

Relative toxicity of selected pesticides to the squash
bug, Anasa tristis DeGeer (Hemiptera: Coreidae) and
its egg parasitoid Gryon pennsylvanicum (Ashmead)
(Hymenoptera: Scelionidae)

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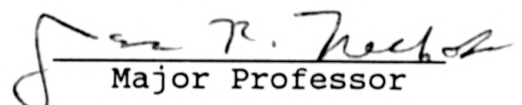
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Introduction and Purpose

The squash bug (hereafter, SB), Anasa tristis DeGeer (Hemiptera: Coreidae), is a serious pest of squash, pumpkin and related cucurbitaceous crops (Knowlton 1933, Beard 1940, Benepal & Hall 1967a, Borrer et al. 1981). Annually, SB is responsible for depredations to cucurbits in many parts of the United states (Wadley 1920, Worthley 1923, Wright & Decker 1955), including the Midwest (Nechols 1987). Nechols (1985) reported the native SB as one of the most important insect pests of vine crops in Kansas. Severe infestations of leaves, petioles, and vines cause plants to rapidly wilt. Piercing injury to plant cells also reduces photosynthetic ability of the plant. Feeding on fruits results in cosmetic damage in addition to creating infection courts for pathogens such as bacterial wilt (Beard 1940b, Nechols 1985).

Production of squash, pumpkins, and melons in Kansas has increased from 2,000 acres to the present 5,000 acres in less than five years (U.S. Dept. Commerce 1987, C. W. Marr [personal communication]). Since these products are becoming more extensively grown for fresh market, control of major insect pests of cucurbits is essential.

There has been some interest in the use of alternative control tactics for SB such as biological control, cultural control and host plant resistance. However, an integrated

pest management program that involves these nonchemical alternatives has not yet been developed. Therefore, chemical control continues to be the predominant means of controlling SB despite its general ineffectiveness (Wadley 1920, Beard 1940b, Wright & Decker 1955, Novero et al. 1962, Nechols 1985, Fargo et al. 1988, Palumbo & Fargo 1989, Nechols et al. 1989, Paige et al. 1989).

Biological control is an attractive alternative because it is an efficient, selective, natural control which may be very economical and self-sustaining. This method of pest control is also environmentally safe since it does not produce pollution and is not ecologically disruptive. Biological control may be highly compatible with other pest control alternatives, particularly host plant resistance and cultural control.

In Kansas, the naturally-occurring egg parasitoid, Gryon pennsylvanicum (Ashmead), has been shown to have relatively greater potential for use in a SB biological control program than other naturally-occurring enemies that have been studied (Tracy & Nechols 1987, 1988; Nechols et al. 1989). Currently this egg parasitoid is being evaluated for augmentative release against the SB.

Because pesticides adversely affect most natural enemies (Bartlett 1964, Hassan et al. 1988, Theiling & Croft 1988), the degree of compatibility between biological and chemical control (e.g., reducing pest populations while

causing minimal disruption to natural enemies), is an important consideration when developing an integrated pest management program. The integration of natural enemies into an agricultural cropping system that periodically is subjected to pesticide applications requires a detailed understanding of how these pesticides affect both pest and beneficial species throughout their life cycles. This includes a quantitative assessment of pesticide toxicity on natural enemies (Croft & Brown 1975, Messing et al. 1989).

Therefore, the overall objective of this thesis was to determine the relative toxicity of various selected pesticides on different developmental stages of the SB and its egg parasitoid Gryon pennsylvanicum. This study was divided into two parts. In part I, I established the relative toxicity of different pesticides on adult SBs in the field, and on immature and adult SBs under greenhouse conditions. In part II, I quantified the effects of the pesticides on different life stages of G. pennsylvanicum under field and greenhouse conditions. These investigations involved both immediate and delayed exposure to pesticides.

Overview of the biology of the squash bug, Anasa tristis

The squash bug, Anasa tristis DeGeer, is a member of the Coreidae, a large and widely-distributed family of insects in the order Hemiptera, suborder Geocorizae (Borror et al. 1981). This serious pest of cucurbits was first described as Cimex tristis in 1773. From its original description until the late 1800s, several workers have referred to this species by various names which since have been synonymized (Beard 1940b). The present nomenclature has persisted for over a hundred years since its clarification by Glover (Glover 1887).

Early studies of the bionomics and seasonal occurrence of the SB were reported in various states including New Hampshire (Weed & Conradi 1902), Kansas and Iowa (Wadley 1920), Massachusetts (Worthley 1923), Utah (Knowlton 1933), Washington (Eichmann 1945), and Connecticut (Elliot 1935, Beard 1940b). Of these, Beard's work was the most comprehensive.

More recently, several researchers have investigated the biology and management of SB in the midwestern part of the United States. These include studies of the pest's regional dormancy and seasonal biology (Nechols 1987, 1988; Fielding 1988), temperature effects on development (Fielding & Ruesink 1988; Fargo & Bonjour 1988), and reproduction and oviposition behavior (Nechols 1987, Paige et al. 1989).

Management tactics for the SB were reviewed by Nechols (1985).

The SB life cycle has been described in detail by Wadley (1920), Knowlton (1933), and Beard (1940b). There are five nymphal instars, in addition to the egg and adult stage. According to Beard, eggs hatch within 10-14 days under field temperatures. Individual eggs are small (1.48 mm length x 1.02 mm width) and triangular in shape. When newly oviposited, eggs are cream to orange-colored; gradually they turn bronze and, finally, brick red shortly before eclosion. Females typically deposit 10 to 20 eggs at a time in compact clusters which are positioned between the raised vein angles along the undersurface of leaves. Occasionally, eggs are laid on upper leaf surfaces or along leaf petioles. Within a cluster (egg mass), individual eggs are arranged in very regular rows and are firmly glued together.

The first instar nymph is 2 to 2.5 mm in length and has a lime-green abdomen. Head, antennae, thorax, and legs are red when newly-hatched, later turning black. Second and third instar nymphs have a light gray coloration. Fourth and fifth instar nymphs differ from the earlier instars in that they are larger (6-7 and 9-10 mm, respectively), darker gray, and have externally visible wing pads. Fifth instar nymphs closely resemble adults in body shape.

Adult SBs are about 14-16 mm in length; females are slightly larger than males. The body is brownish-gray; the fully formed wings are darker gray to black in coloration. The adult head is narrower and shorter than the pronotum and bears four-segmented antennae.

A. tristis, like most other coreids, overwinter as adults. Overwintering adults can be found in crop debris within fields, or in fence rows, building foundations, or other protected habitats (Nechols 1985). Beard (1940b) indicated that female SBs are able to deposit 250-400 eggs in their lifetimes; the reproductive period for overwintered females may last 75 days. Mating pairs join abdomens with the heads facing opposite directions.

Nechols (1985, 1987) reported that initial oviposition by overwintered SB occurs in northeastern Kansas during late May or early June. First generation offspring from overwintered females complete their life cycle in 5 to 6 weeks with adults emerging in July. Reproduction overlaps with overwintered adults. A partial second generation occurs with emergence occurring in late August or early September. This compares to 2-3 generations reported by Fargo et al. (1988) in Oklahoma.

In Kansas, part of the first, and all of second, generation of SB adults undergo a reproductive diapause (Nechols 1987). Diapause is induced and maintained, at least in part, by short (or decreasing) daylengths. It

terminates sometime during mid- to late-Spring, apparently in response to increasing daylengths (Nechols 1988).

Overview of the biology of the egg parasitoid, Gryon pennsylvanicum

Gryon pennsylvanicum (Ashmead) is an egg parasitoid of the family Scelionidae, superfamily Proctotrupoidea. This parasitoid was first described as Hadronotus ajax by Girault in 1920 based on specimens collected from eggs of the squash bug, Anasa tristis (Beard 1940b, Schell 1943). Masner (1983), who revised the genus Gryon in North America, synonymized G. atriscapus Gahan as G. pennsylvanicum. Mitchell & Mitchell (1986) also suggested that G. pennsylvanicum (=H. ajax) and G. atriscapus (=H. atriscapus) are color morphs of the same species. In Texas, the atriscapus morph has brown antennal scapes and femora, whereas these appendages are light colored in the ajax morph.

Most scelionids are egg parasitoids of the Lepidoptera and Hemiptera (Waage 1982). Known hosts of G. pennsylvanicum are eggs of hemipterous insects in the family Coreidae (Masner 1983). Waage (1982) reported that G. pennsylvanicum (=G. atriscapus) parasitizes eggs of Leptoglossus phyllopus L. in Texas. This scelionid has been reported as an egg parasitoid of other species of Leptoglossus in Texas, Louisiana, and Georgia (Masner 1983, Mitchell & Mitchell 1983, 1986), as well as A. tristis.

Very few studies of G. pennsylvanicum's biology have been made. Apart from an early investigation of the

bionomics of this egg parasitoid by Schell (1943), nothing was published until a recent paper by Nechols et al. (1989). Based on a comparative laboratory evaluation of indigenous SB egg parasitoids, these authors reported that G. pennsylvanicum may have relatively greater potential than various encyrtid wasp species as an augmentatively released biological control agent of the squash bug in Kansas.

Schell (1943), indicated that G. pennsylvanicum (=H. ajax), a solitary egg parasitoid, develops from egg to adult within the SB host egg. This author indicated that during development this parasitoid undergoes three larval instars before pupation. The first instar larva is extremely active and has a spherical abdomen, very large mandibles, and a sclerotized caudal-horn. The mandibles move up and down freely. This first instar larva molts ca. 50 hours after hatching. The second instar larva has a flattened, bag-like body without segmentation. The stadial duration is ca. 24 hours. The third instar larva is hymenopteriform with visible segments. This stadium lasts ca. 48 hours. The total larval period is ca. 6 days from hatching. The early pupa is white, later becoming black; the pupal stadium is about 10 days long (Schell 1943). The complete life cycle (egg to emerged adult) takes ca. 18 days at 27°C (Nechols et al. 1989).

As is the case for most scelionid parasitoids, G.

pennsylvanicum adults do not require a specialized diet (Orr 1988). For example, they appear to require only a carbohydrate and water source to produce a large number of eggs under laboratory conditions (Nechols et al. 1989). Host feeding has not been observed (Vogt & Nechols 1991). In nature, nectar, aphid honeydew, and water from dew or precipitation often are the main nutritional sources for scelionids (Orr 1988). Under laboratory conditions, pure honey has been added as a dietary supplement for several parasitic wasps, including G. pennsylvanicum (Taylor 1975, Waddil 1978, Nechols et al. 1989).

Mitchell & Mitchell (1986) found that G. pennsylvanicum located and parasitized host eggs equally well on host plants and on artificial substrates.

Nechols et al. (1989) investigated various biological attributes and environmental responses of G. pennsylvanicum under laboratory conditions. They reported that peak oviposition was ca. 17 eggs per female during the first two days, declining thereafter. The total ovipositional period lasted about 23 days, during which time ca. 80 SB eggs were parasitized per female. Each female parasitoid produces ca. 75% female offspring during its adult life. This sex ratio is slightly lower than that reported by Schell (1943). Parthenogenetic reproduction (arrhenotoky) is used by G. pennsylvanicum. Males emerge from unfertilized eggs; females from fertilized eggs (Schell 1943).

Nechols et al. (1989) also reported the effects of different constant temperatures on the development and survival of G. pennsylvanicum. They found that the lower threshold temperature for development was ca. 12°C. The range of temperatures that resulted in the highest laboratory survival (ca. 90%) was 26.7-29.4°C. On the other hand, extreme temperatures of 18 and 33°C resulted in low survival. These authors also found that rate of parasitization decreased in very old SB eggs. However, if successfully parasitized, preimaginal survival was unaffected by host age.

Squash bug-resistant cultivars did not adversely affect preimaginal attributes of G. pennsylvanicum (Nechols et al. 1989). However, other scelionid species have been reported to be negatively affected by some host plants (Orr et al. 1989).

Overview of alternative management tactics for Anasa tristis.

Several alternative pest management tactics are available for use on cucurbits crops to reduce SB infestations. These include cultural, mechanical, and biological controls, and host plant resistance. At the present time, all of these tactics are being developed but, with the exception of cultural control, none are currently used in a well-defined, deliberate way to manage SB.

Cultural methods suggested for protecting cucurbits from SB include tillage and clean cultivation after the growing season to reduce overwintering populations (Nechols 1985) and early planting (Wadley 1920, Knowlton 1933, Hoerner 1938, Eichmann 1945, Paige et al. 1989).

