

Oviposition Behavior of *Coleomegilla maculata* (Coleoptera: Coccinellidae): Diel Periodicity and Choice of Host Plants

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Environ. Entomol. 34(2): 440–445 (2005)

ABSTRACT Previous research has shown that more *Coleomegilla maculata* (DeGeer) eggs occur on plants with glandular trichomes than on plants without such trichomes. Those studies also revealed that *C. maculata* eggs suffer greater predation (mostly cannibalism) on plants lacking glandular trichomes than on plants with glandular trichomes. Those results, however, left open the question of whether differences in observed egg densities on the two types of plants were caused by oviposition preference or differential removal of eggs by predators. Our primary objective was to determine whether *C. maculata* preferentially oviposit on plants with glandular trichomes. We also determined the diel periodicity of *C. maculata* oviposition; in addition to its intrinsic value in understanding the predator's behavior, this knowledge facilitated our study of oviposition preference. Diel periodicity of oviposition was studied in the laboratory and in the field. In the laboratory, most oviposition occurred in the afternoon and early evening. In the field, the pattern was even more distinct, with all oviposition occurring between 1200 and 2000 hours and >80% occurring between 1400 and 1800 hours Eastern Daylight Time. To determine oviposition preference, we placed equal numbers of velvetleaf, *Abutilon theophrasti* (Medicus), and smooth pigweed, *Amaranthus hybridus* L., plants (with and without glandular trichomes, respectively) in the field and observed them continuously during the afternoon and early evening. Oviposition events by naturally occurring (i.e., wild, uncaged) *C. maculata* were recorded. The beetles clearly preferred to oviposit on plants with glandular trichomes, laying 51 of 52 egg clusters on velvetleaf, despite the fact that they visited smooth pigweed plants more often and spent more time per visit on those plants than on velvetleaf. We hypothesize that egg predation, especially cannibalism, has selected for this oviposition behavior.

KEY WORDS predator, glandular trichomes, sweet corn, smooth pigweed, velvetleaf

THE IMPORTANCE OF THE 12-spotted lady beetle, *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae), as a predator of pest species has been well documented. This native lady beetle occurs in a variety of North American crops and feeds on many types of prey, as well as pollen and fungal spores (Cottrell and Yeargan 1998a). Its prey include eggs of lepidopteran pest species such as the European corn borer, *Ostrinia nubilalis* (Hubner) (Conrad 1959, Coll and Bottrell 1991), and the corn earworm, *Helicoverpa zea* (Boddie) (Pfannenstiel and Yeargan 2002), as well as eggs of coleopteran pests such as the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Hazard and Ferro 1991). In the eastern United States, from Nebraska to Kentucky to New York, *C. maculata* is the most common lady beetle species found on corn, *Zea mays* L. (Cottrell and Yeargan 1998a, Wright and DeVries 2000, Musser and Shelton 2003).

Predation by adult *C. maculata* in sweet corn primarily occurs diurnally, whereas larvae feed both diurnally and nocturnally (Cottrell and Yeargan 1998a, Pfannenstiel and Yeargan 2002). Benton and Crump (1981) observed a marked tendency of *C. maculata* adults to climb up host plants during the morning hours, whereas in the afternoon, many of the beetles climbed down. Female *C. maculata* tend to oviposit in the lower portion of corn plants (Schellhorn and Andow 1999), but the diel periodicity of *C. maculata* oviposition has not been reported.

