

BIOLOGY AND MANAGEMENT OF TWOLINED SPITTLEBUG, *PROSAPIA BICINCTA*  
(SAY) (HEMPITERA: CERCOPIDAE) IN TURFGRASS

by

PUNYA NACHAPPA

(Under the direction of John N. All)

ABSTRACT

Potential predation on twolined spittlebug, *Prosapia bicincta* (Say) eggs, nymphs and adults was investigated in the laboratory by entomophagous arthropods commonly found in turfgrass. Eggs were most vulnerable to attack from predators. Nymphs are protected in the spittlemass from predation, but are susceptible to attack when, mechanically removed from their spittlemass. *P. bicincta* adults were also killed by arthropod predators evaluated in the study. The functional response of the tiger beetle, *Megacephala carolina carolina* L. to varying prey densities of *P. bicincta*, and fourth instar fall armyworm, *Spodoptera frugiperda* (J.E. Smith) larvae in single-prey and two-prey systems was assessed in the laboratory. Like most insect predators, *M. carolina carolina* demonstrated a type II functional response in both situations. An alternate management strategy for *P. bicincta*, with low-risk selective insecticides against nymphs and adults was evaluated in the field. Number of general use insecticides were effective in controlling *P. bicincta* nymphs and adults.

INDEX WORDS: Centipedegrass, Biological control, Functional response, Chemical control, Integrated pest management

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PUNYA NACHAPPA

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PUNYA NACHAPPA

Major Professor: John N. All

Committee: Kristine S. Braman  
Lee P. Guillebeau

Electronic Version Approved:

Maureen Grasso  
Dean of the Graduate School  
The University of Georgia  
December 2004

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CHAPTER 1  
INTRODUCTION



This project concerns an economic pest of turfgrass and ornamentals in the family Cercopidae in Hemiptera. Members of the family Cercopidae are commonly called froghoppers or spittlebugs. The spittlebug genus, *Prosapia* Fennah, occurs in North America. Most species are found in Mexico and Central America (Hamilton 1977). *Prosapia bicincta* (Say), the twolined spittlebug (TLS) is reported from Florida to Maine and as far west as Arkansas and Texas in the United States (Byers 1965). Nymphs and adults are xylem feeders and can feed on almost all plants that provide fluids to meet its requirements (Pass and Reed 1965). TLS has a broad range of grass hosts, including centipedegrass (*Eremochloa ophiuroides* Munro. Hack) (Beard 1973, Vittum et al. 1999, Braman 1995, Shortman et al. 2002), coastal bermudagrass, a cultivar of (*Cynodon dactylon* (L.) Pers (Byers and Wells 1966, Taliaferro et al.1969), panolagrass, (*Digitaria decumbens* Stent), and St. Augustinegrass, (*Stenotaphrum secundatum* Walt.) Kuntze (Genung et al. 1954). Seashore paspalum (*Paspalum vaginatum* Swartz), zoysiagrass (*Zoysia* Willd), and tall fescue (*Festuca arundinaceae* Schreb) are also susceptible to TLS (Shortman et al. 2002); as well as other graminaceous crops. Of late, TLS adults have become economic pests on ornamental hollies, *Ilex opaca* L. and *I. cornuta Burfordii* De France (Pass and Reed 1965, Braman and Ruter 1997).

There have been ambiguous reports on the function of spittlemass. The conventional view is that the spittle provides protection against desiccation for the nymph (Weigert 1964a, b, Wigglesworth 1972, Kuenzi and Copel 1985). Another explanation is that the spittlemass may function as an osmoregulatory device, such as a plastron would for an aquatic insect (Turner1994). It is also thought to serve as protection from predator, parasitoids, bacterial and fungal pathogens (Guilbeau1908, Weaver and King1954, Whitaker 1970, William and Ananthasubramaium1989). Adults do not produce spittlemass, but are aposematic and have the

ability to reflex bleed and jump high to evade natural enemies (Peck 1998, Peck 2000). The nature and origin of the precise defensive factor in the hemolymph remains undetermined.

Despite a high pest status, there is a paucity of information about the basic biology and ecology of TLS. Insect-host-habitat associations vary with geographical range, climatic factors, and management strategy. Integrated Pest Management strategies for the management of spittlebugs are rudimentary. Given the taxonomic diversity of the group and the range of habitats they exploit, there is a need to acquire new bio-ecological information about this species. Sound background knowledge about this pest complex is vital for the development and implementation of a detailed, site-specific, biologically based pest management program for the control of TLS. Other factors limiting the development and implementation of integrated pest management for turfgrass systems include the lack of knowledge of the basic biology and ecology of the beneficial arthropod communities in turfgrass habitats (Potter and Braman 1991).

