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Development and Impact of Geocoris punctipes (Say) (Hemiptera: Lygaeidae) on Selected Pests of Greenhouse Ornamentals

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To the Graduate Council:

I am submitting herewith a thesis written by Nicole D. Pendleton entitled "Development and Impact of Geocoris punctipes (Say) (Hemiptera: Lygaeidae) on Selected Pests of Greenhouse Ornamentals." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Jerome F. Grant, Major Professor

We have read this thesis and recommend its acceptance:

Paris L. Lambdin, Kenneth D. McFarland

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Dr. Jerome F. Grant, Major Professor

We have read this thesis and recommend its acceptance:

Paris L. Lambdin

Kenneth D. McFarland

Accepted for the Council:

Anne Mayhew

Vice Provost and Dean of Graduate Studies

(Original signatures are on file with official student records.)

Development and Impact of *Geocoris punctipes* (Say) (Hemiptera: Lygaeidae) on Selected Pests of Greenhouse Ornamentals

A Thesis
Presented for the
Master of Science Degree
University of Tennessee, Knoxville

Nicole Diane Pendleton

August 2002

Dedication

This thesis is dedicated to my grandparents, John and Dorothy Coffey of Edinburg, Virginia, my lifelong mentors and friends. Their guidance has helped develop me into the person I have become. It is the many skills they have taught me that enabled me to have the organization and discipline necessary to complete the requirements for this degree.

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Dr. Brad Reddick and Ledare Habera provided pepper plants and allowed us to collect insects from their plots. Ball Seed Company provided seeds for our cut flower crop. Allen Cohen and Gay McCain of USDA-ARS provided insects to start our colony of *Geocoris punctipes* and technical suggestions.

Abstract

The big-eyed bug, *Geocoris punctipes* (Say) (Hemiptera: Lygaeidae), a generalist insect predator common in several agricultural systems, is explored as a biological control agent against pests of ornamentals in greenhouses. This research consists of three components: 1) Evaluation of development and survival of the predator (egg through adulthood) when reared on six diets, including greenhouse pests, a combination of greenhouse pests and plant material, and a meat-based artificial diet that has been developed for *G. punctipes,* 2) Assessment of predation rates of mass-reared big-eyed bugs by investigating the number of prey (three prey species common to greenhouse and ornamental crops) killed by newly eclosed, mass-reared, adult big-eyed bugs and comparing the predation of mass-reared and field-collected individuals of the same species, and 3) Determination of the effectiveness of *G. punctipes* in supressing populations of greenhouse whitefly, *Trialeurodes vaporariorium* (Westwood), and western flower thrips, *Frankliniella occidentalis* (Pergande), on a cut flower crop, *Ageratum houstonium* Miller, in the greenhouse. The hypothesis of this research is that the development, survival, and predation efficiency of big-eyed bugs reared on artificial meat-based diet are similar to those of insects reared on live prey. If the hypothesis is true, then mass-reared big-eyed bugs may have potential as a biological control agent of pests in greenhouse Integrated Pest Management programs. This research contributes to our understanding of beneficial insects and their impact on pest species, and to pest management programs that allow growers of ornamental plants to maximize economic profitability while minimizing environmental impacts by reducing pesticide use.

Table of Contents

List of Tables

List of Figures

Figure Page

Chapter I

Literature Review

Geocoris punctipes (Say), the big-eyed bug (Hemiptera: Lygaeidae), is a generalist predator commonly found in agricultural cropping systems, such as corn, cotton, soybean, and alfalfa (Crocker and Whitcomb 1980, Richman et al. 1980, Hagler and Cohen 1991, Campbell and Cone 1994). It is also associated with peanuts, alfalfa, lettuce, and sugar beets and is distributed throughout the southern half of the United States (Ward 1982). Other members of the genus associated with agricultural crops include the western big-eyed bug, *G. pallens* Stål, *G. uliginosis* (Say), and the large big-eyed bug, *G. bullatus* (Say) (Ward 1982). *Geocoris* spp. feed upon a wide range of arthropods, including aphids, tarnished plant bug, whiteflies, lepidopteran larvae, spider mites, and many kinds of insect eggs (Champlain and Sholdt 1967a, Dunbar 1971, Tamaki and Weeks 1972a, Crocker and Whitcomb 1980, Gonzalez et al. 1982, Cohen 1992, Eubanks and Denno 2000a). *Geocoris punctipes* was reported to reduce pest populations of the European red mite, *Panonychus ulmi* (Koch), in 1917 (Champlain and Sholdt 1967a). In an extensive field survey, Crocker and Whitcomb (1980) recorded 67 different prey species for the three predators *G. punctipes, G. bullatus,* and *G. uliginosis.* By reason of omnivory, *Geocoris* spp. have long been considered beneficial insects in field agroecosystems.

i. Life History

The biology and life history of *G. punctipes* in the laboratory are well documented. Less is known of them in the field, particularly during the fall and winter seasons (Ruberson et. al 2001). At least some *Geocoris* spp. overwinter as adults (Ruberson et. al 2001), while in other locations overwintering may take place in the egg stage (Tamaki and Weeks 1972a). At least some overwintering adults undergo reproductive diapause, which varies geographically and is linked to day length and temperature (Ruberson et al. 1998, 2001).

The overall goals of mass-producing *Geocoris* spp., and the prospect of development of biological control programs using this insect, have led to considerable research to understand its biology and development. Two of these studies are summarized in Table 1.1. Champlain and Sholdt (1967a) conducted a detailed study of the duration of life stages, incubation period, and numbers of eggs and egg-laying days for adult female *G. punctipes* (Table 1.1). Dunbar (1971) determined that at 30º C, eggs hatched in about 7 days, with most eggs hatching in morning hours, and immatures undergoing five nymphal instars (Table 1.2). Both Champlain and Sholdt (1967a) and Dunbar (1971) found that the first and fifth instars require the longest development times. The remaining (second- fourth) instars are roughly equal to one another in length (see Table 1.1). Nymphal development lasts, on average, 21-28 days, while the complete life span from egg through adult mortality averages from 77 to 191 days for males, and 105 to 147 days for females (Champlain and Sholdt 1967a, Dunbar 1971).

Upon reaching adulthood, females have a pre-ovipositional period of 1-5 days, and males must be at least 3 days old before they can fertilize females (Dunbar 1971). Males initiate sexual activity (Dunbar 1972), and couples face in opposite directions while mating, which may last for as long as 45 min to 3 hours (Dunbar 1972). Often individuals engage in other activities, such as eating or drinking, while copulating (Dunbar 1972, Crocker and Whitcomb 1980, personal observation).

The ovipositional preferences of female *Geocoris* spp. are relatively unclear. Most references indicate that adult females oviposit primarily on the undersurfaces of leaves (McGregor and McDonough 1917, Van den Bosch and Hagen 1966, Tamaki and Weeks 1972a, Wilson and Gutierrez 1980). Other ovipositional sites recorded are plant terminals (Van den Bosch and Hagen 1966) and soil duff (Tamaki and Weeks 1972a). In laboratory cage tests on soybeans

Table 1.1. Comparison of effects of two laboratory environments and prey on development times and life span of *Geocoris punctipes.*

Stage of development	Development time ($\bar{x}$ ±SD) (d) on each prey	
	Larvae of beet armyworm, *Spodoptera exigua* Hübner[a]	Larvae of potato tuberworm, *Phthorimaea operculella* (Zeller)[b]
Egg	9.9±0.1	7.3±0.5
1st instar	♀8.2±0.1, ♂7.6±1.5	♀6.0±1.0, ♂5.6±1.01
2nd instar	♀5.2±0.8, ♂4.8±0.3	♀3.7±0.8, ♂3.7±1.0
3rd instar	♀4.1±0.4, ♂4.1±0.4	♀3.1±0.4, ♂3.7±0.5
4th instar	♀4.1±0.5, ♂4.0±0.8	♀3.5±0.7, ♂3.6±0.5
5th instar	♀6.1±0.3, ♂6.3±0.3	♀5.5±1.±3, ♂5.6±0.7
Nymphal development time	♀27.7±1.7, ♂26.8±1.6	♀21.8±1.7, ♂22.2±1.9
Egg to adult development time	♀ 37.8, ♂36.7	♀29.1, ♂29.5
Adult longevity	♀67.7±24.5 ♂41.5±30.4	♀118.0±30.70 ♂161.6±33.70
Total life span (egg to death)	♀105.3±24.6 ♂77.4±30.4	♀146.9±30.70 ♂191.2±33.50

[a] Data from Champlain and Sholdt 1967a (25.5° ± 1.7° C, 14L:10D, 50% RH).

[b] Data from Dunbar 1971 (30.0° ± 2.0° C, 14L:10D, 60% ± 5% RH).

aimed at clarifying the preferences of egg-laying female *G. punctipes*, 77% deposited eggs on the cage surface, yielding little evidence about egg-laying preference in field situations (Naranjo 1987). When given a choice of soybean or an associated weed as ovipositional sites, the females that oviposited on plants (ca. 23%, n=83) preferred soybean over any of the ten weed species tested. Most eggs on soybean were laid among the trichomes on the undersurfaces of soybean leaves (Naranjo 1987).

The variation in number of eggs laid by female *Geocoris* spp. is considerable. Female *G. punctipes* laid an average of 496.5 eggs over a period of 62.1 days (Dunbar 1971), while female *G. bullatus* averaged only 75 eggs over their life span (Tamaki and Weeks 1972a). A single female *G. punctipes* can lay up to 20 eggs in a single day (Dunbar 1971). The type of plants that female adult *Geocoris* spp. feed upon can significantly ($p<0.05$) influence the number of eggs laid per female and the duration of oviposition over the lifetime, but not necessarily the daily rate of oviposition (Naranjo and Stimac 1985). Because the types of plants big-eyed bugs encounter in their environment may affect their ability to reproduce, it is useful to understand the interactions between beneficial insects and the crop plants they are to protect.

ii. Diet, Nutrition, and Prey

Because *Geocoris* spp. are known to feed on a wide array of pest species (Crocker and Whitcomb 1980), they have long been considered to be generalist predators. Observations of plant feeding by *Geocoris* spp. led to further investigation into their nutritional needs. Varying theories on whether big-eyed bugs are gaining nutrition or simply moisture from plant parts have been proposed (York 1944, Crocker and Whitcomb 1980, Naranjo and Stimac 1985, Eubanks and Denno 2000b). Sweet (1960) reported that adult *G. punctipes* could survive for several months on sunflower seeds alone, while York (1944) reported that plant material was necessary for the survival of *Geocoris* spp. even in the presence of prey.

Plant material without insect nutrition is insufficient for full development of nymphs (Eubanks and Denno 1999). Tamaki and Weeks (1972a) found that no *G. pallens* or *G. bullatus* nymphs reared solely on sunflower seeds or solely on pea aphids, *Acyrthosiphon pisum* (Harris), survived to adulthood. When a combination of both aphids and sunflower seeds was offered, approximately 10% of *G. pallens* and 20% of *G. bullatus* reached the adult stage and survived long enough to lay eggs. Addition of green bean, *Phaseolus vulgaris* L., improved survival on each diet. Its addition to an aphid and sunflower seed diet increased survival of *G. pallens* from about 58 to more than 120 days, and survival of *G. bullatus* increased from about 85 to 100 days (Tamaki and Weeks 1972a). In another laboratory study, 52% of an experimental colony reared on pea aphids and green bean reached adulthood, but nymphs fed only pea aphids had 100% mortality (Dunbar 1971). This information supports the theory that a combination of both plant and animal nutrition is ideal (Tamaki and Weeks 1972a, Naranjo and Stimac 1985, Eubanks and Denno 1999, 2000b). Plant feeding by *Geocoris* spp. causes little apparent damage, and no conclusive evidence suggests that the omnivorous habits of *Geocoris* spp. are destructive to crop plants (York 1944).

Additional evidence that big-eyed bugs are receiving nutrition from plants is provided by researchers investigating experimental diets. The two most successful (= highest survival to adulthood) experimental diets (before the introduction of meat-based artificial diet) both include plant and insect material. Survival was 64% for big-eyed bugs reared on larvae of the potato tuberworm, *Pthorimea operculella* (Zeller), and green beans. A similarly successful diet included lygus bug eggs and green beans, with survival to adulthood at 58% (Dunbar and Bacon 1972a). Adult *G. punctipes* feed more frequently on nectaried than nectariless cotton in the absence of prey, further suggesting that the predators are receiving nutrition from the plants (Thead et al. 1985). However, the relationship between plant and prey nutrition is apparently a complex one. When fed moth eggs, the addition or removal of pea pods did not effect survival of big-eyed bugs, but survival was dramatically improved by the addition of pea pods for big-eyed bugs reared on aphids (Eubanks and Denno 1999).

iii. Potential as a Biological Control Agent

Several aspects of the biology of *G. punctipes* suggest that they are potential candidates for use in biological pest control programs. These include: 1) wide prey range, 2) long life span, 3) facultative omnivore, and 4) all life stages are predaceous. The wide prey range of *Geocoris* spp. includes many common pests of both agricultural and ornamental crops (see Tamaki and Weeks 1972a, Crocker and Whitcomb 1980) (Table 1.2). Because the insect and mite pests that big-eyed bugs prey upon in the field crops are familiar ornamental pests and similar species, big-eyed bugs may be a good match for use as a biological control agent in ornamental crops. Because big-eyed bugs feed upon a wide range of species, they may be effective against a single greenhouse pest or a combination of pest species at one time.

The long life span is suggestive of persistence in the cropping system. On average, males can live as long as 191 days from egg to adult death and females about 147 days (Table 1.1). Because *G. punctipes* is a facultative omnivore (Stoner 1970, Crocker and Whitcomb 1980, Eubanks and Denno 1999), it could likely survive in the cropping system for a short time in the absence of prey. Continued presence of predators may prevent outbreaks of pest, or at least assure that the predators will already be in the system when an outbreak occurs. Lastly, *Geocoris* spp. are predatory in all life stages, in contrast to some other commonly available polyphagous predators, such as predatory lacewings (*Chrysoperla* spp.). This combination of favorable factors suggests the potential usefulness of big-eyed bugs as agents of biological pest control.

Table 1.2. Prey species recorded for *Geocoris punctipes* in laboratory and field studies. Bold print letter following each author indicates location of study: **F**=field, **FC**= field cages, **L**= laboratory.

Common and scientific name of prey	Reference
ant *Crematogaster clara* Mayr. **F**	Crocker and Whitcomb 1980
beet leafhopper, *Circulifer tenellus* (Baker) **F**	Knowlton 1937
big-eyed bug, *Geocoris punctipes* (Say) **F**	Crocker and Whitcomb 1980
corn earworm, *Helicoverpa zea* (Boddie) **L**	Eubanks and Denno 2000a
cotton aphid, *Aphis gossypii* Glover **F**	Weathersbee and Hardee 1994
cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) **F**	Tamaki and Weeks 1972a
European red mite, *Panonychus ulmi* (Koch) **F**	McGregor and McDonough 1917
fall armyworm, *Spodoptera frugiperda* (J.E. Smith) **F**	Bugg et al. 1991
flea beetle, *Altica* spp. **F**	Crocker and Whitcomb 1980
flea beetle, *Epitrix* spp. **F**	Tamaki and Weeks 1972a
flower thrips, *Frankliniella bispinosa* (Morgan) **F**	Crocker and Whitcomb 1980
garden fleahopper, *Halticus bractatus* (Say) **F**	Crocker and Whitcomb 1980
green peach aphid, *Myzus persicae* (Sulzer) **L**	Tamaki and Weeks 1972a,b, Tamaki et al. 1981
hop aphid, *Phorodon humuli* Scrank **FC**	Campbell and Cone 1994
insidious flower bug, *Orius insidiosus* (Say) **F**	Crocker and Whitcomb 1980
pea aphid, *Acyrthosiphon pisum* L. **F, L**	Dunbar 1972, Tamaki and Weeks 1972a,b, Crocker and Whitcomb 1980, Losey and Denno 1998, Eubanks and Denno 2000a

Table1.2. Continued.