Cottrell and Yeargan (1998b) found higher numbers of *C. maculata* eggs on hophornbeam copperleaf, *Acalypha ostryaefolia* (Riddell), a weed in corn fields, than on nearby corn plants. They also showed that *C. maculata* eggs suffer higher mortality, primarily because of cannibalism, on corn plants than on hophornbeam copperleaf plants. In a study of nine weed species, Griffin and Yeargan (2002a) found more *C. maculata* eggs on velvetleaf, *Abutilon theophrasti* (Medicus), than on any other plant species tested. They also found that *C. maculata* eggs suffered less mortality

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on velvetleaf than on corn or on smooth pigweed, *Amaranthus hybridus* L. Both hophornbeam copperleaf and velvetleaf possess glandular trichomes, whereas corn and smooth pigweed do not, which suggests the glandular trichomes provide protection for *C. maculata* eggs from predation. While those studies (Cottrell and Yeargan 1998b, Griffin and Yeargan 2002) found far more eggs on plants with glandular trichomes than on plants without such trichomes, this does not necessarily mean that more eggs were laid on plants with glandular trichomes. Eggs were sampled in those studies at 24- (Cottrell and Yeargan 1998b) or 48-h (Griffin and Yeargan 2002a) intervals, making it difficult to differentiate between ovipositional preference and differential loss of eggs because of predation between sampling times.

Our first objective was to determine the diel periodicity of *C. maculata* oviposition. This information facilitated accomplishment of our second and primary objective, which was to determine in the field whether naturally ovipositing *C. maculata* preferentially oviposit on plants with glandular trichomes over plants without such trichomes.

Materials and Methods

Field experiments were conducted from late June to mid-August at the University of Kentucky's Spindletop Farm. For all field experiments, 'Golden Queen' sweet corn was planted in rows spaced 0.9 m apart. In each of 2 yr, a total of five plots of sweet corn, 6 by 61 m with seven rows, were planted with 3-m alleys between adjacent plots. The five plots were planted at \approx 2-wk intervals to create a succession of plots in which the corn would reach anthesis (pollen shed) at different times, thus extending the time for experiments. In 2001, the first planting occurred on 1 May and the final planting on 26 June; in 2002, the first planting occurred on 20 May and the final planting on 14 July. Corn plots and alleys between plots were treated with the pre-emergence herbicide S-metolachlor + atrazine (1.34 kg S-metolachlor/ha, 1.74 kg atrazine/ha). Supplemental weeding was done by hand as needed to maintain weed-free plots. We chose to use sweet corn plots as the setting for our field studies of diel periodicity and oviposition preference because such plots are attractive to *C. maculata* adults, especially during a 2-wk period around the time of corn anthesis. Experiments were conducted during corn anthesis to maximize chances that wild (i.e., naturally occurring, uncaged) *C. maculata* would oviposit on our experimental plants. We used velvetleaf for the field study of diel periodicity, and we used velvetleaf and smooth pigweed for the study of oviposition preference. These species were selected because previous research with several weed species consistently found more *C. maculata* eggs on velvetleaf than on smooth pigweed (Griffin and Yeargan 2002a, b). Velvetleaf has fewer and larger leaves than pigweed, but the two species have similar total leaf area per plant (Griffin and Yeargan 2002a). Both weed species were used when they were \approx 20 cm tall.

Diel Periodicity of Oviposition. A laboratory experiment and a field experiment were performed to determine the diel periodicity of *C. maculata* oviposition. For the laboratory study, a colony of *C. maculata* was started from insects collected at the University of Kentucky's North Farm near Lexington. The colony was reared according to the methods described by Cottrell and Yeargan (1998a). Before use in the laboratory experiment, females and males from the *C. maculata* colony were allowed to mate in 9-cm plastic dishes (one of each sex per dish). After mating, the females were placed individually into 9-cm plastic dishes with a water-moistened dental wick and frozen *H. zea* eggs for food and allowed a 12-h acclimation period before any data were collected. All beetles (colony, acclimation, and experimental) were kept in an incubator at $27 \pm 1^\circ\text{C}$ with a photoperiod of 15:9 (L:D) h. Lights came on in the incubator at 0600 hours EDT and went off at 2100 hours. The acclimation period began at 1000 hours, and the first data were collected at 2200 hours. At 2100 hours, a 10.5-cm circle of florist's paper (green) was placed inside the lid of the petri dish, providing an oviposition substrate that could be easily removed and replaced with a new one. Starting at 2200 hours, the females were monitored hourly for 24 h, and the number of egg clusters and the number of eggs per cluster were recorded. Oviposition substrates were replaced with new ones when eggs were found. During the 9 dark h (2100–0600 hours), the hourly monitoring was conducted under a red light. Food was replenished if it was depleted. A total of 105 females was used for this laboratory study.