Host plant resistance is an important practice that has potential for reducing SB populations. For example, Benepal & Hall (1967b) reported that various cultivars of squash and pumpkins (Cucurbita pepo L.) differed in their resistance to field populations of SB. These differences were assumed to be related to qualitative differences in the chemical constituents of the plants but this has not been tested experimentally. Studies by Novero et al. (1962) also demonstrated that SB adults and nymphs responded differently to the same varieties of C. pepo. Differences in survival of SB have been confirmed for different cultivars of C. pepo (Vogt & Nechols, unpublished). Bonjour & Fargo

(1989) found that survival of nymphs was significantly affected by the host type. Despite the identification of resistant sources of host plants, no active breeding program for SB resistance exists, and host plant resistance is not a component of current integrated pest management programs for SBs.

The presence of natural biological control agents of the SB has long been known (e.g., Worthley 1924, Beard 1940b, Schell 1943, Eichmann 1945). However, with few exceptions (Beard 1940b, c; Dietrick & van den Bosch 1957), little has been done historically to evaluate or implement this important control method.

Early studies by Beard (1940b) provided an excellent review of the biology and ecology of the tachinid fly Trichopoda pennipes Fab., a parasitoid of late nymphal and adult SBs. He also listed 3 species of hymenopteran egg parasitoids of the family Scelionidae, Hadronotus ajax Girault (= Gryon pennsylvanicum [Ashmead]), H. anasae Ashmead (= G. anasae), and H. carinatifrons Ashmead (= G. carinatifrons), and the encyrtid, Ooencyrtus anasae Ashmead. Schell (1943) studied the general biology of Hadronotus ajax Girault (= G. pennsylvanicum [Ashmead]) in parasitized SB eggs. SB.

Recent studies in Kansas have evaluated various attributes and ecological responses of selected indigenous

SB egg parasitoids, some of them new to science (Tracy & Nechols 1987, 1988; Nechols et al. 1989). Based on these studies, Nechols et al. (1989) suggested that the solitary parasitoid, G. pennsylvanicum, may have a relative greater potential for use in a biological control program of SB in Kansas. Currently, this egg parasitoid is being evaluated for augmentative release against SB (J. R. Nechols, unpublished).

Part I

Effect of pesticides on immature and adult life
stages of the squash bug, Anasa tristis

Literature Review

Previous chemical control investigations for SB have documented the relative ineffectiveness of a wide variety of insecticides against different life stages of this pest. The first chemical control attempts for SB involved field applications of soap solution and sulfur which were shown to provide better control of immature and adult SBs than nicotine sulfate (Wadley 1920, Worthley 1923). Natural pyrethrins and calcium cyanide were used by Knowlton (1933), and subsequently by Moore (1936), Hoerner (1938), and Eichmann (1945), for the control all SB life stages, especially adults. However, these insecticides gave unsatisfactory results because dust formulations were used so heavily and frequently that burning was noticeable on field-treated plants. Walton (1946) reported that using the botanical, sabadilla, at concentrations of 5 to 10% controlled most immature and adult life stages in the field and laboratory.

With the advent of synthetic insecticides such as DDT circa. 1940, a range of stable and more efficient insecticides became available against SB. Walton (1946) reported that DDT provided effective control (ca. 88% mortality) of young instar nymphs. This author further noted considerably high toxicity of older instar nymphs and adults several days post-treatment. Watkins (1946) also reported that DDT (5% spray) effectively controlled SB

nymphs. Young nymphs also were controlled by sprays of pyrethrins, amine salt, rotenone, and nicotine sulfate. However, none of these chemicals were effective ovicides.

Harries & Matsumori (1955), demonstrated that topical applications in the field and greenhouse of the "new" organophosphates, parathion and malathion provided effective short-term control of adults, while dieldrin gave effective long-term control. Wright & Decker (1955) also found parathion to be highly toxic to adult SBs, but only under laboratory conditions.

First instar SB nymphs are the most susceptible stage to treatment with insecticides (Nechols 1985), while later immature stages and adults tend to be more tolerant (Fargo & Bonjour 1988). These reports affirm the early recommendation by Watkins (1946) that cucurbitaceous crops should be treated with insecticides when young SB nymphs first appear.

Recently, a number of workers have compared the efficacy of various contact insecticides against immature and adult SBs. Beevers & Santoro (1985) reported that cypermethrin, permethrin and methomyl caused greater mortality to SB nymphs and adults than malathion or diazinon. In another study, McLeod (1987) reported that the pyrethroid, esfenvalerate, provided more effective control of immature SB (ca. 60% mortality) than carbaryl, endosulfan or methomyl, which caused ca. 5 to 47% mortality. Sorensen &

Kidd (1988) found that treatments with esfenvalerate, endosulfan and carbaryl produced high mortality of SB nymphs while adults were susceptible only to the pyrethroids, esfenvalerate and fenvalerate. Palumbo and Fargo (1989) established that treatment of SB nymphs with methomyl and permethrin caused ca. 93% mortality as compared to ca. 50% mortality with carbaryl.

Although insecticides are still the primary means of controlling SB, few comprehensive studies have been made of the relative efficacy of currently registered pesticides on the spectrum of SB life stages. For example, most previous field investigations reported only the effects of insecticides on adult SBs in the field (McLeod 1986, Sorensen & Kidd 1988). A few studies have concerned the impact of insecticides on SB nymphal stages (Beevers & Santoro 1985, Palumbo & Fargo 1989, Sorensen & Kidd 1988). However, with the exception of the work of Beevers & Santoro (1985), none of these specified which nymphal instars were tested. Sorensen & Kidd (1988) and McLeod (1987) are the only investigators to have tested esfenvalerate, a relatively new insecticide. However, they did not test the effects of other pesticides, including fungicides, on SB. Therefore, my investigations were conducted with the overall goal of studying the relative toxicity of both insecticides and fungicides on specific SB life stages under field and greenhouse conditions.

Objectives

My specific objectives were 1) to determine the mortality of adult SBs when exposed to the median field recommended rates of selected insecticides, and a widely used fungicide; 2) to determine the mortality of SB first and fourth instar nymphs, and adults, when treated with the lowest and median concentrations of these same pesticides in the greenhouse; and 3) to determine the survival and rate of hatching of SB eggs following application of the lowest and median concentrations of these same pesticides in the greenhouse.

Materials and Methods

Techniques for rearing A. tristis. Colonies of SBs were established from field-collected, unparasitized, SB egg masses during July and August of 1988 near Manhattan, Kansas. Nymphs and adults were maintained on greenhouse-grown, potted, squash plants (ca. 80 days old) of Cucurbita pepo L. cv. "Early Prolific Straightneck", inside screened, metal, frame cages (76 x 91 x 91 cm) using rearing conditions similar to those described by Tracy & Nechols (1987). Adult colony cages contained ca. 130 adult SBs per cage; newly-emerged adults were added as needed to obtain a female:male ratio of 5:1. Cages used to culture immature SBs were supplied with ca. 150 newly hatched first instar nymphs per week. Rearing room temperatures were maintained at $25 \pm 1.3^{\circ}\text{C}$ with a photoperiod of 16:8 (L:D). Squash plants were added to the cages as needed.

Pesticide effects on immature and adult A. tristis in the greenhouse. Lowest and median concentrations of the equivalent field recommended rates of the insecticides, carbaryl (0.1% and 0.2% [Sevin 50WP]), endosulfan (0.1% and 0.2% [Thiodan 50WP]), esfenvalerate (0.003% and 0.005% [Asana 1.9EC]), and malathion (0.1% and 0.2% [Cythion 57EC]), and the fungicide, triadimefon (0.01% [Bayleton 50WP]), were chosen for this study. Formulations of all

pesticides were diluted in tap water to obtain the desired concentrations (see Table 1).

Prior to treatment, 20 newly-molted first and fourth instar SB nymphs, and 1 week-old adults, were placed onto leaves of ca. 2 month-old potted squash plants with a fine-haired brush. Twenty SB eggs (0-6 hours old), glued onto paper strips, also were attached to the plant's upper leaf surfaces using insect pins. Topical applications of each pesticide or a tap water control were then made to all life stages using a 700 ml hand sprayer (Continental GMF, Co.), until run-off (ca. 41 ml of diluted material per plant).

Treated plants and SBs were placed randomly into screened, metal, frame cages (45 x 45 x 45 cm) which were arranged in a split plot design with three replications. To aid observations, white sand was used to cover the soil surface of potted plants, and a white plastic sheet was spread on the bottom of each cage. Temperature, relative humidity, and photoperiod during the experiment were $24.6 \pm 1.2^{\circ}\text{C}$, $65 \pm 4\%$ R.H. and 16:8 (L:D), respectively.

Pesticide effects on SB nymphs and adults were determined by counting the number of dead insects 24 hours post-application. Any insect found walking with uncoordinated movements was categorized as "dead". [This standard was applied in all subsequent experiments.] Egg masses were allowed to air-dry for 1 hour before transfer to

Table 1. Pesticides, pesticide classes, formulations, field recommended rates and concentrations tested against Anasa tristis and Gryon pennsylvanicum.¹

Common name (pesticide class)	Formulation (manufacturer)	Rate (lb. [AI] per acre)		Concentration (% [AI])	
		Lowest-Median		Lowest-Median	
carbaryl ² (carbamate)	Sevin 50 WP Union Carbide	1.0	2.0	0.1	0.2
endosulfan ² (organochlorine)	Thiodan 50 WP FMC Corporation	1.0	2.0	0.1	0.2
esfenvalerate ² (pyrethroid)	Asana 1.9 EC E. I. Du Pont	0.025	0.0375	0.003	0.005
malathion ² (organophosphate)	Cythion 57 EC American Cyanamid	1.25	1.56	0.1	0.2
triadimefon ³ (triazol)	Bayleton 50 WP Mobay Corporation	0.06	0.18	0.008	0.01

¹ The use of trade names does not imply endorsement of any product nor is any criticism implied of products not mentioned. Off-label uses are presented for informational purposes and not as recommendations.

² insecticide

³ fungicide

screen-ventilated plastic vials (15 x 55 mm [d x l]). Vials were then placed in a closed plastic box (32.5 x 25 x 10 cm) with a saturated NaCl solution to maintain ca. 75% R.H. inside an incubator at $26.7 \pm 0.5^{\circ}\text{C}$, and 16:8 (L:D). Numbers of first instar nymphs eclosing from eggs, and the number of days to hatch were recorded.

Percentage data were arcsine-transformed before statistical analysis. An ANOVA test (SAS Institute 1985) was conducted to determine if differences existed in mortality among pesticide treatments and insect life stages. A Fisher's Least Significant Difference (LSD) comparison test was used to separate means for dead SB adults, nymphs and eggs between treatments. The mean square error and approximate degrees of freedom for the LSD were calculated by pooling the main unit (treatments) and subunit (experimental life stage) errors.

Pesticide effects on adult A. tristis in the field.

Commercial field plots of C. pepo L. cv. "Early Prolific Straightneck" were planted on May 17, 1988 at the KSU Ashland Horticulture Research Farm, near Manhattan, Kansas. Individual plots consisted of one row of seven seed-sown squash plants spaced 0.9 m apart within the row, and separated by 1.8 m alleys. Each block consisted of seven plots, and treatments were randomly assigned to the plots within the block. This study was arranged in a randomized

complete block design with three replications.

Standard horticultural practices were used to establish and maintain squash plants. Fertilizer was applied at a rate of 89.6 kg N (1/2 sidedressed) and 50.4 kg P (preplanted) per hectare (ha). The herbicide, chloramben (Amiben 2L), was applied (preemergence) to the sandy loam soil plots at a rate of 2.8 kg [AI]/ha. Field plots were irrigated using a surface trickle irrigation system as needed. Fruits were harvested twice a week.

Each plant in every plot was inspected for adult SBs. These surveys revealed that natural infestations of the pest were very low. Therefore, one day before the experiment began, field-reared SB adults were distributed in all plots to obtain approximately 50 to 70 adults in each plot.

On August 9, squash plants and insects were sprayed with the median field-recommended rate of each pesticide as follows: Insecticides-- carbaryl (Sevin 50WP), 2.12 kg [AI]/ha; endosulfan (Thiodan 50 WP), 2.12 kg [AI]/ha; esfenvalerate (Asana 1.9EC), 0.033 kg [AI]/ha; malathion (Cythion 57EC), 1.68 kg [AI]/ha. Fungicides--triadimefon (Bayleton 50WP), 0.10 Kg [AI]/ha (Table 1). A complete coverage of the foliage, including both sides of the leaf surface, was accomplished. Applications were made with a 15 l. backpack hand sprayer (Solo model 425) delivering 209.9 gal. of water per ha at ca. 60 psi. A tap water

control (wet) and an untreated control (dry) were included in this study.

