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**OVIPOSITIONAL BEHAVIOR OF THE 12-SPOTTED LADY BEETLE,  
*COLEOMEGILLA MACULATA*: CHOICES AMONG PLANT SPECIES  
AND POTENTIAL FACTORS INFLUENCING THOSE CHOICES**

Marisa Lynn Griffin  
*University of Kentucky*

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## ABSTRACT OF THESIS

### OVIPOSITIONAL BEHAVIOR OF THE 12-SPOTTED LADY BEETLE, *COLEOMEGILLA MACULATA*: CHOICES AMONG PLANT SPECIES AND POTENTIAL FACTORS INFLUENCING THOSE CHOICES

*Coleomegilla maculata* is a beneficial coccinellid commonly found in sweet corn fields in Kentucky. Previous work on *C. maculata* has shown an ovipositional preference for the weed *Acalypha ostryaefolia*, compared to three selected weed species and corn. Also, predation of *C. maculata* egg clusters on *A. ostryaefolia* was less compared to clusters on corn and the presence of *A. ostryaefolia* led to higher densities of *C. maculata* larvae on corn.

I determined *C. maculata* ovipositional preference among weed species in field tests using nine common weeds. I also examined ovipositional preference using just *A. ostryaefolia* and *Abutilon theophrasti*. I assessed the roles of potential prey densities, plant structures, and weed attractiveness to adult *C. maculata*. Finally, I examined diurnal and nocturnal predation of *C. maculata* eggs on corn, *A. ostryaefolia*, *A. theophrasti*, and *Amaranthus hybridus*. Significant ovipositional preference was always observed for *A. theophrasti*. *C. maculata* egg clusters on *A. theophrasti* and *A. ostryaefolia* were preyed upon less frequently than clusters on *A. hybridus* and corn.

**Key Words:** *Coleomegilla maculata*, *Abutilon theophrasti*, Velvetleaf, oviposition, sweet corn, weed

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Marisa Lynn Griffin

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Decemeber 15, 2000

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OVIPOSITIONAL BEHAVIOR OF THE 12-SPOTTED LADY BEETLE,  
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THESIS

Marisa Lynn Griffin

The Graduate School  
University of Kentucky

2000

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*COLEOMEGILLA MACULATA*: CHOICES AMONG PLANT SPECIES AND  
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THESIS

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of  
Science at the University of Kentucky

By

Marisa Lynn Griffin

Lexington, Kentucky

Director: Dr. K.V. Yeargan, Professor of Entomology

Lexington, Kentucky

2000

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File name	Description	File type	Size
mgthesisI	Abstract-List of files	PDF	35KB
mgthesisII	Chapter I-Chapter III (up to table 6)	PDF	268KB
mgthesisIII	Chapter III (con't)-Vita	PDF	122KB

## Chapter 1

### Background and Literature Review

The importance of lady beetles as regulators of pest populations was recognized as early as the late 19<sup>th</sup> century when the cottony-cushion scale outbreak in California was controlled by the introduction of the vedalia beetle, *Rodolia cardinalis* (Mulsant) (van den Bosch et al. 1982, Maredia et al. 1992). Another coccinellid species, *Delphastus pusillus* (LeConte) (Coleoptera: Coccinellidae), has been used to control *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), a common whitefly pest in Florida (Hoelmer et al. 1993). Other common species, such as *Harmonia axyridis* (Pallas) and *Coccinella septempunctata* L., are well known for their ability to control aphid pests on various agricultural crops (Harmon et al. 1998). In surveys of aphid predators, these species and others are often listed as strict aphidophages (Benton and Crumb 1979). However, the coccinellid, *Coleomegilla maculata* (DeGeer) is an exception. *Coleomegilla maculata* not only consume various aphid pests, but members of this species are also efficient predators against tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) larvae and 1<sup>st</sup> instars, which are common pests of cotton (Lopez et al. 1976). In Louisiana, *C. maculata* have been found feeding on all life stages of the bandedwing whitefly, *Trialeurodes abutiloneus* Haldeman (Homoptera: Aleyrodidae), in cotton and soybeans (Watve and Clower 1976). Hodek and Honěk (1996) cite *C. maculata* as important predators of European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae), Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), and corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), eggs. Conrad (1959) found that *C. maculata* adults consumed 16% of European corn borer eggs laid on waxed paper placed on corn. Predation by *C. maculata* adults can significantly reduce population densities of Colorado potato beetle eggs and small larvae on potato plants (Grodén et al. 1990). Studies also have demonstrated the efficiency of *C. maculata* larvae as predators of this potato pest (Giroux et al. 1995). Based on sampling and direct observations, *C. maculata* is one of the predominant predators of corn earworm eggs on sweet corn in Kentucky (Cottrell and Yeargan 1998b). In their study, *C. maculata* (adults and larvae) accounted for more than half of the predation of corn earworm eggs. *C. maculata* larvae, which accounted for

56.8% of *C. maculata* predation on corn earworm eggs, fed both diurnally and nocturnally, while *C. maculata* adults fed primarily during the day (Cottrell and Yeargan 1998b).

*Coleomegilla maculata* also are known to feed on eggs laid by their own species (Cottrell and Yeargan 1998a, 1998b, Schellhorn and Andow 1999). Newly hatched *C. maculata* can feed on *C. maculata* eggs laid on corn and will continue to develop normally (Warren and Tadic 1967). Cottrell and Yeargan (1998a) found that *C. maculata* larvae and adults accounted for 89% of the predation of *C. maculata* eggs in corn.

*Coleomegilla maculata* have a strong tendency to feed on plant pollen, especially corn pollen. Several studies have shown *C. maculata*'s ability to survive solely on pollen from various plant species. Smith (1960, 1961) tested *C. maculata* survival on pollen from many plants, including corn (*Zea mays* L.) (Poaceae). Pfannenstiel (1995) also tested *C. maculata* development on corn pollen, finding it to be a suitable food source for the larvae.

*Coleomegilla maculata* are quite widespread throughout the Nearctic region. They have been found on cole crops, snap beans, cucurbits, alfalfa and sweet corn in Rhode Island, where they are also the most abundant coccinellid species on potatoes (Grodén et al 1990). In the Midwest, they are the most prevalent coccinellid predators in alfalfa and corn (Phoofolo and Obrycki 1998). *Coleomegilla maculata* also are the most abundant coccinellid species on corn in Kentucky and New York (Pfannenstiel 1995, Hoffman 1997). *Coleomegilla maculata* colonize corn several weeks after planting, near the end of the vegetative stage (Coderre et al. 1987). In Delaware, adult densities in corn increase in late June, and taper off around mid-August (Udayagiri et al. 1997). Population peaks appear to coincide with corn tasseling and pollen shed (Coll and Bottrell 1991, Hoffman et al. 1997). While population densities for some coccinellid species increase with prey densities, *C. maculata* densities seem to be less affected by prey abundance (Wright and Laing 1980).

Oviposition is an important aspect of the coccinellid life cycle. Females possess the ability to influence egg and larval survival by selecting ovipositional sites that are beneficial to hatching prey (e.g., nearby food sources, favorable environmental conditions, and refuge from predation). Adult coccinellids are able to mate several days

after spring emergence and one mating is usually sufficient to supply females with sperm to produce a lifetime of fertilized eggs (Hodek and HonØk 1996). Most coccinellid eggs are typically attached to a substrate by the narrow end, near prey, providing newly hatched larvae with an immediate food source (Hodek 1973). For example, cassava, *Manihot esculenta* Crantz (Euphorbiaceae), provides a substrate for the eggs of three species of coccinellids which feed on the eggs of the cassava mealybug, *Phenacoccus herreni* Cox and Williams (Homoptera: Pseudococcidae). Some of these coccinellid species have even adopted a parasitoid-like oviposition habit in which eggs are laid inside mealybug ovisacs, which provide a nutrition source to hatching larvae (Sullivan et al. 1991). Other species are known to oviposit near aphid clusters. *Adalia bipunctata* L., a common predator in Minnesota corn fields, will often oviposit close to aggregations of the corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae) (Schellhorn and Andow 1999). Coderre et al. (1987) examined oviposition of two coccinellid species and found that while *Hippodamia tredecimpunctata* Say typically laid their eggs amid an abundance of aphids, *C. maculata* did not.

*Coleomegilla maculata* typically lay eggs in clusters directly on their foraging surface (e.g., corn plants). Their oviposition period begins once they colonize corn and lasts throughout the season, ending around late August (Coderre et al. 1987). Oviposition generally occurs on the underside of corn leaves at the lower end of the plant (Foott 1973, Coderre et al. 1987). However, Cottrell and Yeargan (1998a) found higher proportions of *C. maculata* eggs on hophornbeam copperleaf, *Acalypha ostryaefolia* Riddell (Euphorbiaceae), an endemic weed commonly found in Kentucky corn fields, than on nearby corn plants. *Coleomegilla maculata* larval densities were also greater on corn in weedy plots compared with weed-free plots. While more *C. maculata* eggs were laid on *A. ostryaefolia*, larvae appeared to disperse to nearby corn plants soon after hatching (Cottrell and Yeargan 1999). Cottrell and Yeargan (1998a) also observed that among various weed species in and around the sweet corn plots, *C. maculata* eggs appeared to occur more frequently on *A. ostryaefolia* than on other nearby weed species [e.g., lambsquarters, *Chenopodium album* L. (Chenopodiaceae), prickly sida, *Sida spinosa* L. (Malvaceae), and pigweed, *Amaranthus hybridus* L. (Amaranthaceae)]. In a preliminary experimental study, *C. maculata* showed ovipositional preference for *A. ostryaefolia* over

prickly sida, pigweed, and ivyleaf morningglory, *Ipomoeae hederacea* L. (Convolvulaceae) (K. V. Yeargan and B. L. Newton, unpublished data).

The underlying reason(s) for *C. maculata*'s apparent preference for oviposition on *A. ostryaefolia* is (are) unknown. There are many factors, both physical and chemical, that can elicit ovipositional responses in insects. Many phytophagous insects are attracted by hair-like structures, called trichomes, found on plants (Benedict et al. 1983). Trichomes can be simple (i.e., nonglandular) or they can be stalked glands that exude chemical substances (Radford et al. 1968). The tobacco budworm, *H. virescens*, has shown a preference for oviposition on tobacco, *Nicotiana tabacum* (Solanaceae), and velvetleaf, *Abutilon theophrasti* Medic (Malvaceae) surfaces containing glandular trichomes (Navasero and Ramaswamy 1991). A study with soybean leaves (Powell and Lambert 1993) revealed that although larvae of soybean-defoliating pests such as corn earworms show a non-preference for pubescent soybean plants as opposed to more glabrous cultivars, adults of these species show a definite ovipositional choice for the hairy plants.

Coccinellid females may select oviposition sites based on microclimatic factors (e.g., humidity or light intensity) (Iperti 1966), prey abundance (Coderre et al. 1987), or larval protection from predators and parasitoids (Hodek and Honøk 1996). McMurtry et al. (1974) observed that increased day length led to an increase in oviposition of *Stethorus picipes* Weise in a laboratory study. Females of this species also have been observed to preferentially oviposit on mite-infested foliage over plants lacking mites (Putman 1955). Hodek and Honěk (1996) also state that some coccinellid females are less likely to oviposit in the presence of conspecific predators. *Adalia bipunctata* females exhibit reduced oviposition when confined with larvae or other females (Hemtinne et al. 1992). Research on the effects of pubescent potato plants on some coccinellids (e.g., *Hippodamia convergens* Guèrin-Meneville and *C. maculata*) indicated an ovipositional preference for potato clones bearing glandular trichomes compared with clones lacking glandular trichomes (Obrycki and Tauber 1985). The authors suggested that glandular trichomes on the potato clones provide refuge for coccinellid eggs from larval cannibalism. Interestingly, hophornbeam copperleaf houses both simple and glandular trichomes, either of which may contribute to *C. maculata*'s ovipositional preference.