The field study of diel periodicity was conducted in the corn plots described above. During corn anthesis, 20 pots containing single, greenhouse-grown velvetleaf plants were placed in the plot at 0900 hours. Plants were placed in the middles between corn rows, with two to four plants in each of the six middles; positions were randomly selected, with the restriction of no more than four plants per middle. On a total of six dates between 30 June and 16 August 2001, beginning at 1000 hours, the velvetleaf plants were monitored for the presence of *C. maculata* eggs once every hour for 24 h. The number of egg clusters and the number of eggs per cluster were recorded. Eggs and a small amount of adjacent plant tissue were removed at each hourly observation and placed in plastic dishes. These eggs were subsequently taken to the laboratory, where they were incubated until hatching to verify coccinellid species identity. Daylength during the field study was similar to that used in the laboratory study described above. Over the six dates, the average period between sunrise and sunset was 14.3 h, with the average period from the beginning to end of civil twilight being 15.3 h.

Plant Preference of *C. maculata*. We conducted a field experiment to determine if velvetleaf, which possesses glandular trichomes, is preferred for oviposition by *C. maculata* over smooth pigweed, a species without such trichomes. Velvetleaf and smooth pigweed were monitored by continuous direct observation to

determine the relative frequency of oviposition on these plants by wild *C. maculata*.

During anthesis within a given sweet corn plot, four pots containing individual velvetleaf plants and four pots containing individual smooth pigweed plants were placed within one of the sweet corn plots described above 1 h before the start of the *C. maculata* diel oviposition period. Plots were used in succession as corn plants in each plot reached anthesis, and the area to be used within a plot was chosen randomly. The eight experimental plants were arranged in a semicircle in front of an observer between two rows of corn so they could be continuously watched. The sequence of the velvetleaf and smooth pigweed plants within the semicircle was randomly determined, with a newly randomized sequence used for each replication. On each observation date, the eight potted plants were continuously watched between 1200 and 1900 hours in 2001 and between 1200 and 2000 hours during 2002. This procedure was repeated on 29 different dates, 16 during 2001 and 13 during 2002. A new set of velvetleaf and smooth pigweed plants was used on each date. Each time oviposition was observed on one of the experimental plants, the exact location of that egg cluster and the number of eggs in the cluster was recorded. This was done so the precise site of each egg cluster could be located again 24 h later, thereby allowing us to differentiate the observed cluster from any eggs that might have been laid on the plants after observations ceased. After the 7-h observation period, the plants were left in the field for 24 h, after which the eggs were checked for predation. Only those egg clusters oviposited within the observation period were checked for predation.

During the 2001 season, no data were collected on the relative number of visits to smooth pigweed and velvetleaf or on the amount of time spent on each plant species. During 2002, while gathering additional data on oviposition preference, we also recorded the number of visits and time spent per visit on each plant species. As described below, we also collected a portion of the visiting beetles to obtain an estimate of their sex ratio, because it is not possible to determine the sex of *C. maculata* in the field without disturbing the beetles' behavior. The procedures for determining ovipositional preference in 2002 were the same as those used the previous year, with the following two exceptions. The observation period was extended by 1 h, running from 1200 to 2000 hours. Second, during each replication, we collected every other beetle when it first entered the observation area—i.e., when it first landed on one of the eight plants. Beetles were collected with an aspirator and later returned to the laboratory for dissection to determine their sex. We recorded the plant species on which each collected beetle had first landed. The other arriving beetles were observed but not collected. As in the previous year, we recorded data on oviposition by these beetles. Additionally, for each of these beetles we recorded the number and species of plants visited and the amount of time spent per visit. Several of the beetles visited

multiple plants in the semicircle, and each visit to a different plant was recorded as a separate event. After the observation period, the plants were left in the field for 24 h, after which the eggs were checked for predation. The numbers of *C. maculata* visits to each of the two weed species per trial ($n = 13$ trials) were compared with a paired *t*-test. The average amounts of time spent per visit on each weed species were compared with a two-sample *t*-test ($n = 100$ visits to pigweed and 35 visits to velvetleaf).

