EVALUATION OF POPULATION SUPPRESSION BY IRRADIATED LEPIDOPTERA AND THEIR PROGENY

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Lepidopteran species are among the most important pests of major annual and perennial crops, forests, and stored products throughout the world. More than 25% of the species that appear on a list of the 300 most important exotic insects that threaten the United States are in the order Lepidoptera (ESA 2001). In a supplement to that list, where the 30 most serious threats to Agriculture are named, 50% of the species are lepidopterans (ESA 2001). Unfortunately, control of lepidopteran pests worldwide is achieved almost entirely through the use of synthetic insecticides. This dependence on insecticides has contributed to the development of insecticide resistance in many of the most serious pests. Relevant examples include the codling moth, Cydia pomonella (Lepidoptera: Tortricidae) (Varela et al. 1993) and the diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae) (Shelton et al. 1993), where resistance has developed even against the microbial insecticide Bacillus thuringiensis (Tabashnick et al. 1990). Heavy reliance and frequent indiscriminant use of pesticides also has had a significant negative impact on the environment. Of particular importance to agriculture is the destruction of crop pollinators and natural enemies that keep secondary pests in check (Edwards 2000). Development of alternative tactics to the unilateral use of insecticides is a major emphasis of most local, national and international research organizations concerned with pest control.

The Sterile Insect Technique and Lepidoptera

Genetic pest suppression is unique among biological methods in that it involves the release of genetically modified insects to control the same species (LaChance 1985). Sterile Insect Technique (SIT) programs have been quite successful against a number of pest Diptera (including the screwworm fly, Cochliomyia hominivorax, and the Mediterranean fruit fly, Ceratitis capitata), and numerous mass rearing facilities have been constructed worldwide to support these programs. However, compared to diptera, lepidopterans generally are more expensive to rear and have a propensity to fly greater distances. Additionally, lepidopterans are more resistant to the effects of ionizing radiation than diptera. As a consequence, the greater amount of radiation required to completely sterilize lepidopterans negatively impacts their competitiveness and performance in the field. Nevertheless, two SIT programs are currently operating against pest Lepidoptera, namely the pink bollworm, Pectinophora gossypiiella (Saunders), program in the USA (Staten et al. 1993), and the codling moth program in Canada (Dyck et al. 1993; Bloem & Bloem 2000), and both of these programs have been very successful.

One approach to reduce the negative effects of radio-resistance in Lepidoptera has been the use of inherited or F₁ sterility. Proverbs & Newton (1962) first documented F₁ sterility during the course of their studies on the codling moth. Subsequently, investigators have reported F₁ sterility in many lepidopteran species of economic importance (LaChance 1985). Like SIT, F₁ sterility involves the mass rearing and release of genetically altered insects to insure that when matings occur in the field, a significant proportion of matings involve a treated, released insect. However, F₁ sterility takes advantage of two unique genetic phenomena in Lepidoptera. First, lepidopteran females generally are much more sensitive to radiation than are males of the same species. This may allow the dose of radiation to be adjusted so that treated females are completely sterile and males are partially sterile. Second, when these partially sterile males mate with fertile females the radiation-induced deleterious effects are inherited by the F₂ generation. As a result, egg hatch is reduced and the resulting (F₂) offspring are both highly sterile and predominately male. The lower dose of radiation used in F₁ sterility increases the quality and competitiveness of the released insects (North 1975). In addition, because F₂ sterile progeny are produced in the field, the release of partially sterile insects offers greater suppressive potential than the release of fully sterile insects (LaChance 1985) and is more compatible with other pest control mechanisms or strategies (Carpenter 1993).

Knipling (1970) explored the theoretical application of F₁ sterility for control of lepidopteran pests. Using mathematical models, he suggested that when releasing partially sterile insects, the sterile-to-wild overflooding ratio could be as low as ¼ of what is normally required for fully sterile insects. Population models developed by other researchers using data collected from several pest species (Carpenter 1993; Anisimov 1998) corroborate Knipling’s findings.

Field releases of partially sterile insects have demonstrated the potential of using F₁ sterility to
control many lepidopterans, including the cabbage looper, *Trichoplusia ni* (North & Holt 1969), the corn earworm, *Helicoverpa zea* (Carpenter et al. 1987; Carpenter & Gross 1993), the gypsy moth, *Lymantria dispar* (Mastro 1993) and the codling moth, *Cydia pomonella* (Proverbs et al. 1978; Bloem et al. 1999b; Bloem et al. 2001). In addition, many studies have shown that F₁ sterility can be effectively combined with other biological controls such as pheromone disruption (Bloem et al. 2001), entomopathogens (Hamm & Carpenter 1992a, b) and natural enemies (Carpenter et al. 1996; Greany & Carpenter 1999). As a result of these many studies, F₁ sterility is regarded as the most favorable genetic method for most applications against Lepidoptera (for a review, see Carpenter & Bartlett 1999).

**The FAO/IAEA Sponsored Coordinated Research Programs (CRP’s)**

The Joint Division of Nuclear Techniques in Food and Agriculture of the Food and Agriculture Organization (FAO) and the International Atomic Energy Agency (IAEA) promotes agricultural development through the peaceful use of atomic energy. This mission is accomplished through their Technical Cooperation Projects, Coordinated Research Projects, publications, and meetings and training courses. In response to the recommendations of a group of consultants that met at the IAEA in Vienna in 1984, the Insect and Pest Control sub-program of the Joint FAO/IAEA Division designed and initiated the first five-year (1987-1991) Coordinated Research Program (CRP) on “Radiation Induced F₁ Sterility in Lepidoptera for Area-Wide Control.” Research by CRP participating scientists focused largely on modeling the effects of releasing partially sterile moths on the field dynamics of feral populations, conducting laboratory studies to evaluate the relationship between radiation dose and sterility and conducting selected field-cage evaluations. Scientists from ten countries participated in this CRP, and the research results were published by the IAEA in 1993 (Anonymous 1993). As a result of the research progress during the CRP, the participants recommended to the FAO/IAEA that a second Coordinated Research Program should be considered which would emphasize field applications of inherited or F₁ sterility for lepidopteran pests.

A second CRP entitled “Evaluation of Population Suppression by Irradiated Lepidoptera and Their Progeny” was therefore initiated in 1995 with the objective of assessing the potential for controlling populations of pest Lepidoptera by releasing irradiated moths and/or their progeny in combination with other biological control methods. This CRP concluded in 1998. During this time period, three Research Coordination Meetings (RCM) were held to allow participants to discuss initial results and share ideas. The first RCM was held in Jakarta, Indonesia (24-28 April, 1995), the second meeting was held in Vienna, Austria (2-6 September, 1996), and the final meeting was held in Penang, Malaysia (28 May-2 June, 1998) in conjunction with the FAO/IAEA International Conference on “Area-Wide Control of Insect Pests Integrating The Sterile Insect and Related Nuclear and Other Techniques.”

Twenty-five scientific teams from twenty-two different countries (Bangladesh, China, Pakistan, Myanmar, Syria, India, Java, Philippines, Mauritius, Vietnam, Tunisia, Bulgaria, Romania, Czech Republic, Russia, Ukraine, Iran, Austria, United States, Brazil, Cuba and Canada) participated in this second CRP. Participants conducted research on important pests of annual and perennial crops and stored-product pests (see Tables 1 and 2). The research findings have been published in three separate venues: as refereed publications in scientific journals, as part of a Technical Document or meeting proceedings published by the International Atomic Energy Agency, and as a block of four manuscripts following this introductory article. The manuscripts in this volume report important research findings from four different countries and on four different species. Ocampo (2001) describes the effects of a substerilizing dose of gamma radiation (100 Gy) on the mating competitiveness and mating propensity of the Old World cotton bollworm, *Helicoverpa armigera*, in the Philippines. Seth & Sharma (2001b) report on the effects of different doses of radiation on the common cutworm, *Spodoptera litura* reared on two different diets in India. Koudelova & Cook (2001) examine the competitiveness of mutant and irradiated males of the Mediterranean flour moth, *Ephestia kuehniella*, in the laboratory by counting euphyrene (fertile) and apyrene (non-fertile) sperm transferred to the female during copulation. Finally, Nguyen Thi & Nguyen Thanh (2001) report on the potential of combining F₁ sterility and the parasitoid *Cotesia plutellae* in a system to manage the diamondback moth, *Plutella xylostella*, in Vietnam.

**Major Findings and Impact of the Research Conducted During the F₁ Sterility CRP**

The research conducted during this CRP revealed principles that were common to all species studied. These can be summarized into two major points: (1) F₁ sterility is an effective and environmentally safe tactic for lepidopteran pest suppression that is useful under a variety of environments and crop production strategies. (2) F₁ sterility is compatible with all pest control tactics. The combination of F₁ sterility with pheromones, natural enemies, host plant resistance, entomopathogens and insecticides results in synergistic pest population suppression.
This CRP also highlighted several areas that would benefit from further research and development to increase the economic viability of F₁ sterility programs. Development of diets using locally available ingredients would reduce rearing costs, especially in locations with developing economies. Improvements in mass rearing are needed to take advantage of the economy of scale as evidenced in dipteran SIT programs. Development of genetic sexing techniques, especially those that would eliminate females at the egg or early larval stage, would reduce rearing costs, would increase the efficiency of rearing, irradiation and release by 100% and would eliminate assortative mating of released moths in the field.

The FAO/IAEA sponsored CPR's have had major impacts in the direction of future research for the control of lepidopteran pests. For example, expanded research and implementation programs on F₁ sterility in combination with natural ene-
mies are underway in Tunisia for suppression of the carob moth, *Ectomyelois ceratoniae*, and on the island of Mauritius for control of the diamondback moth, *Plutella xylostella*. F₁ sterility programs for other lepidopteran pest species also are being considered. Furthermore, the research from the F₁ sterility CRP's contributed to the development of a new Coordinated Research Program on

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EFFECT OF GAMMA RADIATION AND SEX-LINKED RECESSIVE LETHAL MUTATIONS ON SPERM TRANSFER IN EPHESTIA KUEHNIELLA (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

Sperm quality and sperm competition play important roles in determining the efficiency of genetic methods for suppression of lepidopteran populations. Herein we have examined competitiveness of mutant and irradiated males of Ephestia kuehniella by counting eupyrene (fertile) and apyrene (non-fertile) sperm transferred to the female during copulation. Mutant BL-2 males, trans-heterozygous for two sex-linked recessive lethal mutations sl-2 and sl-15, produced 50% fewer of both types of sperm as compared to with WT-C (wild-type) males. However, the ratio of apyrene to eupyrene sperm remained the same in both male types (9.5:1). Irradiation of mature male pupae, heterozygous for either sl-2 or sl-15 mutations, with doses between 150 and 350 Gy showed dose-dependent effects on the amount of sperm transferred and on the total length of mating times. As the treatment dose increased the volume of sperm transferred by the male decreased and the mating times got longer. In the F1 descendants of the treated males, males were found to transfer either a relatively normal or a very small volume of sperm, which could reflect changes in gamete segregation and in chromosomal aberrations that are inherited. The dose of 175 Gy is suggested as optimal for irradiation of Ephestia kuehniella.

Key Words: irradiation, sperm transfer, eupyrene, apyrene, sperm ratio, sperm competition, Ephestia kuehniella

RESUMEN

La calidad y la competitividad del esperma es de suma importancia en la determinacion de la eficacia de los metodos geneticos destinados a la supresion de poblaciones de Lepidoptera. En este articulo hemos examinado la competitividad de machos de una sepa mutante asi como la competitividad de machos irradiados de la especie Ephestia kuehniella utilizando como indicador el numero total de esperma nucleado (eupyreno) y ancleado (apyreno) que estos machos transfieren durante la copula. Los machos de la sepa mutante (BL-2) que son trans-heterocigotos para dos mutaciones letales recesivas ligadas al sexo (sl-2 y sl-15), produjeron 50% menos cantidad de los dos tipos de esperma que los machos de tipo salvaje (WT-C). Sin embargo, la relacion entre la cantidad de esperma apyreno y eupyreno se mantuvo constante en ambos tipos de macho (9.5:1). La irradiacion de pupas maduras, heterocigotas para las mutaciones sl-2 o sl-15 con dosis entre 150 y 350 Gy mostró efectos dependientes de la dosis en cuanto a la cantidad de esperma transferido y el tiempo que los machos permanecen en copula. A medida que la dosis de irradiacion se incrementa, el volumen de esperma transferido decrece y el tiempo que los machos permanecen en copula aumenta. Asimismo, se encontro que los descendientes de machos irradiados (F1) transfieren ya sea una cantidad normal de esperma o una cantidad muy reducida de esperma lo cual indica que los cambios en la segregacion de gametos en cromosomas irradiados son heredados. La dosis de 175 Gy parece ser optima para la irradiacion de Ephestia kuehniella.

Substerilizing doses of radiation induce inherited sterility in Lepidoptera (Anisimov et al. 1989; Carpenter 1991), and the impact of gamma rays at the chromosomal level is well known (Astaurov & Frolova 1935; LaChance 1967; Carpenter 1991). The effectiveness of radiation-induced inherited sterility in the Mediterranean flour moth (Ephestia kuehniella), an extremely radio-resistant species, is enhanced by the use of a genetic sexing technique (Marec & Mirchi 1990; Marec et al. 1999). However, when evaluating the potential of inherited sterility for population suppression it is important to quantify the competitiveness of the released males.

In Lepidoptera, the amount of sperm transferred during mating may affect reproductive success (Cook et al., in press), and as such has important consequences in the success of a sterile male release program. Females that receive insufficient amounts of sperm during mating may remate in order to secure enough sperm to fertilize their complement of eggs. Even if females have received enough sperm to fertilize the eggs, they might be able to perceive that insufficient
sperm quantities were transferred during mating. In the Indian meal moth (*Plodia interpunctella*), females that received smaller amounts of sperm did not exhibit reduced fertility, even at the end of their life (Cook 1999). Nonetheless, these females were more likely to remate than females receiving adequate sperm numbers (Cook & Gage 1995). In experiments with *Plodia interpunctella*, where females were mated twice to males that differed in the amount of sperm transferred Cook et al. (1997) found that males that transferred more sperm were able to fertilize a higher proportion of the females eggs. Similar results were reported by Wedell & Cook (1998) for the pierid butterfly *Pieris rapae*. In accordance with the sperm competition theory (Parker 1970), male Lepidoptera have the capacity to increase sperm numbers when sperm competition risk is high (Cook & Gage 1995; Cook & Wedell 1996; Wedell & Cook 1999a). This phenomenon has been reported in other invertebrates (Gage 1991; Gage & Baker 1991; Simmons et al. 1993) and vertebrates (Baker & Bellis 1989; Bellis et al. 1990).

Several other factors affect sperm numbers. For example, in *P. interpunctella*, males transfer fewer sperm in their second and third matings (Gage & Cook 1994), whereas males of *P. rapae* transfer more sperm in the second when compared to the first mating (Cook & Wedell 1996; Wedell & Cook 1999b). Gage & Cook (1994) examined the effect of high versus low protein larval diets on sperm number and size in *P. interpunctella*. They found that the number but not the size of sperm was reduced when male larvae suffered resource restrictions. However, in a study of sublethal viral larval infection in the same species, Sait et al. (1998) found that sub-lethally infected males, despite having lower fertility, did not have significantly fewer or smaller sperm.

Lepidopterans produce two types of sperm cells, nucleated eupyrene (Figs. 1, 2) and smaller, anucleated apyrene sperm (Fig. 3). The two sperm types result from two distinct modes of spermatogenesis (Friedländer 1997), with apyrene outnumbering eupyrene sperm by about ten to one (Gage & Cook 1994; Cook & Wedell 1996, 1999; Marec et al. 1996). Both types are transferred to the female during copulation via the spermatoaphore and both reach the site of sperm storage, the spermatoheca. Apyrene sperm bundles dissociate and become motile prior to male ejaculation while eupyrene sperm remain in bundles (Fig. 1). Until recently, the function of apyrene sperm was unknown although several hypotheses have been postulated (reviewed by Silberglied et al. 1984). Osanai et al. (1987, 1989) suggested that apyrene sperm aid in the motility of eupyrene sperm bundles inside the female. They characterized apyrene function as that of promoting dissociation of eupyrene bundles and separation of individual eupyrene sperm both mechanically and by biochemical degradation. It has also been suggested that apyrenes play a role in sperm competition (Silberglied et al. 1984; Cook & Gage 1995). Also, there is good evidence that they protect a males reproductive investment by delaying female remating (Cook & Wedell 1999). Thus, apyrene sperm, as well as eupyrene sperm, must be considered when investigating male reproductive success.

In this study we examined the effects of mutation and doses of gamma radiation on the number of eupyrene and apyrene sperm transferred by males of *E. kuehniella* in the P and F1 generations in an effort to assess the sperm competitiveness of the released males used in an F1 sterility management program for *Ephestia kuehniella*.

**Materials and Methods**

*Insects*

Insects were reared and experiments carried out in rooms held at constant temperature (25 ± 1°C), 12L:12D photoperiod and ambient relative
humidity. The Mediterranean flour moth, *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae) has been in laboratory culture since 1984 and the strain is considered to be wild-type (WT-C) (for details see Marec 1990). A balanced lethal strain, BL-2, was constructed in 1990 (Marec 1991) and has been reared since 1990 in single pair cultures. BL-2 males are trans-heterozygous for two non-allelic sex-linked recessive lethal mutations, *sl-2* and *sl-15*. The genetic structure of the BL-2 strain is described in Marec et al. (1999). The number and ratio of eupyrene and apyrene sperm (see below) and the total time in copula for BL-2 and WT-C males mated to WT-C females was recorded in the laboratory.

BL-2 males were individually mated to virgin WT-C females one generation before irradiation. Heterozygous males of two genotypes (*sl-2* or *sl-15*) were produced (both male types are designated here as *sl/+*), whereas female zygotes died during embryogenesis (since they were hemizygous for the lethal *sl-2* or *sl-15* mutations). Six to eight day-old pupae were removed from their cocoons and irradiated.

**Irradiation**

Irradiation was performed at the Entomology Unit of the FAO/IAEA Laboratories in Seibersdorf, Austria, using a Co\(^{60}\) Gammaxcell 220 (AE of Canada Ltd.) with dose rates ranging between 50-60 Gy/min. The *sl/+* male pupae were placed in plastic petri-dishes (5 cm diam.) In the first experiment we examined the effect of dose on the F\(_1\) generation. Pupae were treated with 150, 175, 200, 250 or 350 Gy of gamma radiation. In the second experiment we examined the effect on the F\(_1\) generation. A sixth dose was added in this experiment (300 Gy). As a control for the irradiation procedure, untreated *sl/+* and WT-C males were subjected to the same handling as irradiated males. Pupae were placed in containers at the conditions mentioned above and adults were collected as they emerged.

**Sperm Counts**

Males were allowed to mate with WT-C virgin females. For each mating, the total time in copula

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**Fig. 2.** Eupyrene sperm of *Ephesia kuehniella* after dissociation from the sperm bundle and seen in Nomarski interference contrast using Aristoplan microscope. Scale bar = 100 mm.

**Fig. 3.** Apyrene sperm of *Ephesia kuehniella* taken from the spermatophore and seen in Nomarski interference contrast using Aristoplan microscope. Scale bar = 100 mm.
and the number of eupyrene and apyrene sperm transferred was recorded. Sperm were counted using the methods outlined in Gage & Cook (1994) and Cook & Wedell (1996). Immediately after mating, the female was dissected and the spermatophore removed from the bursa copulatrix and placed in a drop of modified Barth saline solution on a glass slide (Gurdon 1991). Using a dissecting microscope (40× magnification), the spermatophore was ruptured to release the sperm. The eupyrene sperm bundles were counted and the number of eupyrene sperm was obtained by multiplying the number of bundles by 256 (which is the number of sperm per bundle present after both mitotic and meiotic divisions are completed). To count the apyrene sperm, the sample was washed off from the glass slide with more Barth saline and diluted with distilled water. Six 10 µl sub samples were placed on slides and allowed to dry. Each was examined using dark-field phase contrast microscopy (100× magnification) and the number of apyrene sperm in each dried sample was counted. The sperm ratio was expressed as a quotient of apyrene to eupyrene sperm.

Statistical Analysis

Data was analyzed using Instat and GraphPad Prism software. The number and ratio of eupyrene and apyrene sperm and the total time in copula for the BL-2 and WT-C males was analyzed with one way analysis of variance (ANOVA). Data collected for the F1 generation appeared to deviate from normality. As such, Kruskal-Wallis one-way analysis of variance by ranks was used to decide if samples came from populations with the same median (Siegel & Castellan 1988). This non-parametric method has the advantage of not requiring the assumption of normally distributed error. Total time in copula for the F1 generation was compared with one-way ANOVA.

RESULTS

Data on the number of eupyrene and apyrene sperm, sperm ratio and the total time in copula for BL-2 and WT-C males is presented in Table 1. The number of both eupyrene and apyrene sperm were significantly lower in males from the BL-2 strain. However, the ratio of apyrene to eupyrene sperm was the same in both male groups (9.5:1). The total time in copula was slightly (although not significantly; P = 0.0774) longer for the mutant male strain (BL-2) than for the wild type (WT-C) males.

Radiation dose significantly affected both eupyrene (Fig. 4-P < 0.05, F = 3.844) and apyrene (Fig. 5-P < 0.05, F = 2.953) sperm numbers in the P generation as males were treated with increasing doses of gamma radiation. When males were treated with 175 Gy the number of eupyrene (Fig. 4) and apyrene (Fig. 5) sperm was higher than for both controls (sl/+ and WT-C). At doses above 175 Gy the amount of sperm decreased significantly to reach a 25% reduction at 350 Gy for both sperm types (F = 3.85, P = 0.013 and F = 2.95, P = 0.009 for eupyrene and apyrene sperm, respectively).

There were no significant differences in the apyrene to eupyrene sperm ratios among treatments (Fig. 6) (F = 1.25, P = 0.28). The total time in copula for control and treated P generation males is shown in Figure 7. There was a significant treatment effect, with males treated at 350 Gy remaining in copula for the longest period of time (F = 5.55, P = 0.0001).

No adult progeny developed from crosses involving P males treated with 300 and 350 Gy. As such, no data is included for the F1 generation at these doses. In the remaining F1 male groups (150-250 Gy), no dose-response was evident when the mean numbers of sperm in the spermatophore were compared. However the numbers of eupyrene to apyrene sperm suggested a bimodal distribution but could not be tested for normality because of insufficient sample size. Therefore, a Kruskall-Wallis test was used to compare medians (Table 2). The number of sperm transferred by F1 generation males was highly variable. Figures 8 and 9 illustrate the distribution of numbers of both sperm types at various doses. These distributions clearly show the difference between WT-C control and untreated sl/+ control in the

<table>
<thead>
<tr>
<th>Strain</th>
<th>Eupyrene sperm (no.)</th>
<th>Apyrene sperm (no.)</th>
<th>Sperm ratio</th>
<th>Total time in copula (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n Mean ± SD</td>
<td>n Mean ± SD</td>
<td>n Mean ± SD</td>
<td>n Mean ± SD</td>
</tr>
<tr>
<td>BL-2</td>
<td>21 6644 ± 4146**</td>
<td>23 6086 ± 28793**</td>
<td>16 9.5 ± 3.3</td>
<td>21 121.9 ± 32.5*</td>
</tr>
<tr>
<td>WT-C</td>
<td>22 14604 ± 7028</td>
<td>26 112182 ± 75046</td>
<td>19 9.5 ± 2.9</td>
<td>25 106.2 ± 26.4</td>
</tr>
</tbody>
</table>

*The difference between strains was not significant at P = 0.05 (compared using ANOVA).
**Marginally significant at P = 0.0774.
***P < 0.001.
numbers of both sperm types transferred. Apyrene to eupyrene sperm ratios in the F1 generation were lower in the controls (sl+/+ and WT-C) than in the treatments (Table 2). Some of the males from each treated group exhibited very high apyrene to eupyrene ratios as shown in Fig. 10. The total time in copula in F1 males did not follow a dose-dependent pattern (Fig. 11-F = 4.605, P = 0.0009). When compared with one-way ANOVA, significant differences were found among controls and treated groups. Males treated with 175 Gy remained in copula for the longest time (the mean was 160 minutes).

In addition, some F1 males exhibited various abnormalities in spermatophore production, including spermatophore bulb formation, sperm transfer to the spermatophore and accessory gland fluid transfer to the bursa copulatrix. We recognized three types of copulatory defects in F1 males: (1) no accessory gland fluid present in the bursa copulatrix; (2) no spermatophore or sperm present in bursa copulatrix, and (3) no spermatophore or sperm present. These defects were observed in 7.3% of the total (110 males) studied and occurred only in treated males.

**DISCUSSION**

In order to predict the amount of sperm transferred by *Ephestia kuehniella* males, we analyzed sperm numbers in parents of irradiated sl/+ males, heterozygous for either the sl-2 or sl-15 mutations, and in BL-2 males which carried both mutations. We discovered that mutations had an effect on the amount of sperm in the spermatophore but not on the apyrene to eupyrene sperm ratio (Table 1). A 9.5:1 apyrene to eupyrene ratio was found in the control (WT-C) males and in the BL-2 mutant males, and this ratio is similar to that reported for other Lepidoptera. For example,

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![Fig. 4. Eupyrene sperm numbers transferred in P generation of irradiated sl/+ males of *Ephestia kuehniella*. Untreated controls: sl/+, WT-C. Bars indicate standard deviation.](image)

![Fig. 5. Apyrene sperm numbers transferred in P generation of irradiated sl/+ males of *Ephestia kuehniella*. Untreated controls: sl/+, WT-C. Bars indicate standard deviation.](image)

![Fig. 6. Apyrene to eupyrene sperm ratio in the P generation for irradiated sl/+ males of *Ephestia kuehniella*. Untreated controls: sl/+, WT-C. Bars indicate standard deviation.](image)

![Fig. 7. Total time in copula in the P generation for irradiated sl/+ males of *Ephestia kuehniella*. Untreated controls: sl/+, WT-C; bars indicate standard deviation.](image)
a 9:1 ratio was reported for *Plodia interpunctella* by Gage & Cook (1994); a 5:1 ratio for *Spodoptera litura* by Etman & Hooper (1979) and a 9.6:1 ratio for *Manduca sexta* by J. G. Shepherd, unpublished data in Silberglied et al. (1984).

Several authors have suggested that anucleate apyrene sperm play a role in sperm competition (Silberglied et al. 1984; Osanai et al. 1987; Cook & Gage 1995). Cook & Weddel (1999) found that female *Pieris napi* that had more apyrene sperm in their storage organ were more likely to delay remating than females with less apyrene sperm. Marec et al. (1999) examined the effect of irradiation on mating success and fecundity of males. Holt & North (1970) also found that radiation dose did not produce any major physiological changes that would decrease reproductive capacity in treated males.

Holt & North (1970) studied the effect of gamma radiation on sperm distribution and copulation in *Trichoplusia ni*. They reported that there was no difference in sperm volume ejaculated but found differences in the number of sperm present in the spermatophore and concluded that irradiation affects the complex process of copulation. In the present study, gamma radiation reduced the number of sperm transferred in treated *E. kuehniella* males of the F generation at all doses except for 175 Gy. At higher doses, the number of eupyrene and apyrene sperm were reduced and the total time in copula for these males was increased. We suggest that total time in copula was dose and sperm volume-dependent (i.e., the higher the treatment dose and the lower the sperm number, the longer the total time in copula). Holt & North (1970) also found that irradiated males copulated for longer times than did untreated controls.

We suggested that the impact of irradiation would be greater in the progeny (F<sub>1</sub>) of treated *sl/+ E. kuehniella* males than in the parental (P) generation. North (1975) suggested that F<sub>1</sub> males originating from a partially sterile male parent in *Pectinophora gossypiella* or from apyrene sperm present in the spermatophore and contributed reduced eupyrene and increased apyrene sperm when compared to controls. These results agree with those of a cytogenetic study (Tothová & Marec, subm.) which showed variability in the numbers of sperm transferred to females (Table 2). In the range of doses tested (150-250 Gy) some males showed sperm numbers that were similar to those found in the untreated controls. However, at the same doses, some males transferred reduced eupyrene and increased apyrene sperm when compared to controls. These results agree with those of a cytogenetic study (Tothová & Marec, subm.) which showed variability in the number and type of chromosome aberrations inherited from irradiated male parents in F<sub>1</sub> males of *E. kuehniella*. This variability was magnified by abnormal pairing of chromosomes during meiosis. This abnormal pairing most probably influenced chromosome segregation in meiosis I, finally resulting in either genetically unbalanced or balanced gametes. However, the level of sterility predicted according to the observed frequency of chromosome aberrations was much higher than the level of inherited sterility found by Marec et al. (1999). Thus, it has been suggested that there is a regulation mechanism which enables the moths to correct the predicted unbalanced state towards balanced segregation of chromosomes during meiosis I (Tothová & Marec, subm.).

In the F<sub>1</sub> males we observed no clear dose-dependence between sperm transfer and the dose of gamma radiation. In general, the mean number of eupyrene sperm transferred decreased, whereas the mean number of apyrene sperm transferred increased. This resulted in a high ratio of apyrene to eupyrene sperm, which fluctuated between values similar to those of controls.
Fig. 8. Frequency distribution of eupyrene sperm numbers transferred by control (sl/+, WT-C) and treated males of F₁ generation *Ephestia kuehniella*.
Fig. 9. Frequency distribution of apyrene sperm numbers transferred by control (sl/+ WT-C) and treated males of F1 generation Ephestia kuehniella.
and those which could be clustered into four groups: (1) higher than 10:1, (2) higher than 20:1, (3) higher than 30:1 and (4) the ratios that exceeded the value 100:1. At 200 and 250 Gy the most frequent ratios fluctuated around the value 30:1. Several authors have reported reduced eupyrene sperm transfer in Lymantria dispar. Twenty-five percent of the females that mated with F₁ males from 100 Gy treated male parents contained no eupyrene sperm in their spermatheca. Similarly, Cheng & North (1972) found that nearly half of normal P. gossypiella females that mated with F₁ males from 150 and 200 Gy treated male parents had only apyrene sperm in their spermathecae. LaChance et al. (1977) suggested that females with no eupyrene sperm in their spermathecae behaved as virgins and re-mated. However, apyrene sperm can influence sperm competition as reported for Pieris napi by Cook & Wedell (1999). They found that more apyrene sperm is stored in females that do not re-mate. As such, males that are able to transfer more apyrene sperm might reduce the possibility of sperm competition. We observed that some treated F₁ males were able to transfer large amounts of apyrene sperm. Matings with these F₁ males may suppress female remating.

An interesting result was obtained when Ephestia kuehniella males were treated with 175 Gy. In both the P and the F₁ generation, this dose caused an increase in the number of eupyrene sperm transferred compared to the sl/+ control (but not WT-C control in the P generation). In F₁ males, the number of apyrene sperm was higher than in both controls and this number had a broader frequency distribution. Therefore we suggest that 175 Gy may be the best dose to treat Ephestia kuehniella for F₁ sterility induction.

We conclude that males inheriting many chromosomal aberrations may change either the mechanisms regulating spermatogenesis or those underlying copulation and sperm transfer to the spermatophore. In this study we found that apyrene sperm are transferred in higher numbers in irradiated males and in very high numbers in their progeny. Given that apyrene sperm numbers are related to female remating in untreated
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Lepidoptera, the effect of receiving such high apyrene sperm numbers on female behavior merits further study.

ACKNOWLEDGMENTS

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INHERITED STERILITY BY SUBSTERILIZING RADIATION IN *SPODOPTERA LITURA* (LEPIDOPTERA: NOCTUIDAE): BIOEFFECTIVITY AND POTENTIAL FOR PEST SUPPRESSION

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Department of Zoology, University of Delhi, Delhi - 110 007, India

**ABSTRACT**

*Spodoptera litura* reared on host plants and on synthetic diet were irradiated with two sub-sterilizing doses of gamma radiation, 100 Gy and 130 Gy, and examined for inherited sterility. Irradiation affected mating success in the parental (P) and F, generations. F1 sterility was higher than P sterility, and F1 males inherited more sterility than did F1 females. F1 progeny developed at a slower rate compared with controls. F1 survival to adulthood decreased with increasing dose of radiation. Sex ratio in F1 moths was skewed towards male. Life tables were constructed for *S. litura* reared on host plant and synthetic diets, and the impact of radiation on population characteristics was ascertained. Reproductive rate (R0) was significantly decreased as a consequence of irradiation, and the effect was more severe in F1 crosses than in P crosses. There was a negative correlation between the dose of radiation and the percent embryonic formation in P crosses. Whereas in F1 crosses, radiation dose (given to male parents) was positively correlated with the percent unhatched embryonated eggs. Early mortality of eggs prevailed in unexisting eggs derived from P crosses, and late embryonic lethality was the major cause of F1 sterility. Effects of irradiation are discussed with an emphasis on assessing the potential of the inherited sterility principle for pest control.

Key Words: common cutworm, irradiation, F1 sterility, synthetic diet, embryonic lethality, population dynamics

**RESUMEN**

Insectos de la especie *Spodoptera litura* provenientes de colonias mantenidas en plantas huéspedes o en dieta artificial se irradiaron a dos dosis substerilizantes de radiación gamma, 100 Gy y 130 Gy, y fueron examinados para detectar la presencia de esterilidad hereditaria. La irradiación afectó la habilidad de copula en las generaciones P y F1. Se encontró que la esterilidad en la generación F1 es más alta que en la generación P y los machos F1 heredaron más esterilidad que las hembras de la misma generación. Asimismo, se encontró que la velocidad de desarrollo de la generación F1 es más lenta que en el grupo control (no irradiado), y la supervivencia al estado adulto se redujo a medida que los insectos fueron expuestos a dosis de radiación más altas. Finalmente, la tasa sexual se vio favorecida hacia el sexo macho en la generación F1. Se construyeron tablas de vida para ambas colonias y se investigó el efecto de la radiación sobre varios parámetros de estas poblaciones. La tasa reproductiva (R0) se redujo significativamente y el efecto fue más severo en parejas de insectos de la generación F1. Asimismo, se encontró una correlación negativa entre la dosis de radiación y la formación de huevos embriónados en la generación P. Esta correlación fue positiva en la generación F1, cuando la radiación se aplicó solamente a los machos. Se detectó mortalidad temprana dentro de los huevos embriónados no eclosionados provenientes de crías en la generación P y se detectó muerte tardía de los huevecillos embriónados en crías en la generación F1. En este artículo discutimos estos efectos con enfoque de la aplicación de la esterilidad F1 como método de control para esta especie.

*Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), the common cutworm, is an economically serious and polyphagous pest in India. This pest is reported to attack a wide range of food plants (112 cultivated plants belonging to 44 families worldwide and 60 plants in India) (Lefroy 1908; Moussa et al. 1960; Thobbi 1961; Chari & Patel 1983). A multifaceted approach is required for the control of this pest because it has developed resistance against a range of insecticides and because other control measures are inadequate when applied alone (Ramakrishnan et al. 1984; Armes et al. 1997). The sterile insect technique (SIT) has been used for Lepidoptera but insects in this order are radio-resistant, presumably due to their holokinetic chromosomal configuration (Bauer 1967). Therefore, lepidopterans require large doses of radiation for sterilization, leading to somatic damage and reduced competitiveness in the irradiated insect.

A favored alternative to using fully sterile moths in SIT is the use of F1 sterility. F1 survivor progeny of sub-sterile parental (P) males results when sub-sterilizing doses of radiation are applied to the P males. The resulting F1 progeny are more sterile than the irradiated parent, and the irradiated moths are more competitive as a result of receiving a lower dose of radiation. Inherited
sterility in the progeny of treated males has been shown to have potential in suppressing populations of lepidopteran pests (North & Holt 1969; Knipling 1970; North 1975; LaChance 1985).

Previous studies of substerilizing gamma-radiation doses on the growth, bioenergetics and reproductive behavior of *S. litura* in the F₁ progeny of treated moths indicated the potential of managing this pest by using inherited sterility (Seth & Sehgal 1993). In this study we evaluated the reproductive performance and mating behavior of *S. litura* in response to two substerilizing doses (100 Gy and 130 Gy) when reared on two different diets.

**MATERIALS AND METHODS**

Insect Rearing

*S. litura* was reared on two diets, the natural food, castor leaves (*Ricinus communis*) and a meridic diet containing chickpea seeds and sinigrin as a phagostimulant (Table 1). Insects were reared on the castor leaves were allowed to pupate in moist, loose soil. Larvae developing on the castor leaves were allowed to pupate in moist, loose soil. Larvae developing on the chickpea diet pupated in the diet container.

Irradiation of Insects

Irradiation of 0-24-h old adult males was conducted in the Genetics Division, Indian Agricultural Research Institute, New Delhi, using a Co⁶⁰ source at the dose rate of about 13.5 Gy/min. On the basis of our initial studies (Seth & Sehgal, 1993), two gamma-radiation doses, 100 Gy and 130 Gy, were selected for this study.

Reproductive Performance and Viability of Irradiated Moths and Their Progeny

Various reproductive parameters were assessed by pairing treated insects (irradiated P males and F₁ moths derived from the treated P males crossed with normal females) with their normal (N) counterparts from the stock culture. Eggs from single-pair matings were counted daily and the number hatching was monitored in 10 replicated samples of 80-100 eggs each (up to first 3 days of egg laying). Corrected sterility and control of reproduction of insect population due to irradiation were determined according to the methods described by Abbott (1925) and Seth & Reynolds (1993).

Experiments on mating success were conducted in laboratory cages (each cage having 10-15 pairs, comprising one replicate). The mating success of moths was assessed by dissection of females immediately after death. The presence of a spermatophore in the bursa copulatrix indicated that the female had mated; the number of spermatophores indicated the mating frequency.

The viability of treated moths, and the survival and developmental pattern of F₁ progeny were determined. Diurnal observations on the insect behavior and the growth index for each treatment was calculated.

**Population Characteristics**

Various features of population dynamics were studied by constructing life tables for *S. litura* on castor leaves and chickpea diet. The female life tables of P and F₁ generation were constructed as described by Birch (1948), and elaborated by Howe (1953), Morris & Miller (1954), and Atwal & Bains (1989). For ascertaining the life table characteristics, a defined size of population was established in field-cages for a particular cross. Then seven batches of 200-250 eggs collected from each cross were reared to determine age specific mortality in different life stages and successful adult emergence. The life tables gave the probability at birth of a female being alive at age *x*, designated as *l₀* (*l₀* = 1). The age schedule for female births denoted the mean number of female offspring produced per unit time by a female of age *x*, designated as *mₙ*. The net reproductive rate (*Rₙ*) was calculated from *l₀* and *mₙ*. Mean length of generation time (*Tₙ*), innate capacity for increase in number (*rₙ*) and finite rate of increase (*λₙ*) were calculated from the data generated in the life tables.

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**Table 1. Constituents of the semi-synthetic diet proposed for rearing of *Spodoptera litura*.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>25 g</td>
</tr>
<tr>
<td>Deionized water</td>
<td>750 ml</td>
</tr>
<tr>
<td>Casein</td>
<td>44 g</td>
</tr>
<tr>
<td>Ground chickpea seeds</td>
<td>93.50 g</td>
</tr>
<tr>
<td>Wessons salts</td>
<td>12.50 g</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.25 g</td>
</tr>
<tr>
<td>Yeast (dried, brewer’s)</td>
<td>19 g</td>
</tr>
<tr>
<td>Methyl-p-hydroxybenzoate</td>
<td>1.25 g</td>
</tr>
<tr>
<td>Sugar</td>
<td>39 g</td>
</tr>
<tr>
<td>Sorbic acid</td>
<td>2 g</td>
</tr>
<tr>
<td>Deionized water</td>
<td>400 ml</td>
</tr>
<tr>
<td>4 M KOH</td>
<td>6.25 ml</td>
</tr>
<tr>
<td>Corn oil</td>
<td>2.50 ml</td>
</tr>
<tr>
<td>Linseed oil</td>
<td>2.50 ml</td>
</tr>
<tr>
<td>Formaldehyde (10%)</td>
<td>5.50 ml</td>
</tr>
<tr>
<td>Sinigrin (1%)</td>
<td>3.53 ml</td>
</tr>
<tr>
<td>Antibiotic and vitamin mixture*</td>
<td>7.50 g</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>1.25 g</td>
</tr>
</tbody>
</table>

*Composition: chloramphenicol (2 g), streptomycin (4 g), tetracycline (36 g), ascorbic acid (80 g), Evion (vitamin E; 0.2 g; Merck Co.), vitamin mixture (2 g; Roche Co.).
Embryonic Development

The embryonic development in F₁ and F₂ eggs was studied to understand the stage at which irradiation induced lethality was manifested. The developmental state of embryos was assessed in dechorionated eggs. The eggs were treated with 3-5% NaClO for 30-40 min, fixed with 10% formalin for 8-12 h, and stained with lactoacetic-orcein or Feulgen stain. The stained embryos were examined under the microscope. The embryonic development was classified as stage I (cleavage/early development), stage II (germ band stage), and stage III (embryo stage). Some embryos were recorded as abnormal.

Statistical Analysis

The effect of radiation on various parameters in the two diets was subjected to analysis of variance (ANOVA). Data were usually obtained in replicates of ten, unless otherwise specified in the text. Percentage data were transformed using arcsine √x before ANOVA. Means were separated at the 5% significance level by least significant difference (LSD) test (Snedecor & Cochran 1989).

RESULTS

Reproductive Performance and Viability of Irradiated Moths and Their Progeny

For the untreated controls, the pre-oviposition period ranged from 1.65 to 1.72 d on the castor leaf diet and 1.69 to 1.70 d on the chickpea diet. The dose of radiation did not significantly affect pre-oviposition period for the P and F₁ crosses in either diet. The oviposition period was not affected in P crosses, but was significantly reduced in most F₁ crosses (Table 2). Also, the radiation treatments caused a significant reduction in fecundity of the mated female during P crosses and F₁ crosses, with the greatest reduction in F₁ crosses. Even normal females mated with treated males (P or F₁) showed a reduction in fecundity.

Table 2. Effect of substerilizing gamma-radiation doses and diet on the ovipositional behavior and longevity of Spodoptera litura parents and F₁ progeny.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Dose (Gy)</th>
<th>Cross type</th>
<th>Preoviposition period (days)</th>
<th>Oviposition period (days)</th>
<th>Eggs per female (no.)</th>
<th>Life span (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>P crosses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Castor leaf</td>
<td>0</td>
<td>Nm × Nf</td>
<td>1.65 ± 0.07 a</td>
<td>7.23 ± 0.32 a</td>
<td>1893 ± 59 a</td>
<td>10.0 ± 0.4 a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Pm × Nf</td>
<td>1.68 ± 0.17 a</td>
<td>7.25 ± 0.17 a</td>
<td>1847 ± 68 b</td>
<td>9.4 ± 0.7 a</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>Pm × Nf</td>
<td>1.89 ± 0.13 a</td>
<td>7.21 ± 0.25 a</td>
<td>1759 ± 93 b</td>
<td>9.4 ± 0.4 a</td>
</tr>
<tr>
<td>F₁ crosses</td>
<td>0</td>
<td>Nm × Nf</td>
<td>1.72 ± 0.08 a</td>
<td>7.62 ± 0.31 a</td>
<td>1990 ± 49 a</td>
<td>10.3 ± 0.4 a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>F₁m × Nf</td>
<td>1.62 ± 0.14 a</td>
<td>6.71 ± 0.11 b</td>
<td>1659 ± 78 b</td>
<td>9.4 ± 0.6 a</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>F₁m × Nf</td>
<td>1.72 ± 0.19 a</td>
<td>7.01 ± 0.09 a</td>
<td>1693 ± 80 b</td>
<td>9.8 ± 0.2 a</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>Nm × F₁f</td>
<td>1.60 ± 0.24 a</td>
<td>6.22 ± 0.49 b</td>
<td>1520 ± 56 b</td>
<td>9.6 ± 0.4 a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>F₁m × F₁f</td>
<td>1.78 ± 0.16 a</td>
<td>6.22 ± 0.25 b</td>
<td>1464 ± 85 b</td>
<td>9.1 ± 0.4 b</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>Nm × F₁f</td>
<td>1.73 ± 0.11 a</td>
<td>6.29 ± 0.42 b</td>
<td>1499 ± 69 b</td>
<td>8.8 ± 0.3 b</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>F₁m × F₁f</td>
<td>1.85 ± 0.24 a</td>
<td>6.22 ± 0.59 b</td>
<td>1219 ± 30 c</td>
<td>8.8 ± 0.3 b</td>
</tr>
<tr>
<td>Chickpea diet</td>
<td>0</td>
<td>Nm × Nf</td>
<td>1.70 ± 0.09 a</td>
<td>7.40 ± 0.23 a</td>
<td>2011 ± 70 a</td>
<td>10.1 ± 0.4 a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Pm × Nf</td>
<td>1.80 ± 0.10 a</td>
<td>7.16 ± 0.11 a</td>
<td>1886 ± 84 b</td>
<td>9.3 ± 0.1 a</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>Pm × Nf</td>
<td>1.88 ± 0.11 a</td>
<td>6.66 ± 0.42 a</td>
<td>1727 ± 69 b</td>
<td>8.9 ± 0.3 a</td>
</tr>
<tr>
<td>F₁ crosses</td>
<td>0</td>
<td>Nm × Nf</td>
<td>1.69 ± 0.09 a</td>
<td>7.33 ± 0.24 a</td>
<td>2155 ± 74 a</td>
<td>9.8 ± 0.5 a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>F₁m × Nf</td>
<td>1.63 ± 0.17 a</td>
<td>7.09 ± 0.08 a</td>
<td>1649 ± 70 b</td>
<td>8.8 ± 0.4 a</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>F₁m × Nf</td>
<td>1.62 ± 0.16 a</td>
<td>6.90 ± 0.37 ab</td>
<td>1592 ± 84 b</td>
<td>8.6 ± 0.4 a</td>
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<tr>
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<td>Nm × F₁f</td>
<td>1.69 ± 0.13 a</td>
<td>6.34 ± 0.10 bc</td>
<td>1492 ± 59 bc</td>
<td>7.2 ± 0.2 c</td>
</tr>
<tr>
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<td>F₁m × F₁f</td>
<td>1.89 ± 0.17 a</td>
<td>6.40 ± 0.13 bc</td>
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<td>Nm × F₁f</td>
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<tr>
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<td>F₁m × F₁f</td>
<td>1.84 ± 0.20 a</td>
<td>5.83 ± 0.22 c</td>
<td>1099 ± 53 e</td>
<td>7.0 ± 0.3 c</td>
</tr>
</tbody>
</table>

* N, normal; P, treated parent; F₁, progeny of treated males; m, male, f, female. Means ± SE followed by the same letter in a column in each generation in case of each diet are not significantly different at P < 0.05 (ANOVA followed by LSD posttest); n = 10.
ample, the reduction in oviposition in P crosses, with respect to untreated crosses, was 6.2% at 100 Gy and 14.1% at 130 Gy on the chickpea diet. This reduction in oviposition was further increased in the F1 crosses (23-30% at 100 Gy, and 39-49% at 130 Gy on chickpea diet). Both doses significantly reduced F1 male and female longevity when the chickpea diet was used. Although the same trend was evident when moths were reared on castor leaves, the reduced longevity for males at the 100 Gy dose was not significant.

A significant, dose-dependent effect was observed on the mating success of P and F1 moths reared on both diets. The treated males were less successful at mating than untreated males (Table 3). For example, at 130 Gy on chickpea diet, the mating success of P males and F1 males was 76.4% and 72.7%, respectively, as compared with 89.6% in the control. The mating percentage was successful at mating than untreated males (Table 3). At 100 Gy on chickpea diet, the F1 male × N female cross gave 76.5% of the total eggs laid by controls (see Table 2) and resulted in 28.3% egg hatch as compared with 78.6% hatch in controls. Therefore, this cross exhibited 63.9% sterility, and 72.3% control of reproduction. For both the diets tested, the control of reproduction in F1 males (mated with normal females) was more than 71% at 100 Gy and about 83-87% at 130 Gy.

**Growth and Survival of F1 Progeny**

The developmental time of F1 eggs, larvae, pupae and adults reared on the two diets were significantly affected by the radiation treatment given to the male parent (crossed with a normal female). Their developmental rate was slower than that of the control progeny (Table 4); the total developmental time between eggs and adults was in-

<table>
<thead>
<tr>
<th>Diet</th>
<th>Dose (Gy)</th>
<th>Cross type</th>
<th>Mating frequency (no. ± SE)</th>
<th>Mating success¹</th>
<th>Fertility²</th>
<th>Corrected Sterility</th>
<th>Control of Reproduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castor leaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>Nm × Nf</td>
<td>1.7 ± 0.1 a</td>
<td>92.8 ± 2.2 a</td>
<td>89.7 ± 2.1 a</td>
<td>0</td>
<td>0</td>
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<td>Pm × Nf</td>
<td>1.9 ± 0.3 a</td>
<td>84.0 ± 3.1 b</td>
<td>52.3 ± 2.0 b</td>
<td>41.1 ± 1.6</td>
<td>48.0 ± 1.9</td>
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<td>130</td>
<td>Pm × Nf</td>
<td>1.9 ± 0.3 a</td>
<td>72.6 ± 3.8 c</td>
<td>44.8 ± 2.5 c</td>
<td>49.5 ± 2.8</td>
<td>56.6 ± 3.2</td>
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<td>88.9 ± 1.4 a</td>
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<td>80.9 ± 3.1 b c</td>
<td>31.6 ± 2.1 ed</td>
<td>64.4 ± 4.3</td>
<td>71.7 ± 4.8</td>
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<td>Nm × F1f</td>
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<td>73.2 ± 2.2 ed</td>
<td>44.5 ± 2.9 b</td>
<td>49.9 ± 3.3</td>
<td>59.4 ± 3.9</td>
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<td>F1m × F1f</td>
<td>1.7 ± 0.3 a</td>
<td>71.4 ± 2.9 d</td>
<td>28.3 ± 3.1 d</td>
<td>68.2 ± 7.4</td>
<td>76.8 ± 8.4</td>
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<tr>
<td></td>
<td>130</td>
<td>F1m × F1f</td>
<td>2.0 ± 0.2 a</td>
<td>75.6 ± 3.5 bc</td>
<td>21.8 ± 1.8 de</td>
<td>75.5 ± 6.2</td>
<td>82.8 ± 6.8</td>
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<td>Nm × F1f</td>
<td>1.7 ± 0.3 a</td>
<td>66.0 ± 2.4 ef</td>
<td>35.9 ± 2.2 c</td>
<td>59.6 ± 3.7</td>
<td>71.0 ± 4.4</td>
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<td></td>
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<td>F1m × F1f</td>
<td>1.9 ± 0.5 a</td>
<td>59.0 ± 4.0 f</td>
<td>20.2 ± 1.1 e</td>
<td>77.3 ± 4.2</td>
<td>86.7 ± 4.7</td>
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<tr>
<td>Chickpea diet</td>
<td></td>
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<td></td>
<td></td>
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<td>Nm × Nf</td>
<td>1.7 ± 0.1 a</td>
<td>89.2 ± 2.2 a</td>
<td>84.4 ± 2.9 a</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Pm × Nf</td>
<td>1.8 ± 0.3 a</td>
<td>81.5 ± 2.2 b</td>
<td>46.2 ± 4.1 b</td>
<td>41.2 ± 3.7</td>
<td>48.6 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>Pm × Nf</td>
<td>1.8 ± 0.2 a</td>
<td>76.4 ± 3.2 b</td>
<td>41.6 ± 2.1 b</td>
<td>47.1 ± 2.4</td>
<td>57.6 ± 2.9</td>
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<td>F1 crosses</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>Nm × Nf</td>
<td>1.7 ± 0.2 a</td>
<td>89.6 ± 2.4 a</td>
<td>78.6 ± 3.0 a</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>F1m × Nf</td>
<td>1.5 ± 0.3 a</td>
<td>84.0 ± 3.1 ab</td>
<td>28.3 ± 2.3 c</td>
<td>63.9 ± 5.4</td>
<td>72.4 ± 6.1</td>
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<tr>
<td></td>
<td></td>
<td>Nm × F1f</td>
<td>1.6 ± 0.1 a</td>
<td>75.4 ± 1.5 bc</td>
<td>43.2 ± 2.9 b</td>
<td>45.0 ± 3.0</td>
<td>59.4 ± 4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F1m × F1f</td>
<td>1.5 ± 0.1 a</td>
<td>65.9 ± 2.8 de</td>
<td>22.3 ± 2.0 ed</td>
<td>71.6 ± 6.4</td>
<td>80.4 ± 7.1</td>
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<tr>
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<td>F1m × F1f</td>
<td>2.1 ± 0.1 a</td>
<td>72.7 ± 3.1 ed</td>
<td>17.5 ± 1.8 de</td>
<td>77.8 ± 7.9</td>
<td>86.5 ± 8.8</td>
</tr>
<tr>
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<td></td>
<td>Nm × F1f</td>
<td>2.4 ± 0.1 a</td>
<td>60.9 ± 1.3 ef</td>
<td>26.7 ± 2.2 c</td>
<td>66.1 ± 5.4</td>
<td>78.5 ± 6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F1m × F1f</td>
<td>2.0 ± 0.2 a</td>
<td>56.1 ± 3.1 f</td>
<td>14.6 ± 2.1 e</td>
<td>81.4 ± 8.7</td>
<td>90.6 ± 8.1</td>
</tr>
</tbody>
</table>

¹N, normal; P, treated parent; F1, progeny of treated males; m, male, f, female.
²For statistical analysis by ANOVA, the percentage data were transformed using arcsine √x. Means ± SE followed by the same letter in a column in each generation in case of each diet are not significantly different at P < 0.05 (ANOVA followed by LSD posttest); n = 10.
TABLE 4. DEVELOPMENTAL PROFILE AND SURVIVAL OF F1 PROGENY OF IRRADIATED *SPODOPTERA LITURA* REARED ON TWO DIETS.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Dose (Gy)</th>
<th>Developmental period (days)</th>
<th>% pupation</th>
<th>Adult eclosion</th>
<th>Growth index</th>
<th>Sex ratio (M:F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Egg</td>
<td>Larva</td>
<td>Pupa</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Castor leaf</td>
<td>0</td>
<td>3.6 ± 0.1 a</td>
<td>15.8 ± 0.3 a</td>
<td>7.9 ± 0.2 a</td>
<td>27.2 ± 0.4 a</td>
<td>89.3 ± 4.6 a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3.9 ± 0.1 b</td>
<td>16.7 ± 0.5 ab</td>
<td>8.5 ± 0.2 ab</td>
<td>29.2 ± 0.6 ab</td>
<td>71.2 ± 2.7 b</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>4.2 ± 0.1 b</td>
<td>17.3 ± 0.4 b</td>
<td>8.6 ± 0.2 b</td>
<td>30.1 ± 0.8 b</td>
<td>62.8 ± 2.9 c</td>
</tr>
<tr>
<td>Chickpea diet</td>
<td>0</td>
<td>3.6 ± 0.1 a</td>
<td>16.2 ± 0.4 a</td>
<td>8.4 ± 0.2 a</td>
<td>28.3 ± 0.7 a</td>
<td>90.2 ± 3.3 a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3.9 ± 0.1 b</td>
<td>17.9 ± 0.4 b</td>
<td>8.5 ± 0.3 ab</td>
<td>30.7 ± 1.0 ab</td>
<td>68.4 ± 4.5 b</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>4.0 ± 0.1 b</td>
<td>18.7 ± 0.3 b</td>
<td>9.1 ± 0.3 b</td>
<td>31.5 ± 0.3 b</td>
<td>58.8 ± 2.1 b</td>
</tr>
</tbody>
</table>

1 Observed in group of 25 individuals comprising each replicate, n = 7. For statistical analysis by ANOVA, the percentage data were transformed using arcsine √x.