Chemical attraction may play an important role in ovipositional choice for some insects, due to plant volatiles or exudates (Peterson et al. 1994). Peterson et al. were able to elicit pickleworm, *Diaphania nitidalis* (Stoll) (Lepidoptera: Pyralidae), oviposition on glass fiber pads brushed with trichome exudate extracted by an n-pentane wash from yellow-squash leaves, *Cucurbita pepo* (L.) (Cucurbitaceae). In a similar study, Peterson and Elsey (1995) obtained plant extracts from whole squash leaves washed with ethanol and hexane in order to elicit pickleworm oviposition. Another study utilized only hexane as a chemical wash on several tomato species, *Lycopersicon* spp. (Solanaceae) (Juvik et al. 1988). The extracts were put onto filter paper and allowed to dry in a fume hood. Extracts obtained from the species *Lycopersicon hirsutum* (L.) elicited the most *H. zea* oviposition when compared to the other tomato species (Juvik et al. 1988). Smith et al. (1973) applied various extracts from red cedar, *Juniperus virginiana* (L.) (Cupressaceae), to artificial substrates, and found the extracts evoked *C. maculata* oviposition. Zhu et al. (1999) studied *C. maculata* electroantennogram responses to pea aphid, *Acyrtosiphon pisum* Harris (Homoptera: Aphididae), and catnip, *Nepeta cataria* L. (Lamiaceae), extracts. Their research indicated that *C. maculata* could possibly use chemicals from potential prey and their host plants to locate prey. The characteristic allelochemicals of *A. ostryaefolia*, or other weeds like it, could possibly explain *C. maculata*'s ovipositional preference.

In order to use *C. maculata* as an effective biological control agent in sweet corn, it would be worthwhile to determine any ovipositional behaviors that enhance this predator's densities. Intercropping or adjacent habitats containing preferred ovipositional sites could help to increase *C. maculata* densities on desired crops. Polycultures of corn and preferred weed species could offer *C. maculata* increased densities of potential prey and refuge from predation and cannibalism of *C. maculata* eggs. Therefore, understanding which factor(s) make the greatest contribution to *C. maculata* ovipositional preferences could provide the basis for manipulation of this natural enemy's densities in corn ecosystems.

## Objectives

The overall objectives of this research were to examine ovipositional preferences of *Coleomegilla maculata* for selected weed species in sweet corn fields in Kentucky, and possible reasons for those preferences. Specifically, my objectives were:

1. To determine the ovipositional preferences of *C. maculata* for nine-selected weed species found in or around sweet corn fields in Kentucky.
2. To determine *C. maculata* ovipositional preferences for *A. ostryaefolia* plants compared with *A. theophrasti* plants.
3. To determine *C. maculata* ovipositional preference for *A. theophrasti* plants with and without immature whitefly prey.
4. To assess the attractiveness of selected weed species to adult *C. maculata*.
5. To examine the influence of potential prey, in addition to whiteflies, on *C. maculata* ovipositional site selection
6. To examine the influence of fruit and flowers of *A. ostryaefolia* on *C. maculata* ovipositional preferences.
7. To examine predation of *C. maculata* eggs on selected plants.

## Chapter II

### *C. maculata* ovipositional preference for selected weeds in Kentucky sweet corn fields

#### Introduction

The 12-spotted lady beetle, *C. maculata*, is one of the most abundant coccinellids found in corn in North America (Hodek and Honěk 1996). Though considered polyphagous, (i.e., not highly aphidophagous like some coccinellid species), *C. maculata* are efficient predators of the corn leaf aphid, *R. maidis* (Schellhorn and Andow 1999). They also have been found feeding on eggs of pests such as the European corn borer and the Colorado potato beetle (Coll and Bottrell 1991, Hazzard and Ferro 1991). A substantial portion of *C. maculata*'s diet in cornfields also consists of corn pollen when it is seasonally available (Smith 1960, 1961).

Adult and larval *C. maculata* feed on similar prey. Adult females may influence larval feeding by ovipositional site selection (Hodek 1973). Many predaceous coccinellids are known to oviposit near food sources (e.g., aphid clusters), providing larvae with immediate prey (Coderre et al. 1987). *Coleomegilla maculata*, however, have been shown to be less likely to oviposit amidst prey aggregates (Schellhorn and Andow 1999). In fact, Cottrell and Yeargan (1998a) observed greater *C. maculata* oviposition on *A. ostryaefolia*, a weed commonly found in corn fields in Kentucky, than on nearby corn plants, yet larvae spent most of their time on the corn plants.

Cottrell and Yeargan (1998a) found significantly higher numbers of *C. maculata* larvae on corn plants in the presence of *A. ostryaefolia* than on corn without *A. ostryaefolia*. Interestingly, they noted that although many *C. maculata* eggs were laid on the weeds, upon hatching, larvae readily dispersed to nearby corn plants. *Coleomegilla maculata*'s quick dispersal from *A. ostryaefolia* to corn plants might be due to the presence of simple and glandular trichomes, which cover the stems and petioles of the plant, possibly causing difficulty for foraging larvae. The effect of trichomes on natural enemies has been found to be both beneficial and detrimental (Obrycki and Tauber 1984). Leaf surfaces with trichomes can provide refuge for the eggs and early instars of beneficials from other predaceous insects, or can impede the searching ability of

predators and parasitoids due to sticky exudates (Lucas and Brodeur 1999). Gruenhagen and Perring (1999) found reduced parasitism of whitefly pests on velvetleaf when compared with glabrous and pubescent melon plants. In their experiment, parasitoids spent more time grooming after contact with velvetleaf's trichome exudates than searching for hosts.

My primary objective was to determine ovipositional preference(s) of *C. maculata* for selected weed species, including *A. ostryaefolia*, in sweet corn plots. In addition, I examined *C. maculata* ovipositional preference(s) for the weed *A. theophrasti* in the presence and absence of whitefly prey.

### **Materials and Methods**

For all experiments, data that failed to conform to the assumptions of an analysis of variance were transformed. Mean separation tests (LSD) were performed only if F-values were significant (i.e., protected LSD test).

#### Corn plots:

Field experiments were conducted in sweet corn plots at the University of Kentucky North Farm. 'Golden Queen' sweet corn was used for all plots; corn rows within plots were always spaced 0.9 m apart. After the corn was planted, all plots were treated with alachlor (3.9kg [AI]/ha). Naturally germinating weeds not controlled by herbicides were manually removed until experiments were completed. Planting dates and other information specific to each experiment are provided in the experimental details that follow.

#### 9-weed-species study:

Four plots of corn (10 m by 10 m; 12 rows), were planted on 11 May and 3 May in 1999 and 2000, respectively. Greenhouse-grown weeds (approximately 20 cm tall) were later transplanted into the corn plots, between corn rows, at two meters apart in a randomized complete block design. Each plot contained two blocks of nine weed species (9 weed species per block, 18 weeds per plot, and 72 plants overall). Within each plot, blocks of weed species were separated from one another by four corn rows in the center of the plot. For one block within a given plot, five weed species were transplanted between rows 3 and 4, and four weed species were transplanted between rows 4 and 5.

Weeds for the other block within a given plot were transplanted between rows 8 and 9 and 9 and 10, again with five and four weed species per row, respectively.

Weeds were transplanted into the plots on 3 July 1999 and 26 June 2000 by placing the pots containing weeds in a hole such that the top of each pot was level with the soil surface. The weed species were transplanted into the corn plots just as the corn began to tassel. Every other day from 5 July until 21 July in 1999 and from 28 June until 14 July in 2000, coccinellid eggs were sampled from the transplanted weed species. Sampling consisted of counting the number of egg clusters and the number of eggs within each cluster, then carefully removing the clusters found on each plant. The clusters were taken back to the laboratory for incubation in order to determine species identification. A Bartlett's test of homogeneity of variance was performed (Analytical Software, 1992). Data were transformed (square root of the number of egg clusters found per plant) and were analyzed using a two-way analysis of variance (ANOVA). The mean numbers of egg clusters were compared among weed species using a protected least significant difference test (LSD). Data are presented as untransformed means  $\pm$  SE.

At the conclusion of this experiment, all 72 weeds were removed from the field and taken to the laboratory for leaf area determination. Leaves from each plant were removed at the petiole and placed in a leaf area meter in order to measure the total area available for *C. maculata* oviposition (Li-cor, model LI-3000, Lambda Instrument, Lincoln, NE, USA). Data were analyzed using a two-way analysis of variance (ANOVA). Means were separated using a protected (LSD) test.

Nine species of weeds were tested (Table 1). These species were selected primarily because they are common in central Kentucky and/or have some physical resemblance to *A. ostryaefolia*, at least in their early growth stages.

As a supplement to these two experiments, on 11 August, 2000, 100 naturally occurring plants (20 of each species), representing five of the nine used in the experiments and which had germinated after the experiment was completed in 2000, were randomly selected and pulled out of the ground. These weeds were found along the

Table 1: Weed species used in the 1999 and 2000 9-weed-species experiments on *C. maculata* ovipositional preference.

<b>Common Name</b>	<b>Scientific Name</b>
Pigweed	<i>Amaranthus hybridus</i>
Hophornbeam copperleaf	<i>Acalypha ostryaefolia</i>
Velvetleaf	<i>Abutilon theophrasti</i>
Virginia copperleaf	<i>Acalypha virginica</i>
Lambsquarter	<i>Chenopodium album</i>
Hairy galinsoga	<i>Galinsoga ciliata</i> <sup>a</sup>
Eastern black nightshade	<i>Solanum ptycanthum</i>
Prickly sida	<i>Sida spinosa</i>
Cocklebur	<i>Xanthium strumarium</i>

<sup>a</sup> In the 2000 experiment, specimens of *Galinsoga ciliata* and *Galinsoga parviflora* Cavanilles were accidentally used because they were not properly identified until the experiment was underway.

perimeter surrounding each of the four test plots. Species represented in this sample were: hophornbeam copperleaf, smooth pigweed, common lambsquarters, prickly sida, and eastern black nightshade. The leaves of the pulled weeds were examined for coccinellid egg clusters. The total numbers of egg clusters and eggs per cluster were recorded.

*A. ostryaefolia* vs. *A. theophrasti* experiment:

In the 1999 nine-weed-species experiment, *C. maculata* significantly chose *A. theophrasti* as an ovipositional substrate over all other selected weed species, including *A. ostryaefolia*. In the Cottrell and Yeargan (1998a) study and a preliminary ovipositional preference experiment (K.V. Yeargan and B.L. Newton, unpublished data), *A. theophrasti* was not examined as a potential ovipositional site. In order to determine *C. maculata*'s ovipositional preference between *A. ostryaefolia* and *A. theophrasti*, I examined *C. maculata* preference for these two species in sweet corn.