Results

Diel Periodicity of Oviposition. In the laboratory study, most oviposition occurred in the afternoon and early evening, although a few eggs were laid at other times (Fig. 1). A total of 124 egg clusters was laid by *C. maculata* in the laboratory study. In the field, all oviposition occurred between 1200 and 2000 hours (Fig. 1). A total of 60 egg clusters was oviposited in the field study, with an average of 12.5 ± 0.74 (SE) eggs per cluster.

Plant Preference of *C. maculata*. On 29 dates over two field seasons, 52 *C. maculata* (30 in 2001 and 22 in 2002) were observed ovipositing on velvetleaf and smooth pigweed plants in the field. Only a single egg cluster was oviposited on smooth pigweed, whereas 51 clusters were oviposited on velvetleaf. The egg cluster laid on smooth pigweed contained three eggs, whereas the clusters oviposited on velvetleaf contained an average of 13.3 ± 0.63 eggs per cluster. When the eggs were checked 24 h after oviposition, the single cluster on smooth pigweed was gone, presumably because of predation, whereas only 3 of the 51 clusters on velvetleaf were missing.

Data on number of visits to, and time spent on, each plant species were recorded only during the second season (13 dates). *C. maculata* adults made significantly more visits to smooth pigweed than to velvetleaf (paired *t*-test, $t = 3.59$, $df = 12$, $P < 0.01$; Fig. 2A). When a beetle oviposited, its visit to a plant lasted almost 10 min (9.8 ± 1.4 min; $n = 22$), which was much longer than the average time spent per visit by beetles that did not oviposit (Fig. 2B). Because of this difference and the fact that virtually all oviposition occurred on velvetleaf, we omitted the 22 beetles that oviposited from the analysis of time spent per visit. Excluding those cases, the average time spent per visit on smooth pigweed was more than twice that spent on velvetleaf (two-sample *t*-test, $t = 2.1$, $df = 133$, $P < 0.05$; Fig. 2B).

During the second season, every other beetle was collected when it first arrived at the set of plants being observed in the field, and those beetles were subsequently dissected to determine their sex. The overall sex ratio of the 77 collected beetles was 39:38 (male:female). More of the newly arrived beetles landed on smooth pigweed (54 beetles) than on velvetleaf (23 beetles) even though equal numbers of each plant species were available. Sex ratios of the collected beetles were independent of the plant species from which they were collected ($\chi^2 = 0.45$, $df = 1$, $P = 0.5$).