2 Growth index = % adult eclosion/total developmental period.

Means ± SE followed by the same letter in a column within each diet are not significantly different at P < 0.05 (ANOVA followed by LSD posttest).
creased by about 7-9% at 100 Gy and 10-13% at 130 Gy. The F₁ insects experienced significantly more larval and pupal mortality than the controls, and also exhibited a higher rate of pupal and adult malformation than the controls. The proportion of F₁ insects surviving to adults decreased with the increasing dose on both diets tested. About 61-64% of the F₁ progeny of 100 Gy treated male parents emerged as adults, whereas 79-82% adults emerged in the controls. The F₁ growth index was significantly decreased according to the dose of radiation administered to the male parent. The sex ratio of the F₁ generation was significantly skewed towards males as a result of the radiation treatments. For example, F₁ females constituted about 41% of the population at 100 Gy and about 50% of the population in the control.

Population Characteristics

Increase in the total developmental period of F₁ progeny delayed the commencement of oviposition with further debilitating effects on adult survival, life expectancy, oviposition rate and female births with respect to the pivotal age group. The reproductive rate (R₀) was significantly decreased as a consequence of radiation treatment, with the effect being more pronounced in F₁ crosses than in P crosses (Table 5). For instance, R₀ for untreated insects was 785.2 on castor and 740.2 on chickpea diet. On chickpea diet, R₀ was decreased by 16.4% at 100 Gy and 36.6% at 130 Gy in P generation. R₀ was further reduced by about 70% at 100 Gy and about 80% at 130 Gy in F₁ crosses, with respect to the control.

The mean generation time (Tₑ) was 31.3 days on castor and 32.3 days on chickpea diet for untreated insects. In F₁ crosses, Tₑ was significantly higher, indicating a protraction of 2-4 days as compared with the control. The Tₑ delay showed a positive correlation with doses of radiation (Table 5). The intrinsic rate of increase (rₑ) in untreated insects was 0.21 on castor and 0.20 on chickpea diet. The effect of irradiation on the intrinsic rate of increase was not apparent in P crosses, but was significant in F₁ crosses, where there was a reduction in the rₑ value (23-25% at 100 Gy and 31-36% at 130 Gy). Irradiation also affected the finite rate of increase of this insect population. For example, on chickpea diet at 130 Gy, it was reduced to 1.15 in F₁ male crosses from 1.23 in the control. A similar pattern was observed on castor leaves.

Embryonic Development of F₁ and F₂ Zygotes

In F₁ and F₂ generations, the embryonic development was disrupted at different stages that reflected a specific pattern of radiation-mediated lethality in P and F₁ moths (Table 6). The reduced egg hatch from P and F₁ males crossed with normal females may have been caused by infertility of eggs or embryonic death. Certain fertilized eggs exhibited embryonic development but were incapable of hatching. The development of such zygotes was arrested at different levels of embryogenesis showing partial to complete embryonation. Some of these eggs could reach the "black head" stage wherein, blackish brown colored head cuticle of the pharate 1st instar larva was visible through translucent chorion of the egg. This type of egg was categorized as an unhatched embryonated egg. At 130 Gy on the chickpea diet, early mortality (EM) of F₁ eggs (derived from P crosses) was 36.9% with no detectable embryonic development. Late embryonic lethality (LEL) was 22.4%, mainly due to embryonic stages blocked at cleavage or early development stage. In F₁ eggs (from F₁ crosses) at the same dose, EM constituted 16.8% and LEL constituted 65.8%. Notably, more than 75% of LEL was restricted to germ band and embryo stages (Table 6, Fig. 1). The formation of embryos was significantly reduced in F₁ eggs (100 Gy and 130 Gy), but was not statistically different from the controls in F₁ eggs at both doses. However, the percentage zygotic viability in P and F₁ crosses was significantly reduced by the dose of radiation (Table 6). EM appeared predominantly in F₁ eggs, whereas F₂ eggs showed sterility mainly due to LEL.

DISCUSSION

The phenomenon of F₁ sterility in S. litura was examined over a range of substerilizing doses (Seth & Sehgal 1993). As a result of the reproductive performance and somatic damage caused by the radiation, we selected two doses (100 Gy and 130 Gy) for a more in-depth study. The reduced reproductive performance of the treated moths resulted from the combined effects of reduced longevity, fecundity, mating success, and fertility. The mating percentage was more adversely affected in F₁ females crossed with normal males and in F₁ self crosses as compared with the F₁ males paired with normal females. A greater reduction in reproductive performance was observed at the 130 Gy dose than the 100 Gy dose. The effects of radiation dose on reproductive performance were not significantly influenced by diet. Similar debilitating effects of irradiation on the reproduction of moths have been reported by several workers (North & Holt 1968; Proshold & Bartell 1970; Cheng & North 1972; North 1975; LaChance et al. 1976; Carpenter et al. 1983; LaChance 1985; Sallam & Ibrahim 1993; Omar & Mansor 1993; Ismail 1994; Carpenter et al. 1987; Makee & Saour 1997). Poor reproductive performance and low fertility in treated S. litura could be due to one or more of the following reasons: (i) poor ability to mate (El-Sayed & Graves 1969), (ii) failure to produce and transfer as many spermatophores as normal males (Rule et al. 1965; Flint & Kressin 1969), (iii) transfer spermato-
<table>
<thead>
<tr>
<th>Diet</th>
<th>Dose (Gy)</th>
<th>Cross type</th>
<th>Net reproductive rate$^2$</th>
<th>Mean generation length (days)</th>
<th>Innate capacity for increase $^3$</th>
<th>Finite rate of increase $(\lambda)$ = antilog$_{e}$rm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$R_0 = \sum l_x m_x$</td>
<td>$T_c = \sum l_x m_x/R_0$</td>
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<td>rm = log$_{e}R_0/T_c$</td>
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<td>Castor leaf</td>
<td>0</td>
<td>Nm × Nf</td>
<td>785.2 ± 27.4 a</td>
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<td>Pm × Nf</td>
<td>602.4 ± 25.4 b</td>
<td>18643.5 ± 774.2 b</td>
<td>30.94 a</td>
<td>0.2068 a</td>
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<td>Fm × Nf</td>
<td>227.4 ± 10.2 c</td>
<td>7489.2 ± 292.0 c</td>
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<td>0.1648 bc</td>
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<td>Nm × Ff</td>
<td>212.8 ± 10.3 c</td>
<td>7262.3 ± 355.1 c</td>
<td>34.12 c</td>
<td>0.1571 bc</td>
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<td>Pm × Nf</td>
<td>591.4 ± 27.0 b</td>
<td>18279.8 ± 835.0 b</td>
<td>30.90 a</td>
<td>0.2065 bc</td>
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<td>Fm × Nf</td>
<td>167.7 ± 7.6 d</td>
<td>5805.4 ± 150.2 d</td>
<td>34.62 c</td>
<td>0.1479 cd</td>
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<td>Nm × Ff</td>
<td>161.8 ± 7.3 d</td>
<td>5626.6 ± 153.1 d</td>
<td>34.76 c</td>
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<td>111.1 ± 12.1 e</td>
<td>3819.7 ± 110.7 e</td>
<td>34.36 c</td>
<td>0.1371 d</td>
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<td>Chickpea diet</td>
<td>0</td>
<td>Nm × Nf</td>
<td>740.2 ± 38.7 a</td>
<td>23913.1 ± 738.8 a</td>
<td>32.30 a</td>
<td>0.2045 a</td>
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<td>Pm × Nf</td>
<td>618.1 ± 28.5 b</td>
<td>19898.6 ± 693.4 b</td>
<td>32.19 a</td>
<td>0.1996 a</td>
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<td>235.6 ± 21.4 d</td>
<td>8207.3 ± 220.7 d</td>
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<td>0.1568 b</td>
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<td>Nm × Ff</td>
<td>220.5 ± 19.3 d</td>
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<td>34.76 bc</td>
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<td>469.0 ± 36.7 c</td>
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<td>152.0 ± 13.1 e</td>
<td>5461.3 ± 120.1 f</td>
<td>35.92 c</td>
<td>0.1399 c</td>
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<td></td>
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<td>Nm × Ff</td>
<td>145.0 ± 11.1 e</td>
<td>5226.3 ± 101.9 f</td>
<td>35.97 c</td>
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<td>101.9 ± 8.9 f</td>
<td>3602.7 ± 76.4 g</td>
<td>35.34 c</td>
<td>0.1308 c</td>
</tr>
</tbody>
</table>

1N, normal; P, treated parent; F1, progeny of treated males; m, male, f, female.

2$lx$, female survival; $mx$, female offsprings produced per unit time; $x$, pivotal age.

Means ± SE followed by the same letter in a column within each diet are not significantly different at $P < 0.05$ (ANOVA followed by LSD posttest); $n = 7$. 
<table>
<thead>
<tr>
<th>Diet</th>
<th>Dose (Gy)</th>
<th>Cross type</th>
<th>Eggs per female (no.)</th>
<th>Eggs showing (%)</th>
<th>Embryonic stage of unhatched eggs</th>
<th>Embryo formation (%)</th>
<th>Unhatched embryonated eggs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No development</td>
<td></td>
<td>Stage I</td>
<td>Stage II</td>
<td>Stage III</td>
</tr>
<tr>
<td>Castor leaf</td>
<td>0</td>
<td>Nm × Nf</td>
<td>2088 (88.9%)</td>
<td>6.5 ± 0.2</td>
<td>2.8 ± 0.1</td>
<td>0.9 ± 0.0</td>
<td>0.7 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Pm × Nf</td>
<td>1847 (52.3%)</td>
<td>26.3 ± 1.7</td>
<td>13.4 ± 0.5</td>
<td>2.5 ± 0.1</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>Pm × Nf</td>
<td>1755 (31.6%)</td>
<td>11.5 ± 0.6</td>
<td>8.4 ± 0.3</td>
<td>7.6 ± 0.4</td>
<td>37.0 ± 2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F₁,m × Nf</td>
<td>1775 (44.8%)</td>
<td>33.1 ± 1.6</td>
<td>9.4 ± 0.2</td>
<td>6.4 ± 0.4</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F₁,m × Nf</td>
<td>1464 (21.8%)</td>
<td>13.2 ± 0.7</td>
<td>7.5 ± 0.2</td>
<td>10.3 ± 0.6</td>
<td>39.4 ± 2.2</td>
</tr>
<tr>
<td>Chickpea diet</td>
<td>0</td>
<td>Nm × Nf</td>
<td>2155 (78.6%)</td>
<td>11.2 ± 0.7</td>
<td>5.0 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Pm × Nf</td>
<td>1886 (46.2%)</td>
<td>28.9 ± 1.5</td>
<td>14.1 ± 0.7</td>
<td>4.2 ± 0.2</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>Pm × Nf</td>
<td>1750 (30.7%)</td>
<td>11.6 ± 0.6</td>
<td>7.7 ± 0.4</td>
<td>9.6 ± 0.4</td>
<td>36.5 ± 1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F₁,m × Nf</td>
<td>1727 (41.6%)</td>
<td>36.9 ± 1.4</td>
<td>14.3 ± 0.8</td>
<td>3.1 ± 0.1</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F₁,m × Nf</td>
<td>1394 (17.5%)</td>
<td>16.8 ± 1.0</td>
<td>5.5 ± 0.3</td>
<td>6.5 ± 0.2</td>
<td>47.0 ± 2.3</td>
</tr>
</tbody>
</table>

1N, normal moth; P, treated parent; F₁, progeny of treated males; m, male; f, female.
2Ovipositional data computed out of mated females; data in parentheses represent % egg hatch.
3Data represent % inviable eggs in the particular stage out of total eggs laid; data in parentheses represent % inviable eggs in the stage out of total unhatched eggs; Stage I, cleavage or early developmental phase; Stage II, germ band phase; Stage III, embryonic phase.
4Percentage data were transformed using arcsine \(\sqrt{x}\) before ANOVA, but data shown are back transformations; means ± SE followed by the same letter in a column in case of each diet are not significantly different at \(P < 0.05\) (ANOVA followed by LSD post-test); n = 7; each replicate comprising of 80-100 eggs.
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...phores that contain little or no sperm; (iv) abnormal sperm structure, which fails to fertilize the eggs (Ashrafi & Roppel 1973), or (v) inheritance of special chromosome rearrangements (LaChance 1985; Anisimov et al. 1989).

The developmental rate of *F*₁ larvae originating from the crosses between treated males and normal females of *S. litura* was slower than that of controls, and this delay in development was greater when males were treated with 130 Gy. Because insect development and differentiation are controlled by hormones (Gilbert 1964), the protracted development of *F*₁ larvae might be due to alteration in hormonal or enzymatic production caused by chromosomal rearrangements, as indicated by Proshold & Bartell (1970, 1972). The *F*₁ growth index showed a decrease as a consequence of irradiation of male parents. The sex ratio in *F*₁ generation was skewed towards male as compared with a 1:1 ratio in the control group of insects reared on castor leaves as well as chickpea diet. Sex distortion appears to be general phenomenon occurring in the progeny of irradiated male lepidopterans (Proverbs 1962; Carpenter et al. 1986), probably resulting from the expression of recessive lethal mutations on the single *Z* chromosome in females but not in *ZZ* males (Marec 1990).

The effect of irradiation on population characteristics was not observed in the *F*₁ generation, especially in case of mean generation time, survival and finite rate of increase, because the treatment was given in the adult stage and it could manifest its impact only in the first filial generation. However, irradiation significantly affected population characteristics in *F*₂ and *F*₃ generations, particularly the production of females. The production of more males than females, and the reduction in female longevity due to sublethal radiation ultimately reduced the net reproductive rate and the potential rate of increase.

Our data suggest that both inability of irradiated males to fertilize eggs and dominant lethal mutations (DLM) induced in sperm were responsible for reduced egg viability in *P* crosses. This is because both unhatched embryonated eggs (LEL) and eggs showing no development (EM) were induced by irradiation. The eggs marked in the category of early mortality (EM) could be unfertilized, indicating physiological damage in irradiated males that reduced the ability to transfer sperm. Alternatively, these eggs could have ceased their development at an early stage due to induced DLM (see discussion in Marec et al. 1999). Whereas in *F*₁ crosses, sterility was largely a result of induced chromosomal aberrations. Therefore, eggs from these crosses showed embryonic development but were unable to hatch, indicating late egg lethality (LEL) as the main cause of egg inviability. Similar findings were made by Proshold & Bartell (1970) in the tobacco budworm, *Heliothis virescens*, and by Bugbio (1988) in *Chilo partellus*. LEL was clearly expressed by *F*₁ males, whereas EM was expressed less, unlike in *P* males of the codling moths (Anisimov et al. 1989). Similarly, Seth & Reynolds (1993) reported that the main cause of sterility in *F₁* generation of *Manduca sexta* was the induction by radiation of lethal mutations that arrested the development in late embryonic life. Also, Marec et al. (1999) observed sterile eggs (eggs with early embryonic lethality as well as unfertilized eggs) and inviable eggs (in which embryos died during different stages of embryogenesis) while studying the gamma radiation induced sterility combined with genetic sexing in *Ephestia kuehniella*.

In view of the overall reproductive performance of *P* and *F₁* moths, and developmental be-

![Fig. 1. Effect of substerilizing gamma-radiation doses on the development of eggs derived from the crosses of treated *Spodoptera litura* males and their *F₁* progeny.](image-url)

Bars show mean ± SE; EM, early mortality in eggs; LEL, late embryonic lethality. Standard error of the mean (SE). N, normal moth; P, treated parent; *F₁*, progeny of treated males; m, male; f, female. ns, non-significant at P < 0.05; *, significant at P < 0.05; **, significant at P < 0.01 (t-test conducted between and LEL in each cross).
behavior of F1 insects, our findings suggest the use of 100 Gy as an effective dose for the suppression of Spodoptera litura populations by the release of partially sterile males. This dose gives better viability of F1 insects among normal population and thus, a higher inherited sterility effect. Although the higher dose (130 Gy) could be more effective in P crosses, it would less effective in the F1 crosses. It is worth noting that degree of irradiation impact on the net reproductive rate of P and F1 crosses observed in their life tables was almost similar to the control of reproductive potential calculated. Proper sperm competition is required for the treated insects to induce reproductive suppression of the feral pest population by using F1 sterility. Studies on sperm formation, transfer and their competitiveness are in progress (RKS).

ACKNOWLEDGMENTS
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EFFECT OF A SUBSTERILIZING DOSE OF RADIATION ON THE MATING COMPETITIVENESS OF MALE AND ON THE MATING PROPENSITY OF FEMALE HELICOVERPA ARMIGERA (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

In this article we report the results of experiments conducted with Helicoverpa armigera (Hubner) to determine the effects of a substerilizing dose of gamma radiation (100 Gy) on the mating competitiveness of treated males and the effect on the mating propensity of females with which they mate. Mating competitiveness of treated and untreated male moths was measured at two different release ratios inside field-cages in a cabbage field. A 1:1 and a 4:1 ratio were used while keeping a constant density of moths per cage. The mean number of matings recorded was not significantly different at either ratio, suggesting that treated males of this species are equally as competitive as their untreated counterparts. In the mating propensity studies, virgin female H. armigera were first mated to treated or untreated males and then re-exposed to untreated males 24 hours later. No statistical differences were found in the number of females that re-mated from either group. Thirty point eight percent of the females first mated with treated males and 29.17% of the females first mated to untreated males re-mated in this study. When both types of females were re-exposed to untreated males in the same field-cage, a higher percentage (38.3%) of females that had initially mated with a treated male re-mated than those initially mated with an untreated male (31.7%), although the differences were not significant.

Key Words: F₁ sterility, partial sterility, remating, corn earworm

RESUMEN

En este artículo se reportan los resultados de experimentos con la especie Helicoverpa armigera. El objetivo de estos experimentos era determinar el efecto de una dosis sub-esterali-

zante de radiación gamma (100 Gy) en la competitividad de copula de machos irradiados y el efecto indirecto sobre la propensidad de re-copula de las hembras con las cuales copularon estos machos. La competitividad de machos fertiles e irradiados se evaluó a dos diferentes tasas de liberación dentro de jaulas de campo colocadas en una plantacion de repollo. Las tasas de liberacion utilizadas fueron 1 macho:1 hembra y 4 machos:1 hembra mientras se mantiene el número de insectos constante dentro de las jaulas. El número promedio de copulas que se recobraron en cada jaula no fue significativamente distinto, lo que sugiere que los machos irradiados pueden competir efectivamente con los machos fertiles. En estudios sobre propensidad de re-copula, hembras virgenes fueron apareadas con machos fertiles o irradiados y luego fueron re-expuestas a machos fertiles despues de 24 horas. No se encontró una diferencia estadística en el porcentaje de re-copulas entre los grupos de hembras. Treinta punto ocho porciento de las hembras que primero se aparearon con machos irradiados re-copularon despues de 24 horas mientras que 29.17% de las hembras primero apareadas con machos fertiles re-copularon despues de 24 horas. Cuando se expusieron estas hembras (inicialmente apareadas con distintos tipos de machos) simultaneamente a machos fertiles despues de 24 horas, un porcentaje mas alto (38.3%) de hembras inicialmente apareadas con machos irradiados se re-aparearon que las hembras apareadas con machos fertiles (31.7%), pero las diferencias no fueron estadisticamente significativas.

The corn earworm, Helicoverpa armigera (Hubner), is one of the most destructive pests of agricultural crops in the Philippines. It is a highly polyphagous insect, feeding on 84 different plant species (Gabriel 1997). One environmentally acceptable control strategy currently being explored for the control of this insect is the release of irradiated partially sterile males capable of transferring sterility to the next generation. The advantages of using partial, inherited or F₁ sterility over classical sterile insect release methods is that partial sterility produces a more competitive insect that will actively mate with wild females and, as such, will effectively introduce heritable sterility into the native population (Mastro & Schwalbe 1988). Inherited sterility has been investigated in a number of lepidopteran pests such as Helicoverpa zea (Carpenter & Gross 1993), Spodoptera frugiperda (Carpenter et al. 1997), Manduca sexta (Seth & Reynolds 1993) and Ephestia kuehniella (Marec et al. 1999).

Helicoverpa armigera, a closely related species of H. zea (Laster & Hardee 1995), is a potential candidate for population suppression by means of
the F\textsubscript{1} sterility technique. Ocampo et al. (1996) were able to induce partial sterility in this species by irradiating pharate adult males with 100 and 150 Gy. When the treated males were mated to untreated females, female fertility was greatly affected. Percentage egg hatch in females mated to 100 Gy or 150 Gy treated males was only 40-60% while that of females mated to untreated males was 80-100% (V. Ocampo, unpublished data). In a related study, Bella et al. (1997) reported that when 100 Gy was used to treat \textit{H. armigera} males, the dose caused chromosomal translocations in 86% of the spermatocytes examined while 150 Gy caused chromosomal translocations in 98% of the spermatocytes in the testes of treated males. Similarly, 100 Gy has been shown to be most efficacious in the coding moth, \textit{Cydia pomonella} (Anisimov 1993) and the fall armyworm, \textit{Spodoptera frugiperda} (Carpenter et al. 1997). In these species, the dose induced a high level of inherited sterility in the F\textsubscript{1} generation without reducing competitiveness of parental males treated.

Mating competitiveness is essential to the effectiveness of a sterile insect release program. However, the refractory period of wild females must not be adversely affected by mating with the released males (Snow 1988). An irradiated male must be able to transfer a full complement of sperm and accessory gland fluid to the female, and this complement must reach the spermatheca to elicit the female refractory period. Giebułtowicz et al. (1990) showed that presence of sperm in the spermatheca is necessary to induce the switch from virgin to mated behavior in the gypsy moth, \textit{Lymantria dispar}. During the refractory period, pheromone production by the female remains low, oviposition behavior is triggered and the female will refrain from remating. If sperm and accessory gland fluid do not reach the spermatheca, pheromone production will increase and the female will resume calling for mates.

In this article we report the results of experiments conducted with \textit{H. armigera} to determine the effects of 100 Gy of gamma radiation on the mating competitiveness of treated males and the effect on the mating propensity of the females with which they mate.

**Materials and Methods**

\textit{Heliothis armigera} used in these experiments came from a laboratory colony maintained at the Department of Entomology, University of the Philippines Los Baños. Larvae were reared under ambient laboratory conditions (27 ± 2°C) in 50 ml plastic cups and fed a soybean-corn based diet as described in Ocampo et al. (2000). Pupae were collected and sexed and stored at ambient conditions until needed. Late pupae containing pharate males were irradiated at a dose of 100 Gy using a Cobalt\textsuperscript{60} Gammacell 220 irradiator delivering a dose of 4 Gy/min. Treated and untreated pupae were held separately until adult emergence. Newly emerged (<24 h-old) virgin male and female moths were used in all mating experiments.

Mating Competitiveness of Males

Male competitiveness was assessed in field-cages (\(n = 4\); 6m × 5m × 2.15m) placed over cabbage plants within 1-ha cabbage field. Three types of males were used in the experiments. Males treated with 100 Gy (T\(\delta\)), as indicated above; F\(\textsubscript{1}\) progeny males (TF\(\delta\)), obtained from crossing treated (100 Gy) P\(\delta\) males to untreated virgin females, and untreated males (N\(\delta\)). In order to distinguish the male “types” the wing tips of treated and F\(\textsubscript{1}\) males were differentially colored with a felt-tip marker and untreated males were left unmarked. Moths were allowed to acclimate to field conditions for 3 hours (from 1700 hours Philippine Standard Time (PST) until they were released at 2000 hours PST). Males and females were released at opposite ends of the cages. In each cage mating pairs were collected once every hour for five hours, from 2100 to 0200 hours PST, coinciding with the peak of mating activity as reported by Morallo-Rejesus and Alcala-Carilo (1981). These workers also report that copulation lasted an average of 30 minutes.

Mating competitiveness was measured at two different release ratios. A 1:\(\delta:\varphi\) ratio, maintaining equal numbers of male “types” while keeping a density of 60 moths per cage. Field-cages in this study received 30 N\(\varphi\) each, and the following male treatments: 30 T\(\delta\); 30 N\(\delta\); 15 T\(\delta\) + 15 N\(\delta\); 10 T\(\delta\) + 10 TF\(\delta\) + 10 N\(\delta\). The next set of experiments was conducted at a 4:\(\delta:\varphi\) ratio, while keeping a constant density of 50 moths per cage. Field-cages in this study received 10 N\(\varphi\) each, and the following male treatments: 40 T\(\delta\); 40 N\(\delta\); 30 T\(\delta\) + 10 N\(\delta\). All combinations were replicated once per night for four nights. Male types with the highest number of recorded matings were considered the most competitive. Data were subjected to analysis of variance and differences between means were tested for significance using Waller-Duncan’s K-ratio t-test (Ott 1993).

Mating Propensity of Females

Mating propensity was assessed inside field-cages (see above). Insects were released into separate cages in the following combinations: 100 untreated \textit{H. armigera} females (N\(\varphi\)) + 100 treated males (T\(\delta\)-100 Gy), and 100 N\(\varphi\) + 100 untreated (N\(\delta\)) males. Pairs in copula were collected once per hour as described above. Females that mated with treated (N\(\varphi\)-T\(\delta\)) and untreated (N\(\varphi\)-N\(\delta\)) males were differentially colored using a felt-tip marker as above. On the following
evening, the mated females (from both groups) were given the opportunity to remate with untreated males (N-Day) by releasing them into field-cages in the following combinations (at 1♀:1♂ ratio): 30 N♀·N♂, 30 N♀·T♂, 30 N♀·T♂, 30 N♀·T♂, 30 N♀·T♂, 15 N♀·N♂, 15 N♀·T♂, 15 N♀·T♂, 30 N♀. Pairs in copula were collected as above. All treatments were replicated four times. Females were then dissected to determine the number of spermatophores in the bursa copulatrix. A twice-mated female should have two spermatophores to confirm two successful matings/copulations.

RESULTS

Mating Competitiveness of Males

The results of the mating competitiveness tests are summarized in Table 1. At the 1♀:1♂ ratio, the mean number of mating pairs collected in cages containing treated (T♂) and untreated (N♂) males was not significantly different (F = 1.07, d.f. = 6, α = 0.05). In addition, when both male types were together in the same cage (30 N♀·T♂), both T♂ and N♂ males appeared to be equally competitive. The mean number of matings for N♀ was 4.25 and this number was 3.76 for T♂ (F = 0.17, d.f. = 6, α = 0.05). Mating competitiveness of F♂ male offspring (TF1♂) was also evaluated. Results show that the mean number of matings for TF1♂ was 4.76, which did not differ statistically from the number of matings with other male types (F = 0.21, d.f. = 9, α = 0.05).

When experiments were conducted at a 4♀:1♂ ratio, there was no significant difference in the mean number of mating pairs collected in cages containing T♂ (7.00) or N♂ (6.00) males (F = 0.46, d.f. = 6, α = 0.05). When three times more treated males than untreated males were released into the field-cage (10 N♀·10 N♂·T♂) the number of observed matings for the treated males significantly exceeded the expected number (F = 216, d.f. = 6, α = 0.05).

Mating Propensity of Females

The results of the mating propensity studies, where virgin female H. armigera were first mated to treated or untreated males and then re-exposed to untreated males 24 hours later are shown in Table 2. Thirty point eight percent of the females first mated with treated males (N♀·T♂) and 29.17% of the females first mated to untreated (N♀·N♂) males re-mated in this study. The difference was not statistically significant (F = 0.04, d.f. = 6, α = 0.05). When both types of females were re-exposed to untreated males in the same field-cage (15 N♀·T♂ + 15 N♀·N♂), a higher percentage (38.3%) of females that had initially mated with a treated male re-mated than those initially mated with an untreated male (31.7%), although the differences were not significant (F = 0.25, d.f. = 6, α = 0.05). Carpenter et al. (1987) report similar trends for Helicoverpa zea, a closely related species to H. armigera.

DISCUSSION

An important concern in sterile insect release programs is that treated males destined for release retain the ability to perceive/orient to pheromone signals from females and, as such, are able to compete with the wild males in locating and mating with calling females in the field. Data presented herein suggest that partially sterilized male H. armigera and their male progeny were as competitive as untreated males in seeking and securing mates in a field-cage situation. Males treated with 100 Gy, F♂ males (from 100 Gy treated fathers) and untreated males appeared to be equally as competitive in mating with virgin females when placed together in field-cages. It appears that a dose of 100 GY of gamma radiation does not cause sufficient physiological damage to alter male mating behavior, but induces sufficient genetic damage in the spermatocytes to reduce sperm viability, as reported by Bella et al. (1997).

Table 1. Mating Competitiveness of Treated (100 GY-T♂), Untreated (N♂) and F1 Offspring (TF1♂) of Helicoverpa armigera Males for Virgin Females Inside Field-Cages (6M·5M·2.15M) in a Cabbage Field at UPLB, Laguna, Philippines, 1996.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Number and type of moths</th>
<th>N × N</th>
<th>N × T</th>
<th>N × TF1♂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1♀:1♂</td>
<td>30 N♀·30 N♂ or 30 N♀·30 T♂</td>
<td>10.00 ± 2.00a</td>
<td>12.75 ± 4.92 a</td>
<td>-2</td>
</tr>
<tr>
<td></td>
<td>30 N♀·15 T♂ + 15 N♂</td>
<td>4.25 ± 2.22a</td>
<td>3.76 ± 0.96 a</td>
<td>-2</td>
</tr>
<tr>
<td></td>
<td>30 N♀·10 T♂ + 10 TF1♂ + 10 N♂</td>
<td>6.25 ± 4.50a</td>
<td>5.25 ± 3.30 a</td>
<td>4.76 ± 1.26 a</td>
</tr>
<tr>
<td>4♀:1♂</td>
<td>10 N♀·40 N♂ or 10 N♀·40 T♂</td>
<td>6.00 ± 2.31a</td>
<td>7.00 ± 1.82 a</td>
<td>-2</td>
</tr>
<tr>
<td></td>
<td>10 N♀·30 T♂ + 10 N♂</td>
<td>0.50 ± 0.58a</td>
<td>6.50 ± 0.58 b</td>
<td>-2</td>
</tr>
</tbody>
</table>

1 Means ± SD on the same row followed by the same letter are not significantly different (K ratio = 500).
2 Not applicable.
and refractory period. Thus, the type of male with which the female mated during mating affects the quality of the sperm complement and accessory fluid transferred by males. These findings agree with an earlier study (Ocampo et al. 2000) that found 31-36% of mated females re-mated regardless of the type of male with which they had first mated (Table 2). In our experiments, about 30% of reared insects in their ability to survive under field conditions.

Helicoverpa armigera has a tendency to mate more than once. In our experiments, about 30% of mated females re-mated regardless of the type of male with which they had first mated (Table 2). These findings agree with an earlier study (Ocampo et al. 2000) that found 31-36% of H. armigera females mated twice. Our data also suggests that females mated to treated (100 Gy) males were no more attractive to untreated males on the night following the first mating than were females mated to untreated males. One explanation for this finding might be that there is no discernible difference in the quality of the sperm complement and accessory fluid transferred by untreated and treated males during mating. Thus, the type of male with which the female mates first does not affect her mating propensity and refractory period.

ACKNOWLEDGMENTS

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### Table 2. Mating propensity of once-mated female Helicoverpa armigera mated to either untreated (N♂) or treated (N♂-T) males and exposed to untreated males after 24 hours inside field-cages (6M×5M×2.15M) in a cabbage field at UPLB, Laguna, Philippines, 1996.

<table>
<thead>
<tr>
<th>Moths released into field-cages</th>
<th>N♂-N♂ × N♂</th>
<th>N♂-N♂ × N♂</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 N♂-N♂:30 N♂ or 30 N♂-T:30 N♂</td>
<td>8.75 ± 3.86 (29.17) a</td>
<td>9.25 ± 2.99 (30.83) a</td>
</tr>
<tr>
<td>15 N♂-T:15 N♂-N♂:30 N♂</td>
<td>4.75 ± 2.87 (31.67) a</td>
<td>5.75 ± 2.75 (38.33) a</td>
</tr>
</tbody>
</table>

Means ± SD (percentage) on the same row followed by the same letter are not significantly different (K ratio = 500).

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RADIATION INDUCED $F_1$ STERILITY IN PLUTELLA XYLOSTELLA (LEPIDOPTERA: PLUTELLIDAE): POTENTIAL FOR POPULATION SUPPRESSION IN THE FIELD

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ABSTRACT
The potential of using $F_1$ sterility in a system to manage the diamondback moth (DBM), Plutella xylostella (L.) (Lepidoptera: Plutellidae), was investigated in the laboratory and in field-cages. When 6-day old male pupae were treated with 200 Gy of gamma radiation, 71.5% developed into normal adults. However, radiation-induced reductions in fecundity and viability were expressed during the $F_1$, $F_2$, and $F_3$ generations. Sterility exceeded 60% in the $F_1$ and $F_2$ generations and 90% in the $F_3$ generation. The sex ratio was skewed in favor of males among $F_1$ and $F_2$ progeny. The percentages of metaphase spermatogonial cells with chromosomal aberrations were 86.9, 21.5 and 9.7 in the $F_1$, $F_2$, and $F_3$, respectively. No differences were observed in the sperm transfer between irradiated and unirradiated males. When treated males were released into field-cages at either a 5:1 or a 10:1 overflooding ratio with untreated DBM, the decrease in $F_1$ adult emergence was not significantly different than for the control. However, adult emergence in the $F_2$ generation was reduced by almost 90%. This degree of suppression was significantly greater than that achieved in cages where only irradiated males had been released. The use of $F_1$ sterility in combination with releases of the parasitoid, Cotesia plutellae (Kurdjumov) (Hymenoptera: Braconidae), in field-cages resulted in a 40% decrease in the DBM population in the $F_1$, and more than 90% in the $F_2$ generation. Nevertheless, additional research is needed to develop this system into an economically feasible strategy for managing early season populations of DBM.

Key Words: diamondback moth, Cotesia plutellae, inherited sterility, black stripe pupal mutant

RESUMEN
El uso potencial de la esterilidad $F_1$ como método de control para Plutella xylostella (L.) (DBM, “diamond back moth”) se investigo en estudios de laboratorio y de campo. Cuando pupas macho de 6 días de edad se irradiaron con 200 Gy de radiación gamma, el 71.5% se desarrollaron como palomillas aparentemente normales. Sin embargo, en las generaciones $F_1$, $F_2$ y $F_3$, las reducciones en fecundidad y viabilidad fueron obvias. El nivel de esterilidad excedió el 60% en las generaciones $F_1$, $F_2$ y $F_3$ y el 90% en la $F_3$. En la progenie $F_1$ y $F_2$, la tasa sexual favoreció a los machos. El porcentaje de espermatozoides en metáfase con aberraciones cromosómicas fue de 86.9, 21.5 y 9.7 en las generaciones $F_1$, $F_2$ y $F_3$, respectivamente. No se observaron diferencias entre machos irradiados y no irradiados en su habilidad en transferir esperma. Cuando se liberaron machos irradiados en jaulas de campo junto con palomillas fértiles en las tasas 5:1 o 10:1, el número de adultos en las generaciones $F_1$ y $F_2$ se redujo significativamente. La reducción fue de 50-60% en la generación $F_1$ y 59-68% en la generación $F_2$. Cuando se liberaron insectos irradiados de ambos sexos en la tasa 5:5:1:1 con palomillas fértiles, la reducción en el nivel poblacional de la generación $F_1$ no fue diferente de lo observado en la jaula control. Sin embargo, se observó una reducción del 90% en la generación $F_2$. El uso combinado de la esterilidad $F_1$ con liberaciones de Cotesia plutellae (Kurdjumov) (Hymenoptera: Braconidae) en jaulas de campo causaron que la población de DBM disminuyera mas del 40% en la $F_1$ y mas de 90% en la generación $F_2$. Sin embargo, sugerimos que es necesario continuar las investigaciones para lograr que este sistema sea económicamente factible para el control de las infestaciones tempranas de DBM.
males. Sutrisno et al. (1993) and Sutrisno & Houdaya (1993) suggested that doses of 175 Gy or 200 Gy could be considered in a DBM suppression program. Preliminary results relating to the potential suppression of DBM populations in the field by the F1 sterility technique are reported herein.

**MATERIALS AND METHODS**

**Laboratory Colonies**

Diamondback moth. Field-collected DBM pupae from cabbage were used to found the colony. Emerging adults were paired in oviposition cages and provided with a 10% sucrose solution. Grooved aluminum foil egg sheets that had been dipped in autoclaved cabbage juice were used as oviposition substrates. Egg sheets were changed daily. Neonates were reared on leaves of *Tropaeolum majus* L. (Rhoideas: Tropaeolaceae), a wild host of DBM. The colony was maintained at 20 ± 2°C, 70-80% RH and a 14L:10D photoperiod. The black stripe pupal mutant (BSP) is a marker present in our DBM laboratory colony (see below).

Parasitoids. A colony of *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae), reared on DBM larvae is maintained at our laboratory in Dalat. Laboratory conditions are the same as for the DBM colony. The colony was founded with field-collected material.

**Effects of Gamma Radiation on DBM**

Six day-old colony pupae were irradiated with a dose of 200 Gy in a Co60 irradiator at the Dalat Nuclear Research Institute (dose rate 43.2 Gy/min). Insects were used in laboratory and field-cage tests. Percent emergence, percentage of moths without deformities and longevity of irradiated versus untreated DBM adults were recorded in the laboratory. An average of 200 pupae were used in each of three replicates for the first two parameters. A subset of 100 adults in each of three replicates were used in the longevity study.

Treated DBM males and females (from the F1, F2 and F3 generations) were crossed with untreated counterparts and allowed to mate and lay eggs. Fecundity, fertility (percent sterile eggs), percent surviving to adulthood and sex ratio were recorded for all crosses. For fecundity and fertility data, an average of 20 moth pairs were used per replicate and three replicates were completed. For percent survival, an average of 100 larvae per replicate were used.

**Chromosomal Aberrations**

Tests of fourth instar DBM larvae from the F1, F2 and F3 generations were dissected in Belar’s saline solution and then squashed to release the germ cells. The slides were stained with aceto-orcein, sealed with clear nail polish and stored in a covered container in the refrigerator until examined for the presence of chromosomal aberrations.

**Sperm Transfer**

Irradiated and unirradiated DBM adult males were allowed to mate with unirradiated virgin females inside laboratory cages. Mating pairs were collected and mating was allowed to proceed until the insects separated. Males were reintroduced into the cages and exposed to virgin females after 24 h for 5 consecutive days. Females from each mating pair were dissected after oviposition to determine the presence of a spermatophore and to assess the ratio of eupyrene to apyrene sperm present in the spermatheca. The normal ratio of eupyrene to apyrene is considered to be 2:1 for *Tri-choplusia ni* according to North & Holt (1971). Any ratio deviating from this is considered abnormal.

**Effect of Mating Status and Type** and **Ratio of Sperm on DBM Oviposition**

Unirradiated males were allowed to mate with unirradiated virgin females in laboratory cages. Mating pairs were removed from the cages. After separation, females were allowed to lay eggs in the laboratory. When oviposition was completed females were dissected to determine the presence of sperm and the ratio of apyrene:eupyrene sperm in the spermatheca. Virgin females were also held for oviposition as controls.

**BSP Mutant**

Individuals in our DBM laboratory colony possess the black stripe pupa or BSP mutant. We have isolated and maintained a vigorous and homozygous mutant culture using the method of Bartlett & Raulston (1982). Mutant individuals were inbred and a small colony was established. We attempted to determine the mode of inheritance of the BSP mutant using the method of Walder (1988). Reciprocal crosses were made between mutant (BSP) females and males, and wild type stock (BSP-). Some F1 adults from the initial crossed were used in reciprocal back-crosses with the mutant strain and the rest were inbred.

**Evaluation of F1 Sterility** for **Suppression of DBM in Field-Cages**

Cabbage was planted inside four field-cages (2m x 2m x 4m); plants were irrigated regularly and received no other treatments during the experiments. Six-day old colony (BSP) pupae were irradiated as indicated above and pupae were sexed and allowed to emerge into separate containers. A wild strain (BSP+ = pupae with no black stripe) was collected from the field as 4th instar
larvae and allowed to pupate and emerge in the laboratory. One-day old adults from both groups were used in the experiment.

Field-cages received the following ratios of irradiated BSP colony females (IF), irradiated BSP colony males (IM), feral BSP + females (UF) and feral BSP + males (UM), respectively: Treatment A = 0:0:1:1 (control), Treatment B = 0:5:1:1, Treatment C = 0:10:1:1 and Treatment D = 5:5:1:1. Cages were checked every two days and eggs deposited on the cabbage were tagged and counted. The population increase in each field-cage was determined by counting the number of eggs, pupae and adults for two generations. Three replications were completed.

Combined Releases of Irradiated DBM and Cotesia plutellae in Field-Cages

*Cotesia plutellae* (CP) parasitoids from our colony (see above), one day-old 200 Gy treated colony (BSP) DBM adults (I) and unirradiated wild (BSP +) moths (U) were released into field-cages in the following ratios: Treatment 1 = 0 CP: 0 I: 1 U (control), Treatment 2 = 5 CP: 0 I: 1 U, Treatment 3 = 0 CP: 5 I: 1 U, and Treatment 4 = 2.5 CP: 2.5 I: 1 U. In Trt. 4, the release of one day-old irradiated moths occurred at day one and was followed ten days later with the release of one-day old parasitoid adults. Field-cages were checked every two days and the cabbage leaves on which eggs had been deposited were tagged and eggs were counted. The size of the DBM population inside the field-cages was determined by counting DBM eggs, pupae and adults. The size of the parasitoid population was determined by counting the number of parasitoid cocoons present in each cage.

Statistical Analysis

Data were subjected to either the pooled t-test or to analysis of variance. Differences between means were tested for significance using Tukey’s Honest Significant Difference (HSD) (Minitab 9.2; Minitab Statistical Software). Deviations from expected ratios were tested by chi-square analysis.

RESULTS AND DISCUSSION

Effects of Gamma Radiation on DBM

The results of our study on the effect of 200 Gy on percent emergence, percentage of adults without deformities and longevity of DBM are presented in Table 1. In general, DBM females appear to be more sensitive to gamma radiation than males. When 6-day old pupae were treated with 200 Gy, 71.5% of the male pupae developed as normal adults and 65.3% of these survived to the 6th day, while the corresponding percentages for treated females were 70.6 and 46.5. Adult longevity of both males and females was reduced by about one-third as compared to controls.

Table 2 shows the results of measuring fecundity, sterility, percent survival and sex ratio of DBM irradiated with 200 Gy and their F1, F2, and F3 progeny. Significant differences were found in all measured parameters when compared to controls. These significant effects persisted in the F1, F2, and F3 progeny. In the F1 generation, the mean number of eggs laid by the controls was 154. This number decreased to an average of 84 eggs when the female was crossed to an irradiated male. Further, the number of eggs produced by untreated females mated to F1 males was reduced to about one-fifth the number laid by the controls. However, when F2 males were mated to untreated females, the number of eggs produced per female was similar to the number produced when irradiated males were mated to normal females. The sterility of treated males was 62.1%, while that of F1 males was 94.8% and that of F2 females was 91.1%. In the F2 generation sterility ranged from

<table>
<thead>
<tr>
<th>Sex</th>
<th>Percent emergence I</th>
<th>Percent adults without deformities I</th>
<th>Percent survival to day 6 I</th>
<th>Longevity (days) I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>94.1 ± 3.1°</td>
<td>89.3 ± 2.7°</td>
<td>84.2 ± 5.3°</td>
<td>12.3 ± 2.4°</td>
</tr>
<tr>
<td>Female</td>
<td>95.2 ± 1.8°</td>
<td>91.1 ± 5.8°</td>
<td>85.1 ± 4.6°</td>
<td>11.9 ± 1.4°</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>85.4 ± 6.3°</td>
<td>71.5 ± 7.2°</td>
<td>65.3 ± 7.4°</td>
<td>8.9 ± 0.8°</td>
</tr>
<tr>
<td>Female</td>
<td>83.2 ± 4.1°</td>
<td>70.6 ± 7.8°</td>
<td>46.5 ± 8.5°</td>
<td>7.9 ± 1.0°</td>
</tr>
</tbody>
</table>

Table 1. Emergence, Percentage of Adults without Deformities and Longevity of *Plutella xylostella* adults which had been irradiated with 200 Gy as 6-day-old pupae.

Means followed by the same letter within a column for each treatment are not significantly different (P > 0.05; pooled t-test).

1Average of 200 pupae, 3 replications.

2Moths, which did not survive up to the sixth day usually, were not able to mate.

3Average of 100 moths, 3 replications.
Survival to adulthood in progeny of untreated moths varied between 75-85%, while it was 57.8% in the F1 generation, from 11.1% to 32.1% in the F2 and from 62.9% to 70.1% in the F3. The sex ratio was biased in favor of males among F1 and F2 progeny.

These results differ from those reported by Sutrisno et al. (1993) and Sutrisno & Hoedaya (1993). We report higher levels of sterility than those reported by these authors. In addition, the fecundity of untreated females mated to irradiated, F1 and F2 males was lower than for untreated controls in our experiments, while Sutrisno et al. (1993) and Sutrisno & Hoedaya (1993) report that fecundity in these crosses was equal to that observed in the untreated controls. The reasons for these differences remain unclear, but might be explained by differences in DBM strain, rearing methods and dose calibration.

### Chromosomal Aberrations

Cytological examination of chromosomal aberrations in the primary spermatocytes in 4th-instar larvae (Table 3) showed abnormal chromosomes evident in 86.9%, 21.5% and 9.7% of larvae from the F1, F2 and F3 generations respectively, while none were evident in the untreated controls. DBM have 31 pairs of chromosomes, individually recognizable during metaphase-I. Reciprocal translocations in the form of rings or chains, involving four or six chromosomes were observed in the F1 generation. These particular aberrations are the main cause of inherited sterility.

### Table 2. Fecundity, Sterility, Percent Survival and Sex Ratio of Plutella xylostella Irradiated with 200 Gy as 6-Day-Old Pupae and their F1 and F2 Progeny.

<table>
<thead>
<tr>
<th>Mating type</th>
<th>Fecundity (total # of eggs)</th>
<th>Sterility (% of eggs that failed to hatch)</th>
<th>Survival (%)</th>
<th>Ratio of males/females</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM × UF</td>
<td>156 ± 10^a</td>
<td>12.7 ± 7.8^a</td>
<td>78.5 ± 8.9^b</td>
<td>1.01 ± 0.01^a</td>
</tr>
<tr>
<td>IM × UF</td>
<td>84 ± 3^a</td>
<td>62.1 ± 6.8^b</td>
<td>57.8 ± 7.6^a</td>
<td>1.98 ± 0.07^a</td>
</tr>
<tr>
<td>UM × UF</td>
<td>145 ± 8^b</td>
<td>10.3 ± 0.6^e</td>
<td>85.1 ± 2.2^c</td>
<td>0.97 ± 0.05^e</td>
</tr>
<tr>
<td>^bF1M × UF</td>
<td>33 ± 4^c</td>
<td>94.8 ± 1.3^f</td>
<td>11.1 ± 3.7^g</td>
<td>1.78 ± 0.20^h</td>
</tr>
<tr>
<td>^bF1F × UM</td>
<td>28 ± 2^c</td>
<td>91.1 ± 2.0^d</td>
<td>32.1 ± 10.5^b</td>
<td>1.63 ± 0.17^b</td>
</tr>
<tr>
<td>UM × UF</td>
<td>140 ± 7^b</td>
<td>11.4 ± 3.4^a</td>
<td>75.7 ± 78^a</td>
<td>1.03 ± 0.14^c</td>
</tr>
<tr>
<td>F1M × UF</td>
<td>84 ± 3^c</td>
<td>63.5 ± 7.1^b</td>
<td>64.7 ± 9.7^c</td>
<td>1.18 ± 0.11^d</td>
</tr>
<tr>
<td>F1F × UM</td>
<td>80 ± 7^a</td>
<td>61.8 ± 8.2^b</td>
<td>65.3 ± 13.3^c</td>
<td>1.42 ± 0.13^d</td>
</tr>
<tr>
<td>F2M × UF</td>
<td>87 ± 6^a</td>
<td>64.8 ± 11.8^d</td>
<td>62.9 ± 12.3^a</td>
<td>1.25 ± 0.12^h</td>
</tr>
<tr>
<td>F2F × UM</td>
<td>86 ± 3^c</td>
<td>62.4 ± 5.6^b</td>
<td>70.1 ± 10.6^b</td>
<td>1.34 ± 0.10^c</td>
</tr>
</tbody>
</table>

^I = irradiated, U = unirradiated.

Means followed by the same letter within a column for each treatment are not significantly different (P = 0.05; Tukey’s Honest Significant Difference).

^Average of 20 pairs of moths, 3 replications.

^Percent survival of 100 neonate larvae to adult, 3 replications.

^Male and female progeny from each F1 cross were followed separately.

### Table 3. Percentage of Metaphase Nuclei with Visible Chromosomal Aberrations in Progeny of Plutella xylostella Males which had been Irradiated with 200 Gy as 6-Day-Old Pupae and their F1, F2 and F3 Male Progeny.

<table>
<thead>
<tr>
<th>Generation</th>
<th>No. of nuclei examined</th>
<th>No. with chromosomal aberrations</th>
<th>Frequency of aberrations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>92</td>
<td>80</td>
<td>86.9</td>
</tr>
<tr>
<td>F2</td>
<td>79</td>
<td>17</td>
<td>21.5</td>
</tr>
<tr>
<td>F3</td>
<td>72</td>
<td>7</td>
<td>9.7</td>
</tr>
<tr>
<td>Control</td>
<td>77</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

^Testes from mature larvae were dissected in Belar’s solution and then squashed to release the germ cells. The slides were stained with aceto-orcein, sealed and stored in the refrigerator until examined.
Carpenter (1991) and Zhang et al. (1993) report that incidence of visible chromosomal aberrations in F₁ and F₂ larvae of *Helicoverpa zea* and *Ostrinia furnacalis* is dose dependent, and that aberrations occur most frequently in the F₁ and progressively less frequently in subsequent generations. North (1975) reported that male progeny of *H. zea* treated with 200 Gy showed at least one translocation. Our observations on DBM agree with results reported by these authors for other Lepidoptera.

**Sperm Transfer**

Table 4 shows the percentage of matings on each of five consecutive days by irradiated (200 Gy) and unirradiated DBM males. This percentage diminished for both groups with each successive mating. Lepidopteran males produce apyrene and eupyrene sperm that is transferred to the female during mating. Eupyrene sperm are nucleate and therefore capable of fertilization, while apyrene sperm are smaller and anucleate. Both types are present in the spermatophore and migrate to the spermathecae in the female after copulation is complete (North & Holt 1971). The percentage of treated and untreated DBM males that transferred sperm in each of five consecutive matings is given in Table 4. No reduction in sperm transfer was evident among the first, second and third matings for either male group. The percentage of males that transferred a normal ratio of eupyrene to apyrene sperm remained fairly high during the first three consecutive matings for both groups.

In *Trichoplusia ni* Holt & North (1970) report that the ability of fully sterile males to transfer sperm was considerably lower than in unirradiated males. They found that when sperm were deposited into the bursa copulatrix, the eupyrene sperm lacked motility, while the apyrene sperm were able to migrate to the spermatheca. In contrast, treating DBM pupae with a substerilizing dose of radiation (200 Gy) resulted in males able to transfer normal ratios of eupyrene:apyrene sperm. Our results for DBM are similar to those reported by El-Naggar et al. (1984) on *Agrotis ypsilon*, Carpenter et al. (1987) on *Helicoverpa zea*, and Sallam & Ibrahim (1993) on *Spodoptera littoralis*.

**Effect of Mating Status and Type and Ratio of Sperm on Oviposition**

North & Holt (1971) reported that when *T. ni* females mated with an irradiated male they often failed to deposit a normal number of eggs. They suggested that this might be caused by inadequate sperm or accessory gland fluid transfer to the female during mating. Table 5 shows the effect of mating status and type and ratio of sperm on oviposition in DBM. Females that received a normal sperm complement laid significantly (twice as many) more eggs than those receiving an abnormal sperm ratio. It appears that type and quantity of sperm transferred significantly influences fecundity in DBM females.

Holt & North (1970) reported that early in copulation the spermatophore in *Trichoplusia ni* becomes filled with a clear fluid even in females

**Table 4. Ability of Plutella xylostella males irradiated with 200 Gy of to transfer sperm.**

<table>
<thead>
<tr>
<th>No. of consecutive matings</th>
<th>No. males tested</th>
<th>Percent mated</th>
<th>% of mated males which transferred sperm</th>
<th>Percent of males which transferred normal ratio eupyrene:apyrene sperm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92</td>
<td>91.3</td>
<td>92.8</td>
<td>89.7</td>
</tr>
<tr>
<td>2</td>
<td>84</td>
<td>75.0</td>
<td>92.2</td>
<td>83.3</td>
</tr>
<tr>
<td>3</td>
<td>63</td>
<td>69.8</td>
<td>90.9</td>
<td>80.0</td>
</tr>
<tr>
<td>4</td>
<td>44</td>
<td>34.1</td>
<td>53.3</td>
<td>32.5</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>33.3</td>
<td>0.0</td>
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</tr>
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</table>

<table>
<thead>
<tr>
<th>Irradiated male</th>
<th>1</th>
<th>127</th>
<th>88.9</th>
<th>92.1</th>
<th>88.9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>113</td>
<td>74.3</td>
<td>92.7</td>
<td>83.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>84</td>
<td>65.4</td>
<td>91.1</td>
<td>79.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>55</td>
<td>32.7</td>
<td>51.2</td>
<td>31.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>18</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Irradiated and unirradiated males were allowed to mate with unirradiated virgin females on each of five consecutive days. Mating pairs were removed from the cages. Mated females were dissected after oviposition to determine the presence of a spermatophore and the ratio of apyrene to eupyrene sperm in the spermatheca.

²The normal ratio of eupyrene to apyrene is considered to be 2 eupyrene:1 apyrene. Any ratio below 1:1 is considered abnormal (North & Holt 1971).
that receive no sperm, and that this may elicit the laying of a significant number of eggs. This observation appears to hold true for DBM. In our experiments mated females that received no sperm laid significantly more eggs than virgin females, which suggests that fecundity in DBM is also influenced by the secretions from the accessory gland transferred during mating.

