susceptible to imidacloprid, fipronil, and to the pyrethroids cyfluthrin and »-cyhalothrin. Field collected animals exhibited high, preexisting resistance to the biorational insecticides spinosad, indoxacarb, tebufenozide, and methoxyfenozide, indicating compatibility with existing IPM practices. Stringent laboratory selection resulted in heritable resistance to imidacloprid, but susceptibility to imidacloprid was restored by the synergist piperonyl butoxide. Seven generations of laboratory selection increased resistance to cyfluthrin and »-cyhalothrin, but susceptibility was restored by application of the synergist piperonyl butoxide. Resistance to cyfluthrin conferred cross-resistance to »-cyhalothrin and also to bifenthrin. Selection over eight generations did not increase resistance to fipronil, and analysis of field collected insects for fipronil susceptibility or resistance failed to show any resistance alleles in these populations.

ENHANCEMENT OF MICROBIAL AGENTS

Without substantial insecticide use, significant crop losses in cotton and corn result from insects belonging to the *Heliothis/Helicoverpa* complex. Because of increased resistance of these insects to insecticides and the negative environmental impacts of traditional chemical insect control, alternatives to these methods are needed. One demonstrated effective technology is the use of insect baculoviruses, which are cost-effective and can be used with current methods of application. However, the use of baculoviruses for biological control is limited by the time required for activity, environmental instability, host insect specificity, lack of information on timing of application, and information on whether resistance to recombinant baculoviruses will develop. In order to address these issues, research was undertaken (1) to genetically modify baculoviruses to increase *in vivo* activity and UV stability and persistence, and (2) to determine the potential for development of resistance of *Heliothis/Helicoverpa* spp. to wild-type and recombinant baculoviruses.

Enhancement of *in Vivo* and *in Vitro* Activity of Baculoviruses

The goals of this project were to generate new recombinant baculoviruses with quicker kill, sunlight-UV stability and longer environmental persistence, to engineer genes (e.g., toxins, anti-feedants) into single or multiple sites in the genome of selected baculoviruses, and to evaluate recombinants *in vivo* and *in vitro*.

One example of this research was the isolation (U.S. Patent 6,042,843) of a baculovirus (PxMNPV) from the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Kariuki and McIntosh, 1999), and characterization of its *in vivo* and *in vitro* activity. PxMNPV was the most effective baculovirus against DBM in comparison to the baculoviruses AcMNPV and AfMNPV, and was also infectious to six other species. PxMNPV replicated in six lepidopteran cell lines, with BCIRL-HvAM1 producing the greatest number of occlusion bodies and the highest viral titer of extracellular virus. Restriction endonuclease profiles of PxMNPV showed similarities to two other wide host-range baculoviruses, AcMNPV and AfMNPV, but could be readily distinguished from these two. PxMNPV has a similar *in vitro* and *in vivo* host range to AcMNPV and AfMNPV for the lepidopteran species under study and was the most effective against DBM larvae.

Baculovirus Resistance

The goals of this project were to determine if pest insects can develop resistance to either wild-type or recombinant baculoviruses under laboratory conditions and, if so, to elucidate the mechanism(s) of resistance on cellular, physiological and behavioral levels.

Heliothis virescens larvae were selected for resistance to wild-type (wtHzSNPV) and recombinant (recHzlqh, which expresses the lqh neurotoxin) baculoviruses over numerous generations. Data did not, in general, indicate that resistance to the viruses had developed after six to seven generations. In *in vitro* studies, later passages of the *H. virescens* cell line, BCIRL-HvAM1, were permissive to infection by both baculoviruses, whereas earlier passages were relatively refractory to the viruses. This observation is unusual, with the opposite frequently being observed (i.e., later passages becoming less permissive than earlier passages). Additionally, these refractory cells from earlier passages were challenged with the viruses over numerous subsequent passages with the goal of increasing their resistance to the baculoviruses, which initially appeared to be achieved, but was later shown to result in the production of persistently infected cells.

These results indicate that *H. virescens* did not become resistant to HzSNPV under laboratory conditions after six to seven generations, suggesting that the development of resistance to this virus by this insect species may not be a concern in the use of this virus in the field, although these experiments will be repeated for validation. Additionally, an *H. virescens* cell line was found to have subpopulations of cells (at earlier passages) that were less permissive to baculovirus infections than other subpopulations (at later passages) and, therefore, could be used in comparative studies to elucidate the cellular mechanism(s) of resistance. Finally, two persistently infected cell lines were developed which may aid in the understanding of persistent baculovirus infections.

DISCUSSION

The use of biological control is an attractive alternative to the use of chemical insecticides, particularly in greenhouse or organic production systems, or as a component of biologically based integrated pest management systems. The improved production and efficacy of pathogens and predators used in biological control will increase the availability and cost effectiveness of these agents and will facilitate the development of sustainable pest management systems that integrate the use of natural enemies with other practices. In addition, increased implementation of biological control tactics will reduce the need for chemical inputs and will support precision agricultural practices, while reducing contamination of air, soil and groundwater by chemical compounds.

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EVALUATING EFFECTIVENESS OF MASS RELEASES OF THE VINE MEALYBUG (*PLANOCOCCUS FICUS*) PARASITOID *COCCIDOXENOIDES PEREGRINUS* IN WESTERN CAPE PROVINCE VINEYARDS, SOUTH AFRICA

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INTRODUCTION

Chemical applications are currently used to control *P. ficus*. Several attempts of classical biological control have been made with importation and release of *Chrysoplatycerus splendens* Howard (Joubert, 1943), *Cryptolaemus montrouzieri* Mulsant (Greathead *et al*., 1971), *Scymnus guttulatus* (Le Conte) and *Scymnus sordidus* (Horn) (Joubert, 1943), *Pseudaphycus angelicus* (Howard), and *Anagyrus pseudococci* (Girault) from Israel (Urban, 1985). In a survey of natural enemies associated with vine mealybug (Urban, 1985), it was found that the parasitoids *Coccidoxenoides peregrinus* (Timberlake), *Anagyrus* spp. and *Leptomastix dactylopii* (Howard) and predatory beetles in the genus *Nephus* were the dominant natural enemies. In addition, it was found that these parasitoids played an important role in biological control of *P. ficus*. However, the level of biological control was not sufficient to keep *P. ficus* infestations below economically acceptable levels.