To minimize interplot effects caused by pesticide drift (see Reed et al. 1985), a 1.5 m x 7.6 m (h x w) plastic sheet was used as a protective barrier between treatments while spraying. Temperature, relative humidity and natural daylengths during the experiment were, respectively, 28°C (avg.) Range = 22-35°C; 61.5 ± 4%; 14:10 (L:D).

Pesticide effects on adult SBs were evaluated by counting the number of dead and live insects 24 hours post-application. Plants were pulled from each plot and placed on a 3 x 3 m. plastic sheet to aid observation. Percentage mortality data were arcsine-transformed before analysis. An ANOVA test (SAS Institute 1985) was conducted to determine if differences existed among pesticide treatments. A Duncan's multiple range test was used to separate means among treatments.

Results

Pesticide effects on immature and adult A. tristis in the greenhouse. Both the lowest and median concentrations of all insecticides were highly toxic to first instar SB nymphs (95 to 100% mortality) (Table 2). In addition, both concentrations of esfenvalerate and, and the median concentration of malathion (0.2%), resulted in a significantly (LSD, $P \leq 0.05$) greater mortality to fourth instar nymphs (ca. 92-97%) than all other pesticides. Fourth instar mortality following treatment with endosulfan was only about half that associated with the esfenvalerate treatments. Insecticides caused significantly greater mortality to all SB instars than did the control (Table 2).

Treatments of both concentrations of esfenvalerate resulted in significantly ($P \leq 0.05$) greater mortality to adult SBs (91.6 and 90% respectively), than all other pesticide treatments. The lowest mortality was associated with the endosulfan treatments (ca. 37%), but differences were significant at the 5% level only from the esfenvalerate and malathion (0.02%) treatments.

Treatment with the fungicide triadimefon (0.01%) had no significantly deleterious effect on either immature or adult SB instars as compared to the control, although mean percentage mortality was somewhat higher for first instar nymphs (range of mortality, 1.6 to 16%) (Table 2).

Table 2. Percentage mortality of squash bugs treated in the greenhouse with the lowest and median recommended concentrations of each pesticide.¹

Treatment	% Concent.	% Mortality_ in each instar X \pm S.D.		
		1 st	4 th	Adult
esfenvalerate	0.005	100 a ²	96.6 \pm 5.7 a	91.6 \pm 7.6 a
esfenvalerate	0.003	100 a	91.6 \pm 7.6 ab	90.0 \pm 5.0 a
malathion	0.2	100 a	95.0 \pm 8.6 a	70.0 \pm 18.0 b
carbaryl	0.2	100 a	83.0 \pm 7.6 b	53.0 \pm 20.0 bc
malathion	0.1	100 a	76.6 \pm 14.0 c	56.6 \pm 20.0 bc
carbaryl	0.1	98.3 \pm 2.8 a	63.3 \pm 20.0 c	53.3 \pm 11.5 bc
endosulfan	0.2	98.3 \pm 2.8 a	56.6 \pm 7.6 c	38.3 \pm 10.4 c
endosulfan	0.1	95.0 \pm 5.0 a	51.6 \pm 10.4 c	36.6 \pm 22.2 c
triadimefon	0.01	16.6 \pm 5.7 b	1.6 \pm 2.8 d	1.6 \pm 2.8 d
water control	0.0	6.6 \pm 2.8 b	1.6 \pm 2.8 d	0.0 d

1 Test performed at 24.6 \pm 1.2°C, 65% \pm 3.8 R.H., and 16:8 (L:D).

2 Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$, ANOVA (SAS), LSD comparison test).

3 There were three replications per treatment, and 20 individuals per instar.

The mean percentage of first instar SB nymphs that hatched from treated eggs, and their respective days to hatching, are shown in Table 3. None of the pesticide treatments differed significantly from one another ($P \leq 0.05$), or from the untreated control. Pesticide treatment had no significant ($P > 0.05$) effect on the percentage of SB eggs that hatched (range: 90 to 100%), or their incubation period (8-9 days) at 26.7°C. (Table 3).

Pesticide effects on adult A. tristis in the field. The insecticides esfenvalerate and malathion resulted in significantly ($P \leq 0.05$) greater adult SB mortality (61.6 and 48.7%, respectively) than did carbaryl or endosulfan (33.4 and 24.2% mortality, respectively) (Table 4). In contrast, mortality associated with the fungicide, triadimefon was very low (9%), and did not differ significantly from that of the water and untreated controls (4.8 and 4.4%, respectively (Table 4). No phytotoxicity was observed in any of the treatments.

Table 3. Percentage hatch and days to hatch for first instar squash bug nymphs from pesticide treated or untreated eggs.¹

Treatment	% Concentration	% Eclosion 1 st instar nymph ($\bar{X} \pm$ S.D.)		Avg. no. days to hatch
carbaryl	0.2	90.0 \pm 8.6	a ²	8.5
malathion	0.2	93.3 \pm 7.6	a	8.5
endosulfan	0.2	93.3 \pm 7.6	a	8.0
carbaryl	0.1	96.6 \pm 2.8	a	9.0
esfenvalerate	0.005	96.6 \pm 5.7	a	8.0
esfenvalerate	0.003	96.6 \pm 2.8	a	8.5
endosulfan	0.1	98.3 \pm 2.8	a	8.0
triadimefon	0.01	98.3 \pm 2.5	a	9.0
malathion	0.1	100.0	a	9.0
water control	0.0	100.0	a	8.5

1 Eggs treated in the greenhouse and then maintained in growth chamber at 26.7°C, 75% R.H., and 16:8 (L:D).

2 Means within column followed by the same letter are not significantly different at ($P > 0.05$, ANOVA (SAS), LSD comparison test).

3 There were three replications per treatment, and 20 individuals per instar.

Table 4. Percentage mortality of adult squash bugs treated with the median recommended rates of each pesticide in the field on August 9th, 1988.¹

Treatment	Rate (AI/acre)	Total tested SB ²	% SB mortality $\bar{X} \pm$ S.D.
esfenvalerate	0.03 lb.	188	61.6 \pm 7.0 a ³
malathion	1.56 lb.	169	48.7 \pm 9.2 a
carbaryl	2.0 lb.	140	33.4 \pm 13.4 b
endosulfan	2.0 lb.	161	24.2 \pm 1.1 b
triadimefon	0.18 lb.	159	9.0 \pm 3.2 c
water control	0.0	164	4.8 \pm 2.5 c
untreated control	0.0	159	4.4 \pm 2.5 c

1 Test performed at Avg. Temperature= 28°C (range: 22-35°C), Avg. R.H.=61.5 \pm 4.2%, and 14:10 (L.D.)

2 Number SB tested for each treatment after 24 hrs. post-application.

3 Means followed by the same letter are not significantly different ($P \leq 0.05$), ANOVA(SAS), Duncan's multiple range test. Each treatment was replicated three times.

Discussion

Both the field and greenhouse results were consistent in demonstrating that esfenvalerate was, comparatively, the most effective insecticide of those tested against A. tristis adults. This pyrethroid also was the most effective in controlling fourth instar SB nymphs in the greenhouse. Esfenvalerate was slightly more toxic to fourth instar nymph and adult SBs than was malathion. It was about two times more effective than carbaryl or endosulfan.

My results agree with a recent study by McLeod (1987) which showed that esfenvalerate was relatively effective against field populations of SB in Arkansas. The level of mortality in this study was similar to that found in my experiment. Likewise, Sorensen & Kidd (1988) found that field applications of esfenvalerate were highly toxic to SB nymphs and adults in North Carolina. In Oklahoma, other pyrethroids (e.g., fenvalerate, permethrin) have been reported to provide significant control of field populations of SB nymphs (Palumbo & Fargo 1989). And, in California, nymphal and adult stages of the SB were more effectively controlled using synthetic pyrethroids than were other classes of insecticides (Beevers & Santoro 1985). In contrast, studies by McLeod (1986) in Arkansas and Roberts & Saluta (1985) in Virginia have shown resmethrin and permethrin to be extremely ineffective against SB.

Esfenvalerate was more effective, and at lower concentrations, than were the other insecticides whose range of standard recommended dosages was 3 to 6 times greater than that of esfenvalerate (Table 1). In general, pyrethroids have been reported to be more effective at lower rates, and persist longer, against adult and immature stages of different hemipterans than standard treatments of other classes of conventional insecticides (Elliot et al. 1978, Brader et al. 1985).

The development and use of new synthetic pyrethroids is based on their ability to control pests that have become resistant to older-class insecticides (Elliot et al. 1978). However, because pyrethroids have been used for a relatively shorter period of time as compared to other insecticides, there has been less time for resistance to develop in SBs and other insect pests.

Although my results indicated that malathion was less effective than esfenvalerate, it still provided a moderate degree of control of adult SB, both in the field and greenhouse. Also, it effectively controlled nymphs (Tables 2 and 4). These findings agree with those of Beevers & Santoro (1985) who also reported that malathion was less effective than esfenvalerate in the field, but still provided moderate control (ca. 55-70% mortality) of SB nymphs and adults. Harries & Matsumori (1955) also found that malathion provided a moderate level of control (ca.

61%) of SB.

On the other hand, carbaryl and endosulfan had very little effect on SB fourth instar nymphs and adults. Toxicity was significantly lower than that observed for esfenvalerate and malathion (see Tables 2 and 4). My findings are supported by recent studies that showed low efficacy of these insecticides against SB (McLeod 1986, 1987; Sorensen & Kidd 1988; Palumbo & Fargo 1989). Sorensen & Kidd (1988) reported that carbaryl provided much better control of SB nymphs and adults than did endosulfan. However, in my experiment there were no significant differences in levels of SB mortality between these two insecticides.

Not surprisingly, all insecticides caused a high rate of mortality (95-100%) in first instar nymphs. Thus, I conclude that this SB life stage is the most vulnerable to insecticides. These findings reaffirm the general supposition that first instar nymphs are very susceptible to insecticides (Nechols, 1985). On the other hand, Beevers & Santoro (1985) reported that seven-day-old insecticide residues did not cause high mortality to first instar SB nymphs. These data suggest that residue levels declined during that time.

My results demonstrated that neither the insecticides, nor the fungicide, triadimefon, had an observable adverse

effect on SB eggs. Uniformly high rates of hatching were observed in all treatments. This may indicate that pesticides did not penetrate the chorion, and that residues that may have been present outside the egg did not affect first instar nymphs during eclosion. Apart from my experiments, there have been no previous studies of fungicidal effects on SB eggs. Watkins (1946) reported that of 26 insecticides tested, only amine salts and light petroleum oil were effective against SB eggs.

Based on my field and greenhouse data, the fungicide, triadimefon, appeared to have no deleterious effect on nymphal or adult SBs. These results are consistent with the data of Watkins (1946) who reported that two other fungicides were nontoxic to SB eggs and nymphs. However, my findings differ from those of Babu & Azam (1987), who showed that triadimefon was highly toxic to a wide range of adult arthropods.

Squash bug mortality was consistently lower for all insecticides in the field than in the greenhouse (cf. Tables 2 and 4). That is, even the most effective insecticide, esfenvalerate, allowed about 40% survival of adult SB in the field. These differences may be attributed to several factors. First, meteorological conditions in the field are different from those in the greenhouse. For example, during field tests high temperatures (22 to 35 °C) could have resulted in volatilization and evaporation of insecticide

residues. In addition, light intensity and quality (e.g., ultraviolet radiation) may enhance photodegradation of pesticide residues. Wind also may have dispersed sprays, resulting in less contact with SB.

Second, it has often been suggested that SB behavior may reduce efficacy of insecticides. This insect has a characteristic mobility that is combined with its habit of hiding at the base of host plants (under leaves or fruits). This behavior may make effective coverage with pesticides difficult to achieve (Beard 1940b, Nechols 1985, Fargo & Bonjour 1988, Paige et al. 1989).

A third factor may be differences in cuticle thickness between greenhouse and field adults. Overwintered adult SBs have been reported to have a thickened cuticle, especially the thorax which becomes heavily sclerotized (Worthley 1924, Beard 1940b). Most of the insects used in the field test were taken from an overwintered population. In contrast, the greenhouse test was done with laboratory-reared SBs with presumably thinner cuticles. The insect cuticle acts as a protective barrier to the penetration of insecticides (Richards 1958, Ebeling 1964).

Another possible factor is physiological resistance. Although there is no experimental evidence for metabolic resistance to insecticides involving detoxification or other biochemical mechanisms in SB (Metcalf 1989), empirical data

from earlier studies suggest that SB may have developed resistance to some of most commonly used contact insecticides (Knowlton 1933, Watkins 1946).

Based on my comparative data, and those of others, it would appear that esfenvalerate is likely to supplement or replace established insecticides (e.g., carbaryl, endosulfan and malathion) for control of SB. This pyrethroid proved to be relatively more effective in controlling this insect pest than the other insecticides. However, to minimize the probability that SB will develop resistance to esfenvalerate, the susceptibility of local SB populations to other insecticides should be investigated before large-scale applications are made. Finally, like other new pyrethroids, esfenvalerate should be applied only when needed (see Ruscoe, 1977).

