The oviposition behavior of the predator *Orius insidiosus*: acceptability and preference for different plants

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Abstract. The ability of natural enemies to reproduce within cropland and effectively suppress pests depends on the presence of plants on which to oviposit within the agro-ecosystems. Our research investigates the acceptability and preferences of a range of plants for oviposition by the predatory bug *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) in the laboratory. Within-plant preferences on pole beans as oviposition sites were evaluated in laboratory choice tests. The acceptability and preference of *O. insidiosus* females for pole bean, soybean, redroot pigweed, and velvetleaf were evaluated in choice and no-choice tests (respectively) in the laboratory. Observations on the acceptability of green foxtail, orchardgrass, buffalograss, smooth brome, redtop grass, blue grama, and tall fescue for oviposition were also conducted. *O. insidiosus* preferred to lay its eggs on the petiole and leaflet petioles of pole beans, and did not distinguish among nodes or petioles of different lengths. Although all broadleaved plants were suitable for egg development, the acceptability of these plants differed significantly, with pole beans being most acceptable and almost no eggs being laid on velvetleaf. Preference tests supported the results of the no-choice tests, with pole bean being the most preferred, and no eggs being laid on the velvetleaf. Green foxtail and orchardgrass were the only grass species found to be acceptable to *O. insidiosus*. The implications of soybean monocultures on the reproductive capacity of and biological control by *O. insidiosus* are discussed, as are possible mechanisms underlying the decision-making process for oviposition.

Key words: *Aphis glycines*, biological control, *Glycine max*, *Orius insidiosus*, reproduction, soybean, weed

Introduction

One of the benefits of polycultures in agricultural systems is that they often foster greater levels of biological control and lower levels of pests (Andow, 1991; Bugg and Waddington, 1994; Norris and Kogan,

2000). There are numerous studies citing the benefits of maintaining a polyculture in and around cropland. These benefits include providing non-prey foods, favorable microhabitats for beneficials, alternative prey, and overwintering habitat for biological control agents of key pests (reviewed in Landis et al., 2000). The preference for or suitability of different plants that occur within cropland as oviposition sites for predators are issues seldom addressed. However, oviposition preferences are critical to conservation biological control, since the ability of natural enemies to respond quickly to pests is dependent in part on whether they can reproduce in cropland (Cottrell and Yeargan, 1998, 1999; Shaltiel and Coll, 2004).

Insect predators use a number of cues to determine whether a site is suitable for oviposition. Many of the cues investigated are prey-mediated, and involve responding to prey densities or semiochemicals emitted by prey (Anderson, 1962; Coderre et al., 1987; Lucas and Brodeur, 1999). In addition to prey factors, predaceous insects also use plant characteristics to select oviposition sites, and in some cases plant characteristics can overwhelm cues derived from prey (Griffin and Yeargan, 2002a, b). Anthocorids, including *Orius insidiosus* (Say) (Heteroptera: Anthocoridae), display distinct oviposition preferences for specific plant species and sites within plants (Askari and Stern, 1972; Isenhour and Yeargan, 1982; Coll, 1996; Richards and Schmidt, 1996; Shaltiel and Coll, 2004). However, the behavior of and cues responsible for oviposition by most anthocorids, and the implications for polycultures on a population's ability to reproduce and suppress prey, remain poorly understood.

Orius insidiosus is an important native predator of crop pests in a number of agronomic systems, and is one of the dominant predators of soybean aphid (*Aphis glycines*; Homoptera: Aphididae) in North America (Fox et al., 2004; Rutledge et al., 2004). Soybeans (*Glycine max* L.) were harvested from nearly 30 million hectares in the USA during 2004 (http://www.usda.gov/nass), and the predominant pest of soybeans, *A. glycines*, has become an annual problem in soybeans since it was first discovered in North America in 2000. Soybeans have been genetically modified for herbicide resistance, and consequently this crop is produced in a monoculture devoid of many other within-field plants. Consequently, one factor that will determine the ability of *O. insidiosus* to regulate populations of *A. glycines* is the relative ability of this predator to use soybean plants as oviposition sites. In the current research we document where on plants *O. insidiosus* prefers to lay eggs, which agronomically relevant plants are acceptable

for oviposition by *O. insidiosus*, and which plants (weeds and crops) *O. insidiosus* prefers to lay eggs on, when given a choice.