Common and scientific name of prey	Reference
pink bollworm, *Pectinophora gossypiela* Saunders **F**	Hagler and Naranjo 1994
potato tuberworm, *Phthorimaea operculella* (Zeller) **L**	Dunbar 1972
psyllid, *Trioza maura* Forster **F**	Knowlton 1942
southern garden leafhopper, *Empoasca solana* DeLong **F**	Tamaki and Weeks 1972a
soybean looper, *Pseudoplusia includens* (Walker) **L**	Crocker et al. 1975, Richman et. al 1980
spider mites, *Tetranychus spp*. **F**	Gonzalez et al. 1982, Crocker and Whitcomb 1980
spider mite, *Petrobia apicalis* (Banks) **F**	Crocker and Whitcomb 1980
spotted alfalfa aphid, *Therioaphis maculata* (Buckton) **F**	Tamaki and Weeks 1972a
sweetpotato whitefly, *Bemisia tabaci* (Gennadius) **L, F**	Cohen 1992, Hagler and Naranjo 1994
threecornered alfalfa hopper, *Spissistilus festinus* (Say) **L**	Crocker and Whitcomb 1980, Medal et al. 1997
tobacco budworm, *Heliothis virescens* (F.) **L, FC**	Lawrence and Watson 1979, Chiravathanapong and Pitre 1980, Crocker and Whitcomb 1980, Hutchison and Pitre 1983
two-spotted spider mite, *Tetranychus urticae* Koch **L, F**	Dunbar 1972, Tamaki and Weeks 1972a,b, Colfer et al. 1998
western tarnished plant bug, Lygus hesperus Knight L	Champlain and Sholdt 1966, Dunbar 1972

To exploit the potential of *Geocoris* spp. or other predators in agricultural or horticultural systems, a great deal of preliminary research must be conducted before effective programs of pest management can be established. For instance, cannibalism is mentioned as a problem in almost every published report on mass rearing *G. punctipes*. However, no references made any suggestion regarding how many big-eyed bugs should be confined in a given space to reduce cannibalism. The following material outlines various contributions that have been made thus far to understand the biology and impact of *G. punctipes* in the field, and to further implement its use as a biological control agent.

iv. Studies of *Geocoris* spp. in Agricultural Crops

The prey range of big-eyed bugs and their ability to reduce pest populations in agricultural crops have been researched extensively (Table 1.2). Early observations of feeding patterns of *Geocoris* spp. led to the idea that these insects were primarily predators of lepidopteran eggs and larvae, i.e., the corn earworm, *Helicoverpa zea* (Boddie). Early investigations of the ability of *Geocoris* spp. to reduce pests included studies of their predation on soybean looper, *Pseudoplusia includens* (Walker) (Richman et. al 1980), and the tobacco budworm, *Heliothis virescens* (F.) (Hutchison and Pitre 1983). When *G. punctipes* was compared to other predators of *H. zea* and *H. virescens*, big-eyed bugs were more efficient predators of eggs and first instar larvae than the other three adult predators, *Chrysoperla carnea* Stephens, *Coleomegilla maculata* (DeGeer), and the spined soldier bug, *Podisus maculiventris* (Say) (Lopez et al. 1976). However, in one series of observations of prey of *Geocoris* spp. in the field, lepidopteran eggs and pupae accounted for only 3% of their total prey (Crocker and Whitcomb 1980).

When *Geocoris* spp. in a soybean field were tested using the ELISA (enzyme linked immunosorbent assays) method, almost 40% tested positive for whitefly, but only 4.1% of the predators were positive for pink bollworm, *Pectinophora gossypiella* Saunders (Hagler and Naranjo 1994). Other laboratory choice tests demonstrated that *G. punctipes* consistently chose the western tarnished plant bug, *Lygus hesperus* Knight, over eggs of both the beet armyworm, *Spodoptera exigua* (Hübner), and *H. virescens* (Hagler and Cohen 1991). No significant (p>0.10) differences among the prey choices of laboratory-reared and field-collected *G. punctipes* were documented (Hagler and Cohen 1991).

Geocoris spp. are also known to be effective in suppressing several non-lepidopteran pests. For instance, *G. bullatus* can be effective in reducing the population growth rate of green peach aphid, *Myzus persicae* (Sulzer), in field cages containing sugarbeet plants (Tamaki and Weeks 1972b, Tamaki et al. 1981). About one-third of *G. punctipes* collected in cotton fields tested positive for remnants of whitefly eggs using the ELISA method, and the predators showed a preference for whitefly compared to eggs of the pink bollworm (Hagler and Naranjo 1994). *Geocoris* spp. also can be significantly (p<0.05) effective in reducing populations of two-spotted spider mite, *Tetranychus urticae* Koch, and the predatory mite *Galandromus occidentalis* (Nesbitt), in cotton.

Because temperature influences development time of nymphs of *Geocoris* spp. (Table 1.1), populations of big-eyed bugs are likely to develop more rapidly in warm spring seasons than in cold ones. The rates at which different insects develop within a given temperature range may impact the effectiveness of a predatory insect as a biological control agent.

In Arizona crops where both *G. punctipes* and *L. hesperus* are present, predation by *G. punctipes* does not begin to impact lygus bug populations until temperatures exceed 35° C (95°F). At temperatures lower than 35°C, lygus bugs develop more quickly than, and prey upon, *Geocoris* nymphs (Champlain and Sholdt 1967b). Temperature and timing also influence other pest/predator relationships. *Geocoris* spp. prefer eggs and early instars of tobacco budworm, and prey upon mature larvae at consistently lower rates than on eggs and early instars (Dunbar and Bacon 1972b, Chiravathanapong and Pitre 1980). *Geocoris* spp. are unlikely to be effective against early-hatching lepidopteran larvae that emerge and mature before *Geocoris* nymphs develop. Other variables influencing the ability of a predator to suppress pests include: 1) the crop, 2) the crop's level of maturity, 3) flora surrounding the crop, 4) interactions with other predatory insects in the complex, and 5) intercropping practices.

Many factors, including plant structure, leaf surface area, and agroecosystem diversity, can influence the ability of *G. punctipes*, as well as other predators, to suppress pest insect populations (Sheehan 1986). Evidence suggests that *Geocoris* spp. are likely to be more useful as biological control agents in some crops than others. They are typically more abundant in tobacco and soybean than in corn and tomato (Pfannenstiel and Yeargan 1998). Depending upon the crop, variety may be an important factor in abundance, and development of, *Geocoris* spp. (Rogers and Sullivan 1986). For example, both adult and immature *Geocoris* were more abundant on varieties of tobacco with lower levels of exudates (Crutchfield 1990), and more *Geocoris* were found on cotton with extrafloral nectaries than on varieties without them (Thead et al.1985). In another study with glabrous and pubescent commercial cotton cultivars, no significant ($p<0.05$) differences were found in the overall numbers of individual adult or immature *G. punctipes* among cultivars over the growing season (Weathersbee and Hardee 1994). In addition, numbers of *G. punctipes* were higher on tomatoes resistant to lepidopteran pests than on susceptible varieties (Barbour et al. 1997).

The stage of maturity of a crop has a direct impact on the density of *G. punctipes* in the field. For example, *G. punctipes* had higher populations and better dispersal in fields of lima beans in pod stage than in less mature fields. In the laboratory, predation on pea aphids was lower on lima bean plants with pods, but the predators were effective in suppressing the pea aphid in the field when the plants were in pod stage (Eubanks and Denno 1999, 2000b). Pods apparently provide secondary nutrition sources for big-eyed bugs when low numbers of prey are available (Eubanks and Denno 1999, 2000b).

The presence of cover and/ or trap crops can impact densities of big-eyed bugs in the field. *Geocoris punctipes* density is affected by the presence of a trap crop even where there are no significant (p=0.0001) effects on aphids or whitefly density (Bugg et al. 1991). In an investigation of cover crop influence on *G. punctipes* in cantaloupe, big-eyed bug populations were lowest when rye was used, and highest when subterranean clover, *Trifolium subterranean* L., was the cover crop. Other legumes such as "Vantage" vetch, *Vicia sativa* L. x *V. cordata* Wulf, and weedy fallow control plots also had large populations of *G. punctipes*. Early cover crops may provide a suitable habitat and attract prey for beneficial species while primary crops are in early stages of development. In cotton fields in Texas, densities of *Geocoris* spp. and other predators were different among cover crops and nearby cotton early in the growing season, but those differences diminished as the season progressed (Parajulee and Slosser 1997). When alfalfa strips were used as trap crops for western tarnished plant bug in cotton systems, alfalfa cutting practice had an effect on the ratio of predators (*Geocoris* and other species) in those strips. While uncut alfalfa had the highest actual numbers of predators, the best predator/prey ratio was observed when the alfalfa was cut every 28 days (Godfrey and Leigh 1994).

Timing the plantings of cover strips and crops, as well as the cropping method (i.e. conventionally plowed vs. drill-planted) plays an important role in the ability of *Geocoris* spp. and other predators to persist in the field (Ferguson et al. 1984). Early cover crops provide habitat and food (via both plant moisture and as a source of insect prey) to predators, allowing populations of predators to become well established early in the season. If a population of predators is already present, the time needed to colonize a new crop may be reduced (Parajulee and Slosser 1997). The relationships between cover crop and predator density need to be better understood if cover crops are to be successfully used to improve soil quality and/or provide harborage for beneficial insects.

Weeds and other plants adjacent to agricultural crops may have an impact on *Geocoris* spp. This impact may be related to nutritional value of the weed or the level of favorable habitat it provides for big-eyed bugs. Six weedy plants associated with soybeans were used as diet supplements for *Geocoris* nymphs fed one of three species of early instar lepidopteran larvae: fall armyworm, *Spodoptera frugiperda* (J.E. Smith), cabbage looper, *Trichoplusia ni* (Hübner), or beet armyworm. The nymphs fed various diets had similar development times, but significant ($p<0.01$) variation existed in the number of eggs laid and duration of ovipositional period among adult females fed different weeds (Naranjo and Stimac 1985). Females laid the highest number of eggs on Florida beggarweed, *Desmodium tortuosum* (Sw.) Dc, and green bean. Only one-half as many eggs were laid on sicklepod, *Cassia obtusifolia* L., and goldenrod, *Solidago fistulosa* Mill. (Naranjo and Stimac 1985). Understanding the intricacies of these and other plant-insect relationships will lead to more successful use of predatory insects as biological control agents.

v. Studies using *Geocoris* spp. in the Greenhouse

Relatively few instances of the use of *Geocoris* spp. as a biological control agent in a greenhouse setting have been reported. *Geocoris bullatus* suppressed population growth of green peach aphid on sugarbeet plants in the greenhouse (Tamaki and Weeks 1972a). In a separate study comparing *G. bullatus* to other insect predators against three pests on sugarbeets in the greenhouse, *G. bullatus* effectively reduced aphid populations when released before aphid densities exceeded 14 individuals per plant (Tamaki and Weeks 1972a). These few data do little to establish the usefulness of this predator in more common greenhouse crops or on ornamental plants in a greenhouse.

Geocoris spp. may be incompatible with hydroponic greenhouse systems without a soil substrate due to the lack of suitable ground-level habitat for the predators. The Latin roots of the genus name, geo- and cori-(s) mean "the earth" and “the bug”, respectively (Borror 1960), reflect the tendency of *Geocoris* spp. to spend a good deal of their time foraging in soil near the base of plants. In an experimental release of *G. punctipes* into a hydroponic tomato crop, no live *Geocoris* spp. were found after 48 hours, perhaps reflecting the importance of soil debris as a place for foraging and hiding by *G. punctipes* (Cohen, personal communication).

Whiteflies, aphids, and mites are all common greenhouse pests included in the host range of *Geocoris* spp. A diet of only one of these aforementioned pests alone may be insufficient nutritionally for the predator (Cohen 1985a, Cohen and Brummett 1997, Cohen and Smith 1998, Cohen and Byrne 1992). However, in a choice test, big-eyed bugs chose aphids over corn earworm eggs (Eubanks and Denno 2000a). Because it has been shown that plant tissue and/or sunflower seeds added to an artificial diet increases survival of *Geocoris* spp., the addition of these materials into a greenhouse setting may help to offset any nutritional problems associated with a limited range of prey species.

Despite the focus on lepidopteran eggs and larvae as prey and nutrition for big-eyed bugs, the predators feed on a wide variety of other pest types common to greenhouses. Gonzalez et al. (1982) found that mites were a primary food of *G. punctipes* and the minute pirate bug, *Orius tristicolor* (White), in California cotton (see also Wilson et al. 1991). *Geocoris* spp. are also frequent predators of sweetpotato whitefly, *Bemisia tabaci* (Gennadius): 39.4% of adults sampled from a sweet potato field tested positive for whitefly and whitefly egg antigen using the ELISA method (Hagler and Naranjo 1994). Despite the evidence that big-eyed bugs feed readily on pests of ornamental plants, little is known about their effectiveness in suppressing pests in greenhouse systems.

vi. Artificial Diet

Early laboratory diets for *G. punctipes* usually consisted of lepidopteran larvae and eggs. Live prey diets are inefficient in a mass-rearing facility because of increased labor, materials, and space costs associated with maintaining multiple colonies, which are difficult to reliably maintain concurrently (see Cohen et al. 1999, Smith and Nordlund 2000). The possible development of a reliable, nutritious, synthetic diet, readily accepted by predators, has long been considered as a potential tool for effectively mass rearing predators (Simmonds 1966, Cohen et al. 1999). Artificial diet is a crucial component of needed automated systems in insect rearing to reduce costs and handling of the insects by humans (Smith and Nordlund 2000).

A meat-based, artificial diet for *G. punctipes,* developed in the laboratories of USDA-ARS, has been used to rear this insect species for more than 10 years and 100 generations (Cohen 1985b, 1993, 2000). The meat-based diet, a paste designed to mimic the nutrition in and consistency of lepidopteran larvae (Cohen 1985a,b), is mainly composed of ground beef, beef liver, and hen's eggs. Two versions of the diet have been used to successfully rear multiple generations of *G. punctipes*: 1) the original version contained two kinds of meat and sugar water (Cohen 1985b), and 2) a later version incorporated hen's eggs, yeast, and antibiotics, and was originally developed for the lacewing *Crysoperla rubafilis* Burmeister (Cohen and Smith 1998). It is unclear if any performance advantage is associated with one version of the meat-based diet over the other for *G. punctipes* (Cohen, personal communication).

vii. Current Use and Availability of *Geocoris punctipes* as a Biological Control Agent

Recent developments in rearing technology, primarily the development of a suitable artificial diet, have enabled some commercial facilities to mass produce *G. punctipes* for consumers. As of 2002, the number of suppliers offering this insect for sale is small. An extensive internet search for beneficial insect suppliers, aided by the publication Suppliers of Beneficial Organisms in North America (Hunter 1997), yielded four companies in the United States which sell *G. punctipes,* with prices ranging from $21-43 dollars per 100 individuals (Table 1.3). Current augmentative use of *G. punctipes* is primarily in

Table 1.3. Commercial insect suppliers offering *Geocoris punctipes* for sale, 2002. (Hunter 1997 and multiple internet searches).

Company Name	Location	Cost of *G. punctipes*
Applied Bio - Pest	Oxnard, CA	price unavailable on web
Arizona Biological Control	Tucson, AZ	100 adults $40.65
Biofac Crop Care	Mathis, TX	100 adults $21.95
Rincon-Vitova Insectaries	Ventura, CA	100 adults $42.50

cotton crops (Wood 1998) and in field strawberries (Wood 1998, Biofac Crop Care, personal communication).