On 11 June, 1999, a single corn plot (8 m by 12 m; 10 rows) was planted for this experiment. On 2 August 1999, field-grown *A. ostryaefolia* and *A. theophrasti* (approximately 20 cm tall) were transplanted ( $\approx$  two meters apart) into the plot between corn rows in a randomized complete block design with nine replications (18 weeds total). Pots with plants were placed into holes so that the tops of the pots were flush with the surrounding soil. Blocks 1 to 3 were planted between corn rows 3 and 4; blocks 4 to 6 between rows 5 and 6; blocks 7 to 9 between rows 7 and 8. The weed species were transplanted into the corn plot just as the corn had begun to tassel. The number of coccinellid egg clusters and number of eggs per cluster found on the transplanted weeds were recorded every other day from 4 August until 20 August 1999. Eggs were collected and incubated for species verification. A Bartlett's test indicated heterogeneous variance; therefore, data were transformed (square root of the number of egg clusters per plant) (Analytical Software 1992). A two-way ANOVA was used to compare the mean number of *C. maculata* egg clusters found on each species. Data are presented as untransformed means  $\pm$  SE. At the conclusion of this experiment, all transplanted weeds were removed and taken to the laboratory for leaf area measurements, as described earlier.

Whitefly-infested velvetleaf vs. non-infested velvetleaf:

During the 1999 ovipositional preference experiment, immatures of the bandedwing whitefly, *T. abutiloneus* were observed to be more abundant on velvetleaf

plants than on the other weed species. Thus, in order to determine the possible influence of these whiteflies on *C. maculata* ovipositional preference, an experiment was conducted in corn during 2000 using velvetleaf with and without immature whiteflies.

A single plot of corn (8 m by 12 m; 13 rows) was planted on 3 May, 2000. Twenty-four velvetleaf plants were grown in the greenhouse from seeds collected the previous summer. Approximately one week prior to transplantation into the field, the pots containing velvetleaf plants were equally divided and placed into two wooden, screened cages in the greenhouse. Plants in one cage were exposed to field-collected adult whiteflies; plants in the other cage remained whitefly free. The adult whiteflies were allowed to oviposit on velvetleaf for approximately five days, after which they were aspirated off the plants and destroyed. The developmental period of the whitefly is such that the experiment was completed before any immature whiteflies on the infested plants could emerge and cross-infest non-infested plants. Once the corn had begun to tassel, all 24 velvetleaf plants (20 cm tall) were transplanted ( $\approx$  2 meters apart), on 7 July, between corn rows in a randomized complete block design; there were twelve blocks total. Pots were placed into holes so that the top of each pot was flush with the surrounding soil. Within a given block, the two types of plants were separated from one another by a single row of corn. Blocks 1 to 4 were planted between corn rows 3 and 5; they were separated by corn row 4. Blocks 5 to 8 were planted between rows 6 and 8, separated by row 7. Blocks 9 to 12 were planted between rows 9 and 11, separated by row 10.

Coccinellid egg clusters were sampled every other day for seven days (from 8 July to 14 July). In order to minimize natural whitefly infestation of the experimental plants in the field, any adult whiteflies found on non-infested plants during coccinellid egg sampling were aspirated and destroyed. The total numbers of egg clusters and numbers of eggs within each cluster were recorded. After recording, eggs were collected and taken back to the laboratory for species verification. The mean number of *C. maculata* egg clusters was compared using a two-way ANOVA. At the conclusion of this experiment, plants were brought back to the laboratory for leaf area measurements as described earlier, and to determine the total number of immature whiteflies on each plant. The latter was accomplished by counting nymphs and pupae with the aid of a microscope.



## Results

### 9-weed-species study:

The mean number of *C. maculata* egg clusters was significantly higher on *A. theophrasti* than on all other weed species in 1999 ( $F=19.8$ ,  $df=8,36$ ,  $P<0.05$ ) (Figure 1a). In 2000, *A. theophrasti* had a higher mean number of egg clusters than all other weed species, except *Galinsoga* spp. ( $F=23.5$   $df=8,36$ ,  $P<0.05$ ) (Figure 1b). The average number of eggs per cluster  $\pm$  SE varied among weed species (Table 2). When the mean leaf area available for *C. maculata* oviposition on each weed species was analyzed with an analysis of variance, only *A. ostryaefolia* (in 1999) and *X. strumarium* (in 2000) had mean leaf areas that were significantly greater than the other weed species ( $F=10.5$ ,  $df=8,71$ ,  $P<0.05$ ) and ( $F=10.7$ ,  $df=8,71$ ,  $P<0.05$ ), respectively (Table 3).

### *A. ostryaefolia* vs. *A. theophrasti* experiment:

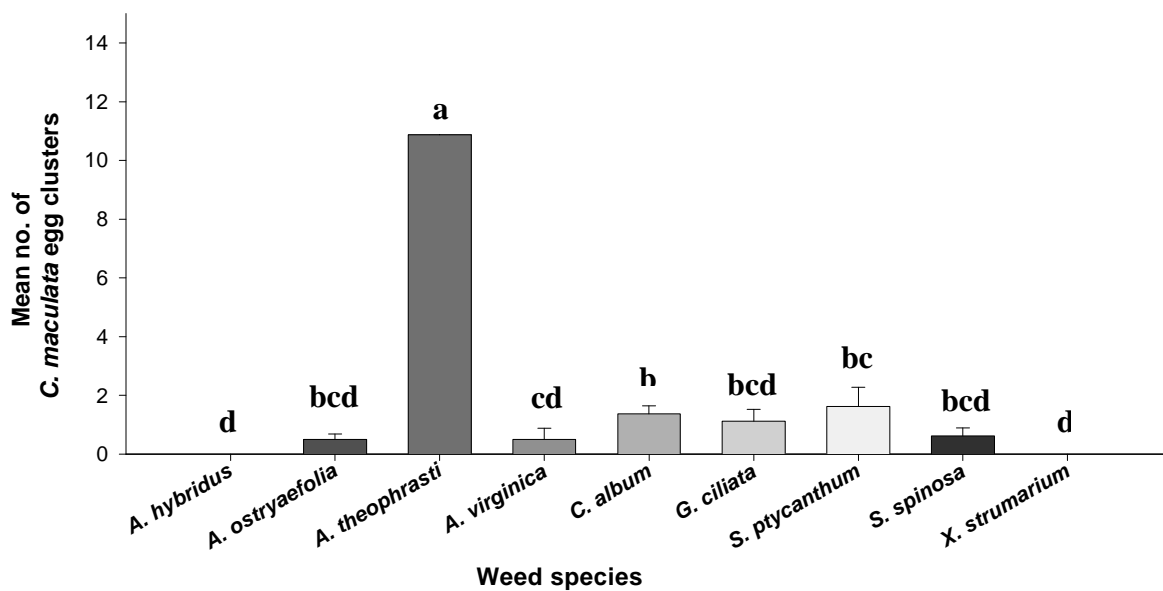
Significantly more *C. maculata* egg clusters were oviposited on *A. theophrasti* than on *A. ostryaefolia* ( $F=165.8$ ,  $df=1,8$ ,  $P<0.05$ ) (Figure 2). The average number of eggs per cluster  $\pm$  SE was  $10.9 \pm 0.7$  ( $n=33$ ) for *A. ostryaefolia* and  $8.7 \pm 0.3$  ( $n=205$ ) for *A. theophrasti*. *Acalypha ostryaefolia* had significantly more leaf area than *A. theophrasti* ( $F=6.2$ ,  $df=1,8$ ,  $P<0.05$ ) (Table 4).

### Whitefly-infested vs. non-infested *A. theophrasti* experiment:

The mean number of egg clusters found on *A. theophrasti* plants infested with whiteflies was not significantly different from the mean number of egg clusters found on non-infested plants ( $F=0.05$ ,  $df=1, 23$ ,  $P>0.05$ ) (Figure 3). The mean number of eggs per cluster  $\pm$  SE was  $13.3 \pm 1.1$  ( $n=29$ ) for whitefly-infested *A. theophrasti* and  $13.5 \pm 0.7$  ( $n=33$ ) for non-infested plants. The leaf areas of plants in this experiment also were not significantly different between treatments ( $F=3.7$ ,  $df=1,11$ ,  $P>0.05$ ) (Table 5). The mean number of immature whiteflies was more than 50 times greater on infested plants compared to non-infested plants ( $F=41.4$ ,  $df=1,11$ ,  $P< 0.05$ ) (Table 5).

Figure 1: Comparison of mean ( $\pm$  SE) number of *C. maculata* egg clusters per plant, per weed species across the 9-weed-species studies in a) 1999 and b) 2000. Means sharing the same letter are not significantly different ( $P>0.05$ , Fisher protected LSD test).

a) 1999



b) 2000

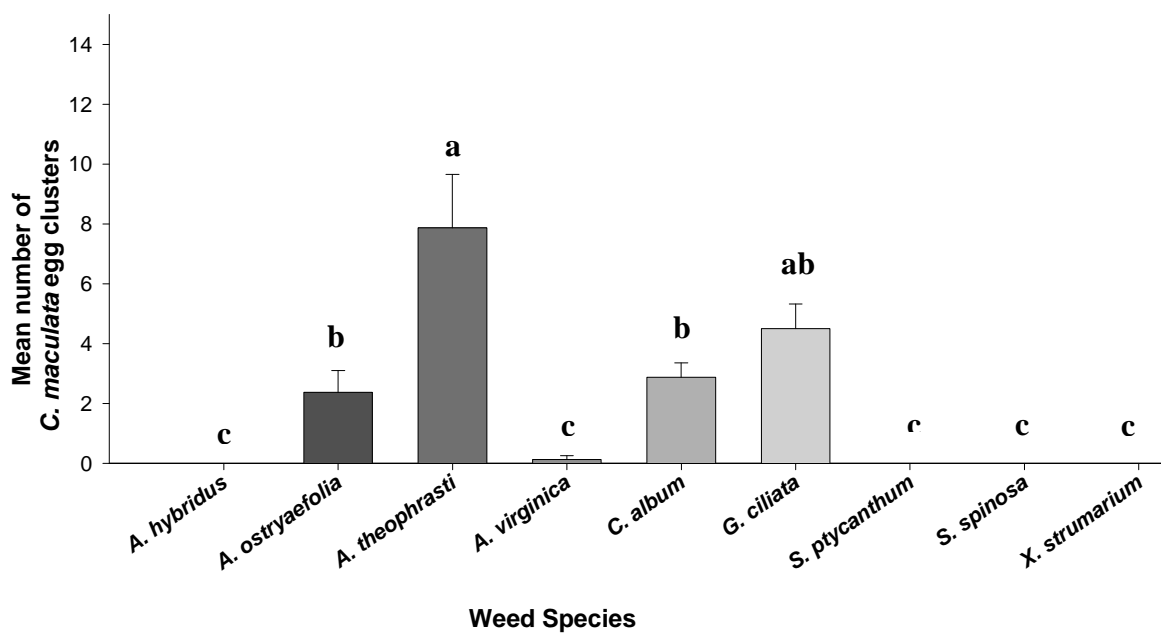


Table 2: Average number ( $\pm$  SE) of *C. maculata* eggs per cluster, per weed species across the 9-weed-species (1999 and 2000) experiments.