Methods

Insects and plants. *Orius insidiosus* adults were received weekly from BioBest Biological Systems (Westerlo, Belgium). Adults were allowed to acclimate to ambient temperature and photoperiod (approximately 25 °C, 12:12 L:D) for 48 h before experiments were initiated. Soybean (var. Surge; SD(M)92-1233) seed was obtained from South Dakota State University Seed Foundation. Pole beans (*Phaseolus vulgaris* L., var. 'Kentucky Blue') (J. W. Jung Seed Co., Randolph, WI), velvetleaf (*Abutilon threophrasti* L.), redroot pigweed (*Amaranthus retroflexus* L.) (velvetleaf and pigweed: F & J Seed Service, Woodstock, IL), green foxtail (*Setaria viridis* (L.) Beauv.), orchard grass (*Dactylis glomerata* L.), buffalograss (*Buchloe dactyloides* [Nutt.] Englem. var. '100'), smooth brome (*Bromus inermis* Leyss.), redtop grass (*Agrostis alba* L.), blue grama (*Bouteloua gracilis* Lag. ex Griffiths, var. 'Custer'), and tall fescue (*Festuca arundinacea* Schreb.) (all grasses from Dr. Leo Schleicher, SDSU, Brookings, SD) were grown in the greenhouse, watered daily, and fertilized at planting (1.6 g/l soil; Osmocote®, Scotts Sierra Horticultural Products Company, Maysville, OH). Broadleaved plants had at least five leaves when experiments were conducted. Green bean (*Phaseolus vulgaris*) plants and pods are commonly used for rearing *O. insidiosus* in the laboratory (Isenhour and Yeargan, 1981; Ruberson et al., 1991; Richards and Schmidt, 1996), and the remainder of the plant species was selected because they are prevalent in South Dakota agroecosystems.

Within-plant oviposition preferences. Because of their ovipositional preference for pole beans (see below), this species was used to examine within-plant site preferences for oviposition by *O. insidiosus*. Pots of pole beans (2–3 plants each, plants with six or fewer nodes) were placed in cylindrical cages (43 cm tall, 23 cm diam.) covered with mesh (0.33 mm squares) (similar cages were used throughout the experiments). The soil within the pot was covered with tinfoil, and the cage sat snugly onto the pot to prevent escape of the insects. Five *O. insidiosus* females that had been reared in the mixed-sex ratio colony were placed into each cage, and were kept on the plants for 48 h at 28 °C, 50% relative humidity (r. h.), and 16:8 L:D photoperiod. The experiment was conducted in nine cages containing a total of 20 plants. After 48 h, *O. insidiosus* remaining alive were tabulated and the plants

were dissected for eggs (data from empty cages were discarded). This predator lays its eggs within the plant tissue, such that only a small (< 1 mm) white operculum extends outside the epidermis of the plant. The number of eggs laid on the petiole, leaflet petiole, leaflets, and internodes was recorded for each plant (terminology adapted from Anonymous, 1997). Furthermore, the distance from the base of the plant to each node was recorded, and the greatest length and width for each leaflet was recorded for each node.

The number of eggs laid per plant on the petioles, leaflet petioles, leaflets, and internodes were compared by ANOVA, and means were separated using LSD test (SYSTAT Software Inc., 2004). The number of eggs laid was compared among the nodes using ANOVA. On pole beans, each petiole leads to a trifoliate; on each trifoliate there exist two leaflets with short leaflet petioles, and one that is comparatively longer. These leaflet petioles were categorized as short or long, and the mean number of eggs laid on each petiole type was compared by ANOVA.

Plant acceptability. The relative acceptability of soybean, pole bean, velvetleaf, and pigweed plants as oviposition sites for *O. insidiosus* was assessed in no-choice tests. Single pots (n = 16 per species) were caged with five *O. insidiosus* females. Each pot had plants with comparable foliage height and number of nodes. After being caged for 48 h at 28 °C, 50% r. h., and 16:8, L:D, the females were removed from the plants. Plants were then dissected at 50× and the number of eggs laid per pot, and the plant regions where the eggs were laid, were recorded.