Biological control by mass releases of natural enemies has contributed to the control of several pseudococcid pests (Mineo and Viggiani, 1977; Longo and Benfatto, 1982; Summy *et al*., 1986; Smith *et al*., 1988; Nagarkatti *et al*., 1992; Smith, 1991; Reddy and Bhat, 1993; Smith *et al.*, 1996; Fronteddu, 1996; Raciti *et al.*, 1997). *Coccidoxenoides peregrinus* is a parasitoid of *P. ficus* (Trjapitsin, 1989), but no reference could be found on biological control of *P. ficus* by mass releases of this parasitoid. However, *P. citri* has been successfully controlled using mass releases of *C. peregrinus* on citrus (Hattingh *et al*., 1999). This study was conducted to investigate the effectiveness of mass releases of *C. peregrinus* as an alternative to chemical control of *P. ficus*.

MATERIALS AND METHODS

The nine experimental vineyard locations were divided equally among one table grape area (the Hex River Valley) and two wine grape areas (Stellenbosch and Robertson), all in South Africa. Each vineyard consisted of a release block (1 ha), an adjacent buffer block (1 ha), and a control block (1 ha) adjacent to the buffer block. No pesticide treatments were allowed in the release blocks, except for ant control. Stem barrier treatments of alpha-cypermethrin SC at 20 ml/liter (Table 1) were used for this purpose. All vines and trellis systems were treated with 50 ml of this pesticide (Ueckermann, 1998). Dormant IPM-compatible ant and mealybug treatments (100-200 ml/liter chlorpyrifos two weeks apart before bud burst) were applied in the buffer and control areas (Table 1). These treatments were applied before the first parasitoid releases. The normal fungicide treatments were applied in all blocks.

Table 1. Insecticide treatments applied in the nine trial sites

Management Practices	Mealybug Management Programs, by Plot Type		
	Parasitoid Release Areas	Buffer Zones	Control Plots
Chemicals used	No insecticide applications	Dursban EC (chlorpyrifos) 100-200 ml.L	Dursban EC (chlorpyrifos) 100-200 ml.L
Time of treatment		2 weeks before bud burst (September)	2 weeks before bud burst (September)
Method of treatment		broadcast spray of bare vines	
	Ant Management Programs, by Plot Type		
	Parasitoid Release Areas	Buffer Zones	Control Plots
Chemicals used	Fastac (Alpha-cypermethrin) SC at 20 ml/L	Fastac (Alpha-cypermethrin) SC at 20 ml/L	Fastac (Alpha-cypermethrin) SC at 20 ml/L
Time of treatment	Early season (October)	Early season (October)	Early season (October)
Method of treatment	applied to form a stem barrier	applied to form a stem barrier	applied to form a stem barrier

Parasitoids were reared and placed in the field in paper bags by stapling one bag in the crown of the vine. Twenty bags were spaced evenly in an experimental block. Six releases were made at monthly intervals starting in November. Release of ± 20,000 parasitoids each were made at all sites on November 11 and December 7, 1999; on January 4, February 3, March 8, April 6, November 1, and December 27, 2000; and January 31, February 28, and March 30, 2001.

Evaluation of Parasitoid Releases

Effectiveness of released parasitoids was evaluated by determining levels of vine mealybug stem infestation, crop loss due to vine mealybug, monthly *C. peregrinus* counts on yellow sticky traps, and monthly percentage parasitism of mealybugs on infested pumpkins deployed in vineyards as baits.

Stem infestation levels. Sampling in the blocks was done in 20 evenly spaced plots, each consisting of five vines. A central systematic sampling system was used. The lateral branches of each of these vines were inspected for *P. ficus* in the area closest to the main stem (up to 20 cm from the main stem) where new growth occurred. One basal leaf in the same area was inspected for mealybugs on the same vine. All bunches on the fifth vine in each of these plots were inspected for the presence of *P. ficus*. The proportion of each infested plant part (lateral branches, leaves and bunches) was recorded in each block. Therefore, in each plot, five vines, five leaves, and all bunches on the fifth vine were classified as infested or uninfested. Sampling was conducted throughout the year for two seasons at intervals of one to four weeks depending on the time of year.

Infestation at harvest (crop loss). The three assessments of bunch infestation closest to harvest were summed and averaged as an estimate of crop loss for the season.

Yellow sticky traps. Yellow sticky traps were used to sample adult *C. peregrinus*. Two sticky traps were used, one on the edge and one in the middle of each trial block. These were left in the field for one month, after which they were replaced.

Percentage parasitism. Sampling natural enemies was done on a monthly basis using two mealybug-infested butternut squash, one placed on the edge and one in the middle of each trial block. Each squash bore at least 100 mealybugs at various stages of development. Squash were placed in polystyrene containers with entry holes smeared with petroleum jelly which effectively excluded ants. After exposure in the field, squash were taken to the laboratory and placed in emergence cages and held for one and two months, after which emerged natural enemies were identified and counted. Accuracy of the calculation of percent parasitism was compromised, but this method enabled the identification of natural enemies. Parasitoid identification was done by G. Prinsloo at the Plant Protection Research Institute in Pretoria.