BSP Mutant

Reciprocal crosses made between BSP and/or BSP⁺ virgin females and males confirm that the gene responsible for the mutation black stripe pupa (BSP) is dominant and most likely located on an autosome.

Evaluation of F₁ Sterility for Suppression of DBM in Field-Cages

Evaluating the results of inherited sterility as a method for population suppression is particularly complex. Released partially sterile individuals mate with released untreated individuals to produce partially sterile progeny. The progeny continue to do the same until the experiment is stopped and effects are ascertained. Several marking techniques (such as radioisotopes, externally applied fluorescent powders and internal oil soluble dyes) have been used to identify released Lepidopteran insects. Unfortunately, these markers do not allow the descendants of a released insect to be tracked through subsequent generations. Bartlett (1967) suggested that mutations could be used as biological markers. Furthermore, he suggested that dominant and co-dominant mutants are most useful, as they can identify not only the released individuals but also their progeny and descendants in subsequent generations. The presence of the BSP mutant in our DBM colony made it possible for us to examine the mating interactions of the irradiated DBM through several generations.

The results of our field-cage experiments are shown in Table 6. When treated males (Trts. B and C) were released at either a 5:1 or a 10:1 over-flooding ratio with unirradiated moths, there was a significant reduction in the number of F₁ and F₂ adults emerging in the field-cages as compared to the control (Trt. A). A 50-60% reduction in the F₁ and 59-68% in the F₂ generation were observed for field-cages receiving Trt. B and C, respectively. When irradiated females and males were released at a 5:5:1:1 overflooding ratio (Trt. D), the decrease in F₁ adult emergence was not significantly different than for the control (<3%). However, adult emergence in the F₂ generation was reduced by almost 90%. This degree of suppression was significantly greater than that achieved in cages where only irradiated males had been released. Nonetheless, 1.4 times more neonates were produced by Treatment D in the F₁ generation as compared to the control, and these neonates caused greater host plant damage.

Several investigators have formulated hypotheses concerning the role that released irradiated females might play in the suppression of populations subjected to the sterile insect technique (Whitten & Taylor 1970, Allam & Galun 1976). If partially sterile females are released they could contribute a significant fraction of the progeny produced in the target population. This increase in the number of F₁ progeny may benefit a release program when the released females carry lethal chromosomal changes that will be transferred to the target population. However, the major suppression of the target population is deferred (but greatly enhanced in) the second generation. North & Holt (1971) suggested that the maximum economic efficiency in the use of inherited sterility for the suppression of lepidopteran populations requires the release of both sexes of partially sterile moths. Our results support the above analyses and findings.

In our field-cage studies BSP individuals were identified among the progeny in all cages except the control. The ratio of BSP to BSP⁺ in the F₁ generation was 2.21, 3.41 and 8.94 in Treatments B, C, and D, respectively. The ratio of BSP to BSP⁺ in the F₂ generation was 0.11, 0.09 and 0.07 in Trts. B, C, and D, respectively (Table 6). All individuals expressing the black stripe pupal mutant were

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**Table 5. Effect of Mating Status and Type and Ratio of Sperm on Plutella xylostella Oviposition in the Laboratory.**

<table>
<thead>
<tr>
<th>Sperm transferred</th>
<th>No. females</th>
<th>No. eggs/female (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal eupyrene:apyrene¹</td>
<td>77</td>
<td>234 ± 26</td>
</tr>
<tr>
<td>Abnormal eupyrene:apyrene¹</td>
<td>55</td>
<td>112 ± 16</td>
</tr>
<tr>
<td>No sperm¹</td>
<td>32</td>
<td>73 ± 14</td>
</tr>
<tr>
<td>None (virgin females)</td>
<td>42</td>
<td>47 ± 11</td>
</tr>
</tbody>
</table>

Means followed by a different letter within a column are significantly different (P = 0.05; Tukey's Honest Significant Difference).

¹Unirradiated males were allowed to mate with unirradiated virgin females in laboratory cages. The mating pairs were removed from the cages. When the mated females had finished oviposition, they were dissected to determine the presence of sperm and the ratio of apyrene and eupyrene sperm in the spermatheca. Virgin females were held for oviposition as controls.
descendants of released DBM, and it was these individuals that transmitted the sterility factors into subsequent generations. In fact, the ratios of BSP to BSP+ in the various field-cages are the ratios of substerile to fertile DBM in the F1 and F2 generations. The presence of BSP-marked individuals is the most compelling evidence of the mating interaction between released substerile and wild moths. The usefulness of mutant markers as a tool to monitor the outcome of sexual interactions between irradiated (BSP) and unirradiated (BSP+) DBM has been demonstrated in these experiments.

Even though our results would suggest that male only releases should be considered for DBM, no efficient technique is currently available to separate large numbers of male and female pupae. As a consequence, simultaneous release of treated males and females is unavoidable at this time. In our experiments, population suppression in the F2 generation for bisexual releases was significantly higher than that observed in the F1 for male only releases, even at a ratio of 10:1. However a higher amount of crop damage by F1 larvae was observed when both irradiated substerile male and female DBM were released into the field-cages. One potential solution to avoid excessive crop damage would be to rear the DBM F1 generation in the laboratory and release F1 adults instead of their irradiated parents. This would reduce crop damage because of the lower fertility in the F1 as compared to the F2 generation. However, the lower fertility in the F1 generation would call into question the economic feasibility of this option.

Combined Releases of Irradiated DBM and Cotesia plutellae in Field-Cages

Knipling (1979) suggested that combining parasitoid releases with sterile insect release might yield both additive and synergistic effects. Although the modes of action of both tactics are different, the effectiveness of the sterile insect technique increases the ratio of adult parasitoids to adult hosts, while the action of parasitoids increases the ratio of sterile to fertile insects. Even greater suppression could be expected if parasitoids are combined with the releases of partially sterile insects. Carpenter (1993) demonstrated that the economic benefits of combining inherited sterility and parasitoids would be greatest when the ratio of irradiated to unirradiated moths is ≥10:1 and the ratio of parasitoid to hosts is ≤5:1.

Our objective was to investigate the potential of using combined releases of partially sterile DBM with releases of Cotesia plutellae, a specific larval parasitoid, to suppress wild DBM populations. The results are summarized in Table 7. In cages receiving partially sterile DBM (Trts. 3 and 4), the number of F1 eggs was significantly (1.5

<table>
<thead>
<tr>
<th>Treatment (No. of IF:IM:UF:UM per cage)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1 generation</td>
</tr>
<tr>
<td></td>
<td>No. eggs</td>
</tr>
<tr>
<td>A-control (0:0:50:50)</td>
<td>3243 ± 767a</td>
</tr>
<tr>
<td>B (0:250:50:50)</td>
<td>2653 ± 667a</td>
</tr>
<tr>
<td>C (0:500:50:50)</td>
<td>2397 ± 645a</td>
</tr>
<tr>
<td>D (250:250:50:50)</td>
<td>8781 ± 1138b</td>
</tr>
</tbody>
</table>

Means followed by the same letter within the same column are not significantly different (P = 0.05; Tukey’s Honest Significant Difference).

Values in parentheses indicate the percent decrease in the moth population as calculated by the formula: ((U-R)/ U) where U is the number of emerged moths in the control population, and R is the number of moths which emerged in the treated population.

BSP = wild moth and BSP+ = mutant moth.

Three replications were completed.

Combined Releases of Irradiated DBM and Cotesia plutellae in Field-Cages

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in the F2 generation the number of eggs in Trts. 3 among treatments were not significant. However, lowest in Treatment 4, although differences most identical. The number of adult moths was caused by larvae in all four populations was al-

terence was found in the number of neonates embryons during development, no significant dif-
mutations in the irradiated moths killed many times) higher. However, because dominant lethal mutations in the irradiated moths killed many embryos during development, no significant difference was found in the number of neonates among treatments. Thus, the level of host damage caused by larvae in all four populations was almost identical. The number of adult moths was lowest in Treatment 4, although differences among treatments were not significant. However, in the F1 generation the number of eggs in Trts. 3 and 4 was significantly lower than the number of eggs in Trts. 1 and 2. Treatment 4 had the lowest number of eggs at 843. The number of neonates for each of the treatments was significantly lower than the number of neonates in the control. Furthermore, the number of neonates in Trt. 3 (288) and Trt. 4 (195) was significantly lower than the number of neonates in Trt. 2 (2534). As a result, the level of damage on host plants was the lowest in cages treated with both irradiated males and females and parasitoids.

Each of the treatments significantly suppressed population growth as compared to the control. However, population suppression was significantly greater in cages receiving a combination of irradiated (200 Gy) DBM males and females followed by a single release of *C. plutellae*. When only one tactic was used, population suppression was significantly higher in cages receiving irradiated DBM than in those receiving parasitoids.

The numbers of parasitoids, BSP, and BSP+ marked moths recovered from each of the treat-
ments are summarized in Table 8. In Treatment 2 (parasitoids only), the population of *C. plutellae* decreased by about 20% in the F1 generation and increased by about 75% in the F2 generation. However, in Trt. 4 (irradiated DBM + parasitoids) the size of the parasitoid population increased by about 50% in F1 generation and declined by about 15% in the F2. These observations might suggest that *C. plutellae* may have had a higher survival rate on irradiated F1 (BSP) larvae than on the wild type (BSP+) larvae. However the greater number of *C. plutellae* in the F2 generation in Trt. 2 is largely due to lower number of DBM larvae in Trt. 4 (195 neonates).

Our data suggest that the use of both tactics, inherited sterility and releases of the parasitoid, *C. plutellae*, may be feasible for managing early-season populations of DBM. Partially sterile DBM adults could be released to produce large numbers of F1 sterile larvae on early-season annual hosts. These F1 larvae could in turn serve as hosts for *C. plutellae* and other parasitoids present in the field. In this way the next generation of parasitoids would be increased, and any surviving partially sterile larvae would become
Table 8. Number of Parasitoids, BSP and BSP+ Marked Plutella xylostella observed in four populations subjected to one release of irradiated BSP moths and of the parasitoid, Cotesia plutellae.

<table>
<thead>
<tr>
<th>Population (CP:I:U)</th>
<th>Parasitoids</th>
<th>F₁ generation</th>
<th>BSP</th>
<th>BSP+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment 1 (0:0:10)</td>
<td></td>
<td>108 ± 25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment 2 (50:0:10)</td>
<td></td>
<td>78 ± 17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment 3 (0:50:10)</td>
<td>41 ± 8</td>
<td>93 ± 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment 4 (25:25:10)</td>
<td>38 ± 3</td>
<td>56 ± 13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment 1</td>
<td>70 ± 6</td>
<td></td>
<td>1431 ± 214</td>
<td></td>
</tr>
<tr>
<td>Treatment 3</td>
<td>13 ± 7</td>
<td></td>
<td>1002 ± 86</td>
<td></td>
</tr>
<tr>
<td>Treatment 4</td>
<td>29 ± 4</td>
<td></td>
<td>246 ± 45</td>
<td></td>
</tr>
</tbody>
</table>

₁BSP+ = wild moth; BSP = mutant moth; I = irradiated BSP moth; U = unirradiated BSP+ moth; CP = Cotesia plutellae.
Three replications were conducted.


KNILLING, E. F. 1979. The basic principles of insect population suppression and management. USDA, Agricultural Handbook No 512.


ABSTRACT

Mole crickets of the genus *Scapteriscus* were accidentally introduced into the southern United States almost a century ago and are considered to be economically important pests in southern U.S. regions. Mole crickets were sampled using acoustic traps in Baton Rouge and New Orleans, Louisiana in the fall of 1998, the spring and fall of 1999, and the spring of 2000. In southeastern Louisiana *Scapteriscus borellii* has a seasonal flight period starting in late February and continuing into June. A lesser flight period occurs in the fall, starting in mid-September and continuing into November. *S. vicinus* was captured only from late February to late April. We determined that *S. borellii* was being parasitized inside acoustic traps by the tachinid fly, *Ormia ochracea* and that *S. vicinus* was parasitized by an anthomyiid fly, *Acridomyia* sp.

Key Words: acoustic attraction, mole crickets, *Ormia*, Tachinidae, *Acridomyia*

RESUMEN

Los grillos topo del genero *Scapteriscus* fueron introducidos accidentalmente en el sur de los Estados Unidos hace un siglo y se consideran ser parásitos importantes económicamente en regiones sureñas de EE.UU. Los grillos topo fueron muestreados usando trampas acústicas en Baton Rouge y New Orleans, Louisiana en el otoño de 1998, la primavera y otoño de 1999, y la primavera del 2000. *Scapteriscus borellii* tiene un periodo de vuelo estacional comenzando tarde en febrero y continuando hasta junio. Un periodo de vuelo mas corto ocurre en el otoño, comenzando a mediados de septiembre y continuando hasta noviembre. *S. vicinus* fue capturado solo entre fines de febrero y fines de abril. Determinamos que *S. borellii* estaba siendo parasitado dentro de las trampas acústicas por la mosca tachinida *Ormia ochracea* y que *S. vicinus* fue parasitado por la mosca esp. *Acridomyia*.

Three species of mole crickets, *Scapteriscus vicinus* Scudder, *S. borellii* Giglio-Tos and *S. abbreviatus* Scudder, were accidentally introduced into the southern United States from South America from around 1899 to 1926 (Walker & Nickle 1981). *Scapteriscus vicinus* and *S. borellii* have since spread throughout the coastal plain from southeastern Texas to southeastern North Carolina (Parkman et al. 1996), with isolated populations of *S. borellii* reported from Arizona (Nickle & Frank 1988) and California (Frank 1994). *Scapteriscus* spp. mole crickets, especially *S. vicinus*, are considered to be serious pests of turf and pasture grasses throughout the southeastern U.S. (Hudson et al. 1988).

Using traps with artificial calling songs of *S. borellii* and *S. vicinus*, surveys of these two species were conducted in Baton Rouge and New Orleans, Louisiana, in the fall of 1998, the spring and fall of 1999, and the spring of 2000. This was done in order to ascertain the population levels in these areas and to determine the flight periods of introduced mole crickets in southeastern Louisiana prior to release of *Ormia depleta* (Wiedemann) (Diptera: Tachinidae) in these cities for biological control. This fly is native to South America, where it attacks mole crickets of the genus *Scapteriscus* (Fowler 1987). It has been released at many sites in Florida and is established there (Frank et al. 1996).

MATERIALS AND METHODS

Acoustic traps that use artificially produced calling songs of mole crickets capture large numbers of these insects, and they are used for population surveys and behavioral studies (Walker 1988). Traps in this study were constructed according to details given in Parkman and Frank (1992) with some modifications. Our traps consisted of 1 m diameter sheet metal funnels suspended from metal yokes. The yokes were supported by 10 cm × 10 cm × 100 cm wooden posts buried into the ground to a depth of 30 cm. A single 20 l plastic bucket was attached to the bottom of each funnel to hold captured insects. Buckets were suspended above ground level to minimize predation of captured mole crickets by red imported fire ants (*Solenopsis invicta* Buren). Holes were drilled through the bottom of the buckets to allow rainwater to drain. Two sound traps were deployed at Burden Research Station...
in Baton Rouge (31°24.46' N, 91°06.74' W) during fall 1998, the spring and fall of 1999 and spring 2000. A second set of traps was also deployed in City Park, New Orleans (29°59.70' N, 90°05.33' W) during fall 1999 and spring 2000. A different song was played over each of the traps; one synthesized the song of *S. borelli* males and the other the song of *S. vicinus* males. The traps were separated by at least 2 m as suggested by Walker (1982). Artificial crickets were obtained from Night Caller Artificial Crickets (Eco-Sim, Gainesville, FL), and they were powered by 12V, 7.0 amp-hour, lead acid gel-cell rechargeable batteries (Power Sonic PS-1270). Batteries were recharged once per week.

Traps were serviced at least every other day. Captured adults of *Scapteriscus* spp. were placed in zippered plastic bags which were placed in a cooler for transport to the laboratory. Adults were held individually in 15 dram snap-lid, plastic vials (WWR Scientific, West Chester, PA). Each vial contained at least 4 cm (~35 ml) of moist sand. The mole crickets were checked each day for parasitism. Dead mole crickets were removed from vials and the sand searched for fly puparia. Larvae of *O. ochracea* Bigot require approximately one week to complete development in the laboratory at 23-25°C (Wineriter & Walker 1990). After three weeks, all puparia were removed from the sand and placed inside 15 dram snap-lid vials onto 3 cm × 3 cm moist paper towels until emergence of adults. Adult flies were identified using keys in Sabrosky (1953). Voucher specimens of *O. ochracea*, *G. rubens* Scudder, *S. borelli* and *S. vicinus* were deposited in the Louisiana State University Arthropod Museum. Larvae of Acridomyia were identified using keys to immature Diptera in Teskey et al. (1991).

**RESULTS AND DISCUSSION**

Seasonal Distribution

Numbers of *S. borelli* and *S. vicinus* captured in fall 1998, spring and fall 1999, and spring 2000 are shown in Figures 1 and 2. The flight period for *S. borelli* in southeastern Louisiana begins in late February and lasts into June. A lesser flight period occurs in the fall, starting in mid-September and lasting into November. For *S. vicinus*, the flight period in southeastern Louisiana also begins in late February and continues into late April. No *S. vicinus* were captured at either of the two trapping locations during fall of the years 1998 or 1999. Flight periods of *S. borelli* and *S. vicinus* in southeastern Louisiana are similar to those reported by Walker et al. (1983) for *S. borelli* and *S. vicinus* at Gainesville, Florida (29°40'N), except that we never captured *S. vicinus* in our traps during the fall months. In addition, numbers of mole crickets captured in our sound traps were much lower than those reported in Florida.

Trap abundance cycles of 7-12 days were observed during the fall 1998 and 1999, and spring 1999 and 2000 trap data (Figs. 1 and 2). Ngo and Beck (1982) also observed trap abundance cycles lasting ca. 9 days for *S. borelli* in Florida and attributed this phenomenon to egg laying cycles of *S. borelli* females. Walker and Nation (1982) reported that some *S. borelli* males will call during the fall months and females can respond in significant numbers. They found that some *S. borelli* females do mate during the fall, since 7 out of 25 females attracted to synthetic calling song in October to December had sperm in their spermathecae. It is not generally believed, however, that egg laying occurs during the fall.

Parasitism of *S. borelli* by *O. ochracea*

*Ormia ochracea* adults were commonly collected in the *S. borelli* traps but were never collected from the *S. vicinus* traps during fall 1998 and 1999. *O. ochracea* were often observed resting inside trap buckets and caller housings. No *O. ochracea* were reared from ca. 200 *Scapteriscus* spp. collected during each spring of 1999 and 2000. This was expected because *O. ochracea* adults were never observed at our traps during spring 1999 and 2000. Of 88 *S. borelli* captured at New Orleans and Baton Rouge between 7 October and 18 October 1999, 18 were parasitized by *O. ochracea*, yielding 34 puparia (range 1-4 puparia per host), (Table 1). A total of 14 adults of *O. ochracea* eclosed from the puparia. Flies (n = ca. 5-10) were regularly observed resting inside the caller housing during the day. When the *S. borelli* trap buckets were opened to inspect the contents, several dozen flies would escape. We were often able to collect flies, usually 10-20, from inside the trap buckets that were either dead or too weak to fly. Walker (1993) found that calls of *S. borelli* and *S. vicinus* attracted few and zero *O. ochracea*, respectively.

Tachinid flies of the tribe Ormiini are orthopteran parasites specializing on crickets and katydids (Walker 1986, 1989). They are known to occur throughout the southeastern United States from Florida to Texas (Walker 1989; Robert & Hoy 1994). Females of the phonotactic tachinid fly *O. ochracea* are larvivorous (Frank 1994), are attracted to artificially produced songs of its host, *Gryllus integer* and larviposit in the vicinity of calling males (Cade 1975; Walker 1989). Walker (1986, 1989) captured large numbers of *O. ochracea* at traps using artificially produced *Gryllus rubens* Scudder songs. Mangold (1978) found that *O. ochracea* also was attracted to synthesized songs of *S. borelli* males and was able to successfully rear *O. ochracea* to adulthood on 1 of 5 *S. borelli* and on one *G. rubens* artificially infested. Wineriter and Walker (1990) also reared...
Fig. 1. (A) Numbers of *S. borellii* captured in sound traps at Burden Research Station, Baton Rouge, LA. Traps were operated from 16 September to 4 November, 1998, (B) Numbers of *S. borellii* and *S. vicinus* captured in sound traps at Burden Research Station, Baton Rouge, LA. Traps were operated from 16 February to 2 June, 1999, (C) Numbers of *S. borellii* captured in sound traps at Burden Research Station, Baton Rouge, LA. Traps were operated from 14 September to 21 October, 1999.
O. ochracea from S. borellii. According to Hudson et al. (1988), larvae of O. ochracea have been collected in the field only from crickets of the genus Gryllus. As Mangold (1978) points out, the rearing of O. ochracea on S. borellii does not indicate that this is a natural host. Instances of parasitism of S. borellii by O. ochracea are probably an artifact of the trapping technique (Walker & Wineriter 1991). Confinement of O. ochracea females with S. borellii for several days in our traps invariably resulted in a portion of the mole crickets being parasitized. Hundreds of first-instar larvae of O. ochracea also were collected from the trap buckets, suggesting that larvipositing by O. ochracea females occurred inside the trap buckets. Our results are in contrast to data reported in Frank et al. (1996), where only one out of tens of thousands of S. victinus mole crickets captured...
in a survey was parasitized, presumably by *O. ochracea*. The authors did not state what species of *Scapteriscus* was parasitized.

Three female *G. rubens* were collected in the *S. borellii* sound trap at Baton Rouge between 14-18 October 1999. Mangold (1978) also reported that several *Gryllus* spp. were attracted to artificial songs of *S. borellii* in Florida. Of particular interest is the attraction of *O. ochracea* and *G. rubens* to artificial songs of *S. borellii* but not of *S. vicinus*. The calling songs of *S. borellii* and *G. rubens* are similar (Burk 1982). Males of *S. borellii* broadcast songs at a carrier frequency of 2.7 kHz, with a pulse rate of ~50 pulses s⁻¹ (Walker 1982). Males of *G. rubens* broadcast at a carrier frequency of 4.8 kHz, also with ~50 pulses s⁻¹ (Walker 1986). In addition, calling songs of *S. vicinus* have a carrier frequency that falls in between that of *S. borellii* and *G. rubens* (3.3 kHz), but have a much faster pulse rate of ~130 s⁻¹ (Walker 1993). Robert et al. (1992) found that the hearing organ of *O. ochracea* females is most sensitive to frequencies in the range of 4 to 6 kHz. There is, however, no reason to suggest that *O. ochracea* females would not respond to carrier frequencies outside these ranges. In fact, Walker (1993) demonstrated that *O. ochracea* females are attracted to carrier frequencies between 2.4 and 6.8 kHz. According to Chapman (1982) there is no evidence that frequency discrimination alone is especially important to the insect. The pulse repetition frequency of insect songs is probably a more important factor in their recognition because impulses occur in the auditory nerves in synchrony with sound pulses. This may explain why *O. ochracea* and *G. rubens* were attracted to artificial calling songs of *S. borellii* at our trapping stations but not to those of *S. vicinus*. Walker (1986), *O. ochracea* females were attracted to synthesized calls of *G. rubens* but not *G. firmus*. The carrier frequencies of these two species fall within the most sensitive range of *O. ochracea* females (4-6 kHz), but songs of *G. firmus* have a much slower pulse rate of ~17 s⁻¹ (Walker 1986). The pulse rate of *S. vicinus* (~130 s⁻¹) is much faster than both *S. borellii* and *G. rubens*. In addition, the carrier frequencies of *S. borellii* and *S. vicinus* are much lower than *G. rubens*. As Walker (1993) points out, our knowledge of cricket communication, and that of their acoustically attracted parasitoids, is far from complete and future studies should examine other components of sound that may be important.

### Parasitism of *S. vicinus* by *Acridomyia* sp.

In late March 2000, two *S. vicinus* that were captured in an acoustic trap in New Orleans were found to have been parasitized by *Acridomyia* sp. (Diptera: Anthomyiidae). One of the parasitized *S. vicinus* contained six maggots, the other contained only one. Unfortunately, no adult flies were obtained. Larvae of flies in this genus are parasitoids of grasshoppers (Acrididae) (Dahlem & Thompson, 1991). Little else is known about members of this genus. Are *Acridomyia* sp. attracted to the calling songs of *S. vicinus* or were these two mole crickets accidentally parasitized?

### Acknowledgments

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EVALUATION OF *SERANGIUM PARCESETOSUM* (COLEOPTERA: COCCINELLIDAE) FOR BIOLOGICAL CONTROL OF SILVERLEAF WHITEFLY, *BEMISIA ARGENTIFOLII* (HOMOPTERA: ALEYRODIDAE), ON POINSETTIA

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**ABSTRACT**

Control of silverleaf whitefly (*Bemisia argentifolii* Bellows & Perring) on greenhouse poinsettia with biological agents has been unreliable. *Serangium parcesetosum* Sicard, a coccinellid predator, appears to have great potential for silverleaf whitefly control. In our study, dynamic changes in *B. argentifolii* populations on caged poinsettia in response to *S. parcesetosum* were monitored. Silverleaf whiteflies were introduced to caged poinsettias at 1 or 10 adults per plant and 6 weeks later *S. parcesetosum* were introduced at 0, 2 or 4 adults per plant. Within 2 weeks of *Serangium* release whitefly mortality increased dramatically, and for the ensuing 10 weeks whitefly levels remained at or near those observed at time of predator release. Beetle larvae were observed 2 to 10 weeks after *Serangium* release when prey was initially high but not when prey was initially low. Thus, whitefly control was primarily due to prolonged survival and continuous feeding of individual beetles. Our data suggest that *Serangium* may work well in a multiple species biological control program for whiteflies on poinsettia. However, further study is needed on multiple species interactions within the host (pest/plant) species, and on release management strategies.

Key Words: Population dynamics, caged study, predator, prey

**RESUMEN**

El control de la mosca blanca (*Bemisia argentifolii* Bellows & Perring) en poinsettia con agentes biológicos ha sido errático. *Serangium parcesetosum* Sicard, un coccinéldido predador, parece tener gran potencial para el control de *B. argentifolii*. En nuestro estudio, cambios dinámicos en poblaciones de *B. argentifolii* en poinsettias enjauladas en respuesta a *S. parcesetosum* fueron observados. Las moscas blancas fueron liberadas en poinsettias enjauladas de 1 a 10 adultos por planta y 6 semanas después *S. parcesetosum* fueron liberados de 0, 2, o 4 adultos por planta. Dentro de 2 semanas desde la introducción de *Serangium* la mortalidad de la mosca blanca incremento dramáticamente, y por las próximas 10 semanas los niveles de moscas permanecieron en o cerca de aquellos observados al momento de introducción del predador. Larvas de escarabajos fueron observadas de 2 a 10 semanas después de la liberación de *Serangium* cuando el número de presa estaba inicialmente alto pero no cuando el número de presa estaba inicialmente bajo. Por lo tanto, el control de la mosca blanca fue debido principalmente a supervivencia prolongada y alimentación continua de escarabajos individuales. Nuestros datos sugieren que *Serangium* pudiera servir bien en un programa de control de especies múltiples de moscas blancas en poinsettia. Sin embargo, más investigación es necesaria sobre las interacciones de especies múltiples dentro la especie (plaga / planta), y en estrategias de control de liberación.

Silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae, also known as the sweetpotato whitefly, *B. tabaci* (Gennadius) Biotype B), is the most important arthropod pest on greenhouse grown poinsettias (Ecke et al. 1990). Poinsettia cuttings often arrive infested with whitefly nymphs at levels well below economic thresholds (Helgesen & Tauber 1977; Hoddle et al. 1999) but whitefly populations rapidly increase to exceed economic thresholds in the absence of effective controls.

Poinsettia is the single largest potted flowering greenhouse crop grown in the U.S. in terms of both number of pots produced (>59 million) and annual wholesale value (>$220 million dollars) (USDA 1997). It appears to be a good candidate crop for biological control because it is produced as a monoculture and has few serious pest problems other than *Bemisia argentifolii* (Parrella et al. 1991). In practice however, economic biological control systems capable of suppressing *B. argentifolii* to the low thresholds required for ornamental crops have been elusive (Parrella et al. 1991). For example, Hoddle et al. (1997a) reported that when *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) was used as the control agent, 23 to 70% of poinsettia plants were infested with immature whitefly at the end of the season. By com-
parision they observed 30% of poinsettias in commercial retail outlets were infested with immature whitefly. With the *E. formosa* Beltsville strain, end-of-season whitefly infestation ranged from 77 to 100% of plants under various release rates compared to 28% of plants observed in commercial retail outlets (Hoddle et al. 1997b). Heinz & Parrella (1994) observed satisfactory whitefly control with a combination of *Encarsia luteola* Howard and *Delphastus pusillus* Le Conte (*Coccoptera: Coccinellidae*) but at a cost 5-times higher than insecticide-based control. With weekly releases of *Eretmocerus emericus* n. sp. Rose & Zolnerowich (Hymenoptera: Aphelinidae), Hoddle et al. (1998) reported that 73 and 83% of plants were infested with immature whitefly at the end of the season compared to only 28% of plants in commercial retail outlets. These studies underscore the need for continued evaluation of promising new biological control agents.

*Serangium parcesetosum* Sicard is a coccinellid predator that has demonstrated potential for the biological control of silverleaf whitefly (Legaspi et al. 1996). This species was originally collected from India in 1929 for release as a biological control agent of citrus whitefly, *Dialeurodes citri* Ashmead (*Aleyrodidae*), in the Union of Soviet Socialist Republics (Kapur 1954; Timofeyeva & Nhuan 1979). As a result of the success in that biological control program, and because of its rediscovery during foreign exploration in Podumbu, India, *S. parcesetosum* is currently being researched as a predator of silverleaf whitefly. *Serangium parcesetosum* (herein referred to as *Serangium*) has been known from the available literature to feed mainly on citrus whitefly, although in field trials in the U.S. it has also attacked silverleaf whitefly (M. C., unpublished). All of the known coccinellids belonging to the tribe Serangiini are obligate predators of whiteflies, or in a few cases, scale insects (Gordon 1985).

Laboratory studies to date show that both larvae and adults of *Serangium* are voracious feeders, capable of consuming large numbers of immature silverleaf whiteflies in short periods of time. Legaspi et al. (1996) showed adults consumed approximately 400 whitefly nymphs in a 24h period. *Serangium* larvae consumed 25 to 50 whitefly eggs or nymphs in 24 h, depending on the larval stage (M. C., unpublished). Furthermore, Legaspi et al. (1996) determined the cumulative lifetime predation rate to be approximately 5,000 whitefly nymphs per adult *Serangium*. These data suggest that *Serangium* may have the potential to control silverleaf whitefly at moderate to high levels. However, low whitefly infestation levels may not be adequate to sustain *Serangium* reproduction or even adult survival.

In this study we investigate the effects of *Serangium* release rates on the population dynamics of *B. argentifolii* on caged poinsettia.

**Materials and Methods**

Four rooted poinsettia (*Euphorbia pulcherrima* Willd. Ex. Klotzsch. cv. ‘Freedom Red’) cuttings were transplanted into individual 30 cm pots on 16 July 1997 in the University of Connecticut research greenhouse range. Each exclusion cage was constructed of a white organdy sleeve supported by 75 cm bamboo plant stakes and placed around each pot. Cages were sealed above the four poinsettia plants and below the lip of each pot. Velcro strips were used on two vertical seams, one on each side of the cage, to facilitate access to the plants.

After plants were established in the pots, silverleaf whiteflies were introduced on the caged poinsettia plants on 23 Aug. 1997 at a rate of either 1 or 10 adults per plant (equivalent to 4 or 40 adult whiteflies per cage). On 3 Oct., *Serangium parcesetosum* were introduced into cages at 0, 2 and 4 adults per plant (equivalent to 0, 8 and 16 *Serangium* per cage, respectively). The result was a 2 x 3 factorial design with initial levels of either 1 or 10 whiteflies per plant and 0, 2, or 4 *Serangium* per plant. Treatments were arranged in a randomized complete block with five replications. Poinsettias in separate cages, but without either prey or *Serangium*, were used to evaluate the effects of whitefly on plant growth. Prey and *Serangium* were shipped overnight from the USDA APHIS Mission Plant Protection Center (Mission, TX) in insulated containers with ice packs. A small Hibiscus plant infested with whitefly pupae was shipped just prior to emergence of the adults. Whiteflies were held in a controlled temperature chamber until adults emerged within 48 h. Newly emerged whitefly adults were aspirated and then transferred into the treatment cages. *Serangium* were shipped as adults in paper cartons with organza lids for ventilation. Each container held 25 adults. A Hibiscus leaf with whiteflies was included in each container for *Serangium* feeding in transit. Immediately upon arrival, *Serangium* beetles were introduced into treatment cages using a fine camelhair paintbrush.

Monitoring Silverleaf Whitefly and *Serangium* Populations

Two leaves per plant (8 leaves per cage) were harvested weekly from 3 Oct. to 5 Dec. 1997. Leaves were selected from the strata of the plant canopy with the greatest number of late instar whitefly nymphs; the sample strata was determined each week just prior to leaf harvest. A 25 cm² section of each leaf was examined under a dissecting microscope and the number of whitefly eggs and the number of live and dead nymphs and pupae were recorded. Immature whitefly were judged dead when they appeared discolored or desiccated, or when the empty integument...

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showed evidence of *Serangium* feeding. As leaf samples were harvested they were visually checked for *Serangium*, and all larvae and adults were returned to their respective cages.

At the conclusion of the study, 8 Dec. 1997, a stratified leaf sample was collected from each cage and whitefly population and percent mortality were determined. The stratified sample consisted of two leaves per cage from each of four locations on the plant: bracts, upper canopy, middle canopy and lower canopy. The two leaves per stratum were collected randomly and examined for whitefly life stages with a dissecting microscope. The poinsettia plants were then destructively harvested and total laminar surface area (leaves plus bracts) was determined for all plants in each treatment using a LI-COR 1600 leaf area meter (LI-COR, Inc. Lincoln, NE). Plants were separated into bracts, leaves and stems and dried in a forced-air oven at 70°C for one week. Plant dry mass was then recorded.

**Data Analysis**

The mean density of whitefly eggs, nymphs, and pupae (per 25 cm²) was calculated for each treatment. Percent whitefly mortality was calculated as follows [1]:

\[
\frac{(\text{dead nymphs} + \text{dead pupae})}{(\text{live nymphs} + \text{live pupae} + \text{dead nymphs} + \text{dead pupae})} \times 100
\]

Equation [1]

Bartlett’s test for homogeneity of variance was conducted on all data (Bartlett, 1937). Non-normal data for whitefly and *Serangium* counts and for plant measurements were transformed using the formula: \(\log_{10}(x + 1)\), with \(x\) representing dependent variables. Non-normal data for whitefly mortality were transformed using the formula: \(\text{Arcsine}\left(\frac{x}{100}\right)^{\frac{1}{2}}\), with \(x\) representing percent whitefly mortality. Analysis of variance was performed using the Statistical Analysis System (SAS Institute 1995). Insect population data were analyzed as a factorial in a randomized complete block design with five replicates. Plant data (laminar surface area and shoot dry mass) were subjected to a two-way analysis of variance with seven treatments and five replicates. The treatments included the six whitefly-*Serangium* combinations and a control with no predators or prey.

**The Greenhouse Environment**

Plants were grown under standard cultural practices for poinsettias (Ecke et al. 1990). Environmental conditions were recorded on a Campbell Scientific 21x Datalogger (Campbell Scientific, Logan, UT) at 1-minute intervals. LI-COR 190-SA quantum sensors (LI-COR, Inc. Lincoln, NE) were used to monitor photosynthetic photon flux and copper-constantan thermocouples were used to monitor temperature. Light intensity was monitored in the greenhouse immediately above the exclusion cages, and also above the plant canopy within the cages. Air temperature was monitored within the cages in the plant canopy and above the canopy, and directly outside of the cages. For the duration of the study, daily photosynthetic photon flux (±SE) averaged 9.1 ± 0.5 mol·m⁻²·day⁻¹ in the greenhouse above the cage and 8.0 ± 0.5 mol·m⁻²·day⁻¹ above the plant canopy in the cage. Air temperature in the cage and in the plant canopy (in the cage) averaged 21.3 ± 0.1°C and 21.4 ± 0.2°C (respectively) and the ambient air temperature in the greenhouse (outside the cage) averaged 22.4 ± 0.2°C.

**RESULTS**

Initial silverleaf whitefly release rates greatly affected final population densities of all whitefly life stages (Figs. 1 & 2; df = 1,16; eggs \(F = 12.3; P = 0.025\), nymphs \(F = 32.8; P = 0.005\), pupae \(F = 10.7; P = 0.031\)). This effect was most evident when whitefly populations were left uncontrolled. For example, the final density of nymphs was approximately 10-times higher in cages with initial release rates of 10 whitefly adults per plant than in cages with initial release rates of 1 whitefly adult per plant (Fig. 1b). A similar pattern was observed

![Fig. 1a-c. Changes in silverleaf whitefly life stages on poinsettia plants over time in the absence of a biological control agent. Plants were initially inoculated with either 1 or 10 whitefly adults per plant on 23 August. Stages include; a) eggs, b) nymphs, and c) pupae. Vertical bars denote standard error of the mean.](image-url)
with respect to both whitefly eggs (Fig. 1a) and pupae (Fig. 1c) at the 1 and 10 whitefly release rates.

A single release of adult *Serangium* beetles was extremely effective at stopping the growth of whitefly populations on poinsettias (Fig. 2). Six weeks after *Serangium* were introduced (13 Nov.), whitefly population densities were dramatically higher in cages without *Serangium* (Fig. 1) than in cages with *Serangium* (Fig. 2) \( \text{F} = 13.9; P = 0.0003, \text{F} = 19.3; P = 0.0001, \text{F} = 9.4; P = 0.002 \) . *Serangium* effectively maintained immature whitefly densities at or near those observed at the time of predator introduction (Fig. 2). For example, nymphal prey densities were 0.7 and 8.0 per 25 cm\(^2\) of leaf surface in the 1 and 10 whitefly cages (respectively) when *Serangium* were introduced (Fig. 2b). These populations remained nearly constant when exposed to either the 2 or 4 beetles per plant release rates (Fig. 2b), while nymphal densities increased up to 70 fold in the ensuing 10 week period without *Serangium* (Fig. 1b). Within both the 1 and 10 whitefly treatments, similar final prey densities were observed for the high and low *Serangium* treatments (Fig. 2).

A dramatic increase in prey mortality was observed within 14 days (16 Oct.) of *Serangium* release (Fig. 3; \( \text{df} = 2,16; F = 49.5; P = 0.0001 \)). In cages with an initial release rate of 10 whitefly adults per plant, mortality reached 57 and 69% for the 2 and 4 *Serangium* treatments (respectively) on 16 Oct. (Fig. 3a). Whitefly mortality in these treatments peaked at about 85% during the 23 Oct. to 6 Nov. time period and was approximately 55% at final harvest. Over the 10 week experimental period, prey mortality averaged 60% (± 4.4% SE) with *Serangium* present in cages with the 10 whitefly treatments.

In cages with an initial whitefly release rate of 1 adult per plant, the increase in prey mortality was less dramatic than in the 10 whitefly treatments (Fig. 3a). For example, mortality was only 10 and 38% for the 2 and 4 *Serangium* treatments (respectively) on 16 Oct. but reached approximately 50 and 80% on 30 Oct. At final harvest in the 1 whitefly treatment, prey mortality rates were about 20 and 60% with 2 and 4 *Serangium* (respectively). Over the 10 week experimental period, whitefly mortality averaged 24% (± 3.9% SE) with an inoculation of 2 *Serangium* per plant and 52% (± 3.9% SE) with 4 *Serangium* per plant. In contrast, mortality in cages without *Serangium*
averaged 4.2% (±0.6% SE) in the 10 whitefly cages and 6.8% (±2.3% SE) in the 1 whitefly cages during the final 10 weeks of the study (Fig. 3b).

At final harvest, live immature whiteflies were observed throughout the plant canopy (Table 1). In cages without *Serangium*, approximately 70% of whiteflies were located in the upper leaf strata and on the red-colored poinsettia bracts. With *Serangium* present prey were more uniformly distributed in the leaf/bract strata in the 1 whitefly cages than in the 10 whitefly cages. This may indicate that *Serangium* needed to move more quickly to the upper canopy to find prey when prey populations were low but not when populations were high. In the 1 whitefly treatment cages with *Serangium*, the average live immature whitefly counts observed throughout the canopy were about 16% of those without predators (Table 1). In the 10 whitefly treatment cages with *Serangium*, the average live immature prey counts were about 10% of those without predators. Average live immature prey counts were similar for both the 2 and 4 *Serangium* treatments in all four strata of the plant canopy with an initial prey release of 1 per plant (Table 1). Only small differences in the number of live immature whiteflies in the middle and lower leaf strata were observed between the 2 and 4 *Serangium* treatments with an initial whitefly release of 10 per plant.

At final harvest, the highest prey mortality was observed in the lower half of the plant canopy (middle and lower leaf strata v. upper leaf and bract strata) (Table 1). Throughout the plant canopy, the highest mortality consistently occurred in cages with *Serangium* and in cages with high initial whitefly release rates (Table 1).

Very few *Serangium* were recovered from cages at time of final harvest and the total number (larvae + adults) of *Serangium* recovered did not vary with treatment (P≤0.05). In cages with an initial inoculation rate of 1 prey, only adult *Serangium* were recovered and at an average density of 0.2 and 1.2 per cage for the 2 and 4 *Serangium* release rate treatments, respectively. In cages with an initial inoculation rate of 10 prey, 2 and 2.4 *Serangium* per cage were recovered from the 2 and 4 *Serangium* release rate treatments (respectively) and *Serangium* larva (1.2 per cage) were only observed in the 2 *Serangium* release rate treatment. During the final weeks of this study, whitefly mortality rates declined in all *Serangium* treatments (Fig. 3a) and simultaneously whitefly populations increased (Fig. 2). These data suggest that low *Serangium* counts at final harvest were not indicative of the predator levels that prevailed during the first eight weeks following *Serangium* release when maximum prey control was observed (Fig. 2).

Poinsettia growth was unaffected by whitefly populations in this study. Total laminar surface area (1.6 ± 0.03 m²/cage, df = 6, 23; F = 1.99; P = 0.11) and shoot dry mass (84.7 ± 1.9 g/cage, df = 6, 23; F = 0.86; P = 0.54) were similar for plants in all treatments.

**Discussion**

Consumers have a low tolerance for insect pests on greenhouse ornamentals like poinsettia. Consequently, high standards must be set when evaluating the effectiveness of natural enemies and their management. Inundative releases of *Encarsia formosa* can produce satisfactory results if introduced in sufficient numbers before whitefly populations begin to build (Hoddle et al. 1997a,b). However, in instances where *Encarsia* fail to control whiteflies, alternative measures are required in order to maintain a salable plant (Parrella et al. 1991; Heinz & Parrella 1994).

In our study, silverleaf whitefly reached damaging populations 6–8 weeks after introduction when left uncontrolled even when initial populations were low (1 adult per plant). Heinz & Parrella (1994) observed a dramatic increase in whitefly populations on greenhouse grown plants (both inside and outside of exclusion cages) after nine weeks exposure to whiteflies even in the presence of weekly releases of *E. luteola*. However, a series of three weekly releases of the predatory beetle *Delphastus pusillus* (1 beetle per plant per week) effectively checked whitefly population growth until the study was ended 3 weeks after the final release. In our study, a single release of 2 *Serangium* per plant effectively checked further increases in prey population for up to 10 weeks.

Heinz & Parrella (1994) recovered several adult *D. pusillus* 3 weeks after the last release, but no evidence of successful predator reproduction was reported. Hoelmer et al. (1993) reported that *D. pusillus* required 100-150 whitefly eggs per day to initiate and sustain oviposition. In our study, *Serangium* larvae were first observed 2 weeks after adults were released in cages with high initial whitefly levels (data not shown) but not in cages with low initial whitefly levels. In cages with high initial prey levels, *Serangium* larvae were recovered as late as 10 weeks after adults were introduced. Cohen & Brummett’s (1997) data suggest that *Serangium* could consume a sufficient number of whitefly immatures daily 10 hour feeding to meet the minimum methionine requirement for normal growth and development. This calculation assumes an average handling time of 1 minute per prey item, and with *D. pusillus* (Hoelmer et al. 1993), it appears from our study that *Serangium* can only reproduce (without nutritional augmentation) under high prey populations.

Legaspi et al. (1996) reported that the average life-span of *Serangium* ranged from 75 days at 20°C to 25 days at 30°C. In our study, typical commercial cropping practices were used, poinsettia...
TABLE 1. DENSITY OF LIVE IMMATURE SILVERLEAF WHITEFLY AND INCIDENCE OF WHITEFLY MORTALITY AT FOUR LEVELS IN THE POINSETTIA CANOPY AT FINAL HARVEST.

<table>
<thead>
<tr>
<th>Initial release rate treatments</th>
<th>Sample strata in poinsettia canopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whitefly</td>
<td>Bracts</td>
</tr>
<tr>
<td>(No. per plant)</td>
<td>Whitefly</td>
</tr>
<tr>
<td></td>
<td>(No. ± SE)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>F-value (P-value)</th>
<th>F-value (P-value)</th>
<th>F-value (P-value)</th>
<th>F-value (P-value)</th>
<th>F-value (P-value)</th>
<th>F-value (P-value)</th>
<th>F-value (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whitefly</td>
<td>1</td>
<td>17.2</td>
<td>(0.014)</td>
<td>1.2</td>
<td>(0.334)</td>
<td>32.4</td>
<td>(0.005)</td>
<td>7.1</td>
</tr>
<tr>
<td>Serangium</td>
<td>2</td>
<td>6.5</td>
<td>(0.009)</td>
<td>1.3</td>
<td>(0.308)</td>
<td>22.42</td>
<td>(&lt;0.001)</td>
<td>4.7</td>
</tr>
<tr>
<td>Whitefly × Serangium interaction</td>
<td>2</td>
<td>0.9</td>
<td>(0.426)</td>
<td>0.4</td>
<td>(0.704)</td>
<td>14.6</td>
<td>(&lt;0.001)</td>
<td>5.2</td>
</tr>
</tbody>
</table>

*Silverleaf whitefly counts are expressed per 25cm² of leaf surface.
canopy temperature averaged 21.6°C and the crop matured in a normal time period. Legaspi et al. (1996) reported a mean life-time cumulative predation of 4909 whiteflies (eggs and immature stages) for Serangium at a mean temperature of 20°C. Even without reproductive success, the single Serangium release in our study effectively prevented prey populations from increasing over a 10-week period (Fig. 1). It appears that this success was largely due to the prolonged survival and continuous feeding of individual adult beetles.

Due to the relatively high number of whiteflies needed to sustain Serangium reproduction and the extremely low pest levels tolerated on ornamental crops, it is unlikely that Serangium could function effectively as the sole biological control agent on a crop like poinsettia. However, Serangium would be especially useful for suppressing localized pest population increases or ‘hot spots’ in the greenhouse, or as the primary biological control agent on crops such as greenhouse tomato where pest population tolerance levels are higher than for ornamental crops. Based on our data it appears that Serangium might be best suited for inclusion in a multiple species biological control approach to silverleaf whitefly management on ornamental crops. As an obligate whitefly predator with a voracious feeding potential, Serangium is capable of checking rapid increases in whitefly populations (based on the caged studies herein), thus potentially enabling whitefly parasitoid species such as Eretmocerus or Encarsia to suppress whiteflies to acceptable thresholds. Heinz & Nelson (1996) found that D. pusillus provided the greatest suppression of silverleaf whitefly when used in conjunction with one or more species of Encarsia. In order to determine if Serangium would be effective in such a role, interspecific interactions between predator and parasite within the host species (pest/plant), as well as release management strategies, must be investigated.

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SURVEY OF TERMITES IN THE DELTA EXPERIMENTAL FOREST OF MISSISSIPPI

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ABSTRACT

Termites were surveyed in the Delta Experimental Forest in west central Mississippi in 1998. Logs, branches, and stumps along three 200-m long, 6-m wide transects were investigated at each of the three study plots. Two subterranean termite species in the family Rhinotermitidae, viz., *Reticulitermes flavipes* (Kollar) and *Reticulitermes virginicus* (Banks), were recorded. *Reticulitermes flavipes* was the common species and constituted 81.3% of the termite occurrences. Of the 685 pieces of wood surveyed, 16.5% had termites. The percentage of the two termite species varied among plots. The percentage of wood materials with signs of termite activity or foraging termites present was positively correlated with the diameter of the wood materials (R = 0.85). The chances of a log, branch, or stump being attacked by these termites increases by 1.3% as the diameter of the wood material increases 1 cm. The percentage of dead wood with sign of termite activity ranged from 11.6% to 67.2% among the sampled plots. Termites were significantly less abundant at Plot 3, which might correspond to a lower elevation and a higher soil moisture.

Key Words: *Reticulitermes flavipes*, *Reticulitermes virginicus*, forest, relative abundance

RESUMEN

Una inspección de termitas se llevo a cabo en el bosque del Delta Experimental Forest al centro-oeste de Mississippi en 1998. Se seleccionaron 3 lotes de 200 m de largo por 6 m de ancho y en cada uno de ellos se colectaron troncos, ramas y tocones. Se anotaron dos especies de termitas subterráneas en la familia Rhinotermitidae: *Reticulitermes flavipes* (Kollar) y *Reticulitermes virginicus* (Banks). *R. flavipes* fue la especie más común y constituyó el 81.3% de las ocurrencias de termitas. De los 685 pedazos de madera inspeccionados, 16.5% tenían termitas. El porcentaje de las dos especies de termitas varió entre lotes. El porcentaje de materiales de madera con señas de actividad de termitas o la presencia de termitas forrajeras fue correlacionada positivamente con el diámetro de los materiales de madera (R = 0.85). La probabilidad de un tronco, rama o tocón ser atacado por estas termitas incremento un 1.3% al incrementar el diámetro de estos materiales por 1 cm. El porcentaje de madera muerta con señas de actividad de termitas vario entre 11.6 a 67.2% entre los lotes muestreados. Las termitas fueron significativamente menos abundante en el Lote 3, lo cual puede corresponder a una elevación mas baja y una humedad de suelo mas alta.

Termites play an important role in forest ecosystems, especially in tropical rain forests, where they occur in extremely dense populations (Harris 1966; Gray 1972; Matsumoto 1976). Termites promote organic matter decomposition, alter soil properties, and provide food to other animals (Wood & Sands 1978; La Fage & Nutting 1978). Although their ecological importance is well known, termites have rarely been quantitatively studied in forests of the United States (Gentry & Whitford 1982; Howard et al. 1982).

Like any other insects, the abundance of termites is influenced by the distribution and quality of their food materials. However, there is very little information regarding the relationship between termites and size or distribution of wood materials (Gentry & Whitford 1982; Jones et al. 1995).

In Mississippi, *Reticulitermes flavipes* (Kollar) and *Reticulitermes virginicus* (Banks) appear to be the most common termite species. In 1998, we conducted a survey of termites in an experimental forest. In this study, we tried to determine the species composition and their foraging activities associated with dead wood in a forest environment. The objectives of this study were to quantitatively describe termite distribution patterns and feeding activities in different forest ecosystems.

MATERIALS AND METHODS

Study Sites

This study was carried out at the Delta Experimental Forest near Stoneville, Mississippi. The study site is mostly level with gentle slopes (<2°) at some sections. The soil type is fine-textured Sharkey clay soil with clay particle content varies from 60 to 85% (Krinard & Johnson 1985). It expands considerably when wet in the winter and...
cracks profusely when dry in the summer. The study sites were sometimes under water in the wet winter months (December-February). Average air temperature in January and July from 30-year data is 5.1°C and 27.6°C, respectively (Boykin 1995). The average annual precipitation is 1326 mm.

Three 200 ha experimental plots were delineated for the survey based on differences in tree stand.

Plot 1 is a sweetgum (Liquidambar styraciflua L.) plantation established in 1964-65 at 3 x 3-m spacing. The diameter at breast height is 23.4 ± 1.2 (mean ± standard error) cm. Some sugarberries (Celtis laevigata Willdenow) have grown into small to large trees. Common shrubs were: sugarberry, hawthorn (Crataegus spp.), willow oak (Quercus phellos L.), Chinese privet (Ligustrum sinense Loureiro), and blackberry (Rubus sp.).

Plot 2 is a naturally regenerated tree stand. Major trees species were: red oaks (Quercus spp.), sweetgum, sugarberry, overcup oak (Quercus lyrata Walter), green ash (Fraxinus pennsylvanica Marshall), and elms (Ulmus spp.). Common understory shrubs were: Chinese privet, swamp dogwood (Cornus stricta Lamarck), Eastern swampprivet (Forestiera acuminata (Michaux) Poir), American snowball (Styrax americana Lamarck), green ash, elms, deciduous holly (Ilex decidua Walter), and blackberry.

Plot 3 is an even-aged stand of natural regeneration resulting from a clearcut in 1937. Major tree species were: green ash, cottonwood (Populus deltoides Marshall), sugarberry, sweetgum, red oaks, black willow, elms, and white oaks. Understory shrubs were similar to Plot 2 but at a much lower density. This plot is at a slightly lower elevation than plots 1 and 2 and is more likely to be flooded in wet winter months.

Soil water content of the plots was measured on September 15, 1999. The precipitation during one month prior to the sampling date was 35.3 mm. The precipitation during the same period of 1998 was 68.1 mm. Nine soil samples were taken from 0-9 cm of the “A” layer. Then they were dried in an oven at 105°C for 48 hrs. Soil moisture content was determined by the difference between fresh soil weight and dry soil weight over fresh soil weight.

Survey

A survey of termites was conducted from October 20 through November 25, 1998 in plots 1, 2, and 3, in that order. Weather data in the three plots during the survey were: average maximum air temperature 22.9, 29.5, and 17.6°C, respectively; average minimum air temperature 8.6, 13.6, and 8.1°C, respectively; average daily rainfall 0.3, 1.5, 6.4 mm, respectively (Stoneville weather station, http://www.deltaweather.msstate.edu/). At each plot, three 200-m long, 6-m wide transects in north-south direction were delineated. Diameter at the large end of all logs, branches, and stumps >3 cm in diameter and partially or entirely within each transect was measured. Each was examined for the presence of termites or signs of termite activity. Termite galleries, fecal debris, and/or body parts were noted as signs of termite activity. Determining whether termite galleries exist in wood was usually not difficult. Galleries made by other arthropods had a more regular shape and not connected to each other into great lengths or width. Soil particles often exist in termite galleries or on surface of the wood. There were occasions when the wood materials were too decayed to determine whether or not termites had existed before. These wood materials were not included in the analysis. Results were recorded as three categories: I) with live termites, II) with sign of termite activity, or III) without termites and no sign of termite activity. When live termites were found, soldiers were collected for species determination. Host trees were not identified. Termite soldiers were examined under an Olympus SZX12 dissecting scope. Typically, two or three termite soldiers which are representative of the soldiers in size and morphology were examined. More soldiers were examined if necessary to determine the species. Termites were identified to species using the key provided by Scheffrahn & Su (1994), and identifications of representative specimens were verified by Rudolf Scheffrahn. Voucher specimens have been deposited in collections of Stoneville Research Quarantine Facility, USDA Agricultural Research Service, Stoneville, MS.