Observations on the acceptability of the vegetative stages of seven different grasses for *O. insidiosus* were also recorded. Potted plants were grown in the greenhouse, and were clipped to approximately 12 cm in height to equalize the aboveground leaf tissue among the species. Soybean plants were also examined concurrently as a control species that is known to be acceptable to *O. insidiosus*. Grass bunches (approximately 1 cm diam. at the crown) were loosened from the soil, and the roots placed into a water-filled cup (150 ml). Each cup was placed into a separate cage (n = 2 cages for each grass species, except n = 3 for green foxtail). Soybean stems (possessing four nodes) were clipped at the soil line and placed into water-filled cups and then into isolated cages (n = 2). Five *O. insidiosus* females were placed into each cage, and were allowed to oviposit for 48 h at 28 °C, 50% r. h., and 16:8, L:D. After this period, the number of surviving *O. insidiosus* and the number of eggs laid per cage were counted for each species. Due to the small sample sizes, no statistics were applied to these observations.

Plant preferences. Choice tests were used to examine whether *O. insidiosus* preferred to lay eggs on soybean, pole bean, pigweed, or velvetleaf. Single stems containing 5–7 nodes or leaves of each species were clipped and placed through a hole in a tight-fitting plastic lid on a single container (150 ml) full of water. Five *O. insidiosus* females were placed into each cage (n = 24 cages) for 48 h at 28 °C, 50% r. h., and 16:8, L:D. After this period, the females were removed and the number of eggs laid and the location of the eggs were recorded for each plant species. Comparisons in the number of eggs laid among the species were made by ANOVA, and means were separated with a LSD test.

Results

Within-plant oviposition preferences. *Orius insidiosus* showed a clear preference for laying eggs on certain plant structures ($F_{3,76} = 7.45$, $p < 0.0001$), with most preferred sites being on the petiole and leaflet petioles of pole beans (Figure 1). There were no obvious preferences for specific nodes along the stem ($F_{5,79} = 0.513$, $p = 0.77$). *Orius insidiosus* did not distinguish among leaflet petioles of different lengths ($F_{1,159} = 0.87$, $p = 0.35$).
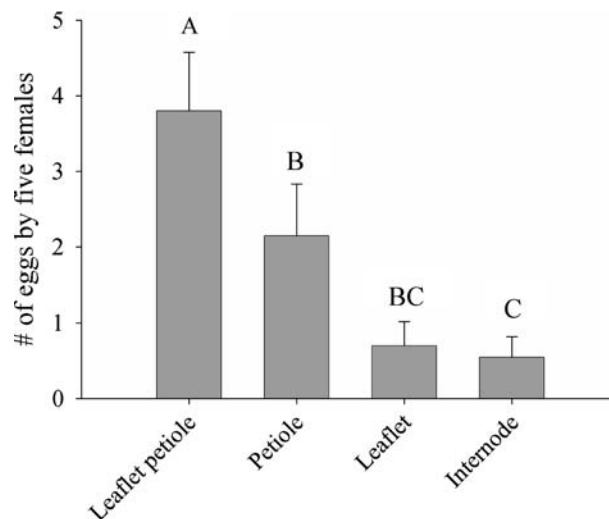

Figure 1. Within-plant preferences for oviposition sites by *Orius insidiosus* on pole bean (n = 20). Columns with different letters are significantly different from each other (LSD test, α = 0.05). Values represent the mean + SEM.

Plant acceptability. There was a clear difference in the relative acceptability of the different plant species when offered in no-choice tests ($F_{3,60} = 11.82$, $p < 0.0001$) (Figure 2). On pole bean, pigweed, soybean, and velvetleaf, there were 213, 147, 111, 8 eggs laid, respectively. Similar to the previous experiment, the petiole and leaflet petioles were the preferred oviposition sites on pole beans, accounting for 53.5% of oviposition sites; the axillary buds accounted for an additional 21.6% of oviposition sites. On pigweed, 85.7% of eggs were laid on the petiole or leaf veins. On soybeans, 82.0% of eggs were laid on the leaf veins, petioles, and on the site where the cotyledon leaves had dehisced. Of the eight eggs laid on velvetleaf, all were laid in the leaf veins.

In the observations of grass plant acceptability, *O. insidiosus* laid 18 eggs per cage on soybeans, 20 eggs per cage on green foxtail, and 12 eggs per cage on orchardgrass (Table 1). None of the other grass species were acceptable as oviposition sites (Table 1). On the grasses, eggs were frequently encountered on the crown, the leaf collars, and infrequently on the leaf veins.