After this period at least 100 mealybugs or mummies were randomly selected on each of the butternut squash samples. Parasitism was determined by looking for mealybugs with emergence holes (mummies) and dissecting the remainder of mealybugs (looking for the remaining live immature parasitoid stages). No eggs and newly emerged crawler stages were used in the determination of percentage parasitism. No counts were made of small hosts that died during the rearing. Percent parasitism (%PA) was estimated using the following formula (Van Driesche, 1983):

$$\%PA = \frac{EMP + LP}{EMP + LP + UMH}$$

where EMP = emerged parasitoid species, LP = all live parasitoid stages, and UMH = unparasitized mealybug hosts.

ANALYSIS OF DATA

Data from stem infestation, percentage parasitism, and trap catches were transformed by averaging data from two consecutive sampling dates and multiplying by the number of days between these dates. The resultant figures from these were summed to give the total number of insect days (Ruppel 1985). Insect days represented the area under each of the data curves. These data were used in a split plot analysis with the three areas as the main plots and treatments and years as the main effects in the sub-plots.

Data pertaining to percentage crop loss were analysed in the same way.

RESULTS

Stem Infestation

Stem infestation by *P. ficus* was lower in the Hex River Valley than in Stellenbosch and Robertson ($P < 0.01$) (Fig. 1).

Infestation at Harvest (Crop Loss)

There were significant differences in *P. ficus* bunch infestations ($P< 0.01$) (Table 2) between treatments. There were also differences between areas with less crop loss due to *P. ficus* infestations in the Hex River Valley than in Stellenbosch and Robertson ($P < 0.01$) (Fig. 1; Table 2). There were also interactions between areas and treatments ($P < 0.01$) with less crop loss due to *P. ficus* infestations in the Hex River Valley than in Stellenbosch and Robertson (Table 4).

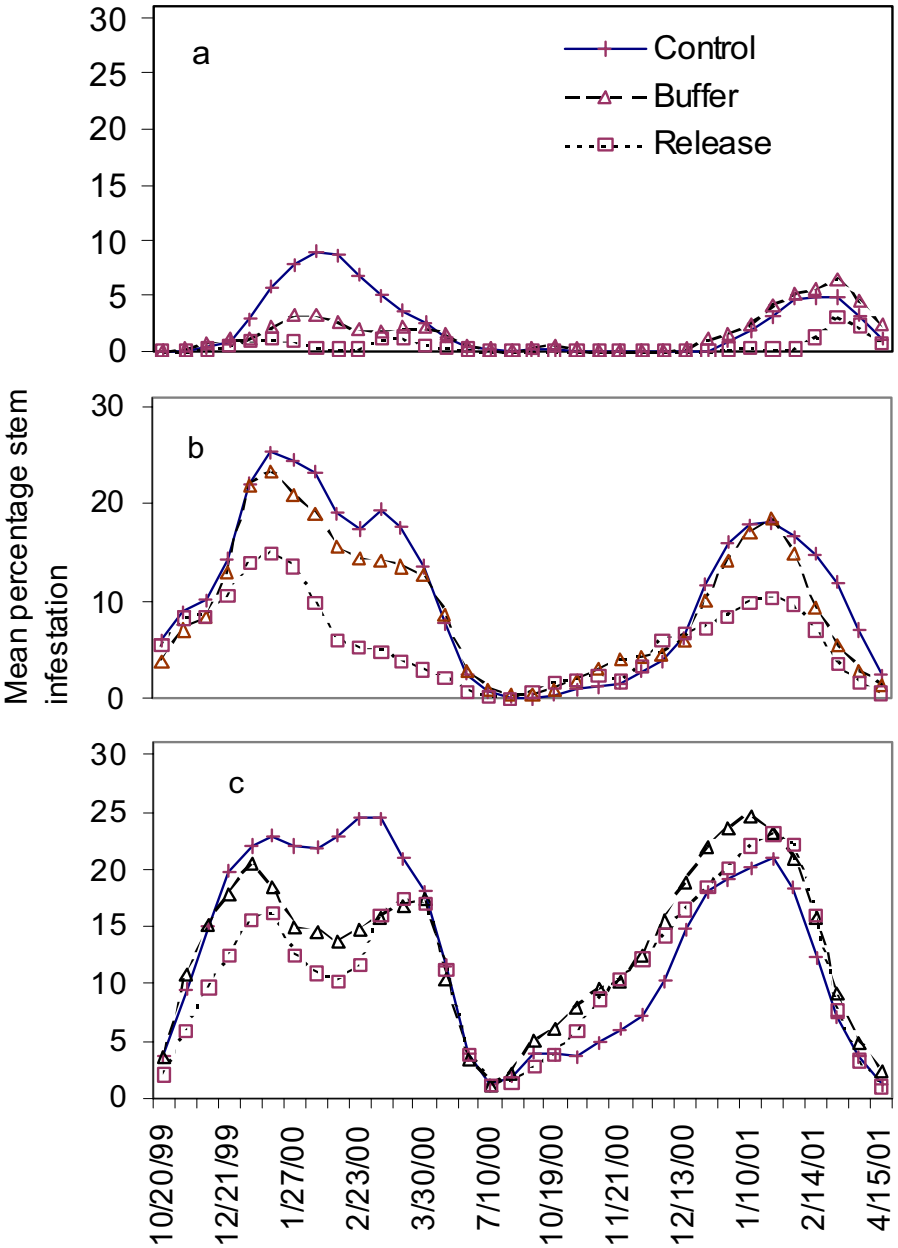

Figure 1. Average stem infestations by *Planococcus ficus* during two seasons in blocks into which *Coccidoxenoides peregrinus* was released and in buffer and control blocks in three vineyards in the Hex River Valley (a); Stellenbosch (b); Robertson (c).