Weed Species	1999 <i>C. maculata</i> eggs per cluster, mean $\pm$ SE	2000 <i>C. maculata</i> eggs per cluster, mean $\pm$ SE
<i>A. hybridus</i>	(n=0)	(n=0)
<i>A. ostryaefolia</i>	8.5 $\pm$ 1.0 (n=4)	14.9 $\pm$ 1.1 (n=19)
<i>A. theophrasti</i>	10.5 $\pm$ 0.5 (n=88)	11.4 $\pm$ 0.6 (n=63)
<i>A. virginica</i>	10.8 $\pm$ 1.6 (n=4)	26.0 (n=26)
<i>C. album</i>	11.5 $\pm$ 1.8 (n=11)	13.6 $\pm$ 1.4 (n=23)
<i>G. ciliata</i>	10.0 $\pm$ 2.3 (n=9)	12.6 $\pm$ 0.7 (n=34)
<i>S. spinosa</i>	8.2 $\pm$ 0.9 (n=13)	(n=0)
<i>S. ptycanthum</i>	14.0 $\pm$ 2.3 (n=5)	(n=0)
<i>X. strumarium</i>	(n=0)	(n=0)

Table 3: Mean leaf area (cm<sup>2</sup>) (MLA), per plant, for all weed species in the 9-weed-species study. Means within a column sharing the same letter are not significantly different ( $P>0.05$ , Fisher protected LSD test).

Weed Species	1999 MLA (cm <sup>2</sup> ), mean $\pm$ SE	2000 MLA (cm <sup>2</sup> ), mean $\pm$ SE
<i>A. hybridus</i>	182.9 $\pm$ 24.5 <b>b</b>	361.6 $\pm$ 18.6 <b>cd</b>
<i>A. ostryaefolia</i>	270.7 $\pm$ 18.8 <b>a</b>	317.9 $\pm$ 27.0 <b>cde</b>
<i>A. theophrasti</i>	146.9 $\pm$ 26.7 <b>bc</b>	483.1 $\pm$ 23.5 <b>b</b>
<i>A. virginica</i>	83.5 $\pm$ 18.5 <b>d</b>	233.5 $\pm$ 28.0 <b>e</b>
<i>C. album</i>	123.3 $\pm$ 10.1 <b>cd</b>	285.7 $\pm$ 53.6 <b>cde</b>
<i>G. ciliata</i>	126.8 $\pm$ 11.9 <b>cd</b>	266.7 $\pm$ 84.9 <b>de</b>
<i>S. spinosa</i>	115.9 $\pm$ 8.1 <b>cd</b>	389.4 $\pm$ 41.2 <b>bc</b>
<i>S. ptycanthum</i>	149.3 $\pm$ 25.9 <b>bc</b>	402.2 $\pm$ 74.9 <b>bc</b>
<i>X. strumarium</i>	127.0 $\pm$ 12.0 <b>cd</b>	678.4 $\pm$ 40.9 <b>a</b>

Figure 2: Mean ( $\pm$  SE) of *C. maculata* egg clusters per plant on *A. ostryaefolia* compared to the mean number of egg clusters found on *A. theophrasti*. Means sharing the same letter are not significantly different ( $P > 0.05$ , Fisher protected LSD test).

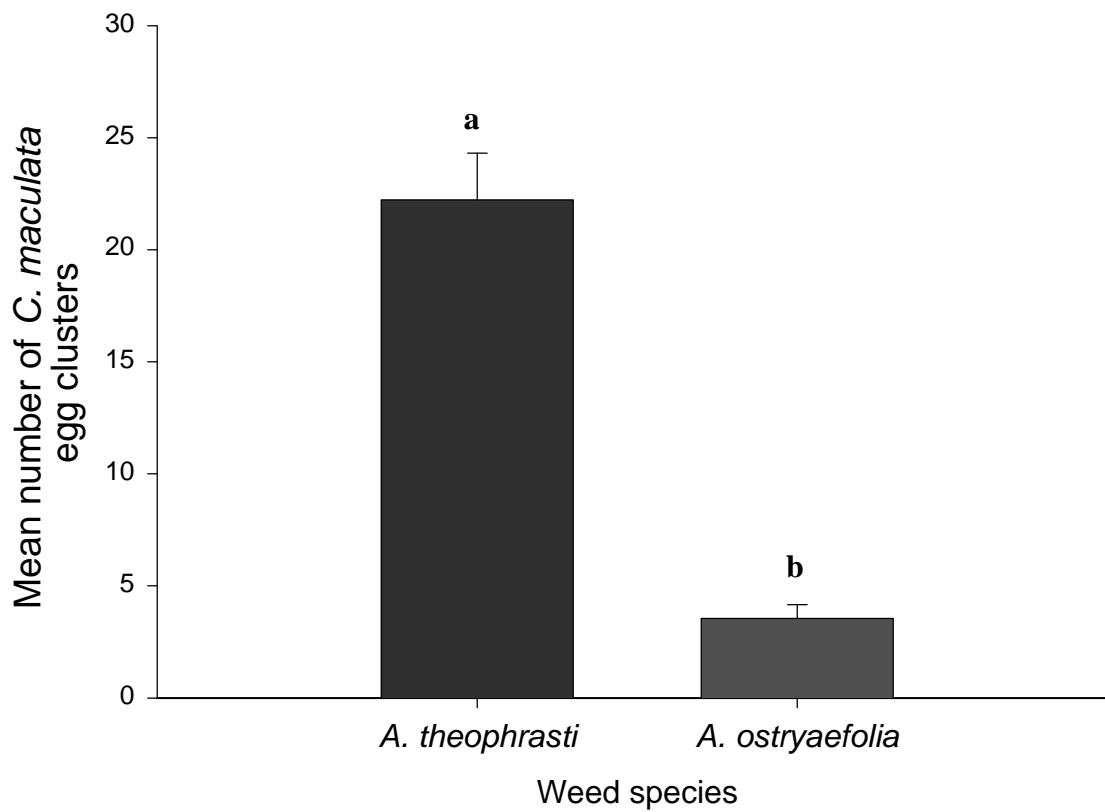


Table 4: Mean leaf area (cm<sup>2</sup>) of *A. ostryaefolia* and *A. theophrasti*. Means sharing the same letter are not significantly different ( $P > 0.05$ , Fisher protected LSD test).

Weed species	Mean leaf area (cm <sup>2</sup> ), mean $\pm$ SE
<i>A. ostryaefolia</i>	975.7 $\pm$ 164.3 <b>a</b>
<i>A. theophrasti</i>	489.4 $\pm$ 77.6 <b>b</b>

Figure 3: Mean ( $\pm$  SE) number of *C. maculata* egg clusters per plant on whitefly-infested *A. theophrasti* compared to the mean number of egg clusters found on non-infested *A. theophrasti*. Means sharing the same letter are not significantly different ( $P>0.05$ , Fisher protected LSD test).

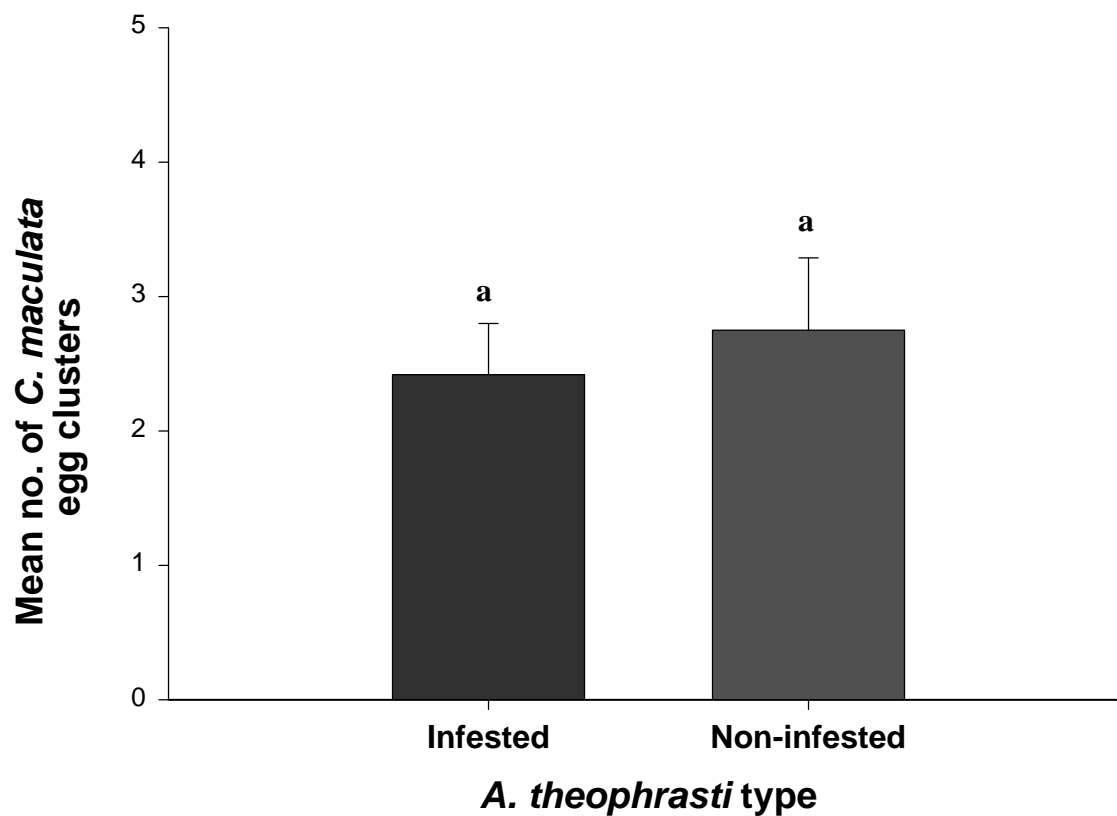


Table 5: Mean leaf area (cm<sup>2</sup>) and mean number of immature whiteflies in the whitefly-infested vs. non-infested *A. theophrasti* experiment. Means within in a column sharing the same letter are not significantly different ( $P>0.05$ , Fisher protected LSD test).

<i>A. theophrasti</i> type	Mean leaf area (cm <sup>2</sup> ), mean $\pm$ SE	No. of immature whiteflies, mean $\pm$ SE
Whitefly-infested	348.4 $\pm$ 22.2 <b>a</b>	257.9 $\pm$ 69.8 <b>a</b>
Non-infested	267.2 $\pm$ 19.5 <b>a</b>	5.2 $\pm$ 4.3 <b>b</b>



## Discussion

Cottrell and Yeargan (1998a) found that when given a choice between ovipositing on corn plants and the endemic weed, *A. ostryaefolia*, *C. maculata* females appeared to prefer *A. ostryaefolia*. I examined *C. maculata*'s ovipositional preferences when presented with a wider selection of common weed species. Out of nine selected weed species (including *A. ostryaefolia*), *C. maculata* appeared to prefer *A. theophrasti* as an ovipositional site. When beetles were given a choice between only *A. ostryaefolia* and *A. theophrasti*, females again readily chose *A. theophrasti* over *A. ostryaefolia*. Measurements of leaf area indicated that the amount of available leaf area was not the reason that more *C. maculata* eggs were laid on *A. theophrasti*. It should be noted that when *A. theophrasti* was not present, Coccinellidae laid far more eggs on *A. ostryaefolia* than on four of the other weed species in my experiments, namely smooth pigweed, lambsquarter, prickly sida, or eastern black nightshade (Appendix 1). Velvetleaf and three other species used in my 9-weed-species experiment were not present in this particular stand of naturally occurring weeds. This preference for *A. ostryaefolia* over several other weed species is in agreement with the results reported by Cottrell and Yeargan (1998a).