Development of a field-dispersal system for *Geocoris* spp. by growers is a product called the Bugslinger, or Aerodynamic Transport Body, devised by USDA-ARS in California, enables big-eyed bugd to easily be dispersed into fields with reduced time and labor (Wood 1998). The transport body, a modification of a target used in skeet shooting, can be filled with beneficial insects and launched into a field from its perimeter. The system, and a slower moving "Mite Meter" which dispenses the insects behind a tractor in a grit-like carrier material, have been tested using both *G. punctipes* and the western predatory mite, *Galandromus occidentalis* (Nesbitt), with about 95% of the predators surviving the launch episode (Wood 1998).

viii. Pesticide Compatibility

Compatibility with chemical pesticides is a major factor in determining the success of predators to persist and to suppress pests in agricultural systems. Beneficial insects may come into contact with pesticides in several ways including: 1) direct topical contact with the chemical, 2) tarsal contact with treated leaves, and 3) ingestion of a pesticide-contaminated prey insect (Herbert and Harper 1986). Because they are facultative omnivores, *Geocoris* spp. may be more susceptible to systemic pesticides than those applied to leaf surfaces (Stoner 1970). The compatibility of *G. punctipes* with pesticides is of crucial importance to its successful use in an IPM program, but is outside the scope of this research. Various authors have investigated this area for both *G. punctipes* and *G. pallens;* an encapsulated review of their findings, expressed as percent mortality of adult *Geocoris* spp. exposed to pesticide residues, is presented in Appendix A.

ix. Exploring the Potential of *G. punctipes* in Greenhouse Systems

Numerous facets of an insect's biology, and its interactions with other organisms, must be understood before it can successfully be used in an Integrated Pest Management (IPM) program. For *G. punctipes*, some facets, such as understanding of basic biological needs, prey range, and insecticide compatibility, have been relatively well developed. Other facets, such as prey preference, numbers of prey species killed by individual *Geocoris* spp., and the ability of *Geocoris* spp. to suppress pest populations under controlled conditions in a greenhouse environment such as interactions with other predators that may occur in a system, have not been sufficiently explored.

The development of a suitable artificial diet for this insect has vastly improved the potential for mass rearing *G. punctipes*, but to date no automated method for packaging this diet has been developed (Cohen, personal communication). Further research is necessary before *G. punctipes* can be reared efficiently and economically for their subsequent use in agricultural or horticultural systems. Currently, *G. punctipes* is more expensive than several other predatory insects available from mass rearing facilities (Table 1.4). Of primary concern is automation of the rearing process, because it is believed that automation is key to keeping production costs low enough to make the product attractive to potential consumers (see Cohen 1993, Cohen et. al 1999, Smith and Nordlund 1999, 2000). A second concern is the availability of consistent, quality artificial diet (Cohen et al. 1999).

The potential for use of *G. punctipes* as a biological control agent in greenhouse systems is far less understood than its role in many field crops. Lack of interest in development of *Geocoris* spp. for use in greenhouses could be partly due to historical interest in other predators and parasitoids, which have been more fully explored in this respect. Another possible explanation is that the development of laboratory techniques to successfully rear *Geocoris* spp. is too

Table 1.4. Sources and prices of other insect predators available for retail sale (generated from internet searches).

Predator	Unit and price	Company name
Chrysoperla spp.	1,000 larvae $16.50	Rincon-Vitova
	1,000 larvae $20.00	Greenfire
	500 larvae $32.50	Heath's Organic
	650 larvae $32.00	IPM of Alaska
Coleomegilla maculata (De Geer)	250 adults 43.00	Rincon-Vitova
Cryptolaemus montrouzeri Mulsant	100 adults $35.95	Heath's Organic
	100 adults $41.40	IPM of Alaska
Harmonia axyridis (Pallas)	100 adults $48.00	Rincon-Vitova
	100 adults $49.00	IPM of Alaska
Hippodamia convergens Guérn-Méneville	500 adults $12.00	Rincon-Vitova
	9,000 adults $29.50	Heath's Organic
Rhyzobius lapanthae (Blaisdell)	100 adults $66.00	IPM of Alaska

recent for full exploration of this potentially useful predator to have been completed.

The overall goal of this research was to contribute to the development of *G. punctipes* as a possible biological control agent of selected pests of greenhouse crops. The specific questions addressed towards reaching this goal were: 1) How does the development and survival of *G. punctipes* reared on meat-based diet compare to its development on more traditional food alternatives?, 2) How many prey individuals of selected greenhouse pests will an adult *G. punctipes* consume in a given period?, and 3) Can *G. punctipes* be effective in suppressing populations of greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), in an ornamental crop in the greenhouse?

Chapter II

Development and Survival of *Geocoris punctipes* Reared on Selected Diets

i. Introduction

The importance of a nutritious and consistent artificial diet that produces quality predators is a key component in the automation of rearing predatory insects (Cohen 1993, Cohen et al. 1999, Smith and Nordlund 2000). Development of a suitable artificial diet is considered to be a major advance to the widespread use of a predatory big-eyed bug, *Geocoris punctipes* (Say), as a biological control agent (Cohen 1993). A meat-based artificial diet has recently been developed for *G. punctipes* by Allen Cohen and staff (USDA-ARS), and has been used to rear *G. punctipes* for more than 10 years and 100 generations (Cohen 1993, 2000), with greater numbers of adults resulting from each successive generation (Cohen 1985b, 1993).

Meat-based artificial diet is used in at least one of the commercial rearing facilities that currently offer *G. punctipes* for sale, where survival from egg to adult is estimated to be 40-50% (Biofac Crop Care, personal communication). When compared to the survival (68%) of *G. punctipes* reared on potato tubermoth, *Phthorimaea operculella* (Zeller) (Dunbar and Bacon 1972a), questions arise regarding the tradeoffs between ease of handling a diet and quality of the predator reared on the diet. To determine whether the artificial diet is truly reducing costs, the savings in time and labor associated with artificial diet must be compared to the cost (in lost product and sales) of the insects that die before completing development. A higher rate of survival on a traditional diet, resulting in greater quantities of salable product, may offset the costs associated with extra handling time and labor.

Previous to the development of meat-based artificial diet, laboratory colonies of *G. punctipes* were typically reared on lepidopteran eggs and early instar larvae (Champlain and Sholdt 1967a, Dunbar and Bacon 1972a, Crocker et al. 1975, Cohen 1984). *Geocoris* spp. have been reared successfully on other hosts. For example, the western big-eyed bug, *Geocoris pallens* (Stal), has been successfully reared on nymphs of the large milkweed bug*, Oncopeltus fasciatus* (Dallas), and also on sunflower seeds (Yokoyama 1980).

Aphids and whiteflies are not traditionally used to rear *G. punctipes* in laboratory colonies. While big-eyed bugs are known to feed on aphids and whiteflies, little information on the predator's biology when these pests are its sole source of nutrition is available (see Cohen 1992). It is unclear at this time whether *G. punctipes* can obtain all needed nutrition from a diet consisting solely of whiteflies, despite the fact that they may prey upon whiteflies over other insects when presented with a choice (Hagler and Naranjo 1994, Cohen and Brummett 1997). Evidence also suggests that *Geocoris* spp. may prefer some aphid species over other ones (Hagler and Cohen 1991). Therefore, incompetence of *G. punctipes* as a control agent of one aphid species should not preclude investigations of the effectiveness of big-eyed bugs in controlling other species of aphids.

Evidence suggests that the addition of sunflower seeds may improve the survival of big-eyed bugs on artificial diets. Plant materials including green bean and sunflower seed have been incorporated into artificial diets, sometimes resulting in better survival rates (Dunbar 1971, Tamaki and Weeks 1972a, Naranjo and Stimac 1985, Yokoyama 1980).

If an artificial diet is to be widely used to mass rear predatory insects, success in rearing the insects in the diet's laboratory of origin must be replicated in other environments. This research included both evaluation of the development and survival of *G. punctipes* reared on meat-based diet alone, as well as a comparison of *G. punctipes* reared on meat-based diet to those reared on other diets. In this study, various aspects of the big-eyed bug's biological development were evaluated for *G. punctipes* reared on meat-based diet under laboratory conditions modeled after those of Champlain and Sholdt (1967a,b) and Dunbar (1972). A second facet of the research compares development and survival of big-eyed bugs reared on meat-based diet to those reared on other diets including prey insects, plant tissue (sunflower seed), and a combination of both prey and plant tissue. Eggs of the beet armyworm, *Spodoptera exigua* Hübner, represent the traditional lepidopteran laboratory diet.

The specific objectives of this research were to compare the biological development of *G. punctipes* reared on six experimental diets including: 1) meat-based artificial diet, 2) beet armyworm eggs, 3) aphids, 4) whiteflies, 5) sunflower seed, and 6) whiteflies and sunflower seed. Emphasis was placed on the following biological characteristics: 1) development time for each immature instar, 2) survival through each instar, 3) overall survival to adulthood, and 4) total development time from hatch to adult eclosion. The initial objectives of this research also included evaluation of the fertility and fecundity of *G. punctipes* reared on the different diets in addition to the previously listed characteristics.

ii. Materials and Methods

Maintenance of Colonies. Five colonies of live insects were maintained during this research. In addition to the maintenance of big-eyed bugs, colonies of beet armyworms were reared to provide a traditional laboratory diet of lepidopteran eggs. Aphids and whiteflies were also offered as experimental diets. Aphids and whiteflies were collected from infested plants, or reared on host plants in field cages for future use. Specific information regarding the maintenance of insect colonies is outlined below.

Rearing Geocoris punctipes. Starter individuals for the *G. punctipes* colony were obtained from the Biological Control and Mass Rearing Research Unit of the USDA-ARS in Stoneville, MS, September 2000. These were later supplemented with insects purchased from Biofac Crop Care, Mathis, TX (May-July 2001), and field-collected individuals from experimental soybean and pepper plots (various varieties) on the University of Tennessee Plant Sciences Farm, Knoxville (June-December 2001). Insects were collected early in the season in soybean by use of a beat sheet (approx. 1 m^2) and aspirator (approx. 25 ml). *Geocoris* spp. were abundant throughout the growing season both on soybean and pepper plants, on the ground near the base of the plants, and on weeds near the crops in buffer strips.

Few *Geocoris* spp. were found in traditionally planted (bare soil, plants in rows) soybeans after the first hard frosts of the fall season. They continued to be abundant, however, in nearby soybean and pepper plots that had been planted with strips of black plastic as ground cover and mulch. Numerous individuals were found at the junction of the plastic strip and the soil line, around the bases of plant stems, and just under the plastic near the plant base. It was not necessary to use a beat sheet when collecting in the plastic-mulched crops.

Adults were housed in plexiglass cages (31 cm wide x 31 cm deep x 41.5 cm tall) with pieces (4 cm x 6 cm) of moistened sponge in either half of a petri dish (8 x 100 mm) as a water source (Figure 2.1). Two sections (approximately 10 cm^3) of Verticel, a corrugated material used in greenhouse cooling systems, were placed into each cage. The Verticel blocks provided hiding places for individuals to reduce cannibalism.

Small pieces of cotton ball were initially provided as an oviposition substrate. Because large numbers of hatching nymphs became entangled in the fibers of the cotton ball and died before freeing themselves, cotton balls were replaced with patches (5-6 cm^2) of white flannel fabric. Flannel patches were changed three times each week (daily during periods of peak populations, again to reduce cannibalism). Flannel patches proved to be a more suitable ovipostion substrate than cotton balls.

After ovipostion, flannel patches with eggs were placed into smaller cages, which served as a place for egg incubation, and later housed the hatching nymphs. Two sizes of small cages were used interchangeably: 1) 18 cm long x 12.8 cm wide x 7 cm tall, and 2) 20 cm long x 14 cm wide x 10 cm tall. Both adult

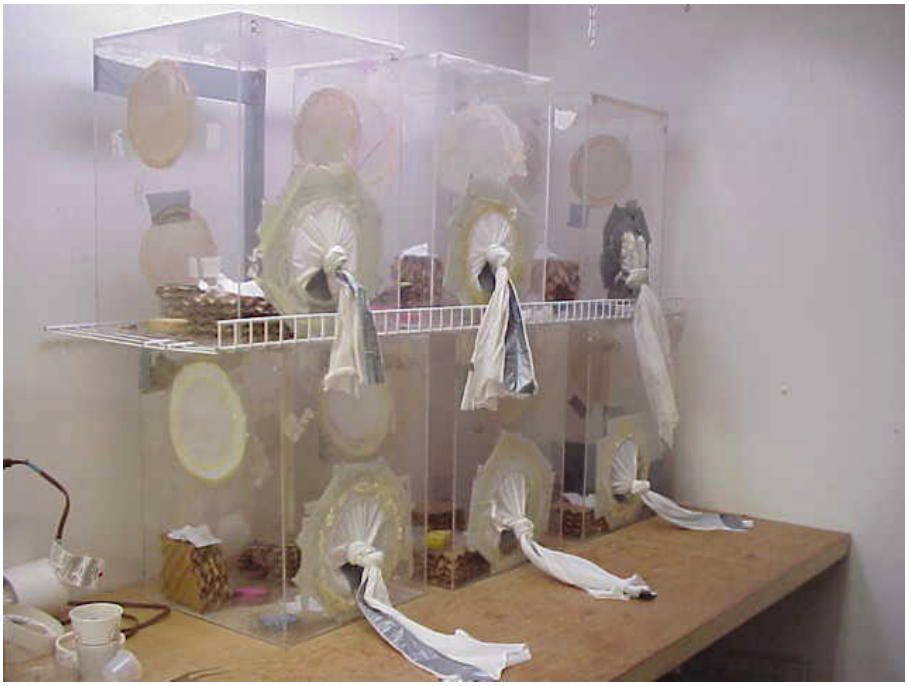

Figure 2.1. Laboratory environment for mass-rearing *Geocoris punctipes* on meat-based artificial diet.

and nymph cages had round holes cut in the sides (adult) or top (nymph), and covered with fine mesh cloth to allow ventilation. Holes in adult cages were approximately 12 cm in diameter, in nymph cages approximately 6 cm. As they hatched, the nymphs were divided into smaller groups to reduce cannibalism. Several densities of nymphs (beginning with 40 per cage, and adjusting down to 30, 20, and finally 15 nymphs per cage) were attempted, but cannibalism was observed at each density, including 15 per cage. Time and space constraints did not allow for separate cages for each individual nymph. Typically, the first individuals within a cohort to progress to a later instar preyed upon smaller individuals before or during the molt of the smaller insect.

Water for nymphs was initially provided by pieces (4 cm x 6 cm) of cut sponge, and later vials (10 ml) of water with cotton wicks, both of which presented problems for early instars. First and second instars, especially, seemed to drown on or near sponges if too much water was applied to the sponges and later condensed onto the cage itself. Small vials (10 ml) of water with cotton wicks were tried as an alternative. Each vial was wrapped with a small overlapping wire, providing “legs” intended to keep the vial from rolling around in the cage (Yokoyama 1980). The wire legs were not sufficient to prevent the vial from rolling on and crushing small *G. punctipes* if the cage was jarred or moved too quickly. The final and most successful water delivery method involved affixing the vial to the cage floor with a small piece of non-toxic modeling clay, which allowed the nymphs access to the water and prevented the vial from rolling over the insects.

***Geocoris punctipes* Reared on Meat-Based Diet.** Artificial meat-based diet was prepared every few weeks as outlined in Cohen and Smith 1998, frozen, and thawed before presentation to the insects. Batches of 1,500 ml of diet were prepared by using 4x the volume of all ingredients listed in the article. Originally the diet was presented to the insects in parafilm “envelopes” as described in Cohen and Smith 1998 (Figure 2.2). These packets were stretched after thawing,

Figure 2.2. Presentation of meat-based artificial diet as outlined in Cohen and Smith (1998).

upon presentation of the diet to the insect, to facilitate insertion of the insects' stylets. The packets were difficult to seal well enough to prevent leaking, which caused pools of liquid to settle near the food packets and contamination (i.e., mold) in the cages. Leaking was fatal to early instars, as they often became stuck in the leaked diet. These problems, as well as numerous packets breaking during the stretching process resulting in an unacceptable amount of wasted diet, led to the development of an alternative method of presenting the diet without changing the ingredients or the process by which the diet was made.