Yellow Sticky Traps

There was no significant difference in the number of *C. peregrinus* caught on yellow sticky traps between the three areas ($P = 0.21$) or between the treatments ($P = 0.19$). There were differences between seasons ($P < 0.01$). More parasitoids were caught on the yellow sticky traps during the second season than during the first (Table 3, Fig. 2). The differences were not as marked in the Hex River Valley as in the Stellenbosch and Robertson areas. This discrepancy resulted in interactions between area and season ($P<0.01$) (Table 3, Fig. 2).

Table 2. Mean percentage crop loss in release, buffer and control vineyards due to vine mealybug (*Planococcus ficus*) infestation at harvest in three grape growing areas during two seasons

Grape growing area	Mean % crop loss					
	Control		Buffer		Release	
	1999-2000	2000-2001	1999-2000	2000-2001	1999-2000	2000-2001
Hex River	2.3	0.03	1.11	0.03	0.05	0
Stellenbosch	8.6	7.3	4.05	3.9	6.5	5.8
Robertson	8.5	8.2	8.01	8.5	6.9	8.3
Average	6.47	5.18	4.39	4.14	4.48	4.7

Table 3 Cumulative yellow sticky trap counts of *Coccidoxenoides peregrinus* starting from September and ending in April during 1999-2000 and 2000-2001

Area	Control		Buffer		Release	
Season	1999-2000	2000-2001	1999-2000	2000-2001	1999-2000	2000-2001
Hex River	22	31	51	63	44	76
Robertson	1	136	18	264	18	332
Stellenbosch	55	50	84	169	93	179
Total	78	217	153	496	115	587

Table 4. Average percentage *Planococcus* ficus parasitism for the control, buffer and release treatments during the two seasons

Area	Control		Buffer		Release	
Season	1999-2000	2000-2001	1999-2000	2000-2001	1999-2000	2000-2001
Hex River	35.4	25.4	42.4	32	44.3	32.7
Robertson	28.7	19.2	37.2	31.2	37.6	32.7
Stellenbosch	20.7	22.6	25.2	37.9	26.2	38.4
Total average	28.3	22.4	34.9	33.7	36	34.6

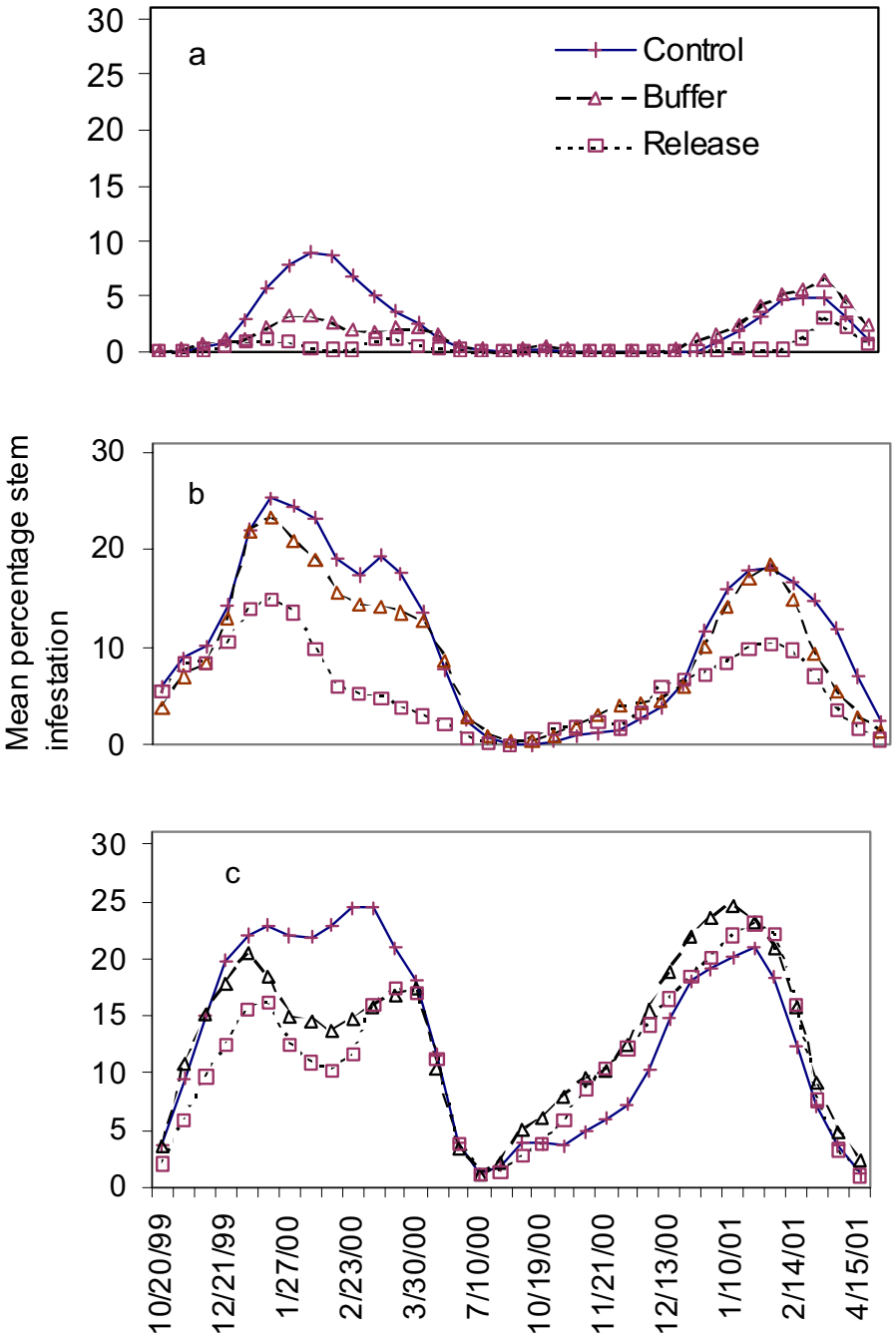

Figure 2. Average number of *Coccidoxenoides peregrinus* caught on yellow sticky traps during two seasons in blocks in which *C. peregrinus* was released and buffer and control blocks in three vineyards in (a), Hex River Valley; (b), Stellenbosch; (c), Robertson. Arrows indicate the release of 20000 *C. peregrinus*/ha.