The bandedwing whitefly was observed more abundantly on *A. theophrasti* than on any of the other selected weed species. It was hypothesized that the presence of the bandedwing whitefly on *A. theophrasti* could influence *C. maculata* ovipositional site selection. Although numerous *C. maculata* eggs were oviposited on the two types of *A. theophrasti*, there was no significant difference in the mean number of *C. maculata* eggs laid on either type. This finding lends support to the work of Coderre et al. (1987) which states that *C. maculata* adults were less likely than other coccinellids to lay eggs near a food source.

Cottrell and Yeargan (1999) found that *C. maculata* egg cannibalism was lower on *A. ostryaefolia* than on nearby corn plants. They hypothesized that the presence of glandular trichomes on the stems and petioles of the plants affected the foraging ability of larval *C. maculata*. Similar to *A. ostryaefolia*, *A. theophrasti* is densely covered with glandular trichomes.

In my ovipositional preference experiments, I have shown that *C. maculata* prefer *A. theophrasti* over the other selected weed species. Even when paired with a species shown to be preferred from previous studies, *C. maculata* readily chose *A. theophrasti* as an ovipositional site. While some coccinellids lay their eggs near food sources, the presence of whitefly prey did not appear to influence *C. maculata* ovipositional selection in my experiments.

## Chapter III

### Potential factors influencing ovipositional site selection of *C. maculata* in sweet corn

#### Introduction

*Coleomegilla maculata* is a polyphagous predator common to corn fields in the United States and Canada (Hodek 1973). They are often found feeding on eggs of the Colorado potato beetle and European corn borer, as well as various life stages of several aphid species (Coll and Bottrell 1991, Hazzard and Ferro 1991, Hodek and Honěk 1996, Schellhorn and Andow 1999). *Coleomegilla maculata* are also well known for their pollen-feeding (Smith 1960, 1961).

Feeding preferences can influence ovipositional choices in insects. Females of the predatory midge, *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae), typically oviposit on pubescent potato plants near large aphid infestations (Lucas and Brodeur 1999). Similarly, several coccinellid species are known to oviposit on plants close to clusters of prey (Coderre et al. 1987, Schellhorn and Andow 1999). Coccinellid beetles are known to use visual cues or color in order to locate prey, and thus, ovipositional substrates. In a study comparing the role of visual cues among common coccinellid species, Harmon et al. (1998) found adult *C. septempunctata* to be highly affected by both light and color. Adult *C. maculata*, however, appeared not to rely on visual or color cues to locate prey (Harmon et al. 1998).

Coccinellids also can utilize olfactory cues to detect prey, overwintering sites, and ovipositional sites. Positive chemotaxes direct some species to overwintering aggregation sites (Benton and Crumb 1979). Several experiments have been conducted using olfactometers to test coccinellid olfactory detection of aphid prey and/or host plants. Hamilton et al. (1999) found that *H. convergens* possess the ability to detect aphids and their host plants. Similarly, Raymond et al. (2000) examined responses of *A. bipunctata* and found that females were more attracted to aphid-infested plants than to those lacking aphids.

The physical makeup of a plant plays a role in oviposition for some insects. The presence of trichomes for example, can influence oviposition. A higher degree of pubescence on soybean plants increases oviposition of *Bemisia argentifolii* Bellows and

Perring (Homoptera: Aleyrodidae) (McAuslane 1996). Similarly, increased oviposition by this pest occurs on tomato leaves covered with dense trichomes (Heinz and Zalom 1995). The effect of trichomes is unknown for many coccinellid species. In field studies, coccinellid oviposition on plants with dense glandular trichomes was greater than on more glabrous plants; however, percentages of beetles were higher on the more glabrous plants (Obrycki and Tauber 1985). Cottrell and Yeargan (1998a) found that *C. maculata* oviposited more on *A. ostryaefolia* (whose petioles and stems possess simple and glandular trichomes) than on nearby corn plants, which possess only simple trichomes. They also observed decreased cannibalism and other predation of *C. maculata* eggs on *A. ostryaefolia* as compared with corn.

My objectives were to examine factors that could influence *C. maculata* oviposition on selected weeds in Kentucky corn fields. Specifically, I assessed the attractiveness of selected weed species to adult *C. maculata*. I also examined potential prey densities on different weed species and the possible role of *A. ostryaefolia* flowers and fruit. In addition, I examined the influence of selected plants on predation of *C. maculata* eggs.

## Materials and Methods

For all experiments, data that failed to conform to the assumptions of an analysis of variance were transformed.

### Corn plots:

For all field experiments, 'Golden Queen' sweet corn was planted in rows spaced 0.9 m apart. All plots were treated with alachlor (3.9 kg [AI]/ha) at planting. Supplemental weeding was done manually.

### Assessment of weed attractiveness to adult *C. maculata*:

In order to test adult *C. maculata* attraction to specific weed species from afar, an experiment was conducted in corn from 3 July until 19 July, 1999, using two species of greenhouse-grown weeds (*A. ostryaefolia* and *S. spinosa*) and a bare-soil control. The weeds were selected based on a preliminary study from the previous summer (K.V. Yeargan and B.L. Newton., unpublished data) in which *A. ostryaefolia* was clearly

preferred for *C. maculata* oviposition over *S. spinosa*. The two weed species and bare-soil controls were allocated to a single corn plot (12 m by 12 m; 14 rows of corn; planted 11 May). Weeds were transplanted (or controls marked) approximately two meters apart between corn rows into randomized complete blocks on 3 July. Pots with weeds were transplanted so that the top of each pot was flush with the surrounding soil. Each block contained two *A. ostryaefolia*, two *S. spinosa*, and two controls; there was a total of 6 blocks, 24 plants and 12 controls. Hardware cloth cages (30 cm by 30 cm by 30 cm; 0.6 cm mesh size) were placed over the transplanted weeds and bare soil controls (36 cages total) on 4 July. Tassels began to shed pollen about 3 or 4 days after cages had been installed over the weeds and controls. To catch any visiting adult *C. maculata*, each cage was coated with adhesive (Stikem Special, Seabright Enterprises, Emeryville, CA, USA). *Coleomegilla maculata* adults were collected every four days (7 July to 19 July) from the cages, and taken back to the laboratory for gender determination (by dissection).

A second experiment was performed from 2 August until 14 August 1999 in a manner similar to the experiment just described (i.e., 36 sticky cages over selected weed species and bare soil; adult *C. maculata* were collected every 4 days). However, due to results obtained in the 9-weed-species study (Chapter II), this second experiment used three weed species: *A. ostryaefolia*, *A. theophrasti*, and *A. hybridus*, along with bare-soil controls. Weeds used in this experiment were transplanted from the field into pots. As in the first experiment, a single plot of corn was used (12 m by 12 m with 14 rows; planted on 11 June). Between every other corn row, a row of weeds (or bare-soil controls) was transplanted (or marked with stakes in cases of bare soil controls) on 2 August; each row consisted of six cages (over weed or bare soil) as in the previous experiment, with treatments randomly assigned within each row. Due to the addition of a third weed species in this experiment, however, each row contained only one entire block (consisting of three plants and one control) and one half block (consisting of either two plants of the selected weed species or a plant and control); each half block was continued into the next row of treatments. Cages coated with adhesive (Stikem Special) were placed over the transplanted weeds and bare-soil controls on 3 August. Tassels began to shed pollen about 2 or 3 days after cages had been installed over the weeds and controls.

*Coleomegilla maculata* adults were collected every 4 days for 12 days (6 August to 14

August) and taken to the laboratory for gender determination by dissection. In both experiments, Bartlett's test for homogeneity of variance was performed (Analytical Software 1992). If necessary, data were transformed (square root of the number of adult *C. maculata* found on cages associated with each treatment). Data were analyzed using a two-way ANOVA. Means were compared among treatments using an LSD test. Data are presented as untransformed means  $\pm$  SE.

#### Densities of potential prey on different weed species:

Because the presence of prey found on a plant might influence *C. maculata* oviposition, densities of potential prey were recorded on three dates (July 9, 15, and 21, 1999) during the 9-weed-species experiment (Chapter II). While sampling the 72 plants for *C. maculata* egg clusters, I also examined plants for arthropod prey on those dates. Any arthropods not recognized in the field were collected and taken back to the laboratory for identification. A Bartlett's test was used to determine homogeneity of variance and data were transformed (square root of the number of prey found on each plant). A two-way ANOVA was used to determine whether potential prey densities varied among weed species. The mean numbers of arthropod prey were compared among weed species using a protected LSD. Data are presented as untransformed means  $\pm$  SE.

Due to an abundance of immature whiteflies observed on *A. theophrasti*, the level of immature whitefly infestation on all the weed species was assessed at the end of two types of experiments (i.e., the 9-weed-species studies, and the *A. ostryaefolia* vs. *A. theophrasti* experiment; Chapter II). Before leaves were removed from the plants for leaf area measurements, the lowest, full-grown, non-senescent leaf from each plant from those experiments was marked with corrective fluid (Wite Out, Bic Corp., Miford, CT, USA). After the leaf area measurements were taken, the marked leaves were retrieved and examined under a microscope. All immature whiteflies (i.e., nymphs or pupae) on each leaf were counted and recorded. Adults were not counted after the plants were removed from the field because of their ability to easily move from one plant to another or escape. Bartlett's test indicated heterogeneous variances, so data were transformed (square root of the number of immature whiteflies found on the selected leaf from each weed specimen) and analyzed using a two-way ANOVA. In the 9-weed-species experiments, means were separated using a protected LSD.

Possible role of *A. ostryaefolia* flowers and fruits in *C. maculata* oviposition:

In order to determine the possible importance of non-animal food resources to ovipositing *C. maculata* females, forty greenhouse-grown *A. ostryaefolia* were transplanted on 3 July into a single corn plot (10 m by 18 m; planted 11 May 1999) just as the corn had begun to tassel. Eight weeds were placed two meters apart into five rows. Pots with weeds were placed into holes so that the top of each plot was flush with the surrounding soil. Each block of weeds was separated by a row of corn; there were twelve total rows of corn in the plot. One half of the *A. ostryaefolia* plants in each block were randomly selected and all fruits and flowers were removed from those plants, with a scalpel. This procedure was repeated on those same plants at two-day intervals throughout the duration of the experiment. The number of coccinellid egg clusters and the number of eggs within each cluster were recorded and collected every other day for 17 days. Sampling began on 4 August and ended on 20 August. All eggs collected were incubated in the laboratory in order to verify species. Data were transformed (square root of the number of egg clusters found on each plant) and analyzed using a two-way ANOVA.

Predation of *C. maculata* eggs on selected weed species and corn:

In July and August 2000, I examined predation of *C. maculata* eggs on selected weed species in corn. A laboratory colony of *C. maculata* was started from adults collected near Lexington, KY. The beetles were kept in an environmental chamber at  $27 \pm 1^\circ \text{C}$  and 15:9 L/D. Mated females were placed singly into 9 cm petri dishes and provided food and water. Food consisted of *H. zea* eggs obtained from a laboratory colony, similar to the one described by Cottrell and Yeargan (1999). A water source was provided by placing a moistened cotton dental wick into each petri dish. Circles of green floral paper (10.5 cm in diameter) were used to line the lids of the petri dishes, thus allowing *C. maculata* females an ovipositional substrate that could be removed and replaced easily. Egg clusters were collected daily for five days prior to each run of this experiment and stored at  $15^\circ \text{C}$ . Because the number of eggs per cluster varies in the field, a constant number of eggs was not predetermined for this experiment. The number of eggs in each cluster was counted and recorded prior to placing them into the field. Before sheets containing egg clusters were placed on the specified plants, they were

trimmed to approximately 6 cm<sup>2</sup> and coded according to date on which they were laid. The mean number of eggs per cluster ( $\pm$ SE) used in this experiment was 12.5  $\pm$  0.4 (n= 235 clusters).