Plant preferences. There was a strong preference for pole beans as an oviposition site by *O. insidiosus* ($F_{3,156} = 8.41$, $p < 0.0001$) (Figure 3). When given a choice among plants, *O. insidiosus* laid 66.4% of eggs on pole beans. On the pole beans, 36.4, 31.2, and 19.5% of oviposition sites were on the leaf veins, petioles, and leaflet petioles.
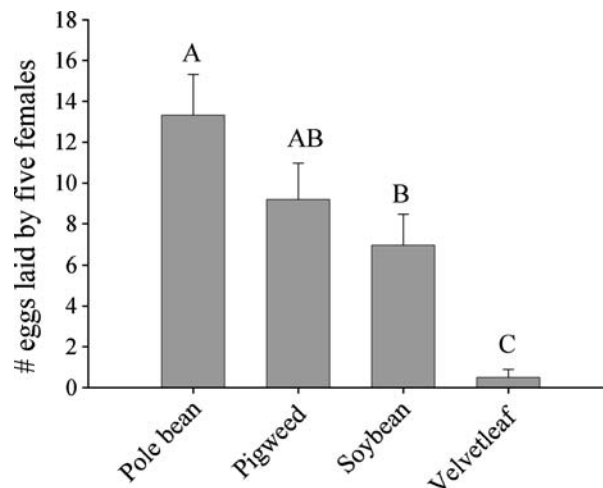

Figure 2. Relative suitability of plants as oviposition sites for *Orius insidiosus* in no-choice tests (n = 16). Columns with different letters are significantly different from each other (LSD test, $\alpha = 0.05$). Values represent the mean + SEM.

Table 1. The number of eggs laid per plant by five *O. insidiosus* females on soybean and grass species. Two plants were used for each species, with five females confined per plant for 48 h.

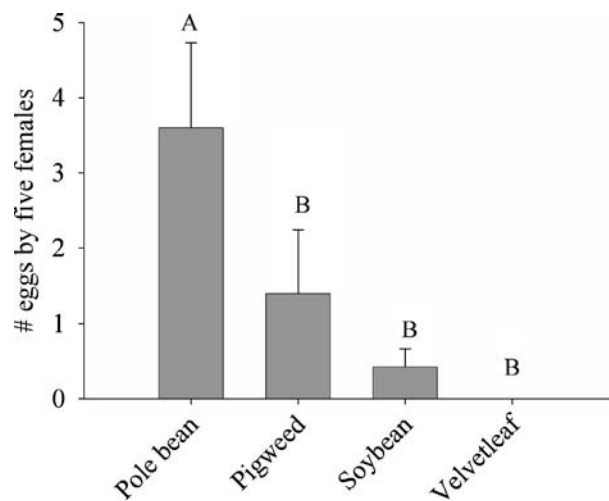| Species	Mean number of eggs laid per plant (individual plant observations)
Green foxtail	21 (30, 12)
Soybean	18 (22, 14)
Orchardgrass	6 (12, 0)
Buffalograss	0
Small brome	0
Redtop grass	0
Blue grama	0
Tall fescue	0

Figure 3. Preferences for plants as oviposition sites by *Orius insidiosus* in choice tests (n = 40). No eggs were laid on velvetleaf. Columns with different letters are significantly different from each other (LSD test, $\alpha = 0.05$). Values represent the mean + SEM.

Discussion

Orius insidiosus displayed clear preferences for certain plant species as oviposition sites, in spite of many of the plants being suitable for egg development. Furthermore, *O. insidiosus* distinguished among plant structures on individual plants, and the preference for oviposition

sites on pole bean changed depending on whether pole beans were offered singly or with other plant species. When plants were offered singly, *O. insidiosus* seldom oviposited in the leaf veins of pole beans, but in the presence of other plants, females laid the highest proportion of their eggs on the leaf veins. Coll (1996) was also able to demonstrate preferences for certain plant species in choice tests, but found similar acceptability among plants in no-choice tests. Our results in acceptability may have differed because we offered entire plants versus only the leaves, and we found that leaves were often not the preferred site for egg laying. Coll (1996) also found that *O. insidiosus* does not prefer to lay its eggs on the vegetative structures of sweet corn (*Zea mays* [L.]) when given a choice among lima beans (*Phaseolus lunatus* [L.]), peppers (*Capsicum anuum* [L.]), tomatoes (*Lycopersicum esculentum* [Miller]), and sweet corn. Our observations support this conclusion in that many monocot plants are unacceptable oviposition sites for *O. insidiosus*. However, green foxtail and orchardgrass had substantial numbers of eggs laid on them, and the silks of corn are acceptable oviposition sites (Barber, 1936). Thus, it does not seem that grasses are universally unacceptable for *O. insidiosus*. Other data suggest that not all *Orius* spp. are as discerning as *O. insidiosus* when it comes to oviposition sites. Guo and Wan (2001) found no preferences in egg-laying by *Orius sauteri* when given a choice among *Kalanchoe blossfeldiana*, *Glycines max* (soybeans), and *Crassula portulacea*. This being said, our results show that *O. insidiosus* does distinguish between egg-laying sites, but the mechanisms behind the oviposition decision and its implications for biological control remain to be investigated.