Statistical Analysis

The presence or absence of live termites or signs of termite activity in dead wood materials were recorded as 1 or 0, respectively. The wood materials were grouped into 6 categories according to diameter. The actual diameter of the 6 categories ranged from 3-5.9, 6-8.9, 9-11.9, 12-14.9, 15-17.9, and ≥18 cm, respectively. Analysis of Variance (ANOVA) was performed to test for differences between diameter groups and plots using PROC MIXED of the SAS software (SAS Institute 1999). Then the data were further analyzed for the trend between diameter of the wood materials and presence of termites or signs of termite activity. R² of the regression equation was determined by the ratio of the sum of squares for diameter groups as a trend with 1 degree of freedom divided by the sum of squares for diameter groups with 5 degrees of freedom. Soil moisture content data of the plots were compared by Tukey’s Studentized Range Test after ANOVA. All analyses were performed by SAS software (SAS Institute 1999).
RESULTS AND DISCUSSION

Species Composition and Distribution of Termites

Among the 685 branches, logs, and stumps examined, 114 (16.5%) had live termites. Two termite species were found, Reticulitermes flavipes (Kollar) and R. virginicus (Banks). Species were not identified in 7 samples because no soldiers were collected. Among the 107 identified termite samples, R. flavipes and R. virginicus were found in 12.7% and 2.9% of the samples, respectively (Table 1). Reticulitermes flavipes was most frequently encountered and occurred in 81.3% of the termite samples. This is similar to observations by Howard et al. (1982) in southern Mississippi. The relative abundance of R. virginicus in plots 1-3 was 7.7%, 21.4%, and 53.8%, respectively. The high percentage in Plot 3 was not precise because termites in 7 of the 20 samples were not identified. However, the percentage of R. virginicus in Plot 3 was at least 35.0%, which was still higher than the other plots. This higher percentage might relate with higher soil moisture. Soil moisture in Plot 3 (21.4 ± 0.8%) was significantly higher than that in Plot 1 (19.8 ± 0.8%) measured on September 15, 1999 (F = 12.90; df = 2, 24; P = 0.0002). As reported by Howard et al. (1982), R. flavipes tends to occupy higher, more arid places in southern Mississippi.

The percentage of samples with signs of termite activity in plots 1-3 was 60.0%, 67.2%, and 11.6%, respectively. The percentage in plots 1 and 2 was significantly higher than Plot 3 (F = 132.76; df = 2, 508; P < 0.0001) (Fig. 1). The much lower termite activity that observed in Plot 3 might in part be because termites in 7 of the 20 samples were not identified. However, the percentage of R. virginicus in Plot 3 was at least 35.0%, which was still higher than the other plots. This higher percentage might relate with higher soil moisture. Soil moisture in Plot 3 (21.4 ± 0.8%) was significantly higher than that in Plot 1 (19.8 ± 0.8%) measured on September 15, 1999 (F = 12.90; df = 2, 24; P = 0.0002). As reported by Howard et al. (1982), R. flavipes tends to occupy higher, more arid places in southern Mississippi.

The percentage of samples with signs of termite activity in plots 1-3 was 60.0%, 67.2%, and 11.6%, respectively. The percentage in plots 1 and 2 was significantly higher than Plot 3 (F = 132.76; df = 2, 508; P < 0.0001) (Fig. 1). The much lower termite activity that observed in Plot 3 might indicate unsuitable conditions for termites, especially for R. flavipes. Plot 3 tended to be flooded for a longer period of time and more frequently than plots 1 and 2 during the winter months. This might have created an unsuitable condition for termites to survive in the winter.

Live termites were found in 24.8%, 22.2%, and 7.0% of the logs, branches, and stumps in plots 1-3, respectively. The percentage of wood materials with termites in Plot 3 was significantly lower than that in plots 1 and 2 (Fig. 1) (F = 23.04; df = 2, 674; P < 0.0001). This pattern is same as the result on the percentage of wood with signs of termite activity. The lower temperatures during the survey might have an impact on the low number of termite occurrences in Plot 3. However, the difference between percentage of wood with termites and that with signs of termite damage in Plot 3 is proportionally smaller than those in plots 1 and 2 (Fig. 1). This suggests that the influence of temperature on termite occurrences in Plot 3 was not significantly higher than that in plots 1 and 2.

Relationship between Termite Activity and Size of Wood Materials

Most of the wood samples in the three plots were <12 cm in diameter. The percentage of wood samples with diameter ≥12 cm in the plots 1-3 was 1.8%, 5.1%, and 19.7%, respectively. A cause-effect relationship existed between percentage of samples with signs of termite activity and diameter of the wood (F = 20.34; df = 1, 508; P < 0.0001; R² = 0.73) (Fig. 2). The chance of a sample being infested by termites increased 1.3 ± 0.3% as the diameter of the wood increased 1 cm. Perhaps this is because larger wood materials tend to maintain moisture, which is important for termite survival and foraging. According to the regression equation, Percentage = 0.327 + 0.013 diameter, even a very small dry branch such as 1 cm in diameter will have a 34% chance of having been attacked by termites.

The percentage of samples with termites present was also positively correlated with diameter of the wood material (F = 30.82; df = 1, 674; P < 0.0001; R² = 0.72) (Fig. 3). The chance of a sample with termites present increased 1.3 ± 0.2% as the diameter of the wood materials increased 1 cm.

In conclusion, we found two species of termites in the Delta Experimental Forest near Stoneville, Mississippi. Reticulitermes flavipes was the most common termite species. The occurrence of termites varied with site conditions. Selection of

Table 1. Relative abundance of 2 termite species in 3 plots of the Delta Experimental Forest, Mississippi.

<table>
<thead>
<tr>
<th>Plot</th>
<th>Number of wood materials examined</th>
<th>R. flavipes</th>
<th>R. virginicus</th>
<th>Reticulitermes spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percentage (%)</td>
<td>Number</td>
<td>Percentage (%)</td>
</tr>
<tr>
<td>1</td>
<td>210</td>
<td>48</td>
<td>22.9</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>189</td>
<td>33</td>
<td>17.5</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>286</td>
<td>6</td>
<td>2.1</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>685</td>
<td>87</td>
<td>12.7</td>
<td>20</td>
</tr>
</tbody>
</table>

Wood materials with termites
wood materials by the two termite species was positively correlated with the diameter of the wood materials. This has practical significance for termite control and baiting studies. It provides an estimate of the probability of termite attack on wood materials.

Gentry & Whitford (1982) sampled lowland hardwood forests and pine plantations in South Carolina (similar latitude as in our study location), and found that the occurrences of *R. flavipes* and *R. virginicus* varied among different habitats. *Reticulitermes flavipes* represented 26.6-60% of the termites, which was lower than what we observed on the 3 sites on the Delta Experimental Forest. In their study, nearly all the wood greater than 2 cm diameter had signs of termite usage. The termite population or activity in their study location was much higher than in our study location.

We measured the length of every piece of wood in this survey. The wood materials were grouped into 4 categories according to their length. The actual length of the 4 categories ranges from <3, 3-5.9, 6-8.9, and ≥9 m, respectively. Although the length of wood had significant effect on the percentage of wood with live termites (F = 11.23; df = 3, 666; P < 0.0001), it did not have significant effect on the percentage of wood with signs of termite activity (F = 0.67; df = 3, 502; P = 0.57). A long branch with small diameter may be less likely to be attacked by termites than a short branch with large diameter. Therefore, length alone is not a good predictor for estimating the probability of termite attack on the wood materials.

Jones et al. (1995) found a positive relationship ($R^2 = 0.50$) between volume of wood and dry wood termite colony size on Mona Island, Puerto Rico. In a laboratory experiment, Hedlund and Henderson (1999) found termite consumption rate increased as the wood volume increased. Termites foraged less actively when larger wood existed. These findings support our conclusion that larger wood tends to be more attractive for termites to feed on. So, size of the wood materials measured by diameter or volume is important for termite foraging activities.

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SURVIVAL OF *DIAPHORINA CITRI* (HOMOPTERA: PSYLLIDAE), AND ITS TWO PARASITOIDS, *TAMARIXIA RADIATA* (HYMENOPTERA: EULOPHIDAE) AND *DIAPHORENCYRTUS ALIGARHENSIS* (HYMENOPTERA: ENCYRTIDAE), UNDER DIFFERENT RELATIVE HUMIDITIES AND TEMPERATURE REGIMES

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ABSTRACT

The ability of an exotic citrus pest, *Diaphorina citri* Kuwayama, and its two parasitoids, *Tamarixia radiata* (Waterston) and *Diaphorencyrtus aligarhensis* (Shafee, Alam & Agarwal), to survive under different relative humidities (7%, 33%, 53%, 75% and 97%) at 25 and 30°C was compared. The data obtained may help to predict potential climatic limitations to their establishment in Florida and indicate whether the pest has different climatic tolerances compared to these parasitoid populations. Adult survival was evaluated under relative humidities maintained by saturated salt solutions. *D. citri* survived longer than the parasitoid populations at all experimental conditions, suggesting it has a lower net water loss rate. The *T. radiata* (Taiwan) population showed the greatest moisture requirement at all temperatures and relative humidities tested. The *T. radiata* (Vietnam) population survived longer than the Taiwan, suggesting that the two populations may perform differently in different geographical regions. *D. aligarhensis* and *T. radiata* (Vietnam) survived similar lengths of time, except at the higher relative humidities, so the moisture requirements for these two populations are comparable.

Key Words: Asian citrus psylla, relative humidity tolerances, saturated salt solutions, biological control

RESUMEN

La habilidad de la plaga exótica del cítrico, *Diaphorina citri* Kuwayama, y sus dos parasitoides, *Tamarixia radiata* (Waterston) y *Diaphorencyrtus aligarhensis* (Shafee, Alam & Agarwal) de sobrevivir bajo diferentes humedades relativas (7%, 33%, 53%, 75% y 97%) a 25 y 30°C fueron comparadas. Los datos obtenidos pueden ayudar a predecir las limitaciones climáticas a su establecimiento en la Florida e indican si la plaga tiene diferentes tolerancias climáticas en comparación a estas poblaciones parasitoides. La supervivencia de adultos fue evaluada bajo humedades relativas mantenidas por soluciones de sal saturada. *D. citri* sobrevivió más tiempo que las poblaciones parasitoides bajo todas las condiciones experimentales, sugiriendo que tiene una velocidad más baja de pérdida de agua neta. La población *T. radiata* (Taiwán) demostró el mayor requerimiento de humedad en todas las temperaturas y humedades relativas probadas. La población *T. radiata* (Vietnam) sobrevivió mas que la de Taiwán, sugiriendo que las dos poblaciones pueden ejecutar de manera distinta en diferentes regiones geográficas. *D. aligarhensis* y *T. radiata* (Vietnam) sobrevivieron duraciones de tiempo similares, excepto bajo humedades relativas altas, indicando que los requerimientos de humedad para estas dos poblaciones son comparables.

The Asian citrus psylla, *Diaphorina citri* Kuwayama, was found for the first time in June 1998 by personnel in the Division of Plant Industry, Florida Department of Agriculture and Consumer Services (Hoy & Nguyen 1998; Halbert 1998; Mead 1977). It has successfully established in Florida and it will probably colonize all citrus-growing areas in Florida and may spread to Louisiana, Texas, Arizona and California citrus.

*D. citri* damages citrus by depleting sap from the plant and, because its saliva is toxic, growing shoots are distorted and may die. It also causes damage by excreting honeydew, which allows the growth of sooty mold (Chien & Chu 1996). However, the worst threat is that *D. citri* is an efficient vector of the bacterium, *Liberobacter asiaticum*, which causes greening disease. This is the most serious disease of citrus in the world, causing reduced production and eventual death of the trees (McClean & Schwarz 1970).

No effective eradication efforts are known for *D. citri*, but classical biological control of the psyllid vector should contribute to suppression of their populations. Two parasitoids have been imported (Hoy et al. 1999; Hoy & Nguyen 2000). *Tamarixia radiata* (Waterston) is an ectoparasitoid imported from Taiwan and Vietnam, while *Diaphorencyrtus aligarhensis* (Shafee, Alam &
Agarwal) is an endoparasitoid of D. citri obtained from Taiwan. Approximately 12,000 T. radiata were released into Florida between 15 July and 1 December 1999. D. aligarhensis was approved for release on 15 March 2000 and 5000 were released during the 2000 growing season (Hoy, unpublished).

The success of classical biological control programs may be determined by the use of the appropriate natural enemy ecotypes. Moisture requirements may be especially important in determining suitability of an imported biological control agent for release and establishment in a new geographic area. Only a few experiments have been conducted to compare the water balance or the temperature-relative humidity relations in a pest and its parasitoids. Yoder & Hoy (1998) found the citrus leafminer and two populations of its endoparasitoid Ageniaspis citricola Logvinovskaya both require a moisture-rich environment although the citrus leafminer is less dependent on high relative humidities than its parasitoid, A. citricola.

The objective of this study is to compare, under laboratory conditions, the effect of temperature and relative humidity on the survival of an exotic citrus pest, D. citri and its two parasitoids T. radiata and D. aligarhensis. This information will help in mass rearing these parasitoids and may help to predict potential climatic limitations to their establishment in Florida.

MATERIALS AND METHODS

Rearing

D. citri was reared on Murraya paniculata (Chien et al. 1989), ornamental orange jasmine, in the quarantine facility at the University of Florida, Gainesville (Skelley & Hoy, unpublished). The plants were pruned, fertilized and held under a photoperiod of 16:8 (L:D) at 21 to 24°C to stimulate the growth of new flushes because adult psyllids lay eggs only on newly sprouted leaf buds. Trees that had aphid or scale infestations were sprayed with an application of oil or pyrethrum (Prentiss, Inc., Sandersville, GA).

D. citri was reared in quarantine under a photoperiod of 18:6 (L:D) at 26°C and 60% relative humidity (RH). T. radiata colonies from Taiwan and Vietnam and D. aligarhensis from Taiwan were reared in separate cages and rooms in quarantine to reduce the risk of contamination. Both parasitoid species were reared at 27°C and 60% RH under a photoperiod of 18:6 (L:D) in cages (76 cm x 46 cm x 66 cm) constructed with a 1.27 cm diameter polyvinyl chloride pipe and covered with a fine nylon mesh.

Specimen Collection

Adults used for experiments were collected at 5 PM on the day of their emergence. To ensure that all four populations emerged on the same day, the psyllids, T. radiata and D. aligarhensis were set up 15 days, 12 days and 18 days, respectively, before the start of the experiments. The day before starting an experiment, all old psyllids and parasitoids were collected to ensure that only new individuals would be present in the cages. D. citri is collected by aspirating adults off the plant, while T. radiata can be collected off the top of the mesh cage. D. aligarhensis is aspirated most often from the bottom of the cage. Individual adults were placed in groups of five into 28.35 g condiment cups (Plastics, Inc. St. Paul, MN). The lid of the cup was covered with mesh glued to it with clear nail polish. All containers were cleaned with 70% EtOH before being reused.

Pretreatment Conditions

After collection, all individuals were pretreated for 21 hours by placing them into bell jars placed in an incubator (Percival I-30B) programmed with a photoperiod of 18:6 (L:D) at 17°C. RH within the bell jars was maintained by a saturated salt solution, Mg(NO₃)₂.6H₂O, which yields a 56% RH (Winston & Bates 1960; O’Brien 1948; Carr & Harris 1949). Specimens were given no water or honey. Pretreatment was expected to remove adsorbed water from the cuticular surface and to ensure a uniform physiological state. Pretreatment minimizes the effects of ingestion, reproduction, excretion and defecation (Arlian & Ekstrand 1975). The survival rate should reflect the relative changes in water balance of the insects before desiccation (Wharton 1985).

Experimental Conditions

At 2 PM specimens were transferred into stainless steel chambers (30.5 cm x 30.5 cm x 30.5 cm) (Fisher Scientific, Pittsburgh, PA) held within a single incubator (Percival I-35 series) at either 25 or 30°C with a photoperiod of 18:6 (L:D). Plastic cups containing five adults were placed into a chamber with either 7, 33, 53, 75 or 97% RH maintained by saturated salt solutions. Reagent grade salts (Sigma, St. Louis, MO) dissolved in distilled water were used: 200 ml of saturated salt solution was placed into chambers with the three higher RH, and 100 ml of salt solution was used in the two lower RH because they are hygroscopic substances and should be formulated as a paste to maintain the desired RH. These salts were chosen because they maintain the same RH at 25 and 30°C (Winston & Bates 1960). The chemicals used were sodium hydroxide (7% RH), magnesium chloride hexahydrate (33% RH), magnesium nitrate hexahydrate (53% RH), sodium chloride (75% RH) and potassium sulfate (97% RH). RH in each chamber was confirmed by a hygrometer (Thomas Scientific, Swedesboro, NJ), as well as the salt crystal method outlined by Winston & Bates (1960), which utilizes the fact...
that a salt crystal will pick up moisture and deliquesce in relative humidities which are at or above that maintained by its saturated solution. A very small crystal is introduced into the closed chamber and observed under magnification; if the RH is below that for the salt, there will be no change. If the RH is very close, the deliquescent will be only partial; if just above there will be a narrow rim of liquid around the mass. Experimental units were placed randomly into each RH chamber.

Survivability Tests

Survival of individuals was recorded every three hours. RH chambers were sampled separately and specimens were out of the chamber for a maximum of ten minutes. Insects found on their dorsal surface that failed to respond to probing were considered dead. All dead specimens were sexed.

Statistics

Four replicates were tested at 25°C under each RH on separate dates, while three replicates were run at 30°C and each RH condition. At 25°C, a total of 400 individuals (100 per experiment) were tested for D. citri and the two T. radiata populations. Only 300 individuals (75 per experiment) of the D. aligarhensis population were tested at 25°C. At 30°C, a total of 300 individuals (100 per experiment) were tested for all four populations. Mean percentage survival (±SEM) was calculated for all four populations at each temperature-RH combination. LT50 values were calculated by adding the time it took to get 50% mortality under each condition on each date. A grand mean and SEM was determined for each population at each temperature-RH combination. LT50 values were compared statistically by z test statistics.

RESULTS AND DISCUSSION

The survival of D. citri, D. aligarhensis and T. radiata populations increased with increasing RH (Figs. 1, 2). When the temperature was increased from 25 to 30°C, adult survival of all populations decreased, as expected (Figs. 1, 2). At 75 and 97% RH at both temperatures, D. citri survived longer than the parasitoid populations, suggesting it has a lower net water loss rate (Figs. 1D, 1E, 2D, 2E).

The T. radiata (Taiwan) population showed the greatest moisture requirement at all temperatures and RH tested. The T. radiata (Vietnam) population survived longer than the Taiwan population at 25°C and 53, 75 and 97% RH as well as all RH tested at 30°C (Figs. 1C, 1D, 1E, 2). This suggests that the two populations may perform differently in different geographical regions.

D. aligarhensis and T. radiata (Vietnam) survived similar lengths of time under all RH at 25 and 30°C, so the moisture requirements for these two populations appear comparable (Figs. 1, 2).

LT10 and LT50 values can be found easily from the figures, but the differences are small and not many inferences about the populations can be made at the ends of the curves. The LT50 values of the pest D. citri were always higher than the LT50 values of its parasitoid populations at 25°C (Fig. 1).

LT50 values at 25°C for D. aligarhensis are different from LT50 values for the T. radiata (Taiwan) population (P < 0.05) (Table 1). D. aligarhensis and T. radiata (Vietnam) had different LT50 values, except at 53 and 97% RH (z = 0.10, P > 0.05; z = 0.75, P > 0.05) (Table 1). The LT50 values of the two T. radiata populations are not different at 7 and 33% RH (z = 1.55, P > 0.05; z = 0.10, P > 0.05) (Table 1).

All parasitoids held at 25°C were dead by 40.5 hours, whereas a few psyllids survived up to 94.5 hours (Fig. 1). There were no differences between survival of males and females of the D. citri and T. radiata populations, perhaps because there is no apparent difference in size. The D. aligarhensis population is made up of females only.

The LT50 values of the pest were equal to or greater than the LT50 values of its parasitoids at 30°C (Table 1). As the RH increased to 97%, the LT50 of the D. citri population was different from that of D. aligarhensis and the T. radiata (Vietnam) population (P < 0.05) (Table 1). The LT50 values of the T. radiata (Taiwan) population were different (P < 0.05) from the D. citri, D. aligarhensis and T. radiata (Vietnam) populations at all RH tested (Table 1). Again, there were no differences in survival rates of males and females. All parasitoids held at 30°C were dead by approximately 40.5 hours, but a few psyllids managed to survive up to 52.5 hours (Fig. 2).

The data suggest that, especially at 75 and 97% RH at 25°C and 97% RH at 30°C, some D. citri will have up to two times longer to find food and water in the field compared to its parasitoids (Figs. 1D, 1E, 2E). D. citri also survived well at the lower RH of 7 and 33%, suggesting that it could disperse and survive in the more arid conditions found in western citrus-growing regions in the United States (Figs. 1A, 1B, 2A, 2B).

The RH responses of the Vietnam population of T. radiata were different from those of the Taiwan population, especially at 30°C, suggesting that the two populations of T. radiata may be ecotypes (populations varying in their ability to survive in different climates).

This information is helpful in rearing these parasitoids for release in biological control programs because it indicates there are no large differences in pest and natural enemy responses to the RH and temperatures likely to be encountered in the rearing facility. The ability of D. citri
Fig. 1. Survival curves of the Asian citrus psylla, *Diaphorina citri*, and its two parasitoids, *Diaphorencyrtus aligarhensis* imported from Taiwan and *Tamarixia radiata* imported from Taiwan and Vietnam, at 25°C and 7% RH (A), 33% RH (B), 53% RH (C), 75% RH (D), and 97% RH (E).
Fig. 2. Survival curves of the Asian citrus psylla, *Diaphorina citri*, and its two parasitoids, *Diaphorencyrtus aligarhensis* imported from Taiwan and *Tamarixia radiata* imported from Taiwan and Vietnam, at 30°C and 7% RH (A), 33% RH (B), 53% RH (C), 75% RH (D), and 97% RH (E).
to survive longer at 97% RH than its parasitoids is interesting and difficult to interpret (Figs. 1E, 2E). It could mean that D. citri is better adapted to very high RH than its natural enemies. The data obtained compare the intrinsic water loss rates of these four populations under extreme conditions of no food or water, and the ability of D. citri, D. aligarhensis and T. radiata populations to disperse to more optimal sites was precluded. In citrus groves D. citri can feed on host plants to obtain water and food. Likewise, the parasitoids can host feed for moisture and nutrients, feed on honeydew, drink droplets of water and disperse to find microhabitats more suitable for survival.

Yoder & Hoy (1998) found two populations of A. citricola and their host, the citrus leafminer, both require a moisture-rich environment although the citrus leafminer is less dependent on high RH than its parasitoids. The results of this study were similar, with D. citri and its parasitoids requiring a moisture-rich environment, although D. citri does not depend on high RH as much as its parasitoids, especially the T. radiata (Taiwan) population. D. citri survived longer at higher temperatures and lower RH than the T. radiata (Taiwan) and the D. aligarhensis populations (Figs. 2A, 2B).

Hoy et al. (2000) found slight physiological differences in the Taiwan and Australian ecotypes of A. citricola, which indicated genetic differences exist. Subsequent analysis of Random-Amplified-Polymorphic-DNA Polymerase-Chain-Reaction (RAPD-PCR) and DNA sequence analyses of two Actin genes led to the conclusion that the two populations actually are cryptic species, even though these two populations of A. citricola can not be separated on the basis of morphological characteristics (Hoy et al. 2000). Additional analysis of the two populations of T. radiata could establish whether they are cryptic species.

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FALL DRAGONFLY (ODONATA) AND BUTTERFLY (LEPIDOPTERA) MIGRATION AT ST. JOSEPH PENINSULA, GULF COUNTY, FLORIDA

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ABSTRACT

I describe the fall 1999 migration of 5 Lepidoptera and 4 Odonata species north along St. Joseph Peninsula, Gulf County, in the Florida Panhandle. Highest counts were for the Gulf fritillary butterfly (Agraulis vanillae (L.), Lepidoptera: Nymphalidae) which accounted for 58% of the insects counted; the highest rate was 3,162/h, with an estimate of total season migration of over 250,000 individuals. The common green darner dragonfly (Anax junius (Drury 1773) Odonata: Aeshnidae) was the next most common with a maximum rate of 3,297/h. The median and peak period for these two species was the first week in October. The observed flight pattern may demonstrate a reluctance to cross open water.

Key Words: Odonata, dragonfly, Anax junius, common green darner, butterfly, Lepidoptera, Gulf fritillary, Agraulis vanillae, migration, dispersal

RESUMEN

Se describe la migración de 5 especies Lepíptera y 4 especies Odonata en el otoño de 1999 hacia el norte a lo largo de la península de St. Joseph, condado del Golfo, en la región noroeste de la Florida. Las cuentas más altas fueron de la mariposa Agraulis vanillae (L.) (Lepidóptera: Nymphalidae), la cual constituyó el 58% de los insectos contados; la cuenta más alta fue de 3,162/h, con un estimado de migración estacional total de más de 250,000 individuos. La libélula Anax junius (Drury 1773) (Odonata: aeshnidae) le sigue en número con una cuenta total de 3,297/h. El medio y periodo cumbre para estas dos especies fue la primera semana de Octubre. El patrón de vuelo observado puede demostrar una aversión a cruzar el agua.

MATERIALS AND METHODS

Both butterflies (Lepidoptera) and dragonflies (Odonata) were counted on St. Joseph Peninsula, Gulf County, Florida from 14 Aug through 7 Dec, 1999. The peninsula is 24 km long, oriented north-south, roughly parallel to the mainland (Fig. 1). The count location was on an elevated, 50 m long boardwalk bisecting the peninsula, at Eagle Harbor (29°45.98′N, 85°24.29′W) in St. Joseph Peninsula State Park. At the boardwalk, the peninsula has a width of 200 m and is 11 km from the north tip (Fig. 1).

There are only a few small freshwater wetlands on the peninsula that support small breeding populations (<50 pair) of Odonata including A. junius, black saddlebags (Tramea lacerata Hagen 1861, Odonata: Libellulidae) and Carolina saddlebags (Tramea carolina (L.), Odonata: Libellulidae). There were low numbers of breeding butterflies including D. plexippus and Gulf fritillary butterfly (Agraulis vanillae (L.), Lepidoptera: Nymphalidae) on the peninsula.

Counts were made in 2, 5-minute periods per hour, 1-2 days per week, from 3 to 11 hours each day. All species with ≥3 cm wing span were counted. Flight direction, estimated height, predation, perching, tandem flights, and copulation, were recorded. Weather conditions, particularly frontal boundary, and wind direction were recorded.

Hourly flight rates (in any flight direction) were calculated from the average of the 2, 5-minute counts in an hour. Highest observed hourly migration rates for the fall for each species were reported. Linear interpolation between the observed hourly flight rates, first between hours in a day,
and then between days, gave an hourly rate for each unobserved daylight hour from 14 Aug to 7 Dec. All observed and interpolated hourly rates were summed to get an index of total fall flight magnitude. The date ranges when the middle 50% of the individuals of a species migrate were calculated (that is, the period between the dates on which migration was 25 and 75% complete).
RESULTS AND DISCUSSION

A total of 12,616 insects were counted in 222, 5-minute count intervals on 19 days from 14 Aug to 7 Dec. Most (98.7%) were heading north along the peninsula (Fig. 1), with the remainder either patrolling (Odonata) or heading south (both Odonata and Lepidoptera). The most common migrant was the Gulf fritillary butterfly, *A. vanillae*, followed by the common green darner dragonfly, *A. junius* (Table 1). All insects appeared to use powered flight rather than soaring. Although there were peak flights during the first week of October (Fig. 2), there was a steady and persistent flight on each clear day.

One possible flight pattern is that the insects head south down the continent, fly west when reaching the Gulf, and are then funneled north via the peninsula. The northeast prevailing wind direction in the fall (Winsberg 1990) would provide a further push from the mainland onto the peninsula (Fig. 1). Local sea breezes, would complicate this general pattern. The insects may exhibit some risk avoidance by not crossing the open Gulf. This migration route has been documented for 3-5,000 hawks annually, with the heaviest flights 3-4 days after the passage of northern cold fronts when the wind is from the northeast (Stedman 1984). After reaching the north tip of the peninsula, it is unknown whether the insects fly the 2-km distance to the mainland or head southwest over the Gulf (to uncertain landfall). The low number of south-flying insects counted suggests that they are not back-tracking down the peninsula. Observations from the peninsula may not necessarily reflect the direction of a potentially larger migration on the mainland.

There have been observations from boats (e.g., Lowery 1946) and oil platforms (Baust et al. 1981, Russell 1999) of some dragonflies and butterflies flying over the Gulf. My observations of monarch migration match previously recorded western movement of eastern populations (Urquhart & Urquhart 1978; Van Hook & Hermann 1999); and destinations may be either the Gulf Coast states, or Mexico. These data do not suggest a peninsular Florida destination for all Gulf fritillary (*A. vanillae*) and cloudless sulphur butterflies (*Phoebis sennae* (L) Lepidoptera: Pieridae), as suggested by other studies (Walker 1979; Walker 1991). Previous reports of the long-tailed skipper (*Urbanus proteus* L., Lepidoptera: Hesperiidae) at Carrabelle Beach (80 km east), indicated flight directions in both coastal directions (roughly east and west, Walker and Littell 1994). Destination and migration patterns of Odonates are generally unknown (May 1992) though northward movement of *A. junius* in summer and scarcity of adult *A. junius* and *T. lacerata* in winter in Florida have been suggested (Paulson 1966; Dunkle 1989).

The peak day on 2 Oct (Figs. 2, 3) was 3 days after the passage of a cold front. Some studies have reported the passage of cold fronts as an impetus for migration (Russell et al. 1998), but the following weekend also had high numbers, despite no frontal activity. During drizzle the flight dropped off to zero, but on the 2 overcast days (18 Sep, 27 Sep) the high *A. vanillae* rates were still 48 and 2,472/h; while for *A. junius* the high rates were 336 and 252/h respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>Highest hourly rate¹ (date and hour)</th>
<th>% total²</th>
<th>50% period³ (median)</th>
<th>Est. total⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Odonata</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Anax junius</em></td>
<td>3,297 (2 Oct, 7 pm)</td>
<td>23.2%</td>
<td>27 Sep-6 Oct (1 Oct)</td>
<td>77,970</td>
</tr>
<tr>
<td><em>Tramea lacerata</em></td>
<td>228 (2 Oct, 11 am)</td>
<td>3.3%</td>
<td>13 Sep-16 Oct (Sep 30)</td>
<td>20,938</td>
</tr>
<tr>
<td><em>Pantala flavescens</em></td>
<td>138 (16 Oct, 4 pm)</td>
<td>1.8%</td>
<td>17 Sep-8 Nov (2 Nov)</td>
<td>9,052</td>
</tr>
<tr>
<td><em>Tramea carolina</em></td>
<td>108 (11 Sep, 1 pm)</td>
<td>1.0%</td>
<td>12 Sep-2 Nov (2 Oct)</td>
<td>4,762</td>
</tr>
<tr>
<td><strong>Lepidoptera</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Agraulis vanillae</em></td>
<td>3,162 (3 Oct, 10 am)</td>
<td>57.8%</td>
<td>28 Sep-17 Oct (6 Oct)</td>
<td>273,201</td>
</tr>
<tr>
<td><em>Urbanus proteus</em></td>
<td>522 (21 Oct, 2 pm)</td>
<td>7.4%</td>
<td>2 Oct-23 Oct (14 Oct)</td>
<td>43,908</td>
</tr>
<tr>
<td><em>Phoebis sennae</em></td>
<td>114 (3 Oct, 10 am)</td>
<td>2.2%</td>
<td>11 Sep-11 Oct (1 Oct)</td>
<td>11,447</td>
</tr>
<tr>
<td><em>Danaus plexippus</em></td>
<td>138 (6 Nov, 12 pm)</td>
<td>2.1%</td>
<td>2 Oct-8 Nov (22 Oct)</td>
<td>12,244</td>
</tr>
<tr>
<td><em>Junonia coenia</em></td>
<td>42 (2 Oct, 2 pm)</td>
<td>1.0%</td>
<td>8 Oct-9 Nov (23 Oct)</td>
<td>6,088</td>
</tr>
</tbody>
</table>

¹Average rate from 2.5-minute counts/h, and date and hour of the count in parenthesis.
²Percentage of total count of all insects for this species.
³The 50% period is the period between 25% and 75% completion of seasonal migration.
⁴Estimated total is based on linear interpolation between observed hourly rates both between hours and days.

Table 1. Migration levels during fall 1999 of dragonflies (common green darner, black saddlebags, wandering glider, Carolina saddlebags) and butterflies (gulf fritillary, long-tailed skipper, cloudless sulfur, monarch, buckeye) at St. Joseph Peninsula, Florida.
For both the Lepidoptera and Odonata the period when the middle 50% of the seasonal flight occurred varied by species (Table 1) with the earliest being *P. sennae* and the latest being the buckeye, *Junonia coenia* (L., Lepidoptera: Nymphalidae). The whole extent of the flight period was much larger. For example, the flights ranged from 14 Aug to 28 Nov for the dragonflies *A. junius* and the wandering glider *Pantala flavescens* (F., Odonata: Libellulidae). Flights began on 5 Sep for all butterflies and extended to 28 Nov for *D. plexippus* and *J. coenia*. By 7 Dec, *A. vanillae* was the only species observed.

Flight height of the Odonata was about 2-m over the boardwalk, for a total of 9-m over the base of the fore dune. The Lepidoptera were within 2-3 m of the ground, but on encountering the boardwalk most flew over it. The flights were normally on the leeward side of the fore dune.

Though there wasn’t a consistent daily pattern, flights usually began increasing by 9 am (e.g., Fig. 3) The occasional peak hourly rates of *A. junius* in the evening (e.g., Fig. 3) may represent an evening descent from above the height of direct observation (Corbet 1999).

Behaviors other than flight included 51 instances of predation by *A. junius* on *A. vanillae*, stable fly (*Stomoxys calcitrans* (L), Diptera: Muscidae, see Wright 1945; Fye et al. 1980), mosquitoes (Diptera: Cilicidae), long-tailed skipper (*U. proteus*), buckee butterfly *J. coenia*, and black saddlebags dragonfly *T. lacerata*. The only other instances of predation were twice by *T. lacerata* on stable flies, and once by a wandering glider dragonfly (*P. flavescens*) on the buckeye butterfly (*J. coenia*). No predation on dragonflies was observed despite abundance of potentially predatory birds including merlin (*Falco columbarius*), American kestrel (*Falco sparverius*) (e.g., Walter 1996) barn swallow (*Hirundo rustica*), tree swallow (*Tachycineta bicolor*), and loggerhead shrike (*Lanius ludovicianus*). Only dragonflies were observed preying on Lepidoptera, as described above. There was some mortality from vehicles on the peninsula road of both butterflies and dragonflies. Some butterflies paused within 100 m north of the boardwalk on flowering plants.

There were 28 pairs of *A. junius* flying in tandem (latest date 3 Oct). There were 9 pairs of *T. lacerata* flying in tandem (as late at 20 Nov) and one instance of flying in the wheel position indicating copulation (20 Nov). There was one instance of a paired *P. flavescens* in the wheel position on 6 Nov. This indicates that at least some Odonates were reproductively mature. No mating among the butterflies was observed.

The estimated seasonal totals ranged from 4,700 for *T. carolina*, to 273,000 for *A. vanillae* (Table 1). There was an average 40% variance of the two counts in a given hour. The temporal variability within the day (e.g., Fig. 3) and sharp peaks between days (Fig. 2), indicate that the linear interpolation method should not be viewed as an absolute number, but rather as an index of the relative abundance of the migrants. These migration levels are less than the millions in massive migratory swarms (May 1995; Russell et al. 1998) of Odonates, and less than the estimated 30 million seasonally migrating Lepidoptera in peninsular Florida (Walker 1991). This study does indicate a slow and steady migration for both dragonflies and butterflies at this location. Although none of these species are considered threatened (Logan 1997) or rare (Deyrup & Franz
their migrations may be endangered (Brower & Malcom 1991). Further studies along obvious migration corridors, and observations from the tip of St. Joseph Peninsula, would help piece together flight patterns. More intensive surveys over multiple years would help determine if there were synoptic or local weather correlates to migration. Protection along migration paths might include avoiding use of insecticides, and not mowing nectar plants. The impact of coastal development on these routes is unknown.

ACKNOWLEDGMENTS

Michael May alerted me to the general interest in these phenomena. Wendy Brill, Jerrell J. Daigle, Michael May, Tom Walker, and George Wallace reviewed earlier drafts of this manuscript.

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COMPARISON OF SOME LIFE HISTORY PARAMETERS BETWEEN ALATE AND APTEROUS FORMS OF TURNIP APHID (HOMOPTERA: APHIDIDAE) ON CABBAGE UNDER CONSTANT TEMPERATURES

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Abstract
Development, longevity, survivorship and fecundity of the alate and apterous forms of the turnip aphid, *Lipaphis erysimi* (Kaltenbach), were studied on cabbage under constant temperatures in the laboratory. The developmental durations for alate nymphs were 15.8, 9.5, 8.0 and 5.4 d at 15, 20, 25 and 30°C, respectively, and those for apterous nymphs were 13.9, 6.8, 6.1 and 5.0 d. Alate nymphs developed 1.9-3.0 d longer at 15, 20 and 25°C than the apterous nymphs, but the developmental durations between the alate and apterous forms were not significantly different at 30°C. The longevities of alate adults were 12.6, 17.7, 17.6, and 17.2 d at 15, 20, 25, and 30°C, respectively, compared with 25.3, 21.3, 17.5 and 11.7 d, respectively, for apterous aphids under the corresponding temperature regimes. Fecundity was also significantly less for alate adults than for apterous adults. Alate adults produced an average of 7.9, 37.9, 39.0, and 11.9 nymphs in their lifespan at 15, 20, 25 and 30°C, respectively, compared with 52.5, 90.8, 83.0 and 29.7 nymphs per apterous adult at the same temperature regimes.

Key Words: *Lipaphis erysimi*, turnip aphid, alate aphid, apterous aphid, development, reproduction, cabbage, vegetable

Resumen
Bajo temperaturas constantes en laboratorio, se estudió el desarrollo, la longevidad, la supervivencia y la fecundidad de las formas alada y áptera del áfido del nabo *Lipaphis erysimi* (Kaltenbach). El tiempo de desarrollo en ninfas aladas fue de 15.8, 9.5, 8.0 y 5.4 días a 15, 20, 25 y 30°C respectivamente, mientras que en ninfas ápteras fue de 13.9, 6.8, 6.1 y 5.0 días. Las ninfas aladas se desarrollaron de 1.9-3.0 días más rápido a 15, 20 y 25°C que las ninfas ápteras, pero el tiempo de desarrollo entre las formas alada y áptera no fue significativamente diferente a 30°C. La longevidad de los adultos alados fue de 12.6, 17.7, 17.6 y 17.2 días a 15, 20, 25 y 30°C respectivamente, comparada con 25.3, 21.3, 17.5 y 11.7 días para los áfidos ápteros bajo los regímenes correspondientes de temperatura. La fecundidad también fue perceptiblemente menor para los adultos alados que para los adultos ápteros. Los adultos alados producen un promedio de 7.9, 37.9, 39.0 y 11.9 ninfas en su lapso de vida a 15, 20, 25 y 30°C respectivamente, comparado con 52.5, 90.8, 83.0 y 29.7 ninfas por adulto áptero bajo el mismo régimen de temperaturas.

The turnip aphid, *Lipaphis erysimi* (Kaltenbach), is a worldwide pest on *Brassica* crops (Begum 1995; Liu et al. 1997; Prasad 1988; Yue & Liu 2000). The biology of apterous *L. erysimi* on several *Brassica* vegetables were well documented (Ahlawat & Chenulu 1982, Amjad and Peters 1992; Chander & Phadke 1994; Castle et al. 1992; Prasad & Phadke 1984; Edelson et al. 1993; Setokuchi & Muma 1993; Singh et al. 1983; Singh & Sachan 1995). The nymphs and adults suck the sap from leaves, young shoots, inflorescence and young pods, resulting in chlorophyll reduction or even plant death. Additionally, alate *L. erysimi* can transmit some important plant virus diseases, such as sugar cane mosaic virus, cucumber mosaic virus and bean yellow mosaic potyvirus (Ahlawat & Chenulu 1982; Castle et al. 1992).

The life history parameters, such as development, longevity, survivorship and fecundity of the alate form of *L. erysimi* were not well documented, and these biological characteristics and parameters are essential for effective aphid management (Halbert et al. 1981). In this paper, we report the effects of four different constant temperature regimes on development, survivorship, longevity and fecundity of both the alate and apterous forms of *L. erysimi* on cabbage in the laboratory.

Material and Methods
Host Plants
Cabbage, *Brassica oleracea* var. *capitata* L. (Grand Slam Hybrid), was seeded in styrofoam
germination trays with 5 seeds per cell (2.5 by 2.5 by 7.5 cm) in a greenhouse. Seedlings were thinned when the plants were 2.5-cm high leaving one healthy plant per cell. These seedlings were transplanted individually to plastic pots (15 cm in diam.) when they were 8-cm high with 5-6 leaves. Some of these seedlings were maintained in the greenhouse, and others were maintained in an insectary for feeding the aphid colony.

Development, Survivorship, Longevity and Fecundity

The aphid colony has been maintained on cabbage in a greenhouse for >1 year. Both alate and apterous adult aphids were collected from the greenhouse colony, and were transferred onto potted cabbage plants in an air-controlled insectary at 25 ± 2°C and 55-60% relative humidity (RH) under a photoperiod of 12:12 (L:D) h. Detached cabbage leaves were used to rear L. erysimi in all experiments. Clear plastic petri dishes (12.5 cm by 1.2 cm) were used as aphid rearing arenas. Eight layers of paper tissues were put on the bottom of the petri dishes and the paper tissues were saturated with water for sufficient moisture. A cabbage leaf disk (~8.0 cm in diam) with the adaxial surface facing up was placed on the water-saturated paper tissue in each petri dish. At the time of the experiment, neonate nymphs (<24 h old 1st instars) were collected from the laboratory colony using a small camel hair brush (#000) and placed into each rearing arena. Thirty to 40 aphids were used for each treatment. Four constant temperatures, 15, 20, 25, and 30°C, were maintained in growth chambers (Percival, Boone, IA) with a photoperiod of 14:10 (L:D) h and 50-75% RH. Development, molting, survival and number of newborn nymphs were recorded daily until the females died. Newborn nymphs were removed after the daily recording until the death of the adult. Leaf disks were replaced at the first sign of deterioration, normally at 4-5 d intervals.

Data Analysis

Aphids were excluded from the data set if they died within 24 h or they were never observed feeding. Developmental duration, longevity and reproduction of both alate and apterous L. erysimi under the four constant temperatures were analyzed using a two-way analysis of variance (ANOVA) (temperatures × aphid forms), and the means were separated using the least significant difference (LSD) test at $P = 0.05$ upon a significant $F$-test (SAS Institute 1996).

RESULTS AND DISCUSSION

Nymph Development

Temperature significantly affected the development for both alate and apterous nymphs (Fig. 1). At the four temperatures, alate nymphs developed significantly faster at higher temperatures than at lower temperatures ($F = 18.6; df = 3, 102; P = 0.0001$). The developmental duration was shortest at 30°C (5.4 d), followed by at 25°C (8.0 d), at 20°C (9.5 d), and the longest at 15°C (13.9 d). Similarly, apterous nymphs also developed faster at higher temperatures than at lower temperatures ($F = 9.3; df = 3, 101; P = 0.0002$). The developmental duration was shortest at 30°C (5.0 d), followed by at 25°C (6.1 d), at 20°C (6.8 d), and the longest at 15°C (13.9 d). Alate nymphs developed significantly longer than apterous nymphs at 15, 20 and 25°C ($F = 11.2-23.14; df = 1, 102; P = 0.0025-0.0001$). Those under 30°C did not show significant differences in developmental durations ($F = 1.13; df = 1, 102; P = 0.5741$).

Survivorship and Longevity

All nymphs in both alate and apterous forms survived to adulthood (Figs. 1 and 2). The longevities of both alate and apterous adults were significantly affected by temperature. The longevity of alate adults was significantly shorter at 15°C than those at the other three temperatures ($F = 7.13; df = 3, 104; P = 0.0471$). In contrast, the lon-
gervities of apterous adults declined as the temperatures increased from 15°C to 30°C. The longest longevity (25.3 d) was obtained at 15°C, followed by 21.3 d at 20°C, 17.5 d at 25°C, and the shortest, 11.7 d at 30°C. Between the two forms, alate adults lived a significantly shorter period than apterous adults ($F = 9.87-12.23; df = 1, 102; P = 0.0012-0.0001$) at 15, 20 and 30°C except for those at 25°C ($F = 0.97; df = 1, 102; P = 0.8754$). The largest difference in longevity between the two forms was found at 15°C at which the apterous adults lived =2-fold longer than that of alate adults (25.3 d vs. 12.6 d respectively). At 20°C, the apterous adults lived 3.6 d longer than the alate adults. The longevities of both forms were almost the same at 25°C. In contrast, the apterous adults lived 5.5 d shorter than the alate adults (17.2 d vs. 11.7 d) at 30°C.

Similar effects were also reported by DeLoach (1974) and Liu (1991). Aphid development and reproduction typically increase from zero at a low temperature threshold, reach a maximum at the most favorable temperature, then decrease rapidly to zero at a lethal threshold. Like other aphids in general, *L. erysimi* reared at temperatures above the upper or below the lower thresholds develop more slowly than those under the most favorable conditions. The nymphs developed fastest at 30°C, and slightly slower at 25 and 20°C. DeLoach (1974) found that *L. erysimi* on turnip could continue to reproduce at 30°C, but failed to do so at 35°C. Similarly, Liu (1991) found the most favorable temperature for *L. erysimi* was 26°C. At 8.3 and 35°C, few nymphs developed to adults, but none produced any offspring. At 11.3 and 32.8°C, few adults successfully reproduced. Therefore, the lower and upper threshold temperature for *L. erysimi* should be higher than 8°C and lower than 33°C, with the most favorable temperature between 25 and 27°C. DeLoach (1974) also reported great mortality for some young *L. erysimi* nymphs because these young nymphs failed to become well-established on the new host plants after having been transferred from the original host plants.

**Fecundity**

Temperature played a significant role for aphid reproduction (Figs. 1 and 2). Alate adults produced the most nymphs at 25°C (39.0 nymphs/adult) and 20°C (37.9 nymphs/adult), followed by these (11.9 nymphs/adult) at 30°C, and the fewest at 15°C (7.9 nymphs/adult). Apterous adults produced most nymphs at 20°C (90.8 nymphs/adult), followed by 83.0 nymphs/adult at 25°C, 52.5 nymphs/adult at 15°C, and the fewest, 29.7 nymphs/adult at 30°C. Between the two forms, apterous adults produced 6.6-, 2.4-, 2.1- and 2.5-fold more nymphs per adult than alate adults of the corresponding temperature.

Both alate and apterous adults started to produce nymphs 1 or 2 d after the last molting, so the adults from last molting to the first reproduction were only 1-2 d old (Fig. 2). Both fecundity and reproductive period varied at the four temperature regimes. Generally, the alate aphids had lower daily reproductive rates and shorter period of reproduction than the apterous forms at the same temperature. At 15°C, reproductive periods lasted 12 d at 15°C, compared with 24 d at 20 and 25°C, respectively, and only 11 d at 30°C. Daily fecundity at the peak reproduction period was 3-4 nymphs at 20 and 25°C, <2 nymphs at 30°C and 15°C.

The reproductive periods of apterous lasted 34 d at 15°C, compared with 24 d at 20°C, 25 d at 25°C, and only 11 d at 30°C. An aphid could produce 8-9 nymphs per d at the peak reproduction period at 20 and 25°C, 6-7 nymphs at 30°C, and 2-3 nymphs at 15°C.

Our results clearly indicate that many biological parameters, including nymph development, adult longevity and fecundity differed between alate and apterous forms. Similar results were reported by Takaoka (1973) who reviewed that longer nymph period, longer adult longevity and lower fecundity of alate virginoparae were found in many species of aphids compared to those of
their apterous form. Information from this study will aid in predicting the population dynamics of \textit{L. erysimi} on cole crops in south Texas. The susceptibility to low (<10°C) and high temperature (>35°C) may present a partial explanation for the low field populations of \textit{L. erysimi} in the summer and in the winter months when temperatures are often >30 and <10°C, respectively. Meanwhile, the information on \textit{L. erysimi} obtained under constant temperatures may not be directly applicable for field populations that may be affected by fluctuating temperatures (Liu & Meng, 1990) and other biotic and abiotic factors.

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MOTH EXPERIENCE AND NOT PLANT INJURY AFFECTED FEMALE CABBAGE LOOPER MOTH (LEPIDOPTERA: NOCTUIDAE) ORIENTATION TO POTATO PLANTS

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ABSTRACT

Naive mated female cabbage looper moths, *Trichoplusia ni* Hübner, responded in a flight tunnel to potted potato plants (*Solanum tuberosum*). Percentages of moths attracted to uninjured potato plants, mechanically-damaged potato plants, and potato plants treated with regurgitant from larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* Say, were similar, indicating no effect of plant treatment. Attraction of female cabbage looper moths to potato plants was increased following prior contact (experience) by themoth with a potato plant. This increase in responsiveness to potato plants with experienced moths occurred whether the plants were uninjured, mechanically damaged, or treated with Colorado potato beetle larval regurgitant. Moths preconditioned on potato plants treated with regurgitant exhibited similar rates of attraction to mechanically-damaged plants and to regurgitant-treated plants. However, moths preconditioned on mechanically-damaged plants were more responsive to mechanically damaged plants compared to regurgitant-treated plants.

Key Words: *Trichoplusia*, attraction, host-finding, learning, kairomone

RESUMEN

Polillas de repollo *Trichoplusia ni* Hübner, sin experiencia previa, respondieron en un túnel de vuelta a plantas de papa en tiestos (*Solanum tuberosum*). Los porcentajes de polillas atraídas a plantas de papa sin daño, plantas de papa con daño mecánico, y plantas de papa tratadas con regurgitante de larvas del escarabajo de papa de Colorado, *Leptinotarsa decemlineata* Say, fueron similares, indicando la falta de efecto al tratamiento de la planta. La atracción de hembras de polilla a plantas de papa fue incrementada siguiendo contacto previo (experiencia) de la polilla con la planta de papa. Este incremento en respuesta a plantas de papa con polillas con experiencia ocurrió aun si las plantas no tenían daño, tenían daño mecánico, o tratadas con regurgitante larval del escarabajo de papa de Colorado. Polillas preacondicionadas a plantas tratadas con regurgitante exhibieron tiempos similares de atracción a plantas mecánicamente dañadas y a plantas tratadas con regurgitante. Sin embargo, polillas preacondicionadas a plantas mecánicamente dañadas respondieron mas a plantas mecánicamente dañadas en comparación con plantas tratadas con regurgitante.

The cabbage looper, *Trichoplusia ni* (Hübner) is a polyphagous herbivore that can feed on a diversity of plant taxa (Sutherland & Greene 1984). The cabbage looper moth exhibits weak attraction responses to a wide range of host plants, a behavior that may bring it to a host habitat (Landolt 1989). This response is enhanced by conspecific larval feeding or mechanical damage to the plant (Landolt 1993). Attraction of cabbage looper moth to plants is also enhanced following contact with a host plant (experience), indicating learning of host plant odors (Landolt & Molina 1996). Cabbage looper moth to celery (*Apium graveolens*) or cotton (*Gossypium hirsutum*) were more likely to orient to that same species of plant rather than to the other species of host plant (Landolt and Molina 1996). These previous studies did not consider possible differential roles of constitutive (stored and released) and induced (produced de novo) odor chemistry produced by plants in response to injury. That is, we do not know if cabbage looper moths are more strongly attracted to injured plants because of constitutive or induced plant odors, or how cabbage looper moth learning of host plant odor (Landolt & Molina 1996) might be affected by these changes in host plant chemistry.

The cabbage looper larvae are found on potato plants and complete development on potato foliage (Sutherland & Greene 1984; Landolt, unpublished data), qualifying potato as a host plant, although it is not considered to be a common pest of potato. Potato plants respond to mechanical injury, feeding by Colorado potato beetle, *Leptinotarsa decemlineata* (Say), and applications of Colorado potato beetle regurgitant, in part by increasing the release of volatile chemicals (Bolter et al. 1997). Treatment of potato foliage with regurgitant from larvae of the Colorado potato beetle or from cabbage looper larvae makes potato plants more attractive to Colorado potato beetle females (Landolt et al. 1999). The Colorado potato beetle is a spe-
cialist on species of \textit{Solanum} and might be expected to be adapted to the defensive chemicals, including volatiles, produced by induced potato plants. Karban and Baldwin (1997) suggest that specialist herbivores might respond positively and generalist herbivores might respond negatively to induced host plant kairomones. It would be of interest then to determine how a polyphagous herbivore such as the cabbage looper responds to plants following such treatments.

The objectives of these studies were to determine if cabbage looper moths are attracted to potato plants, if they are attracted more strongly to potato plants that are mechanically injured (to release constitutive odorants), and if they are more weakly attracted to potato plants that are treated to produce and release induced odorants. Also, effects of previous exposure or experience with plants on cabbage looper moth attraction to uninjured and injured potato plants was examined, with the expectation that moth attraction responses might be enhanced following experience with undamaged and mechanically-damaged plants, but might not be enhanced following experience with induced plants.

**MATERIALS AND METHODS**

General Methods

Russet-Burbank potato plants were used in all experiments. Plants were grown in a glass greenhouse from September through April in Yakima, Washington, but with supplemental lighting from sodium lamps and 400 watt metal halide lamps providing a 14:10 L:D photoperiod. Plants were started with potato eyes in soil in 15 cm diam pots. Soil was a mix of sand, peat moss and fertilizer. Plants were used in experiments when 4 to 6 weeks old (30-35 cm in height) and before blooming.

Three to five days before experiments, plants were placed in a controlled environment room at 25°C and 50% RH. Lighting was supplied with overhead 400 watt metal halide lamps on a light cycle coinciding with the light cycle for the cabbage looper moth.