Part II

Effect of pesticides on immature and adult life
stages of the egg parasitoid, Gryon pennsylvanicum

Literature Review

Many studies have examined the influence of pesticides on arthropods, but most have been directed toward pest species. Relatively little emphasis has been placed on the effects of pesticides on natural enemies, including parasitoids (Croft & Brown 1975, Messenger et al. 1976, Manson & Johnson 1988).

The widespread use of pesticides, and resulting toxicity to parasitoids and other natural enemies, has produced target pest resurgences and secondary pests outbreaks (Waddil 1978, Metcalf 1986). The ecological consequences of this environmental disruption have been increased crop damage, unnecessary addition of insecticides, increased pest resistance, and environmental contamination (Metcalf 1986, Rosenheim & Hoy 1988). For these reasons, investigations of interactions among hosts (pests), parasitoids, and pesticides are an important part of the development of integrated pest management programs (Bartlett 1964, Hassan et al. 1988).

The degree to which pesticides have a detrimental effect on parasitic Hymenoptera depends on the compound used, and on the sensitivity of the particular species of parasitoid (Croft & Brown 1975, Elliot et al. 1978, Theiling & Croft 1988). Another important determinant is the stage of the life cycle that is exposed to the pesticide. In general, immature stages of hymenopteran endoparasitoids

are well protected from several toxic compounds (Bartlett 1964, Bull & Coleman 1984). However, Flanders et al. (1984) reported that the prepupal and early pupal stages of the eulophid parasitoid, Pediobius foveolatus, were vulnerable to the insecticides methyl parathion and acephate. More recently, Obrycki et al. (1986) reported that the preimaginal stages of the eulophid egg parasitoid, Edovum puttleri, were highly susceptible to permethrin, a pyrethroid. Culin & Dubose III (1987) found that parathion had the most deleterious effect on development of the braconid, Microplitis demolitor, within its host larvae.

In contrast to the immature life stages, free-living adults of most parasitic Hymenoptera are generally highly susceptible to broad-spectrum pesticides (Bartlett 1964, Hoogcarspel & Jobsen 1984). These adverse effects are most apparent when adults are exposed immediately after treatment (e.g., see Harbaugh & Mattson 1976, Orr et al. 1989). Short-term residual effects, defined by Ripper (1956) as those which occur within one day of application, also may have quite severe effects on adult parasitoids. Harbaugh & Mattson (1976) reported that immediate applications of resmethrin, malathion, and endosulfan were highly toxic to the aphelinid scale parasitoid, Encarsia formosa. Orr et al. (1989) also found that the scelionid, Trissolcus basalis, was susceptible to methyl parathion immediately after

application.

Long-term residual effects of pesticides on natural enemies may pose a significant barrier to the implementation of biological control. In particular, the organophosphates and carbamates seem to have prolonged residual toxicity to adult parasitoids. For example, Bartlett (1963) measured the effects of 4-day-old residues of 61 insecticides on various species of aphelinid, pteromalid, and encyrtid wasps. Results revealed that carbaryl and malathion were among the more toxic of the insecticides tested to these parasitoids. Harbaugh & Mattson (1976) reported that malathion was highly toxic to adult Encarsia formosa after 7 days post-treatment. In addition, Bull et al. (1987) confirmed that the braconid larval parasitoid, Microplitis croceipes, was extremely susceptible to malathion. Meyerdirk et al. (1982) found that carbaryl and phosmet had a residual toxicity that lasted up to 30 days post-treatment for a majority of the encyrtid parasitoids he examined. Flanders et al. (1984) also found that carbaryl caused high adult mortality in the eulophid, Pediobius foveolatus, for 6 to 9 days following application.

Various pyrethroid insecticides, especially those with high stability and broad-spectrum activity, also have been shown to possess a long residual toxicity to beneficial insects, including bees and parasitic Hymenoptera. The kind of formulation, and application procedures used,

have been shown to diminish or increase these adverse effects (Elliot et al. 1978). Wilkinson et al. (1979) reported that low and high rates of permethrin resulted in high adult mortality of the braconid, Apanteles marginiventis, 5 days post-application. Bull & Coleman (1984) also reported that permethrin adversely affected survival of adult Trichogramma spp. when exposed within five days of treatment. Hatbu & Samsoe-Petersen (1987) reported that permethrin and cypermethrin were highly toxic to the adult stage of the aphelinid, Encarsia formosa, and that fenvalerate produced only moderate toxicity after 3 days post-application.

The detrimental effects of organophosphates on adult parasitoid wasps have been well-documented. For example, Harbaugh & Mattson (1976) demonstrated that applications of malathion were highly toxic to the aphelinid, Encarsia formosa. Plapp & Vinson (1977) reported that the ichneumonid parasitoid, Campoletis sonorensis, was adversely affected by ethyl parathion and methyl parathion. Bull & Coleman (1984), who evaluated the effect of various insecticides on Trichogramma pretiosum, found that methyl parathion caused high mortality of adult parasitoids. O'Brien et al. (1985) found that azinphosmethyl was highly toxic to the braconid, Bracon mellitor. The survival of another braconid, Microplitis croceipes, also was moderately affected by malathion and methyl parathion (Bull et al. 1987). Obrycki

et al. (1986) reported that methamidophos was highly toxic to the eulophid parasitoid, Edovum puttleri. Peter & David (1988) found methyl parathion and quinolphos to be more toxic to the adult braconid, Apanteles taragamae, than pyrethroids. Orr et al. (1989) reported that methyl parathion has short-term adverse effects on adults of the scelionid, Trissolcus basalis. Hoy & Cave (1989) found chlorpyrifos to be the most toxic insecticide to the adult stage of the aphidiid, Trioxys pallidus, of five insecticides tested.

Many carbamates have been found to have adverse effects on adult parasitoids. Plapp & Vinson (1977) reported that carbaryl caused moderate toxicity to the ichneumonid, C. sonorensis. Similarly, Meyerdirk et al. (1982) found this insecticide to be extremely toxic to the aphelinid red scale parasitoid, Aphythis melinus. Waddil (1978), who evaluated the effects of various pesticides on the encyrtid, Capidosoma truncatellum Dalman, and the scelionid, Telenomus remus Nixon, found that methomyl produced high mortality to free-living adults of both parasitoids. More recently, Elzen et al. (1987) reported that the same insecticide caused 100% adult mortality in adults of the braconid, Microplitis croceipes.

Many of the more recently-developed insecticides are synthetic pyrethroids which vary in their degree of toxicity

to natural enemies (Elliot et al. 1978). For example, Waddil (1978) suggested that fenvalerate may have good potential in IPM programs because it was less toxic to various parasitoids over a short period as compared with insecticides in other classes. However, Manson & Johnson (1988) reported that fenvalerate was moderately toxic to the eucolid parasitoid, Chrysonotomyia punctiventris Crawford, and to the pteromalid, Halticoptera circulus (Walker). Harbaugh & Mattson (1976) found that resmethrin produced a moderate adverse effect on the aphelinid, E. formosa. Plapp & Vinson (1977) reported that the ichneumonid, C. sonorensis, was highly toxic to permethrin.

Although fungicides are widely use to control mildews and antracnose infections, their influence on natural enemies appears to have been little studied (Babu & Azam 1987). In fact, reports of insecticidal activity of foliar fungicides on any kind of beneficial species have been rare (Vorley & Wratten 1985). An exception is a study by Obrycki et al. (1986) who reported that triphenyltin hydroxide was highly toxic to the eulophid, E. puttleri, 24 hrs post-treatment. In addition, Teague et al. (1985) found that the carbamate fungicide benomyl reduced emergence of the parasitoid Cotesia margineventis Cresson from its lepidopterous host. This fungicide also caused lower emergence of the braconid, Apanteles aristoteliae, at several concentrations.

While the above-cited studies have been instructive in reporting the toxicity of specific pesticides to certain parasitoids, there is no information available on the toxicity of insecticides or fungicides on the squash bug egg parasitoid, Gryon pennsylvanicum. Thus, the overall goal of this part of the thesis was to study the effects of the same pesticides tested on the squash bug (see Part I) on G. pennsylvanicum.

Objectives

The specific objectives of this study were 1) to determine the effects of pesticides on survival of selected parasitoid immature life stages; 2) to investigate the immediate and short-term effects of the pesticides on mortality of adult parasitoids; 3) to determine the long-term residual effects of pesticides on mortality of adult parasitoids; and 4) to determine dose-mortality relationships for adult parasitoids in response to selected insecticides.

Materials and Methods

Parasitoid cultures. G. pennsylvanicum colonies were initiated at various times between 1987 and 1988 by collecting parasitized SB egg masses from summer squash and pumpkin fields near Manhattan, Kansas. Rearing techniques were similar to those described by Tracy & Nechols (1987).

Emerged adults were placed into ventilated plexiglass boxes (30 x 30 x 30 cm) with a 10 x 15 cm organdy-covered hole on the bottom of the box to allow water vapor from a saturated NaCl solution to maintain a relative humidity of about 75%. The plexiglass boxes were held under $26.7 \pm 0.5^{\circ}\text{C}$ and a photoperiod of 16:8 (L:D), in Forma environmental growth chambers in the Department of Entomology, Kansas State University.

Colonies were supplied every other day with six SB egg masses (each one with 18 eggs ≤ 24 hour old, glued with honey on a 2 x 15 cm index card strip). Fine droplets of pure honey were provided as food for adult parasitoids.

Percentage of survival of immature G. pennsylvanicum.

Greenhouse-laboratory test. First instar larval and pupal life stages of the egg parasitoid were exposed to the lowest recommended concentrations of the insecticides carbaryl (0.1%); endosulfan (0.1%); esfenvalerate (0.003%); malathion (0.1%); and the fungicide, triadimefon (0.008%).

Pesticides were diluted in tap water to obtain the desired concentrations (Table 1).

To determine the effects of these pesticides on each developmental stage of the parasitoid, individual pairs (female and male) of newly emerged adult parasitoids (≤ 12 hour old), were held for 24 hrs inside glass vials (6 cm high x 1.5 cm diameter) that contained fine droplets of pure honey. Male parasitoids then were removed and a cut piece of squash leaf containing a cluster of six SB eggs (≤ 6 hr old) was inserted into each vial. These eggs were exposed to female parasitoids for ca. 6 hr. These procedures were similar to those described by Tracy and Nechols (1987) and Nechols et al. (1989).

Subsequently, SB eggs containing either larval (45 ± 4.5 hrs old) or pupal (154 ± 6.5 hr old) developmental stages of the egg parasitoid were sprayed in the greenhouse at 24°C , and 70% R.H., with a pesticide or water control with a 700 ml hand sprayer (Continental GMF, Co.) until run off. Treated eggs 45 ± 3 [$\bar{X} \pm \text{S.D.}$] per replicate were allowed to air dry and then were glued onto a 1 x 2 cm index card strip using Elmer's Glue-All (Borden, Inc). After one hour, the eggs from each replication were transferred to a clean plastic vial (4 cm high x 1.5 cm diameter) with top and bottom lids covered with organdy cloth to provide ventilation. Plastic vials then were placed inside a NaCl humidity box in a growth chamber at $25.5 \pm 0.5^{\circ}\text{C}$, 16:8

(L:D), and held until adult emergence.

About two to three days before expected first parasitoid emergence, half of the treated host eggs (22 ± 1.4) were rinsed for 10 sec in a 95% acetone solution to remove pesticide residues, followed immediately by a distilled water rinse to remove acetone residues. Preliminary experiments (with and without acetone treatment) indicated that neither SB embryogenesis nor preimaginal parasitoid development were affected by this procedure (100% survival). Treatments were arranged in a split plot design and replicated three times across incubators (blocks). Water and acetone controls were used in this experiment.

Field test. Larval and pupal stages of G. pennsylvanicum were obtained using parasitization procedures described above but with the following differences. Groups of 10-24 SB eggs, ≤ 12 hrs old, were exposed for 24 hrs in a plexiglass colony box that contained ca. 300 adult parasitoids (female:male ratio ca. 5:1).

Parasitoids were exposed to the lowest concentrations of the insecticides carbaryl (0.1%), esfenvalerate (0.003%), and malathion (0.1%) in the field. Because large numbers of immature parasitoids of the required age were difficult to obtain, only three insecticides were tested in this experiment.

SB eggs containing larval (ca. 48 hrs old) and pupal

(ca. 168 hrs old) stages of the parasitoid were treated with an insecticide or water control as described in the greenhouse-laboratory test. Treatments were arranged in a 3 x 2 factorial design and replicated three times across plants (blocks).

