

# Susceptibility of Twolined Spittlebug (Hemiptera: Cercopidae) Life Stages to Entomophagous Arthropods in Turfgrass

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**ABSTRACT** *Prosapia bicincta* (Say) (Hemiptera: Cercopidae), the twolined spittlebug, is an economic pest of turfgrass in the southeastern United States. No data concerning natural enemies of *P. bicincta* in turfgrass have been reported previously. We compared predation of spittlebug eggs, nymphs, and adults in the laboratory by potential generalist predators commonly found in turfgrass: bigeyed bugs *Geocoris uliginosus* Say and *Geocoris punctipes* Say; red imported fire ant, *Solenopsis invicta* Buren; wolf spiders (*Lycosa* sp. Walckenaer); carabid beetles *Harpalus pensylvanicus* DeGeer and *Calosoma sayi* Dejean; and tiger beetles *Megacephala carolina carolina* L. Eggs were readily consumed by generalist predators. *S. invicta* consumed 100% of the eggs offered. *H. pensylvanicus* and *C. sayi* were also significant predators of *P. bicincta* eggs. Nymphs live in spittlemasses that protect them from attack by predators, but exposed nymphs were susceptible to attack when mechanically removed from their spittlemasses. *S. invicta* and *M. carolina carolina* caused significant mortality of exposed nymphs. *P. bicincta* adults are aposematic and have the ability to reflex bleed; however, reflex bleeding did not prevent attack by predators. *S. invicta* and *M. carolina carolina* killed 100% of the adult spittlebugs offered in laboratory bioassays. *Lycosa* sp. are less voracious predators of adults. Sound background knowledge about *P. bicincta* and its potential natural enemy complex is important for the development and implementation of a detailed, site-specific, biologically based pest management program in turfgrass.

**KEY WORDS** *Solenopsis invicta*, *Lycosa* sp., *Calosoma sayi*, *Geocoris uliginosus*, *Megacephala carolina carolina*

*Prosapia bicincta* (Say) (Hemiptera: Cercopidae), the twolined spittlebug, is reported from Florida to Maine and as far west as Arkansas and Texas in the United States (Byers 1965). Both adults and nymphs are polyphagous xylem feeders (Pass and Reed 1965) with a broad range of grass hosts (Vittum et al. 1999). Nymphs and adults also are known to feed on numerous annual or perennial hosts (Pass and Reed 1965). Females lay eggs in hollow stems, under leaf sheaths, at the base of the soil line, and in debris (Fagan and Kuitert 1969). Eggs overwinter and hatch in March-April; newly emerged nymphs find a suitable host and produce a spittlemass within 5 min of feeding on the host (Fagan and Kuitert 1969, Pass and Reed 1965).

Several hypotheses have been proposed about the function of spittlemass: protection against desiccation (Weigert 1964, Wigglesworth 1972); an osmoregulatory device (Turner 1994); and protection from predators, parasitoids, and bacterial and fungal pathogens (Guilbeau 1908, Whitaker 1970, William and Ananth-subramaium 1989). Adults do not produce spittlemass,

but they are aposematic (black with red variations) and have the ability to reflex bleed and jump high to evade natural enemies. Reflex bleeding in *Prosapia* nr. *bicincta* showed no clear evidence of mechanical or chemical deterrence to predators (Peck 2000).

Attempted control of *P. bicincta* has included mechanical and chemical measures. Burning of the previous year's refuse and mowing height of grass are some mechanical control options (Beck 1963). However, chemical control has been most successful in controlling spittlebugs (Beck 1963). There is paucity of information on natural enemies of the New World spittlebugs. There are no records of parasites or predators of the eggs, nymphs, or adults of *P. bicincta* (Braman 1995).

Previous studies have documented the wealth of beneficial arthropods, such as carabids, staphylinids, mites, spiders, tiger beetles, and ants in turfgrass habitats (Reinert 1978, Cockfield and Potter 1984, Braman et al. 2003). These indigenous predators help regulate pest outbreaks in lawns, golf course, and urban landscapes (Reinert 1978, Cockfield and Potter 1984, Terry et al. 1993). Seasonal activity of these predators often coincides with the activity of one or both generations of *P. bicincta* in turfgrass (Braman and Pendley 1993),

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suggesting a potential interaction among predators and spittlebugs. The objective of our study was to investigate predation on all life stages of *P. bicincta* by entomophagous arthropods common in turfgrass. Additionally, we examined the role of spittlemass in providing protection against predators.