In the alternative presentation, diet was pushed through the cut corner of a heavy-duty plastic bag (2 l) onto a round cardboard disc (Figure 2.3a). Parafilm was stretched over the diet and disc (Figure 2.3b), then smoothed evenly over the disc, creating a "plate" of the diet, which could be presented to the insects or frozen for later use (Figure 2.3c). The alternative presentation resulted in more uniformly stretched parafilm, greatly reduced leaking, and took 50% less time to produce than the original method. When the parafilm occasionally ripped during the wrapping process, it was possible to patch tears easily without leaking or loss of diet material (Figure 2.3c).

Two disc sizes were used. Cardboard coasters (10 cm diameter), obtained from a local beverage distributor, were used for the large adult cages. The large discs held approximately 15 g of diet and were covered with a piece of parafilm (7.5 x 5 cm before stretching). Smaller discs (4.5 cm diameter) were formed from disposable, thin cardboard lids for beakers (available from Fisher Scientific Products), and were used for feeding cohorts of immature *G. punctipes*. A small pull-tab was removed from the side of each small disc before it could be used to present the diet. Small discs received about 4 g of diet before being covered with a piece of parafilm (3.75 x 5 cm before stretching). Parafilm was stretched to approximately 3x its original size before being wrapped around the diet and disc, then sealed around the bottom of the disc. Wax or glassine weighing paper

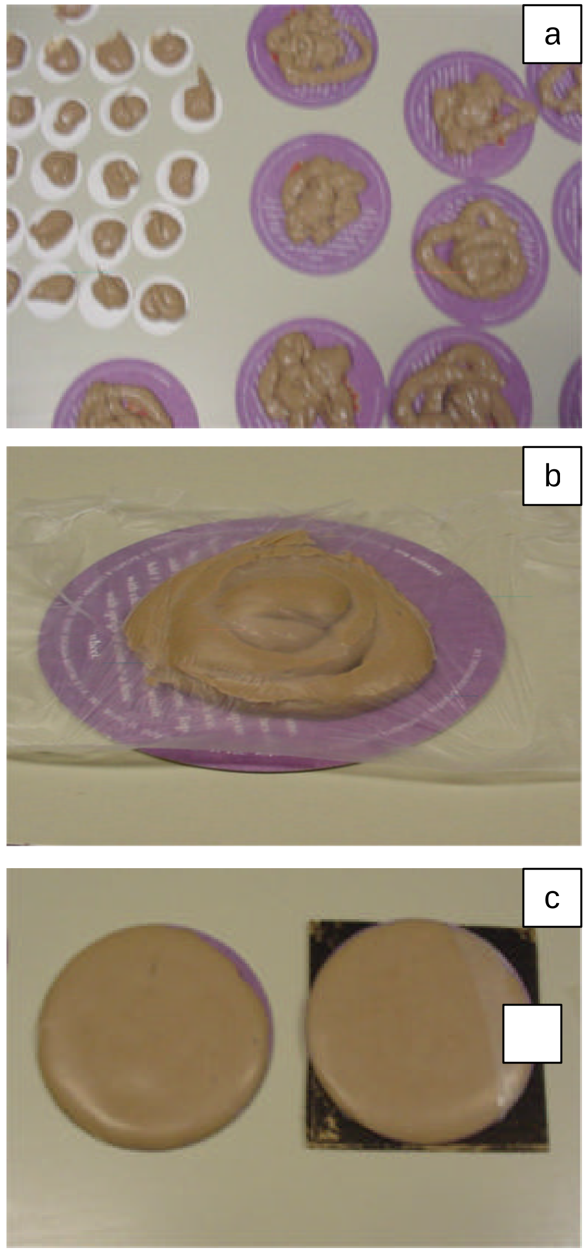

Figure 2.3. Presentation of artificial diet material used to rear *Geocoris punctipes*: a) Diet material squeezed onto cardboard discs of various sizes, b) Pre-stretched parafilm over diet and cardboard disc, and c) Complete diet disc ready for presentation to insect (left) and small tear has been repaired with additional strip of parafilm (right).

was placed between the layers of discs before freezing to keep them from adhering to one another. Details of this alternative presentation were made available to Dr. Cohen and co-workers.

Artificial diet was presented to *G. punctipes* in our colony three times weekly. The literature on meat-based artificial diet is vague about how much diet material should be provided for each insect. In our insectary, one large diet disc (15 g) was used for each 50 adults in a large cage (up to approximately 150 individuals per large cage) to maintain the general colony. Small diet discs (4 g) were used in the nymph cages at the rate of one disc per eight early (first and second) instars and one disc per five later instars. Diet was provided three times each week.

Rearing the Beet Armyworm, Spodoptera exigua. A colony of beet armyworm was reared to provide eggs as an experimental diet for *G. punctipes*. Starter insects for this colony were provided by John Ruberson (University of Georgia, Entomology Department, Athens, TN) in Fall 2000, and multiple, consecutive generations were reared for the duration of this research. Beet armyworms were reared in a growth room where the temperature was maintained at 25 ±5°C (77º F), with a photoperiod of 14L:10D.

Larvae were reared on a modified version of the pinto bean diet described in Greene et al. (1976). One batch (3,000 ml) of bean diet provided enough diet for 40-45, 190 ml, waxed paper cups cups with approximately 70 ml of diet in each. About 15-20 newly-hatched beet armyworm larvae were placed into each cup.

As they developed, pupae were collected from the bean diet (28-35 days after being placed into the cups), and transferred into 3.8 liter glass jars. Approximately 40 pupae were put into each jar, which served as the adult habitat. When the adults emerged about 7-10 days later from the pupal cases, they were provided cotton balls saturated with honey water (10% honey in water solution) for moisture and food. Paper towels lined the bottom of each jar. Two strips (25 cm long x 11.5 cm wide) of paper towel were hung from the top of the jar to provide oviposition sites. Paper towel was also used to cover the jar and provided additional oviposition substrate. The covering towel and oviposition strips were secured to the lip of the jar with a rubber band.

Jars were checked at least three times each week for eggs, which were collected to serve as an experimental diet. Eggs collected from the paper towel strips and jar cover were frozen until needed to feed nymphs of *G. punctipes*. Other eggs, especially those laid on the sides of the glass jars and on the dishes containing cotton balls, were allowed to hatch, and these larvae were collected and transferred to bean diet to perpetuate the colony.

Rearing Aphids and Whiteflies. Green peach aphids, *Myzus persicae* (Sulzer), red aphids, *Uroleucon nigrituberculatum* (Olive), oleander aphids, *Aphis nerii* Boyer de Fonscolombe, and greenhouse whiteflies, *Trialeuroides vapoariorum* (Westwood), were collected from greenhouses and ornamental plantings on the campus of the University of Tennessee and from local greenhouse growers. Several short-lived colonies of green peach aphids and whiteflies were maintained on caged plants inside our insectary on the University of Tennessee Plant Sciences Farm during Fall 2000 and Spring 2001. In June 2001, three field cages (122 cm wide x 152.5 cm deep x 244 cm tall) were erected outside the insectary at the University of Tennessee Plant Sciences Farm. Two cages contained snapdragons, *Antirrhinum majus* L., and a third cage contained tomato plants, *Lycopersicon esculentum* L. variety “Roma”. The snapdragons sustained a small number of green peach aphids until September 2001, but numbers of caged aphids were not always sufficient to meet all research needs and had to be supplemented, when possible, with green peach aphids collected from greenhouse infestations on the University of Tennessee campus. Tomato plants served as a whitefly habitat and sustained a population of whitefly throughout

Summer 2001. Occasionally, whiteflies collected from greenhouse infestations were added to the cage to augment the population.

Evaluation of Development and Survival of Geocoris punctipes Reared on Meat-based Diet. The objective of this research was to evaluate several aspects of the biology of *G. punctipes* reared on meat-based artificial diet. Over 3,780 individual *G. punctipes* were evaluated in this study between April and July 2001. Nymphs were housed in plexiglass cages (either 18 cm long x 12.8 cm wide x 7 cm tall or 20 cm long x 14 cm wide x 10 cm tall). The two sizes were used interchangeably as there were not enough of either size to accommodate all the insects. Nymphs were housed in these plexiglass cages for the duration of the experiment and were the offspring of *G. punctipes* reared on artificial diet in our original laboratory colony. Nymphs were housed in groups of 15, 20, 30, and 40. Cannibalism was a problem at every density, but lack of space and cages made it necessary to house more than the optimal number of nymphs in a single cage.

For each cage of *G. punctipes* nymphs, the following information was recorded: number of days to hatch, number of days spent in each instar, percent of individuals that survived to each instar, number of individuals to reach adulthood, and total development time. Cages were monitored at least five times each week. A cage was recorded as having reached the next instar when greater than 50% of the individuals in the cage had progressed to the next instar. Because of budgetary, space and time constraints it was impossible to maintain individual cages for each insect so that the exact length of progression to the next instar could be determined for each individual.

Comparative Assessment of Geocoris punctipes Reared on Meat-based and Alternative Diets. The objective of this research was to compare the survival, fertility, and fecundity of *G. punctipes* reared on different diets. The diets used included : 1) meat-based artificial diet, 2) beet armyworm eggs, 3) green peach aphid, 4) whitefly, 5) whitefly and sunflower seed, and 6) sunflower seed only.

Individual *G. punctipes* were hatched from eggs collected from the laboratory colony reared on meat-based artificial diet. Upon hatching, nymphs were divided into groups of 15 and housed in white cardboard cylindrical containers (90 mm wide x 100 mm tall). A light, tightly woven mesh fabric, which allowed air circulation and light penetration and prevented the escape of nymphs from the experimental chamber, was placed over each container and secured with the outer portion of the original paper lid of the container. Water was provided in vials (10 ml) with cotton wicks and wire stabilizers to prevent the vials from rolling over small nymphs. Flannel patches were provided to provide hiding places for the nymphs.

One of the six experimental diets was assigned to, and placed in, each container of nymphs. Each diet was replaced every 2-3 days as it was consumed. For artificial meat-based diet, two small (4 g, 4.5 cm) discs were provided in each cage and replaced three times each week. Beet armyworm eggs were presented on the small pieces of paper towel on which they had been laid. Approximately ten average size egg masses (approximately 100 eggs per mass) were presented in each cardboard container. This number varied slightly when egg masses were especially small or large.

Tomato leaves with whitefly pupae were collected every 2-3 days and presented to the nymphs in the cardboard containers. Sunflower seed was shelled, raw, and unsalted. For the diets containing both insects and sunflower seed, approximately 5 g (one tablespoon) of sunflower seeds were added to the bottom of each cardboard container and were not replaced during the duration of the experiment. For the diet consisting only of sunflower seeds, approximately 10 g of sunflower seed were placed into the bottom of each container and were not replaced for the duration of the experiment.

Each cylindrical carton of nymphs was monitored at least five times a week and food replenished as needed. The following information was recorded: 1) number of days spent in each instar, 2) percent of individuals that survived each development stage, 3) number of individuals to reach adulthood, and 4) total development time. A cohort of nymphs was considered to have reached the next development stage (instar) when greater than 50% of the individuals in the cage had changed to the next instar. Because of budgetary, space and time constraints, it was impossible to maintain individual cages for each insect so that exact times could be determined.

The intended scope of this experiment included comparing the fecundity of the adult females that developed on the various feeding regimens. However, several diets only too few adults for a sound statistical analysis. Among the problems encountered were: 1) the few males and females did not eclose close enough to one another in time, 2) one of a mated pair died before the female laid any eggs (preovipostional period lasts several days), 3) Only a single adult, or 2 of the same sex, were produced. On one diet, whitefly and sunflower seed, adults produced viable eggs that resulted in offspring, but too few eggs or young were produced to provide information regarding fertility or fecundity of *G. punctipes* reared on these diets.

Data Analysis. Analysis of variance (ANOVA) (SPSS 2001) was used to detect differences among the diets. Tukey's HSD was used to compare the means. General linear model was used to determine instar lengths, and ANOVAs were used to detect differences in development times for the different instars and diets. Separate ANOVAs were run for development from hatch through each successive instar (second, third, fourth, and fifth) and total development time from hatch to adult eclosion.

iii. Results and Discussion

Evaluation of Development and Survival of Geocoris punctipes Reared on Meat-based Diet. Survival to adulthood of *G. punctipes* reared on meat-based was poor (ranging from 2 to 20% over the four-month period) (Figure 2.4). This survival rate would not be sufficient to allow for profitable commercial production of this insect. However, mortality of *G. punctipes* was attributable to several factors that were not related to diet. Numerous early instars drowned in condensation or water droplets within the cages. Cannibalism was a major problem, and larger individuals within a cohort were frequently seen with a smaller nymph impaled on the stylets, or feeding upon its carcass. Cannibalism is a recurring problem in other reports on mass-rearing of *G. punctipes.* Even though artificial meat-based diet is nutritionally designed to meet the needs of this insect, perhaps instinct dictated that the big-eyed bugs pursue moving prey over choosing artificial food.

Inexperience of the insect-rearing staff also played a role in the high mortality of big-eyed bugs reared on this diet. Combined with mortality due to drowning and cannibalism, these data probably do not reflect an accurate picture of the potential for *G. punctipes* to survive on an artificial meat-based diet. In addition, other laboratories report at least a 40% success rate on the diet, indicating that a better success rate is possible (Biofac Crop Care, personal communication).

Because of the overall low survival and mortality due to factors other than diet, no further evaluation was made of the survival and development of this series of *G. punctipes* nymphs. Further investigations into the survival and development of big-eyed bugs reared on artificial meat-based diet should begin with a water source that is safe for young instars, and an ideal situation would include individual cells or cages, or at least low densities of the insect, to reduce cannibalism.

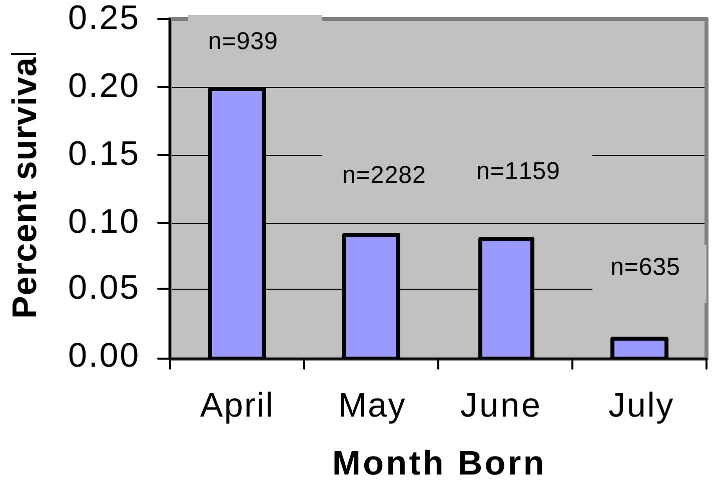

Figure 2.4. Percent survival (to adulthood) of *Geocoris punctipes* reared on meat-based artificial diet (April- July, 2001).

Because of the overall low survival and mortality due to factors other than diet, no further evaluation was made of the survival and development of this series of *G. punctipes* nymphs. Further investigations into the survival and development of big-eyed bugs reared on artificial meat-based diet should begin with a water source that is safe for young instars, and an ideal situation would include individual cells or cages, or at least low densities of the insect, to reduce cannibalism.

Survival to Adulthood. Survival to adulthood varied significantly (p<0.001, df=5, 65, F=14.05) among the six diets. The combination of whitefly and sunflower seed was the most successful diet with an average of 40% of the nymphs surviving to adulthood, and was significantly different from all other diets (Figure 2.5). Survival to adulthood for the five remaining experimental diets was extremely low (from 0.01% for sunflower seed to 5.10% for aphid), and were not significantly different from one another (Figure 2.5).