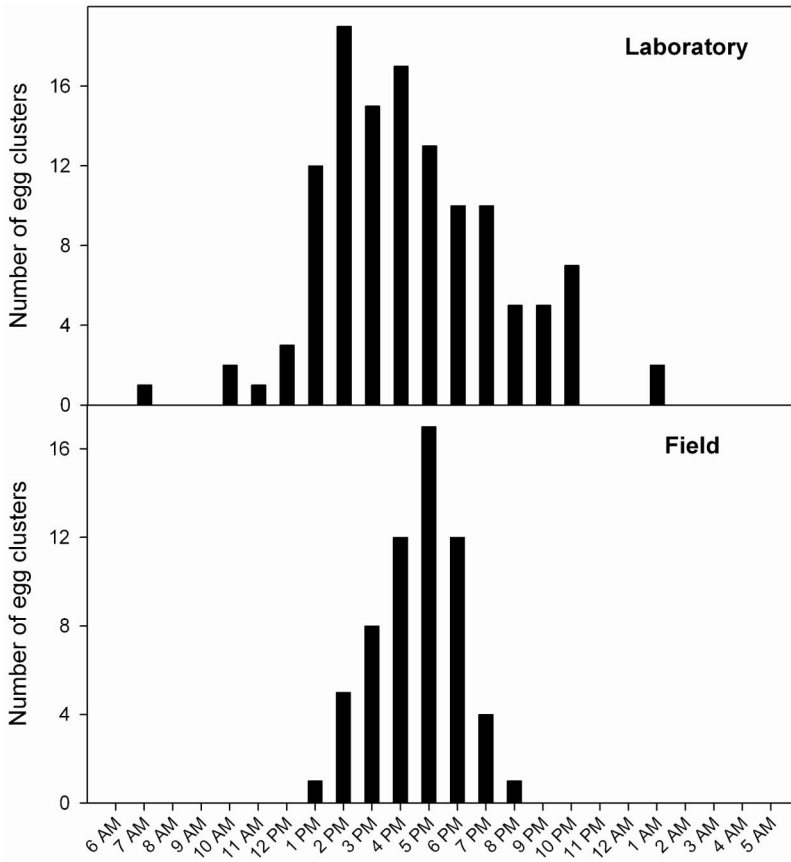


Fig. 1. Diel periodicity of *C. maculata* oviposition in the laboratory and in the field. Bars represent number of egg clusters laid during the hour that ended at the time shown on the x-axis.

Discussion

Our laboratory and field results showed that *C. maculata* primarily oviposit in the afternoon and early evening. The diel pattern of oviposition was especially distinct in the field, with all oviposition occurring within an 8-h period that began at noon; >80% of the oviposition occurred during a 4-h period between 1400 and 1800 hours. Knowledge of this pattern allowed us to concentrate our observations in the plant-choice experiment during a period when naturally occurring beetles would be ovipositing.

In previous studies, far more *C. maculata* eggs were found on plant species with glandular trichomes than those without such trichomes (Cottrell and Yeargan 1998b, Griffin and Yeargan 2002a), but it was unclear from those studies whether this represented ovipositional preference or differential survival of the eggs between sampling times. The results reported here clearly show that *C. maculata* preferentially oviposit on velvetleaf, a plant species with glandular trichomes, compared with smooth pigweed, which lacks glandular trichomes. Nearly all (98%) of the 52 observed cases of oviposition occurred on velvetleaf, despite the fact that *C. maculata* visited smooth pigweed plants more frequently and spent more time on them per visit.

The pigweed and velvetleaf plants used for our field observations of *C. maculata* oviposition were virtually free of prey throughout the study. Furthermore, Griffin and Yeargan (2002a) found that *C. maculata* oviposition was not affected by large differences (>50-fold) in whitefly densities, and other studies have found that *C. maculata* generally oviposit away from concentrations of aphids rather than near them (Schellhorn and Andow 1999). Thus, *C. maculata* seem to select oviposition sites based on criteria other than proximity to high prey densities. Previous studies (Cottrell and Yeargan 1998b, Griffin and Yeargan 2002a, b) and the results reported here all strongly suggest that *C. maculata* prefer to oviposit on plants with glandular trichomes.

We hypothesize that predation, especially cannibalism, has selected for *C. maculata* behavior of preferentially ovipositing on plants with glandular trichomes. Earlier studies showed that predation rates on *C. maculata* eggs were very high on corn and smooth pigweed plants, often approaching 100% mortality (Cottrell and Yeargan 1998b, Griffin and Yeargan 2002b). Cottrell and Yeargan (1998b) found, over a 3-yr period, that cannibalism accounted for 88% of 289 cases of predation on *C. maculata* eggs on sweet corn plants, with the remaining 12% of the cases being