Cabbage looper moths were obtained from a colony established in 1997 at the Yakima Agricultural Research Laboratory, with stock that originated from northern Florida in 1987. Pupae were sorted by sex and placed in cages with dishes of sugar water on cotton balls and with water dispensers on the cage tops. Pupae were moved daily to new cages to provide cohorts of moths of discrete ages. All pupae and adult moths (including mating cages, see below) were kept in an environmentally controlled room with reversed light cycle (lights off at 0900 h and lights on at 1700 h), 24°C, and 60% RH.

To obtain mated females, groups of 30 males (3-6 d old) and 25 females (2-3 d old) were placed together in screened cages (45 x 45 x 45 cm) at the beginning of the scotophase. Moths were provided sugar water and water as in the emergence cages. Females were removed from mating cages near the end of the subsequent photophase and were placed in another cage. Assays were conducted during the first 3 h of the following scotophase. For each set of assays, a subset of female moths were recaptured and were dissected to verify that they mated, evidenced by the presence of a spermatophore in the bursa copulatrix.

In all experiments, mated female moth responses to potted plants were evaluated in a flight tunnel that was similar to that described by Landolt and Molina (1996). Charcoal-impregnated fiberglass filters were used at both ends of the tunnel to minimize contamination of the tunnel and experimental room with plant and other odors. The experimental room was equipped with overhead red incandescent lamps to facilitate observation of moths. Flight tunnel room conditions were 22-24°C and 50-70% RH. Plants tested for attractiveness to moths were placed at the center of the upwind end of the flight tunnel. Moths were released from open 30 ml polystyrene vials near the center of the downwind end of the flight tunnel and were observed for two minutes. Released moths were scored for upwind oriented flight (zig-zagging upwind flights within the probable boundaries of the odor plume) and for contact with the plant. A replicate consisted of a series of 5-10 moths tested per plant. No plant was used more than once either in a conditioning treatment or in an assay.

Four experiments were conducted to evaluate moth attraction to potato plants.

**Naive Moths to Uninjured Potato Plants**

The first experiment tested the hypothesis that naive moths are attracted to uninjured potato plants. Naive mated female moths were assayed for attraction to an uninjured potato plant or to a pot of soil (as an experimental control). Ten replicates were conducted, with 80 moths tested to plants and 80 moths tested to pots of soil.

**Naive Moths to Injured Potato Plants**

The second experiment evaluated the effects of plant injury on attraction of naive moths to potato plants. Two non-competitive tests were conducted using naive female moths. These two tests were 1) a comparison of moth responses to uninjured potato plants and to potato plants that were damaged mechanically and 2) a comparison of moth responses to uninjured potato plants and potato plants treated with regurgitant from larvae of the Colorado potato beetle. To incur mechanical damage to the potato plants, three leaves on a potato plant were cut with scissors, lengthwise about 3 cm.
along the main leaf axis. This was done one hour before assays were started. Treatment of plants with beetle regurgitant were made to three leaves of each plant. Leaves were first scraped with a razor blade (about one cm²). A fourth or fifth instar beetle larva was gently squeezed until it produced a droplet of regurgitant at the mouthparts which was then applied to the scraped area of leaf. This was done 24 h before assays were started.

Nine bioassay replicates were conducted, with 90 moths tested per treatment and 90 per control. In both studies, the sequence of plants tested (treatment and control) was reversed daily for the 9 days.

Experienced Moths to Uninjured and Injured Potato Plants

The third experiment evaluated the effects of prior contact (experience) with potato plants on moth attraction to potato plants. Three tests were conducted to compare the responses of naive versus experienced mated female moths to potato plants that were either uninjured, were mechanically-damaged, or were treated with Colorado potato beetle regurgitant. For each assay, mated females were divided into 2 equal groups when separated from males in the mating cages. One group was placed in a larger screened cage (45 × 45 × 45 cm) with a potted potato plant, at the onset of the scotophase (experienced moth group). The other group was placed in a similar cage with no plant (naive moth group). Both cages were kept in the controlled environment room used to hold plants for bioassays, on a 14:10 L:D light cycle, 50% RH and 25°C for 24 h. Female moths were then removed from these cages and held in 30 × 30 × 30 cm screened cages in the controlled environment room holding moths and no plants, with water and sugar water, until used in bioassays in the following scotophase. A comparison was first made of naive versus experienced moths to an undamaged plant. Naive moths were tested for attraction to and contact with an uninjured potato plant, followed by the testing of experienced moths tested to the same plant. The following day, using a new plant, a set of experienced moths were tested for responses to an uninjured plant, followed by the testing of naive moths. This was continued for 12 assay sets, with 80 naive and 80 experienced moths tested to uninjured potato plants. This protocol was then followed to compare naive versus experienced moth responses to a mechanically damaged plant, with a total of 80 naive and 80 experienced moths tested to mechanically damaged plants in 16 bioassay sets. A comparison was then made of naive versus experienced moth responses to regurgitant treated potato plants, with 60 naive and 60 experienced moths tested to regurgitant treated potato plants in 10 bioassay sets.

Discrimination of Uninjured vs Injured Potato by Experienced Moths

The fourth experiment evaluated the ability of experienced moths to discriminate between mechanically-damaged plants and plants that had been treated with Colorado potato beetle regurgitant. The objective of this experiment was to determine if moths preconditioned on one type of injured potato plant (mechanically-damaged or regurgitant-treated) would respond better to the type of plant they were preconditioned with, compared to the other type of plant. There were 4 treatment regimes: 1) moths that were placed with mechanically-damaged plants in one scotophase and were then tested for responses to mechanically-damaged plants in the following scotophase, 2) moths that were placed with mechanically-damaged plants in one scotophase and were then tested for responses to regurgitant-treated plants in the following scotophase, 3) moths that were placed with regurgitant-treated plants in one scotophase and were then tested for responses to mechanically-damaged plants in the following scotophase, and 4) moths that were placed with regurgitant-treated plants in one scotophase and were then tested for responses to regurgitant-treated plants in the following scotophase.

Mated females were subdivided into 2 equal groups when initially separated from males in the mating cages, near the end of a photophase. These female moths were placed either in a cage with a mechanically-damaged plant (cut with scissors as before) or in a cage with a regurgitant-treated plant (Colorado potato beetle larval regurgitant applied to scrapes on 3 leaves 24 h before). After 24 h, during the last 2 h of the photophase, these moths were then removed from the cages containing plants and were placed in 2 clean cages and held another 2-4 h until they were used in bioassays. For the bioassay, a mechanically-damaged plant was placed in the flight tunnel and 5 moths that had been preconditioned with a mechanically-damaged plant and 5 moths that had been preconditioned with a regurgitant-treated plant were then tested alternately for responses to the same plant. A regurgitant-treated plant was then placed in the flight tunnel and 5 moths that had been preconditioned with a mechanically-damaged plant and 5 moths that had been preconditioned with a regurgitant-treated plant were tested alternately for responses to this plant. This protocol was followed on 10 different days, providing a total of 50 moths tested for each treatment category.

For experiments 1, 2 and 3, percentage response data for treatment pairs were analyzed by a paired t test to determine if responses differed between treatments or between treatment and control, with a significance level of P ≤ 0.05. Per-
Results of experiment 4 were compared using Tukey's Test following an ANOVA.

Results

Naive Moths to Uninjured Potato Plants

The percentages of naive moths that flew upwind towards uninjured Russet Burbank potato plants were significantly greater than the percentages of moths responding to a pot of soil (Table 1). A significant percentage of moths tested also landed on uninjured potato plants and none landed on pots of soil (Table 1).

Moths to Injured Potato Plants

Percentages of naive moths attracted to mechanically-damaged and uninjured plants were not significantly different (Table 1). Percentages of naive moths attracted to uninjured plants were not significantly different from those attracted to regurgitant-treated plants. There were also no significant differences between percentages of moths landing on plants, for either of the treatment comparisons (Table 1).

Experienced Moths to Uninjured and Injured Potato Plants

Moths preconditioned (experienced) on potato plants were attracted significantly more often to potato plants, than were naive moths (Table 2). Increased attraction of preconditioned moths to potato plants was observed when those plants were uninjured, mechanically-damaged, or treated with Colorado potato beetle regurgitant (Table 2). Additionally, increased landing was observed in moths that were preconditioned either on uninjured plants or on plants with mechanical damage (Table 2). Percentages of preconditioned moths landing on regurgitant-treated plants were numerically but not statistically greater than percentages of naive moths landing on those same plants.

Discussion

Results indicate significant but weak attraction of naive cabbage looper moths to Russet Burbank potato plants. This finding is similar to the weak attraction responses of naive cabbage looper moths to cabbage (*Brassica oleracea*), celery, tomato (*Lycopersicon esculentum*), and soybean (*Glycine max*) plants (Landolt 1989). This low level attraction to a wide variety of plants (Landolt 1989) may be a host-habitat finding strategy rather than a host-finding behavior, as was suggested previously (Landolt 1993).

There was no significant enhancement of attraction of cabbage looper moths to potato plants cut with scissors to produce mechanical damage. Because mechanical damage causes short term increased emission of volatile chemicals from potato (Bolter et al. 1997), a heightened response by cabbage looper moths was expected. Similar mechanical damage to foliage of cotton plants (*Gossypium hirsutum*) increased their attractiveness to cabbage looper moths (Landolt 1993) and similar damage to cabbage plants increased their attractiveness to *Mamestra brassicae* (L.) moths (Rojas 1999). However, this effect was not observed.

Table 1. Mean (±SE) Percentages of Cabbage Looper Females That Were Attracted To and Contacted Potato Plants in a No-Choice Test Conducted in a Flight Tunnel.

<table>
<thead>
<tr>
<th>Treatment comparisons</th>
<th>n*</th>
<th>x̄ ± SE</th>
<th>t</th>
<th>p</th>
<th>x̄ ± SE</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pot of Soil</td>
<td>10</td>
<td>0.0 ± 0.0</td>
<td>2.58</td>
<td>0.01</td>
<td>0.0 ± 0.0</td>
<td>2.14</td>
<td>0.03</td>
</tr>
<tr>
<td>Untreated plant</td>
<td>10</td>
<td>20.0 ± 7.7</td>
<td></td>
<td></td>
<td>14.0 ± 6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated plant</td>
<td>9</td>
<td>25.7 ± 3.7</td>
<td>1.25</td>
<td>0.24</td>
<td>5.8 ± 2.7</td>
<td>0.90</td>
<td>0.39</td>
</tr>
<tr>
<td>Mechanically-damaged plant</td>
<td>9</td>
<td>32.1 ± 5.9</td>
<td></td>
<td></td>
<td>8.1 ± 2.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated plant</td>
<td>9</td>
<td>20.0 ± 6.7</td>
<td>0.96</td>
<td>0.37</td>
<td>6.7 ± 1.7</td>
<td>1.04</td>
<td>0.33</td>
</tr>
<tr>
<td>Regurgitant-treated plant</td>
<td>9</td>
<td>14.4 ± 3.8</td>
<td></td>
<td></td>
<td>10.2 ± 4.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*n = number of replicates with 5 moths tested per replicate.
with cabbage looper attraction to mechanically-damaged cabbage plants (Landolt 1993). It is not yet evident why orientation to mechanically-damaged plants may be enhanced in some cases and not in others.

There was also no enhancement of moth attraction to potato plants treated with regurgitant of larvae of the Colorado potato beetle. This treatment is known to stimulate the production and prolonged release of odorants from potato (Bolter et al. 1997) and makes potato plants more attractive to female Colorado potato beetle (Landolt et al. 1999). In previous studies, conspecific larval damage made cotton plants more attractive to cabbage looper moths (Landolt 1993) and made cabbage plants more attractive to *M. brassicae* moths (Rojas 1999). However, such damage to cabbage plants made them less attractive to cabbage looper moths (Landolt 1993) and such damage to chrysanthemum plants made them less attractive to *M. brassicae* moths (Rojas 1999). As with the varying responses of moths to mechanically-damaged plants, the varying responses of moths to insect damaged and regurgitant-treated plants calls for an explanation.

Results of experiments 3 and 4 indicate a positive effect of cabbage looper moth experience with potato plants prior to testing moths in flight tunnel assays. This enhancement of attraction responses occurred regardless of whether the plant was uninjured, mechanically-damaged or regurgitant-treated. Similarly, greater numbers of moths contacted the plants if they had prior experience with the same type of plants, compared to moths with no such experience, with the exception of regurgitant treated plants. Such an effect of preconditioning is considered a form of learning, as described by Papaj and Prokopy (1989). Landolt and Molina (1996) reported strong enhancement of host attraction responses by mated female cabbage looper moths following prior experience with host plants, using cotton, celery, and soybean plants. In those studies it was shown that brief contact with the plant was all that was required to strongly increase the subsequent response of the moth to that plant species. Evidence of learning of host plant odor chemistry also exists for the moths *Helicoverpa armigera* (Hübner) (Cunningham et al. 1998), *Heliothis virescens* (Hartlieb 1996) and *Spodoptera littoralis* (Boisd-

### Table 2. Mean (±SE) Percentages of Naive and Experienced Cabbage Looper Females Attracted to and Contacting Potato Plants in a No-Choice Flight Tunnel Assay.

<table>
<thead>
<tr>
<th>Moth treatment</th>
<th>n*</th>
<th>x ± SE</th>
<th>t</th>
<th>p</th>
<th>x ± SE</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Attracted</td>
<td></td>
<td></td>
<td>Contact</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undamaged plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naive</td>
<td>12</td>
<td>25.9 ± 6.6</td>
<td>3.12</td>
<td>&lt;0.01</td>
<td>9.2 ± 5.4</td>
<td>2.30</td>
<td>0.02</td>
</tr>
<tr>
<td>Experienced</td>
<td>12</td>
<td>44.3 ± 5.7</td>
<td>3.12</td>
<td>&lt;0.01</td>
<td>21.9 ± 6.6</td>
<td>2.30</td>
<td>0.02</td>
</tr>
<tr>
<td>Mechanically-damaged plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naive</td>
<td>16</td>
<td>27.5 ± 4.9</td>
<td>7.25</td>
<td>&lt;0.01</td>
<td>8.8 ± 4.1</td>
<td>4.48</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Experienced</td>
<td>16</td>
<td>55.4 ± 4.2</td>
<td>7.25</td>
<td>&lt;0.01</td>
<td>24.8 ± 4.2</td>
<td>4.48</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Regurgitant-treated plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naive</td>
<td>15</td>
<td>42.5 ± 6.0</td>
<td>2.19</td>
<td>0.03</td>
<td>25.1 ± 5.1</td>
<td>1.58</td>
<td>0.07</td>
</tr>
<tr>
<td>Experienced</td>
<td>15</td>
<td>62.1 ± 4.1</td>
<td>2.19</td>
<td>0.03</td>
<td>40.0 ± 7.4</td>
<td>1.58</td>
<td>0.07</td>
</tr>
</tbody>
</table>

*n* = number of assay replicates with 5 moths tested per assay replicate.

Fig. 1. Mean (±SE) percentages of mated female cabbage loopers attracted to (solid bars) and contacting (open bars) Russet Burbank potato plants in a flight tunnel, after preconditioning contact with other potato plants during the previous scotophase. The first treatment letter indicates the type of plant the moths were preconditioned with and the second letter indicates the type of plant the moths were presented with in the flight tunnel assays. Plants for preconditioning and for flight tunnel assays were either mechanically-damaged (C) or were regurgitant-treated (R). Bars with the same letter are not significantly different by Tukey’s Test (P ≤ 0.05).
val) (Fan et al. 1997) and for the phytophagous beetles *L. decemlineata* (Say) (Visser & Thiery 1986) and *Diaprepes abbreviatus* (L.) (Harari & Landolt 1999). In contrast, Rojas and Wyatt (1999) noted decreased responsiveness to host plants by *Mamestra brassicae* L. following earlier contact with host plants.

In previous studies (Landolt and Molina 1996), it was shown that cabbage looper moth learning of host plant odor was specific to the plant species used in preconditioning. When moths had prior contact with cotton plants, they were more strongly attracted to other cotton plants and not to celery plants. Similarly, when moths had prior contact with celery plants, they were more strongly attracted to other celery plants and not to cotton plants. In the studies described herein, this discrimination between plants did not occur when moths had prior contact with mechanically-damaged potato plants or regurgitant-treated potato plants and were tested for attraction to both types of potato plants. There was some enhancement of response to those plants following moth preconditioning on mechanically-damaged plants, but moths responded similarly to the two types of injury. Apparently, cotton and celery plants have strongly divergent odor chemistries with little, if any, qualitative overlap, making it more likely that an insect could learn and discriminate separate odors. However, the odor chemistries of mechanically-damaged (to release constitutive odors) and regurgitant-treated (to release induced odors) potato plants overlap extensively (Bolter et al. 1997) and moths that are preconditioned on potato plants with one type of injury may be unable to discriminate between plants with either type of injury.

It was hypothesized that the cabbage looper moth, as a generalist herbivore, might show enhanced attraction to mechanically-damaged plants because of increased emission of volatiles and might show decreased attraction to Colorado potato beetle regurgitant-treated plants because of the induction of defensive chemistry associated with that treatment to potato (Bolter et al 1997) and similar treatments with lepidopterous larvae to other plants (Tumlinson et al. 1992; Turlings et al. 1995). In the case of cabbage looper orientation to potato odor, those expectations appear incorrect because observed response rates were similar to uninjured and injured potato, both by naive and by experienced moths.

Host-finding by the cabbage looper moth appears to involve several behavioral strategies, host-habitat location, responses to some plant injury volatiles, and learning of host plant odor. Evidence indicates a weak response to a variety of plant species that may bring them into the vicinity of possible hosts (Landolt 1989). Injury to plants may (cotton) or may not (cabbage, potato) increase a plant’s attractiveness to cabbage looper (Landolt 1993). Additional work is needed to determine the chemical bases for the response differences observed among plants. Prior contact with a plant increases the subsequent responsiveness of the moth to the same type of plant, indicating associative learning of host plant odor when contact is made with plant foliage. The inability of the moth to selectively respond to potato plants with different types of injury still leaves open the question of the relative significance of constitutive versus induced odorants to cabbage looper host finding with other species of plants.

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EFFECTS OF FIRE ANTS (HYMENOPTERA: FORMICIDAE) ON HATCHING TURTLES AND PREVALENCE OF FIRE ANTS ON SEA TURTLE NESTING BEACHES IN FLORIDA

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ABSTRACT

Red imported fire ants (Solenopsis invicta Buren) have increasingly been observed in loggerhead (Caretta caretta L.) and green (Chelonia mydas L.) sea turtle nests in Florida, and in the nests of freshwater turtles. They may be attracted to the disturbance, mucous and moisture associated with turtle nesting and establish foraging tunnels into turtle nests shortly after egg-laying, thus increasing the vulnerability of hatchlings to fire ant predation. We conducted experiments on a freshwater turtle (Pseudemys nelsoni Carr) to determine the potential impacts of S. invicta on turtle hatchlings. Over 70% of hatchlings were killed by S. invicta during pipping or shortly after hatching. To determine the extent of S. invicta infestation of sea turtle nesting beaches, we sampled known nesting beaches throughout the state of Florida. Beach surveys indicated that S. invicta are present and often abundant on most dunes and dunes along the Florida coast.

Key Words: Caretta caretta, endangered species, fire ant, invasive species, Pseudemys nelsoni, Solenopsis invicta, turtles

RESUMEN

Se han observado hormigas bravas (Solenopsis invicta Buren) cada vez mas en nidos de tortugas marinas (Caretta caretta L.) y (Chelonia mydas L.) en la Florida, y en los nidos de tortugas de agua dulce. Estas hormigas pueden ser atraídas al disturbio, mucosidad y humedad asociada con anidaje de tortugas y establecen túneles de forraje hacia nidos de tortuga poco después de la puesta de huevos, así incrementando la vulnerabilidad de los recién nacidos a predación por la hormiga brava. Llevamos a cabo experimentos con una tortuga de agua dulce (Pseudemys nelsoni Carr) para determinar el impacto potencial de S. invicta en tortugas recién nacidas. Mas del 70% de los recién nacidos fueron muertos por S. invicta durante el proceso de salir del cascaron o poco después de salir. Para determinar la extensión de infestación de S. invicta en playas donde anidan tortugas marina, muestreamos playas conocidas por tener nidos por todo el estado de la Florida. Exámenes de playas indicaron que S. invicta esta presente y mucha la mayoría de las playas y dunas a lo largo de la costa Floridana.

There has been considerable concern and debate over the potential impact of fire ants on nesting sea turtles, but little quantitative evidence exists. Information on the impact of Solenopsis invicta (Buren) on hatching turtles has been largely incidental and anecdotal. Fire ants may impact turtle populations directly by preying on hatchlings and/or indirectly by stinging hatchlings, resulting in reduced weight gain and survival.

Wilmers et al. (1996) documented an increase in the presence of fire ants in green (Chelonia mydas L.) and loggerhead (Caretta caretta L.) turtle nests on undeveloped island beaches off Florida. Red imported fire ants were observed feeding on pipped eggs, and stinging, killing and subsequently feeding on turtle hatchlings (Wilmers et al. 1996). Moulis (1997) documented a 15% decrease in hatching release rate for loggerhead sea turtles emerging from nests infested with fire ants as compared to uninfested nests. The ultimate effect of this predation on sea turtle populations and the magnitude of the problem else-where is unknown. The nesting period (May-August) for loggerheads in the eastern United States (Johnson et al. 1996) corresponds with a concentrated period of brood production in S. invicta. During that
time, protein needs for fire ants are maximal (Sorensen et al. 1983). For secure turtle populations, high juvenile mortality may not affect population size (Congdon & Gibbons 1990), but for small populations, decreases in annual cohort size may affect population viability (Heppell et al. 1996).

Our objectives were to assess the potential impacts of the invasive non-indigenous ant, *S. invicta*, on hatching turtles by using the eggs of a freshwater species (Florida red-bellied turtle, *Pseudemys nelsoni* Carr) in a controlled experimental setting. *Pseudemys nelsoni* often lays its eggs in alligator nests, approximately 20% of which are infested with fire ants in central Florida (Allen et al. 1997). In addition, we determined the geographic extent of *S. invicta* occurrence on sea turtle nesting beaches throughout the state of Florida by sampling beaches with baits attractive to ants.

**Materials and Methods**

To assess the potential for *S. invicta* impact on the eggs and hatchlings of turtles we conducted a laboratory experiment using *P. nelsoni* eggs. The eggs of this freshwater turtle species are elliptical and approximately 2.5 cm long, occur in clutches of about 15, and are found regularly in American alligator nests in Florida. Although the shape of eggs in this species is different from the generally round shape of sea turtle eggs, and egg and clutch size vary among turtle species, we have observed no differences in attractiveness among eggs of several different species regardless of size or shape. *Pseudemys nelsoni* and many other turtle species, including sea turtles, share the trait of emerging from the nest only after most or all of the clutch has hatched.

Ten clutches of *P. nelsoni* eggs were collected (1996) from Lake Apopka in central Florida and transferred to ten 61 × 36 × 13 cm enclosures at the U.S.D.A.-A.R.S. Imported Fire Ant Laboratory, in Gainesville, Florida. Five clutches served as controls. For both control and treated clutches, eggs were placed in sphagnum nesting material adjacent to a shallow pan of water, which allowed individual hatchlings immediate access to water upon emergence. Treated clutches were maintained identically to control clutches, but were exposed to a field-collected mound of *S. invicta* at the opposing end of each enclosure. This controlled situation simulated the natural conditions for the many *P. nelsoni* clutches which share alligator nests with fire ant mounds (Allen et al. 1997). The enclosures allowed fire ants to forage among the clutch as may occur within natural turtle nests (Wilmers et al. 1996; Allen et al. 1997). Fire ants were provided with honey as a food source. Eggs were observed twice daily as they approached hatching and constantly as pipping commenced. Surviving turtles were transferred to the Florida Game and Fresh Water Fish Commission incubation facilities (Gainesville, FL) with food supplied *ad libitum*, and measured weekly for 6 weeks to determine if differences in weight gain between treated and untreated groups existed (Allen et al. 1997).

To determine the presence of *S. invicta* on sea-turtle nesting beaches, we sampled for fire ants at 18 known sea turtle nesting beaches throughout Florida. Collection localities are given in Table 1. Transects consisting of approximately 20 samples at 10-m intervals were established along dune lines. Multiple transects (2 to 7) were sampled at each locality. Baits on all transects consisted of ground beef, except for those in Duval, St. Johns and Volusia counties, which consisted of a sugar-based bait attractive to a variety of ants, newly formulated by the U.S.D.A.-A.R.S. Baits were left in the field for approximately 1 h before being collected and transported to U.S.D.A.-A.R.S. facilities in Gainesville, Florida, for sorting and identification of ant species.

**Results**

*Pseudemys nelsoni* clutch size varied from 6-16 eggs, but only 2-11 of the eggs in a given clutch ultimately hatched. In control groups, 59% of the eggs did not hatch, and in those exposed to fire ants 37% did not hatch. The cause or causes of inviability were not determined but may be attributed to flooding or crushing by the attendant female alligator prior to collection. During and after hatching, 100% of hatching turtles in control groups (17) survived, and were eventually released. The proportion successfully hatching in clutches exposed to fire ants (10 of 35) was significantly less (median = 33%, range 0-55%; Mann-Whitney Rank Sum Test t = 40.0, df = 8, P = 0.008). Approximately half of the mortalities occurred while the hatchlings were still in the egg, while most others died less than 1 h after emergence. Fire ants did not breach turtle eggs, but entered the eggs as soon as a pipped hole was present. Too few individuals exposed to fire ants survived to assess differences in weight gain between the two groups.

We collected 734 ant bait samples and a total of 31,392 ants from Florida sea turtle nesting beaches. About 40% of the collected ants (12,658) were *S. invicta*. Fire ants were detected foraging along dune lines on sea turtle nesting beaches in all regions of the state, and were detected on 13 of 18 specific sites (Table 1). Within those 18 sites, fire ant occurrence on baits varied from 0 to 63%, and represented from 0 to 97% of the individuals collected. Fire ant occurrence followed no obvious geographic pattern. Ants were abundant at some very remote locations (e.g., Boca Grande Key near Key West) but were uncommon on beaches at some locations that have undergone extensive human disturbance (e.g., northeastern Florida beaches).
DISCUSSION

Our surveys found *S. invicta* present on most beaches, at both the wrack and dune lines. Our and other observations indicate that fire ants often are present in sea turtle nest cavities (e.g., Wilmers et al. 1996; Parris et al. 2001). The egg-laying process may initially attract fire ants because it represents a local disturbance and food source. Mucous associated with the egg laying process is an attractive food for fire ants and sea turtle nest cavities provide a desirable micro-climate for fire ants. It appears that fire ants cannot breach intact sea or freshwater turtle eggs (Wojcik & Allen, unpublished data). However, once fire ants build subterranean foraging trails to a site that has provided food, such as turtle nest cavities, they maintain those foraging tunnels. Additionally, post-laying disturbances caused by predators such as raccoons (*Procyon lotor* L.) or ghost crabs (*Ocyypode* sp.) that fracture some eggs may attract fire ants to nest cavities. Thus, fire ants may maintain a presence in the nest cavity until hatching.

Our experiments with *P. nelsoni* eggs indicate that turtle hatchlings are both highly attractive and vulnerable to fire ants. Presumably, endangered species such as sea turtles or gopher tortoises attempting to hatch from nests with established fire ant foraging tunnels are equally as vulnerable. Fire ants often use the burrow aprons of gopher tortoises as colony sites (personal observation). Nearly half of the *P. nelsoni* killed by *S. invicta* successfully exited from their eggs and reached the water before succumbing to the effects of envenomization. In a natural setting, these individuals would have been considered to have hatched successfully, thus under-estimating fire ant-induced mortality by about 50%. Our laboratory research only documented direct mortality of hatchlings. However, other research has shown serious indirect effects on individual animals (American alligators, *Alligator mississippiensis* Daudin, Allen et al. 1997; northern bobwhite, *Colinus virginianus* L., Giuliano et al. 1996) stung non-lethally by *S. invicta*. These effects included the loss of digits and appendages, and reduced weight gain, both likely to affect survival in the wild.

This work indicates that hatching turtles are very vulnerable to predation by *S. invicta* and that *S. invicta* is now a common component of the ant community of sea turtle nesting beaches. However, population-level impacts are unknown. Moulis (1997) documented a 15% decrease in hatchling release rate for sea turtles (*C. caretta*) emerging from nests infested with fire ants as compared to uninfested nests, but predation by vertebrate predators (e.g., raccoons) can vary between 5 to 90% (Ratnaswamy et al. 1997).

Fire ant populations are increasing in terms of both the spatial extent of infestation (Cokendol-
POTENTIAL FIRE ANT (HYMENOPTERA: FORMICIDAE) IMPACT ON THE ENDANGERED SCHAUS SWALLOWTAIL (LEPIDOPTERA: PAPILIONIDAE)

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ABSTRACT

The Schaus swallowtail, *Papilio aristodemus ponceanus*, historically occurred in tropical hardwood hammocks from South Miami to the upper Florida Keys and is currently listed as federally endangered. Much of the remaining hardwood hammock habitat is fragmented by roads and human development that may alter the microhabitat within the hammocks and increase the probability of invasion by non-native predators and competitors. One non-indigenous species that has recently invaded the Florida Keys, and that may impact the Schaus swallowtail is the red imported fire ant (*Solenopsis invicta* Buren). We estimated abundance of red imported fire ants in Schaus swallowtail habitat on Key Largo, and the decrease in red imported fire ants resulting from the application of chemical ant baits. In addition, we conducted laboratory experiments to determine how vulnerable swallowtail life stages are to red imported fire ant predation. We found red imported fire ants at 50% of transects in the hardwood hammock, up to 40 m from hammock edge. Chemical treatments were only partially effective in decreasing red imported fire ant abundance, and the effect was short-lived. All immature swallowtail life stages were vulnerable to predation by red imported fire ants. Habitat restoration that decreases red imported fire ant abundance may be the most cost-effective and long-term method of decreasing impacts from red imported fire ants.

Key Words: Florida, habitat loss, invasive species, non-indigenous species, *Papilio aristodemus*, *Solenopsis invicta*

RESUMEN

El *Papilio aristodemus ponceanus* ocurria historicamente en bosques tropicales de madera dura ("tropical hardwood hammocks") desde el sur de Miami hasta en norte de los Cayos de la Florida, y esta actualmente listado como bajo peligro por el gobierno federal. La mayoria de habilitat de este bosque tropical esta fragmentado por calles y desarrollo humano que puede alterar el microhabitat dentro de los bosques e incrementar la probabilidad de invasión por predadores y competidores exóticos. Una especie no indigena que recientemente ha invadido los Cayos Floridano, y que puede impactar a *P. aristodemus* es la hormiga brava roja (*Solenopsis invicta* Buren). Estimamos la abundancia de *S. invicta* en habitats de *P. aristodemus* en Cayo Largo, y la reduccion en *S. invicta* como resultado de la aplicacion de trampas quimicas de hormigas. En adicion, llevamos a cabo experimentos de laboratorio para determinar que tan vulnerable son las etapas de vida de *P. aristodemus* a predacion por *S. invicta*. Encontramos *S. invicta* en un 50% de lotes en los bosques, hasta 40 m del borde del bosque. Tratamientos quimicos fueron solo parcialmente efectivos para disminuir la abundancia de *S. invicta*, y el efecto fue de corta duracion. Todas las etapas de vida de *P. aristodemus* fueron vulnerables a predacion por *S. invicta*. La restauracion del habilitat que reducen la abundancia de *S. invicta* puede ser el metodo mas efectivo en costo y a largo plazo para reducir los impactos de *S. invicta*.

The Schaus Swallowtail, *Papilio (Heraclides) aristodemus ponceanus*, is a large dark brown and yellow butterfly that historically occurred in hardwood hammocks from South Miami to the upper keys of Florida (Emmel 1995). Hardwood hammocks are closed-canopy broad-leaved forests with a high diversity (>150 species) of both evergreen and semi-evergreen tropical tree species. Adult swallowtails spend most of their time within the hardwood hammock, but will fly in clearings and along roads (Rutkowski 1971, Brown 1976). Nectaring activity occurs on >30 species of wild plants along the margins of the hammock but rarely occurs in areas open to direct sunlight (Rutkowski 1971). Adult females lay eggs on a small number of host tree species that occur primarily on the edges and in tree gaps of hardwood hammocks in portions of Monroe and
Dade Counties, Florida, including torchwood, *Amyris elemifera* (L.), and wild lime, *Zanthoxylum fagara* (L.) Sarg., (Emmel 1986, 1995). Both of these trees are relatively small and tend to produce suckers around the base. It appears that leaves on these suckers may be the preferred oviposition locations for Schaus (Bagget 1982; Emmel 1995). Young torchwood and wild lime leaves are the primary food of most Schaus caterpillars. Like other butterflies, the Schaus is an important pollinator of native plants, serves as a food source for insectivorous species, and contributes to the biological diversity of the Florida Keys.

The Schaus swallowtail was listed as Federally endangered in 1984 because of population declines caused by the destruction of its tropical hardwood hammock habitat, mosquito control practices, and over-harvesting by collectors. Reintroductions occurred between 1995 and 1997 (U.S. Fish and Wildlife Service 1999) and in 1998 the Schaus swallowtail was documented on 13 areas on the mainland and the Upper and Middle Keys.

Currently, efforts are underway to protect the remaining hardwood hammocks in south Florida from commercial and residential development as well as from pesticide spraying (U.S. Fish and Wildlife Service 1999). Even if these measures are successful, much of the existing habitat is already fragmented by roads and human development that may alter the microhabitat within the hammocks (Saunders et al. 1991) and increase the probability of invasion by non-native predators and competitors (Usher 1988).

An invasive non-native species that may have a negative impact on the Schaus swallowtail is the red imported fire ant (*Solenopsis invicta* Burken). Red imported fire ants were first recorded in the upper Florida Keys in 1976 (Callcott & Collins 1996), but were considered to be restricted to disturbed areas (Deyrup et al. 1988; Porter 1992). During a Keys-wide ant survey in 1996, *S. invicta* was identified on 10 of the 14 major keys (Forys et al. 1999). *Solenopsis invicta* was found in every major habitat type including hardwood hammocks, pinelands, salt and freshwater marshes, and disturbed areas (e.g., roadsides, parking lots) (Forys et al. 1999). While little research has been conducted on red imported fire ant predation on Lepidoptera, the presence of *S. invicta* in the tropical hardwood hammocks is of particular concern for the Schaus swallowtail because red imported fire ants are known to prey on a wide range of other invertebrates (Porter & Savignino 1990). Schaus swallowtail eggs, larvae, and pupae may be vulnerable because they occur on tree species (e.g., torchwood, wild lime) that generally occupy habitat edge where red imported fire ant infestations tend to be the highest.

The objective of this study was to 1) measure the abundance of red imported fire ants in Schaus swallowtail habitat, 2) determine if swallowtail eggs, larvae and pupae are readily discovered and consumed by red imported fire ants, and 3) explore the effectiveness of fire ant control in areas important to Schaus swallowtail reproduction.

**MATERIALS AND METHODS**

**Red Imported Fire Ant Foraging**

To determine fire ant abundance and ability to forage into closed canopy tropical hardwood hammock, we established bait transects on North Key Largo, Florida. North Key Largo is a long and narrow (<1 km) island at the northernmost portion of the Florida Keys. There are several residential developments, but most of North Key Largo is federally and state owned. In the center of the key, a well-traveled highway (SR 905) bisects the hardwood hammock. Recently, Schaus swallowtails have been successfully reintroduced in this area (U.S. Fish and Wildlife Service 1999).

Twenty transects were placed perpendicular to SR 905 into the Key Largo hammock. Ten of the transects were on the federally-owned north side of SR 905 and the other 10 were directly across the road on the state-owned south side of SR 905. Habitat on both sides of SR 905 was similar. Transects were separated by 100 m and consisted of 10 sampling stations spaced 5 m apart beginning at known fire ant infested areas (the roadside) and continuing perpendicular into the intact hardwood hammock.

At each of the 10 sampling stations along all 20 transects, we placed two terrestrial and two arboreal baits, with members of each pair separated by >1 m. The paired terrestrial baits (one honey, one hamburger meat) were placed directly on the ground on pieces of aluminum foil. The paired arboreal baits were placed in plastic condiment cups with 5-10, 3-6 mm holes punched throughout the cup. Both arboreal cups were placed 1-1.5 m from the ground in a tree as close to the terrestrial bait as was possible.

**Predation on Swallowtails**

To determine the attractiveness and vulnerability of Schaus swallowtails to red imported fire ants we conducted an experiment using eggs, larvae, and pupae of the giant swallowtail (*P. creophilotes*) Cramer as a surrogate species. The giant swallowtail is a common Key’s resident that is similar to the Schaus swallowtail in its distribution and natural history. Both species of swallowtails occur in the Florida Keys, utilize species of Rutaceae as host plants, and lay eggs singly on leaves. The larvae of both eat new leaves, do not use nests, pupae of both hibernate and are similar in structure and appearance (Scott 1986). The giant swallowtail reproduces more frequently (has multiple broods in one year) and adults can be
found throughout the year, while the Schaus swallowtail has only one set of brood a year and adults are found mid March through mid September (Emmel 1995).

Ten eggs, larvae (third and fourth instar), and pupae were purchased from a commercial butterfly farm (Robert Brown, Butterfly Paradise, 19940 Adams Rd., Ft. Myers, FL). Each egg, larva, and pupa was individually attached 1 m off the ground on a wild lime tree placed in enclosures with active red imported fire ant colony. For comparison we also placed 10 balls of hamburger meat of equal size to the larvae and pupae one meter off the ground on a wild lime tree in an additional 10 enclosures with an active red imported fire ant colonies. The eggs had been laid on wild lime leaves and we attached these leaves to the wild lime tree using gardeners wire. The pupae were placed in small porous cups and were attached to the wild lime tree. The larvae were directly placed on leaves. The enclosures were monitored for three hours and both the length of time for the fire ants to discover (make first contact) and entirely consume the hamburger balls and swallowtail eggs, larvae, and pupae were recorded. A t-test was used to compare the time to discovery for each swallowtail life stage and the hamburger meat. No statistical comparisons were made between time to consumption for the life stages and hamburger meat because the life stage differed in shape and consistency from the meat.

Effectiveness of Fire Ant Control

Five, 50-m sections of the shoulder of the main road that bisects the Key Largo hammock (SR905) were treated in July, 1997, using a fire ant bait, Amdro®, broadcast from the back of a four-wheeler and leaving 5, 50-m untreated areas as controls. Areas >100 m were left between treatment and control areas. The active ingredient in Amdro® is hydramethylnon, a metabolic inhibitor that usually kills queens, workers and the colony within 2-4 weeks (Williams 1994).

To measure the effectiveness of this treatment, red imported fire ant abundance was monitored by placing hamburger and honey baits every 5 m along 50-m transects extending into the hammock at the midpoint of each treatment and control areas before and after treatment. These transects were surveyed twice before treatment in March and July 1997, and three times after treatment in October and December 1997 and March 1998. We compared the number of red imported fire ants collected at each hammock transect in the treated and untreated areas for each survey using a Mann-Whitney U Test because the data was not normally distributed and/or variances were not equal.

**RESULTS**

Red Imported Fire Ant Foraging

In the North Key Largo hammock, red imported fire ants were identified on 8 of the 10 transects from the south side of SR905 and 2 of the 10 transects from the north side of SR905. Most of the baits with red imported fire ants were near the road. The maximum foraging distance into the hammock was 40 m. Red imported fire ants were detected arboreally at 3 transects on the south side of the road, up to a distance of 25 m into the hammock.

Predation on Swallowtails

Red imported fire ants predated all of the immature swallowtail life stages. Fire ants discovered the butterfly stages faster than the hamburger meat although this difference was significant only for the larval life stage (t = 4.66, d.f. = 18, P < 0.0001) (Table 1). The larval stage was the first to be discovered by fire ants and was consumed the fastest (Table 1). However, three larvae escaped predation during the three h experiment by moving to higher branches of the wild lime tree after being initially detected by a fire ant. All of the pupae and eggs were discovered and consumed by fire ants. It took ants the longest to consume the pupal life stage because the fire ants had to first breach the hard exterior of the pupa.

<table>
<thead>
<tr>
<th>Life stage (or meat)</th>
<th>Time to discovery</th>
<th>Time to full consumption</th>
<th>Percent escaping discovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td>30.8 (37.2)</td>
<td>18.2 (4.8)</td>
<td>0</td>
</tr>
<tr>
<td>Larvae</td>
<td>6.7* (5.4)</td>
<td>10.3 (1.9)</td>
<td>30</td>
</tr>
<tr>
<td>Pupae</td>
<td>46.2 (22.5)</td>
<td>93.8 (28.6)</td>
<td>0</td>
</tr>
<tr>
<td>hamburger meat</td>
<td>48.3 (27.7)</td>
<td>32.2 (5.6)</td>
<td>10</td>
</tr>
</tbody>
</table>

*P < 0.05.
Effectiveness of Fire Ant Control

Before treating with Amdro®, the average number of red imported fire ants in the proposed treatment areas and untreated areas did not differ significantly (March 1997: \( T = 92.5, P = 0.36, n_1 = 10, n_2 = 10 \); July 1997: \( T = 101.0, P = 0.78, n_1 = 10, n_2 = 10 \)). In October, 1997, three months after the July treatment, there were no red imported fire ants at any of the bait transects in the treated area and this was significantly fewer than in the untreated areas (\( T = 75.0, P = 0.03, n_1 = 10, n_2 = 10 \)). However, five and eight months after the treatment, the number of red imported fire ants was not significantly lower in the treated areas (December 1997: \( T = 92.0, P = 0.34, n_1 = 10, n_2 = 10 \); March 1998: \( T = 109.5, P = 0.76, n_1 = 10, n_2 = 10 \)). Overall, the number of red imported fire ants declined in both treated and untreated areas after the July 1997 treatment, probably due to the unusually dry conditions during that time period (Fig. 1).

**DISCUSSION**

The lab experiment we conducted provides evidence of the potential for red imported fire ant predation on the Giant swallowtail, and suggests that other species of butterflies that occur in the southeastern United States also may be vulnerable, including Schaus swallowtail. Fire ants predated on all terrestrial life history stages of the swallowtail, although the mobility of larvae afforded some protection. The high rate of predation was surprising due to the suite of anti-predator behaviors that swallowtails exhibit such as laying one egg per leaf, secretive behavior of larvae, and the production of foul-smelling scents from the osmeteria when larvae are disturbed (Rutkowski 1971). The ability of the red imported fire ant to penetrate the pupae was particularly disturbing. While these results were found in a laboratory experiment on a related species, it is probable that red imported fire ants impact Schaus swallowtails in nature. A manipulative field experiment with red imported fire ant population reductions and careful monitoring of Schaus swallowtail life stages and populations would provide definitive evidence.

Recently, Schaus swallowtail reintroductions occurred in state and federally owned hammocks on northern Key Largo (Emmel 1995; U.S. Fish and Wildlife Service 1999). We found that red imported fire ants were abundant on both the edges of the north Key Largo hammock and up to 40m into the interior. Fire ants were more abundant terrestrially than arboreally, but fire ants did forage arboreally. Because of the abundance of red imported fire ants and the results of the predation experiment, it is possible that fire ants are a threat to the long-term success of Schaus swallowtail reintroductions.

To further increase the chance of survival of the reintroduced Schaus swallowtail populations, red imported fire ant populations should be reduced either through use of fire ant baits or through habitat restoration. However, treatment of the road shoulders with Amdro® in North Key Largo was not as successful as similar treatments elsewhere (Allen et al. 1995). Red imported fire ants were significantly reduced for only three months in the treated area (Fig. 1). While most of the red imported fire ant mounds were on the road shoulder, there may have been mounds within the hardwood hammock that were not affected by treatments which helped to recolonize the treated areas. Some of these colonies may have occurred on old paved roads that remain in the Key Largo hammock. These roads are abandoned and mostly overgrown with vegetation, but they may still serve as favorable fire ant habitat.

Even mowed paths <2 m in width inside the hammock may increase red imported fire ant densities. The south side of the Key Largo hammock consistently had more red imported fire ants than the north. A utility path that runs between power poles on the south side of SR905 may account for the differences seen in the abundance of red imported fire ants on the south and north sides of the road.

Previous studies in this area have indicated that roads bisecting the hammock are especially attractive colonization sites for fire ants (Forys et al. 1999). The removal and restoration of aban-
doned roads and access paths, and limiting disturbance of road shoulders, will probably lower fire ant populations in the area. Reducing the abundance of red imported fire ants in the Key Largo hammock would be beneficial to the Schaus, as well as a suite of other rare invertebrate and vertebrate species that may be susceptible to predation (e.g., Key Largo cotton mouse, *Peromyscus gossypinus*; Key Largo woodrat, *Neotoma floridana smallii*; Florida tree snails, *Liguus fasciatus*).

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EFFECT OF SWEETPOTATO GENOTYPE, STORAGE TIME AND PRODUCTION SITE ON FEEDING AND OVIPPOSITION BEHAVIOR OF THE SWEETPOTATO WEEVIL, CYLAS FORMICARIUS (COLEOPTERA: APOINIDAE)

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ABSTRACT

The effect of sweetpotato genotype, storage time and production site on Cylas formicarius (Fab.) feeding and oviposition rates was investigated. Sweetpotato genotype had a significant effect on feeding and oviposition rates in both no-choice and choice arenas. Beauregard and Centennial were uniformly susceptible across all age groups. W-250 had the least number of feeding punctures and eggs at 7 and 25 days after harvest. At 85 days after harvest, W-244 had the least number of feeding punctures and eggs, while W-250 was not significantly different from Beauregard and Centennial. Roots of the same genotype grown in different locations differed in the number of feeding punctures and eggs. These results suggest that antixenosis is responsible for at least part of the sweetpotato weevil resistance. Storage time and production sites appeared to affect the expression of the resistance, but the outcomes depended on the genotypes.

Key Words: host plant resistance, antixenosis, storage time, production site

RESUMEN

Fue investigado el efecto de genotipo del camote, la duración de almacenamiento y el lugar de producción sobre la alimentación y oviposición de Cylas formicarius (Fab.). El genotipo del camote tuvo un efecto significativo sobre la alimentación y velocidad de oviposición dadas las opciones de raíz y no raíz. Beauregard y Centennial fueron igualmente susceptibles entre los genotipos evaluados. W-250 tuvo un menor numero de agujeros y huevos a 7 y 25 días después del cosechado. A 85 días después de cosechado, W-244 tuvo menos agujeros y huevos, mientras que W-250 no tuvo diferencias significativas con Beauregard y Centennial. Raíces del mismo genotipo sembradas en diferentes localidades tuvieron diferente numero de agujeros y huevos. Los resultados sugieren que antixenosis es responsable al menos en parte por la resistencia del picudo del camote. La resistencia al picudo del camote parece ser afectada por el tiempo de almacenaje y las localidades de producción, pero los resultados finales dependen mas en los genotipos.

Sweetpotato weevil (SPW), Cylas formicarius (Fab.), is a major constraint to sweetpotato Ipomoea batatas (L.) Lam. production worldwide (Chalfant et al. 1990; Jansson & Raman 1991). It attacks sweetpotato both in the field and during storage. Adults make feeding and oviposition punctures on the root surface that can reduce root quality and market value. Larval tunneling in roots induces terpenoid production that renders even slightly damaged roots unfit for human and animal consumption (Cockerham et al. 1954; Uritani et al. 1975). Due to the concealed nature of the feeding habit, control of SPW is difficult. The use of resistant sweetpotato cultivars is a potentially viable option that could be an economical component in the integrated management of SPW (Martin & Jones 1986; Collins et al. 1991).

Many studies have been conducted on SPW resistance in sweetpotato indicating variable resistance in the field (Rolston et al. 1979; Mullen et al. 1980b, 1981, 1982, 1985; Taleker 1987b) and laboratory (Mullen et al. 1980a; Barlow & Rolston 1981; Nottingham et al. 1987, 1989; Ratnayake 1995; Story et al. 1996, 1999a, b, c). However, little success has been realized in the development of resistant cultivars, partly because of inconsistencies in the performance of selected breeding lines (Talekar 1987a, b) and a lack of understanding of the resistance mechanisms. The expression of insect resistance can be influenced by many environmental factors (Smith 1989). Identification of these factors would help to explain the inconsistent performance of resistant genotypes. Such information would also be useful in facilitating the development of resistant cultivars and understanding the underlying mechanisms of resistance. In temperate growing areas like the United States, storage roots are cured by
keeping them in a specially designed facility maintained at about 30°C and 85% to 90% RH for 4 to 7 days. Cured roots are often evaluated over a period of several months in sweetpotato breeding programs. Thus curing and storage time may affect the outcome of SPW resistance evaluations because physical and chemical changes occur in the roots (Bouwkamp 1985). In addition, sweetpotato is grown throughout a wide geographic range. Wide variations in SPW resistance between production sites have been observed in field plots (Talekar 1987b). However, until this study, no comparative study has been done under controlled laboratory conditions to determine the effect of storage and production site on SPW feeding and oviposition. We evaluated 4 sweetpotato genotypes (“Beauregard”, “Centennial”, “W-244”, and “W-250”) to determine the effects of storage and production site on SPW feeding and oviposition.

MATERIALS AND METHODS

Insect rearing

A SPW colony was established from a field collected population (about 500 insects) and maintained in the laboratory on storage roots of Beauregard in plastic containers (5.6 L) with screen covers at 28 ± 2°C and 85 ± 10% RH. In preparing experimental insects, 5 fresh storage roots (US #1) were exposed to about 1000 adults (male and female) for 5 days, then were removed and kept under the conditions described above. Emerging adults (male and female) were collected weekly and held with fresh storage roots. Female adults 3-4 weeks old were used in the bioassays to ensure adequate egg-laying capability (Wilson et al. 1988).

Bioassay

The assay technique was an adaptation of one previously described by Mullen et al. (1980a) and has been used in several SPW feeding and oviposition studies (Nottingham et al. 1987; Wilson et al. 1988). It consisted of a 24-well tissue culture plate (12.5 × 8.5 × 2.0 cm; Falcon®) placed in a rectangular clear plastic container (17 × 12 × 6 cm). Cores were cut from selected roots with a cork borer (1.6 cm diameter) and inserted into the wells so that only the surface of the root periderm was exposed. The cores had the same diameter as the wells, providing a close fit. Female adults were kept without food for 3 hours before being introduced into the arena at the rate of 2 weevils per root core. A moist cotton ball was placed in the container to maintain 90-100% RH and prevent desiccation of the root cores. After 24 hours the number of feeding punctures on each core was recorded, and after 48 hours the number of eggs was counted. All tests were conducted at 28 ± 5°C, 85 ± 10% RH under total darkness to eliminate light as a variable. Cores from only one genotype were presented to the weevils in no-choice tests. In choice tests, one core from each genotype was randomly arranged on the plate and presented to the insects.

Four sweetpotato genotypes were chosen according to their performance in no-choice whole-root laboratory evaluations (Story et al. 1996). W-244 and W-250 were breeding lines shown to be resistant to SPW. Beauregard and Centennial were two susceptible cultivars. To determine the effect of curing and storage time, bioassays were conducted with roots of the following groups: non-cured 7 days after harvest (DAH), cured 25 DAH, and cured 85 DAH. Storage roots were produced using standard practices at Burden Research Plantation, Baton Rouge, Louisiana. Slips were planted on July 5, 1996 with 0.3 m spacing in 20-plant plots with rows separated by 1.2 m. Storage roots were harvested on November 1, 1996, cured (30°C, 90% RH for 7 days), and stored at 15 ± 2°C. At each test date, both no-choice and choice tests were conducted with complete randomized experimental designs and 8 replications (8 US#1 roots for each genotype).

Three sweetpotato growing regions were chosen to evaluate the effect of production site on the expression of SPW resistance. They were Baton Rouge, Louisiana (LA), Edisto, South Carolina (SC), and Pontotoc, Mississippi (MS). Storage roots were produced at each site using similar production practices. Non-cured 7 DAH roots of all 4 genotypes from LA and MS and cured 25 DAH roots of 3 genotypes (Beauregard, Centennial and W-250) from LA and SC were used. No-choice tests were conducted with 8 replications.

Data Analysis

All data (average number of feeding punctures or number of eggs per root core) were analyzed with the PC SAS General Linear Model (GLM) procedure (SAS Version 6.12 1990), followed by Tukey multiple range tests for mean separations. The effect of storage time was tested as a fixed block effect by pooling data from all 3 age groups. Curing effect was tested using a contrast statement. Production site effect was analyzed as a fixed block effect in a randomized complete block design. In all tests the significance level was α = 0.05.

RESULTS

Genotype, Curing and Storage Time Effects

Significant differences in both choice and no-choice tests in feeding and oviposition were found among the 4 genotypes. In both no-choice and choice tests, W-250 had the lowest number of feeding punctures and eggs at 7 and 25 DAH (Table 1). At 85 DAH, W-244 had the least numbers of feed-
ing punctures and eggs while W-250 was not significantly different from Beauregard and Centennial (Table 1). The curing process did not have a significant effect on feeding and oviposition in both choice and no-choice tests. Storage time had a significant effect on the number of eggs deposited in no-choice tests ($F = 61.52$, $df = 2, 84$, $P = .0001$), but not in choice tests. Storage time did not have an effect on feeding punctures. Cultivar and storage time interaction effect was significant in all cases. W-250 had some resistance relative to the susceptible cultivars when the roots were non-cured 7 DAH and cured 25 DAH, but the resistance factors were diminished in cured 85 DAH roots (Table 1). The opposite trend was found with W-244, in which significant differences in the number of punctures and eggs were detected only with cured 85 DAH roots when they were compared with the susceptible cultivars. Beauregard and Centennial were uniformly susceptible across the three root age groups.

Production Site Effects

Non-Cured Roots. A significant production site effect was found for the number of feeding punctures ($F = 5.72$, $df = 1, 56$, $P = 0.0202$) on the non-cured roots from Louisiana and Mississippi where Mississippi roots received higher number of feeding punctures than Louisiana roots (Table 2). However, the number of eggs deposited was not significantly different ($F = 0.05$, $df = 1, 56$, $P = 0.8915$). The interaction effect of genotype and production site was highly significant for both feeding and oviposition ($F = 4.63$, $df = 3, 56$, $P = 0.0058$, $F = 6.33$, $df = 3, 56$, $P = 0.0009$, respectively), indicating that the feeding and oviposition rates among the 4 genotypes were different.