Percentage parasitism

There were no differences in percent parasitism between areas ($P = 0.55$) or treatments ($P = 0.35$). There was a difference between years ($P < 0.01$), with a slightly higher percent parasitism during the first season than during the second (Fig. 3).

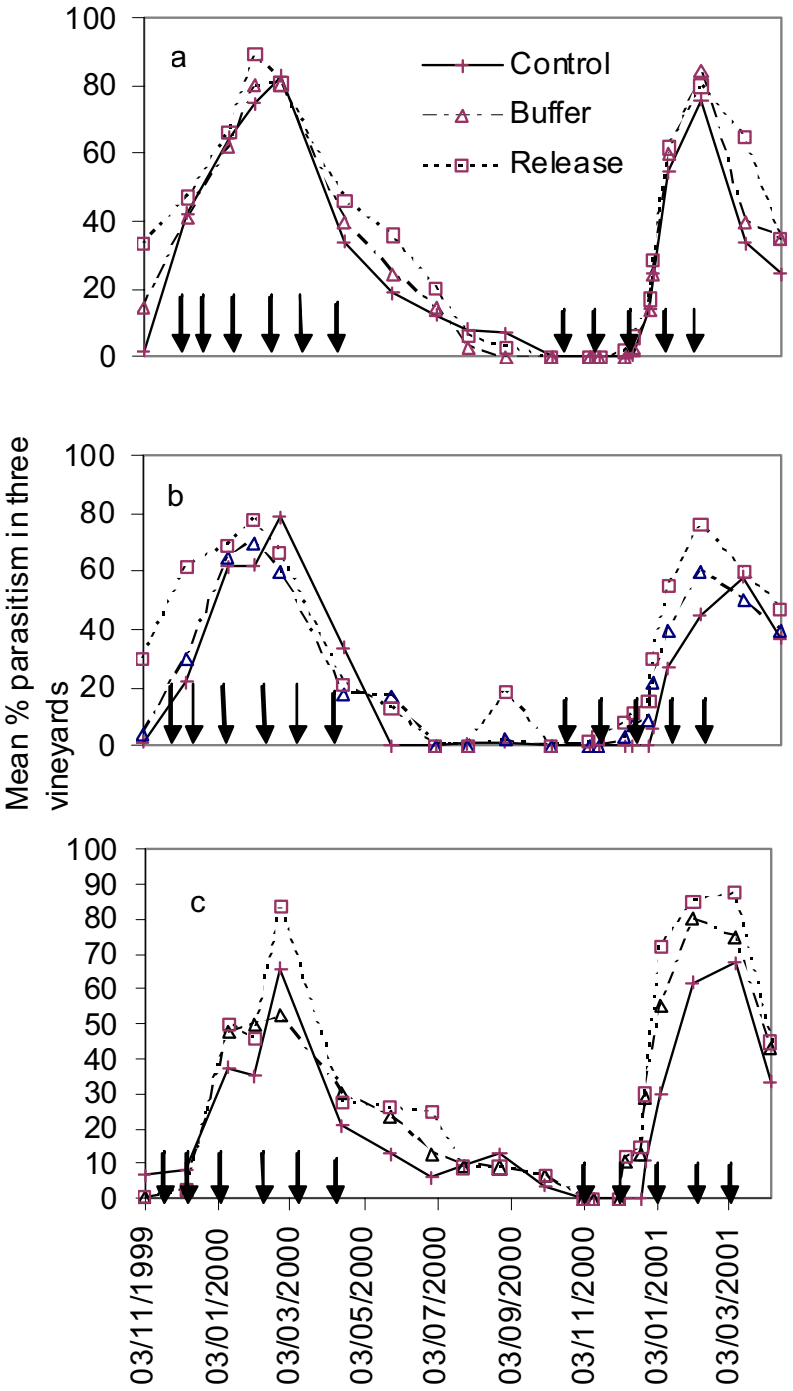

Figure 3. Percentage parasitism during two seasons in blocks where *Coccidoxenoides peregrinus* was released and buffer and control blocks in three vineyards in Hex River Valley (a); Stellenbosch (b); and Robertson (c). Arrows indicate a release of 20,000 *C. peregrinus* per hectare.

DISCUSSION

No differences were detected in the percentage *P. ficus* stem or bunch infestation, *C. peregrinus* counts on yellow sticky traps, or percentage parasitism among the release, buffer, and control blocks. The large plot size meant that the treatments might not have been increasing the variance and, therefore, the experimental error. The large plots also made it logistically difficult to increase the number of replicates, which would have increased the degrees of freedom, providing more sensitive tests. In addition the large plot size may have meant that the treatments were ecologically heterogeneous, increasing the experimental error.

Planococcus ficus stem infestation in the Hex River Valley was significantly lower than in Robertson and Stellenbosch. The lower stem infestations in the Hex River Valley did not influence the number of parasites caught or percent parasitism. Generally *P. ficus* stem infestation levels remained lower in the release than in the buffer and control blocks during both seasons in all areas (Fig. 1), although this was not reflected in the formal analysis. This may indicate that the additional released *C. peregrinus* aided biological control in the release blocks.

Planococcus ficus bunch infestations at harvest (Table 2) in the release and buffer treatments were lower than in the control, suggesting that the releases limited crop loss to a greater extent than the chemical control program. The average crop loss in the buffer and release plots was similar. Therefore, it appeared that the buffer plots also benefited from the parasitoid releases. The higher numbers of *C. peregrinus* and percentage parasitism recorded in the release and buffer blocks may therefore have resulted in the lower overall bunch infestation levels in these treatments at the end of the season.