For this experiment, a single plot (10m x 14m; 12 rows) of corn was planted on 5 June, 2000. Ten field-grown *A. theophrasti*, *A. ostryaefolia*, and *A. hybridus* were transplanted on 26 July ( $\approx$  2 meters apart) between corn rows in a randomized complete block design (30 plants total). Plants were transplanted into five rows within the plot; each row contained two blocks of three weed species. Pots were placed into the ground so that the top of each pot was flush with the surrounding soil. A single corn stalk, in a row adjacent to each block of weeds, was randomly selected and marked with a wooden stake (for a total of 30 weeds and 10 marked corn stalks). At dawn on four dates ( 27 July, 1, 6, and 11 August), one *C. maculata* egg cluster was placed onto each plant. A sheet containing a single egg cluster was stapled to the underside of a randomly chosen leaf on each of the thirty weeds. Insect pins were used to secure sheets with egg clusters to corn stalks at randomly chosen heights ranging from 15-45 cm from the ground, a height range typical of naturally laid *C. maculata* egg clusters (Cottrell and Yeagan 1998b). At dusk, the egg clusters were observed for evidence of predation. Any cluster with eggs that had been fed upon was recorded and replaced with a fresh egg cluster and the number of eggs fed upon was recorded. At dawn the next day, evidence of predation was again recorded and all egg sheets were collected and taken to the laboratory. Any predators observed feeding on *C. maculata* egg clusters were recorded during the dusk and dawn periods when egg clusters were examined. For statistical analysis, the four dates on which this experiment was conducted were considered to be replicates. On each date, the percentage of *C. maculata* egg clusters attacked was calculated for each plant species (=treatment), based on the ten egg clusters that had been exposed on specimens of that plant species. The data were transformed (arcsine of the square root of the proportion attacked) prior to analysis with a one-way ANOVA. A protected LSD test was used to compare mean proportion of clusters attacked among the four plant species. Data are presented as untransformed means  $\pm$  SE. This procedure was used to analyze the proportion of clusters attacked during the day, during the night, and for the entire 24-hour period. To derive the proportion attacked during the 24-hour period, the proportion



attacked during the day was added to the proportion attacked during the night. In the few cases where this value was greater than 1.0, the value was truncated at 1.0. Values greater than 1.0 were possible because attacked egg masses were replaced at dusk and examined at dawn the following day, but I wanted the sum to reflect the probability that an “original” egg mass would have been attacked if it had been left in place for the entire 24 hours. Values greater than 1.0 are meaningless in this context.

## Results

### Assessment of weed attractiveness to adult *C. maculata*:

In both experiments, there was no significant difference among treatments in the number of *C. maculata* adults (female, male, or total) found on cages ( $F=0.3$ ,  $df=2,10$ ,  $P=0.76$ ;  $F=0.3$ ,  $df=2,10$ ,  $P=0.79$ ;  $F=0.5$ ,  $df=2,10$ ,  $P=0.67$ ) (Figures 4a, b, and c) and ( $F=0.9$ ,  $df=3,24$ ,  $P=0.46$ ;  $F=1.6$ ,  $df=3,24$ ,  $P=0.22$ ;  $F=1.5$ ,  $df=3,24$ ,  $P=0.23$ ) (Figures 5a, b, and c), respectively.

### Densities of potential prey on different weed species:

Based on direct-observation sampling of potential arthropod prey in the 9-weed-species study (Chapter II) there were significantly more arthropods found on *A. theophrasti* (excluding immature whiteflies) than on any other weed species ( $F=3.6$ ,  $df=8,36$ ,  $P<0.05$ ) (Table 6). Adult whiteflies composed the majority of arthropods (excluding immature whiteflies) found on *A. theophrasti* plants, and were observed only on plants of this species ( $F=169.0$ ,  $df=8,36$ ,  $P<0.05$ ) (Table 6). Other arthropods observed on the weed species were: various leafhoppers, *Geocoris* spp., and *C. maculata* larvae. Flea beetles were found on all weed species except *A. theophrasti*, with the highest sample mean found on *A. ostryaefolia* ( $F=4.1$ ,  $df=8,36$ ,  $P<0.05$ ) (Table 6). Immature whiteflies were not included in this analysis because they could not be reliably sampled in the field. In assessments at the conclusion of the 9-weed-species experiments (1999 and 2000), *A. theophrasti* had significantly more immature whiteflies present than any other weed species ( $F=55.2$ ,  $df=8,36$ ,  $P<0.05$ ) and ( $F=54.3$ ,  $df=8,36$ ,  $P<0.05$ ) respectively (Table 7). Similarly in the *A. ostryaefolia* vs. *A. theophrasti* experiment, far more immature whiteflies were found on *A. theophrasti* than on *A. ostryaefolia* ( $F=151.8$ ,  $df=1,17$ ,  $P<0.05$ ) (Table 8).

Figure 4: Comparison of mean ( $\pm$  SE) number of adult *C. maculata* a) females, b) males, and c) total, caught per cage across treatment type. Means sharing the same letter are not significantly different ( $P > 0.05$ , Fisher protected LSD test).

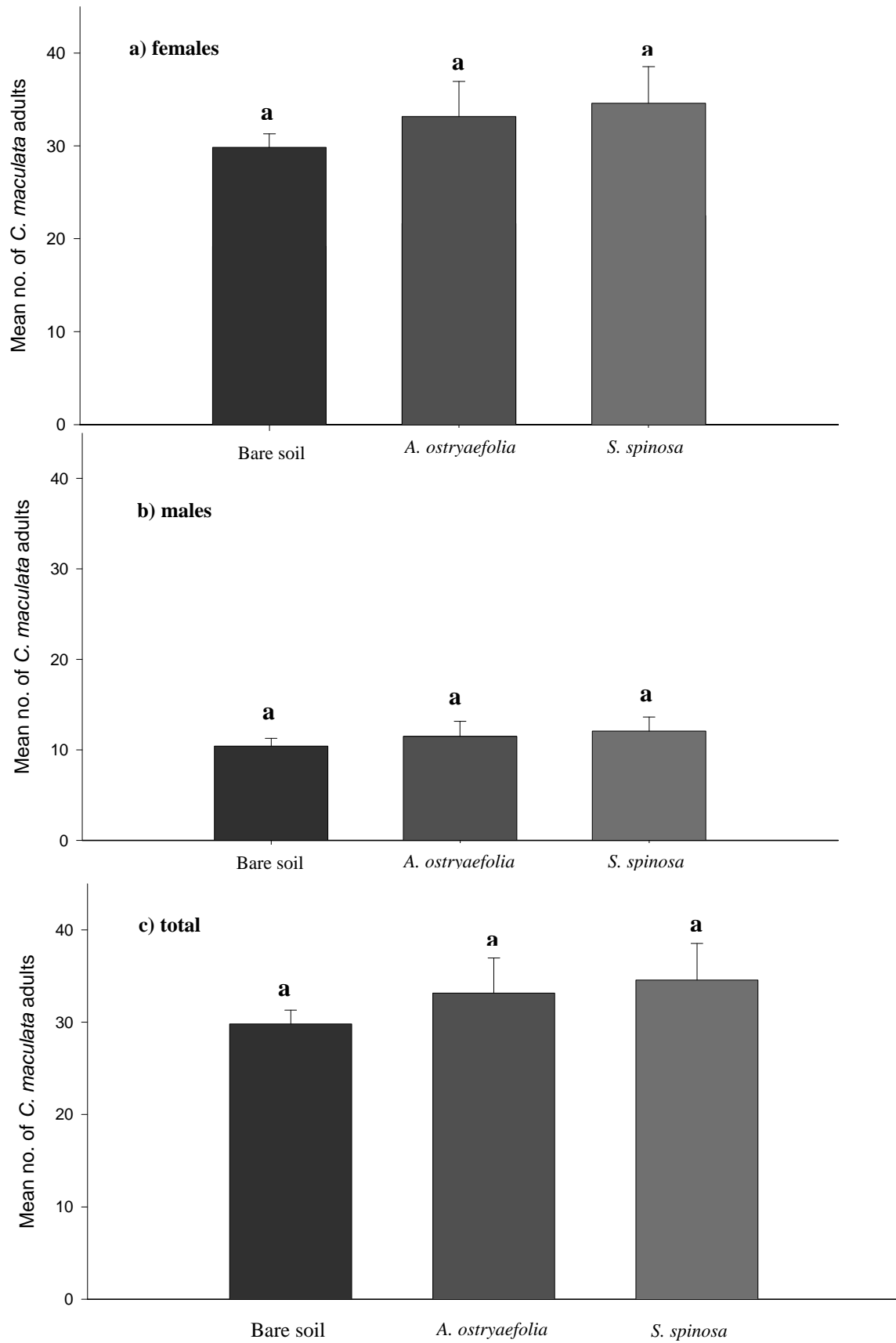


Figure 5: Comparison of mean ( $\pm$  SE) number of adult *C. maculata* a) females, b) males, and c) total, caught per cage across treatment type (second experiment). Means sharing the same letter are not significantly different ( $P > 0.05$ , Fisher protected LSD test).