Table 1. Effect of genotype and storage time (DAH = days after harvest) on the number of feeding punctures and the number of eggs of sweetpotato weevil under no-choice and choice test conditions.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Feeding puncture</th>
<th>Eggs</th>
<th>Choice test</th>
<th>Feeding puncture</th>
<th>Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No-choice test</td>
<td></td>
<td>Choice test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-cured 7 DAH roots</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beauregard</td>
<td>17.8 b</td>
<td>9.8 b</td>
<td>15.8 b</td>
<td>7.6 a</td>
<td></td>
</tr>
<tr>
<td>Centennial</td>
<td>23.1 a</td>
<td>12.4 a</td>
<td>21.8 a</td>
<td>8.9 a</td>
<td></td>
</tr>
<tr>
<td>W-250</td>
<td>9.0 c</td>
<td>5.7 c</td>
<td>3.4 d</td>
<td>2.0 c</td>
<td></td>
</tr>
<tr>
<td>W-244</td>
<td>20.9 ab</td>
<td>9.0 b</td>
<td>9.7 c</td>
<td>3.9 b</td>
<td></td>
</tr>
<tr>
<td>Cured 25 DAH roots</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beauregard</td>
<td>19.2 a</td>
<td>7.7 a</td>
<td>14.5 a</td>
<td>5.5 a</td>
<td></td>
</tr>
<tr>
<td>Centennial</td>
<td>20.4 a</td>
<td>7.6 a</td>
<td>18.3 a</td>
<td>6.6 a</td>
<td></td>
</tr>
<tr>
<td>W-250</td>
<td>11.9 b</td>
<td>4.0 c</td>
<td>4.7 b</td>
<td>1.8 b</td>
<td></td>
</tr>
<tr>
<td>W-244</td>
<td>17.4 a</td>
<td>6.2 b</td>
<td>13.5 a</td>
<td>6.3 a</td>
<td></td>
</tr>
<tr>
<td>Cured 85 DAH roots</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beauregard</td>
<td>23.3 a</td>
<td>10.5 a</td>
<td>20.1 a</td>
<td>7.0 a</td>
<td></td>
</tr>
<tr>
<td>Centennial</td>
<td>24.5 a</td>
<td>12.8 a</td>
<td>18.9 a</td>
<td>7.1 a</td>
<td></td>
</tr>
<tr>
<td>W-250</td>
<td>19.6 a</td>
<td>11.1 a</td>
<td>13.6 a</td>
<td>5.6 a</td>
<td></td>
</tr>
<tr>
<td>W-244</td>
<td>10.0 b</td>
<td>5.6 b</td>
<td>4.5 b</td>
<td>2.7 b</td>
<td></td>
</tr>
</tbody>
</table>

1Means followed by the same letter within a column of each storage time category are not significantly different ($p > 0.05$, Tukey).

Table 2. Effect of genotype and production site (Louisiana, Mississippi) on the number of feeding punctures and eggs of sweetpotato weevil on non-cured roots under no-choice test conditions.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Feeding puncture</th>
<th>Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Louisiana</td>
<td>Mississippi</td>
</tr>
<tr>
<td>Beauregard</td>
<td>15.8 b</td>
<td>24.3 a</td>
</tr>
<tr>
<td>Centennial</td>
<td>21.8 a</td>
<td>16.9 b</td>
</tr>
<tr>
<td>W-250</td>
<td>3.4 d</td>
<td>9.5 c</td>
</tr>
<tr>
<td>W-244</td>
<td>9.7 c</td>
<td>11.2 c</td>
</tr>
</tbody>
</table>

1The tests were conducted using non-cured roots 7 days after harvest.

2Means followed by the same letter within production site are not significantly different ($p > 0.05$, Tukey).
between these two sites (Table 2). For Louisiana grown roots, all 4 genotypes were significantly different from each other in number of feeding punctures, while no significant difference was found between Beauregard and Centennial in the number of eggs laid. Centennial had the most feeding punctures and eggs. For Mississippi grown roots, significant differences were found between the two susceptible cultivars in both number of feeding punctures and eggs. Significant differences were not found between W-244 and W-250 but they were different from susceptible cultivars. Beauregard was the preferred genotype for both feeding and oviposition.

Cured Roots. A significant production site effect was found between the number of eggs deposited \( (F = 4.38, df = 1.42, P = 0.0424) \) in Louisiana and South Carolina cured roots where South Carolina roots had higher number of eggs (Table 3). The number of feeding punctures was not significantly affected by production site \( (F = 1.90, df = 1.42, P = 0.1723) \). Although no statistically significant production site and genotype interaction effects were found, there was a trend toward different performance of W-250 from these two locations. Roots grown in Louisiana had significantly fewer punctures and eggs for W-250 when compared to Beauregard and Centennial. However, no differences were detected among the 3 genotypes grown in South Carolina.

**DISCUSSION**

Plant resistance to insects may be due to antibiosis, antixenosis (nonpreference), tolerance, or escape. All these types have been reported in sweetpotato resistance to SPW (Waddill & Conover 1978; Barlow & Rolston 1981; Mullen et al. 1981; Talekar 1987b; Ratnayake 1995). This study evaluated antixenosis effects (plants lack the characteristics that attract insects and are avoided by insects) on feeding and oviposition by female adult of SPW. We found that Beauregard and Centennial were preferred by SPW with respect to both feeding and oviposition. These results are consistent with previous reports that have shown the susceptibility of Beauregard (Ratnayake 1995; Story et al. 1996) and Centennial (Mullen et al. 1980b; Nottingham et al. 1989; Rolston et al. 1979) in both field and laboratory tests. W-244 and W-250 are two breeding lines with resistance to SPW (Ratnayake 1995; Story et al. 1999a). The lower numbers of feeding punctures and eggs on the roots of these two lines suggest that antixenosis was responsible for at least part of SPW resistance and that the resistant factor(s) may have a broad spectrum. Talekar (1987a) argued against the feasibility of nonpreference in sweetpotato. He pointed out that it had little value because weevils lack choices among sweetpotato genotypes in commercial plantings. We found that SPW exhibited feeding and oviposition differences among sweetpotato genotypes under no-choice conditions, suggesting the possibility of utilizing antixenosis in SPW management.

No-choice and choice are the two experimental settings for evaluating plant resistance to insects. Sometimes, results from these two kinds of tests appear to be contradictory. Resistant genotypes identified under choice conditions can receive more feeding damage than susceptible genotypes when insects are forced to feed on only one genotype (Tingey 1986). Usually, under choice conditions, susceptible plants receive higher levels of damage than resistant plants when compared to the results of no-choice conditions. This results in a larger variance among genotypes under the choice conditions. We found no significant differences in number of feeding punctures \( (F = 1.2513, df = 95, P = 0.13818) \) and eggs \( (F = 1.2781, df = 95, P = 0.11679) \) between choice and no-choice tests when testing for equal variance as described by Sokal and Rohlf (1981).

Curing and storage are common postharvest procedures for sweetpotato in temperate growing areas. During these processes many physical and chemical changes may occur in the roots. For example, curing promotes wound periderm formation on injured surfaces, thus reducing decay and water loss (Bouwkamp 1985). Storage has been reported to induce changes in carbohydrate composition, enzyme activities and cell wall components (Takahata et al. 1995; Walter & Palma 1996). Our study shows that curing had no effect

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**Table 3. Effect of Genotype and Production Site (Louisiana, South Carolina) on the Number of Feeding Punctures and Eggs of Sweetpotato Weevil on Cured Roots Under No-Choice Test Conditions.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Feeding punctures&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Eggs&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Louisiana</td>
<td>South Carolina</td>
</tr>
<tr>
<td>Beauregard</td>
<td>14.5 a</td>
<td>15.5 a</td>
</tr>
<tr>
<td>Centennial</td>
<td>16.3 a</td>
<td>14.2 a</td>
</tr>
<tr>
<td>W-250</td>
<td>4.7 c</td>
<td>13.1 a</td>
</tr>
</tbody>
</table>

<sup>a</sup>The tests were conducted using cured roots 25 days after harvest.

<sup>b</sup>Means followed by the same letter within production site are not significantly different \( (p > 0.05, 	ext{Tukey}) \).
on SPW feeding and oviposition behaviors. However, as storage time lengthened, SPW feeding and oviposition rates changed among genotypes. This suggests that storage time may influence the expression of SPW resistance, but the effect differs with each genotype.

The importance of environmental factors in the expression of SPW resistance has been noted by Talekar (1987 b). Our study also had some significant production site effects and the interactions of genotype and production site on SPW feeding and oviposition. Previous studies have related SPW resistance to the presence and concentration of a pentacyclic triterpene, boehmeryl acetate, in the periderm tissues of sweetpotato roots. This chemical has been identified as a SPW oviposition stimulant (Son 1989; Wilson et al. 1989). Our study suggests the possibility of the presence of deterrent(s) or repellent(s) in the resistant genotypes or a reduction of boehmeryl acetate. Environmental factors very likely influence such phytochemicals and hence alter the level of resistance.

In conclusion, SPW exhibited different feeding and oviposition preferences among sweetpotato genotypes. Curing, storage time, and production site influenced SPW feeding and oviposition behavior. When screening for SPW resistance, all conditions associated with testing materials (storage roots) and environmental conditions should be kept as consistent as possible. Potentially resistant lines should be evaluated under multiple sets of environmental conditions over a period of several years.

ACKNOWLEDGMENTS

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CONTAINER COLOR AND LOCATION AFFECT MACROINVERTEBRATE COMMUNITY STRUCTURE IN ARTIFICIAL TREEHOLES IN PANAMA

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ABSTRACT
I investigated the effects of habitat color and location on community structure in artificial water-filled treeholes in the forest of Barro Colorado Island, Panama. The macroinvertebrate fauna of 9 replications (5 in understory, 4 in tree-fall gaps) of black, blue, red, and green 650 ml plastic cups were censused weekly for 7 wks. Macroinvertebrate abundance and species richness were greater in understory cups than in gap cups. Seven species colonized black cups exclusively. Black cups in the understory, and red and black cups in gaps, attracted more species on average than other colors. Species richness and abundance were consistently lowest in green cups. Species were more broadly distributed among cup colors in the understory, suggesting that diffuse light conditions influenced color perception. There was no overlap in species composition between water in the artificial treeholes and water held by red Heliconia bracts or green tank bromeliads.

Key Words: color, microcosm, mosquitoes, phytotelmata, tree-fall gaps, tropics

RESUMEN
Investigué los efectos del color de hábitat y localidad en la estructura de la comunidad en huecos de árboles artificiales llenos de agua en el bosque de Isla Barro Colorado, Panamá. La fauna macro invertebrada de 9 repeticiones (5 en el “understory”, 4 en espacios abiertos de árboles caídos) de vasos plásticos de 650 ml de color negro, azul, rojo, y verde fueron observadas semanalmente por 7 semanas. La abundancia macro invertebrada y la riqueza de especies fueron mayor en vasos del “understory” que en los vasos de espacios abiertos. Siete especies colonizaron vasos negros exclusivamente. Los vasos negros en el “understory”, y los vasos rojos y negros en los espacios, atrajeron un promedio mayor de especies que otros colores. Riqueza y abundancia de especies fueron mas bajas consistentemente en vasos verdes. Las especies fueron distribuidas mas ampliamente entre colores de vaso en el “understory”, sugiriendo que condiciones de luz difusa influyen percepción de color. No hubo área común en composición de especies entre agua en los huecos de árbol artificiales y agua retenida por brácteas rojas de Heliconia o bromelias de tanque verde.

Container-breeding mosquitoes use a variety of physical and chemical cues when selecting oviposition substrates (Bates 1949; Bentley & Day 1989), and many species show preferences for specific habitat colors (Frank 1985, 1986, and references therein). Most investigations of mosquito response to habitat color have been lab-based, and color preferences have not been studied for other taxa that typically coexist with mosquitoes in the field. Although mosquitoes generally dominate the macrofauna of tropical phytotelmata, several other insect taxa are also common in these habitats (e.g., Fish 1983). The effects of habitat color on colonization by non-mosquito aquatic macroinvertebrates in tropical phytotelmata are not known.

Water-filled treeholes are common phytotelmata in many temperate and tropical forests (Snow 1949; Kitching 1971), and occur in both tree-fall gaps and forest understory. Artificial treeholes (plastic containers containing leaf litter and rain water) are often used for field-based population and community-level studies. These containers provide suitable mimics of the treehole habitat, and typically attract the same fauna found in the natural setting (e.g., Fincke et al. 1997; Yanoviak, in press). Artificial treeholes are usually clear (Pimm & Kitching 1987; Srivastava & Lawton 1998), black or brown (Fincke et al. 1997; Yanoviak 1999a), or blue (O. M. Fincke, Dept. Zoology, Univ. Oklahoma, pers. comm.). How such color differences may affect community structure in these experimental systems has never been investigated.

The potential importance of habitat color extends beyond artificial treeholes; there is considerable variation in color among natural phytotelmata. Common phytotelmata in the lowland forests of Panama include water-filled treeholes, tank bromeliads (e.g., Vriesia spp., Guzmania spp.), fallen palm petioles and spathes (e.g., of Astrocaryum stendleyanum Bailey), and exocarps of
fallen *Tontelea ovalifolia* (Miers) A. C. Smith fruits. Treehole interiors typically appear black or brown; bromeliads are generally green; palm spathes and *T. ovalifolia* husks are initially cream-colored, but gradually become orange, reddish, and eventually dark brown as they age (pers. obs.). In addition, the red and green bracts of *Heliconia* spp. often contain sufficient water to support aquatic macroinvertebrate assemblages (e.g., Seifert & Seifert 1979; Naeem 1988; Lounibos & Machado-Allison 1993).

This study examined the effects of container color and location (tree-fall gap or forest understory) on colonization of artificial treeholes by macroinvertebrates under field conditions. Specifically, I hypothesized that container location and color would affect macroinvertebrate species composition, species richness, and abundance. Earlier work on this system showed that highly exposed treeholes (in forest canopy) contained fewer species than holes in the understory (Yanoviak 1999b). Thus, I predicted that macroinvertebrate species richness and abundance would be lower in gap holes of this study. Based on field observations and other studies showing oviposition preference for dark containers (e.g., McDaniel et al. 1976), I predicted that black cups would attract more species than other colors. Finally, because color is a potentially important cue for species colonizing bromeliads (Frank 1985, 1986), I expected that green cups would attract some taxa (e.g., *Wyeomyia* spp. mosquitoes) that normally inhabit tank bromeliads in Panama.

**MATERIALS AND METHODS**

In mid-July 1997, I established 9 replications of four colored plastic cups (black, blue, green, and red; 650 ml, 8.5 cm diameter X 12 cm height - Churchill Container Corp., Shawnee, KS, USA) in the seasonally wet tropical forest of Barro Colorado Island (BCI), Panama (see Leigh et al. 1996 for a site description). Four replicates were located in tree-fall gaps and 5 in forest understory. I secured each cup to a pole-sized (10-20 cm diameter) tree approximately 1 m above the ground (as described in Yanoviak 1999a). Cups initially contained ca. 400 ml rain water and a strip of balsa wood (0.2 X 4 X 16 cm) as an oviposition site for insect colonists (Novak & Peloquin 1981). The balsa strip was small enough relative to the cup that it had little effect on general color appearance of the habitat. Holes drilled in the cup rims maintained water levels ca. 100 ml below capacity. Thus, any insect entering to oviposit was exposed to the cup color from all sides and from below. Cups within a replication were separated by > 5 m and the distance between replicates was > 100 m. I allowed rain water and detritus to accumulate naturally.

The macroinvertebrate fauna (organisms > 0.5 mm) of all cups was censused weekly for 7 wks beginning 26 July 1997. I occasionally collected subsamples of pupae and late-instar larvae to confirm field identifications. Water temperatures were measured at irregular intervals with a Corning® modular electronic probe. The experiment was terminated after 7 wks because detritus accumulations and fungal or algal growth changed the interior color of some cups to dark brown.

In addition to the fauna of artificial treeholes, I qualitatively sampled macroinvertebrates living in nearby water held by 14 tank bromeliads, 20 *T. ovalifolia* exocarps, 12 fallen palm spathes, and 12 *Heliconia latispatha* Benth. bracts (from 12 different plants) for comparison with the species composition of communities in the plastic cups. Taxa that could not be identified in the field were collected and reared in the lab.

Differences in macroinvertebrate species richness and abundance in the artificial tree holes were analyzed with 2-way repeated-measures ANOVAs using location (gap or understory) and color as main effects. When significantly different, means were compared with Ryan-Einot-Gabriel-Welsch multiple range tests (SAS 1989). Abundance data were log(x+1) transformed prior to analysis to correct variance heterogeneity (Sokal & Rohlf 1981), but all means presented in results were calculated from untransformed data. Sørensen’s (1948) coefficient of similarity \[ C = \frac{2j}{(a + b)} \] where \( j \) = number of species common to two treatments, and \( a \) and \( b \) = the number of species in each treatment was used to quantify overlap in species composition (all replicates pooled within gap or understory locations) among the four cup colors.

Reflectance data for the red, blue, and green cups were obtained with an Ocean Optics® S2000 spectrophotometer. Measurements were taken under dim illumination to reduce the variance attributable to environmental lighting. The probe was dark-spectrum calibrated by placing it into a black plastic bag, and a reference spectrum was obtained by measuring an Ocean Optics reflectance standard (a white piece of plastic). Three separate recordings, each resulting in 1570 data points between 360 and 860 nm, were taken from each cup. Reflectance spectra for the red, blue, and green cups are shown in Fig. 1.

**RESULTS**

Artificial treeholes located in the understory were colonized by more individuals and more species than cups in tree-fall gaps (Tables 1 and 2; compare Figs. 2 and 3). Conditions in the cups differed dramatically between locations. Temperatures in gap cups exceeded 40°C on sunny days, and all gap cups contained abundant filamentous algae by Week 5. In understory cups, temperatures never exceeded 32°C and algae were virtually nonexistent. There were no significant inter-
actions between treehole color and location for both macroinvertebrate species richness and abundance (Table 1), so effects of color were tested separately for gap and understory locations.

Average species richness and abundance differed among cup colors in both gaps and understory (Table 1), but significant effects did not occur in either location until Week 4 (based on Ryan post-hoc tests). Species richness was greatest in black cups in the understory (Fig. 2a) and black and red cups in gaps (Fig. 3a) after 7 wks of colonization. Macroinvertebrate abundance was significantly lower in green cups in forest understory, but did not differ among black, blue, and red cups in gaps contained more individuals than did green cups by the end of the study (Fig. 3b). The significant time*treatment interaction for understory species richness, but not for abundance (Table 1), reflects the early successional status of the artificial treeholes (Yanoviak, in press). Species richness in understory cups increased over time whereas abundance remained relatively constant (Fig. 2). The lack of a similar time interaction in gaps is attributed to high variance (Fig. 3).

Fig. 1. Reflectance spectra for the red, blue, and green cups used in this study. Each point is the mean of 30 data associated with 10 wavelength measurements (3 replicates × 10 adjacent wavelengths), and each mean was plotted against its median wavelength value. Variance was very low; error bars (±1 SD) are shown only for a subset of the means.

Black cups were colonized by the most taxa overall, whereas green cups attracted the fewest species (Table 2). Seven species, including four non-mosquito taxa, occurred exclusively in black cups. Overlap in species composition among different colors was marginally greater in the understory than in gaps (Wilcoxon 2-sample test using Sørensen’s coefficients obtained for each location, P = 0.077), and assemblages in understory black cups showed higher similarity to assemblages in red and blue cups than to those in green cups (Table 3). Several species that avoided blue and/or green cups in gaps colonized one or both of those colors in the understory (Table 2).

There was no overlap between the species composition of water held by bromeliads or H. latispatha bracts on BCI and the cups used in this experiment. However, several treehole species were found in water held by palm spathes and T. ovalifolia exocarps; both were dominated by the mosquitoes Trichoprosopon digitatum (Rondani) and Limatus spp.

**DISCUSSION**

My results show that the location and color of artificial treeholes can influence species richness, abundance, and composition in container communities. As predicted, macroinvertebrate species richness and abundance were lower in artificial treeholes located in gaps. This result is likely due to oviposition preferences for shaded vs. exposed sites. Prior work on this system showed that some species are more common in shaded holes (Yanoviak 1999c) and that highly exposed canopy tree holes contain fewer species than understory holes (Yanoviak 1999b). It is also probable that extremely high water temperatures in gap cups depressed richness and abundance through larval mortality. Larvae of several mosquito species cannot survive temperatures above 40°C for more than a few minutes (Bates 1949). Finally, abundant algae in gap cups may have deterred oviposition by chemical or other means, and occasionally caused larval mortality by restricting their movements within the cups (pers. obs.).

<table>
<thead>
<tr>
<th>Table 1. Repeated-measures ANOVA output. SS = type III sum of squares. Time = P-value for univariate test of time*treatment interaction. * = P &lt; 0.05, ** = P &lt; 0.005.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abundance</strong></td>
</tr>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>Location</td>
</tr>
<tr>
<td>Color</td>
</tr>
<tr>
<td>Location*Color</td>
</tr>
<tr>
<td>Color, Gap</td>
</tr>
<tr>
<td>Color, Underst.</td>
</tr>
</tbody>
</table>
Cup color significantly affected macroinvertebrate species richness and abundance in both locations and, as predicted, black cups attracted the most species overall. Although many taxa colonized more than one color of cup, the general distribution of species among colors suggests that most prefer to oviposit in dark containers or avoid green oviposition sites. This pattern is well documented for several mosquito species (e.g., Williams 1962; Wilton 1968; McDaniel et al. 1976; Hilburn et al. 1983; Beehler et al. 1992; Jones & Schreiber 1994; also reviewed by Frank 1985), and at least partly explains why the green cups of this study contained few species and individuals.

Differences in distributions of species among cup colors between gap and understory locations most likely reflect differences in how colors are perceived in these light environments. (One exception among the taxa found in this study is the annelid *Dero* sp., which is primarily dispersed by phoresy.) Most insects are unable to see red (Chapman 1998), thus red containers probably appear dark gray to potential colonists. Wave-lengths of incident light in forest understory are shifted toward the blue/green end of the spectrum by the surrounding vegetation and toward red under overcast conditions (Endler 1993), thus blue and green cups in forest understory may also

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**Table 2. Distribution of Invertebrate Taxa among Different Colored Cups in Forest Understory and Tree-fall Gap Locations. Values are Maximum Number of Individuals Observed in a Cup within a Treatment. Minimum Abundance was Zero in Most Cases. Mean Abundance and Mean Richness are the Average (+1 SD) Number of Individuals and Species within a Treatment, all Census Dates Combined.**

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Understory</th>
<th>Gap</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black</td>
<td>Blue</td>
</tr>
<tr>
<td>Annelida: Naididae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dero</em> sp.</td>
<td>115</td>
<td>7</td>
</tr>
<tr>
<td>Odonata: Pseudostigmatidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mecistogaster</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Coleoptera: Scirtidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Prionocyphon</em> sp.</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Diptera: Ceratopogonidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bezzia snowi</em> Lane</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td><em>Forcipomyia</em> spp.</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>Diptera: Chironomidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chironomus</em> sp.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diptera: Culicidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aedes terrae</em> (Walker) complex</td>
<td>35</td>
<td>1</td>
</tr>
<tr>
<td><em>Anopheles eiseni</em> Coq.</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><em>Culex conservator</em> D. &amp; K.</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td><em>C. corriganii</em> D. &amp; K.</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td><em>C. urichi</em> (Coq.)</td>
<td>21</td>
<td>59</td>
</tr>
<tr>
<td><em>Haemagogus equinus</em> Theobald</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td><em>H. leucotaeniatus</em> (Komp)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>H. lucifer</em> (Howard, Dyar &amp; Knab)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Limatus assuleptus</em> (Theobald)</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td><em>Orthopodomyia fascipes</em> (Coq.)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Toxorhynchites theobaldi</em> (D. &amp; K.)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Diptera: Psychodidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Telmatoscopus</em> spp.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diptera: Syrphidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Copestylum rafaelanum</em> (Townsend)</td>
<td>69</td>
<td>0</td>
</tr>
<tr>
<td>Diptera: Tipulidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sigmatomera</em> spp.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total no. of taxa represented</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Mean abundance</td>
<td>22.3</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>(31.20)</td>
<td>(16.18)</td>
</tr>
<tr>
<td>Mean richness</td>
<td>2.5</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>(1.71)</td>
<td>(1.10)</td>
</tr>
</tbody>
</table>
have been perceived as gray or reddish. Results of this study support such a conclusion. Blue and red cups attracted more species overall in the understory than in gaps, and species that colonized three or more colors tended to be more abundant in red and black. The mosquitoes *Culex urichii* (Coquillett) and *Limatus assuleptus* (Theobald) are exceptions to the latter, but both are also habitat generalists and will colonize water in almost any container (pers. obs.).

Five of the 7 species found only in black cups occurred in very low abundance. The midge *Chironomus* sp. and the mosquito *Orthopodomyia fascipes* (Coquillett) are normally more abundant in artificial treeholes than was observed here (Yanoviak, in press), so their distribution in this study may not reflect color preferences. Despite its low abundance, the presence of *Anopheles eis- eni* Coquillett only in black cups probably reflects a color preference; several other *Anopheles* species prefer to oviposit in dark containers (reviewed by Frank 1985). *Mecistogaster* spp. and *Toxorhynchites theobaldi* (Dyar & Knab) are top predators in this system, and typically exist in low abundance due to competition and cannibalism (Fincke 1999). Treeholes are a limiting reproductive resource for pseudostigmatids (Fincke 1992a), and the species occurring on BCI avoid other phytotelmata, including bromeliads (Fincke 1992b). Lab studies showed that some *Toxorhynch- ites* species prefer to oviposit in black containers (Hilburn et al. 1983; Jones & Schreiber 1994). Therefore, it is reasonable to conclude that the presence of these two taxa only in black cups reflects a habitat color preference.

### Table 3. Sørensen’s similarity coefficients for macroinvertebrate assemblages occurring among the four cup colors in understory and tree-fall gap locations.

<table>
<thead>
<tr>
<th></th>
<th>Understory</th>
<th></th>
<th></th>
<th>Gap</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black</td>
<td>Blue</td>
<td>Red</td>
<td>Black</td>
<td>Blue</td>
<td>Red</td>
</tr>
<tr>
<td>Blue</td>
<td>0.667</td>
<td>—</td>
<td>—</td>
<td>0.333</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Red</td>
<td>0.609</td>
<td>0.625</td>
<td>—</td>
<td>0.444</td>
<td>0.750</td>
<td>—</td>
</tr>
<tr>
<td>Green</td>
<td>0.444</td>
<td>0.727</td>
<td>0.615</td>
<td>0.333</td>
<td>0.250</td>
<td>0.250</td>
</tr>
</tbody>
</table>

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Fig. 2. Mean (±SE) macroorganism species richness (A) and abundance (B) in different colored cups located in forest understory. N = 5 for each mean. Within a week, means followed by the same letter do not differ based on post-hoc tests (only noted where differences occurred; a single letter was used for overlapping means). Some error bars were omitted for clarity.

Fig. 3. Mean (±SE) macroorganism species richness (A) and abundance (B) in different colored cups located in tree fall gaps. N = 4 for each mean. Within a week, means followed by the same letter do not differ based on post-hoc tests (only noted where differences occurred; a single letter was used for overlapping means). Some error bars were omitted for clarity.
The lack of species overlap between the colored cups and similarly colored phytotelmata on BCI can be explained by physical attributes of these systems other than color. First, the general form of a container may influence colonization. For example, Frank (1986) used artificial bromeliads to show that ovipositing mosquitoes are sensitive to the general shape of plant containers. Second, macroinvertebrate colonization is influenced by the vertical position of phytotelmata. Fallen fruit husks and palm spathes occur at ground level, whereas all of the containers used in this study were ca. 1 m above the ground. Some mosquito species in the BCI forest (e.g., *Limatus* spp., *T. digitatum*) are sensitive to this difference in height (Yanoviak 1999b, in press). Finally, many species respond to chemical cues when selecting oviposition sites (Bentley & Day 1989), and these cues may complement or exceed the effects of color (Lounibos & Machado-Allison 1993).

CONCLUSIONS

My results suggest that differential colonization and harsh environmental conditions in tree-fall gaps tend to reduce the abundance and diversity of macroinvertebrates in artificial tree holes. Container color appears to play a role in habitat selection for several species in this system, but perception of color may be confounded by variable light conditions in forest understory. Additional studies under controlled laboratory conditions are needed to determine oviposition preferences of the many non-mosquito colonists of tropical phytotelmata.

ACKNOWLEDGMENTS

A. Almanza assisted in the field and R. Fuller provided reflectance data for the cups. Comments from L. P. Lounibos, D. Srivastava, and an anonymous reviewer improved the manuscript. This work was supported by funds from the IIE/Fulbright Foundation and the University of Oklahoma.

LITERATURE CITED


PUPATION BIOLOGY OF *FRANKLINOTHrips ORIZABENsIS* (THYSANOPTERA: AEOLOTHRIPIDAE) AND HARVESTING AND SHIPPING OF THIS PREDATOR

MARK S. HODDLE, KIKUO OISHI, AND DAVID MORGAN
Department of Entomology, University of California, Riverside, CA 92521, USA

ABSTRACT
In the laboratory, 82-99% of late second instar *Franklinothrips orizabensis* Johansen larvae abandoned avocado branches and artificial branches constructed of wooden dowels and were recovered below branches trapped on tangle foot coated plastic sheets, suggesting a preference by this life stage for selection of pupation sites beneath host plants. Of three media tested (coarse and fine vermiculite, and parafilm cones) for harvesting *F. orizabensis* pupae in cocoons, parafilm cones were most easily harvestable from colonies, and 44% of deployed late second stage larvae that were recovered used parafilm cones for pupation in experimental cages. Harvesting and shipping trials using aspirated adult *F. orizabensis* or pupae in parafilm cones showed significant differences in survivorship when held in the laboratory or shipped round trip from Riverside, California to Amherst, Massachusetts. Survivorship of aspirated adults was reduced on average by 41% following shipping, and mortality was highest for adult males. Transit survivorship was increased by 53% if *F. orizabensis* were shipped as cocoons in parafilm cones. Inclusion of ice packs in polystyrene boxes did not significantly increase survivorship rates for *F. orizabensis* adults or pupae that were either retained in the laboratory or shipped. This result may have been an artifact resulting from the time of year (i.e., May and temperatures were moderately cool) when shipping trials were conducted.

Key Words: *Franklinothrips*, pupation biology, harvesting, shipping

RESUMEN
En el laboratorio, 82-99% de las larvas en el segundo instar de *Franklinothrips orizabensis* abandonaron las ramas de aguacate y las ramas artificiales construidas de clavijas de madera y fueron recuperadas bajo ramas atrapadas en hojas plásticas con capa pegajosa “tangle foot”, sugiriendo una preferencia de selección de lugares de pupación debajo de plantas huéspedes por esta etapa de vida. De los tres medios probados (vermiculita fina y ordinaria, y conos de parafina) para cosechar pupas de *Franklinothrips orizabensis* en capullos, conos de parafina fueron los más fáciles de cosechar de las colonias, y el 44% de larvas de segunda etapa que fueron recuperadas usaron conos de parafina para pupación en jaulas experimentales. Énsayos de cosecha y embarque usando adultos aspirados de *Franklinothrips orizabensis* o pupas en conos de parafina demostraron diferencias significativas en supervivencia al ser llevadas a cabo en el laboratorio o embarcadas ida y vuelta desde Riverside, California hasta Amherst, Massachusetts. Supervivencia de adultos aspirados fue reducida un promedio de 41% después de embarque, y la mortalidad fue mayor entre machos adultos. Supervivencia de transporte fue incrementada por 53% si *Franklinothrips orizabensis* eran embarcadas como capullos en conos de parafina. La inclusión de bolsas de hielo en cajas de policistireno no incrementó significativamente las cantidades de supervivencia para adultos o pupas de *Franklinothrips orizabensis* que fueron retenidas en el laboratorio o embarcadas. Este resultado puede haber sido un artefacto resultando por el tiempo del año (es decir, Mayo y las temperaturas eran moderadamente frías) cuando los ensayos de embarque fueron llevados a cabo.

Inoculative, augmentative, and inundative biological control programs targeting pestiferous thrips in perennial outdoor crops have seldom been successful (Parker & Skinner 1997). The major limiting factors that have been identified as constraints on successful thrips biological control programs for outdoor crops are: (1) lack of effective resident natural enemies that respond in a rapid density dependent manner to increasing thrips densities. (2) Thrips phenology and life cycle characteristics can result in long periods of low thrips densities. In some instances this may facilitate rapid pest outbreaks as thrips natural enemy densities have declined because of lack of prey. (3) Incompatibility of thrips natural enemies with broad-spectrum insecticides used to control other crop pests. (4) The high cost of insectary-reared natural enemies make large-scale field releases for thrips control into outdoor crops of low value uneconomical (Grafton-Cardwell & Ouyang 1995a; Parker & Skinner 1997; Parrella & Lewis 1997). Natural enemies that have been commonly observed with phytophagous thrips in perennial tree crops are phy-

In some instances, thrips biological control by Type IV phytoseiid mites (i.e., specialized pollen feeders that exhibit generalist predatory activity [McMurtry & Croft 1997]) can be enhanced by pollen bearing wind break trees (Grout & Richards 1990, 1992a,b) or cover crops in orchards (Grafton-Cardwell et al. 1999a). Crop management practices such as pruning that enhances succulent leaf material can also promote increased densities of Type IV phytoseiids (Grafton-Cardwell & Ouyang 1995b; Grafton-Cardwell 1997). Augmentative releases of insectary-reared phytoseiids for thrips control have been shown to significantly reduce densities of Scirtothrips citri Moulton (Thysanoptera: Thripidae) on citrus (Citrus spp.), but, at present, this technology is not cost effective (Grafton-Cardwell & Ouyang 1996a; Grafton-Cardwell et al. 1999b).

Scirtothrips perseae Nakahara (Thysanoptera: Thripidae) was first discovered damaging avocado foliage and fruit in southern California orchards in 1996, and at time of discovery was a species new to science (Nakahara 1997). In 1998, crop losses due to down-graded fruit and increased production costs due to S. perseae feeding damage were estimated to have cost California growers (US) $7-$13 million (Hoddle et al. 1998, 1999). Foreign exploration efforts to determine the native range of S. perseae indicate that this pest is of Central American origin (Hoddle et al. 1999) and associated natural enemies collected concurrently from avocados in Latin America have included Franklinthrips spp. (Hoddle, unpublished).

Surveys in southern California avocado orchards for indigenous natural enemies associated with S. perseae have revealed that an undescribed species of Franklinthrips orizabensis Johanson (Thysanoptera: Aeolothripidae) is the dominant predator where this pest has attained high densities (>10 S. perseae larvae per leaf) (Hoddle, unpublished). Female Franklinthrips spp. lay eggs directly into plant tissue. Developing larvae pass through two instars (second instars are distinguished by red hypodermal pigments), before pupating within protective silk cocoons which are spun from secretions produced from the anal region (Arakaki & Okajima 1998).

Unlike Euseius tularensis Congdon (Acari: Phytoseiidae), which in some instances can regulate citrus thrips, S. citri (Moulton) (Grafton-Cardwell & Ouyang 1995b), E. hibisci (Chant) a common Type IV phytoseiid in southern California avocado orchards, has not been observed to respond in a significant density dependent manner to increasing S. perseae populations (Hoddle unpublished). Because of industry interest, the potential for using F. orizabensis for augmentative releases against S. perseae in avocado orchards is being investigated. Optimal temperature require-

ments and diets for mass rearing this predator have been evaluated (Hoddle et al. 2000, 2001) and limited field trials evaluating releases of F. orizabensis against S. perseae on avocados have been conducted (Silvers 2000).

Augmentative field releases of mass-reared F. orizabensis onto avocado trees failed to significantly suppress S. perseae populations in California. This may have been due more to the poor quality of adults which suffered high mortality (>50%) after shipping from an insectary in Europe rather than the inherent ineffectiveness of F. orizabensis (Silvers, 2000). In order to fully explore the potential of augmentative releases of F. orizabensis for controlling of S. perseae in California avocado orchards, low impact techniques for harvesting and distribution that minimize transit mortality of this predator are required. One possible approach would be to collect and transport F. orizabensis when larvae are pupating within protective silk cocoons as opposed to aspirating and shipping adults. The purpose of this study was to investigate the pupation biology and behavior of F. orizabensis, and to develop techniques based on an understanding of pupation behavior for collecting and shipping pupating larvae and compare survivorship rates to currently employed methods for collecting and shipping adult predators.

MATERIALS AND METHODS

Franklinthrips orizabensis Colony

Franklinthrips orizabensis colonies were maintained in cages in a temperature controlled room (25°C, 60% RH, L:D 14:10) on lima beans (Phaseolus lunatus Linnaeus variety “Baby Fordshook” [plants are needed for oviposition of eggs by female F. orizabensis]) at the University of California Riverside, California, USA. Colony reared F. orizabensis were fed irradiated Epicera kuehniella (Zeller) (Lepidoptera: Pyralidae) eggs (supplied by Beneficial Insectaries, Oak Run, CA, USA) which were liberally deposited on upper surfaces of horizontal bean leaves. Our colony was initiated with adult F. orizabensis collected from an avocado orchard infested with S. perseae in Fallbrook, California, USA. Adult progeny produced by field collected F. orizabensis used to initiate the colony were deposited with the Systematic Entomology Laboratory, USDA-ARS, Beltsville, Maryland, USA and identified as F. orizabensis by Dr. S. Nakahara.

Source of Second Instar Franklinthrips orizabensis Larvae for Experiments

Undersides of mature ‘Hass’ avocado leaves collected from the Biological Control Grove (F. orizabensis has not been collected here) at the University of California, Riverside were pre-
sented to colony-reared female *F. orizabensis* for oviposition (Hoddle et al. 2000). Adult females were confined with male *F. orizabensis* within modified Munger cells (Munger 1942, Morse et al. 1986), fed irradiated *E. kuehniella* eggs, and left to oviposit in temperature cabinets (25°C, L:D 14:10 h).

At the end of the 24 h oviposition period, the leaf area enclosed by the Munger cell which was exposed to ovipositing females was excised from the avocado leaf. Trimmed leaves were labeled, placed on water-saturated foam pads in stainless steel trays, and incubated at 25°C. Leaves were examined daily for emerged *F. orizabensis* larvae. Emerged first instar larvae were collected with a fine camel hair brush, placed individually in 1 dram glass shell vials with irradiated *E. kuehniella* eggs as food, and vials were sealed with parafilm® (American National Can, WI). Larvae were reared in temperature controlled cabinets (25°C, L:D 14:10 h) in shell vials until second instars which were then used for experiments. Late second instar larvae select pupation sites and spin pupation cocoons within which propupal and pupal stages occur.

**Pupation Behavior**

Pupation behavior experiments were conducted in the laboratory at 25°C and ambient light to determine the proportion of *F. orizabensis* larvae that pupate on avocado branches and artificial wooden branches.

**Pupation on avocado branches.** Individual branches were selected on small (1.5-2 m tall) Hass avocado trees planted in 20 liter plastic pots. Tanglefoot was applied at the base of branches at trunk attachment to prevent larvae leaving experimental branches. Clear plastic sheets (60 cm × 40 cm) covered with tanglefoot were placed under each experimental branch and the outline of the overhanging branch was drawn in the tanglefoot. Ten late second instar *F. orizabensis* larvae of known age were placed on avocado leaves and supplied with irradiated *E. kuehniella* eggs as food. Larvae were left for three days and then numbers recovered from tanglefoot covered plastic sheets were recorded, and the perpendicular distance jumped from branches was determined by measuring distances from larvae to drawn branch outlines in tanglefoot. Artificial branches were examined under a dissecting microscope and numbers of larvae pupating on wooden dowels were recorded. Experiments were replicated 10 times for artificial branches with and without refugia.

**Pupal Harvesting Techniques**

The pupation behavior study outlined above indicated that >80% of late second instar *F. orizabensis* larvae would fall or jump from natural and artificial branches to seek pupation sites below branches (see Results Section). Since late second instar larvae spin silk cocoons we sought to identify a media within which larvae would pupate that could allow easy harvesting of *F. orizabensis* in cocoons. Evaluations of pupation media were conducted in temperature controlled cabinets (25°C, L:D 14:10). Five late second instar *F. orizabensis* larvae were placed with pupation media in glass Petri dishes (9.5 cm diameter) with lids which were sealed with parafilm and left for three days in temperature controlled cabinets. Cocoons were then located in Petri dishes and numbers of adult *F. orizabensis* that successfully emerged following harvesting were recorded daily.

**Vermiculite.** Vermiculite (Therm-o-Rock West Inc., Chandler, AZ) was sieved into two grades coarse (all granules that were not retained by 20 mesh USA Standard Testing Sieve) and fine (all granules not retained by 35 mesh USA Standard Testing Sieve). Five ml of each material was placed into glass Petri dishes and the ability to harvest cocoons spun onto vermiculite granules was assessed after three days. Each vermiculite...
treatment was replicated 10 times. Collection of cocoons was attempted by sieving vermiculite with a 20 mesh sieve based on the assumption that cocoons encased with vermiculite would be too large to pass through and would be retained.

**Parafilm cones.** Parafilm cones (diameter 3 mm, height 5 mm) (Fig. 1) were constructed by wrapping strips of parafilm (4 mm wide, 15 mm long) around the tapered end of a camel hair brush. Five parafilm cones were placed in each Petri dish, and this treatment was replicated 10 times. Cone utilization for pupation by *F. orizabensis* larvae was visually assessed after three days.

**Parafilm cones and fine vermiculite.** Five parafilm cones and 5 mls of fine vermiculite were combined in a glass Petri dish. This treatment was replicated 6 times, and utilization of either cones or vermiculite for pupation was assessed after three days.

### Selection of Pupation Sites in Small Rearing Cages

Thirty individually-reared late second instar *F. orizabensis* were hand placed on four lima bean plants contained within rearing cages (30 cm × 30 cm × 37.5 cm), fed irradiated *E. kuehniella* eggs which were liberally deposited on upper surfaces of horizontal bean leaves, and maintained in a temperature controlled room (25°C, 60% RH, L:D 14:10) for three days. Cages were supplied with 50 parafilm cones which were distributed on potting media and cage floors. After three days, numbers of *F. orizabensis* cocoons in parafilm cones, in cage corners and sleeves, on bean plants, and in vermiculite in which bean plants were growing (plants were destructively sampled) were determined. After inspection for cocoons, experimental cages were examined every two days for 10 days for adults that emerged from cocoons that were not detected during initial cage inspection. This treatment was replicated 10 times.

### Harvesting of *Franklinothrips orizabensis* Pupae from Laboratory Colonies

Parafilm cones were readily used as pupation sites by *F. orizabensis* larvae in glass Petri dishes and small rearing cages (see Results Section). The efficacy of using parafilm cones to harvest *F. orizabensis* pupae was determined in laboratory colonies. *F. orizabensis* colonies used for this experiment were fed irradiated *E. kuehniella* eggs and maintained in cages (75 cm × 40 cm × 45 cm) with 9-12 lima bean plants. Plants were raised 3 cm from the cage floor on metal pipes fixed to cage walls. Under these metal supports a clear plastic sheet (70 cm × 30 cm) was positioned holding 100 parafilm cones (Fig. 2). Parafilm cones were left beneath plants for three days before the plastic sheet with cones was removed. Cocoons containing pupae were counted, held in Petri dishes in a temperature controlled cabinet (25°C, L:D 14:10), and number and sex of emerging adult *F. orizabensis* was recorded daily. This trial was replicated 10 times and approximately 300 adult *F. orizabensis* were present in mass-rearing cages each time.

### Shipping Adult and Pupal *Franklinothrips orizabensis*

Thirty individually-reared adult *F. orizabensis* (15 male and 15 female; all were 1-2 days of age) were aspirated into three dram glass shell vials, supplied with irradiated *E. kuehniella* eggs as food, and sealed with a wad of cotton wool. Thirty *F. orizabensis* pupae (1-2 days of age) in parafilm cones were placed in three dram shell vials and sealed with cotton wool. Aspirated adults and pupae in parafilm cones were each subjected to one of the following treatments:

**Aspirated adults and cocoons retained in the laboratory.** The purpose of this experiment was to quantify predator mortality in the absence of shipping stress. Aspirated adults and pupae in parafilm cones were placed in sealed polystyrene foam boxes (20.5 cm × 16.5 cm × 15.5 cm) with or without ice packs. Temperatures and humidities in boxes were recorded every 10 mins with Hobo data loggers (Onset Computer Corp., Pocasset, MA). Survivorship of aspirated adults was recorded after 48 h and pupae in parafilm cones were removed from boxes after 48 h and reared in a temperature controlled cabinet (25°C, L:D 14:10) to determine emergence rates and sex. This treatment was replicated five times.

**Aspirated adults and cocoons shipped to Massachusetts.** The purpose of this experiment was to quantify predator mortality due to shipping stress. Adult and pupal *F. orizabensis* were collected, prepared, and shipped in polystyrene foam boxes with or without ice-packs with Hobo data loggers as described for the laboratory retention study. Boxes were shipped Federal Express prior-
ity overnight to Amherst, Massachusetts and then returned immediately the next day by Federal Express priority overnight to Riverside, California. Upon receipt in Riverside, proportion of adults surviving was determined, and pupae in parafilm cones were removed from boxes and reared in a temperature controlled cabinet (25°C, L:D 14:10) to determine emergence rates and sex. Round trip transit time from California to Riverside was approximately 48 h. This treatment was replicated five times.

Analysis of collecting and shipping data. Analysis of adult and cocoon survivorship data was performed on logit transformed data (ln live/dead) after weighing for sample size. The effects of shipping, cooling, life stage, and sex on survivorship of *F. orizabensis* were tested for significance using Chi-square analysis and pair-wise T-test comparisons (0.025 level of significance).

Analysis of temperature and humidity data. Temperature and humidity recorded for each day of the two day period for both laboratory held and shipped polystyrene foam boxes was analyzed using ANOVA and Tukey’s Studentized range test for means separation (0.05 level of significance).

RESULTS

Pupation Behavior

In all three treatments (i.e., natural avocado branches and artificial branches with and without pupation refugia) >90% of deployed late second instar *F. orizabensis* larvae were recovered (Table 1). On avocado branches and artificial branches with refugia <20% of recovered larvae pupated on branches, and the majority were recovered from tanglefoot coated plastic below branches. This result indicated that late second instar *F. orizabensis* larvae may prefer to pupate below trees, or suitable sites for pupation on branches were either not available or located. Artificial branches without pupation refugia resulted in 100% branch abandonment by larvae (Table 1). Of larvae recovered from plastic sheets, 80-98% were found within 0-2.5 cm of branches, indicating that larvae fall rather than actively jump away from branches when searching for pupation sites below host plants (Table 1). High rates of branch abandonment may make it possible to collect *F. orizabensis* below host plants if suitable pupation sites for cocoon construction can be provided that allow for easy harvesting of this life stage.

Pupal Harvesting Techniques

In coarse vermiculite, 80% of deployed late second instar *F. orizabensis* larvae pupated successfully (Table 2). Use of this media for harvesting cocoons was unsuitable as larvae spun silk cocoons between vermiculite particles and the bottoms and sides of Petri dishes, or crawled into cracks in vermiculite particles making detection difficult. Consequently, a technique for harvesting pupal *F. orizabensis* based on sieving for cocoons encased in vermiculite would not be prac-
tical. The use of fine vermiculite resulted in 100% mortality of larvae as this grade probably acted as an abrasive desiccant (Table 2).

In Petri dishes, parafilm cones were utilized as pupation sites by 80% of deployed late second instar *F. orizabensis* larvae of which 72% emerged successfully. All recovered larvae had spun cocoons inside parafilm cones (Table 2). Parafilm cones combined with fine vermiculite resulted in 67% successful pupation and all recovered pupae were found inside parafilm cones. Provision of parafilm cocoons mitigated the adverse effects of fine vermiculite on pupation success rates (Table 2). Parafilm cones were the most successful media tested for harvesting *F. orizabensis* pupae.

Selection of Pupation Sites in Small Rearing Cages

Of the 300 second instar *F. orizabensis* larvae released into small rearing cages 58% were recovered. The highest numbers of recovered pupae were found inside parafilm cones (Table 3). No pupating larvae were found on bean leaves or stems. Approximately 10% of pupae were found inside seed coats attached to cotyledons, and a similar percentage of cocoons were found in vermiculite potting mix down to a depth of 2 cm (Table 3).

Harvesting of *Franklinothrips orizabensis* Pupae from Laboratory Colonies

Over a three day period, 48% of parafilm cones in cages were used by pupating *F. orizabensis* larvae (Table 4). Greater numbers of females were harvested using parafilm cones, and 0-67% (mean 35% ± 6.50 [SE]) of cones would have more than one pupating larva. Adult emergence rates from parafilm cones placed in colony cages was high, >97% (Table 4).

Shipping Adult and Pupal *Franklinothrips orizabensis*

**Handling and shipping.** Survivorship of adult *F. orizabensis* was significantly affected by shipping ($\chi^2 = 44.39, df = 1, p < 0.0005$) with survivorship rates of adults being reduced on average by 41% in comparison to *F. orizabensis* that were held in the laboratory and not subjected to shipping stress (Fig. 3). Adult male *F. orizabensis* suffered significantly higher mortality rates than females ($\chi^2 = 34.18, df = 1, p < 0.0005$) with survival being reduced on average by 24% (Fig. 3). Survivorship of *F. orizabensis* was significantly increased when predators were shipped as cocoons ($\chi^2 = 185.10, df = 1, p < 0.0005$) and transit survivorship was increased on average by 53% (Fig. 3). Inclusion of icepacks in polystyrene foam boxes that were either retained in the laboratory or shipped roundtrip from California to Massachusetts did not significantly alter survivorship rates in comparison to the same treatments without icepacks ($\chi^2 = 0.62, df = 1, p = 0.43$).

**Temperature and humidity.** Significant differences in temperature ($F = 2627, df = 3, 1448, p < 0.0005$) (Fig. 4A) and humidity ($F = 173, df = 3, 1448, p < 0.0005$) (Fig. 4B) existed between poly-

### Table 1. Mean percentage recovery (± SE) of *Franklinothrips orizabensis* larvae released onto avocado branches, artificial branches lacking pupation sites, and artificial branches with pupation sites.

<table>
<thead>
<tr>
<th>% Total larval recovery</th>
<th>Avocado branches</th>
<th>Artificial branches without pupation refugia</th>
<th>Artificial branches with pupation refugia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>93.00 ± 2.13</td>
<td>98.00 ± 1.33</td>
<td>95.00 ± 1.67</td>
</tr>
<tr>
<td>% Larvae pupating on branch</td>
<td>1.11 ± 0.01</td>
<td>0.00 ± 0.00</td>
<td>17.51 ± 0.01</td>
</tr>
<tr>
<td>% Larvae recovered from sticky traps 0-2.5 cm from branch</td>
<td>97.78 ± 0.01</td>
<td>90.89 ± 0.01</td>
<td>80.38 ± 0.01</td>
</tr>
<tr>
<td>% Larvae recovered from sticky traps 2.5-5.0 cm from branch</td>
<td>1.11 ± 0.01</td>
<td>9.11 ± 0.07</td>
<td>2.11 ± 0.01</td>
</tr>
<tr>
<td>% Larvae recovered from sticky traps &gt;5.0 cm from branch</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

### Table 2. Mean percentage (± SE) of *Franklinothrips orizabensis* in each of four pupation media contained in Petri dishes that either pupated successfully and emerged as adults, died as larvae or pupae, or were not recovered.

<table>
<thead>
<tr>
<th>Pupation Media</th>
<th>% Emerged ± SE</th>
<th>% Dead ± SE</th>
<th>% Unrecovered ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coarse vermiculite</td>
<td>80.00 ± 5.96</td>
<td>6.00 ± 3.06</td>
<td>14.00 ± 6.70</td>
</tr>
<tr>
<td>Fine vermiculite</td>
<td>0.00 ± 0.00</td>
<td>100 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Parafilm cones</td>
<td>72.00 ± 4.42</td>
<td>8.00 ± 4.42</td>
<td>20.00 ± 5.16</td>
</tr>
<tr>
<td>Parafilm cones &amp; fine vermiculite</td>
<td>66.67 ± 4.22</td>
<td>16.67 ± 6.15</td>
<td>16.67 ± 6.15</td>
</tr>
</tbody>
</table>
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styrofoam boxes that were retained in the laboratory. Boxes with ice-packs were significantly cooler and had higher humidity rates, particularly on the second day, presumably resulting from condensation on ice packs. The mean temperature also increased in boxes with ice packs on the second day (Fig. 4A). Significant differences in temperature \( (F = 1422, \text{df} = 3, \ 2672, \ p < 0.0005) \) (Fig. 4C) and humidity \( (F = 513, \text{df} = 3, \ 2672, \ p < 0.005) \) (Fig. 4D) existed between polystyrene foam boxes that were shipped round trip from California to Massachusetts. Boxes with ice-packs were significantly cooler, especially on the first day, and had higher humidity rates, particularly on the second day, probably from condensation on ice packs. Humidity in boxes without ice packs was constant during the transit period (Fig. 4B).

**DISCUSSION**

In the laboratory, the majority (82-99%) of recovered late second instar \textit{Franklinothrips orizabensis} larvae actively abandoned natural and artificial wooden dowel branches to search for pupation sites below host plants. Predatory and phytophagous thrips have been captured emerging from the ground beneath citrus trees (Childers et al. 1994), and pupation beneath host plants is common for pestiferous phytophagous thrips (Grout et al. 1986; Harrison 1963; Okada 1981; Reed & Rich 1975; Schweizer & Morse 1996, 1997; Tsuchida 1997). Host plant abandonment in the field has been inferred from analysis of leaf duff samples from commercial avocado orchards in southern California where \textit{F. orizabensis} specimens have been recovered to a depth of 2.5 cm into the soil (Hoddle et al. 1998).

Host plant abandonment rates by \textit{F. orizabensis} larvae prior to pupation in avocado orchards have not been quantified. Our estimates of larval abandonment on young avocado trees in the laboratory may have been over-estimated as young trees have fewer bark fissures that can be used as pupation refugia in comparison to mature trees in orchards. This suggestion is supported by higher recovery rates of \textit{F. orizabensis} pupae on artificial branches with pupation refugia. In comparison to phytophagous thrips, there is little published information on pupation behavior and site selection by predatory thrips in field situations and this area would benefit from more research.