### Materials and Methods

**Insect Source.** Adult *P. bicincta* were field-collected from local residential areas and commercial landscapes from June to September around Athens (2003) and Griffin (2004), GA. Spittlebugs were maintained using procedures described by Shortman et al. (2002). Adults were maintained on centipede grass, *Eremochloa ophiuroides* (Munro) Hack., in 800-ml mason jars ventilated with 32-mesh screens. The jars were then placed in environmental chambers (Conviro, Manitoba, Canada) and maintained at 24°C, 75–80% RH, and a photoperiod of 15:9 (L:D) h. Adults were provided with moistened filter paper at the base of the jar, which served as an oviposition site. Eggs were collected daily, placed on moistened filter paper in 11.0-cm petri dishes (Pioneer Plastics, Dixon, KY), and incubated until hatching. Three 1-d-old nymphs were placed on centipede grass planted in Stuewe and Sons's single-cell cone-tainers (3.8 cm in diameter by 21 cm in height) with a camel's-hair brush and allowed to complete nymphal stage. Eggs, nymphs, and adults from the laboratory-reared colony were used for experiments. All predation experiments were conducted at room temperature (24 ± 1°C) under fluorescent light set at 15:9 (L:D) h. Relative humidity was maintained at 70%.

Generalist predators used in the study included members of the family Geocoridae (big-eyed bugs), *Geocoris punctipes* Say and *Geocoris uliginosus* Say; Formicidae (red imported fire ant), *Solenopsis invicta* Buren; Carabidae (ground beetles), *Harpalus pensylvanicus* De Geer, *Calosoma sayi* Dejean, and *Megacephala carolina carolina* L. (tiger beetles); and Lycosidae (wolf spiders), *Lycosa* sp. Walckenaer. *G. uliginosus* and *G. punctipes* were collected by sweeping in centipede grass during June to September (2003–2004). *Lycosa* sp., *H. pensylvanicus*, *C. sayi*, and *M. carolina carolina* were collected from pitfall traps inserted into centipede grass or from light traps during June–September (2003–2004). All predators were maintained in the laboratory at room temperature under a photoperiod of 15:9 (L:D) h. They were fed field crickets, *Gryllus rubens* Scudder and fall armyworm, *Spodoptera frugiperda* (J.E. Smith) eggs as food.

**Egg Predation.** *Trial 1.* Potential predation of *P. bicincta* eggs by *G. punctipes* and *H. pensylvanicus* were evaluated in the laboratory. Egg predation studies were conducted with 10–12-d-old twolined spittlebug eggs obtained from the laboratory-reared colony. Predators were held without food for 24 h before testing. Three, five, or 10 eggs were placed on moist filter paper in 11.0-cm-height by 2.4-cm-diameter petri dishes (Pioneer Plastics, Dixon, KY) with a single predator with the exception of red imported fire ants.

Egg consumption was recorded at the end of 24 h. Controls were maintained without predators. There were a total of 45 replicates for *G. punctipes* at all prey egg densities (15 replicates × three egg densities); there were 60 replicates for *H. pensylvanicus* at all prey egg densities (30 replicates × three egg densities).

Potential predation of *P. bicincta* eggs by *S. invicta* was evaluated in a bioassay similar to Brinkman et al. (2001). Four red imported fire ant colonies were excavated from West Brook farm, Spalding Co., GA, and were placed in 15.2-liter plastic buckets coated with Fluon (Northern Products, Woonsocket, RI). Centipede grass plugs were planted in specimen cups (6.5 cm in height by 15.0 cm in diameter) and placed in a plastic cage 8.0 cm in height by 18.0 cm in diameter (Pioneer Plastics), with one specimen cup per plastic cage. *S. invicta* colonies were connected to the plastic cage with 4.0-mm-diameter clear vinyl tubing also coated with Fluon. Eight plastic cages each with specimen cup containing centipede grass plug were connected to four *S. invicta* colonies. Three, five, or 10 eggs were placed at the base of the centipede plug and the plastic container was closed. Observations on the number of eggs consumed were recorded after 1 h. Food and water were removed from the colony during predation studies. This bioassay allowed *S. invicta* workers to forage on spittlebugs but prevented escape from the containers. Containers that were not connected to the *S. invicta* colony were treated as controls. There were a total of 24 replicates for *S. invicta* at all prey egg densities (eight replicates × three egg densities).