The survival of big-eyed bugs to adulthood when reared on meat-based artificial diet was 3.10%. These data suggest that meat-based artificial diet is not superior to other diet alternatives for rearing the predator *G. punctipes*. However, as in the previous experiment, there was some mortality due to cannibalism and the aforementioned problems with water delivery. Further research is needed to clarify the usefulness of meat-based artificial diet for *G. punctipes*. An ideal scenario would include individual cages for each predator or extremely low densities of big-eyed bugs in experimental cages to reduce mortality by cannibalism.

As suggested in other published reports, a combination of plant and insect material seems to be ideal for the survival of *G. punctipes*. It was learned during the course of this experiment that in the laboratory where meat-based artificial diet was developed, green beans are often used as an additional source of moisture for big-eyed bugs. It is possible that the insects are gaining nutrition

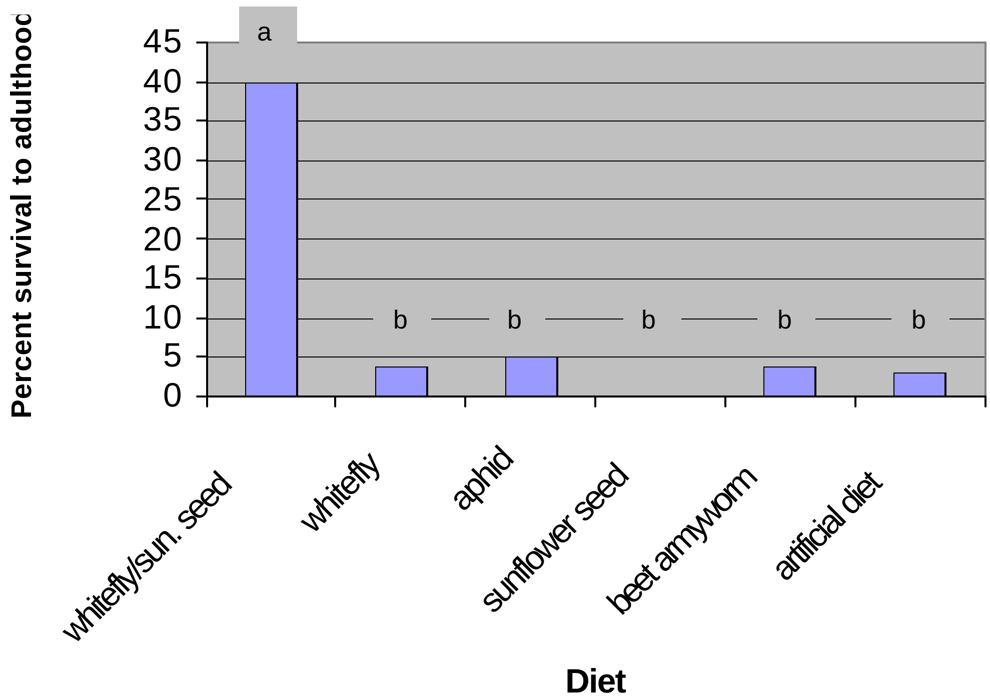

Figure 2.5. Percent survival to adulthood of *Geocoris punctipes* nymphs reared on selected diets. Bars with the same letter are not significantly different from one another (p=0.05).

from the green beans as well as moisture. Further study comparing the survival of big-eyed bugs mass-reared on artificial diet with and without the addition of green beans or sunflower seeds may help to determine if the addition of these items improve survival of big-eyed bugs on artificial diet.

Development Time. Diet played a significant ($p<0.001$, df=5, 21, F=15.63) role in the total development time from hatch to adult. The whitefly and sunflower seed diet again stood out separate from the other diets, with the shortest average total development time (egg hatching to adulthood) of 23.60 days (Figure 2.6). Big-eyed bugs reared on either aphids or sunflower seeds only had the longest overall development times, averaging 35.00 and 30.50 days, respectively, with development times for other diets falling within these two extremes (Figure 2.6).

To further consider the impact of various diets on the development of *G. punctipes,* development times through each successive instar were also recorded. No clear pattern of diet influence on nymphal development was detected when each instar was considered separately. Differences in development time due to diet were detected for some instars (first and fifth), but not for others. Length of the first instar had the greatest variation due to diet type. There were significant ($p<0.001$, df=1,5, F=9.02) differences among the diets, with mean development time to second instar ranging from 4.5 (aphid) to 7.0 (sunflower seed only) days (Figure 2.7). The combination whitefly and sunflower seed diet, while producing the most adults, did not allow a shorter development time for the first instar. Big-eyed bugs fed whitefly and sunflower seed averaged 6 days to the second instar.

No significant differences were detected for development time through the third ($p=0.89$) or fourth ($p=0.19$) instars. However, differences were detected for development time to fifth instar ($p<0.001$, df=1,5, F=8.85). By this ime, big-eyed bugs reared on a combination of whitefly and sunflower seed again showed signs of faster development, with the shortest development time to the fifth instar,

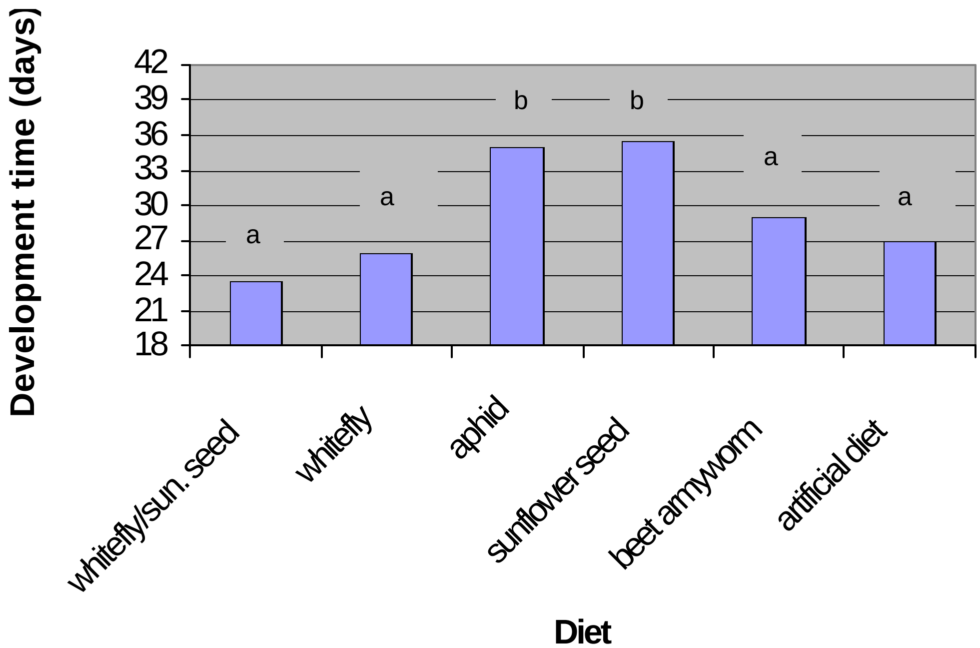

Figure 2.6. Development time (days) of *Geocoris punctipes,* from hatch to adulthood, when reared on different diets. Bars with the same letter are not significantly different from one another (p=0.05).

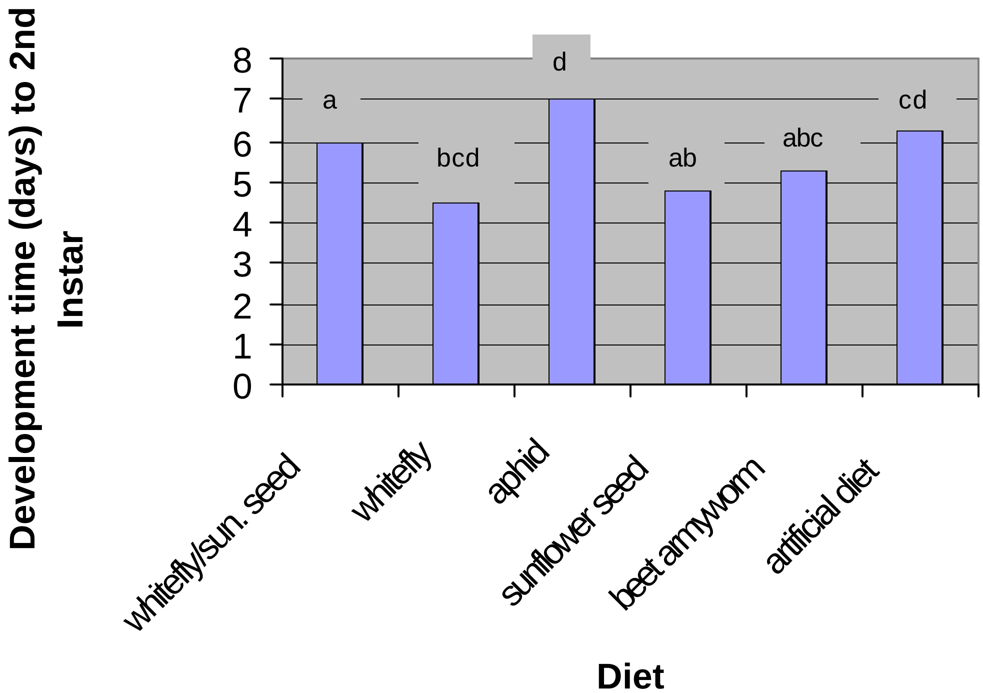

Figure 2.7. Development time (days) to second instar for *Geocoris punctipes* reared on various diets. Bars with the same letter are not significantly different from one another (p=0.05).

17.6 days from hatching (Figure 2.8). This shorter development time was significantly different only from the aphid diet ($p<0.002$) and the sunflower seed diet ($p<0.001$), which had mean development times to the fifth instar of 27.0 and 27.4 days, respectively.

Big-eyed bugs reared on the combination of whitefly and sunflower seed had the fastest overall development time (23.6 days) from hatch to adulthood (Figure 2.6). However, the combination diet was significantly different only from the aphid diet and the sunflower seed diet. Diet significantly influenced the development time of the fourth and fifth instars, but no differences were detected for other instars. Because the addition of sunflower seed caused such a dramatic increase in survival over the diet of whitefly alone (from 3% to 40%), perhaps survival of *G. punctipes* reared on artificial diet could be increased by the addition of sunflower seeds.

Fertility and Fecundity. The original scope of this research included evaluation of the fertility and fecundity of female *G. punctipes* reared on the different diets. However, while all diets produced at least one adult, in most cases there was not a male and female that eclosed as adults close enough to one another in time to be used in the study. In other cases, two adults were paired for evaluation but one or both of the insects died before any eggs were laid. However, 18 adults reared on the combination of whitefly and sunflower seed produced 143 eggs resulting in 86 live nymphs (60% hatch). The production of second generation nymphs on the whitefly and sunflower seed diet, but not on other diets, further supports the superiority of the combination of plant and insect material as the ideal combination for nutrition of *G. punctipes*.

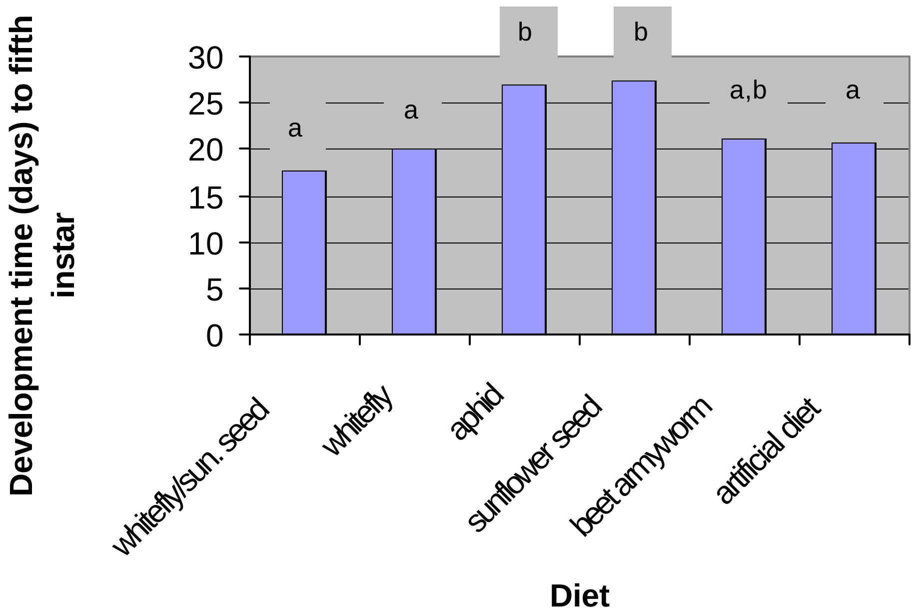

Figure 2.8. Development time (days) to fifth instar for *Geocoris punctipes* reared on various diets. Bars with the same letter are not significantly different from one another.

iv. Summary

The availability of a nutritious, economic, and palatable diet is of major importance to those who would mass rear insects for a profit. Use of an artificial diet eliminates the need for the mass-rearing facility to rear a second insect colony as prey for the predator. Recently, a meat-based artificial diet used for *G. punctipes* was developed by USDA-ARS in Stoneville, MS, and successive generations of the insect have been reared on the diet. However, for the artificial diet to be truly marketable, it must also be usable by mass-rearing facilities and others outside the originating laboratory with similar survival of the insects. In addition, concern that insects reared on artificial diets will not have the same vigor or predatory capabilities as their feral counterparts suggests the need for further investigation.

The objectives of this research were to evaluate the development and survival of G. *punctipes*, a beneficial predatory insect, when reared on artificial meat-based and other experimental diets in the laboratory. Other experimental diets evaluated included lepidopteran eggs (a traditional laboratory diet), and diet regimens of pest insects, sunflower seeds, and combinations of pests and sunflower seeds. Newly hatched big-eyed bugs were separated into cohorts of 15 and each group fed one of six experimental diets. Survival and development time to each successive instar were recorded.

Big-eyed bugs reared on the combination of whitefly and sunflower seed had a much greater survival rate (40%) compared to any other diet. These findings are supported by other research, which indicate that a combination of both plant and insect material is the most suitable for G. *punctipes*. No significant differences were detected among the other diets for survival to adulthood.

Big-eyed bugs reared on meat-based artificial diet in our study did not fare as well as those reared on whitefly and sunflower seed. In fact, the only diet with a survival rate lower than that of artificial meat-based diet was sunflower seed. However, for all diets, some mortality was caused by problems unrelated to diet such that further research is needed to contribute a better understanding of the role of artificial meat-based diet as a food source for mass-reared *G. punctipes*. Further research should also investigate the possible addition of sunflower seed or other plant material (i.e., green bean) to artificial meat-based diet. This combination may increase survival of mass-reared *G. punctipes* or otherwise aid in its speedy development, which, in the case of a mass-rearing facility, could mean better turnover of product and profit potential.

Chapter III

Predation of Mass-Reared *Geocoris punctipes* Against Selected Greenhouse Pests

i. Introduction

The predator *Geocoris punctipes* (Say), a big-eyed bug, has been observed feeding on dozens of different prey species (Crocker and Whitcomb 1980) in field crops. While considered an important predator in many agroecosystems, relatively little information is available regarding the rate of predation of big-eyed bugs against specific pest species. More information is available regarding lepidopteran pests than any other group.

Predation by *Geocoris* spp. on lepidopteran eggs and larvae have been reported in several publications (Table 3.1). While other reports suggest that *Geocoris* spp. are important predators of aphids and whiteflies, few indicate specific predation rates against these pests. Eubanks and Denno (2000a) reported that adult big-eyed bugs consumed 4.5 pea aphids, *Acyrthosiphon pisum* (Harris), in an 18-hour period, while predation against green peach aphid, *Myzus persicae* (Sulzer), was approximately 2.3 aphids per day (Tamaki and Weeks 1972b).