More *C. peregrinus* were caught on yellow sticky traps in the release and buffer blocks than in the control in all three areas (Fig. 2). However, no differences among treatments were detected by the formal analysis. The higher numbers of *C. peregrinus* in release and buffer blocks in all three areas may have added to biological control as was found by Smith *et al.* (1988) using early mass releases of *L. dactylopii* against *P. citri*. The slightly higher *C. peregrinus* counts in the buffer blocks than in the control could be explained by gradual movement of the parasitoid to the surrounding areas over time. Continued higher counts of *C. peregrinus* were made in all release areas compared with the buffer and control blocks throughout the season. Therefore, it appeared as if the releases successfully supplemented naturally occurring *C. peregrinus* populations.

No significant differences were found between percentage parasitism between any of the grapegrowing areas. The general trend in all three areas was that of a higher percent parasitism in the release and buffer blocks (Table 4, Fig. 3) than in the control blocks.

Mass releases of *C. peregrinus* controlled the pest adequately in the Hex River Valley. The low infestation levels of *P. ficus* appeared to be more suitable for biological control than the high *P. ficus* infestation encountered in Robertson and Stellenbosch.

In Stellenbosch and Robertson a measure of control was evident but not sufficient to keep *P. ficus* populations below economic injury levels. High initial *P. ficus* infestation levels appeared to be less suitable for biological control. Future strategies should include more effective ant control by chemical stem barrier treatments, and initial suppression of high mealybug population levels through the use of dormant season chemical treatments.

Mass releases of *C. peregrinus* in all three areas resulted in *P. ficus* control similar to that achieved using chemical sprays. This method of control is therefore at least as effective as chemical control. The main problem encountered in the use of this strategy in the Hex River Valley was the high cost and lack of available high quality parasitoids. Risks using this method of control include the injudicious use of chemicals during the release period, the lack of ant control, and lack of technical support.

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INTRA- AND INTERSPECIFIC COMPETITION BY *BIOSTERES ARISANUS* (HYMENOPTERA: BRACONIDAE) AND *DIACHASMIMORPHA TRYONI* (HYMENOPTERA: BRACONIDAE) IN THE MEDFLY *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE)

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ABSTRACT. Competition between parasitoid species is an important consideration in the selection and management of effective biological control agents. Intra- and interspecifc competition by *Biosteres arisanus* (Sonan) and *Diachasmimorpha tryoni* (Cameron), a solitary egg and a larval endoparasitoid, respectively, of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) were studied in the laboratory. The outcomes of competition among or between the parasitoid progeny were determined by dissection and rearing of parasitized hosts. When superparasitism occurred, *D. tryoni* eliminated conspecific competitors by direct physical attacks as first instar larvae, while *B. arisanus* killed the competitors as eggs (75%) or first instar larvae (25%) through physiological suppression. Superparasitism by *B. arisanus* was low (<1%) when parasitism of cohorts was lower than 50% but increased significantly with rising mean parasitism. Superparasitism in *D. tryoni* was common. However, *D. tryoni* could discriminate parasitized hosts as parasitism of unparasitized hosts was significantly higher than that of hosts previously parasitized by *B. arisanus* (although the number of stings on parasitized and unparasitized hosts was not different). The number of *D. tryoni* eggs per host was positively correlated with frequency of host stinging. Parasitization by *B. arisanus* resulted in prolonged development of host larvae and high mortality of host eggs. Also, multiple stings by *D. tryoni* caused high mortality of host larvae. In multiparasitized hosts, 77.4% *D. tryoni* progeny died as eggs within three days, indicating physiological inhibition of egg hatch in the presence of *B. arisanus* larvae. Only in 2 of 134 dissections in which multiparasitism occurred were *B. arisanus* larvae killed by *D. tryoni* larvae. Rearing results further showed that *B. arisanus* won almost all competitions against *D. tryoni*. *Diachasmimorpha tryoni* is an inferior competitor against the egg-larval parasitoid. Results are discussed in relation to a possible competition-driven host shift by *D. tryoni* onto a non-target host fly in Hawaii.

RESIDUAL TOXICITY OF INSECTICIDES USED IN COTTON TO THE EGG PARASITOID *ANAPHES IOLE* (HYMENOPTERA: MYMARIDAE), AND IMPLICATIONS FOR INUNDATIVE BIOLOGICAL CONTROL

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INTRODUCTION

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is an important pest of cotton, *Gossypium hirsutum* L., in the southcentral United States (Hanny *et al.*, 1977). Reduction in the use of insecticides in cotton for lepidopterous pests and the boll weevil, *Anthonomus grandis* Boheman, (Hardee *et al.*, 2001) has created an opportunity for potential use of biological control for suppression of *L. lineolaris*.

Anaphes iole Girault (Hymenoptera: Mymaridae) is a minute wasp that parasitizes eggs of *Lygus* species (Jackson and Graham, 1983). Parasitism rates are variable but can reach 100% (Graham *et al.*, 1986). Although *A. iole* is native to North America, it is uncommon in the cotton production region of the Mississippi River delta (Williams, unpub.). Inundative releases of *A. iole* might lead to suppression of *L. lineolaris* in cotton where insecticide use has been reduced.

An effective augmentative release program using *A. iole* to manage *L. lineolaris* in cotton will depend in part on timing parasitoid releases to minimize the deleterious effects of insecticides. Because releases of *A. iole* will be made after insecticide applications, detailed information on the effects of insecticide residues of various ages on this wasp is needed to develop appropriate guidelines for timing releases. Such information might permit strategies that minimize the disruptive effects of insecticides, such as the use of selective compounds and altered rates or timing of insecticide applications.