*Trial 2.* Laboratory predation of *P. bicincta* eggs was expanded to include *G. punctipes*, *H. pensylvanicus*, *S. invicta*, *G. uliginosus*, *C. sayi*, and *M. carolina carolina* in the second trial. The experimental procedures were similar to that described in trial 1, including the *S. invicta* bioassay except that we placed five *P. bicincta* eggs per petri dish. There were a total of 60 replicates for *G. uliginosus* at all prey egg densities (20 replicates × three egg densities); 60 replicates for *C. sayi* at all prey egg densities (20 replicates × three egg densities) and 60 replicates for *M. carolina carolina* at all prey egg densities (20 replicates × three egg densities). There were similar number of replicates for *G. punctipes*, *H. pensylvanicus*, and *S. invicta* as in trial 1. Controls were maintained without predator. Egg consumption was recorded at the end of 24 h.

*Trial 3.* Predation of *P. bicincta* eggs was evaluated in a greenhouse bioassay. Centipede grass was planted in Stuewe and Sons's single-cell cone-tainers (3.8 cm in diameter by 21 cm in height). The plants were maintained in the greenhouse under a photoperiod of 16:8 (L:D) h and 22–25°C. The predators used in the greenhouse bioassay were *G. uliginosus*, *G. punctipes*, and *H. pensylvanicus*. Five 10–12-d-old *P. bicincta* eggs were transferred onto crowns of the centipede grass by using moist filter paper wedges along with a single predator to the cone-tainers. The cone-tainers were sealed with fiber sleeves milk-test filters (Kleentest Products, Milwaukee, WI). The milk-test filters were cut to 3 cm in diameter by 6 cm in height and rolled

**Table 1.** Mean  $\pm$  SE of *P. bicincta* eggs consumed by predators during 24 h in trial 1 of egg predation bioassay

Predator	n	Mean $\pm$ SE no. of eggs		
		3	5	10
<i>S. invicta</i>	Colony	3.0 $\pm$ 0.00	5.00 $\pm$ 0.00	10.0 $\pm$ 0.00
<i>H. pensylvanicus</i>	25	2.92 $\pm$ 0.05	4.76 $\pm$ 0.10	9.60 $\pm$ 0.09
<i>G. punctipes</i>	25	2.64 $\pm$ 0.12	4.32 $\pm$ 0.18	9.28 $\pm$ 0.15

**Table 2.** Mean  $\pm$  SE *P. bicincta* eggs consumed by predators during 24 h in trial 2 of the egg predation bioassay

Predator	Mean $\pm$ SE	$\chi^2$	df	P
<i>G. punctipes</i>	3.03 $\pm$ 0.14	95.29	1	<0.0001
<i>G. uliginosus</i>	3.93 $\pm$ 0.11	65.67	1	<0.0001
<i>H. pensylvanicus</i>	4.43 $\pm$ 0.08	294.94	1	<0.0001
<i>C. sayi</i>	4.64 $\pm$ 0.12	153.22	1	<0.0001
<i>S. invicta</i>	5.0 $\pm$ 0.0	155.24	1	<0.0001
<i>M. carolina carolina</i>	2.0 $\pm$ 0.22	9.61	1	0.001

down from top and then sealed with a paper clip. Observations on the number of eggs eaten were recorded 24, 48, and 72 h postexposure to predators. Each *G. punctipes* and *G. uliginosus* greenhouse bioassay was replicated 25 times; *H. pensylvanicus* experiments had 30 replicates. Controls were maintained without predators.

**Nymph Predation. Trial 1.** One-day-old *P. bicincta* nymphs were challenged with *S. invicta* and *H. pensylvanicus*. Experimental procedures were similar to egg predation bioassay, including *S. invicta* predation bioassay where, *P. bicincta* eggs were replaced with nymphs. A single intact *P. bicincta* nymph was placed in a petri dish with moist filter paper, and a blade of centipedegrass as a xylem source for the production of spittlemass during the experiment. Another set of nymphs were lightly washed with water to remove the spittlemass and placed in a petri dish without centipedegrass. Devoid of the xylem source, *P. bicincta* nymphs failed to produce the spittlemass. This was done to confirm the role of the spittlemass as a protective shield against predators. Nymphal mortality was recorded after 24 h. Both intact and exposed nymphs were challenged with the same predators in a similar experimental arena. There were a total of 60 replicates for *H. pensylvanicus* (30 replicates  $\times$  two *P. bicincta* nymph treatments); 16 replicates for *S. invicta* (eight replicates  $\times$  two *P. bicincta* nymph treatments). Controls were maintained without predators.