Efforts to determine if mass-reared big-eyed bugs have similar feeding habits to wild individuals have been conducted using several pest species: lygus bug, *Lygus hesperus* (Knight); oleander aphid, *Aphis nerii* (Boyer de Fonscolombe); pea aphid, *A.pisum*; tobacco budworm, *Heliothis virescens* (F.); corn earworm, *Helicoverpa zea* (Boddie); and beet armyworm, *Spodoptera exigua* (Hübner) (Champlain & Sholdt 1966, Dunbar and Bacon 1972a, Richman et al. 1980, Hagler and Cohen 1991, Cohen 2000). No significant (p=0.10)

Table 3.1. Published reports of predation of lepidopteran eggs and larvae by male and female *Geocoris punctipes* in a laboratory setting.

Prey species	Number prey consumed	Publication
corn earworm, *Helicoverpa zea* (Boddie) (eggs)	♀ 9.3, ♂26.7 per day	Lindgren et al. 1976
soybean looper, *Pseudoplusia includens* Walker (eggs)	9.9±2.0[a] per day	Richman et al. 1980
tobacco budworm *Heliothis virescens* (F.) and *H. zea* combined (eggs)	♀ 30.0±13.8, ♂ 51.0±20.7 per 2 days	Lopez et al.1976
H. virescens (larvae)	62.4±6.4 per day	Chiravathanapong and Pitre 1980
P. includens (larvae)	5.3±0.7 per day	Richman et al. 1980
H. zea (larvae)	♀ 26.5, ♂31.9 per day	Lindgren et al. 1976

[a] $\bar{x}$ ± SE (standard error).

differences between mass-reared and field-collected *G. punctipes* in prey consumption or in prey preference among the consumption or in prey preference among the pest species tested were observed (see Hagler and Cohen 1991). It is desirable for mass-reared predators to have predatory behavior consistent with that of wild individuals if mass-reared insects are to be useful in augmentative releases (Hagler and Cohen 1991).

The objectives of this research were to determine: 1) if *G. punctipes* mass-reared on a meat-based artificial diet will feed upon selected greenhouse and ornamental pests (e.g., green peach aphid, a red aphid, *Uroleucon nigrituberculatum* (Olive), and oleander aphid), 2) the number of selected prey that *G. punctipes* will kill in a 24-hour period, and 3) whether mortality rates for the prey of mass-reared individuals are similar to that of wild individuals for these pest species.

ii. Materials and Methods

Prey Species. Three pest species were evaluated as prey of *G. punctipes* : 1) green peach aphid, 2) *U. nigrituberculatum* (red aphid), and 3) oleander aphid. Green peach aphid was collected from infestations on various ornamental plants and weeds in greenhouses on the University of Tennessee Agriculture Campus. Red aphids were collected from ornamental *Solidago* spp. located outside the Department of Botany at the University of Tennessee. Oleander aphids were collected from milkweed, *Asclepias syriaca* L., plants in the University of Tennessee Institute of Agriculture Gardens and from an unidentified vine in the Department of Botany Gardens at the University of Tennessee. Locating, collecting, transferring the prey species to stock plants, and attempting to maintain colonies of the prey species necessary to complete these experiments, were a limiting factors in the success of this research. Attempts to transfer prey to available host plants were often only partly successful, and limited numbers of aphids were available for the experiment. Stress to the aphids, which often had to be collected during the heat of the day, may have contributed to high numbers of prey mortality. Further details regarding the culturing of prey species for experimental use is provided in Chapter II.

Predation by Mass-reared G. punctipes on Greenhouse Pests. Individual *G. punctipes* for this study were hatched from eggs collected from a laboratory colony, reared on artificial meat-based diet. Detailed information regarding rearing *G. punctipes* on meat-based diet is provided in Chapter II. Individuals were collected as they eclosed as adults and used within three days of eclosion so that individuals of similar ages were compared.

More than 50 repetitions were conducted using the three prey species on 22 days from July through October 2001 (n=642). The number of repetitions conducted on a given day ranged from 1-4 based on the number of predator and prey available. Each repetition included 12-20 *G. punctipes*: at least 6 each, and usually 10 each, male and female, newly eclosed (less than three days) *G. punctipes.* Treatments consisted of either: a) a predator and 15 individual prey or b) a control with 15 prey individuals (no predator). Controls were used to account for any prey that may have expired as a result of the stress of collection, transportation to the laboratory, or transfer to the experimental arena.

Individual *G. punctipes* were sexed and placed into petri dishes (15 x 100 mm), which had been lined with moistened filter paper. Big-eyed bugs were starved in these dishes for 24 hours. Each big-eyed bug was then transferred to a dish containing moistened filter paper and 15 prey individuals. After a second 24-hour period, the big-eyed bug was removed, and the number of prey killed was recorded. In a few cases, some of the aphids produced progeny during the 24-hour experimental period, resulting in greater than 15 aphids. In these cases the newborn aphids were ignored. Only the number of dead aphids was recorded. Because the newly emerged aphids were easy to spot, and were not counted, it is unlikely that their presence impacted the outcome of the experiment. Predation by male and female *G. punctipes* was recorded separately.

Predation by Mass-reared vs. Field-collected G. punctipes on Selected Greenhouse Pests. To compare the predatory rates of *G. punctipes* mass-reared on artificial diet to those of field-collected individuals, feral *G. punctipes* were first collected from soybean fields (several varieties) at the University of Tennessee Plant Sciences Farm by use of an aspirator (approx. 25 ml). Field-collected, late fifth-instar nymphs were held in plexiglass cages (18 cm long x 12.8 cm wide x 7 cm tall or 20 cm long x 14 cm wide x 10 cm tall), and fed meat-based artificial diet for the few days until adult eclosion.

Mass-reared and field-collected adults were then starved for 24 hours and placed into petri dishes (15 x 100 mm) with moistened filter paper and 15 individual green peach or red aphids. Adults were used within three days of eclosion so that similar ages were compared. After 24 hours, the number of prey killed was recorded. Four repetitions, each with 12-20 newly eclosed *G. punctipes* per repetition, were conducted between 4 and 13 September, 2001 (n=71). Predation by males and females was evaluated separately.

Data Analysis. ANOVA (SPSS 2001) was used to test for differences in prey killed among pest types, gender, and origin (field-collected or mass-reared). Tukey HSD was used to compare means. A t-test was used to test for equality of means.

iii. Results and Discussion

Predation by Mass-reared G. punctipes on Greenhouse Pests. *Gecocoris punctipes* mass-reared on an artificial meat-based diet successfully killed all three prey species. Mass-reared *G. punctipes* killed significantly more green peach aphids than red aphids or oleander aphids ($p<0.0001$, df=2, 490, F=21.80), (Table 3.2). No significant differences in prey mortality were detected between the red aphids and oleander aphids. On average and irregardless of sex, big- eyed bugs killed 6.14 green peach aphids, 4.43 red aphids, and 3.62 oleander aphids during a 24-hour period. The lower number of oleander aphids killed is not surprising because these aphids are aposematically colored and contain oleandrin, a distasteful and potentially harmful glycoside (Hagler and Cohen 1991). The significantly larger number of green peach aphids killed is promising from a biological control standpoint because the green peach aphid is a common pest of a large number of greenhouse crops and are resistant to many insecticides (Blackman and Eastop 1984).

Across all prey species, females killed significantly more prey than did males ($p<0.001$, df=1, 490, F=11.51) (Table 3.2, Figure 3.1). For all prey species combined, females and males killed an average of 5.3 and 4.1 aphids, repsectively. Differences in mortality by males and females varied by prey species. The number of green peach aphids killed by males and females was not significantly ($p=0.189$) different. However, female big-eyed bugs killed significantly more red aphids ($p=0.030$) and oleander aphids ($p=0.008$) than did males. These results are expected because female insects are believed to have higher nutritional requirements associated with developing and laying eggs. Crocker et al. (1975) reported that female fifth instar and adult *G. punctipes* consumed significantly ($p=0.0001$) more prey than males, and focused only on female *G. punctipes* in later feeding studies (see also Richman et al. 1980).

Although females consumed more prey, no indication that male and female *G. punctipes* were choosing different prey species to feed upon (i.e., females preferring one species and males preferring another) was detected. No significant ($p=0.634$) differences were found between male and female *G. punctipes* for prey species preference. While females killed numerically more

Table 3.2. Mean number (± SE) of prey consumed by mass-reared *Geocoris punctipes* reared on artificial meat-based diet in 24 hours. *

Prey Species	n	Male *G. punctipes*	Female *G. punctipes*	Combined sexes	p value, difference between sexes
green peach aphid, *Myzus persicae*	69	5.77±0.42a	6.53±0.40a	6.14±0.25a	0.19
red aphid, *Uroleucon nigrituberculatum*	92	3.73±0.48b	5.16±0.40b	4.43±0.34b	0.03
oleander aphid, *Aphis nerii*	92	2.94±0.41b	4.30±0.31b	3.62±0.32b	0.01

* Means within a column followed by different letters are significantly different (p=0.05, t-test); SE= standard error.

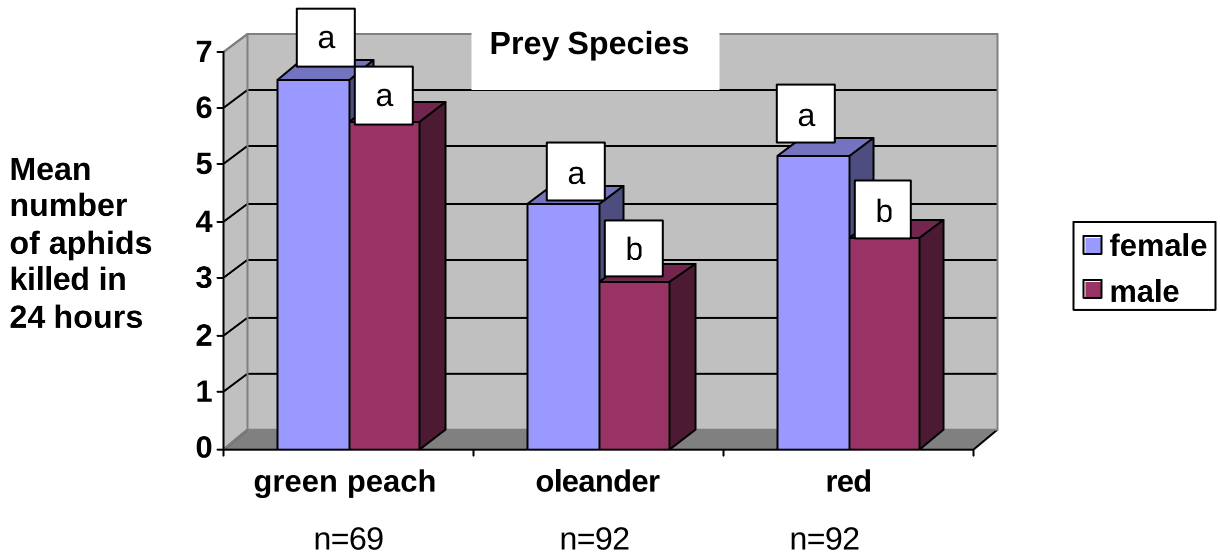

Figure 3.1. Number of aphids killed by female and male mass-reared *Geocoris punctipes* (reared on meat-based artificial diet) in 24 hours in the laboratory (p=0.05, Tukey HSD). (Bars within a prey species with the same letter are not significantly different.)

prey than did males, the overall proportions of green peach aphids to red to oleander aphids killed were similar for both males and females.

Further research is needed to determine the upper limits of the predatory capability of *G. punctipes* against these species. These data suggest that big-eyed bugs may have killed more aphids, if they had been available in the experimental arena. A substantial percentage (18.9%) of the petri dishes contained 15 dead aphids 24 hours after the big-eyed bug was introduced into the dish. Almost one-half (46.8%) of the dishes contained 10 or more dead aphids after the experimental period (although some of these aphids undoubtedly died of stress during collection and transfer to petri dishes). These data suggest that big-eyed bugs in this study may not have realized their maximum predatory potential when only 15 prey individuals were introduced.

Predation by Mass-reared vs. Field-collected G. punctipes on Selected Greenhouse Pests. Analysis of variance revealed no significant (p=0.644, df=1, 426, F=0.21) differences in prey killed by mass-reared or field-collected *G. punctipes* for green peach aphid and red aphid combined. Mass-reared and field-collected *G. punctipes* killed 5.25 and 5.05 aphids, respectively. These results are similar to those reported by other researchers (Cohen 2000, Hagler and Cohen 1991). For example, Hagler and Cohen (1991) found no significant differences (p= 0.10) between mass-reared *G. punctipes* and field-collected individuals in choice tests using several prey species including an aphid and other pests. It is desirable for mass-reared insects to have predation habits similar to their feral counterparts if the mass-reared insects are to be effective as biological control agents.

When the two pest species were analyzed separately using an independent samples test (Figure 3.2), however, statistical differences in predation were found. Mass-reared *G. punctipes* killed significantly (p=0.01,

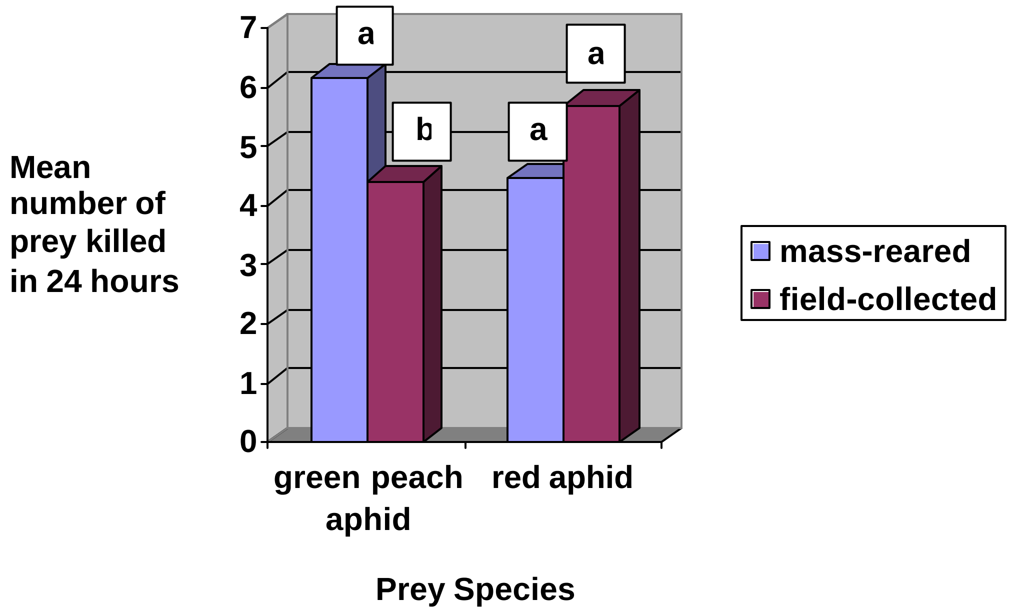

Figure 3.2. Number of prey killed by mass-reared *Geocoris punctipes* reared on meat-based artificial diet (n=340) and field-collected (n=87) *G. punctipes* in 24 hours in the laboratory. Bars within a prey species with different letters are significantly different (p=0.05, Tukey HSD and t-test).

df=266, F=0.24) more green peach aphids than did field-collected individuals. Conversely, field-collected *G. punctipes* killed slightly (p=0.06, df=157, F=0.40) more red aphids than did mass-reared *G. punctipes*. It is possible that these differences would not have been detected if a larger sample size were used. When both prey species were combined, no differences were detected. Further research would likely clarify the differences, if any, in predation by mass-reared and naturally occuring *G. punctipes*.

iv. Summary

Adult big-eyed bugs reared on artificial meat-based diet preyed upon green peach aphid, red aphid, and oleander aphid in the laboratory. Big-eyed bugs killed significantly more green peach aphids than the other two prey species. Because the green peach aphid is a common pest of greenhouse and ornamental crops, these results suggest the possible use of mass-reared big-eyed bugs as biological control agents in ornamental crops against these pests. Big-eyed bugs are already useful predators in agroecosystems. Further research should evaluate predation by *G. punctipes* against these or similar pests in a greenhouse or ornamental crop.