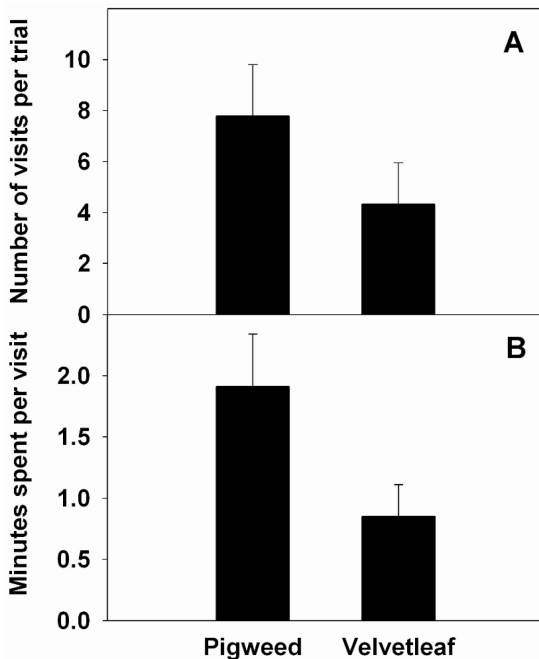


Fig. 2. Behavior of wild *C. maculata* adults in the field when presented equal numbers of smooth pigweed and velvetleaf plants; bars represent means \pm SE. (A) Number of times beetles visited each type of plant ($n = 13$ trials); means are significantly different (paired *t*-test, $P < 0.01$). (B) Minutes spent per visit, excluding beetles that oviposited ($n = 100$ and 35 for pigweed and velvetleaf, respectively); means are significantly different (two-sample *t*-test, $P < 0.05$).

predation by other Coleoptera, Hemiptera, Hymenoptera, Orthoptera, and Opiliones. Most of the cannibalism of eggs was done by *C. maculata* larvae, rather than adults. Cannibalism and other predation on *C. maculata* eggs were much lower on plants with glandular trichomes, namely hophornbeam copperleaf (Cottrell and Yeargan 1998b) and velvetleaf (Griffin and Yeargan 2002b). Cottrell and Yeargan (1999) found that newly hatched *C. maculata* disperse from hophornbeam copperleaf plants by dropping to the ground, rather than crawling down the plants. Adult *C. maculata* are able to land on plants with glandular trichomes, oviposit, and fly away, but foraging for prey on these plants apparently is more difficult than on plants lacking glandular trichomes.

The occurrence of higher *C. maculata* egg densities on plants with numerous glandular trichomes has been well established (Obrycki and Tauber 1985, Cottrell and Yeargan 1998b, Griffin and Yeargan 2002a). While reduced predation of the eggs on these plants may contribute to higher observed densities of *C. maculata* eggs, our results clearly show for the first time that *C. maculata* preferentially oviposit on plants with glandular trichomes. It is noteworthy that previous studies comparing predation rates on plants with and without glandular trichomes (Cottrell and Yeargan 1998b, Griffin and Yeargan 2002b) used equal numbers of

sentinel egg clusters on each type of plant at the start of those experiments. Our results on oviposition preference, however, suggest that, when both types of plants are available, relatively few eggs are laid on plants lacking glandular trichomes, so it is unlikely that predators have many opportunities to feed on *C. maculata* eggs on such plants. Thus, whereas cannibalism and other predation may have selected for oviposition preference and may continue to maintain this behavior, it seems that oviposition preference largely accounts for observed differences in egg densities on the two types of plants.

It may be possible to manipulate densities of *C. maculata* larvae on certain crops by placing plants with glandular trichomes near stands of plants lacking such trichomes. In such an arrangement, one would expect the sticky pubescence to interfere with foraging by predators. Thus, the plants with glandular trichomes would act as ovipositional refuges from cannibalism and other predation on *C. maculata* eggs. It is likely that predation on herbivores on those refuge plants also would be reduced by the presence of glandular trichomes, but the intent would be to augment *C. maculata* larval populations on the crop that lacks such trichomes. An earlier study showed that presence of a weedy species (hophornbeam copperleaf) with glandular pubescence near sweet corn led to increased *C. maculata* larval densities and higher predation rates on corn earworm eggs on the corn associated with those weeds than on monoculture corn (Cottrell and Yeargan 1998b). It remains to be determined whether companion planting of a desirable cultivated species with glandular pubescence might similarly augment larval populations of this beneficial predator on sweet corn or other crops.