**Trial 2.** A single second or third instar of *P. bicincta* was challenged with *C. sayi* and *M. carolina carolina* in addition to *S. invicta* and *H. pensylvanicus*. Experimental procedure was similar to that used in trial 1 of nymphal predation. There were a total of 40 replicates for *C. sayi* (20 replicates  $\times$  two *P. bicincta* nymph treatments) and 40 replicates for *M. carolina carolina* (20 replicates  $\times$  two *P. bicincta* nymph treatments). Nymphal mortality was recorded after 24 h.

**Adult Predation. Trial 1.** A single adult *P. bicincta* was challenged with *Lycosa* sp. and *S. invicta*. Both predators were evaluated in the similar bioassays used for egg and nymphal predation trials. Adult *P. bicincta* mortality was recorded after 24 h. Controls were maintained without predators. There were a total of 30 replicates for *Lycosa* sp. and 16 replicates for *S. invicta*.

**Trial 2.** Adult *P. bicincta* were challenged with *H. pensylvanicus*, *C. sayi*, and *M. carolina carolina*. Tests were conducted in similar bioassays described in egg and nymphal predation trials. Two or four *P. bicincta* adults were placed with a single predator in a petri dish, and observations were recorded after 24 h. There were a total of 60 replicates for *H. pensylvanicus* (30

replicates  $\times$  two adult densities); 40 replicates for *C. sayi* (20 replicates  $\times$  two adult densities); and 40 replicates for *M. carolina carolina* (20 replicates  $\times$  two adult densities). Controls were maintained without predators.

**Statistical Analysis.** Data on the predation of *P. bicincta* eggs, nymphs, and adults was analyzed using Poisson regression. Poisson regression analysis is used when the response variable represents counts. PROC GENMOD (SAS Institute 2001) was used to perform Poisson regression. Comparisons between various predators used in the egg, nymph, and adult predation bioassays were done using Likelihood ratio test for type III analysis. Likelihood ratio statistic has a chi-square distribution with single degree of freedom. Data from three consecutive days of the greenhouse egg predation bioassay used repeated measures analysis with correlation structure where the experimental unit was replicates  $\times$  predators. The dependent variable was the number of eggs eaten each day. The independent variable was the type of predator and the time period (h).

**Results**

**Egg Predation. Trial 1.** There was a significant effect of *P. bicincta* egg density (three, five, or 10 eggs) on egg consumption by the predators ( $\chi^2 = 318.27$ , df = 2,  $P < 0.0001$ ). There was no difference egg consumption by *S. invicta*, *H. pensylvanicus*, and *G. punctipes* ( $\chi^2 = 2.04$ , df = 2,  $P < 0.36$ ) (Table 1).

**Trial 2.** There was a significant effect of predator on *P. bicincta* egg consumption ( $\chi^2 = 34.12$ , df = 5,  $P < 0.0001$ ). *S. invicta*, *C. sayi*, and *H. pensylvanicus* were effective egg predators compared with *G. punctipes*, *G. uliginosus*, and *M. carolina carolina* (Table 2).

**Trial 3.** There was a significant effect of time ( $\chi^2 = 21.75$ , df = 2,  $P < 0.0001$ ), predator ( $\chi^2 = 7.62$ , df = 2,  $P < 0.022$ ), and the interaction of predator  $\times$  time ( $\chi^2 = 12.04$ , df = 2,  $P < 0.01$ ) on *P. bicincta* egg consumption in the greenhouse bioassay. As time (hours) increased, *P. bicincta* egg consumption by the predators also increased up to 48 h. There was a significant difference in the number of eggs consumed by the *H. pensylvanicus* and both species of *Geocoris* during 24 h ( $\chi^2 = 13.36$ , df = 2,  $P < 0.001$ ) and 48 h ( $\chi^2 = 7.14$ , df = 2,  $P < 0.03$ ) in the greenhouse bioassay (Fig. 1). However, there was no significant difference in number of eggs consumed by the predators at 72 h ( $\chi^2 = 4.51$ , df = 2,  $P < 0.12$ ) in the greenhouse bioassay. *H. pensylvanicus* and *G. punctipes* consumed