For all of the prey species evaluated, females killed more prey than did males, which is consistent with findings in other research. Mass-reared and field-collected *G. punctipes* had similar predation habits. No significant differences between the number of prey killed by mass-reared and field-collected *G. punctipes* were observed when both prey species (green peach aphid and red aphid) were considered in combination. However, mass-reared *G. punctipes* killed more green peach aphids than did field-collected big-eyed bugs. These results suggest that mass-reared big-eyed bugs could prey upon pests in a greenhouse or ornamental crop in a manner similar to, or in the case of green peach aphid, more aggressively than, feral *G. punctipes*.

Further research is needed to better define the upper limits of the predatory capability of adult *G. punctipes* against green peach, red, and oleander aphids. Almost 20% of the petri dishes had all dead aphids at the end of the evaluation period, suggesting that some *G. punctipes* could have killed more prey if they had been available. Future studies should evaluate greater densities of prey to determine the maximum number of aphids that big-eyed bugs can kill in a given period.

Because big-eyed bugs are predatory in all life stages, another area of future research should be investigations of the predatory potential of nymphs against aphids. Although some data are available on predation of fifth-instar nymphs on lepidopteran larvae and eggs, predation on aphids, whitefly, and other greenhouse pests by immature big-eyed bugs is largely unexplored.

Two of the three original objectives of this research were met: it was established that *G. punctipes* mass-reared on meat-based artificial diet will kill the greenhouse pests evaluated, and that predation by mass-reared and field-collected big-eyed bugs was similar. The number of the selected prey that *G. punctipes* can kill within a given time needs further study to be answered conclusively.

Chapter IV

Effectiveness of *Geocoris punctipes* in Suppressing Populations of Greenhouse Whitefly and Western Flower Thrips in an Ornamental Crop

i. Introduction

The big-eyed bug, *Geocoris punctipes* (Say), is a common predator of numerous pest species that feed upon ornamental plants including whiteflies, aphids, and spider mites (Champlain and Sholdt 1967a, Dunbar 1971, Tamaki and Weeks 1972b, Crocker and Whitcomb 1980, Gonzalez et al. 1982, Cohen 1992, Eubanks and Denno 2000a) (Table 1.2). *Geocoris* spp. are generalist predators (Crocker and Whitcomb 1980, Bugg et. al 1991, Eubanks and Denno 1999) considered to be important in agricultural crops such as corn, cotton, soybean, and alfalfa (Crocker and Whitcomb 1980, Richman et al. 1980, Hagler and Cohen 1991, Campbell and Cone 1994). *Geocoris punctipes* can also be successfully reared on a diet of a single insect species (Champlain and Sholdt 1967a, Dunbar 1971, Cohen and Debolt 1983), and most research on rearing *G. punctipes* in the laboratory on insect hosts have used lepidopteran larvae and eggs as the diet for the predator.

Most research concerning the predatory capabilities of *G. punctipes* against prey species have focused on agricultural crops (Crocker et al. 1975, Chiravathanapong and Pitre 1980, Crocker and Whitcomb 1980, Richman et al. 1980, Tamaki et al. 1981, Gonzalez et al. 1982, Hutchison and Pitre 1983, Bugg et al. 1991, Campell and Cone 1994, Hagler and Naranjo 1994, Weathersbee and Hardee 1994) (Table 1.2). However, several prey species recorded for *G. punctipes* are also pests of greenhouses and ornamental crops (McGregor and McDonough 1917, Dunbar 1972, Tamaki and Weeks1972a,b, Tamaki et al.1981, Crocker and Whitcomb 1980, Gonzalez et al. 1982, Cohen 1992, Kerns and Gaylor 1993, Hagler and Naranjo 1994, Colfer et al. 1998, Losey and Denno 1998, Eubanks and Denno 2000a).

Two aphid species, the pea aphid, *Acyrothosiphon pisum* (Harris), and the cotton aphid, *Aphis gossypii* Glover, are recorded as natural prey of *Geocoris* spp. (Crocker and Whitcomb 1980). Kerns and Gaylor (1993) found *Geocoris* spp. to be key predators of the cotton aphid in cotton (see also Weathersbee and Hardee 1994). *Geocoris* spp. will also prey upon the green peach aphid, *Myzus persicae* (Sulzer) (Tamaki and Weeks 1972b, Tamaki et al. 1981).

In the field, *G. punctipes* is a frequent predator of other pests that also occur in the greenhouse, for instance, the sweetpotato whitefly, *Bemisia tabaci* (Gennadius)(Cohen and Byrne 1992, Hagler and Naranjo 1994). In field sweet potatoes, 39.4% of adult big-eyed bugs tested positive for whitefly and whitefly egg antigen using the ELISA method (Hagler and Naranjo 1994). Other prey include spider mites (*Tetranychus* spp.) and several species of thrips: Gonzalez et al. (1982) reported that these pests were primary foods for both *G. punctipes* and the minute pirate bug, *Orius tristicolor* (White), in California cotton (see also Wilson et al. 1991). Whitefly, spider mites, and thrips are all common greenhouse pests.

Despite the fact that *Geocoris* spp. prey upon pests of greenhouses and ornamentals, relatively few instances of the use of *Geocoris* spp. as a biological control agent in a greenhouse setting have been reported. *Geocoris bullatus* (Say) suppressed population growth of green peach aphid on sugarbeet plants in the greenhouse (Tamaki and Weeks 1972a). In a separate study comparing *G. bullatus* to other insect predators against three pest species on sugarbeets in the greenhouse, *G. bullatus* effectively reduced aphid populations when released before aphid densities exceeded 14 individuals per plant (Tamaki and Weeks 1972a).

No published reports indicate ability of *G. punctipes* to suppress thrips populations, but several species of thrips are recorded as prey of *G. punctipes* in a field survey (Crocker and Whitcomb 1980, see also Gonzalez et. al 1982). *G. punctipes* is being marketed as a thrips predator by at least one mass-rearing facility on their web site (Biofac Crop Care, http://www.biofac.com/Orders/ BigEyedBug/bigeyedbug.html, May 2002). These few data do little to establish the usefulness of this predator in more common greenhouse systems or on ornamental plants in a greenhouse.

Whiteflies, aphids, and mites are all common greenhouse pests included in the host range of *Geocoris* spp. No published research has been conducted to evaluate the survival and reproduction of big-eyed bugs in an ornamental crop in the presence of aphids, thrips, or whiteflies, or the ability of big-eyed bugs to suppress populations of these pest species on ornamental plants within a greenhouse setting.

The objectives of this investigation were to determine the potential of *G. punctipes* as a biological control agent in an ornamental cut flower crop, *Ageratum houstonianum* Mill, within cages in the controlled conditions of a greenhouse. Potential was evaluated by measuring the following: 1) whether *G. punctipes* significantly reduced populations of the greenhouse whitefly, *Trialeuroides vaporariorum* (Westwood), 2) whether *G. punctipes* significantly reduced populations of western flower thrips, *Frankliniella occidentalis* (Pergande), and 3) whether differences in plant quality among plants with and without *G. punctipes* present can be detected. Plant quality was measured by the number of leaves, number of lateral branches, number of flowers, and height of plant from soil level to tip of plant.

ii. Materials and Methods

Cages and Benches. Cage frames (61 cm wide, 91.4 cm deep, and 122 cm tall) were constructed of 19 mm diameter PVC pipe and fitted with covers made of thrips screen. Each cage had a flap-type opening sealed with Velcro fastener from top to bottom of the center front (Figure 4.1).

Three greenhouse benches (111 cm wide x 300 cm long x 15 cm deep) were first lined with tobacco canvas (Figure 4.2) to prevent soil loss while allowing water to drain freely. Benches were then filled with planting medium (Berger BM6 mix) to a depth of approximately 18 cm. Four cages were placed on each of the three benches for the duration of the experiment. An extra 10 cm of thrips screen at the bottom of the cages was tucked into the soil, and soil mounded up on either side of the screen, in an effort to prevent the escape of insects from inside the experimental arena to the outside, and to keep thrips and other insects from entering or leaving the cages.

Plants and Plant Culture. Seeds of *A. houstonianum* var. 'Leilani', a plant grown commercially as both a cut flower and potted plant (Ball Seed Co., personal communication), were provided by Ball Seed Company. This plant was recommended by the staff of Ball Seed Co. as a suitable plant for this study because: 1) whiteflies are a common pest of greenhouse-grown *Ageratum*, 2) it is relatively easy to grow, 3) it is not photoperiod sensitive (does not require a specific day length to flower), and 4) it does not need supplemental lighting to extend day length.

Ageratum houstonianum seeds (250) were planted in plastic seed trays with Berger BM2 seed mix as substrate on 14 February 2002. The plastic seed tray was covered with a clear plastic lid to retain humidity in the seed environment. Seeds germinated in eight days and were maintained in the trays until reaching a size of approximately 15 cm tall (approximately four weeks after seeds were planted). When the plants reached a height of 15 cm or taller, they were transferred from seed trays into the prepared benches within the cages. Eight *Ageratum* plants were placed into each of the 12 cages, providing spacing comparable to growing recommendations for the crop when grown as a cut flower (15 x 23 cm, Ball 1997). Greenhouse temperature was maintained at

Figure 4.1. Cages constructed of PVC pipe and thrips screen for use in greenhouse experiment.

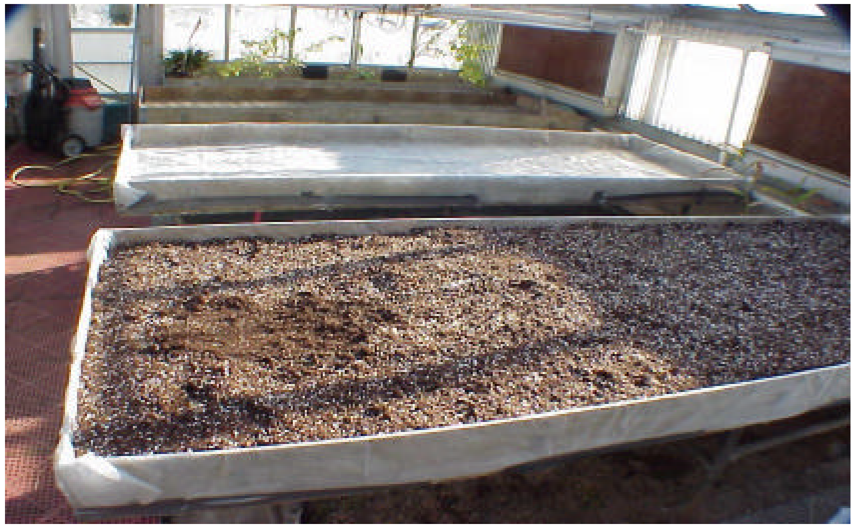

Figure 4.2. Greenhouse benches lined with tobacco canvas (above) and filled with planting medium (below) before addition of cages.

approximately 25ºC. Because *A. houstanianum* is not photoperiod sensitive, no supplemental lighting was necessary.

Insects. Adult greenhouse whiteflies were collected from a greenhouse infestation at the University of Tennessee, Knoxville Agricultural Campus using an aspirator (approx. 25 ml) The whiteflies were collected primarily from the weeds underneath the greenhouse benches where they were most concentrated and where pesticides had not been sprayed. Western fllower thrips, also a recorded prey species of *G. punctipes*, were discovered on the *Ageratum* plants when the plants were transplanted into the experimental cages. Because little information is available regarding the ability of big-eyed bugs to suppress populations of thrips, their presence on the plants presented an opportunity to evaluate an additional prey species. Thus, thrips were included as a target prey in this experiment.

Fifth-instar *G. punctipes* were purchased from Biofac Crop Care (Mathis, TX) and maintained on meat-based artificial diet (described in Chapter II) in an insectary until they became adults to be released into the greenhouse cages. At the time of their release into the experimental cages, each individual had been an adult for less than seven days.

Experimental Design. Three treatments were used to measure the ability of *G. punctipes* to reduce populations of greenhouse whitefly and western flower thrips within a caged arena in the greenhouse. Treatments were: 1) no predators, 2) low predator density (six individual adults, three of each sex), and 3) high predator density (12 adults, six of each sex). All treatments were repeated four times for a total of 12 cages, which were located on three greenhouse benches in a complete randomized block design. Experiments were conducted in Greenhouse 8 on the University of Tennessee Agriculture Campus.

After *A. houstonianum* plants were transplanted into the cages, 20 adult greenhouse whitefly were released into each cage from the aspirator vial into which they had been collected in a nearby greenhouse. After one week, the number of whitefly and thrips within each cage was estimated by choosing one mature leaf (in the upper 1/3 of the plant) from each of three plants. Three leaves per cage were sampled, and the number of whitefly pupae or thrips on the bottom surface of each leaf counted and recorded. Only whitefly pupal cases still containing an insect were counted.

Immediately afterward, 0 (control), 6 (low density) or 12 (high density) adult *G. punctipes* were introduced into the treatment cages. Because the addition of sunflower seeds to a diet of whitefly greatly increased the survival of big-eyed bugs (Chapter III), sunflower seeds were placed into the cages containing big-eyed bugs for additional nutritional support. Approximately 10 g of sunflower seeds were placed onto the plastic lid of a 190 ml waxed paper cup. Three large v-shaped cuts were made around the rim of the cup and it was inverted over the lid and sunflower seeds. The inverted cup acted as a roof over the seeds, allowing them to stay relatively dry when plants were watered. Sunflower seeds were replaced as needed (approximately once per week) when they became moldy or discolored.

The addition of the sunflower seeds and cups provided an unintended benefit. With one or two exceptions, once the big-eyed bugs were released into the cages, they were rarely seen again on plants or soil. They were, however, frequently observed feeding on the sunflower seeds and were often found among the seeds when they were refreshed. The unintended benefit was that the presence of the sunflower seeds allowed us to verify that the big-eyed bugs were still present in the system when they would have been otherwise difficult to observe within the cages.

One week and two weeks following the introduction of the predators, three leaves (mature leaves from the upper portion of the plant) from each cage were collected from three randomly selected plants within the cage. The number of whitefly pupae and thrips visible were counted and recorded once each week.

Three weeks after the introduction of the predators, the *A. houstonianum* plants were cut at the soil line and immediately placed into labeled plastic bags. The plants were then taken to the laboratory for evaluation, and each leaf of each plant was inspected. For each plant, the following information was recorded: number of thrips, number of whitefly (adults and pupae), number of big-eyed bugs (adults and nymphs), and number of big-eyed bug eggs. In addition, information regarding the plant was also recorded: height of plant, number of leaves (leaves greater than 1 cm across), number of lateral branches, and number of flowers.

Data Analysis. ANOVA, Mann-Whitney nonparametric test, and regression (SPSS 2001) were used to determine relationships between the number of big-eyed bugs, the number of pest species, and quality of plants within the cages. Mann-Whitney nonparametric test was used to determine differences in numbers of *Geocoris* eggs in high and low density cages. Analysis of variance was used to determine differences in pest species levels at weekly intervals and at the end of the experiment. Tukey HSD was used to determine differences between the high and low *Geocoris* treatments.

iii. Results and Discussion

Pest Levels. No significant differences in numbers of either whitefly or thrips were detected in cages with and without *G. punctipes* the first week after the big-eyed bugs had been placed into the experimental arena. By the second week, significantly ($p=0.002$, $df=2,32$, $F=7.301$) fewer western flower thrips were found in cages with big-eyed bugs than in cages without big-eyed bugs. The trend continued until the end of the experiment when significantly ($p<0.001$, $df=2,94$, F= 85.61) fewer western flower thrips were found on the *Ageratum* plants in cages containing big-eyed bugs. No significant (p=0.65) difference was detected between the number of thrips in the cages with big-eyed bugs the second week and at the end of the study.