The experiment, which began on September 26, 1989, was conducted in a large garden area under semi-field conditions in Manhattan, Kansas. In each replication, treated eggs (13 to 15 for larval and pupal stages) were air-dried, glued onto index card strips, and then attached with insect pins to the upper (abaxial) surfaces of adjacent leaves in the interior middle canopy of a potted squash plant (1.5 months old) placed outdoors. One potted plant represented one replication for all treatments.

About four days before the first adult parasitoid was expected to emerge, all parasitized SB eggs were placed in vials. Half of them had been rinsed with 95% acetone and then placed in ventilated plastic vials as described earlier. Afterward, vials were attached to the base of the leaves (petioles) with an insect pin. The average environmental conditions during the 25-day test period were: daily temperature, 16.4°C (range = $10.9\text{--}29.7^{\circ}\text{C}$); R.H. $62.2 \pm 8.21\%$ and natural daylengths (including civil twilight) which ranged from ca. 13:11 to 12:12 (L:D).

At the end of the field experiment, unemerged eggs were transferred to glass vials and held in a laboratory growth

chamber at 25.5°C and ca. 75% R.H. Eggs that did not produce SBs or parasitoid adults were dissected.

Statistical analysis. Pesticide effects on survival from the parasitoid's larval and pupal stages were determined by computing the percentage of emergence (no. of emerged adults/no. of parasitized host eggs x 100) for each condition (see Obrycki et al. 1986). Percentages were arcsine-transformed and then subjected to the GLM procedure (SAS Institute 1985) for both the field and greenhouse-laboratory experiments. A Fisher's LSD test was performed to compare pairs of mean percentages between treatments and stages.

Immediate and short-term pesticide effects on adult G. pennsylvanicum in the greenhouse. Groups of twenty adult female parasitoids (1 to 3 days old) were placed into clean glass vials (6 cm high x 1.5 cm diameter) that had been provisioned with fine droplets of pure honey as food. Disposable cardboard ice cream containers (Fonda, Inc.) (8.5 cm high x 8.5 cm diameter) were modified by making organdy-covered holes (4 cm diameter), on the tops, bottoms, and sides, to allow air movement. A 1.5 cm hole was also cut to allow entry of the insects. Before transfer to the greenhouse, the inner surface of each container was treated until run-off with either a pesticide or water control using

a 700 ml hand sprayer. Treatments were arranged in a split plot design and replicated three times across greenhouses (blocks).

Adult parasitoids were released from the glass vials into a container that had been pretreated 0, 1, 6, 10, or 24 hrs prior to the test with the lowest recommended concentrations of each pesticide as follows: carbaryl (0.1%), endosulfan (0.1%), esfenvalerate (0.003%), malathion (0.1%), and the fungicide triadimefon (0.008%) (Table 1). Adult parasitoids were exposed for one hour in each test. Containers were then placed in a greenhouse under conditions of $24.5 \pm 1.3^{\circ}\text{C}$, $65.7 \pm 3.1\%$ R.H., and natural light.

The effect of the pesticides on parasitoid survival was determined by recording the number of adult female parasitoids dead at the end of one hour. Survivors were moved into a clean glass vial and observed for 30-60 min for additional mortality. Statistical analysis was conducted on the percentage of mortality (no. of dead parasitoids/20 parasitoids in a container \times 100). Percentage data were arcsine-transformed before analysis using the GLM procedure (SAS Institute 1985). A Fisher's LSD test was used to make multiple comparisons between mean mortality and other variables in the model (e.g., pesticide treatment, exposure time, etc.) including their interactions.

Long-term residual effects of selected insecticides on adult G. pennsylvanicum under combined field-greenhouse conditions.

Potted squash plants (ca. 1.5 months old) were placed 91 cm apart between and among rows in a large garden area under semi-field conditions in Manhattan, Kansas. Greenhouse-grown plants were sprayed until run off (see procedures above) on September 4, 1989, with one of three insecticides; carbaryl (0.1%), esfenvalerate (0.003%), or malathion (0.1%) (diluted with tap water to obtain the desired concentration), or with a tap water control. Because large numbers of adult parasitoids of the desired age were difficult to obtain, only three insecticides were tested in the field experiment. The insecticides tested were selected on the basis of the frequency and quantity of their present or former use by commercial growers to control SB and also because they represent three different and important classes of insecticides. Treatment and control plots were arranged in a randomized complete block design.

To determine the long-term effects of insecticide residues on G. pennsylvanicum, leaves treated with each insecticide were sampled over a 28-day period from September 4 to October 2, 1989. The average environmental conditions during the 28-day test period were: Temperature = 18.9°C (range: 13.1-25.2°C), R.H.= 74.01% range: 58 to 93%, and natural daylengths (including civil twilight) which ranged

from ca. 13:11 to 12:12 (L:D). None of the plants used in this experiment had been exposed to any pesticide before. On each sample date, four leaf replicates from each treatment were selected randomly from the canopy within squash plants. These were then collected, wrapped with aluminum foil, and transferred to the greenhouse. Leaves were then cut into circular pieces (8.5 cm diameter) and fixed to screen-covered tops and bottoms of cylindrical disposable paper cages (4.5 cm high x 8.5 cm diameter), leaving the upper leaf surfaces exposed to each other inside the cage. Previously, experimental cages were modified from those used by Powell et al. (1986) by making four 1.5 cm holes on the sides. Three holes were covered with organdy cloth to allow air movement; the fourth was fitted with a cotton plug and was used to introduce the parasitoid.

Approximately two hours before the start of each test, fifteen adult female parasitoids (1-3 days old) were aspirated from a laboratory colony into clean glass vials as described previously. Vials with parasitoids were taken to a greenhouse near the experimental site and held at ca. 25°C and 62% R.H., until required for the experiment.

The adult parasitoids were released into the cages and exposed for one hour with treated leaves that had been sprayed 0, 2, 7, 14, 21, or 28 days prior to the test. (A different set of leaves was used for each insecticide

treatment at every time interval.) Cages with leaves and insects were maintained under greenhouse conditions of $24.5 \pm 1.6^{\circ}\text{C}$, $68 \pm 3\%$ R.H., and constant light. Artificial light (fluorescent "coolwhite") was added from tops and bottoms of the cages to attract the parasitoids to the leaf pieces. Treatments were arranged in a split plot design across greenhouses (blocks) and replicated four times. A preliminary test to determine parasitoid distribution inside the cage showed that G. pennsylvanicum adults spent most of their time on the treated leaves ($79 \pm 11\%$). Constant ventilation, through the use of forced air flow, was maintained in the greenhouse during the 1-hr exposure period to minimize insecticide fumigation effects, and also to remove moisture accumulation inside and around the cages.

The residual effect of insecticides on parasitoid mortality was determined by recording the number of adult females dead for each time interval. Survivors were moved to clean glass vials as described previously. A statistical analysis using the arcsine-transformed percentage mortalities (no. of dead parasitoids/15 parasitoids exposed in a cage \times 100), was performed by using the GLM procedure (SAS Institute 1985). Treatment means between and within time intervals were compared for statistical significance using Fisher's LSD comparison test ($P=0.05$).

Dose-mortality relationships between adult G. pennsylvanicum and selected insecticides in the greenhouse. Dose-mortality relationships were established by exposing adult parasitoids to fresh dry film of a graded series of diluted concentrations of the insecticides carbaryl, esfenvalerate and malathion. These compounds were selected for the same reasons described above.

Fifteen adult female parasitoids (1-3 days old) were aspirated from rearing colonies into clean glass vials, as described earlier, and held in a growth chamber at 26.7°C, 75% R.H., and constant light until the experiment began.

Initially, fresh stock solutions of carbaryl (0.1%), esfenvalerate (0.003%), and malathion (0.1%) (Table 1), were prepared with tap water (used as dilution material), on the basis of weight (mg AI) per unit volume (liters). A series of diluted concentrations were first screened to determine the ranges for testing each insecticide (e.g., 5 to 100% mortality) (see Manson & Johnson 1988). A premise application method was used in this experiment by depositing a specific amount of each insecticide inside the experimental arena (cage), on the basis of weight (mg AI) per unit area (m^2) (A. B. Broce, personal communication).

Three ml of each insecticide dilution were sprayed inside a disposable paper cage (4.5 cm high x 8.5 cm diameter or its equivalent of 0.021 m^2) using a hand sprayer

as described earlier. The sprayed material was allowed to dry leaving an insecticide residue on the inner surface of a cage. After ca. 30 min post-application, adult parasitoids were released into the treated or water control cages which then were placed in a ventilated greenhouse under the same conditions described in the previous experiment.

Toxicity of the insecticide was assessed by recording the number of adult parasitoids dead after one hour of exposure to each treatment. Survivors were transferred to clean glass vials provisioned with honey droplets for an additional hour of observation. All tests were replicated three times for each series of insecticide concentrations.

Data were analyzed using a PROBIT analysis package ("Milliken program", Department of Statistics, Kansas State University) to quantify relationships between insecticide concentration and mortality (Finney 1971). Comparisons of differences in insecticide toxicity to adult parasitoids were made by examining the intercepts and slopes of the respective probit regression curves and their LD_{50} values. As there was no mortality in the controls (0%), I regressed the uncorrected mortality values (in probits) against log dose (concentration) to obtain the probit regression equations (intercept + slope [log dose]). From these probit regressions, the LD_{50} values could be estimated (see McGregor & Mackauer 1989).

A difference between two LD_{50} values was considered

significant ($P < 0.05$) if the 95% fiducial limits did not overlap. A difference between two slopes was considered significant ($P < 0.05$) if it was 1.96 times the standard error of either slope (Manson & Johnson 1987, 1988). A chi-square test was used to determine significance of the fitted logit-regression line.

All previous experiments were tested with adult female G. pennsylvanicum only. Large numbers of male parasitoids could not be produced because of the low male:female sex ratio (1:5). However, a separate experiment was run to compare the relative effects of the same three insecticides (one concentration per insecticide) on males and females of G. pennsylvanicum.

In this experiment, concentrations of the insecticides carbaryl (0.14 mg AI/ml), esfenvalerate (0.06 mg AI/ml), and malathion (0.1 mg AI/ml) were tested. These concentrations were chosen from the dilution series used in the previous dose-mortality test that caused ca. 30 to 60% mortality of parasitoids. Each insecticide or a tap water control was applied using the same procedures and cages as in the dose-mortality test. Treatments were arranged in a split plot design and replicated four times across blocks (greenhouse). After insecticide sprays had dried on the inner surface of the cages, five parasitoid adults of each sex (1-3 days after emergence) were released into the treated cages for

one hour of exposure. Toxicity of the insecticides then was assessed by counting the number of dead insects. Abiotic conditions during the greenhouse test were $25.5 \pm 0.5^{\circ}\text{C}$, 64.5 ± 1.5 R.H., and constant light ("coolwhite"). The data were analyzed by a GLM procedure (SAS Institute, 1985). An LSD test was used to separate mean mortality among parasitoid sexes for each insecticide.

Results

Percentage of survival of immature G. pennsylvanicum.

Greenhouse-laboratory test. None of the insecticides or the fungicide, triadimefon, significantly reduced the percentage of adult parasitoid emergence when pesticides were applied to host eggs that contained the parasitoid's first instar larval or pupal stages ($P>0.05$, GLM using arcsine-transformed percentages) (Table 5). Survival rates of both treated and control insects ranged from 92.2 to 98%. Dissections of parasitized SB eggs at ca. 24 hrs post-treatment indicated that all larvae were active; larvae were observed continuously moving their heads and caudal horns.

Acetone treatments applied to remove pesticide residues also had no significant adverse effect on parasitoid emergence when compared to untreated and water controls (Table 5). No mortality was observed in adult parasitoids, most of which emerged 9 to 15 hrs before observations were made. The number of days to adult emergence ranged from 8 to 12 days for the pupal stage, and 16 to 20 days for the larval stage.