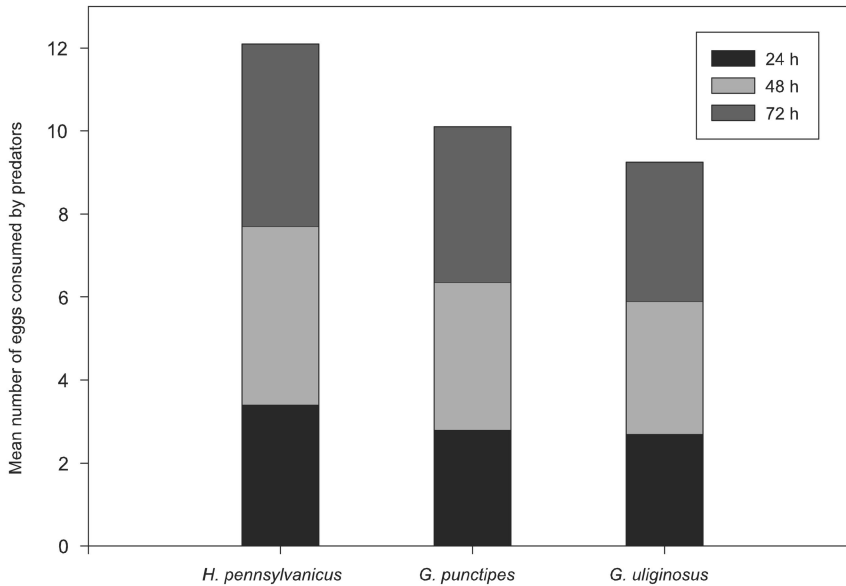


Fig. 1. Mean  $\pm$  SE number of *P. bicincta* eggs consumed by predators in a greenhouse bioassay.

significantly more *P. bicincta* eggs than *G. uliginosus* in the greenhouse bioassay.

**Nymph Predation. Trial 1.** There was a significant effect of *S. invicta* ( $\chi^2 = 6.46$ ,  $df = 1$ ,  $P < 0.01$ ) and *H. pensylvanicus* ( $\chi^2 = 13.49$ ,  $df = 1$ ,  $P < 0.0002$ ) on 1-d-old *P. bicincta* nymphal predation. Newly hatched *P. bicincta* nymphs covered in spittlemass were rarely killed. In some cases, nymphs moved out of their spittlemass and were preyed upon by *S. invicta*. Control mortality was negligible.

**Trial 2.** Second or third instars of *P. bicincta* protected by the spittlemass were rarely eaten by the various predators in the laboratory. There was significant mortality of exposed *P. bicincta* nymphs caused by *M. carolina carolina* ( $\chi^2 = 1,161.5$ ;  $df = 1$ ;  $P < 0.0001$ ) and *S. invicta* ( $\chi^2 = 12.84$ ,  $df = 1$ ,  $P < 0.0003$ ). *C. sayi* and *H. pensylvanicus* did not cause significant mortality of *P. bicincta* nymphs ( $\chi^2 = 0.00$ ,  $df = 1$ ,  $P < 1.0$ ). No mortality was observed in the controls.

**Adult Predation. Trial 1.** When provided live, first generation (June) adult *P. bicincta*, *Lycosa* sp. rejected them as food; however, *Lycosa* sp. consumed crickets as food during this period. Interestingly, *Lycosa* sp. began eating second generation (August) *P. bicincta* adults and continued to do so until the end of September. Hence, no statistical analysis of the data was performed. *S. invicta* consumed 100% of adult *P. bicincta* offered.

**Trial 2.** There was a significant effect of predator on *P. bicincta* adult mortality ( $\chi^2 = 112.52$ ,  $df = 4$ ,  $P < 0.0001$ ). *S. invicta* and *M. carolina carolina* were the most effective predators of adult *P. bicincta*. *H. pensylvanicus*, *C. sayi*, and *Lycosa* sp. also caused significant mortality of *P. bicincta* adults (Table 3). There was negligible mortality in the controls.