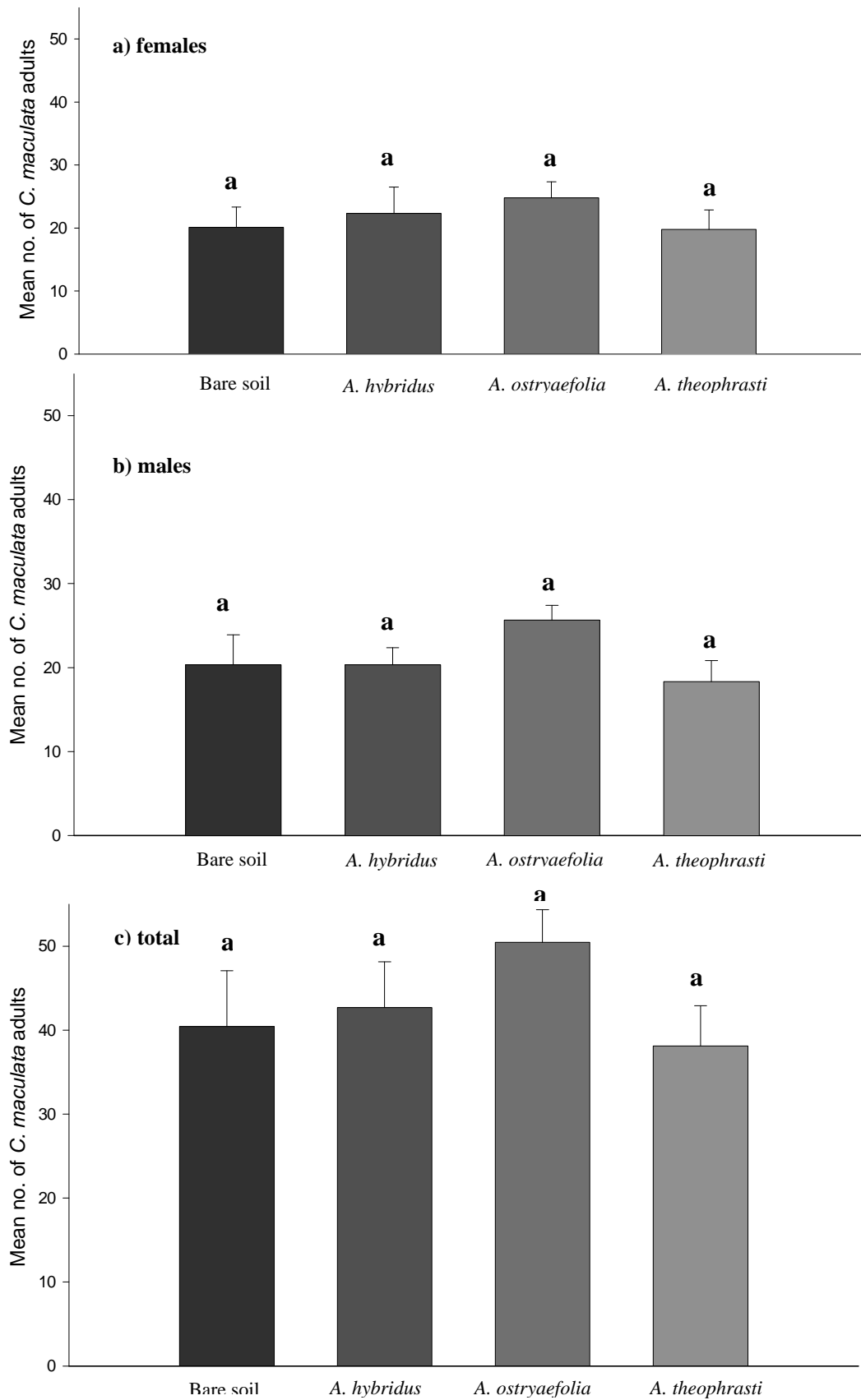


Table 6: Mean ( $\pm$  SE) numbers of arthropods sampled in the (1999) 9-weed-species study. Means within a column sharing the same letter are not significantly different ( $P>0.05$ , Fisher protected LSD test).

Weed species	Total arthropods <sup>a</sup> , mean $\pm$ SE	No. adult whiteflies <sup>b</sup> , mean $\pm$ SE	No. flea beetles <sup>c</sup> , mean $\pm$ SE
<i>A. hybridus</i>	1.6 $\pm$ 0.5bc	0.00	1.6 $\pm$ 0.5abc
<i>A. ostryaefolia</i>	3.0 $\pm$ 0.9ab	0.00	2.9 $\pm$ 0.9a
<i>A. theophrasti</i>	4.9 $\pm$ 0.7a	4.6 $\pm$ 0.7	0.00d
<i>A. virginica</i>	1.5 $\pm$ 0.5bc	0.00	0.9 $\pm$ 0.4bcd
<i>C. album</i>	2.4 $\pm$ 1.1b	0.00	1.0 $\pm$ 0.5bc
<i>G. ciliata</i>	2.4 $\pm$ 0.6b	0.00	2.0 $\pm$ 0.5abc
<i>S. spinosa</i>	1.4 $\pm$ 0.4bc	0.00	1.1 $\pm$ 0.4abc
<i>S. ptycanthum</i>	0.8 $\pm$ 0.4c	0.00	0.5 $\pm$ 0.3cd
<i>X. strumarium</i>	2.4 $\pm$ 0.6b	0.00	1.8 $\pm$ 0.5abc

<sup>a</sup> mean number of all arthropods (excluding immature whiteflies) found per plant and per plant species

<sup>b</sup> mean number of all adult whiteflies found per plant and per plant species

<sup>c</sup> mean number of all flea beetles found per plant and per plant species

Table 7: Mean number of immature whiteflies (WFI) recorded on selected leaves of each plant from each weed species in the 9-weed-species study (1999 and 2000). Selected leaves were always the lowest, full-grown, non-senescent leaf on each plant. Means within columns followed by the same letter do not differ significantly ( $P>0.05$ , Fisher protected LSD test).

Weed Species	1999 WFI, mean $\pm$ SE	2000 WFI, mean $\pm$ SE
<i>A. hybridus</i>	0.0b	0.2 $\pm$ 0.2b
<i>A. ostryaefolia</i>	0.0b	0.0b
<i>A. theophrasti</i>	36.9 $\pm$ 9.4a	40.1 $\pm$ 6.1a
<i>A. virginica</i>	0.0b	0.0b
<i>C. album</i>	0.0b	0.0b
<i>G. ciliata</i>	0.6 $\pm$ 0.4b	0.4 $\pm$ 0.3b
<i>S. spinosa</i>	0.4 $\pm$ 0.4b	0.0b
<i>S. ptycanthum</i>	0.0b	0.6 $\pm$ 0.5b
<i>X. strumarium</i>	0.0b	3.0 $\pm$ 1.0b

Table 8: Immature whitefly assessment for the *A. ostryaefolia* vs. *A. theophrasti* experiment. Selected leaves were always the lowest, full-grown, non-senescent leaf on each plant. Means followed by the same letter do not differ significantly ( $P>0.05$ , ANOVA).

Weed species	No. of imm. whiteflies, mean $\pm$ SE
<i>A. ostryaefolia</i>	5.0 $\pm$ 1.5 <b>b</b>
<i>A. theophrasti</i>	151.7 $\pm$ 15.9 <b>a</b>



Possible role of *A. ostryaefolia* fruit and flowers in *C. maculata* oviposition:

There was no significant difference in the number of *C. maculata* eggs laid on *A. ostryaefolia* plants with or without fruit and flowers ( $F=0.9$ ,  $df=1,34$ ,  $P=0.48$ ) (Figure 6). The mean number of eggs per cluster  $\pm$  SE for *A. ostryaefolia* with fruit and flowers was  $8.4 \pm 1.8$ ; *A. ostryaefolia* plants lacking fruit and flowers had  $10.2 \pm 5.1$  eggs per cluster.

Predation of *C. maculata* eggs on selected weed species and corn:

Predation of *C. maculata* egg clusters occurred on all plant types during the day. Diurnal predation of egg clusters was significantly higher on corn than on *A. theophrasti* ( $F=5.8$ ,  $df=3,12$ ,  $P>0.05$ ) (Figure 7a). During the night, predation of *C. maculata* eggs occurred only on corn and *A. hybridus* ( $F=15.1$ ,  $df=3,12$ ,  $P>0.05$ ) (Figure 7b). When predation of *C. maculata* egg clusters was examined over a 24 hour period, predation was significantly higher on corn and *A. hybridus* than on *A. ostryaefolia* and *A. theophrasti* ( $F=9.6$ ,  $df=3, 12$ ,  $P<0.05$ ) (Figure 7c). Only five predators (i.e., four on corn, one on pigweed) were observed while they were feeding on *C. maculata* eggs; all were larval *C. maculata*.

## Discussion

In each experiment examining *C. maculata* ovipositional preferences for selected weeds (Chapter II), I found that *A. theophrasti* was preferred over all other plants (except *Galinsoga* spp. in 2000). This was true in the 9-weed-species experiments and the *A. ostryaefolia* vs. *A. theophrasti* experiment. Several factors could influence *C. maculata* ovipositional site selection. In the weed attractiveness experiments, I tested whether *C. maculata* adults were attracted from afar to selected weed species. The mean number of *C. maculata* adults recovered from traps over each treatment type was not significantly different among treatments, even for traps over bare soil controls. This suggests that adult beetles are not attracted from afar to their preferred ovipositional sites by visual or olfactory cues from the selected weed species. Harmon et al. (1998) found that although other coccinellid species were influenced (albeit slightly) by visual cues, such as light intensity and color, *C. maculata* were not. While some coccinellids appear to detect

Figure 6: Mean ( $\pm$  SE) of *C. maculata* egg clusters per plant on *A. ostryaefolia* with intact fruits and flowers compared with the mean number of *C. maculata* egg clusters on *A. ostryaefolia* plants lacking fruits and flowers. Means followed by the same letter are not significantly different ( $P > 0.05$ , Fisher protected LSD test).