Field test. None of the insecticides tested significantly affected the percentage of parasitoids that developed and emerged from SB eggs when compared to untreated and water controls ($P>0.05$, GLM using arcsine-transformed percentages) (Table 6). Survival rates ranged

Table 5. Percentage and number of days to adult G. pennsylvanicum emergence from pesticide-treated or untreated SB eggs containing the parasitoid's 1st instar larval or pupal stages. Greenhouse-laboratory test.¹

Treatment ³	% Emergence ($\bar{X} \pm \text{S.D.}$) ²		No. days to adult emergence	
	Larva	Pupa	Larva	pupa
carbaryl (.1)	92.2 \pm 2.6	92.9 \pm 2.5	18-19	9-11
carbaryl (.1) + acetone	98.6 \pm 2.4	94.4 \pm 6.3	17-20	10-12
endosulfan (.1)	95.3 \pm 4.7	95.7 \pm 4.1	17-19	9-11
endosulfan (.1) + acetone	93.8 \pm 5.3	98.6 \pm 2.4	18-19	10-12
esfenvalerate (.003)	98.5 \pm 2.5	95.8 \pm 4.1	16-18	8-11
esfenvalerate (.003) + acetone	97.0 \pm 2.5	94.4 \pm 4.8	17-19	9-10
malathion (.1)	94.2 \pm 2.1	95.8 \pm 4.1	17-18	10-12
malathion (.1) + acetone	94.0 \pm 6.6	94.4 \pm 4.8	16-19	9-11
triadimefon (.008)	97.0 \pm 2.5	98.6 \pm 2.4	17-19	9-10
triadimefon (.008) + acetone	98.4 \pm 2.6	95.3 \pm 4.7	16-18	10-12
water control	97.0 \pm 2.5	95.4 \pm 4.5	17-18	9-12
water control + acetone	98.3 \pm 2.8	97.1 \pm 5.0	16-19	8-11
untreated control	97.0 \pm 2.5	97.0 \pm 2.5	16-18	9-11

1 Eggs ($N = 45 \pm 3$ per replicate) were treated in the greenhouse at 25°C, 70 % R.H., then transferred to growth chambers at 25.5 \pm 0.5°C, ca. 75% R.H, 16:8 (L:D).

2 No significant differences in mean percentages ($P > 0.05$); GLM (SAS), with arcsine

Table 6. Percentage and number of days to adult G. pennsylvanicum emergence from insecticide-treated or untreated SB eggs containing the parasitoid's 1st instar larval or pupal stages. Field test run from September 26 to October 20, 1989.¹

Treatment ²	% Emergence ($\bar{X} \pm$ S.D.) ^{3,4}		No. days to adult emergence	
	Larva	Pupa	larva	pupa
carbaryl (.1)	81.7 \pm 4.3	81.4 \pm 10.7	22-25	13-16
carbaryl (.1) + acetone	84.2 \pm 9.9	90.4 \pm 10.3	21-24	12-14
esfenvalerate (.003)	88.4 \pm 10.8	86.1 \pm 6.4	20-25	13-16
esfenvalerate (.003) + acetone	81.7 \pm 4.3	82.9 \pm 3.8	21-24	15-17
malathion (.1)	88.5 \pm 7.4	85.9 \pm 6.7	22-23	12-15
malathion (.1) + acetone	81.7 \pm 4.3	82.9 \pm 3.8	22-24	13-14
water control	81.7 \pm 4.3	89.7 \pm 11.7	21-24	12-15
water control + acetone	83.9 \pm 8.1	85.4 \pm 8.0	21-25	14-16

1 Eggs treated on September 26 and maintained in the field at avg. temperature = 16.4°C (range = 11-29°C), R.H. = 62.2 \pm 8.2%, and natural daylengths = ca. 13:11 to 12:12 (L:D).

2 Pesticide (concentration %); acetone = 95% concentration.

3 No significant differences in mean percentages ($P > 0.05$); GLM (SAS), with arcsine transformation.

4 There were 3 replications for each treatment. There were 13-15 observations per replicate.

from 81.7 to 90.4% for treated and untreated parasitized host eggs. The number of days to adult parasitoid emergence from treated and untreated SB eggs ranged from 20 to 25 days, and 12 to 17 days, for larval and pupal stages, respectively.

Immediate and short-term pesticide effects on adult G. pennsylvanicum in the greenhouse. Results showed that almost all adult female parasitoids died when exposed directly to pesticide sprays or to dry residues (delayed exposure) \leq 24 hrs post-treatment (Table 7; Fig. 1). This compared to almost no mortality for the controls. Insecticide-related mortality ranged from 90 to 100% and did not differ significantly among chemicals or time treatments ($P > 0.05$, LSD test of arcsine-transformed percentages).

A high percentage of mortality also was observed when adult parasitoids were exposed to the fungicide, triadimefon, at 0 and 1 hr post-treatment (95 and 61.6%, respectively). In contrast, delayed exposure of G. pennsylvanicum adults to triadimefon until 6 hrs or more post-treatment resulted in significantly lower (\leq 15%) mortality than all insecticide treatments (LSD comparison test, $P = 0.05$). However, fungicide-related mortality was significantly greater at each exposure time than that observed for the water control ($P \leq 0.05$ LSD comparison test).

Table 7. Mean (\pm S.D.) percentage mortality of adult female *G. pennsylvanicum* in the greenhouse following immediate or delayed exposure to various pesticides.¹

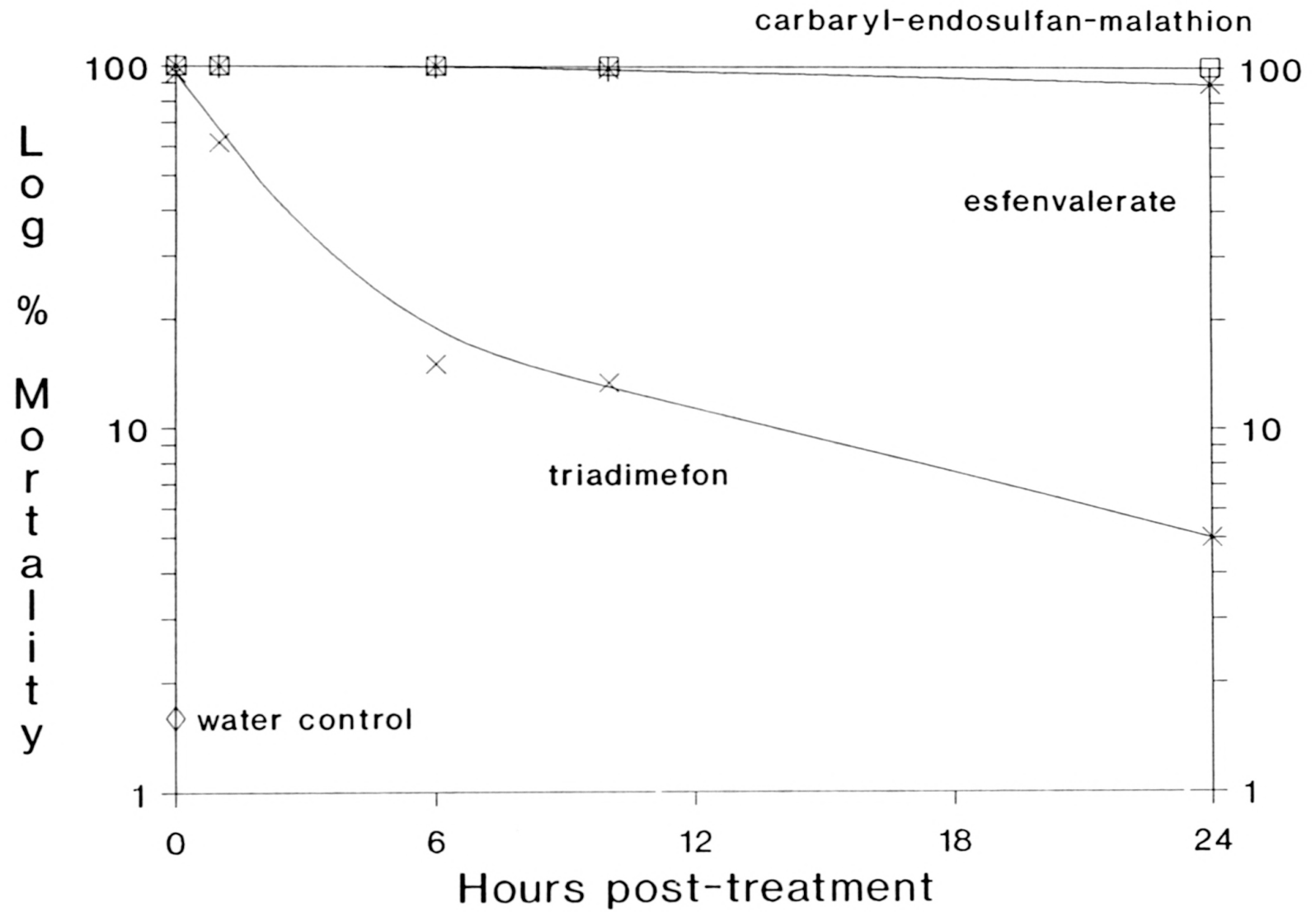
Treatment	% Concentration	% Mortality No. hours post-treatment ²				
		0	1	6	10	24
carbaryl	0.1	100a ³	100a	100a	100a	100a
endosulfan	0.1	100a	100a	100a	100a	100a
malathion	0.1	100a	100a	100a	100a	100a
esfenvalerate	0.003	100aA	100aA	100aA	98.3 \pm 2.8aA	90.0 \pm 10aB
triadimefon	0.01	95.0 \pm 5bA	61.6 \pm 12.5bB	15.0 \pm 5bC	13.3 \pm 5.7bC	5.0 \pm 5.5bD
water control	0.0	1.6 \pm 2.8c	0c	0c	0c	0c

¹ Test performed at 24.5 \pm 1.3°C, 65.7 \pm 3.1% R.H.

² Twenty parasitoids were exposed for one hour in each test. Each treatment was replicated three times.

³ Means within a column followed by different lowercase letters are significantly different ($P \leq 0.05$). Means within rows followed by different capital letter are significantly different ($P \leq 0.05$); GLM (SAS), LSD with arcsine transformation.

Figure 1. Immediate and short-term pesticide effects on adult G. pennsylvanicum. Greenhouse test. No mortality occurred in the water control after 0 hours.



Long-term residual effects of selected insecticides on adult G. pennsylvanicum under combined field-greenhouse conditions. All adult parasitoids died when exposed to wet sprays of each insecticide (Table 8, "0 days post-treatment"). However, at all delayed exposure intervals, there were significant differences among insecticides in the percentage of parasitoid mortality observed ($P < 0.05$) (GLM, LSD comparison test). For example, delayed exposure to carbaryl for two days resulted in 100% mortality as compared to about 37% mortality for esfenvalerate at the same interval. Exposure to carbaryl one week after application still caused 95% mortality; with esfenvalerate, only 5% mortality was observed. Thus, the percentage of mortality was consistently highest with carbaryl, and lowest with esfenvalerate. An intermediate level of mortality was observed at each exposure interval with malathion (Table 8).

The residual effects of each insecticide also differed. For carbaryl, the LT_{50} (time to 50% mortality) was about 21 days. In contrast, the LT_{50} for esfenvalerate was between 0 and 2 days. The LT_{50} for malathion was intermediate, about 7 days (Table 8). These time-related differences in mortality rates are shown clearly in Fig. 2. No parasitoids died in the control treatment throughout the 28-day test period.

Table 8. Mean (\pm S.D.) percentage mortality of adult female *G. pennsylvanicum* in the greenhouse following a delayed exposure to plants treated in the field with various insecticides on September 4, 1989.¹