Acknowledgments

We thank T. Cottrell, M. Klahr, and K. Pitz for reviewing an earlier version of the manuscript. This study (paper 04-08-129) was conducted in connection with a project of the Kentucky Agricultural Experiment Station.

References Cited

- Benton, A. H., and A. J. Crump. 1981. Observations on the spring and summer behavior of the 12-spotted ladybird beetle, *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae). *J. N.Y. Entomol. Soc.* 89: 102-108.
- Coll, M., and D. G. Bottrell. 1991. Microhabitat and resource selection of the European corn borer (Lepidoptera: Pyralidae) and its natural enemies in Maryland field corn. *Environ. Entomol.* 20: 526-533.
- Conrad, M. S. 1959. The spotted lady beetle, *Coleomegilla maculata* (DeGeer), as a predator of Eur. corn borer eggs. *J. Econ. Entomol.* 52: 843-847.
- Cottrell, T. E., and K. V. Yeargan. 1998a. Effect of pollen on *Coleomegilla maculata* (Coleoptera: Coccinellidae) population density, predation, and cannibalism in sweet corn. *Environ. Entomol.* 27: 1402-1410.
- Cottrell, T. E., and K. V. Yeargan. 1998b. Influence of a native weed, *Acalypha ostryaefolia* (Euphorbiaceae), on *Coleomegilla maculata* (Coleoptera: Coccinellidae) pop-

- ulation density, predation, and cannibalism in sweet corn. *Environ. Entomol.* 27: 1375–1385.
- Cottrell, T. E., and K. V. Yeargan. 1999. Factors influencing dispersal of larval *Coleomegilla maculata* (Coleoptera: Coccinellidae) from the weed *Acalypha ostryaefolia* (Euphorbiaceae) to sweet corn. *Entomol. Exp. Appl.* 90: 313–322.
- Griffin, M. L., and K. V. Yeargan. 2002a. Oviposition site selection by the spotted lady beetle *Coleomegilla maculata* (Coleoptera: Coccinellidae): choices among plant species. *Environ. Entomol.* 31: 107–111.
- Griffin, M. L., and K. V. Yeargan. 2002b. Factors potentially affecting oviposition site selection by the lady beetle *Coleomegilla maculata* (Coleoptera: Coccinellidae). *Environ. Entomol.* 31: 112–119.
- Hazzard, R. V., and D. N. Ferro. 1991. Feeding responses of adult *Coleomegilla maculata* (Coleoptera: Coccinellidae) to eggs of Colorado potato beetle (Coleoptera: Chrysomelidae) and green peach aphids (Homoptera: Aphididae). *Environ. Entomol.* 20: 644–651.
- Musser, F. R., and A. M. Shelton. 2003. Predation of *Ostrinia nubilalis* (Lepidoptera: Crambidae) eggs in sweet corn by generalist predators and the impact of alternative foods. *Environ. Entomol.* 32: 1131–1138.
- Obrycki, J. J., and M. J. Tauber. 1985. Seasonal occurrence and relative abundance of aphid predators and parasitoids on pubescent potato plants. *Can. Entomol.* 117: 1231–1237.
- Pfannenstiel, R. S., and K. V. Yeargan. 2002. Identification and diel activity patterns of predators attacking *Helioverpa zea* (Lepidoptera: Noctuidae) eggs in soybean and corn. *Environ. Entomol.* 31: 232–241.
- Schellhorn, N. A., and D. A. Andow. 1999. Cannibalism and interspecific predation: role of oviposition behavior. *Ecol. Appl.* 9: 418–428.
- Wright, R. J., and T. A. DeVries. 2000. Species composition and relative abundance of Coccinellidae (Coleoptera) in south central Nebraska field crops. *J. Kansas Entomol. Soc.* 73: 103–111.

Received for publication 8 September 2004; accepted 26 January 2005.