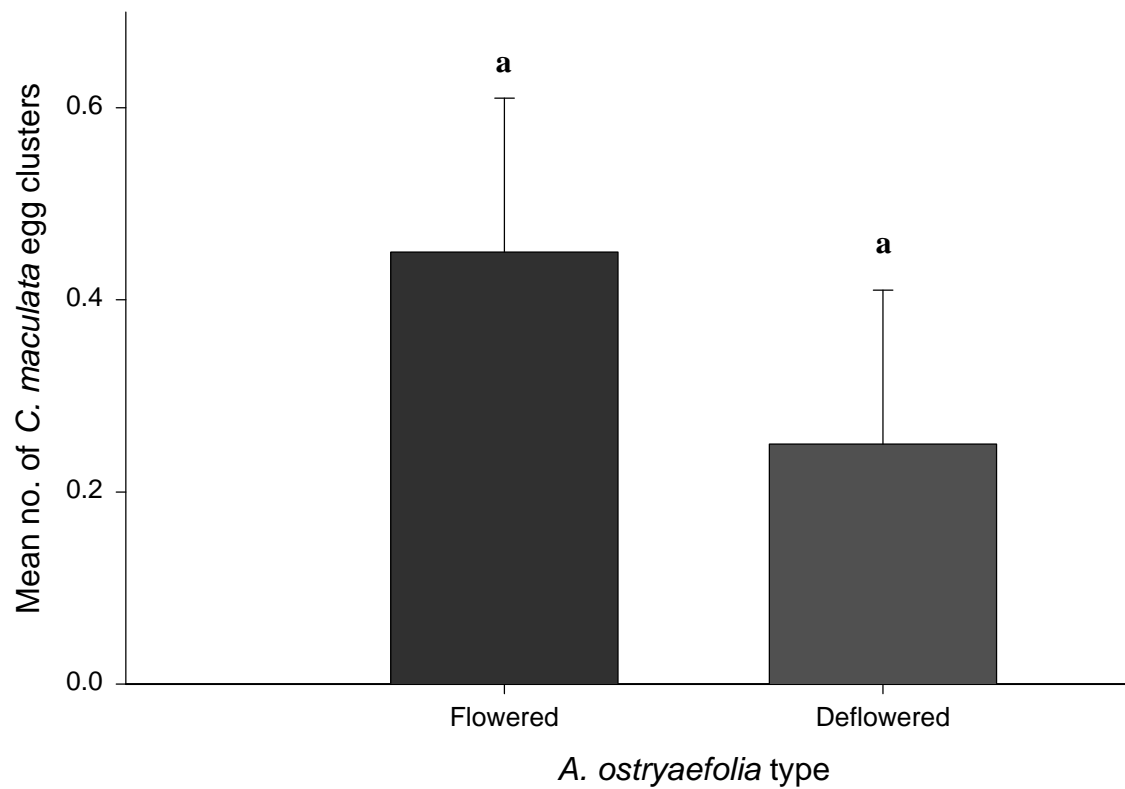
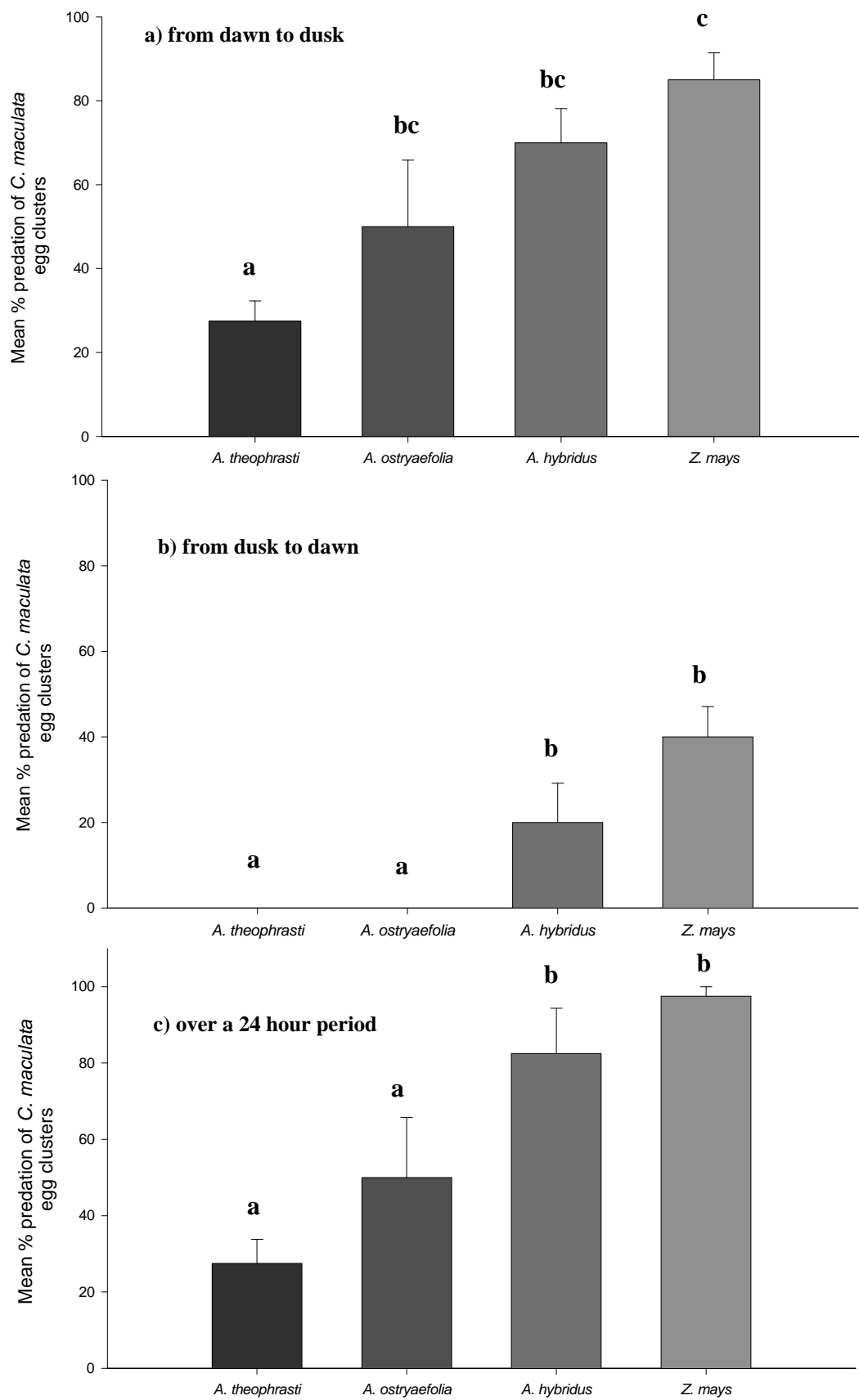


Figure 7: Mean ( $\pm$  SE) percentage of predation of *C. maculata* egg clusters a) from dawn to dusk, b) from dusk to dawn and c) over a 24 hour period, on selected plants. Means followed by the same letter are not significantly different ( $P > 0.05$ , Fisher protected LSD test).



aphid prey and/or host plants through olfaction (Hamilton et al. 1999, Raymond et al. 2000), *C. maculata* did not discriminate between landing on traps over weeds or over bare soil. Because of *C. maculata*'s polyphagous diet, ovipositional preferences for intact *A. ostryaefolia* or *A. ostryaefolia* lacking fruits and flowers, was examined. Although *C. maculata* are well known pollen feeders (Smith 1960, 1961), their ovipositional selections did not appear to be affected by the presence or absence of flowers on *A. ostryaefolia* plants.

Coderre et al. (1987) stated that *C. maculata* are more likely to oviposit away from clusters of prey, compared with other coccinellid species. When I sampled arthropod prey in the first 9-weed-species experiment, the bandedwing whitefly, *T. abutiloneus*, was much more abundant on *A. theophrasti* than any other weed species. Similarly, when immature whitefly infestation was assessed, there were always significantly more whiteflies on *A. theophrasti*. Immature whiteflies could serve as possible food for young *C. maculata* larvae. However, when I examined ovipositional preferences for *A. theophrasti* with or without immature whitefly infestations (Chapter II), there was no significant difference in the mean number of eggs laid on either type of plant, suggesting that whitefly presence did not significantly influence *C. maculata* oviposition.

Egg predation and cannibalism are widespread among coccinellid species (Hodek and Honøk 1996). In my experiment, *C. maculata* larvae were the only predators seen feeding on *C. maculata* eggs. Virtually all egg clusters placed on corn stalks were consumed within 24 hours. *A. hybridus* also suffered a high percentage of predation. Egg clusters placed on *A. ostryaefolia* and *A. theophrasti* were less frequently attacked by predators. More predation on all plant species occurred during the day than at night. While adult *C. maculata* are primarily diurnal feeders, larvae feed both day and night (Cottrell and Yeorgan 1998b).

In this study, I examined various factors that could potentially influence *C. maculata* oviposition on weeds such as *A. ostryaefolia* and *A. theophrasti*. *Coleomegilla maculata* do not appear to be attracted to these weed species from a distance by visual or olfactory cues. Abundance of prey did not seem to serve as an ovipositional stimulus. Glandular trichomes occur on the leaves, stems, and petioles of *A. theophrasti* (Figure 8).

There are two types of glandular trichomes on *A. theophrasti*: one with long stalks ending in a clavate head; the other with a short stalk bearing a capitate gland (Navasero and Ramaswamy 1991). *Acalypha ostryaefolia* also possess glandular trichomes on stems and petioles (Figure 8). However, glandular trichomes are absent from the other seven weed species I tested. *Chenopodium album* does not possess glandular trichomes, but its, stem, petiole, and leaf surfaces are covered with “bladders” which contain vacuolar water (Harr et al. 1991) (Figure 8). Simple trichomes are found on leaves, stems, and petioles of *A. virginica*, *G. ciliata*, and *X. strumarium*, while stems and petioles of *A. hybridus*, *S. spinosa*, and *S. ptycanthum* possess simple trichomes (Figure 8). Thus, there is a correlation between presence of glandular trichomes and selection of ovipositional sites by *C. maculata*. Furthermore, predation on *C. maculata* egg clusters is significantly lower on those plants with glandular trichomes than those without them. Presence of glandular trichomes may be a major factor influencing choice of oviposition sites by *C. maculata*.

Figure 8: Stems and petioles of the weed species used in the 9-weed-species studies.



*Abutilon theophrasti*



*Acalypha ostryaefolia*



*Acalypha virginica*



*Amaranthus hybridus*



*Chenopodium album*



*Galinsoga ciliata*



*Sida spinosa*



*Solanum ptycanthum*



*Xanthium strumarium*

## Chapter IV

### Summary and Conclusions

Previous research revealed a preference by *C. maculata* for ovipositing on the weed *A. ostryaefolia* compared with corn; however, in my study whenever *A. theophrasti* was available to ovipositing *C. maculata* in the field, it was selected more frequently than *A. ostryaefolia* or any other weed tested (except *Galinsoga* spp. in 2000). While there was *C. maculata* oviposition on several of the other selected weed species, the combined mean number of egg clusters on those species was only a fraction of the number on *A. theophrasti*.

Visual cues, either from preferred oviposition plants or potential prey (e.g., whiteflies), did not influence *C. maculata* choices. When plants were covered by sticky traps, or sticky traps were placed over bare soil, there was no difference in the number of adults found on any of the cages. More specifically, adult female *C. maculata* were not attracted to their preferred plant species from afar. This indicates that ovipositional choices are made at close range.

Due to *C. maculata*'s previously reported preference for ovipositing on *A. ostryaefolia* and their polyphagous diet, I examined the influence of *A. ostryaefolia*'s fruit and floral structures on ovipositional choice. Beetles did not show a significant preference between plants with or without those structures.

An abundance of potential prey near an ovipositional site would seem beneficial to a predator. Hatching larvae would have a food supply nearby. In my study, there were significantly more adult and immature whiteflies, *T. abutiloneus*, on *A. theophrasti* plants than on any of the other tested weed species. One might expect that coccinellid oviposition would be influenced by the presence of these whiteflies. *Coleomegilla maculata* are one of five coccinellid species known to feed on *T. abutiloneus* in Louisiana cotton fields (Watve and Clower 1976). However, when I compared *C. maculata* oviposition on *A. theophrasti* with large populations of *T. abutiloneus* to *A. theophrasti* virtually lacking these potential prey, there was no significant difference in the mean number of *C. maculata* eggs oviposited. It appears likely that *C. maculata* oviposits preferentially on *A. theophrasti* for some reason other than prey availability.



*Coleomegilla maculata* egg clusters laid on *A. theophrasti* and *A. ostryaefolia* are better protected from potential predators than clusters on *A. hybridus* and corn. This is especially evident during the day, when most predation occurs. Corn and *A. hybridus* apparently provide excellent foraging surfaces for *C. maculata* larvae because predation rates of *C. maculata* eggs were very high on these plant species. In my study and a previous one (Cottrell and Yeargan 1998b), *C. maculata* larvae were the predominant predators of *C. maculata* eggs (i.e., cannibalism). Larval foraging on *A. theophrasti* and *A. ostryaefolia* may be more difficult because of the glandular trichomes covering the leaves, petioles, and stems of *A. theophrasti* and the stems and petioles of *A. ostryaefolia*.

The generalist predator, *C. maculata*, is an important naturally occurring biological control agent in certain agricultural crops (e.g., corn, potatoes). Discovering methods that could increase densities of this coccinellid could prove beneficial in many agroecosystems. Further investigation of *C. maculata*'s ovipositional preference for *A. theophrasti* is needed. Experiments that directly examine the influence of trichome exudates on *C. maculata* would be beneficial. Weed species can influence biological control by increasing plant diversity for a given crop system, which may lead to an increase in natural enemy populations. While the inclusion of large numbers of weeds in crop systems is undesirable, borders of weeds could serve as alternate sources of natural enemy food, ovipositional substrates, and refugia, thus increasing natural enemy efficiency.

Appendix 1: Supplemental sample of naturally occurring weed species found along corn plot (2000 9-weed-species study) perimeter.

Weed species	Coccinellid egg clusters, mean $\pm$ SE	Coccinellid eggs per cluster, mean $\pm$ SE
<i>A. ostryaefolia</i>	1.0 $\pm$ 0.3 (n=20)	14.3 $\pm$ 0.8 (n=20)
<i>A. hybridus</i>	0.00	0.00
<i>C. album</i>	0.2 $\pm$ 0.1 (n=20)	15.0 $\pm$ 1.7 (n=3)
<i>S. spinosa</i>	0.00	0.00
<i>S. ptycanthum</i>	0.00	0.00

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### **Vita**

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