The mechanisms for oviposition preferences in predatory insects can generally be categorized by the pre- and post-ovipositional consequences of the egg-laying decision. Foraging rates of *O. insidiosus* can be influenced by morphological and physiological plant characteristics. The ease with which females can lay eggs into a substrate seems to affect the acceptability of some plant species. Thickness of the external covering may also render some species unsuitable as oviposition sites (Castañé and Zalom, 1994), but this may have played a minimal role in our experiment with dicotyledons where entire plants were used, which had a variety of possible oviposition sites with varying epidermal characteristics. In our study, eggs were most frequently laid on the areas of the plant where trichomes were less dense, namely the petioles or on other glabrous plant structures. One explanation for this is that the trichomes hindered the egg-laying process, as suggested by Armer et al. (1999). Also, *O. insidiosus* is facultatively phy-

tophagous, feeding on plant tissue even in the presence of prey (Armer et al., 1998). The structural, chemical, and nutritional aspects of the different plants potentially could influence a female's performance on the plants and ultimately affect her decision to lay eggs.

Plant acceptability as an oviposition site may also be affected by the plant's suitability for egg hatching and subsequent immature development. For instance, moisture retention levels of substrates appear to be one characteristic that influences oviposition choices of substrates by *O. insidiosus* (Richards and Schmidt, 1996). Other factors seem to be at play with the unacceptability of velvetleaf in the current study. Judging from the data on plant acceptability and preference (Figures 2 and 3), velvetleaf is clearly not acceptable to *O. insidiosus*. But the avoidance of this plant is in spite of this plant being suitable for egg development; five of six eggs that were laid and monitored on velvetleaf hatched without abnormality. In other predator species, *Coleomegilla maculata*, predation of egg masses is considerably lower on velvetleaf than on other plants occurring in the corn system (Griffin and Yeargan, 2002b). This fact has been observed with coccinellids and other plants possessing glandular trichomes (Obrycki and Tauber, 1985; Cottrell and Yeargan, 1999). Griffin and Yeargan (2002b) showed that predator eggs survived better on plants that possessed glandular trichomes versus glabrous plants, and used this fact to explain *Coleomegilla maculata*'s preference for these species. In the case of *O. insidiosus*, nymphal success does not entirely explain the acceptability of plant species, since nymphs reared on peppers did poorly compared with those reared on tomato, yet these plants were equally acceptable to *O. insidiosus* females (Coll, 1996).

We hypothesize that ovipositional preferences for agronomically relevant plants could have important implications for biological control of soybean aphid in the soybean agroecosystem. Soybeans are produced in large monocultures, and the occurrence of preferred plants along field margins may detract *O. insidiosus* populations from venturing into soybean fields. On the other hand, fostering plant diversity within soybean fields may increase reproduction within fields and encourage *O. insidiosus* populations to more closely track those of *Aphis glycines*. Research on *Anthocoris nemoralis* showed reduced oviposition and lower egg survival on cultivated pear trees relative to surrounding wild trees. Furthermore, this study speculates that control of pear psylla (*Cacopsylla bidens* [Sulc.]) on the cultivated pear trees was dependent on the proximity of wild trees, which facilitated population increases in the predator (Shaltiel and Coll, 2004). A similar relationship was seen with coccinellids, where diversifying the

agroecosystem by allowing weeds as sites for oviposition by predators increased predator densities within cornfields (Cottrell and Yeargan, 1998). From the literature and the current data, the influence of plant architecture on both the adult and offspring fitness may be playing a role in plant acceptability and suitability for oviposition by predators. More research on mechanisms behind oviposition decisions and reproduction by *O. insidiosus* under field conditions will help to identify specific plant qualities that influence the decision-making process for *O. insidiosus* and how these decisions shape the outcome of biological control.

Acknowledgements

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