Coarse and fine vermiculite were unsuitable pupation substrates for harvesting \textit{F. orizabensis} pupae. In Petri dishes with coarse vermiculite, pupating larvae constructed cocoons by attaching silk to walls and floors of dishes, and coarse vermiculite particles were then attached to cocoon surfaces not adhered to the Petri dish. Pupating \textit{F. orizabensis} could not be harvested in this media as cocoons were destroyed (i.e., cocoons were ripped open exposing pupae) when removal of vermiculite granules was attempted. Fine vermiculite was an unsuitable pupation substrate as it resulted in mortality of 100% of second instar

**TABLE 3. MEAN PERCENTAGE (± SE) RECOVERY OF \textit{FRANKLINOTHIRPS ORIZABENSIS} COCOONS FROM DIFFERENT LOCATIONS IN SMALL REARING CAGES.**

<table>
<thead>
<tr>
<th>Location of Cocoons in Cage</th>
<th>% Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cage sleeves</td>
<td>10.40 ± 4.34</td>
</tr>
<tr>
<td>Cage corners</td>
<td>7.01 ± 1.49</td>
</tr>
<tr>
<td>Parafilm cones on potting media &amp; cage floor</td>
<td>44.02 ± 6.86</td>
</tr>
<tr>
<td>Seed coat attached to cotyledons</td>
<td>9.61 ± 5.53</td>
</tr>
<tr>
<td>Potting soil</td>
<td></td>
</tr>
<tr>
<td>0-1 cm deep</td>
<td>7.22 ± 3.52</td>
</tr>
<tr>
<td>1-2 cm deep</td>
<td>3.89 ± 1.24</td>
</tr>
<tr>
<td>&gt;2 cm deep</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Dead larvae &amp; pupae</td>
<td>3.51 ± 1.24</td>
</tr>
<tr>
<td>Adults emerged into cages after inspection for cocoons</td>
<td>13.58 ± 3.10</td>
</tr>
</tbody>
</table>

**TABLE 4. UTILIZATION OF PARAFILM CONES BY \textit{FRANKLINOTHIRPS ORIZABENSIS} IN MASS REARING CAGES.**

<table>
<thead>
<tr>
<th>Measured Variable</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parafilm cones utilized by pupating larvae</td>
<td>48.33 ± 3.43</td>
</tr>
<tr>
<td>Male \textit{Franklinothrips orizabensis} emerging from cones</td>
<td>39.49 ± 1.79</td>
</tr>
<tr>
<td>Female \textit{Franklinothrips orizabensis} emerging from cones</td>
<td>60.51 ± 1.79</td>
</tr>
<tr>
<td>No. adult \textit{Franklinothrips orizabensis} emerging per cone</td>
<td>1.31 ± 0.97</td>
</tr>
<tr>
<td>Pupal mortality in parafilm cones</td>
<td>2.35% ± 0.35</td>
</tr>
</tbody>
</table>
Fig. 3. Survivorship of aspirated adult male and female *Frankliniorthips orizabensis* and pupae in parafilm cones either retained in the laboratory at ambient temperatures or cooled with ice packs, or shipped roundtrip from Riverside, California to Amherst, Massachusetts either with or without ice packs. Mean treatment proportions surviving with the same letters are not significantly different (0.025 level of significance).

Fig. 4. Mean temperatures (A) and humidities (B) in polystyrene foam boxes with and without ice packs when retained in the laboratory for 48 h, and mean temperatures (C) and humidities (D) when polystyrene boxes were shipped round trip from Riverside, California to Amherst, Massachusetts with or without ice packs. Mean temperatures and humidities followed by the same letters are not significantly different (0.05 level of significance).
**F. orizabensis** larvae. Placing parafilm cones in Petri dishes with fine vermiculite significantly increased larval and pupal survivorship.

Parafilm cones were utilized by late second instar **F. orizabensis** larvae as pupation sites in Petri dishes, small rearing cages and laboratory colonies. In small rearing cages where known numbers of second instar larvae were deployed, 44% of recovered **F. orizabensis** pupae were found in parafilm cones. Parafilm cones may have been desirable pupation sites because of the conical design (i.e., the tapering nature of the unit and its horizontal position) resulted in larvae being able to find a desirable wall width within the cone to adhere silk strands during cocoon construction. In commercial mass rearing operations, the use of parafilm cones or some other artificial pupation site under host plants (e.g., small, mass produced clear plastic cones) that are easily harvestable may be cost effective for rapidly collecting and shipping **F. orizabensis** pupae. The use of electronic scanning devices could conceivably assist in sorting cones with and without pupae as cones with cocoons turn a blackish color as adults mature. This color change could aid electronic detection and mechanical sorting (Petitt et al. 1996; Smittle et al. 1986; Whitten 1969; Wolf et al. 1972). Alternatively, weight differences between cones with and without pupae may be amenable to air stream separation (Jackson et al. 1996). Mechanical harvesting and sorting of **F. orizabensis** pupae in artificial pupation media could significantly reduce production costs for this predator. **F. orizabensis** larvae that pupate in sites other than cones could be a source of adults for sustaining cage colonies and may mitigate the need to set aside portions of the harvested product for colony maintenance. It is possible that harvesting pupae in cones and not returning individuals to colonies that exhibit a preference for cones could select for individuals that do not utilize harvestable media for pupation.

Survivorship of **F. orizabensis** during shipping was greatly enhanced if predators were shipped as pupae in parafilm cocoons. Adult **F. orizabensis**, especially males, were extremely sensitive to asperation and transit stress, and high mortality resulted when adult males and females were shipped long distances. Transit mortality of adult and pupal **F. orizabensis** was not significantly reduced by inclusion of ice packs in polystyrene foam boxes. This may have been an artifact resulting from the time of year this shipping trial was conducted as shipments were made during May when weather conditions were cool. Inclusion of ice packs with shipments of **F. orizabensis** pupae would be recommended, especially during summer.

Work is currently underway with commercial insectaries in California to implement mass rearing of **F. orizabensis** for field trials against *S. perseae* in avocado orchards.

**ACKNOWLEDGMENTS**

This work was supported in part by the California Avocado Commission. Dr. Roy Van Driesche, University of Massachusetts at Amherst kindly assisted with the shipping aspects of this trial. We thank Dr. Miwa Takano-Lee for critically evaluating this work.

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EFFECTS OF VEGETATION CONTROL ON PARASITOIDS OF THE NANTUCKET PINE TIP MOTH, RHYACIONIA FRUSRANNA (LEPIDOPTERA: TORTRICIDAE)

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Abstract

The Nantucket pine tip moth (Rhyacionia frustrana (Comstock)) is host to numerous parasitoid species that can cause substantial moth mortality. Little is known regarding the effects of forest management practices on these parasitoids. Abundance of parasitoids and parasitism rates, based on rearing of adult moths and parasitoids, were compared in herbicide-treated and untreated loblolly pine, Pinus taeda L., stands in the southeastern Georgia coastal plain. Three parasitoids, Lixophaga mediocris Aldrich, Eurytoma pini Bugbee, and Hyssopus rhyacioniae Gahan accounted for over 70% of total parasitism. Parasitism rates did not differ significantly between treated and untreated plots. Tip moth damage was higher in the untreated plots. Malaise trapping of parasitoids yielded no significant difference in numbers of tip moth parasitoids or total parasitoids captured in treated and untreated plots, suggesting a functional response of parasitoids to tip moth densities. Higher levels of naturally occurring vegetation did not improve tip moth control through increased parasitism rates.

Key Words: Rhyacionia frustrana, parasitoids, vegetation control, Pinus taeda, parasitism

RESUMEN

La polilla de pino Nantucket (Rhyacionia frustrana (Comstock)) es huésped a numerosas especies parasitoides que pueden causar mortalidad substancial de polilla. Poco se sabe en relación con los efectos de practicas de control forestal en estos parasitoides. La abundancia de parasitoides y las incidencias de parasitismo, basado en el cultivo de polillas adultas y parasitoides, fueron comparadas en áreas de pinos Pinus taeda L., sin tratar y tratados con herbicida, en el plano costeño del sureste de Georgia. Tres parasitoides, Lixophaga mediocris Aldrich, Eurytoma pini Bugbee, y Hyssopus rhyacioniae Gahan resultaron en mas del 70% del parasitismo total. Incidencias de parasitismo no difirieron significativamente entre terrenos tratados y no tratados. Daño de polilla de pino fue mayor en los terrenos no tratados. Capturas de parasitoides no produjeron diferencias significativas en números de parasitoides de polilla o en el total de parasitoides capturados en terrenos tratados y no tratados, sugiriendo una respuesta funcional de parasitoides a densidades de polilla de pino. Niveles mas elevados de presencia de vegetación natural no mejoro el control de polilla de pino a través de cantidades más altas de parasitismo.

The Nantucket pine tip moth, Rhyacionia frustrana (Comstock), is a common insect pest of southern pines, attacking seedlings and saplings of loblolly (Pinus taeda L.), shortleaf (P. echinata Miller), and Virginia (P. virginiana Miller) pines (Berisford 1988). Eggs are laid on needles and shoots. Early instar larvae mine needles and fascicle sheaths. Later instars feed on the meristematic tissue of shoots and buds, causing shoot death and losses in form, wood quality and volume growth (Cade & Hedden 1987). The moth has 2 to 5 generations annually (depending on climatic conditions) and overwinters as a pupa inside of the dead shoot or bud (Berisford 1988).

Damage caused by the Nantucket pine tip moth is highly variable, and may be negligible or high enough to cause tree mortality. Increased tip moth population fluctuations have been associated with intensive forest regeneration practices, including chemical control of competing vegetation to increase seedling growth and survival (Miller & Stephen 1983; Nowak & Berisford 2000). Tip moth populations may be constrained to some degree by natural agents, including parasitoids and predators (Eikenbary & Fox 1968a,b; Freeman & Berisford 1979; Wallis et al. 1980; Gargiullo & Berisford 1983; Warren 1985; McCravy & Berisford 1998, 2000). Approximately 60 species of parasitoids have been associated with the Nantucket pine tip moth (Yates & Beal 1962a,b; Freeman & Berisford 1979; Wallis et al. 1980; Gargiullo & Berisford 1983; Warren 1985; McCravy & Berisford 1998, 2000). However, potential effects of intensive forest management practices on tip moth parasitoids have received little attention. Herbaceous vegetation can be an important
source of food for adult parasitoids (Leius 1961, 1963; Syme 1975), and can also be important as refugia and in maintaining suitable microclimatic conditions (Reed et al. 1970; Powell 1986). Pimentel’s enemy impact hypothesis (Pimentel 1961) suggests that natural enemies of herbivorous insects are more effective in diverse systems than in simple ones. In a review of tests of this hypothesis, however, Russell (1989) found the results to be inconclusive for parasitism.

We initiated a study to determine if chemical control of competing vegetation affects parasitism of the Nantucket pine tip moth. The objective of this study was to compare rates of parasitism and abundance of parasitoids of the tip moth in herbicide-treated and untreated loblolly pine stands.

**Materials and Methods**

The study was conducted in 1996-97 at 2 one-yr-old *P. taeda* plantations in Burke County, in the Georgia coastal plain. Site 1 was 70 ha and Site 2 was 47 ha. Tree density at each plantation was approx. 1500 trees/ha. In spring, 1996, each site was divided into 2 equally sized plots, with 1 randomly selected plot receiving treatment with the herbicides hexazinone (Velpar®, DuPont, Wilmington, DE) and sulfometuron methyl (Oust®, DuPont, Wilmington, DE) at rates of 2.34 and 0.22 liters/ha, respectively. The remaining halves were left as untreated plots. The treatment plots at each site were treated again in fall, 1996 with imazapyr (Arsenal®, American Cyanamid, Princeton, NJ) and glyphosate (Accord®, Monsanto, St. Louis, MO) at 0.58 and 2.38 liters/ha, respectively. All herbicides were applied by helicopter. There was no replication within sites. Rather, sites were treated as replicates to decrease the potential for confounding effects due to parasitoid immigration into small plots. In spring, 1996, 50 pines were randomly chosen in each plot of each study site to serve as permanent study trees. Tip moth infestations were evaluated on these trees by counting numbers of infested shoots in each generation, when tip moths were in the late larval and pupal stages and damas were removed and counted. Recognizable tip moth parasitoids were also separated and counted. Two parasitoids, *Hyssopus rhyacioniae* Gahan and *Pteromalus* Swederus sp., were determined to be gregarious based on dissection of individual tip moth-infested shoots, averaging 12.2 ± 1.20 (mean ± SE, n = 15) and 2.92 ± 0.45 (n = 12) individuals per brood, respectively. Total numbers of individuals reared for these 2 species were divided by these means to provide a more accurate estimate of the number of parasitism events.

At site 1, parasitoids were collected using malaise traps to estimate the relative abundance of parasitoids in the herbicide-treated and untreated plots. Two traps, 1 in each treatment plot, were operated for 8, 5-day trapping periods bi-weekly from mid-June to early October 1996. Traps were randomly relocated for each trapping period. Insects were collected in 70% EtOH. At the end of each trapping period, samples were taken to the laboratory and parasitoids, defined as Parasitica (Huber 1993) plus tachinids (Diptera), were removed and counted. Recognizable tip moth parasitoids were also separated and counted.

Mean percentage ground cover and mean number of flowering plants per plot were compared between treatments using paired t-tests. Two-way repeated measures analysis of variance, with generation as the repeated variable, was used to compare mean numbers of infested shoots per tree between treatments and sites, to compare overall parasitism rates between treatments and sites, and to analyze species-specific parasitism by the most common parasitoid species in relation to treatment. Percent parasitism data were arcsine transformed prior to analysis. Paired t-tests were used to compare numbers of parasitoids captured in malaise traps between treatments. It was noted...
that larger parasitoids appeared to be relatively more common in samples from the untreated plots. Therefore, chi-square tests were used to analyze ratios of ichneumonoids and tachinids to microhymenoptera reared and captured in treated and untreated plots. All analyses were done using SigmaStat Version 2.0 software package (Jandel Scientific Software 1995).

RESULTS

The most common plant species found and months in which flowering individuals were present are shown in Table 1. Mean percent ground cover (±SE) was significantly greater in the untreated plots than in the treated plots (t(7) = 8.48, P < 0.001), indicating that herbicide treatments were effective (Fig. 1). Mean number of flowering plants was also greatest in the untreated plots (t(7) = 2.82, P = 0.03) (Fig. 1).

Tip moth infestation levels, based on numbers of damaged shoots per tree, were higher in the untreated plots than the treated plots (10.89 ± 1.41 vs. 2.64 ± 0.49; F(1,3) = 33.31; P = 0.01). Infestation levels ranged from 7.96 ± 2.50 in the fall generation to 15.41 ± 4.15 in the winter for the untreated plots, and from 1.22 ± 0.18 in spring to 3.79 ± 0.21 in summer for the treated plots (Fig. 2). There was no significant difference between sites (F(1,3) = 1.26, P = 0.34), and no significant interaction (F(1,3) = 1.04, P = 0.38).

Overall, 7,198 parasitoids were reared, including 17 species in 9 families representing 2950 parasitism events. Three species, Lixophaga mediocris Aldrich, Eurytoma pini Bugbee, and Hysopus rhyacioniae Gahan accounted for over 70% of total parasitism. Mean percent parasitism was 47.47 ± 7.94 in the treated plots and 46.65 ± 7.47 in the untreated plots (F(1,3) = 0.01, P = 0.92). Difference in parasitism rates between sites was not significant (F(1,3) = 5.56, P = 0.10), and there was no significant interaction (F(1,3) = 0.24, P = 0.66). Parasitism was highest in spring (73.93 ± 2.94) and lowest in winter (22.36 ± 1.33).

There was no relationship between treatment and contribution to total parasitism among the 3 most common parasitoid species (F(2,6) = 0.06, P = 0.94). However, as a group, ichneumonoids comprised a greater proportion of rearings, relative to microhymenoptera, in the untreated than the herbicide-treated plots at Site 1 (14.27% vs. 8.02%; X^2(1) = 13.53, P < 0.001) and Site 2 (17.39% vs. 11.57%; X^2(1) = 8.57, P = 0.003).

Malaise trapping resulted in 2,981 total parasitoid captures. Mean numbers of captures per trapping period (±SE) were 189.88 ± 49.15 in the herbicide-treated plot and 182.75 ± 36.90 in the untreated plot, and were not significantly different (t(7) = 0.11, P = 0.916; Fig. 3). Ichneumonoids accounted for 9.09% of total captures and 11.77% of total parasitic hymenoptera, while microhymenoptera accounted for 68.13% and 88.23% of

<table>
<thead>
<tr>
<th>Table 1. Common plant species found in quadrat sampling and months in which flowering individuals were found</th>
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<td><em>Tephrosia spicata</em> (Walter) Torrey and Gray</td>
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<td><strong>Hypericaceae</strong></td>
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<td><em>Hypericum denticulatum</em> Humboldt, Bonpland, and Kunth</td>
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<td><strong>Passifloraceae</strong></td>
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<td><strong>Vitaceae</strong></td>
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<tr>
<td><em>Vitis rotundifolia</em> Michaux</td>
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</table>
these totals, respectively. Tachinids comprised 22.78% of total captures. There were no significant between-treatment differences in captures of ichneumonoids ($t_{(7)} = 1.037, P = 0.334$), microhymenoptera ($t_{(7)} = 0.409, P = 0.695$), or tachinids ($t_{(7)} = 0.328, P = 0.753$). However, there was a significantly greater proportion of ichneumonoids and tachinids captured, relative to microhymenoptera, in the untreated (35.29%) than the treated (28.57%) plot ($X^2_{(1)} = 15.199, P < 0.001$). A total of 136 known tip moth parasitoids were captured. Mean numbers captured/trap period ($\pm$SE) were 9.50 $\pm$ 2.84 in the herbicide-treated plots and 7.50 $\pm$ 1.84 in the untreated plots ($t_{(7)} = 0.528, P = 0.613$). *Lixophaga mediocris* (72% of total) and *E. pini* (14% of total) were the most common species captured.

**DISCUSSION**

Most studies of tip moth damage in relation to forest regeneration practices have found an inverse relationship between damage and amount of competing vegetation (Berisford & Kulman 1967; White et al. 1984; Hood et al. 1988; Ross et al. 1990). However, Miller & Stephen (1983) and Nowak & Berisford (2000) found no differences in damage levels between herbicide-treated and untreated plots, but greater temporal variation in tip moth damage levels in the treated plots. We also found greater temporal variation in damage levels in treated plots, at least on a relative scale, with a roughly 3-fold difference between peak and lowest infestation rates in the treated plots, but only a 2-fold difference in the untreated plots (Fig. 2). Nowak & Berisford (2000) also found that infestation levels were generally lower in treated than untreated plots at low tip moth densities, as was the case in our study. There was little difference in variation in parasitism rates between treatments in our study, suggesting that the greater relative fluctuations in tip moth damage in the treated plots, and greater overall damage in the untreated plots, were not due to disruption of parasitoid populations.

The overall tip moth parasitism rate of 47% generally agrees with that of 42% found by Freeman & Berisford (1979) in the Georgia piedmont and 41% found by Eikenbary & Fox (1965) in the South Carolina coastal plain, but is higher than the 26% rate obtained by the latter authors in the South Carolina piedmont. Tip moth parasitism rates were highest in the spring generation and lowest in the winter in our study. Gargiullo & Berisford (1983) found a similar pattern for tip moth parasitism in the Georgia piedmont. If tip moth parasitoids attack specific life stages, the
increased asynchrony of tip moth development in later generations (Fettig et al. 2000) could make it more difficult for the parasitoids to find suitable hosts, since fewer tip moths in the required life stage(s) would be present at any given time.

Tip moth populations were approx. 2 to 5 times higher in the untreated plots, based on damage estimates, but there was no difference in parasitism rates between treatments, meaning that greater total parasitism occurred in the untreated plots. Malaise trapping showed no difference in parasitoid abundance between plots, suggesting a greater number of moths parasitized per parasitoid in the untreated plots, a functional response perhaps resulting from greater parasitoid fecundity or longevity, or less searching time due to higher host densities. The ratio of ichneumonoids and tachinids to microhymenoptera reared and captured in malaise traps was greatest in the untreated plots, suggesting that vegetation control in pine plantations may have a relatively greater impact on populations of the larger parasitoids. Larger-bodied parasitoids may have greater metabolic requirements, which could affect the amount and type of food needed. Small wasps are also probably more affected by wind currents that result from lack of vegetation to act as a windbreak. There is also evidence that some female ichneumonoids must feed at flowers to complete egg development (van Emden 1963). Numerous studies (Syme 1975; Foster & Ruesink 1984; Wäckers & Swaans 1993; Idris & Grafius 1995, 1996, 1997; Johanowicz & Mitchell 2000) have documented the importance of flower availability on fecundity, survival, and parasitism rates by ichneumonoids. Detailed studies of specific plant associations of tip moth parasitoids, and effects of vegetation control practices on these plants, are needed.

In summary, we found that total tip moth parasitism was greater in untreated than in herbicide-treated plots, but parasitism rates were equal between plots. Tip moth damage was substantially higher in untreated than in herbicide-treated stands, suggesting that vegetation management practices do not necessarily affect the role of parasitoids in tip moth population control.

ACKNOWLEDGMENTS

We thank M. Dalusky (University of Georgia) for technical advice, and N. LeCroy, R. Garland, J. Smith, T. Jackson, B. Dorough, M. Morrow, and D. Hart (University of Georgia) for invaluable technical assistance. B. Haynes and the late M. Moore (University of Georgia) provided advice on vegetation sampling and assistance with plant identifications, respectively. J. Seckinger, S. Cameron, and F. Bevan (International Paper) were of great help in providing study sites. A. Braccia (University of Georgia), C. Fettig (University of Georgia), R. Hedden (Clemson University), J. Meeker (Florida Dept. of Agriculture and Consumer Services), J. Nowak (University of Georgia), and S. Salom (Virginia Polytechnic Institute and State University) provided helpful comments on earlier versions of the manuscript, as did the anonymous reviewers. Thanks also to R. Weseloh (The Connecticut Agricultural Experiment Station) for suggesting use of repeated measures ANOVA. L. Coote (Royal Ontario Museum), C. Darling (Royal Ontario Museum), G. Gibson (Agriculture and Agri-Food Canada), E. Grissell (USDA, Systematic Entomology Laboratory), S. Heydon (University of California-Davis), J. Luhan (Minnesota Dept. of Agriculture), A. Menke (USDA, Systematic Entomology Laboratory), M. Schaff (USDA, Systematic Entomology Laboratory), A. Sharkov (Ohio State University), J. Whitfield (University of Arkansas), M. Wood (Agriculture and Agri-Food Canada), and G. Zolnerowich (Texas A&M University) provided positive identifications of parasitoids. This project was supported by the Pine Tip Moth Research Consortium and the Georgia Traditional Industries Program.

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TRAPPING FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) ADULTS IN TRAPS BAIATED WITH PHEROMONE AND A SYNTHETIC FLORAL VOLATILE COMPOUND

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ABSTRACT

Field experiments were conducted to determine the effectiveness of the floral compound phenylacetaldehyde in increasing capture of fall armyworm, Spodoptera frugiperda, males in pheromone-baited traps. Plastic Unitraps were placed in cotton and corn fields in north-central Florida and were baited with commercial sex pheromone and synthetic phenylacetaldehyde released from hollow polyethylene stoppers and glass microcapillary pipets. Addition of phenylacetaldehyde as a lure was not effective in collecting more moths and actually reduced numbers of moths captured compared to pheromone-baited traps. Nontarget Hymenoptera were also collected in traps; more Sphecoidea were found in phenylacetaldehyde-baited traps compared to pheromone-baited traps.

Key Words: insect behavior, Spodoptera, phenylacetaldehyde, monitoring

RESUMEN

Experimentos de campo fueron llevados a cabo para determinar la efectividad del compuesto floral fenilacetaldehído en incrementar la captura de machos del gusano de otoño Spodoptera frugiperda, en trampas con carnada de feromona. Trampas de plástico “Unitraps” fueron colocadas en campos de maíz y algodón en regiones del centro-norte de Florida y se les preparó con feromona de sexo comercial y fenilacetaldehído sintético soltados de tapones huecos de polietileno y de pipetas microcapilares de cristal. La adición de fenilacetaldehído como atrayente no fue efectivo en la colección de más polillas y en realidad redujo el número de polillas capturadas en comparación con trampas con señuelo de feromona. Himenóptera en general también fueron colectadas en trampas; mas Sphecoidea fueron encontradas en trampas con fenilacetaldehído comparadas con trampas de feromona.

MATERIALS AND METHODS

1997

An experiment was conducted in northwestern Alachua County, Florida, from 21 July to 10 October. All-white Universal Moth Traps or Unitraps (Great Lakes IPM, Vestaburg, MI) were placed along pivot roads and edges in an 80-ha field of cotton, Gossypium hirsutum L. This field was sur-
rounded by \(=\) 400 ha of cotton, separated by paved and unpaved roads and forested strips.

Traps were baited with lures containing sex pheromone of the fall armyworm and phenylacetaldehyde. Three treatments were used: (1) Trécé® (Trécé, Inc., Salinas, CA) red septa lures containing components of the *S. frugiperda* pheromone, (2) phenylacetaldehyde (Aldrich Chemical Co., Milwaukee, WI) placed in hollow polyethylene stoppers (Kimble, Vineland, NJ, purchased through Thomas Scientific, Swedesboro, NJ, \#9713-F28), or (3) a combination of both lures. Pheromone lures were attached to the bottom of a cork that was placed in a hole in the canopy of the Unitrap. The stopper with phenylacetaldehyde (0.5 ml per stopper) was hot-gun glued (Arrow Fastener Co., Saddle Brook, NJ) to the bottom of the cork, which was placed in the trap canopy. The combination lure was composed of a cork with attached stopper and the pheromone lure attached to the side of the cork. All traps contained insecticide strips to kill moths that were captured (Hercon® Vaportape II containing 10% 2, 2-dichlorovinyl dimethyl phosphate, Hercon Environmental Co., Emigsville, PA). Trap contents were removed three times per week and pheromone and phenylacetaldehyde lures were replaced every two weeks. The experiment was designed as a randomized complete block with three blocks of the three treatments. The location of each trap within each block was changed weekly.

1998

In late March, fields in the same area in northwestern Alachua county were planted to silage corn (*Zea mays* L.). An experiment was designed to compare capture of fall armyworm males in Unitraps that presented two lower release rates of phenylacetaldehyde in combination with Trécé *S. frugiperda* pheromone lures. Phenylacetaldehyde was injected into disposable 100 µl glass microcapillary pipets (Kimble, Vineland, NJ) that had been flame-sealed at one end, and filled so that there would be a 10-mm length of the vapor-air column above the liquid. Holes were drilled into corks that were placed in the canopy of the Unitrap, and microcapillaries were placed in the corks. The double phenylacetaldehyde release rate contained two microcapillaries of the synthetic compound. Therefore, the three treatments were: (1) Trécé *S. frugiperda* pheromone lures, (2) Trécé lures plus phenylacetaldehyde in one microcapillary pipet, or (3) Trécé lures plus phenylacetaldehyde in two pipets. Traps contained insecticide strips to kill insects. The experiment was designed as a randomized complete block with four blocks of the three treatments, and trap location within a block was randomized weekly. Trapping began 27 April and ended 12 June. Additionally, all aculeate Hymenoptera that were captured were sorted and numbers were compared across treatments.

**Release Rates**

Release rates of phenylacetaldehyde in the stoppers used in the 1997 experiment were determined by the modified techniques of Heath & Manukian (1992). Briefly this system consists of a glass chamber (25.7 cm long and 7.6 cm ID) constructed of Pyrex glass with a glass frit inlet, a ground-glass joint outlet, and a multiport collector base to which the collector traps were connected. Collector traps were made from a 4.0 cm long by 4.0 mm ID piece of glass tubing and contained 50 mg of Super-Q® (Alltech Assoc., Deerfield, IL) as the adsorbent. Two stainless steel frits were used to contain the adsorbent. The collector traps were connected to stainless steel tubing by 0.64 cm unions and 0.64 cm ID Teflon® ferrules. These traps were cleaned by soxhlet extraction with methylene chloride for 24 h and dried in a fume hood prior to use. The airflow rate through the volatile collection system was 1 L/min during a 1-h collection period. Volatiles from the lures collected on the traps were eluted using 100 µl of high purity methylene chloride. Tetradecane was added as internal standard prior to analysis. Release rates were determined from three lures on days 3, 5, 7, and 14. The lures were held in a hood without airflow between analyses.

Gas chromatographic analyses were conducted with a Hewlett-Packard Model 5890A Series II gas chromatograph, equipped with a cool on-column capillary injector (septum injector) and flame ionization detector. Helium was used as the carrier gas at a linear flow of 18 cm/sec. A combination of three fused silica columns connected in series by GlasSeal® connectors (Supelco Inc., Bellefonte, PA) was used. A deactivated fused silica column, 8.0 cm long by 0.55 mm ID, was connected between the injector and the retention gap column. This column permitted the use of 0.4 mm OD stainless steel needles with a septum injector for on-column injections. The retention gap column used was 10 m by 0.25 mm ID deactivated fused silica and the analytical column used for analysis was a 30 m by 0.25 mm ID (0.25 µm film) SE-30.

Release rates of phenylacetaldehyde in the microcapillaries used in the 1998 experiment were determined in a manner similar to that reported by Weatherston et al. (1985a, b), by which rates are estimated mathematically based on size of microcapillary, length of vapor-air column above the liquid, and volatility of the formulated compound.

**Statistics**

For each experiment, insect numbers were compared across treatments in a split block analysis of variance (ANOVA), where treatment was
the main plot and date was the subplot (Steel & Torrie 1980). To satisfy ANOVA assumptions, counts were $\log(x + 1)$ transformed before analysis. Treatment means or treatment combinations were separated by a LSD mean separation test or orthogonal comparisons (PROC GLM, CONTRAST statement, SAS Institute 1996). Untransformed means (±SE) are given in text and figures, whereas statistical results refer to transformed data.

RESULTS

1997

Fall armyworm males were collected from late July through early October, with a peak capture of over 100 moths per night per trap in early September (Fig. 1). Traps baited with pheromone alone consistently captured more moths than traps baited with both pheromone and phenylacetaldehyde, or traps baited with phenylacetaldehyde alone (mean ± SE, moths per night: pheromone alone 21.6 ± 3.0, pheromone + phenylacetaldehyde 13.7 ± 1.6, phenylacetaldehyde alone 0.12 ± 0.03; $F = 196.7$; df = 2, 4; $P < 0.0001$). The release rate for phenylacetaldehyde in the stoppers over the 14-day period averaged 492.9 ± 15.1 μg/h (Fig. 2).

1998

Male fall armyworm numbers in traps were low until late May (Fig. 3). More moths per night were collected from 29 May through 15 June in pheromone-baited traps (27.1 ± 5.0) than pheromone + phenylacetaldehyde-baited traps (12.5 ± 3.4) ($F = 5.1$; df = 2, 6; $P = 0.0511$). Capture in pheromone + double phenylacetaldehyde-baited traps (13.6 ± 2.4) was intermediate. The release rate for phenylacetaldehyde in the microcapillary tubes was initially 230.8 ng/h for the single tube and 461.6 ng/h for the double rate.

DISCUSSION

Previous research showed a numerical increase in captures of males with traps baited with both sex pheromone and phenylacetaldehyde with five moth species (Noctuidae, Plusiinae) (Creighton et al. 1973). In fact, most of the successful studies of attraction to phenylacetaldehyde have been completed with species of Plusiinae (Smith et al. 1943; Creighton et al. 1973). In fact, most of the successful studies of attraction to phenylacetaldehyde have been completed with species of Plusiinae (Smith et al. 1943; Creighton et al. 1973).
However, our research with laboratory-reared fall armyworm (Noctuidae, Amphipyrinae) in a flight tunnel showed increased percentages of upwind flight and source contact in combination with a sex pheromone lure (Meagher & Mitchell 1998). The disappointing results in these field experiments showed that wild *S. frugiperda* moths were not attracted to phenylacetaldehyde-baited traps alone, or in combination with sex pheromone. Landolt et al. (1991) and Heath et al. (1992) reported that cabbage looper, *Trichoplusia ni* (Hübner), moths responded to release rates ranging

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<th>Superfamily/family/tribe/genus/species</th>
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<td>Apoidea</td>
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1973; Cantelo & Jacobson 1979; Haynes et al. 1991; Landolt et al. 1991; Heath et al. 1992). However, our research with laboratory-reared fall armyworm (Noctuidae, Amphipyrinae) in a flight tunnel showed increased percentages of upwind flight and source contact in combination with a sex pheromone lure (Meagher & Mitchell 1998).
from 50 ng/h to 4 μg/h in flight tunnel and screen-cage bioassays. Because counts were lower in the combined phenylacetaldehyde + pheromone-baited traps, it is assumed that fall armyworm moths were able to perceive phenylacetaldehyde at the release rates tested (230 ng/h—500 μg/h), but were repelled from those traps.

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Release rate determination of the phenylacetaldehyde was conducted in the laboratory of R. Heath by B. Dueben (USDA-ARS). Technical support in the field was provided by J. Brady and C. Dillard. N. D. Epsky (USDA-ARS), D. L. Kline (USDA-ARS) and J. Nation (Univ. of Florida) provided helpful reviews of an earlier manuscript.

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MEXICAN SPECIES OF PARASITOID WASPS OF THE GENUS MARIETTA (HYMENOPTERA: APHELINIDAE)

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ABSTRACT

A key to females and notes on 5 species of the Mexican fauna of the genus Marietta are given. Marietta montana n. sp. from the Biosphere Reserve “El Cielo”, State of Tamaulipas, México, is described and illustrated.

Key Words: Marietta n. sp., Tamaulipas, México

RESUMEN

Se presenta una clave y notas acerca de las 5 especies conocidas de la fauna mexicana del género Marietta. Se describe e ilustra Marietta montana n. sp. de la Reserva de la Biosfera “El Cielo” del Estado de Tamaulipas, México.

The world fauna of the genus Marietta includes 19 species (Hayat 1998), seven of which are known in the Nearctic region (Woolley 1997), including three in México. Species of the genus Marietta are almost always hyperparasitoids of Homoptera, including Diaspididae, Coccidae and other families. Beardsley & Tsuda (1990) noted Marietta pulchella Howard as a primary parasitoid of Conchaspis angracei Cockerell (Fam. Conchaspidae), which is common species of scale insect in México (Miller 1996).

The genus Marietta often referred to in earlier literature as Perissopterus, a junior synonym, was first recorded from México by Howard (1895), who described M. mexicana from Guadalajara. In addition to the three species previously known from México, we found M. graminicola Timberlake 1925, collected in the State of Tamaulipas, and a new species, M. montana Myartseva & Ruíz-Cancino, reared from a diaspine scale in Tamaulipas.

MATERIALS AND METHODS

Specimens of the parasitoid genus Marietta were collected by sweeping in several landscapes of México and reared from different hosts of scale insects (Homoptera: Coccoidea). One Marietta species, reared in 1998 in La Perra, Reserve “El Cielo”, Tamaulipas, México, from an unidentified Melanaspis species (Homoptera: Diaspididae) on Pinus trees, including P. patula, is described here as new to science.

Parasitoids were reared under laboratory conditions from scale insects collected on young twigs of Pinus trees in mountain landscapes. Some adult parasitoid individuals were mounted on card points, others were stored in 75% alcohol and later some females and males were mounted on slides in Canada balsam. The morphology of these specimens was studied. The senior author illustrated the morphological structures from slide preparations using a stereoscope and drawing apparatus (by increasing × 280, × 400).

Some Marietta specimens were received for study from Dr. V. A. Trjapitzin. The species identification was carried out using the key of world fauna of the genus Marietta (Hayat 1986). The original description of Marietta mexicana (Howard 1895) and other publications containing information about Mexican species of the genus Marietta were analyzed.

An illustrated key to females and notes on five Mexican species of Marietta are provided, as well as information on the genus Marietta.

Genus: Marietta Motschulsky, 1863;
Type species: Marietta leopardina Motschulsky, 1863;
Synonymy: Perissopterus Howard, 1895;
Pseudaphelinus Brèthes, 1918.

KEY TO FEMALES OF THE KNOWN MEXICAN SPECIES OF MARIETTA

1. Antennal scape subtrapezoidal, less than 2× as long as wide, with irregular fuscous patch in the middle (Fig. 1). Fore wing with apical margin widely hyaline ......................... M. graminicola Timberlake
—Antennal scape narrower, at least 2.5× as long as wide, with one or more narrow blackish longitudinal, transverse bands. Fore wing with apical margin not hyaline. ........................................... 2

2. Antennal scape with one blackish longitudinal band along the middle (Fig. 2). Fore wing with apical margin infuscated ................................................................. M. pulchella (Howard)
—Antennal scape with two blackish longitudinal or oblique transverse bands or with one transverse band (Figs. 3-5). Fore wing with fuscous band not touching apical margin .................................................. 3

3. Antennal scape more than 3× as long as wide, with two blackish longitudinal bands (Fig. 3). Clava with fuscous base and apex. ................................................................. M. mexicana (Howard)
—Antennal scape 3× or less as long as wide, with two blackish oblique transverse bands in the middle, or with one transverse band (Figs. 4-5) ......................................................... 4

4. Antennal scape with two complete oblique transverse bands (Fig. 4). Clava uniformly fuscous
—Antennal scape with one oblique transverse band, not touching its dorsal margin (Fig. 5). Clava with fuscous base and apex ................................................................. M. montana n. sp.

Marietta montana Myartseva & Ruíz-Cancino, n. sp.
(Figs. 6-13)

Description

Female (Figs. 6-11).

Length: 0.80-0.93 mm, mean of 2 specimens in alcohol 0.93 mm and of 3 specimens on pins 0.80 mm.

Coloration: Head yellow, with slight orange tinge; occiput with brown band around foramen, darker on its sides; lower margin of face narrowly brownish; apices of mandibles dark brown to black; maxillary and labial palpi whitish; setae on frontovertex and face fuscous. Scape pale yellowish-white, with a short incomplete brown-blackish oblique transverse band and small blackish spot below on the ventral margin; pedicel pale yellowish-white except brown-blackish basal part, especially dorsally, two funicle segments brownish; first segment of clava in basal ⅕, second segment in basal half and on apex infuscated. Pronotum whitish, with two elongate fuscous spots on each side; mesoscutum and scutellum yellowish or orange-yellowish; mesopleuron, metanotum and propodeum whitish-yellow. Fore wing whitish, ornamented with irregular fuscous pattern as in Fig. 10. Legs pale yellowish-white, with blackish bands: fore tibia with two bands, middle and hind tibia each with three bands; basitarsus, tibial spur, two last tarsal joints of middle leg fuscous, basitarsus and two last tarsal joints of fore and hind legs fuscous. Gaster largely dusky dorsally, sides of tergites whitish-yellow, with black margins and two longitudinal spots on each. Expanded part of ovipositor brownish-black.

Structure: Head of the same width as mesosoma (in dried specimens wider than mesosoma), 1.5× wider than long, and approximately 1.5× wider than high. Frontovertex length slightly more than width (Fig. 6). Ocelli in slightly obtuse triangle, distance from hind ocellus to eye and occipital margin approximately 1.5× the diameter of ocellus. Antennal (Fig. 7) radicle (R), scape (S), pedicel (P), two flagellar segments (F₁, F₂) and two

Figs. 1-5. Antennal scape of Marietta species: 1-M. graminicola Timberlake, 2-M. pulchella (Howard), 3-M. mexicana (Howard), 4-M. picta (André) (by Jasnosh, 1966), 5-M. montana, sp. Nov.
clava segments ($F_3, F_4$) with the following ratios of length to width: R-6.5:6.5, S-45:15, P-25:10, $F_1$-4:6, $F_2$-4:8, $F_3$-25:15, $F_4$-42:16. Flagellar segments $F_5$, $F_6$ with 2 and 2.2 linear sensillae in two rows, respectively. Clava 1.5× longer than scape. Mandibles with two teeth and short truncation (Fig. 8).
Maxillary and labial palpi 2- and 1-segmented, respectively. Mesosoma (Fig. 9) with broad mesoscutum, 1.5 x as wide as long, with 12-14 setae situated in 3 rows symmetrically, with reticulate sculpture; scutellum with 2 pairs of setae and similar sculpture to mesoscutum; the base of each setae on mesoscutum and scutellum surrounded by small brownish spot; each side lobe and axilla with one seta. Fore wing (Fig. 10) 2.6 x as long as wide; the longest marginal cilia more than 5.6 x shorter than the maximum width of wing. Costa cell short; submarginal vein with 3-4 setae; marginal vein a little longer than submarginal vein, with 6-7 setae along anterior margin; stigmal vein very short; basal part of wing glabrous. Disc with 6-7 setae along anterior margin; stigmal cell short; submarginal vein with 3-4 setae; marginal vein a little longer than first segment, the disc of fore wing being 2.6 x as wide, with one seta at apex.

Comments

Marietta montana n. sp. is similar in coloration and structure to Marietta picta (André) and especially to Marietta mexicana (Howard). M. montana can be distinguished from M. mexicana which has one brown transverse band on scape, the apical segment of scape clava more than 2 x longer than first segment, the disc of fore wing before linea clava with two hyaline cells in the middle; a longer marginal fringe; the body not so extensively fuscous; the placoid sensillae on scutellum situated anterior to level of the second pair of setae, which are closer to apex of scutellum; and the propodeum with a distinctly elevated median triangular area. Females and males of M. montana can be distinguished from M. picta which has one brown transverse band on scape, infuscated clava on base and apex, and a different fore wing color pattern.

Holotype: female (card mounted). Paratypes: 2 females and 1 male card mounted; 1 female, 2 males in Canada balsam; 2 females, 2 males in 75% alcohol. México, Tamaulipas, Gómez Fárias, Reserve “El Cielo”, La Perra (1900 m) 23-X-1998, S. N. Myartseva, all reared from diaspine scales Melanaspis sp. on Pinus spp.

Deposition

Female holotype and one male paratype (card mounted) are deposited in the National Museum of Natural History, Washington, D.C., USA; one female paratype (card mounted) is in the Department of Zoology, Institute of Biology, National Autonomous University of México (UNAM), México City; the remaining paratype specimens are deposited in the Insect Museum-UAM Agronomía y Ciencias, Autonomous University of Tamaulipas (UAT), Ciudad Victoria, Tam., México.

Etymology

Marietta montana is named for its inhabitation of the mountains of the Sierra Madre Oriental, at La Perra, Gómez Fárias (1900 m).

Notes on Other Mexican Species of Marietta Examined

1. M. graminicola Timberlake, 1925

   Using Hayat (1986) we determined that specimens collected in México belong to the species M. graminicola. Literature: Coronado-Padilla & Sosa-Esquillano, 1966:43 (Mexico); Contreras-Coronado, 1972:27-30 (Mexico, ex Antonina graminis Maskell); Rivera-Guillot, 1972:15 (Mexico); De Santis, 1979:317 (Mexico); Hayat, 1986:10 (?Mexico).

   Distribution: México, ?USA, Hawaii.

2. M. mexicana (Howard, 1895)
   Material: México, Morelos, Cuernavaca, UAEM: 20-25-VIII-1995, 1 female (V. Trjapitzin); 7-IV-1996, 1 female (E. Chouvakhina); 30-IV-1996, 1 female (G. Peña-Chora); all ex Parasaissezia nigra (Nieter) (Homoptera: Coccidae), new host record for M. mexicana, on Ficus benjamina; San Luis Potosí, 11-XI-1999, 2 females (S. Myartseva), ex white scale; 4 females and 2 males (S. Myartseva) ex Ceroplastes sp.

   Literature: Howard, 1895: 21-23 Guadalajara, ex Ceroplastes sp., Pseudococcus agavis MacGregor as jucca, Coccus hesperidum Linnaeus); García-Martell, 1973: 19 (Morelos, Cuernavaca);

Distribution: Nearctic, Neotropical, Japan.

3. M. picta (André, 1878).

   M. picta has only been reported once in Mexican publications, more than 20 years ago. This species is widely distributed in the Palearctic region.

Distribution: Nearctic, Palearctic, India.

4. M. pulchella (Howard), 1881
   Material: México, Tamaulipas, Ciudad Victoria: 12-X-1998, 1 female; 30-XII-1998, 1 male; 8-I-1999, 1 female (S. Myartseva), all ex Saissetia oleae (Olivier) (Homoptera: Coccidae), new host for M. pulchella, on Nerium oleander.

   Distribution: Nearctic, Neotropical.

ACKNOWLEDGMENTS

We thank Dr. V. A. Trjapitzin (Universidad Autónoma de Tamaulipas and Zoological Institute of the Russian Academy of Sciences), for collecting some specimens and for his suggestions for this study. To Dr. G. Evans for his advise, comments and review of this manuscript and Dr. A. B. Hamon for the identification of Coccoidea hosts (FSCA. Division of Plant Industry, Florida Department of Agriculture and Consumer Services). We also thank biologist Rafael Brito, who organized the trip to the Sierra Madre Oriental, Reserve “El Cielo”. We also thank the Research Center (UAMAC, UAT) for its continuous support for the study of Mexican Hymenoptera. Funding for the senior author was provided by CONACYT—México, through the Program “Cátedras Patrimoniales de Excelencia”.

REFERENCES CITED


EFFECT OF TEMPERATURE ON EGG DEVELOPMENT OF DIAPREPES ABBREVIATUS (COLEOPTERA: CURCULIONIDAE)

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The Diaprepes root weevil, *Diaprepes abbreviatus* (L.), has become a key pest of citrus and ornamental plants in Florida since its introduction to the state in 1964. Adult *D. abbreviatus* feed and oviposit on leaves of many plant species in addition to citrus including crops such as cassava, cotton, peppers, potatoes, sugar cane, and sweet potatoes. As neonate larvae emerge, they drop to the ground and crawl on the surface for a period of up to three hours (Jones & Schroeder 1983) before burrowing into the soil to feed on roots. Eggs are subject to predation by ants and earwigs (Tryon 1986) and parasitization by wasps such as the eulophid *Quadrastichus haitiensis* Gahan. Recent efforts to establish exotic egg parasitoids in Florida have met with some initial success (Hall et al. 2000), but a more complete understanding of host biology may contribute to effective rearing and release.

Lapointe and Shapiro (1999) and Lapointe (2000) described the effects of humidity and temperature on larval and pupal development of *D. abbreviatus*. To complete the description of the thermal requirements for development of *D. abbreviatus*, I report here the response of eggs of *D. abbreviatus* to a range of temperatures and describe the thermal upper and lower limits of egg development.

Male and female adult *D. abbreviatus* were obtained from a laboratory colony at the U.S. Horticultural Research Laboratory, Orlando, FL, now located at Ft. Pierce, FL. Approximately 20 male/female pairs were placed in a screened cage (30 by 30 by 30 cm) and provided with bouquets of citrus foliage as food and strips of wax paper for oviposition (Wolcott 1933). Eggs on wax paper strips were collected from cages twice daily and placed in plastic vials with plastic caps in temperature-controlled growth chambers with a photoperiod of 12:12 L:D and constant temperatures of 12, 15, 18, 19, 21, 22, 26, 30, and 32°C. Vials were misted periodically with sterile water and re-capped to avoid desiccation of the eggs. The wax paper strips were gently separated 2 to 3 d after oviposition to facilitate emergence of the neonate larvae. Vials were checked daily for neonate emergence and time to hatch was recorded. After egg hatch commenced, the number of eggs that failed to hatch after 7 consecutive days of no neonate emergence in the vial was recorded. The mean and median number of days to egg eclosion and 95% confidence intervals were calculated (Snedecor & Cochran 1967). The developmental rate (inverse of median no. days to eclosion) was plotted against temperature and a linear regression was calculated (Legg et al. 2000).

Wolcott (1936) reported that eggs of *D. abbreviatus* hatched 7 d after oviposition and suggested that this was unaffected by temperature. In this study, no eggs hatched when held at constant temperatures of 12 or 32°C. The percentage of unhatched eggs ranged from 6% at 18°C to 43% at 30°C (Table 1). For the constant temperatures regimes tested here, there was a positive linear relationship (y = 0.009x - 0.108, r² = 0.99) between developmental rate based on median values and temperature between 15 and 30°C (Fig. 1). Extrapolation based on the regression equation yielded a median functional lower developmental threshold of 12.0°C. The upper thermal limit for

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>n</th>
<th>Survival (%)</th>
<th>Days to hatch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Median</td>
</tr>
<tr>
<td>12</td>
<td>1150</td>
<td>0.0</td>
<td>27.0 ± 0.03</td>
</tr>
<tr>
<td>15</td>
<td>736</td>
<td>no data</td>
<td>28.1 ± 0.03</td>
</tr>
<tr>
<td>15</td>
<td>818</td>
<td>78.0</td>
<td>18.5 ± 0.01</td>
</tr>
<tr>
<td>18</td>
<td>2367</td>
<td>93.5</td>
<td>14.4 ± 0.05</td>
</tr>
<tr>
<td>19</td>
<td>938</td>
<td>84.6</td>
<td>11.4 ± 0.07</td>
</tr>
<tr>
<td>21</td>
<td>671</td>
<td>87.3</td>
<td>11.4 ± 0.15</td>
</tr>
<tr>
<td>22</td>
<td>622</td>
<td>76.5</td>
<td>7.1 ± 0.06</td>
</tr>
<tr>
<td>26</td>
<td>1045</td>
<td>80.8</td>
<td>5.8 ± 0.12</td>
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<tr>
<td>30</td>
<td>566</td>
<td>57.4</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>750</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Survival of eggs and number of days (median and mean ±95%CI) required to complete development of eggs of the Diaprepes root weevil at 9 constant temperatures.
constant temperatures occurred between 30 and 32°C because no eggs survived at 32°C. This is somewhat surprising since mean daily maximum air temperatures in Florida citrus groves exceed 32°C from May through September (Lapointe 2000). On Puerto Rico, Wolcott and Martorell (1943) observed seasonality in occurrence of egg clusters in sugarcane, with greatest abundance occurring in June and a secondary peak in September and October.

It has been observed that female *D. abbreviatus* prefer to oviposit on mature, fully expanded citrus leaves and avoid flush or tender foliage. Various explanations can be put forth to explain this preference. First, the feeding preference of *D. abbreviatus* for tender foliage may have contributed to a separate preference for oviposition substrate (older leaves) to avoid egg consumption by feeding adults. Second, the shear forces exerted by expanding leaves may dislodge egg masses cemented between leaf surfaces, thereby exposing the egg mass. Finally, temperature experienced by eggs of *D. abbreviatus* sandwiched between actively transpiring citrus leaves may vary from ambient air temperatures, particularly mature leaves within a shaded canopy, thereby protecting eggs from lethal temperature extremes.

Lower thermal limits for neonate larvae and pupae were estimated to be 15°C (Lapointe 2000). In this study, egg survival at 15°C was 78% and development time was 4.8 times greater at 15°C compared with that at 30°C. There appeared to be an increase in mortality at 30°C but the mean time to hatch continued to decrease compared with cooler temperatures (Table 1). Of the temperatures tested, 26°C appears optimal based on survival and development rate. This information should be useful for those interested in rearing the Diaprepes root weevil or its egg parasitoids.

**SUMMARY**

No eggs of the Diaprepes root weevil survived constant temperatures of 12 or 32°C. There was a positive linear relationship (y = 0.009x - 0.108, r² = 0.99) between developmental rate and temperature between 15 and 30°C. Extrapolation based on the regression equation yielded a median functional lower developmental threshold of 12.0°C and the upper thermal limit for constant temperatures occurred between 30 and 32°C.

**REFERENCES CITED**


FIRST RECORD OF \textit{CHRYSOMYA MEGACEPHALA} (DIPTERA: CALLIPHORIDAE) IN GEORGIA, U.S.A.

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Four species of Old World \textit{Chrysomya} Robineau-Desvoidy (Diptera: Calliphoridae) have been introduced to the New World during the past 20 years (Wells 1991). \textit{Chrysomya megacephala} (F.) was first collected from Brazil in 1975 and was thought to have originated from southern Africa (Baumgartner & Greenberg 1984).

\textit{Chrysomya megacephala} has become established in North America since being introduced into Brazil (Baumgartner & Greenberg 1984). The first record of \textit{Chrysomya megacephala} in the United States was from a single specimen collected in Texas (Wells 1991). This species has since been collected in California (Greenberg 1988),

\begin{table}
\centering
\begin{tabular}{l|l|l|l|l}
Species & \# Collected & Date & Location & Bait \\
\hline
\textit{Calliphora vicina} Robineau-Desvoidy & 11 & 28 May & Townes Co, GA & Chicken liver \\
& 01 & 28 May & Oconee Co, SC & Chicken liver \\
& 01 & 01 June & Dekalb Co, GA & Chicken liver \\
\textit{Chrysomya megacephala} (Fabricius) & 07 & 28 June & Tift Co, GA & Poultry facility \\
\textit{Cochliomyia macellaria} (Fabricius) & 01 & 01 June & Anderson Co, SC & Chicken liver \\
& 03 & 14 June & Pickens Co, SC & Dung \\
& 05 & 28 June & Tift Co, GA & Poultry facility \\
& 13 & 23 July & Pickens Co, SC & Human dung \\
& 03 & 25 July & Pickens Co, SC & Human dung \\
& 01 & 26 July & Pickens Co, SC & Human dung \\
\textit{Phaenicia cuprina} (Weidemann) & 43 & 28 May & Jones Co, GA & Chicken liver \\
\textit{Phaenicia sericata} (Meigen) & 01 & 30 March & Oconee Co, SC & Chicken liver \\
& 23 & 28 May & Jones Co, SC & Chicken liver \\
& 02 & 28 May & Pickens Co, SC & Chicken liver \\
& 04 & 28 May & Rabun Co, GA & Chicken liver \\
& 07 & 01 June & Fulton Co, GA & Chicken liver \\
& 07 & 01 June & Townes Co, GA & Chicken liver \\
& 07 & 01 June & Dekalb Co, GA & Chicken liver \\
& 05 & 28 June & Tift Co, GA & Poultry facility \\
\textit{Phormia regina} (Meigen) & 03 & 28 May & Jones Co, GA & Chicken liver \\
& 12 & 28 May & Townes Co, GA & Chicken liver \\
& 01 & 28 May & Pickens Co, SC & Chicken liver \\
& 01 & 28 May & Oconee Co, SC & Chicken liver \\
& 07 & 28 May & Rabun Co, GA & Chicken liver \\
& 05 & 01 June & Anderson Co, SC & Chicken liver \\
& 01 & 01 June & Dekalb Co, GA & Chicken liver \\
& 01 & 15 June & Pickens Co, SC & Sea urchin \\
\end{tabular}
\caption{A summary of Calliphorid species collected at bait stations in Georgia and South Carolina from 30 March through 26 July 1999.}
\end{table}
Florida (Baumgartner 1993), New Mexico (DeJong 1995), and Alabama (Wells 2000). We present a new distribution record for Chrysomya megacephala and associated calliphorid species in Georgia and South Carolina.

We collected adult calliphorids by aerial netting over various baits in Georgia and South Carolina from 30 March through 26 July 1999. Baits were placed in open glass or metal containers at ground level.

The University of Georgia Museum of Natural History and Clemson University Arthropod Collection were examined for unreported records of Chrysomya spp. from Georgia and South Carolina. Voucher specimens of each species were deposited in the University of Georgia Museum of Natural History and the Clemson University Arthropod Collection. Adult calliphorid occurrences at bait stations are summarized in Table 1.

**SUMMARY**

Chrysomya megacephala was only collected in Georgia, while five other calliphorid species were collected in both Georgia and South Carolina. The University of Georgia Museum of Natural History and the Clemson University Arthropod Collection did not contain any Chrysomya specimens collected in either state.

We thank Jeremy Greene and Gary Herzog for their helpful comments on an earlier draft of the manuscript. We would also like to thank Peter Adler for providing transportation and the use of his facilities while conducting this research.