Only limited information is available about the effects of pesticides on *A. iole*. Carrillo *et al.* (1994) reported differential susceptibility of geographically distinct populations of *A. iole* tested with several insecticides. Udayagiri *et al.* (2000) evaluated the susceptibility of *A. iole* to weathered foliar residues of 14 pesticides used in California strawberry production.

Our objectives were to estimate contact toxicity of field-weathered insecticide residues to *A. iole* and identify compounds that might be compatible with inundative releases of this parasitoid in cotton.

MATERIALS AND METHODS

Insects

Anaphes iole used in this study were obtained from a laboratory colony (U.S.D.A., Starkville, Mississippi) maintained on host (*Lygus hesperus* Knight) eggs. Upon emergence, wasps were provided with distilled water and a 1:1 mixture of honey:distilled water for three days, after which time the bioassays were conducted.

Insecticides and Field Conditions

Insecticides were chosen based on their existing and potential importance in cotton production in the southcentral United States. The insecticides tested were oxamyl (Vydate 2 EC, carbamate, 0.28 kg ai/ha, E. I. DuPont de Nemours and Co., Wilmington, Delaware), spinosad (Tracer 4 SC, bacterial fermentation, 0.1 kg ai/ha, Dow AgroSciences, Indianapolis, Indiana), cyfluthrin (Baythroid 2 EC, pyrethroid, 0.045 kg ai/ha, Bayer, Inc., Kansas City, Missouri), lambda-cyhalothrin (Karate 1 EC, pyrethroid, 0.034 kg ai/ha, Syngenta, Wilmington, Delaware), imidacloprid (Provado 1.6 F, chloronicotinyl, 0.053 kg ai/ha, Bayer, Inc., Kansas City, Missouri), thiamethoxam (Actara 25 WG, neonicotinoid, 0.1 kg ai/ha, Novartis, Greensboro, North Carolina), fipronil (Regent 2.5 EC, phenyl pyrazole, 0.043 kg ai/ha, Aventis, Research Triangle Park, North Carolina), acephate (Orthene 75 SP, organophosphate, 0.28 kg ai/ha, Valent USA Corp., Walnut Creek, California), and indoxacarb (Steward 1.25 SC, oxadiazine, 0.123 kg ai/ha, E. I. DuPont de Nemours and Co., Wilmington, Delaware).

This study was conducted using cotton, *G. hirsutum* (DPL var. NuCOTN 33B), in a commercial field in Elizabeth, Mississippi. Weather conditions were monitored daily throughout the study at the National Weather Service, Mid-South Agricultural Weather Service Center 2.8 km west of the cotton field. The study was established as a randomized block design. Each insecticide was included in three to five trials, with each trial constituting a block. In each trial, 4-12 bioassay chambers were set up for each insecticide-time combination. Formulated insecticides were mixed with distilled water according to label specifications and using a coverage rate of 93.5 liters per ha. Insecticide mixtures were prepared immediately before use. Controls consisted of distilled water. Fully expanded mainstem leaves (fifth node beneath the terminal) were hand-sprayed on both surfaces until run-off to ensure complete coverage. At 0 (about 30 min after application), 2, 4, 8, 16, 23, and 30 days after treatment, treated and control leaves were removed from plants and the petioles were placed in water in florist's waterpiks. Leaves were then transported in ice chests to the laboratory where bioassays were set up.

Bioassay Procedure

Bioassay methodology developed for minute Hymenoptera was used in this study (Williams and Price, unpub.). The methodology exposed parasitoids to residues of insecticides on leaf surfaces. Briefly, a bioassay chamber consisted of a piece of transparent flexible tubing (2.54 cm internal diameter, 3.5 cm long) with organdy-covered ventilation holes (1 cm dia), two scintillation vial caps, each containing agar gel and a leaf disk (2.3 cm dia), a piece of dialysis membrane, and a feeding tube. Approximately 25 parasitoids (mixed gender, assumed mated) were aspirated into each chamber. The feeding tube contained ca. 25 ml of a 1:1 honey:distilled water mixture. Bioassay chambers were held at 27 ± 1 °C, 65-85% RH, and 14:10 L:D photoperiod. After a 48-h exposure period, mortality was assessed by observing wasps in each chamber with a dissecting scope (10-50x). Wasps not exhibiting repetitive (nonreflex) movement were scored as dead.

Data Analysis

Due to the importance of female parasitoids in biological control, only data for female *A. iole* were analyzed. Parasitoid survival for each insecticide-time combination was control-adjusted, after which survival over time was estimated by nonlinear regression fitting a Weibull function or by probit analysis using a lognormal distribution (SAS Institute, 2000).

Environmental conditions recorded during the study were compared with 30-year averages for the area. Two-tailed Student's *t*-tests were run for maximum temperature, minimum temperature, precipitation, solar radiation, and wind speed (Zar, 1996).

RESULTS

Control mortality in these trials ranged from 0 to 16.3% (mean = 10.2, SD = 4.2). Parasitoid survival at day 0 was <3% for all insecticides tested, with the exception of indoxacarb (ca. 32%). Subsequent survival varied considerably, both between insecticide classifications (e.g., organophosphate vs. phenyl pyrazole) and within classifications (pyrethroids and nicotinoids). However, survival rates for several compounds were similar. Residue age associated with 50% survival (ST_{50}) was £3 days for indoxacarb, imidacloprid, acephate, and oxamyl (Table 1). Comparison of 95% confidence intervals showed that ST_{50} values were not significantly different for these compounds. For these insecticides, survival of A. iole was approximately 90% when wasps were exposed to 16-day old residues. Estimates of ST_{50} valuses were about six days for L-cyhalothrin and nine days for thiamethoxam. These estimates were significantly different from each other and from the compounds in the previous group. Cyfluthrin, spinosad, and fipronil residues had ST_{50} values ³30 days, which were significantly greater than those of the other compounds.