## Discussion

To our knowledge, this is the first published documentation of potential natural enemies of *P. bicincta* in turfgrass. Predation on all life stages of *P. bicincta*: eggs, nymphs (with and without spittlemass), and adults were demonstrated under controlled laboratory and greenhouse conditions. *P. bicincta* eggs were consumed by *G. punctipes*, *G. uliginosus*, *S. invicta*, *H. pensylvanicus*, *C. sayi*, and *M. carolina carolina*, with *S. invicta* being the most significant consumer of eggs. Ants are the most abundant ground-dwelling insects in turfgrass and are known to reduce densities of eggs and nymphs of turfgrass pests such as black cutworm, *Agrotis ipsilon* (Hufnagel), and Japanese beetle, *Popillia japonica* Newman (Lopez and Potter 2000, Braman et al. 2002). *G. uliginosus*, a polyphagous predator in turf (Reinert 1978, Braman et al. 2003), was also an efficient predator of *P. bicincta* eggs. *H. pensylvanicus* are cosmopolitan beetles that feed on various living and dead insects as well as seeds (Best and Beegle 1977). Our study indicated that *H. pensylvanicus* is also a potential predator of *P. bicincta* eggs in turfgrass.

Our results confirm previous reports of the spittlemass providing protection against predators (Whitaker 1970). However, other functions such as osmoregulation and protection against parasitoids and

Table 3. Mean  $\pm$  SE *P. bicincta* adults killed by predators during 24 h in trial 2 of the adult predation bioassay

Predator	Mean $\pm$ SE	$\chi^2$	df	P
<i>Lycosa</i> sp.	1.55 $\pm$ 0.14	18.69	1	0.001
<i>H. pensylvanicus</i>	0.58 $\pm$ 0.24	4.85	1	0.027
<i>C. sayi</i>	1.37 $\pm$ 0.19	12.56	1	0.001
<i>S. invicta</i>	4.0 $\pm$ 0.00	184.49	1	<0.0001
<i>M. carolina carolina</i>	4.0 $\pm$ 0.00	146.06	1	<0.0001

fungal pathogens, also may play a role. Exposed *P. bicincta* nymphs challenged with predators were consumed almost immediately. Intact nymphs (with spittlemass) were left untouched; predators did not seem to recognize the presence of the nymphs in the spittlemass with the exception of *S. invicta*. *S. invicta* were aggressive toward nymphs in the spittlemass and in some cases forced the nymphs out of their spittlemass and preyed upon them.

*P. bicincta* adults are brightly colored, conspicuous insects with aposematic coloration. Peck (2000) demonstrated the ability of spittlebugs of genus *Prosapia* (Cercopidae) to reflex bleed (emit a fluid from pretarsi) when disturbed. Reflexive bleeders are known to be repulsive to natural enemies through their hemolymph, which acts as a mechanical deterrent or chemical components in the blood that are distasteful or noxious (Blum and Sannasai 1974). However, the aposematic coloration and the ability to reflex bleed did not confer any visual, mechanical or chemical deterrence against arthropod predators. It is thought that the warning coloration and distastefulness could provide protection against vertebrate predators such as birds. We identified several common ground-dwelling arthropods, *S. invicta*, *M. carolina carolina*, *Lycosa* sp., and *C. sayi*, as significant predators of adult *P. bicincta* in the laboratory. However, we have no valid explanation for the temporary rejection of *P. bicincta* adults by wolf spiders during trial 1 of adult predation bioassay. We noticed extensive grooming of mouthparts and antennae of predators when they came in contact with the hemolymph of the adult spittlebug.

In addition to aposematic coloration and the ability to reflex bleed, spittlebugs can jump and fly to avoid predators; this behavior is probably their most important defensive mechanism in the field. The small arena of our experimental setup prevented the spittlebugs from jumping away from the grasp of the predators. However, when *P. bicincta* adults were challenged with tiger beetles in larger experimental arenas they were easily killed by tiger beetles (Nachappa et al. 2006), indicating that they cannot completely avoid being captured and killed by predators in the field.

Although our study was conducted in artificial arenas it is appropriate for simple comparisons of the physiological capacities of the predator to various preys. We identified potential predators of *P. bicincta* eggs, nymphs and adults in turf; additional studies to define and enhance their impact on *P. bicincta* are needed. Conservation of the rich diversity of beneficial population is essential for sustainable management system in turf (Potter and Braman 1991). Insecticidal sprays of organophosphates and carbamates have short-term adverse effects on beneficials, including ants, spiders, beetles, and parasitic Hymenoptera (Terry et al. 1993, Kunkel et al. 2001). Further study to define the role of these predators in spittlebug suppression and to understand the nontarget effects of current management practices on these predators is warranted.

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