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Heath et al. (1996, 1997) described a cylindrical sticky trap for tephritid fruit flies constructed with fruit fly adhesive paper (FFAP) (Atlantic Paste and Glue Co., Inc., Brooklyn, NY) which has an extremely tacky but non-messy adhesive. Capture rates with this trap were twice those of McPhail traps baited with the same lure. Unfortunately, the traps were difficult for users because they stick fast to almost everything they contact, including small birds in some field tests. In this work we describe a sticky trap made with the same paper but which avoids many of these problems.

Experimental traps were constructed from fluorescent light green (E.I.C.MWG 17089) or yellow (fluorescent chartreuse, E.I.C.MWY 16823) FFAP. Green and yellow were chosen because they were the most attractive colors in previous experiments with many species of Tephritidae including the Mexican fruit fly (Katsoyannos 1989; Robacker et al. 1990). Except for the controls, all traps had black plastic mesh (Co-Polymer Gutter Guard, Amerimax Home Products, Lancaster, PA) stapled over the sticky surfaces.

The FFAP was cut into 23 × 14 cm rectangles to equal the size of the Pherocon AM trap (Trece, Inc., Salinas, CA) that was used as the standard trap for this work. Plastic mesh was also cut into 23 × 14 cm rectangles. Mesh size (distance between plastic strands) initially was 0.7 × 0.7 cm. Mesh size was cut to 1.5 × 1.5 or 2.2 × 2.2 cm to test the effect of mesh size on trap efficacy. Thickness of the plastic strands was approximately 1.1-1.4 mm. Two FFAP rectangles placed back to back with sticky surfaces outward, each with a mesh rectangle on its sticky surface, were stapled together as a unit (Fig. 1). Trap lures were AMPu vials (2 ml) containing an agar mixture of ammonium carbonate, methylamine HCl, and putrescine, described previously (Robacker 1995).

Mexican fruit fly (Anastrepha ludens Loew) was used to evaluate the effectiveness of the trap. Flies were from a laboratory culture that originated in Nuevo Leon, Mexico, in 1987. Flies were irradiated with 70-92 Grays (Cobalt 60) before adult eclosion. Mixed-sex groups of 200 flies were kept in 473 ml cardboard cartons with sugar and water until released in test plots 3 to 8 days after eclosion.

Trap tests were conducted in one row of Ruby Red Grapefruit (Citrus paradisi MacFadyen) and one row of Dancy Tangerine (C. reticulata Blanco) in a citrus orchard in Weslaco, TX. Two blocks of 8 consecutive trees were used in each row. AMPu vials were attached to the tops of traps. Traps were hung one to a tree, north of center, at 1-2 m height. Traps were placed in the orchard during the morning and removed for fly counts on the following day. Approximately 2000 flies were distributed equally among the test trees on the day before a test.

The first experiment was a test of the 8 possible trap types made from the 2 colors with the 3 mesh sizes plus no mesh (Table 1). The 8 trap types were randomized in each block. Four repli-
cations of the experiment over time were conducted for a total of 16 tests (4 blocks × 4 replications) of each trap type. Replications over time (test days) were treated like replications over space (blocks of trees) for statistical analyses. Data were subjected to analysis of variance using SuperANOVA (Abacus Concepts 1989).

One experimental trap type was compared with the Pherocon AM (no bait) trap in each of 4 additional experiments (Table 2). Experimental traps and Pherocon AM traps were alternated within blocks. Replications of the 4 experiments were conducted over time for a total of at least 24 tests of each trap type. Data were analyzed by t-tests.

In the first experiment, traps without plastic mesh were much more effective than any traps with mesh (Table 1). This was especially true for yellow traps in which the traps without mesh captured 6x more flies than the most effective trap with mesh. Green traps without mesh captured about 3x more flies than the most effective traps with mesh. Thus, mesh greatly reduced the effectiveness of the FFAP. Observations in flight cages showed equal attraction to traps with or without mesh. Thus, the black mesh was not repellent. However, flies often escaped from the mesh traps by using the plastic mesh to pull themselves from the sticky surface.

Results of experiments to evaluate FFAP traps with different color/mesh combinations against Pherocon AM traps are shown in Table 2. All 4 of the experimental traps were competitive with the Pherocon AM traps.

Unlike Pherocon traps, FFAP traps with mesh are easy-to-handle. Unlike FFAP traps without mesh, they do not trap birds because the mesh prevents feathers from laying across the panel surface. Traps with mesh are also easy to stack into a box because they do not adhere to each other. Also, FFAP is stickier than Tangletrap but the adhesive does not run and leaves very little residue on the skin, much like contacting cellophane tape. Disadvantages of these traps are much reduced efficacy compared with FFAP traps without mesh, and severe damage to FFAP by rain. Further research is needed to optimize trap and mesh colors and sizes for different fruit flies, and to reduce rain damage.

We thank Maura Rodriguez and Jose Garcia for technical assistance. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by the USDA.

Table 1. Numbers of Mexican fruit flies captured on sticky traps with various mesh sizes compared with traps without mesh.1,2

<table>
<thead>
<tr>
<th>Test trap</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow, no mesh</td>
<td>12.0 c</td>
<td>17.9 d</td>
<td>29.9 d</td>
</tr>
<tr>
<td>Yellow, 0.7 × 0.7 cm mesh</td>
<td>0.2 a</td>
<td>0.4 a</td>
<td>0.6 a</td>
</tr>
<tr>
<td>Yellow, 1.5 × 1.5 cm mesh</td>
<td>1.2 ab</td>
<td>1.1 a</td>
<td>2.3 ab</td>
</tr>
<tr>
<td>Yellow, 2.2 × 2.2 cm mesh</td>
<td>2.2 ab</td>
<td>2.9 ab</td>
<td>5.2 ab</td>
</tr>
<tr>
<td>Green, no mesh</td>
<td>9.8 c</td>
<td>17.4 d</td>
<td>27.1 d</td>
</tr>
<tr>
<td>Green, 0.7 × 0.7 cm mesh</td>
<td>2.2 ab</td>
<td>5.1 bc</td>
<td>7.3 bc</td>
</tr>
<tr>
<td>Green, 1.5 × 1.5 cm mesh</td>
<td>2.8 b</td>
<td>5.8 bc</td>
<td>8.6 c</td>
</tr>
<tr>
<td>Green, 2.2 × 2.2 cm mesh</td>
<td>3.5 b</td>
<td>6.6 c</td>
<td>10.1 c</td>
</tr>
</tbody>
</table>

1 All traps were baited with an AMPu vial.
2 Means in the same column followed by the same letter are not significantly different by Fishers protected LSD test (P < 0.05).

Table 2. Numbers of Mexican fruit flies captured on sticky traps with mesh compared with Pherocon AM traps in citrus orchard experiments.1,2

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Test trap</th>
<th>Test trap</th>
<th>Pherocon trap</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Total</td>
</tr>
<tr>
<td>1</td>
<td>Yellow, 1.5 cm</td>
<td>1.7</td>
<td>3.0</td>
</tr>
<tr>
<td>2</td>
<td>Yellow, 2.2 cm</td>
<td>4.3*</td>
<td>4.6</td>
</tr>
<tr>
<td>3</td>
<td>Green, 1.5 cm</td>
<td>2.7</td>
<td>4.4</td>
</tr>
<tr>
<td>4</td>
<td>Green, 2.2 cm</td>
<td>1.6</td>
<td>2.8</td>
</tr>
</tbody>
</table>

1 All traps were baited with an AMPu vial.
2 Within each experiment, means with an * are significantly different from Pherocon trap means at the 5% level by t tests.
SUMMARY

A sticky trap for fruit flies made from fruit fly adhesive paper (FFAP) covered with a plastic mesh of either 1.5 × 1.5 or 2.2 × 2.2 cm mesh size was as effective as Pherocon AM traps in capturing Mexican fruit flies. FFAP traps without mesh captured 3× more flies than the best traps with mesh. However, mesh eliminates many problems associated FFAP traps. The mesh-covered traps are simple, compact, easy to pack, and do not capture birds or leave residue on users’ hands.

REFERENCES CITED


EFFECT OF VARIABLE PHOTOPERIOD ON DEVELOPMENT AND SURVIVAL OF CIRROSPILUS SP. NR. LYNCUS (HYMENOPTERA: EULOPHIDAE), AN ECTOPARASITOID OF PHYLLOCNISTIS CITRELLA (LEPIDOPTERA: GRACILLARIIDAE)

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The eulophids Cirrospilus sp. near lyncus and Pnigalio pectinicornis L. are the most abundant parasitoids of the citrus leafminer, Phyllocnistis citrella Stainton, in Spain (Urbaneja et al. 2000). These were opportunistically recruited onto this pest after its introduction in 1993. C. sp. near lyncus is a late larval solitary idiobiont ectoparasitoid which can also behave as a hyperparasitoid. Its impact on P. citrella relies on both parasitism and host feeding (Urbaneja et al. 1998a). C. sp. near lyncus populations in citrus orchards increase from mid-July until the end of October, but remain almost undetectable during the rest of the year (Urbaneja et al. 1999). A previous study demonstrated that C. sp. near lyncus was very well adapted to temperatures prevailing on the western part of the Mediterranean Basin. That is the range between 7.1°C, mean of minimum temperatures of the coldest month (January), and 29.0°C, mean of maximum temperatures of the hottest one (August) (Urbaneja et al. 1999). To further study the influence of environmental conditions on the biology of this wasp, the effects of photoperiod on development and survival were investigated.

Environmental chambers were used to check the effects of three different photoperiods: 16:8, 12:12 and 8:16 (L:D). Temperature fluctuated from 10°C to 30°C in eight 3h-steps of 5°C (mean temperature 20°C). Highest temperature always coincided with the mid-point of the photophase. This sequence mimics the regime of field temperatures both in spring and autumn. Therefore results obtained under both 16:8 and 8:16 (L:D) photoperiods are presumed to reflect field conditions at those seasons. Insects were reared at the Institut Valencià d’Investigacions Agràries as described by Urbaneja et al. (1998b). Eggs of C. sp. near lyncus were obtained by offering detached citrus leaves containing P. citrella third instar larvae (LIII) to isolated mated females (12 LIII per female). Exposure took place in Petri dishes (140 mm diameter) where leaves were placed on a layer of agar (2% weight) under a temperature of 25°C during 4 hours. After exposure, leaves were checked under a stereoscopic binocular microscope and those containing parasitized hosts (recognized by the presence of C. sp. near lyncus eggs on them) were randomly transferred to the corresponding experimental photoperiod on a layer of agar (2% weight) under a temperature of 25°C during 4 hours.

### Table 1. Mean Development Times (Days) of Male and Female C. Sp. Near Lyncus Under Three Different Photoperiods (Mean ± SE).

<table>
<thead>
<tr>
<th></th>
<th>16:8 (L:D)</th>
<th>12:12 (L:D)</th>
<th>8:16 (L:D)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td>2.16 ± 0.18</td>
<td>2.28 ± 0.22</td>
<td>2.26 ± 0.15</td>
</tr>
<tr>
<td>Larva</td>
<td>5.39 ± 0.32</td>
<td>5.72 ± 0.28</td>
<td>5.74 ± 0.21</td>
</tr>
<tr>
<td>Pupa</td>
<td>8.84 ± 0.31</td>
<td>8.47 ± 0.41</td>
<td>8.50 ± 0.24</td>
</tr>
<tr>
<td>Total</td>
<td>16.39 ± 0.33</td>
<td>16.47 ± 0.24</td>
<td>16.50 ± 0.19</td>
</tr>
<tr>
<td>(n = 14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td>2.25 ± 0.22</td>
<td>2.11 ± 0.12</td>
<td>2.67 ± 0.25</td>
</tr>
<tr>
<td>Larva</td>
<td>5.71 ± 0.38</td>
<td>6.07 ± 0.22</td>
<td>5.88 ± 0.30</td>
</tr>
<tr>
<td>Pupa</td>
<td>9.14 ± 0.28</td>
<td>9.09 ± 0.25</td>
<td>9.29 ± 0.35</td>
</tr>
<tr>
<td>Total</td>
<td>17.11 ± 0.39</td>
<td>17.27 ± 0.36</td>
<td>17.83 ± 0.28</td>
</tr>
<tr>
<td>(n = 19)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 2. STAGE SPECIFIC SURVIVAL (%) OF C. SP. NEAR LYNCUS UNDER THREE DIFFERENT PHOTOPERIODS. INITIAL NUMBER OF REPLICATES WAS 60, BUT CALCULATIONS ARE BASED ON THE NUMBER OF NON-DECAYING LEAVES (=REPLICATES) AT THE END OF EACH PERIOD.

<table>
<thead>
<tr>
<th>Photoperiod</th>
<th>Egg-larva</th>
<th>Larva-pupa</th>
<th>Pupa-adult</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:8 (L:D)</td>
<td>93.3</td>
<td>85.7</td>
<td>91.7</td>
<td>73.3</td>
</tr>
<tr>
<td>12:12 (L:D)</td>
<td>93.0</td>
<td>97.5</td>
<td>97.4</td>
<td>88.4</td>
</tr>
<tr>
<td>8:16 (L:D)</td>
<td>92.5</td>
<td>89.2</td>
<td>87.9</td>
<td>72.5</td>
</tr>
</tbody>
</table>

The absence of this wasp in citrus orchards from autumn until early summer may be related to the extraordinary scarcity of P. citrella during winter months. We hypothesize that C. sp. near lyncus is forced to abandon citrus orchards and does not return until P. citrella populations have already peaked up. For this reason, conservation tactics aimed at favoring the permanence of C. sp. near lyncus could increase the impact of parasitism early in the season. C. sp. near lyncus was first noticed in Spain as a parasitoid of P. citrella, and its original host range remains unknown. Therefore, as a first step to implement conservation strategies, its host range should be determined.

SUMMARY

No differences could be observed neither in development times nor on survival of C. sp. near lyncus exposed to three different photoperiods: 16:8; 12:12 and 8:16 (L:D). Therefore, conservation tactics aimed at favoring the winter permanence of this species in citrus orchards could increase the impact of this opportunistic parasitoid on its host P. citrella.

We thank J. E. Peña (TREC-University of Florida) and C. Rodríguez-Saona (USDA-ARS Phoenix, USA) for their comments on an early draft of this note, and R. Hinarejos, A. Muñoz and J. Torres for technical assistance. A.U. and E.L. were recipients of a predoctoral grant from IVIA.

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EFFECTS OF COOL TEMPERATURES ON OVIPosition AND DEVELOPMENT OF COTESIA MARGINIVENTRIS (HYMENOPTERA: BRACONIDAE)

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C. marginiventris, an abundant parasitoid of noctuids in summer, practically disappears from fall to spring in northern Florida, when the cabbage looper (Trichoplusia ni (Hübner)) can become a serious problem in crucifers. Temperature might be a limiting factor for the efficiency of C. marginiventris. In the present study we attempted to evaluate the oviposition performance of C. marginiventris and its development at different temperatures. We used cabbage looper and beet armyworm (Spodoptera exigua (Hübner)) as hosts to determine if the time of parasitoid development differs in hosts with different growth rates.

C. marginiventris used in this study were obtained from the Mississippi State University rearing facility. Both host species were from 1.5 year-old colonies maintained on pinto bean-based diet (Guy et al. 1985) at USDA-ARS, Gainesville, Florida. First- and second-instar larvae of either beet armyworm or cabbage looper were offered in clusters of 100 to C. marginiventris females. The larvae on individual leaves of pigweed (Amaranthus sp.) were placed in transparent 400 ml plastic containers, with a top of nylon screen. Females of C. marginiventris were chilled to 10°C and a single female was then placed into each container and supplied with a streak of honey and a moist cotton ball (placed on the nylon top). The containers were placed in temperature-controlled environmental chambers (Forma Scientific Diurnal Growth Chambers with a 16 h light : 8 h dark photoperiod) at 10, 15, 20, and 25°C. After 24 h, the wasps were removed, and feeding cups with artificial diet were added to each container. Some containers were held in their respective environmental chambers to determine time of parasitoid development at different temperatures. Other containers were transferred to the 25°C chamber to maximize the rearing of progeny. A total of 60 C. marginiventris females were tested. Containers were checked daily for parasitoid cocoons. Cocoons and larvae with a parasitoid-produced exit hole were removed and counted to assess parasitism. The statistical analyses (ANOVA and t-test) were performed using “JMP” at α = 0.05 (SAS Institute Inc., ©1989-95).

At 10°C, no parasitism occurred. Apparently, the wasps remained completely inactive at this temperature. When oviposition occurred at 15°C, 20°C, and 25°C, a mean (±SE) of 28.4 ± 5.3 (n = 5), 40.2 ± 7.4 (n = 9), and 39.6 ± 8.8 (n = 7) percent of host larvae were parasitised, respectively. The fecundity of C. marginiventris varied greatly and no significant (P > 0.05) differences in mean number of progeny/female were observed.

At 15°C, cabbage looper larvae develop faster than beet armyworm larvae. However, the difference in development time of C. marginiventris larvae was non-significant (P > 0.05) when reared in beet armyworm (47.3 ± 1.5 days SE) and in cabbage looper (45.4 ± 2.6), ranging from 37 to 62 days (n = 23). Eighteen C. marginiventris' cocoons were transferred from 15 to 25°C: 6 took 6 days to eclose; 6 emerged with a two-week delay; and the rest never eclosed. Five cocoons that were left at 15°C never eclosed as well.

At 20°C, mean development time was 17.6 ± 0.3 days in beet armyworm larvae (n = 69, wasp progeny were from six different females). No significant difference was found (P > 0.05) when first instar (17.4 ± 0.3 days (n = 19)) vs. second instar host larvae (17.7 ± 0.3 days (n = 50)) were parasitised. In cabbage looper, mean development time was more rapid (16.1 ± 0.3 days (n = 53, six different females)) than in beet armyworm, and this difference was significant (P < 0.0001). The development time at this temperature ranged from 14 to 21 days. Duration of the pupal stage was 6.6 ± 0.1 days (n = 28), ranging from five to eight days.

At 25°C, development took 8.4 ± 0.1 days in beet armyworm larvae (n = 152, progeny of six different females). Unlike 20°C, at 25°C the parasitoids developed more slowly in cabbage looper (9.7 ± 0.1 days (n = 48, progeny of five different females)) (P < 0.0001). Duration of the pupal stage at this temperature was 4 days.

Maximum life-time fecundity of C. marginiventris was previously found to be 111 ± 16 (Jalali et al. 1987). Here, we report data on maximum 24-h fecundity and how it changes with time. At 22°C, we supplied single C. marginiventris females with ca. 150 one-day-old beet armyworm larvae on young collard leaves in tightly sealed 15 cm Petri dishes. A streak of honey was placed on the inside of the lid. After 24 hours, the wasps were removed and offered a new group of larvae. The old larvae were fed collard leaves for two more days and then dissected for parasitoids. Mean fecundity was 85.8 ± 1.7 eggs on the first day, and 16.3 ± 6.8 eggs on the second day.
Twenty *C. marginiventris* were reared outdoors in November-December, using beet armyworm larvae as hosts. Larvae on cabbage leaves were placed in a 30 × 30 × 30 cm plexiglass cage with two netting sides for ventilation. Thus, the light cycle and temperature inside the cage were ambient. We monitored the cage daily for *C. marginiventris* cocoons and later, for adults. During the test period, temperatures fluctuated between 15-25°C during the day and −2-5°C at night. The average monthly temperature in the region in November and December is 14°C and 16°C, respectively, equal to our coldest rearing setting in the laboratory. Nevertheless, the parasitoids pupated in only 14.2 ± 0.3 days; the duration of the pupal stage was 11.4 ± 0.2 days. During November-January, we also placed ca. 200 beet armyworm larvae on cabbage plants in several cabbage fields in northeastern Florida to determine the presence of naturally-occurring *C. marginiventris*. A mixture of two-to-five day-old larvae were used, which fed on collard leaves prior to release. Larvae were collected after three-four days and dissected for parasitoids. They yielded eggs and larvae of *C. marginiventris* of all instars, which indicated that *C. marginiventris* was successfully developing through.

**SUMMARY**

Host species had no or small effect on the time of *C. marginiventris* development at different temperatures, while temperature at the time of oviposition had no effect on the numbers of resulting progeny. In the laboratory, most eggs (85/female) were laid by wasps during the first 24-hours. In winter, *C. marginiventris* reproduced more rapidly outdoors than at controlled constant temperatures with the same average, and attacked sentinel host larvae in cabbage fields.

**REFERENCES CITED**


The biology of the phorid fly *Pseudacteon* was reviewed by Porter (1998). The female fly oviposits into the thorax of its ant host. After hatching, the larva works its way into the host’s head capsule where it continues to grow and ultimately decapitates the host. The fully grown larva then uses the head capsule of the host as a pupal case. Because of their parasitic lifestyle, flies of this genus are of great interest as potential biocontrol agents against the imported fire ants, *Solenopsis invicta* Buren and *Solenopsis richteri* Forel, in North America.

Four species of *Solenopsis* fire ants are native to North America: *S. amblychila* Wheeler, *S. aurea* Wheeler, *S. geminata* (Fab.) and *S. xyloni* (MacCook). All four of these species overlap in their ranges in the southwestern United States, mainly in Arizona and Texas (Trager 1991). *Pseudacteon crawfordi* was described by Coquillett in 1907 and is known to be parasitic on *S. geminata* (Disney 1994) and *S. xyloni* (Feener 1987). Here we report that *P. crawfordi* attacks *S. aurea*. *Solenopsis aurea* is a species of fire ant that occurs in xeric conditions in the southwestern United States and is found mainly in Texas and Arizona (Trager 1991).

Collection: Near dusk, on August 3, 1999, a nest of *S. aurea* was excavated. The nest was located close to mile marker 13 north of Portal, Arizona on Speed Road. Foragers from the colony were initially found under a pile of semi-moist cow manure. The nest was located close to a pool of water created by a leaking irrigation line. Shortly after the cow manure was disturbed and the ants were uncovered, six female phorid flies were seen hovering around and attacking intermediate-sized workers. A series of the phorid flies was collected with the ants. *Solenopsis aurea* workers were also collected the next day from 0800-1200 h, but no phorid flies were seen. The phorid flies were identified as *Pseudacteon crawfordi* by Sanford Porter (Medical and Veterinary Entomology Research Laboratory, USDA-ARS, Gainesville, Florida) and the fire ants were identified as *S. aurea* by senior author (JPP). This represents a new host record for *Pseudacteon crawfordi*.

Voucher specimens of both the ants and flies are deposited in the University of Georgia, Collection of Arthropods, Athens, Georgia, USA.

**SUMMARY**

*Solenopsis aurea* in Arizona is reported as a new host species for *Pseudacteon crawfordi*.

**REFERENCES CITED**


EFFECT OF SPINOSAD ON ORIUS INSIDIOSUS (HEMIPTERA: ANTHOCORIDAE) WHEN USED FOR FRANKLINIELLA OCCIDENTALIS (THYSANOPTERA: THRIPIDAE) CONTROL ON GREENHOUSE POT CHRYSANTHEMUMS

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2Department of Entomology, College of Agricultural and Environmental Sciences, Griffin Campus University of Georgia, Griffin, GA 30223

Western flower thrips, Frankliniella occidentalis (Pergande), is a major pest of greenhouse vegetables and ornamentals. This is a result of both feeding damage and the thrips ability to transmit tospoviruses (Tommasini & Maini 1995; van de Wetering et al. 1996). Control of this thrips species has been difficult because it has developed resistance to the major classes of insecticides used for its control (Brødsgaard 1994; Zhao et al. 1995). Orius insidiosus (Say) is capable of reducing thrips populations on greenhouse vegetables (van den Meiracker & Ramakers 1991) and ornamentals (Fransen et al. 1993).

Spinosad (Dow AgroSciences, Indianapolis, IN) is a new insecticide derived from the fermentation of Saccharopolyspora spinosa. Spinosad effectively controls western flower thrips under laboratory conditions (Eger et al. 1998). Funderburk et al. (2000) demonstrated that spinosad was more effective than broad-spectrum insecticides in suppressing F. occidentalis populations in field peppers. This suppression was partially due to the spinosad applications not reducing O. insidiosus populations compared to the standard insecticide treatment. Our objective was to evaluate the impact of spinosad on O. insidiosus when used in combination to control F. occidentalis on greenhouse grown pot chrysanthemums.

Three rooted chrysanthemum ‘Charm’, Dendranthema × grandiflora (Ramat.) Kitamura, (Yoder Brothers Inc., Barberton, OH) cuttings were planted per 15 cm plastic pot containing Pro-Gro Professional Growing Medium® 300 (Pro-Gro Products Inc., McCormick, SC). Plant terminals were removed to promote branching two weeks after the plants were potted. A foliar application of 5,000 ppm daminozide (B-Nine®, Uniroyal, Middlebury, CT) was applied four weeks after potting. The western flower thrips population was a natural infestation which occurred prior to the plants being placed into the cages. Screen cages measuring 105 × 57 × 57 cm (L × W × H) were placed over groups of eight plants on cloth covered greenhouse benches. The cages were constructed using PVC pipe frames covered with mesh screen (25 threads per cm). Cages were lifted off of the plants to apply treatments and collect samples. Plants were watered daily with 200 ppm N [20-10-20 Peter’s Peat-lite Special® (Scotts, Marysville, OH)] using a spaghetti drip watering system that entered each cage through a hole in the fabric under the pots.

The cages were arranged in a randomized complete block design with four treatments and six replicates in the first trial and five replicates in the second trial. The four treatments investigated were O. insidiosus (four females and two males per cage), spinosad (0.5 ml / liter), O. insidiosus + spinosad, and a control. Spinosad was applied to runoff using a hand sprayer at 241 kPa. Sampling for thrips and O. insidiosus was conducted weekly by collecting ten flowers per cage and placing them into a jar containing 200 ml of 50% ethyl alcohol. The flowers were then rinsed, removed from the jars and discarded. The number of adult and immature thrips and O. insidiosus were determined by microscopic evaluation.

In the first trial, O. insidiosus adults were released at bud break and again 14 days later. Spinosad was applied approximately 24 h after each O. insidiosus release. In the second trial, an attempt was made to establish O. insidiosus before the plants set bud, but poor establishment occurred. A second O. insidiosus release was made one week after bud formation and spinosad was applied 14 and 28 days after the second O. insidiosus release. Sampling in both trials was conducted every seven days.

A logarithmic transformation [log10 (x + 1)] of the data was used to make the variance independent of the means (Sokal & Rohlf 1995). Data were subjected to Analysis of Variance (GLM procedure). The means were separated using the least significant difference test (LSD) at the P < 0.05 level (SAS Institute 1985). The effects of the treatments were determined by comparing thrips populations in each of the four treatments. The effects of spinosad on O. insidiosus was determined by comparing the O. insidiosus present in the O. insidiosus treatment and the O. insidiosus + spinosad treatment.

In the first trial O. insidiosus, spinosad, and the combination treatments resulted in lower thrips populations (adults + larvae) than the control (Table 1). Both treatments containing spi-
Table 1. Mean densities ± SD of Orius insidiosus (A) and Frankliniella occidentalis (B) per 10 chrysanthemum flowers in the first O. insidiosus and spinosad compatibility trial.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after first spinosad application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td>A (Orius insidiosus)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.2 ± 0.4 a</td>
</tr>
<tr>
<td>Orius</td>
<td>0.7 ± 1.0 a</td>
</tr>
<tr>
<td>Spinosad</td>
<td>0.0 a</td>
</tr>
<tr>
<td>Spinosad + Orius</td>
<td>0.2 ± 0.4 a</td>
</tr>
<tr>
<td>F(8,15) = 2.1</td>
<td>F(8,15) = 2.3</td>
</tr>
<tr>
<td>P = &gt;0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>B (Frankliniella occidentalis)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>124.5 ± 61.5 a</td>
</tr>
<tr>
<td>Orius</td>
<td>60.0 ± 24.4 b</td>
</tr>
<tr>
<td>Spinosad</td>
<td>20.3 ± 21.5 c</td>
</tr>
<tr>
<td>Spinosad + Orius</td>
<td>19.0 ± 17.5 c</td>
</tr>
<tr>
<td>F(8,15) = 8.6</td>
<td>F(8,15) = 7.1</td>
</tr>
<tr>
<td>P &lt; 0.001</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

1 Means in each column for each species followed by the same letters are not significantly different (P > 0.05; LSD).
2 A second treatment spinosad was made on day 14 and Orius release on day 13.

Table 2. Mean densities ± SD of Orius insidiosus (A) and Frankliniella occidentalis (B) per 10 chrysanthemum flowers in the second O. insidiosus and spinosad compatibility trial (Griffin, GA, Spring 1999).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after first spinosad application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>A (Orius insidiosus)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.0 ± 0.0 a</td>
</tr>
<tr>
<td>Orius</td>
<td>0.2 ± 0.4 a</td>
</tr>
<tr>
<td>Spinosad</td>
<td>0.3 ± 0.5 a</td>
</tr>
<tr>
<td>Spinosad + Orius</td>
<td>0.2 ± 0.4 a</td>
</tr>
<tr>
<td>F(8,15) = 1.0</td>
<td>F(8,15) = 1.3</td>
</tr>
<tr>
<td>P &gt; 0.5</td>
<td>P &gt; 0.5</td>
</tr>
<tr>
<td>B (Frankliniella occidentalis)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.3 ± 2.4 a</td>
</tr>
<tr>
<td>Orius</td>
<td>0.8 ± 1.6 c</td>
</tr>
<tr>
<td>Spinosad</td>
<td>4.8 ± 5.7 ab</td>
</tr>
<tr>
<td>Spinosad + Orius</td>
<td>1.5 ± 2.3 bc</td>
</tr>
<tr>
<td>F(8,15) = 5.7</td>
<td>F(8,15) = 6.4</td>
</tr>
<tr>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

1 Means in each column for each species followed by the same letters are not significantly different (P > 0.05; LSD).
2 A second spinosad application was made on day 17.
dates (Table 2). The *O. insidiosus* populations were not significantly different.

Funderburk et al. (2000) demonstrated *Orius* was capable of establishing and controlling thrips in field peppers. Our data obtained in the greenhouse differ from those of Funderburk et al. There may be at least two explanations for this difference. First, *O. insidiosus* was capable of moving between treated and untreated areas in the field pepper trials, while under the greenhouse cage conditions its movement was restricted. Second, although in both studies spinosad was applied at the same rate, there was a difference in the volume of insecticide applied between the two studies. Spinosad was applied at 174 liters per acre in the field studies while in the greenhouse the application rate was approximately 750 liters per acre. Additional studies need to be conducted to evaluate the timing of insecticide applications and predator releases.

We thank Stan Malloy and Sherrie Stevens for assistance with plant maintenance and insect sampling and Jerry Davis for help with data analysis. We also thank Yoder Brothers Inc., Barberton OH, for supplying chrysanthemums and Dow AgroSciences, Indianapolis, IN, for the spinosad.

**SUMMARY**

*Orius insidiosus* and spinosad were used in combination against *F. occidentalis* on greenhouse pot chrysanthemums to test for compatibility. *Orius insidiosus* failed to establish in the first trial when exposed to spinosad, but was more compatible in the second trial. This indicates that spinosad may have an effect on *Orius* populations when there was little free movement of the thrips and *Orius* between plants.

**REFERENCES CITED**


A MORPHOLOGICAL MEANS OF DISTINGUISHING FEMALES
OF THE CRYPTIC FIELD CRICKET SPECIES, GRYLLUS RUBENS
AND G. TEXENSIS (ORTHOPTERA: GRYLLIDAE)

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The field crickets Gryllus rubens Scudder and G. texensis Cade and Otte (previously G. integer from Texas, see Cade & Otte (2000)) are the most commonly collected crickets throughout the southeastern United States from North Carolina to Texas. The two species are sympatric from western Florida to eastern Texas (Walker 1998). These two species are the only known trilling field crickets in the southeastern US, and are currently separated by song differences alone. Male G. rubens produce trilled calling song with pulse rates averaging about 80 p/s at 25°C (Walker et al. 1992, Walker 1998, 2000 Martin et al. 2000). The pulse rates of the two species become more similar at lower temperatures, but provided that the temperature is above approximately 20°C, males can unambiguously be identified to species by song (Walker 1998; Gray & Cade 2000). No morphological means of distinguishing either males or females has previously been reported (see Nickle & Walker (1975) for other species of the southern United States). Here we report that most females of the two species can be separated on the basis of body-size relative ovipositor length; G. rubens have longer ovipositors relative to body size than do G. texensis.

We compared the ovipositor lengths and the pronotal widths of 122 females from several localities across the species’ geographic ranges. Females were either laboratory reared from field-caught nymphs, laboratory reared offspring of field-caught field-inseminated females, or were field caught females. Females from allopatric sites were identified to species on the basis of collection locality alone (G. rubens: Gainesville, FL (n =15); G. texensis: Austin, TX (n = 14), Dallas, TX (n = 6)) or based on the songs of their brothers (G. texensis: Uvalde, TX (n = 2), Austin, TX (n = 1)). Females from sympatric sites (G. rubens: Milton, FL (n = 8), Marianna, FL (n = 12), Pensacola, FL (n = 5), Mobile, AL (n = 1), Decatur, AL (n = 1); G. texensis: Milton, FL (n = 32), Starkville, MS (n = 9), Tuscaloosa, AL (n = 4), Pensacola, FL (n = 4), Mobile, AL (n = 2), Carrollton, GA (n = 6)) were identified to species based on the songs of their brothers.

The crickets were measured as two replicates; replicates 1 and 2 represent crickets in the collections of DAG and the Florida State Collection of Arthropods, Gainesville, respectively. The data are presented together in Figure 1 and both separately and pooled in Table 1. We found that female G. rubens have ovipositors ca. 2.5 to 3 mm longer relative to their body size than do female G. texensis. For the pooled data, we tested the effect of species with pronotal width as a covariate using an ANCOVA. The model r² was 0.88 (i.e., 88% of the variation in ovipositor length was accounted for). Both species (F(1,119) = 170.96, P < 0.0001) and pronotal width (F(1,119) = 440.93, P < 0.0001) were significant predictors. A comparison of the slopes of ovipositor length on pronotal width was made by testing for a species x pronotal width interaction in a separate ANCOVA. The slopes did not differ (G. rubens, slope = 2.285, G. texensis, slope = 2.374; F(1,118) = 0.10, P > 0.7507). The intercepts of the lines were 1.34 mm for G. rubens and -1.31 mm for G. texensis. Thus it should be possible to separate accurately most females of these two species.

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**Table 1. Mean ±SD ovipositor lengths (mm) of G. rubens and G. texensis.**

<table>
<thead>
<tr>
<th>Replicate</th>
<th>G. rubens</th>
<th>G. texensis</th>
<th>t(df)</th>
<th>P -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.42 ± 0.79 (n = 24)</td>
<td>11.46 ± 0.77 (n = 31)</td>
<td>t(53) = 13.91</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>14.7 ± 2.0 (n = 18)</td>
<td>11.2 ± 2.4 (n = 49)</td>
<td>t(65) = 5.55</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Combined data</td>
<td>14.53 ± 1.44 (n = 42)</td>
<td>11.30 ± 1.91 (n = 80)</td>
<td>t(120) = 9.60</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
SUMMARY

Females of the field crickets *Gryllus rubens* and *G. texensis* can generally be distinguished on the basis of their ovipositor length relative to body size. *G. rubens* females have longer ovipositors than do *G. texensis*.

REFERENCES CITED


BEMISIA AFER SENS. LAT. (HOMOPTERA: ALEYRODIDAE)  
OUTBREAK IN THE AMERICAS

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Bemisia tabaci (Gennadius) was reported on sweetpotato (Ipomoea batatas Lam.) from the central coast of Peru in the late 1980s, noting that it was not a significant pest (Redolfi 1989, cited in Nuñez 1995). However, in the 1997-1998 agricultural season, unusually large populations of Bemisia tabaci were reported to be significantly affecting sweetpotato yields in the coastal valleys of Peru (Valencia et al. 2000). In August of 2000, P. Anderson (CIAT) made a field visit to the Cañete Valley, approximately 100 km south of Lima, with Cristina Fonseca of the International Potato Center (CIP) and Ing. Jose M. Valencia of the Cañete Experimental Station, to explore the problem. The nymphs that were actively reproducing on sweetpotato were Bemisia. However, the adult whiteflies, which were abundant on sweetpotato and pepino (Solanum muricatum Ait.) were larger and whiter (more Trialeurodes-like) than typical for Bemisia tabaci. Thus, nymphs were collected from sweetpotato for taxonomic verification.

Whitefly nymphs were slide-mounted and tentatively identified as Bemisia afer, by P. Hernandez at the International Center for Tropical Agriculture (CIAT) in Cali, Colombia. The identification was verified as Bemisia afer sens. lat., by J. Martin at the Natural History Museum in London, UK (BMNH). Voucher specimens were deposited in the BMNH.

This is the first outbreak we have observed of Bemisia afer sens. lat. in an agricultural situation in the Americas. B. afer has been recorded from Egypt, Greece, Sicily, the Middle East, the Ethiopian region, Comoro Islands, India, Pakistan, New Guinea, Fiji, Tonga (Martin 1987), Sudan, Sierra Leone, Cote d'Ivoire, Nigeria, Niger, Chad, Cameroon, Congo, Zaire, Uganda, Rhodesia, Malawi, South Africa (Bink-Moennen 1983), and Australia (Martin 1999). B. afer has hitherto been considered as a common and widespread pest species, feeding on a wide variety of plants (Martin 1987).

In Belize in 1994 and 1996, plants of a papaveraceous host, Bocconia frutescens L., were found to be colonized by very large populations of a species of Bemisia with very large characteristic puparia. This belongs to B. afer sens. lat., but the puparial characteristics fall outside those normally observed in areas of the world where B. afer is widespread. While studying the whitefly collection of the US National Museum of Natural History (housed at USDA, Beltsville, MD), Martin noted a small number of Bemisia afer-group samples that are likely to be conspecific with the samples from B. frutescens in Belize. These samples were either field-collected in, or intercepted by US quarantine authorities from Honduras, Mexico and El Salvador. Quoted host plants include Pouteria sp ( Sapotaceae), Hibiscus sp (Malvaceae), Origanum sp (Labiatae), Ficus sp or ssp (Moraceae), Serjania sp ( Sapindaceae) and Psidium guajava (Myrtaceae). There are also two additional slides from Belize in BMNH, one from an unidentified woody vine and matching the Bocconia puparia, and the other (possibly a smooth-leaf form of the same species) from a wild cassava plant growing on a forest track remote from agriculture. From this material, it appears that this taxon is widespread and oligophagous in Central America.

Bink-Moennen (1983) proposed the synonymy of Bemisia hancocki Corbett (1936) with B. afer (Priesner & Hosny 1934). This synonymy was based on examination of one badly damaged syntype of B. afer deposited in the BMNH. Based on Martin’s subsequent examination of a complete syntype puparium of B. hancocki deposited in USNM, this synonymy may have been premature. However, with the considerable degree of puparial morphological plasticity now becoming evident within the B. afer group, formally resurrecting B. hancocki could cause further nomenclatural confusion at this point.

B. hancocki was first described from cotton (Gossypium hirsutum L.) in Uganda by Corbett (1936). Mound (1965) examined B. hancocki specimens from cotton, peanut (Arachis hypogaea L.), and Vigna (catjang) unguliculata (L.) Walp., and noted B. hancocki collections from cassava (Manihot esculenta Cranz) in Sierra Leone, Nigeria, Cameroon, and Sudan. He further described the variation in the puparial morphology of B. hancocki as being almost as great as that of B. tabaci. Personal observations made by Martin, Estrella Hernández-Suarez (ICIA, Canary Islands, Spain) and by Raymond Gill (CDFA, Sacramento, USA) indicate that the B. afer group actually displays considerably greater puparial morphological variation than does B. tabaci and its forms/biotypes.

Although specimens from the B. afer group have been previously discovered in non-agricultural situations in the Americas, this is the first
report of a *Bemisia afer sens. lat.* outbreak on an important crop host in the New World. The extent of *B. afer* dissemination and its host-associations in Peru need to be investigated. Furthermore, the taxonomy of *Bemisia afer* and *Bemisia hancocki* should be re-visited and, the possible role of *B. afer* in virus transmission needs to be clarified.

**SUMMARY**

The first outbreak of *Bemisia afer sens. lat.* in an agricultural situation in the Americas is reported. *B. afer* was discovered on sweetpotato (*Ipomoea batatas* Lam.) in the Cañete Valley in the central coast of Peru.

**REFERENCES CITED**


Both mature and immature mangoes, *Mangifera indica* L., grown in subtropical Florida are marketed. Food use of immature mangoes includes being cooked for sauce, powdered for a flavoring agent, pickled, and processed into chutney. Immature mangoes are distinguished from mature mangoes in that they do not ripen after harvest (Reid 1992). Immature mangoes have a whitish flesh, lack juiciness, and have a sour flavor, while mature mangoes have yellow flesh, are juicy, and have a sweet flavor.

Mango has been listed as a host of Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Norrbom & Kim 1988). California, where the largest immature mango market is, maintains a Caribbean fruit fly quarantine on both mature and immature imported mangoes. Mangoes can only be shipped from Florida to California if they have undergone an acceptable postharvest treatment that disinfests them of fruit flies.

To our knowledge, there have been no studies to determine to what extent immature mangoes may represent a lower risk for fruit fly infestation than mature mangoes. Peña & Moyhuddin (1997) reviewed mature mango fruit resistance to *Anastrepha obliqua* (Macquart) and infestability differences among cultivars were suggested to be caused by differences in toxic chemicals, nutrients, or resin ducts. Hennessey & Schnell (1995) determined that for mature fruit, some genotypes are more resistant to Caribbean fruit fly than others. They also suggested highly resistant germplasm accessions could be employed in breeding efforts and integrated pest management systems for control of Caribbean fruit flies. The present investigations were conducted to determine if some germplasm accessions in the immature state were more resistant to Caribbean fruit fly than others. They also suggested highly resistant germplasm accessions could be employed in breeding efforts and integrated pest management systems for control of Caribbean fruit flies.

Fruits used in the experiments were from the USDA National Clonal Germplasm Repository at the Subtropical Horticulture Research Station in Miami, FL, where over 150 *Mangifera* accessions are maintained. The accessions comprise a valuable source of genetic diversity utilized by breeders and researchers from all over the world. Fruits from 18 *M. indica* accessions (individual trees) were selected based on availability and inclusion in a previous study of mature mangoes (Table 1). Immature mangoes were available from March 21 to June 23, 1996. Tree cultivation practices included fertilization, pruning, and chemical weed control. No insecticides were applied during testing and for at least four months prior to testing. The methods for rearing flies, determining mango maturity, and bioassaying of fruits were previously described in detail (Hennessey & Schnell 1995). Flies were reared on an agar-based diet (Hennessey 1994) which was used as the control in the present study. Stage of maturity of the mangoes was determined for each accession and harvest date by holding 5-10 fruits in the laboratory and observing flesh color, juiciness, and flavor after 5-8 days. Each accession was bioassayed on three dates: early (March-April), middle (May), and late (June) immature period (Table 1). Slices, including peel, from five fruits per accession were bioassayed for each date. Ten mature Caribbean fruit fly eggs were inoculated on each fruit slice and corresponding agar control portions. The corrected mean percentage adult emergence was the criterion used to evaluate resistance. This value was obtained by dividing the percentage of eggs reaching adulthood from each slice by the mean percentage reaching adulthood from five agar controls of the same date.

The experiment was completely randomized. Corrected mean percentage adult emergence data from the three harvest dates combined (n = 15) were analyzed with PROC ANOVA and compared among cultivars with the LSD test (SAS Institute 1992).

The corrected mean percentage of emergence of adult flies from mangoes varied from 0.0% for ‘Saigon Seedling’ M13269 to 35.7% for ‘Keitt’ (Table 1). The most resistant group included 15 cultivars that supported less than 28.9% emergence. Hennessey & Schnell (1995) observed that the corrected mean percentage emergence varied from 26.6% for mature ‘Tobago Small Red’ to 119.0% (a higher emergence rate than the control) for mature ‘Sabre’. In that study, the group that contained the most resistant cultivar (P = 0.10) consisted of ‘Peach’, ‘Irwin’, ‘Tommy Atkins’, ‘Rumani’, ‘Turpentine’, ‘Keitt’, ‘13-1’, ‘Sandersha’, ‘Zilate’, and ‘Tobago Small Red’ (Table 1). Mature mangoes served as a better substrate for development than did immature mangoes of the same cultivar. Over all cultivars, immature mangoes supported from 2-59% of the emergence compared with mature fruits of the same cultivars did. This could be interpreted to mean that, within cultivars, most immature man-
Immature mango slices were artificially infested with Caribbean fruit fly eggs in the laboratory. The mean percentage of emergence of adults, relative to emergence from control media, varied from 0.0% for ‘Saigon Seedling’ (M13269) to 35.7% for ‘Keitt’. Immature mangoes supported only 2-59% of the emergence compared with mature fruits of the same cultivars. Resistant cultivars may possibly be coupled with preharvest insecticide treatments to reduce fruit fly infestations to below the level of quarantine concern.

### References


Regulatory agencies worldwide take careful note of published literature that describes the distribution of various pests, which they take into account when establishing their quarantine rules for importing agricultural commodities from other countries. Thus, timely and accurate reporting of pest distributions is of great commercial importance.

The West Indian fruit fly, *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae), a serious and highly polyphagous fruit pest, is documented as present in Florida in numerous publications, including some prominent and recent ones. The widely used CABI Distribution Maps of Pests (1988, citing Foote 1965) show *A. obliqua* present in Florida. Likewise, Foote et al. (1993) include the Florida Keys in their distribution map for *A. obliqua*. Weems (1970) wrote that *Anastrepha obliqua* (as *A. mombinpraeoptans*) “apparently . . . exists at a threshold level in Florida. Most recent Florida records are for several adult females in 1957 from Key West and one larva in mango from Ft. Lauderdale, June 25, 1963, which was identified by Dr. R. H. Foote as *Anastrepha* species, possibly *mombinpraeoptans*.” A similar comment, “the West Indian fruit fly . . . occurs in southern Florida” was uncritically repeated by Heppner (1991).

I believe that these publications do not correctly reflect the present situation in Florida, and they likely have been in error for several decades. This is based on an examination of the entire collection of this species from the Florida State Collection of Arthropods (FSCA, Gainesville), the official repository for insect specimens of regulatory importance in Florida, and the National Museum of Natural History (NMNH, Washington DC), plus all of the official identification records of the Division of Plant Industry (DPI) regarding detections of this species from 1915 to the present.

Briefly, the history of *A. obliqua* in Florida is as follows (see Clark et al. 1996 and references therein). It was first discovered in Florida in 1930. As a result of that discovery, a large fruit fly survey and eradication campaign was conducted from 1930 until 1936. Eradication actions began in 1934 and included widespread fruit removal and destruction, and biweekly insecticidal sprays. During this time, numerous *A. obliqua* specimens were collected, all from Key West, excepting a single specimen from mainland Florida. The FSCA has 63 specimens taken in Key West from 1931 to 1935 and one specimen from Redlands [Dade Co.] in 1935. The NMNH has 48 specimens from Key West, which “either have dates from 1931-33 or (the majority) don’t have a date other than ‘Nov’”, but the latter were reared by L. C. McAlister, so they should have originated from the same time period” (A. L. Norrbom, USDA-Systematic Entomology Laboratory, pers. comm.).

The 1957 record noted above by Weems (1970) is doubtful. DPI identification records show that R. H. Foote did identify three specimens of *A. obliqua* in 1957, but these were specimens that had been collected in Key West in 1935. There are no voucher specimens in the FSCA or NMNH to confirm presence of *A. obliqua* in Florida in 1957. Other DPI identification records indicate detection of fruit fly larvae in fruit which were identified as *Anastrepha mombinpraeoptans*: (1) “Tampa, 1946, fruit?, det[ermination] A. Stone?”; (2) “Miami, hog plum, 1947, det Merrill and G.W. Dekle”; (3) “Miami, 1946, guava, det Dr. A. Stone?”; (4) “Miami, 1947, mango, det Merrill?”; (5) “Ft. Lauderdale, 1963, Anastrepha sp. (possibly mombinpraeoptans Sein), mango, det R. H. Foote”. Fortunately, slide-mounted voucher specimens were retained for instances (1) through (4). Label data for each of these show that larvae were taken from intercepted fruit originating in Puerto Rico. The Ft. Lauderdale larva of instance (5) came from a residential address. Foote correctly left its identification as uncertain, as it is not possible to separate larvae of West Indian fruit fly from those of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), and several other species of *Anastrepha* (Steck et al. 1991). In fact this larva may have been a harbinger of the large colonization by Caribbean fruit fly in south Florida, where adults were first detected in 1965. Unfortunately, the 1963 larva was not retained as a voucher in the FSCA. The final DPI identification record of *A. obliqua* was from two adult specimens trapped in Key West, 1971, determined by Weems. These specimens are in the FSCA. I have re-examined them, and found them to be mis-identified *A. suspensa*.

In summary, there is no confirmed evidence of the presence of *A. obliqua* in Florida since 1935. Apparently, the control actions of 1931-1936 indeed eradicated this pest from Florida as no adult *A. obliqua* has ever again been detected in the field, despite the presence of many thousands of fruit fly detection traps that have been run throughout the Keys and peninsular Florida continuously and year-round since 1956. I think it is safe to say that Florida is completely free of *Anastrepha obliqua* and probably has been so for the past 65 years.
Contribution No. 904, Bureau of Entomology, Nematology & Plant Pathology—Entomology Section.

SUMMARY

A comparison of published and other documented identification records of West Indian fruit fly, Anastrepha obliqua, in Florida and their associated museum specimens shows that this pest has not re-established in Florida after its eradication in about 1935.

REFERENCES CITED

ERRATUM

Caymanis, a New Genus of Antillocorini from the Cayman Islands (Hemiptera: Lygaeidae). By R. M. Baranowski and Julieta Brambila 84(1): 117-118.

The name Caymanis should be substituted for Abroxis in the description of the genus Caymanis on page 117, right column.

Holotype deposited in the Florida State Collection of Arthropods.

The role of insects in human culture is a subject of growing interest, and this book treats one of the most interesting elements, insects in mythology. Myths seemingly convey history, but actually represent natural history, and religious, philosophical, psychological aspects of culture. That insects should be important elements of myths is an idea that is quite foreign to modern western cultures, but nevertheless widespread when viewed from an historical and world-wide perspective. Kritsky and Cherry introduce the reader to several cultures where insects occur in myths, and provide insight that allows an appreciation of insects in ancient and diverse civilizations.

There are three major sections to this book: general mythology, old world mythology, and new world mythology. The first section points out that insect symbols are common. For example, ants and bees are often symbols of industriousness and organization, whereas grasshoppers represent destruction. Quite interesting is the observation that there are many parallels in mythology; cultures distant in time or geography often use the same insect symbols. The section on old world mythology consists of chapters on insect names derived from Greek and Roman mythology, insects in Egyptian mythology, insects in the bible, and cicadas in Chinese mythology. The section on new world mythology consists of chapters on insects in Mesoamerican astronomy, insects in the mythology of Native Americans, and mosquito origin myths.

Almost everyone will find something of interest in this little book. Personally, I've often pondered about the origin of some insect family names, so it was interesting to read about some minor Roman and Greek deities, and how their roles in mythology relate to the behavior and morphology of insects. Anyone teaching a course in introductory entomology or insect classification will find numerous tidbits of information to spice up lectures, and make those horrible, unpronounceable order and family names more appealing. The chapter on re-interpretation of the references to insects in the bible was also quite revealing, both because it reinforces the importance of locusts to residents of the Middle East, and because it highlights the tractability of the "gospel."

The major strengths of this little book are that it brings together a diverse set of very interesting literature, and provides an introduction to literature that is scattered and often overlooked. The major weakness is that it truly is a little book, and in most cases the reader hungers for more detail and explanation than is provided. Although some chapters have been published previously, this book brings them together in a concise and useful format. It is a reference that many will want for a quick, enjoyable read, and for a valuable instructional aid. Even if you do not engage in formal teaching, think of the conversational value of being able to explain why mosquito images appear on totem poles!

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Ten well-written chapters with outstanding illustrations quickly give the reader the sense that this author knows his subject and knows how to convey this information. An introductory chapter explains some gross morphological features, discusses numbers and types of spiders, and introduces some of the more common families. The remaining chapters cover anatomy, metabolism, neurobiology, webs, locomotion and prey capture, reproduction, development, ecology, and phylogeny and systematics. The coverage is sufficiently thorough for an introductory text. This book would have to be the primary textbook for an arachnology course, and it would be an excellent supplement for an entomology or invertebrate zoology course.

This second edition has been updated and expanded from the original edition published in 1982. Particularly Chapter 4 on neurobiology has had considerable new material added, such as sections on temperature perception, peripheral nerves, and the sub- and supraesophageal ganglia. Chapter 10 on phylogeny and systematics briefly discusses the most current hypothesis about phylogenetic relationships available at the time. However, it is clear from the coverage that the author’s strengths are in morphology and physiology. Other chapters vary in the amount of updating that has been done, which is reflected in the changes, or lack thereof, made in the nomenclature of suborder, family, genus, and species names. On p. 4, the author explains why the suborder names Orthognatha and Labidognatha are no longer in use, then proceeds to use them on p. 16. Other names no longer in use, such as Eurypelma and Dugesiella among the tarantulas, are still cited on several occasions. Elsewhere, both the old and new names are given (e.g., Lycosa pullata = Pardosa pullata, p. 236). More consistency in making these changes would have been helpful. Distances given for jumping spider (Salticidae) visual and stalking distances (pp. 11, 90) are low and do not reflect recent research. The idea that jumping spider courtship is always primarily visual is maintained, with no mention of the vibratory courtships which can be performed in complete darkness. One jumping spider is misidentified as a lynx spider (Oxyopidae) (Fig. 13b, p. 16); another is misspelled: Phiddipus is correctly Phidippus (p. 197). Another misspelling (p. 5) is Scytotidae (= Scytodidae), the family of spitting spiders. The bolas spider Mastophora is not credited with using pheromones to attract prey (p. 147), which is well-known, but a relative is so credited. Ctenidae (wandering spiders) are surprisingly still sometimes classified as a subfamily of Lycosidae (wolf spiders) (p. 9). The characterization of wandering tarantulas (Theraphosidae) versus climbing tarantulas (Aviculariidae) on p. 165 is simply wrong. Aviculariidae (proper spelling) is a synonym of Theraphosidae. Genera and species names are not italicized on several occasions, especially in figure legends. A glossary would have been useful.

The above criticisms are relatively minor and likely only to be noticed by another arachnologist. They do not detract from the flow of the book, and can be corrected by an instructor or through follow-up with the bibliography provided. Unfortunately, the bibliography does not directly cite some of the more useful volumes (e.g., Ecophysiology of Spiders, 1987, ed. Nentwig; Spider Communication, 1982, eds. Witt and Rovner), so that one has to search for articles cited from these books in order to find the book itself.

No book can be all things to all people, and certainly individual subjects are covered in more depth elsewhere. Nevertheless, this is the most complete summary of what is known of spider biology presently available. I would highly recommend it.

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