Trends observed for ST_{75} values were similar to those for ST_{50} (Table 1). Time for 75% survival (ST_{75}) was <7 days for indoxacarb, imidacloprid, acephate, and oxamyl; and ST_{75} estimates were not significantly different. The ST_{75} estimate for L-cyhalothrin was nearly 8 days, and did not differ from that of acephate. The ST_{75} estimate for thiamethoxam was nearly 14 days and was different from those of other compounds tested.

Weather conditions during the trials were compared to local 30-year averages (Boykin *et al.,* 1995). Two variables deviated significantly from long-term averages. Average daily wind speed and precipitation were significantly lower (P=0.0116 and P<0.0001, respectively) during the course of the study than the 30-year averages.

DISCUSSION

Knowledge of the effects of pesticides on beneficial insects is necessary for successful integration of inundative biological control in agroecosystems (Croft, 1990). Contact toxicity of residues is comprised of two components: initial mortality (incurred soon after application) and the rate of change in mortality as residues dissipate. Udayagiri *et al.* (2000) determined susceptibility of *A. iole* wasps of mixed gender to residues of pesticides used in California strawberries and identified which compounds might be best suited for use with inundative releases of this parasitoid for control of *L. hesperus*. They postulated that compounds with low initial mortality or rapid rates of residual decay offered greatest potential for integration with inundative releases. Highly variable decay rates were observed in the present study (Table 1) and by Udayagiri *et al.* (2000). Our results provide additional information on the toxicity of field-weathered insecticide residues to *A. iole* wasps. Several studies, in addition to the present one, indicate that susceptibility of *A. iole* to pesticides varies greatly between compounds, even those within the same chemical classification (Carrillo *et al.,* 1994; Udayagiri *et al.,* 2000; Williams and Price, unpub.).

Table 1. Survival of female *Anaphes iole* Girault wasps exposed to field-weathered residues of selected insecticides.

Insecticide	ST_{50} days (95% CI)[a,b]	ST75 days (95% CI)[b,c]	Slope±SE	Ho:slope=0 P>X^2 (or *F*)	No. wasps
Organophosphate					
Acephate	2.72 (1.23-4.22)A	6.58 (3.67-9.49)AB	0.79±0.23	0.0017	1626
Carbamate					
Oxamyl	3.18 (2.46-3.89)A	5.11 (3.76-6.46)A	1.46±0.31	0.0001	2565
Oxadiazine					
Indoxacarb	2.34 (1.46-3.22)A	5.12 (3.30-6.93)A	0.89±0.25	0.0010	715
Nicotinoids					
Imidacloprid	2.64 (2.46-2.81)A	4.87 (4.51-5.32)A	4.12±0.25[d]	0.0001	2123
Thiamethoxam	8.94 (7.11-10.77)C	13.79 (10.33-17.24)C	1.60±0.29	0.0001	1854
Pyrethroids					
L-cyhalothrin	6.03 (5.78-6.29) B	7.78 (7.42-8.21)B	9.93±0.56[d]	0.0001	1417
Cyfluthrin	28.62 (24.41>30) D	>30	0.08±0.01	0.0001	3003
Bacterial fermentation					
Spinosad	>30	>30	0.17±0.04	0.0001	2527
Phenyl pyrazole					
Fipronil	>30	>30	0.04±0.01	0.0017	2589

[a] Estimated time (days) for 50% survival of parasitoids.
[b] ST estimates within a column followed by the same letter are not significantly different (95% confidence limits overlap).
[c] Estimated time (days) for 75% survival of parasitoids.
[d] Based on log10 transformed data.

Environmental conditions can greatly influence persistence and degradation of foliar pesticide residues (Willis and McDowell, 1987; Bentson, 1990). In the present study, rainfall and wind speeds were significantly less than normal and may have indirectly affected survival of *A. iole*. Rainfall, especially that which occurs shortly after application, can lead to immediate reduction of insecticide residues (Willis *et al.,* 1994). Effect of wind on persistence of insecticides has been investigated less than rainfall. With the exception of dust formulations (Harper *et al.,* 1983), wind does not appear to be a major factor in dissipation of insecticide residues. These results suggest that under normal conditions, pesticide residues might have decayed more rapidly than occurred in the present study, in turn increasing the survival of *A. iole* wasps. Thus, the present study might be a conservative assessment of the effects of field-weathered insecticides on *A. iole*.

This study helps us select the insecticides likely to be those most compatible with inundative releases of *A. iole* in cotton. Using the 95% CI values for the ST_{50} estimates, we grouped the insecticides into one of three categories. Compounds with upper 95% CI limits <5 days were considered to be of low residual toxicity. This group included indoxacarb, imidacloprid, acephate, and oxamyl. Compounds with upper 95% CI limits encompassing 5£11 days were considered to be of moderate residual toxicity. This group included lambda-cyhalothrin and thiamethoxam. The remaining insecticides, cyfluthrin, spinosad, and fipronil, with 95% CI limits >11 days, were classed as high residual toxicity compounds. Based on this information, insecticides with the most promise for integration with releases of *A. iole* are those classified as low residual toxicity. Parasitoids released >5 days after application of these compounds would not suffer excessive mortality. Compounds considered to be of moderate residual toxicity might be useful under some situations. Relative toxicity of the insecticides to *A. iole* could likewise be grouped using the 95% CI values for the ST_{75} estimates.

Our results suggest that inundative releases of *A. iole* can be effectively integrated with insecticide use in cotton in the southcentral United States. Timing of parasitoid releases might be adjusted to minimize the deleterious effects of insecticides. Compounds with comparatively low residual toxicity allow releases relatively soon after application, thereby increasing the potential for parasitism of *Lygus* eggs laid by bugs not killed by the insecticide or by immigrating adults. Insecticides with moderate residual toxicity dictate higher release rates to compensate for increased wasp mortality. Compounds exhibiting high residual toxicity show limited potential for integration with releases of *A. iole*.

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