Both the low and high (6 and 12 *G. punctipes*) densities of predators were effective against populations of western flower thrips, but the higher concentration of big-eyed bugs was not significantly (p=1.00) more effective in controlling thrips populations. Plants caged without big-eyed bugs had an average of 34 thrips per plant at the end of the experimental period, while plants caged with 6 and 12 big-eyed bugs contained an average of 2 and 4 thrips per plant, respectively (Figure 4.3).

Interestingly, significantly (p=0.002) more *G. punctipes* eggs were found in cages with 6 *G. punctipes* (low density) than in the high density cages. This study contributes some information to the numbers of *G. punctipes* that might need to be introduced into a greenhouse system for them to be effective biological control agents on ornamental crops. Future studies should evaluate different numbers of *G. punctipes* in the experimental arena so that the best combination of effectiveness and economy of insects in a biological control program can be determined.

Big-eyed bugs did not significantly (p=0.161) reduce populations of whitefly during this experiment. However, mean number of whitefly per plant were numerically lower in the cages with big-eyed bugs (Figure 4.4). At the end of the experimental period, cages with 12 big-eyed bugs averaged 16 whitefly pupae plant, compared to 31 per plant in cages with no big-eyed bugs (Figure 4.4).

When the plants were inspected at the end of the experiment, it was noted that some plants had as many as 95 to 100 whitefly pupae while the next closest plant had only a few. This pattern of distribution is consistent with early stages of

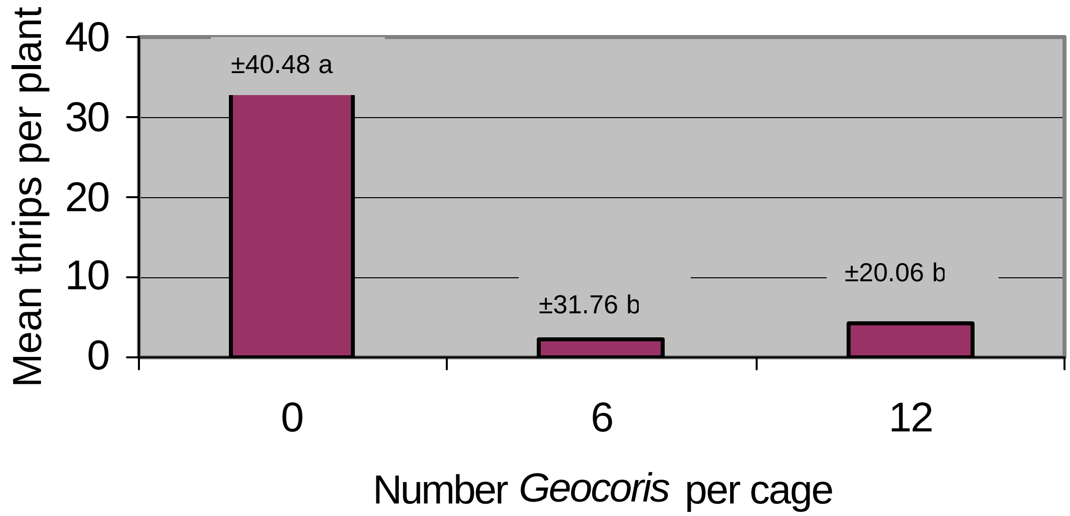

Figure 4.3. Mean number of western flower thrips (± standard deviation) per plant (*Ageratum houstonianum*) after three week experimental period enclosed with the predator *Geocoris punctipes.* Columns with the same letter are not significantly different from one another (p=0.05).

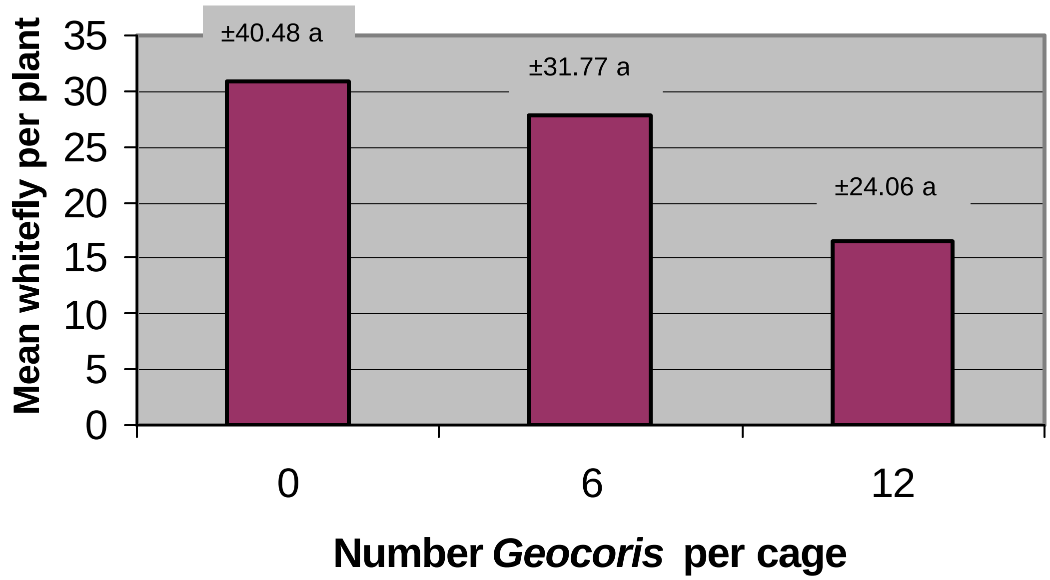

Figure 4.4. Mean number of greenhouse whitefly (± standard deviation) per plant (*Ageratum houstonianum*) after three week experimental period enclosed in cages with the predator *Geocoris punctipes*. Columns with the same letter are not significantly different from one another (p=0.05)

whitefly infestation (Byrne et al. 1990). It is possible that if the experiment had run longer, differences in whitefly populations may have been detected in cages with and without *G. punctipes* as whitefly populations became more distributed throughout each cage.

Plant Quality. Upon visual inspection, plants appeared to be similar to one another in quality at the end of the experimental period. No plants showed the characteristic shriveling of leaf tissue associated with heavy thrips infestation or any other signs of stress due to insect feeding. The only visibly different plants were in the two cages closest to the evaporative cooling unit in the greenhouse, which appeared to be shorter and less vigorous than plants in other locations. Plant quality was statistically evaluated by height of plant, number of leaves (leaves greater than 1 cm across), number of lateral branches, and number of flowers. Several significant interactions were detected when these values were correlated to the numbers of pests and predators in the cages for leaves, laterals, and flower count. Number of leaves and number of whitefly were positively correlated ($p=0.05$). Number of laterals was positively correlated with increasing numbers whitefly ($p=0.05$) and thrips ($p=0.01$), but negatively correlated with the number of big-eyed bugs ($p=0.01$). Flower count was also negatively correlated with number of big-eyed bugs ($p=0.05$) and positively correlated with number of thrips ($p=0.01$).

iv. Summary

Geocoris punctipes were introduced into cages containing *A. houstonianum* plants infested with thrips and whitefly. Leaves were randomly collected from the plants one and two weeks after the introduction of the predators. The number of pest insects on the leaves was counted and recorded.

After three weeks, the plants were cut at the soil level and inspected for pest insects, *G. punctipes* and their eggs, and various indicators of plant quality.

Within greenhouse cages, *G. punctipes* began to reduce populations of thrips on *A. houstonianum* within two weeks of their introduction into the crop system. No statistical differences in the numbers of thrips in cages containing 6 and 12 *G. punctipes* were observed, indicating that the lower concentration of predators was as effective in reducing western fllowers thrips populations on *A. houstonianum*.

Geocoris punctipes did not significantly reduce populations of greenhouse whitefly in the three week experimental period, but in cages with 12 G. *punctipes*, 40% fewer whitefly pupae were found at the end of three weeks. Because big-eyed bugs numerically reduced populations of greenhouse whitefly, these predators may have potential as biological control agents despite these results. For instance, what if the big-eyed bugs had been introduced into the cages at the same time as the whitefly, instead of one week later? What if the experiment had run four or six weeks?

These data suggest that *G. punctipes* may be a useful biological control agent on ornamental crops in greenhouses. Further research into the effectiveness of *G. punctipes* to control pests in *A. houstonianum* and other ornamental crops should include varying numbers of *G. punctipes* to help determine the ideal density of big-eyed bugs to release into crop systems for maximum effectiveness and return on investment to the grower. In addition, future experiments should continue for longer than three weeks to more accurately measure the impact of big-eyed bugs against pest populations.

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Appendix

Pesticide compatibility of *G. punctipes* and *G. pallens*

Appendix. Mortality of adult *G. punctipes* and *G. pallens* exposed to pesticides. When several concentrations were tested, results listed here are for the highest concentration tested. All test took place in the laboratory (see also Tilman and Mulrooney (2000b) regarding a field test of several pesticides.)
* Acaricide, Fungicide, Herbicide, Insecticide. Chemicals followed by (†) were tested against *G. pallens.*

Chemical	A, F, H, I*	Method	% Mortality	Authors	Year
Bentazon	H	residue on cotton plants in greenhouse	♂23.81 ♀16.67	Farlow and Pitre	1983
Azinphos-methyl	I	spray chamber bioassay	10.0	Elzen et al,	1998
Azinphos-methyl	I	applied to eggs of *H. zea*	♂44.5 ♀44.5	Elzen	2001
Baccilus thuringensis	I	tarsal contact		Herbert and Harper	1986
Baccilus thuringensis	I	sprayed leaves in petri dish	1.8±1.9	Boyd and Boethel	1998
Bentazon	H	residue on cotton plants in greenhouse	♂16.67 ♀28.57	Farlow and Pitre	1983
Carbaryl †	I	residue on cotton leaves	11.2±5.0	Yokoyama et al.	1984
Chlorfenapyr	I	spray chamber bioassay	66.7	Elzen et al.	1998
Chlorfenapyr	I	sprayed leaves in petri dish	100	Boyd and Boethel	1998
Chlorfenapyr	I	Residue on cotton leaves	♂55.2±18.7 ♀23.9±11.5	Elzen and Elzen	1999
Chlorfenapyr	I	applied to eggs of *H. zea*	♂61.1 ♀66.7	Elzen	2001
Cyfluthrin	I	spray chamber bioassay	0.0	Elzen et al.	1998
Cyfluthrin	I	applied to eggs of *H. zea*	♂44.5 ♀22.2	Elzen	2001
DEF†	I	residue on cotton leaves	8.8±7.1	Yokoyama and Pritchard	1984

Difocol†	I	residue on cotton leaves	2.9±0.5	Yokoyama et al.	1984
Dipel	I	residue on cotton leaves	0.0	Ali and Watson	1982
Endosulfan	I	spray chamber bioassay	0.0	Elzen et al.	1998
Endosulfan	I	applied to eggs of *H. zea*	♂77.8 ♀94.5	Elzen	2001
Enomectin benzoate	I	sprayed leaves in petri dish	25.6±8.7	Boyd and Boethel	1998
Fenvalerate 24 EC		residue on filter paper	20.6	Wilkinson et al.	1979
Fipronil	I	spray chamber bioassay	86.7	Elzen et al.	1998
Fipronil		applied to eggs of *H. zea*	♂88.9 ♀94.5	Elzen	2001
Glyphosphate †	H	residue on cotton leaves	8.6±3.4	Yokoyama et al.	1984
Imidicloprid	I	spray chamber bioassay	6.7	Elzen et al.	1998
Imidicloprid	I	sprayed leaves in petri dish	36.5±9.0	Boyd and Boethel	1998
Imidicloprid	I	applied to eggs of *H. zea*	♂11.1 ♀50.0	Elzen	2001
Lambda cyhalothrin		sprayed leaves in petri dish	87.5±6.3	Tilman & Mulrooney	2000
Lambda cyhalothrin		topically applied to *Geocoris*	100	Tilman & Mulrooney	2000
Malathion ULV	I	spray chamber bioassay	56.7	Elzen et al.	1998
Malathion ULV		applied to eggs of *H. zea*	♂38.9 ♀66.7	Elzen	2001
Methidathion†	I	residue on cotton leaves	7.9±1.6	Yokoyama et al.	1984
Methomyl †	I	residue on cotton leaves	8.8±5.9	Yokoyama et al.	1984
Methoxyfenozide	I	Residue on cotton leaves	♂0.0±0.0 ♀10.8±10.8	Elzen and Elzen	1999
Methyl parathion	I	sprayed leaves in petri dish	100	Boyd and Boethel	1998
Oxamyl	I	spray chamber bioassay	0.0	Elzen et al.	1998
Permethrin 2.0		residue on filter paper	21.3	Wilkinson et al.	1979

EC					
Permethrin 3.2 EC		residue on filter paper	32.9	Wilkinson et al.	1979
Permethrin	I	sprayed leaves in petri dish	92.3±5.4	Boyd and Boethel	1998
Profenfos 4.0 EC		residue on filter paper	30.7	Wilkinson et al.	1979
Profenofos	I	spray chamber bioassay	0.0	Elzen et al.	1998
Profenofos		applied to eggs of *H. zea*	♂44.5 ♀33.3	Elzen	2000
Propargite †	I	residue on cotton leaves	13.3±4.4	Yokoyama et al.	1984
S 1812		sprayed leaves in petri dish	2.5±2.5	Tilman & Mulrooney	2000
S 1812		topically applied to *Geocoris*	10.0±4.47	Tilman & Mulrooney	2000
Spinosad	I	spray chamber bioassay	0.0	Elzen et al.	1998
Spinosad	I	sprayed leaves in petri dish	69.1±9.7	Boyd and Boethel	1998
Spinosad	I	Residue on cotton leaves	♂0.0±0.0 ♀0.0±0.0	Elzen and Elzen	1999
Spinosad		sprayed leaves in petri dish	7.7±7.5	Tilman & Mulrooney	2000
Spinosad		topically applied to *Geocoris*	93.3±4.2	Tilman & Mulrooney	2000
Spinosad		applied to eggs of *H. zea*	♂16.7 ♀11.1	Elzen	2001
Sulfur †	I	residue on cotton leaves	1.9±0.0	Yokoyama et al.	1984
Sulprofos 6.0 EC		residue on filter paper	68.0	Wilkinson et al.	1979
Tebufenozide	I	Residue on cotton leaves	♂0.0±0.0 ♀0.0±0.0	Elzen and Elzen	1999
Tebufenozide		applied to eggs of *H. zea*	♂11.1 ♀16.7	Elzen	2001
Thiodcarb	I	sprayed leaves in petri dish	21.9±7.8	Boyd and Boethel	1998

Vita

Nicole Pendleton was born in Newport News, Virginia, on May 4, 1971. She grew up in the Hampton Roads area of Virginia, spending summers with her sister and grandparents in a rural part of the state. She graduated from Central High School, Woodstock, Virginia, in May of 1989. Nicole spent several years working in the horticulture industry before returning to school in 1996 when she began classes at Pellissippi State Community College in Knoxville, Tennessee. After completing two years there she transferred to the University of Tennessee, Knoxville, where she received her bachelors' degree (summa cum laude) in Ornamental Horticulture and Landscape Design, business concentration, in May 2000. She was accepted into the Entomology and Plant Pathology department at the University of Tennessee shortly afterwards. Under the direction of Dr. Jerome F. Grant, she completed the requirements for a Master of Science degree in Entomology and Plant Pathology, with a minor in Botany, in August 2002. She is a member of Pi Alpha Xi, an honor society for horticulture students, the Entomological Society of America, Gamma Sigma Delta Agricultural Honor Society, and the American Philatelic Society.