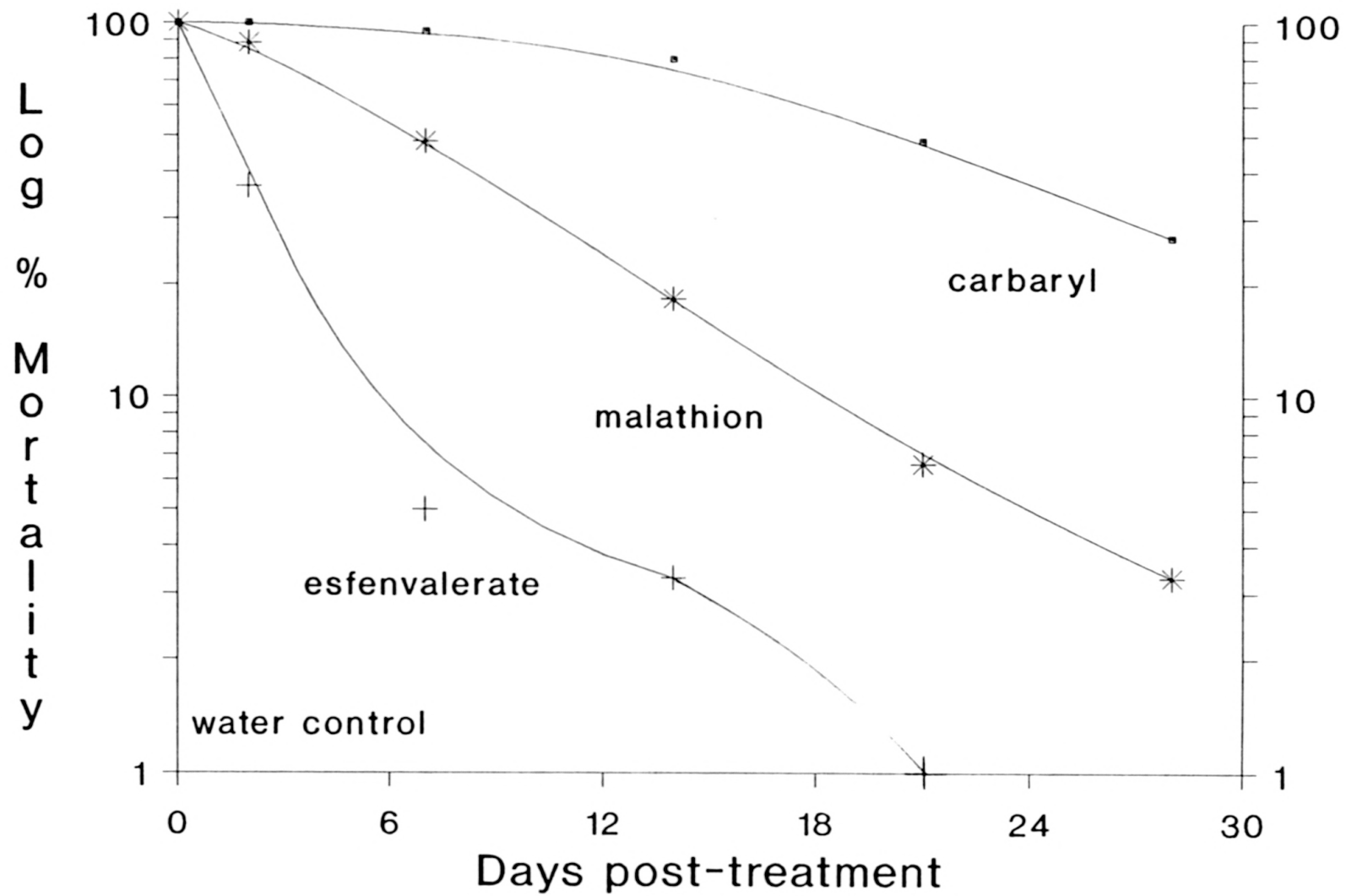
Treatments	% Concentration	% Mortality No. days post-treatment ²					
		0	2	7	14	21	28
carbaryl	0.1	100aA ³	100aA	95.0 \pm 6.3aA	80.0 \pm 5.4aB	48.3 \pm 11.3aB	26.6 \pm 14.4aC
malathion	0.1	100aA	88.3 \pm 10bB	48.3 \pm 12.6bC	18.3 \pm 6.3bD	6.6 \pm 5.4bE	3.3 \pm 3.8bcE
esfenvalerate	0.003	100aA	36.6 \pm 8.6cB	5.0 \pm 6.3cC	3.3 \pm 3.8cdC	0.0cC	0.0cC
water control	0.0	0.0b	0.0d	0.0d	0.0d	0.0c	0.0c

1 Plants treated and maintained in the field at avg. temperature = 18.9^o, avg. R.H. = 74%, and natural daylengths = ca. 13:11 to 12:12 (L:D), and then transferred to greenhouse.

2 Fifteen parasitoids per treatment were exposed for one hour in each test at 24.5 \pm 1.6^oC, 68 \pm 3.1 % R.H. Each test was replicated 3 times.

3 Means within columns followed by different lowercase letters are significantly different ($P \leq 0.05$). Means within rows followed by different capital letters are significantly different ($P \leq 0.05$). GLM (SAS), LSD with arcsine transformation.

Figure 2. Long-term residual effects of three insecticides on G. pennsylvanicum. Field-greenhouse test. No mortality occurred in the water control. There was no mortality in the esfenvalerate treatment after 18 days post-treatment.



Dose-mortality relationships between adult G. pennsylvanicum and selected insecticides in the greenhouse. Table 9 shows intercepts, slopes, and LD₅₀ values of the dose-mortality curves for adult G. pennsylvanicum in response to each insecticide tested. The LD₅₀ values for carbaryl, malathion, and esfenvalerate were 0.0028, 0.0077 and 0.0240 mg(AI)/m², respectively. The data points for all insecticides fit the log dose-probit curve ($P \leq 0.05$, Chi-square test). The LD₅₀ values were significantly different among all insecticides ($P < 0.05$), based on the criterion of non-overlap for the fiducial limits (upper and lower range for LD₅₀ values within 95% C.I.) (see Manson & Johnson 1988, and Powell et al. 1986). The log₁₀ probit values are shown in Fig. 3. The slope for dosage-mortality curves for the esfenvalerate treatment was significantly different from carbaryl and malathion ($P < 0.05$) based on the S.E. criterion between slopes (see Manson & Johnson 1987).

The percentage of adult mortality did not differ significantly ($P > 0.05$) between male and female parasitoids when they were exposed to dry residues of each insecticide (Table 10).

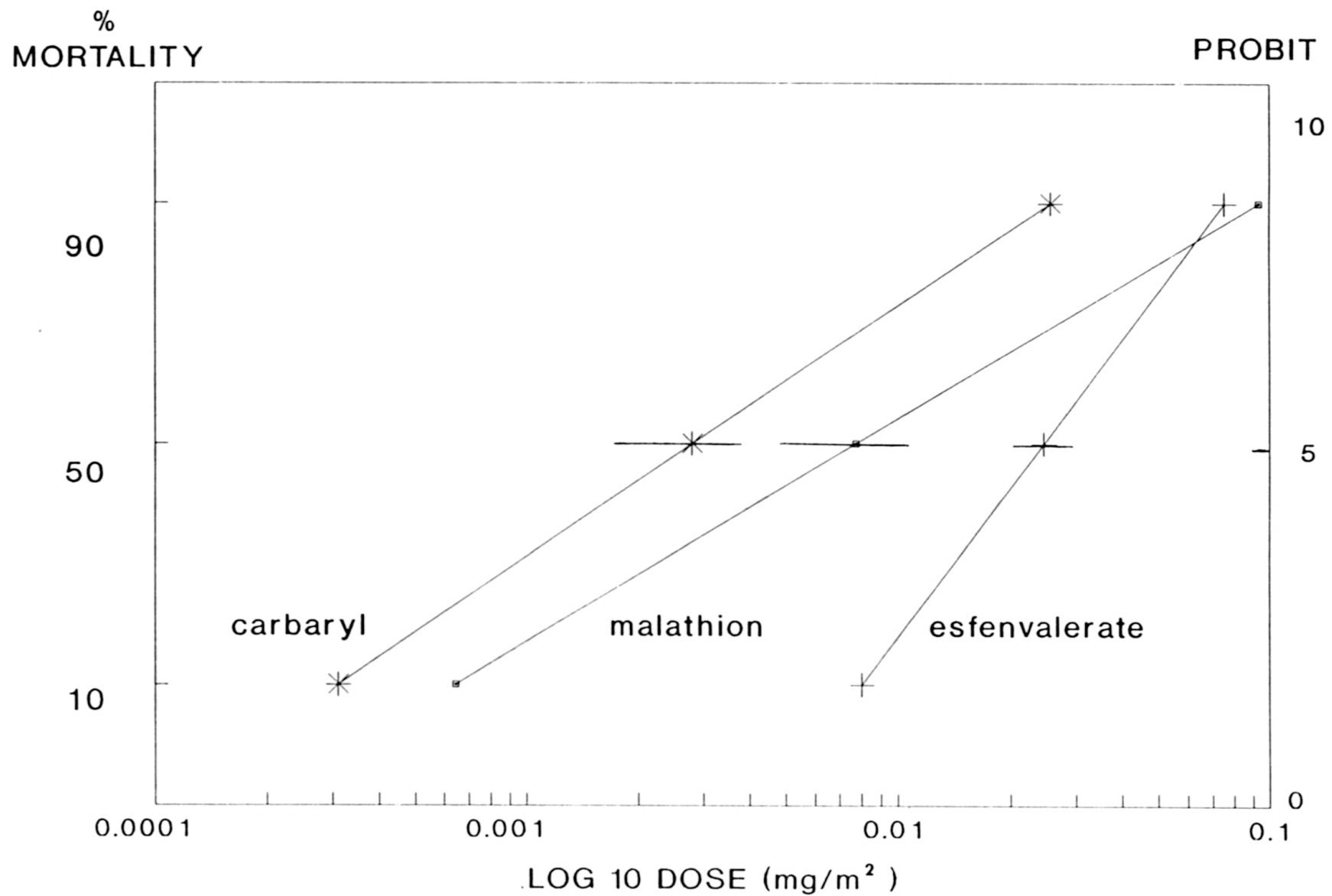
Table 9. Probit regression coefficients and LD₅₀ values for G. pennsylvanicum in response to exposure to various dosages of three insecticides.¹

Compound	Intercept	Slope \pm S.E.	mg (AI)/m ²		
			95% Confidence interval ²		
			LD ₅₀	Lower limit	Upper limit
carbaryl	7.30	1.34 \pm 0.15	0.0028	0.0019	0.004
malathion	6.56	1.18 \pm 0.15	0.0077	0.0053	0.010
esfenvalerate	7.06	2.63 \pm 0.31	0.0240	0.0210	0.029

1 Fifteen adult female parasitoids per treatment were exposed for one hour in each test. Each test was replicated 3 times.

2 Probit analysis (Log₁₀ dose curves) were significantly ($P \leq 0.05$; Chi-square test).

Figure 3. Percentage adult mortality and probit values for G. pennsylvanicum in response to 1-hour exposure to various dosages of three insecticides.



Horizontal lines through LD50 values denote S.D.

Table 10. Mean (\pm S.D.) percentage mortality of adult G. pennsylvanicum males and females following exposure to three insecticides.¹

Treatment	Concentration (mg AI/ml)	% Mortality	
		Male	Female
esfenvalerate	0.06	30 \pm 11a	35 \pm 19a ²
malathion	0.10	55 \pm 20a	55 \pm 19a
carbaryl	0.14	70 \pm 11a	60 \pm 16a
water control	0.00	0	0

¹ Test performed in the greenhouse at 25.1 \pm 0.5 °C, 65.5 \pm 1.5% R.H.

² Means within rows followed by the same letter are not significantly different (P > 0.05), GLM (SAS), Fisher's LSD test.

Discussion

Percentage of survival of immature G. pennsylvanicum.

Results of greenhouse and field experiments indicated that preimaginal survival of G. pennsylvanicum was unaffected by the treatment of host eggs by pesticides. In all treatments, a high percentage of parasitoids emerged, which equaled that of untreated control eggs (Table 5 and 6).

Results similar to those found in my studies were reported by Orr et al. (1989). They found that development of the scelionid, Trissolcus basalis, was unaffected when host eggs were treated with the insecticides permethrin and methyl parathion. The data of Mani & Krishnamoorthy (1986) also showed that various insecticides (e.g., carbaryl, endosulfan) and fungicides (e.g., Dithane) had no adverse effect on the scelionid, Telenomus remus. Studies with trichogrammatid egg parasitoids (Bull & House 1983, Singh 1986, Varma & Singh 1987) also support my findings. In all of these studies, survival of immature egg parasitoids was high following insecticide applications to hosts.

The absence of a negative pesticide effect on first instar and pupal G. pennsylvanicum within SB eggs may be related to the inability of the pesticides to penetrate the chorion of the host egg. For example, Orr et al. (1989) reported that host stink bug eggs provided a barrier to insecticide penetration which protected its egg parasitoid, Trissolcus basalis. Novozhilow et al. (1973) also found

that egg and larval stages of the scelionid egg parasitoid, Trissolcus grandis, were least vulnerable to the insecticide chlorophos. These data differ from recent studies of the eulophid egg parasitoid, Edovum puttleri, which showed that preimaginal stages of the parasitoid are extremely vulnerable to pyrethroids (Obrycki et al. 1986). In that study, however, insecticide effects were indirect in that parasitoid larval mortality appeared to be related to mortality of the host egg. This was not a factor in my experiment where a high rate of hatching was observed in unparasitized SB eggs (Table 3).

The results also indicated that pesticides had no adverse effects on the ability of adult G. pennsylvanicum to successfully emerge. Rates of emergence from pesticide-treated SB eggs compared closely to untreated controls. These data differ from recent studies of other scelionid egg parasitoids which showed that survival of emerging adults was reduced when host eggs were treated with organophosphates (Mani & Krishnamoorthy 1986, Orr et al. 1989). Pyrethroids also were shown to have adverse effects on emergence of certain trichogrammatid egg parasitoids (Bull & Coleman 1984). On the other hand, the fungicides, dithane and fytolan had no toxic effect on emergence of the scelionid, Telenomus remus (Mani & Krishnamoorthy 1986).

The causes for a nontoxic effect of treated SB eggs on

adult G. pennsylvanicum emergence are unknown, particularly since long-term residual effects were noted in other experiments. However, pesticide residues on the outer surface of SB eggs may have been at insignificant levels at the time of emergence (ca. 17 to 25 days post-treatment). Alternatively, parasitoids may not come into direct contact with these residues for long periods during or after eclosion.

Pesticide effects on G. pennsylvanicum following immediate or delayed exposure. My results clearly showed that adult parasitoids were extremely vulnerable to all insecticides (100% mortality) when exposed to them immediately after treatment in the greenhouse (see Fig. 1). This is not unexpected since most insecticides are highly toxic to parasitic hymenopterans when wet residues come into direct contact with the free-living adult stage (e.g., Harbaugh & Mattson 1976, Lasota & Kok 1986). However, the scelionid egg parasitoid, T. basalis, has been reported to be highly tolerant to the pyrethroid permethrin when exposed to the insecticide immediately after application (Orr et al. 1989).

Exposure of G. pennsylvanicum to various insecticides within one day of application also resulted in very high mortality (Fig. 1, Tables 7 and 8). Short-term delays in exposure to insecticides also have been shown to have severe negative effects on other scelionid wasps. For example, the

parasitoid, Telenomus remus, has been reported to be adversely affected by various insecticides (e.g., carbaryl, chlorpyrifos) (Waddil 1978, Mani & Krisnamoorthy 1986). On the other hand, Orr et al. (1989) reported that methyl parathion and permethrin had low and non-toxic effects, respectively, to adult Trissolcus basalis when parasitoids were exposed to the insecticide 6 hrs post-treatment. Generally, most insecticides have been shown to adversely affect parasitoids of the family Scelionidae (Orr 1988). These may have a severe consequences on adult parasitoid activity when they are directly exposed to these chemicals.

Carbaryl is one of the most widely recommended insecticides for the control of SBs. However, my results indicate that residues of this insecticide are extremely toxic to adult G. pennsylvanicum, and they remain so for long periods. For example, carbaryl was 20-times more toxic, after one week, than esfenvalerate (Table 8). In addition, G. pennsylvanicum adults were still affected by this insecticide after four weeks (Table 8). In other studies of parasitic Hymenoptera, carbaryl has been reported to produce high levels of toxicity for long periods (Bartlett 1963, Meyerdirk et al. 1982, Flanders et al. 1984, Morse et al. 1987). In general, carbaryl is one of the insecticides most often cited for its deleterious impact on the parasitic Hymenoptera and other groups of natural enemies (Croft & Brown 1975).

My results indicated that malathion was very toxic to adult parasitoids up to one week post-treatment (LT_{50}) (Fig. 2). If this insecticide is used in an integrated pest management program for squash bug in cucurbits, its residual activity on natural enemies (e.g., egg parasitoids) should be considered carefully. Field-greenhouse experiments indicated that residues of esfenvalerate on squash leaves had the least adverse effect to adult G. pennsylvanicum of all insecticides on a long-term basis (Table 8). Not only was adult survival better at each sample interval, but the relative level of toxicity related to this insecticide decreased at the fastest rate. For example, insignificant mortality occurred 7 days post-treatment with esfenvalerate. In contrast, high mortality was observed with other insecticides up to 2 to 3 weeks post-treatment (Table 8). These results for G. pennsylvanicum are not surprising because earlier studies have shown that various synthetic pyrethroids had moderately low residual effects on adult parasitoids, including other scelionids. Waddil (1978) reported low toxicity to T. remus after 5 days of exposure with fenvalerate and buthrenin. Permethrin was reported safe to T. basalis after the first day (Orr et al. 1989). The successful integration of chemical and biological control, therefore, must take into account the persistence of pesticide residues that are toxic to adult parasitoids.

Greenhouse results indicate that the fungicide, triadimefon, had a high insecticidal effect on G. pennsylvanicum immediately after application. However, toxicity of this fungicide decreased at a relatively faster rate than any of the insecticides. For example, triadimefon was 20-fold less toxic than all insecticides 24 hrs post-treatment (Table 7). This fungicide has been reported to have low toxicity to parasitoids (Theiling & Croft 1988). It has also been shown to be nontoxic to several other groups of beneficial insects (Sotherton & Moreby 1988). The relative safety of fungicides also has been reported for the scelionid, T. remus (Mani & Nagarkatti 1983).

From the dose-mortality data obtained in the greenhouse, it may be possible to extrapolate field concentrations of pesticides that were associated with levels of observed mortality. These data suggest that very low concentrations of insecticides are highly toxic to the parasitoid. However, a more precise determination of dose-mortality relationship would require residue analysis of plants sampled at various times after treatment.

My results indicated that male and female G. pennsylvanicum did not respond differently when they were exposed to three different insecticides (Table 10). These data are consistent with a study by Elzen et al. (1989) of the parasitoid, Microplitis croceipes, which showed that mortality between females and males did not differ, even

when high rates of insecticides were used. In contrast, Orr et al. (1989) observed reduced female mortality in the scelionid, Trissolcus basalis, when adults were exposed to organophosphates. Also, O'Brien et al. (1985) reported that females of the braconid, Bracon mellitor, were more tolerant of organophosphates than were males. In contrast, males of the aphidiid wasp, Aphidius smithi, suffered less from insecticide exposure than females (McGregor & Mackauer 1989).

My experiments revealed that free-living G. pennsylvanicum adults are the most vulnerable life stage to pesticides. Exposure to residues soon after treatment resulted in high mortality. Thereafter, adverse effects decreased at different rates depending on the pesticide. Of all pesticides, the fungicide, triadimefon, and the insecticide, esfenvalerate, had the shortest residual effects. The insecticide, carbaryl, had the longest residual toxicity. Because G. pennsylvanicum is protected within host eggs, the use of pesticides whose residual effects are shorter than the emergence time of adult wasps may allow the partial conservation of this natural enemy.

Summary and Conclusions

Data from both field and greenhouse-laboratory experiments indicated that, of all insecticides tested, esfenvalerate provided the most effective suppression of SBs, with the least deleterious effects to its egg parasitoid, G. pennsylvanicum. In contrast, carbaryl was much less effective on late nymphal and adult SBs than was esfenvalerate, and it was extremely toxic to adult parasitoids for long periods.

Malathion was less effective in controlling adult SBs than esfenvalerate, but it was relatively more toxic to SBs than either carbaryl or endosulfan. Residual effects of malathion on adult parasitoids were significantly longer, and resulted in higher mortality, than those associated with esfenvalerate.

Pesticides had no adverse effects on the preimaginal survival of the parasitoid's larval or pupal stages inside SB eggs in either greenhouse-laboratory or field tests; nor did they affect hatching of unparasitized SB eggs. They did have a dramatic effect on free-living life stages of the pest and natural enemy. For the SB, first instar nymphs were most vulnerable to all insecticides under greenhouse conditions. Toxicity declined with advancing SB age for all insecticides. However, differences in mortality among pest life stages was the smallest for esfenvalerate. No adult G.

pennsylvanicum survived exposure to insecticide residues within 24 hrs of treatment. Thus, it appears that there is little or no opportunity for conserving adult parasitoids present in fields during insecticide applications, or those which emerge soon after treatment. However, delayed exposure of adult G. pennsylvanicum to insecticide residues allowed increased survival. Residual toxicity was substantially shorter for esfenvalerate than for any of the other insecticides. On the other hand, carbaryl residues were the most toxic, and persisted the longest.

The fungicide, triadimefon, had little or no effect on SB's, either in the field or greenhouse. However, it was extremely toxic to adult parasitoids immediately after treatment, and moderately so after one hour. Thereafter, toxicity declined to low, but significant, levels.

These data have potential implications for biological control. For example, in situations where both chemical and biological control (e.g. augmentative release of the parasitoid) are utilized, various steps can be taken to conserve SB natural enemies. First, only the lowest recommended rates of pesticides should be used. Second, if insecticide applications are necessary, esfenvalerate is recommended because of its shorter residual effects on G. pennsylvanicum adults. Third, residue data indicate that insecticide applications with esfenvalerate should be made

no sooner than one week before parasitoid release. Similarly, treatments should be avoided when peak parasitoid emergence is expected to occur within a week of application. In addition, because powdery mildew (Erysiphe cichoracearum De Candolle) is a problem in pumpkins during most seasons, conservation of G. pennsylvanicum may only be possible if the fungicide, triadimefon, is not applied concurrently with parasitoid releases or peak parasitoid emergence. Finally, pesticides should be applied only when economic thresholds warrant their use.

The possible influence of long-term, sublethal, pesticide effects on G. pennsylvanicum (e.g., effects on reproduction [mating, oviposition]; searching ability feeding and longevity) also needs to be examined. Finally, further research is needed to determine the extent to which field-emerging parasitoids are adversely affected by residues of those pesticides present on SB eggs at different intervals after treatment.

From the above findings, I conclude that the insecticide, esfenvalerate, and the fungicide, triadimefon, may be regarded as marginally compatible with G. pennsylvanicum if an augmentation approach is adopted for this natural enemy as part of an integrated pest management (IPM) program for SB. However, because both of these pesticides are highly destructive to adult parasitoids, the repeated use of chemical controls are expected to offset the

effective use of biological control. Therefore, pesticides should be used only when necessary for controlling SBs. A careful monitoring program for SBs and other cucurbit pests would contribute to the reduced use of pesticides.

Although chemical control may be necessary in current integrated pest management programs to suppress periodic pests, including SBs, it is not a viable long-term alternative because all pesticides tested were found to be highly destructive to the adult stage of G. pennsylvanicum a potentially important SB biological control agent. Equally important, even the most effective insecticide (esfenvalerate) did not provide adequate control of adult SBs in the field.

In addition, two major problems related to overreliance and long-term pesticide use are expected for the SB. First, because synthetic pyrethroids such as esfenvalerate have been used for a relatively short time, one would expect to see the eventual development of insecticide resistance in SB. Pesticide resistance has been documented for many other arthropod pests. Although no studies have been made of physiological resistance in SB, this subject should be investigated in light of the generally poor field control of SBs.

Second, environmental contamination (e.g., to groundwater) is an inevitable consequence of increased use

of pesticides and other agrochemicals. This has negative consequences for human safety, wildlife conservation, and agriculture (e.g., pollinators and natural enemies). Therefore, future research should focus on the further development of biological control and other non-chemical methods such as cultural control and host plant resistance.

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Relative toxicity of selected pesticides to the squash
bug, Anasa tristis DeGeer (Hemiptera: Coreidae) and
its egg parasitoid Gryon pennsylvanicum Ashmead
(Hymenoptera: Scelionidae)

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AN ABSTRACT OF A THESIS

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Abstract

A series of experiments were conducted to compare the relative toxicity of the insecticides carbaryl, endosulfan, esfenvalerate, and malathion, and the fungicide, triadimefon, on eggs, first instar nymphs, fourth instar nymphs, and adults of the squash bug (SB), Anasa tristis. Relative toxicity of these pesticides also was determined for the first instar larval, pupal, and adult stages of the egg parasitoid, Gryon pennsylvanicum.

None of the pesticides adversely affected embryogenesis or hatching of SB eggs. However, both nymphal stages of the pest were highly susceptible to all four insecticides in the greenhouse. Esfenvalerate was the only insecticide with high toxicity to adult SBs in the field and greenhouse. This was followed by malathion which caused moderate rates of adult mortality.

Adult emergence of G. pennsylvanicum was tested by exposing SB eggs that contained both larval or pupal stages of the parasitoid to each insecticide or to the fungicide. None of the pesticides tested significantly reduced parasitoid emergence in the greenhouse or field compared with the untreated controls (ca. 82-97% survival).

On the other hand, all insecticides were highly toxic to free-living adult parasitoids when they were exposed directly to wet residues immediately after application, or

to dry (short-term) residues up to 24 hours post-treatment. The fungicide triadimefon was the only pesticide that had a minimal adverse effect on adult parasitoids within 24 hours of application.

In the field, carbaryl residues remained toxic to adult parasitoids for up to 28 days, at which time ca. 27% mortality was observed. In contrast, there was a substantial reduction in adult G. pennsylvanicum mortality after only 2 days post-treatment with esfenvalerate. Thereafter, very low parasitoid mortality was observed.

There were no significant differences in percentage mortality between male and female parasitoids when adults of each sex were exposed to carbaryl, esfenvalerate, or malathion.

These data have potential implications for biological control. Specifically, to partially conserve Gryon pennsylvanicum populations when chemical applications are made, the lowest recommended rates of esfenvalerate should be used since this insecticide has the lowest relative toxicity and the shortest residual effect of any of the chemicals tested. Finally, treatments with insecticides or fungicides should be made only when strictly necessary for the periodic suppression of potentially damaging plant pests.

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