Biological control of urban pests has a broad appeal to management professionals and their clients because of the apparent ecological and environmental safeness of this approach. Classical biological control, augmentation and conservation have been attempted in landscape settings with varying levels of success (Raupp et al. 1992). The sole biological control agent recorded for TLS is the southern meadowlark *Sturnella magna argutulla* (Bangs). A significant number of TLS were recorded in the crop contents of the bird (Genung and Green, Jr. 1974).

Landscapes have a rich diversity of beneficial insects including those found in turfgrasses (Reinert 1978, Cockfield and Potter 1984, Braman and Pendley 1993a). Previous studies have shown the rich diversity of beneficial arthropods such as carabids, staphylinids, mites, spiders and ants in turfgrass habitats (Mailoux and Streu 1981, Cockfield and Potter 1984, Braman and Pendley 1993a). These indigenous predators help regulate pest outbreaks in lawns, golf course

and urban landscapes (Streu and Gingrich 1972, Reinert 1978, Cockfield and Potter 1984, Terry et al. 1993). Seasonal activity of these predators coincides with the emergence of both generations of the TLS in turfgrass habitats (Braman and Pendley 1993a, b). Natural enemies in turfgrass can be conserved to benefit biological control program of TLS.

The first part of this research project entailed laboratory bioassays to investigate predation of TLS eggs, nymphs and adults by generalist predators commonly found in turfgrass: big-eyed bugs (*Geocoris uliginosus* Say, *G. punctipes* Say), red imported fire ants (*Solenopsis invincta* Buren), wolf spiders (*Lycosa* spp. Walckenaer), carabid beetles (*Harpalus pennsylvanicus* DeGeer, *Calosoma sayi* Dejean) and tiger beetles (*Megacephala carolina carolina* L.). Potential predation of twolined spittlebug eggs by *G. punctipes*, *G. uliginosus*, *S. invincta*, *H. pennsylvanicus*, *C. sayi* and, *M. carolina carolina* were determined. Nymph predation trials began with newly hatched nymphs (neonates) or second and third instar nymphs with and without spittlemass being challenged with *S. invincta*, *H. pennsylvanicus*, *C. sayi* and, *M. carolina carolina*. Field collected adult spittlebugs were challenged with *Lycosa* spp., *S. invincta*, *H. pennsylvanicus*, *C. sayi* and, *M. carolina carolina*.

A second component of my thesis involved the determination of functional response of the tiger beetle, *M. carolina carolina* in single-prey and two-prey systems. Functional response relates change in predation rates to changing prey density (Solomon 1949, Holling 1959). The type of functional response is significant as it helps define the effectiveness of the natural enemies. We determined the functional response, handling time, and attack coefficient of *M. carolina carolina* on adult twolined spittlebug, *P. bicincta* and fourth instar fall armyworm, *S. frugiperda*, when prey was offered alone (single-prey) and together (two-prey system) in the same arena.

In the third part of this project, we evaluated effects of low-risk selective insecticide applications on first generation TLS adults and second-generation nymphs. Damage assessment caused by the first generation adults' post-insecticidal application was also recorded. We also surveyed the non-target arthropod abundance. Current chemical control for spittlebugs includes spraying with chlorpyrifos, diazinon, acephate and cyfluthrin. However, the Environmental Protection Agency (EPA) has phased out chlorpyrifos and diazinon for homeowner use, limiting chemical control options for spittlebugs. Effects of chemical management on beneficial invertebrates in turf have also been assessed (Cockfield and Potter 1983, Potter et al. 1990, Vavrek and Niemczyk 1990, Braman and Pendley 1993a, Terry et al. 1993). Short-term insecticidal sprays for spittlebugs have adverse effects on collembolans, ants, spiders and parasitic hymenoptera (Braman and Pendley 1993a). Concerns over potential risks to humans and the environment have led the public demand for critical reassessment of current management tactics, but the demand for high-quality landscapes are still on the increase (Braman et al. 1998). Research on compatibility of pesticides with the beneficial fauna is essential to the development of ecologically sound pest management program for turf (Potter and Braman 1991). An evaluation of products for spittlebug suppression was undertaken to identify replacement technology for chlorpyrifos and diazinon.

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CHAPTER 2  
LITERATURE REVIEW

### I. Taxonomic Classification and Distribution of *Prosapia bicincta*

Members of the family Cercopidae in Hemiptera are commonly called the spittlebugs or froghoppers. Moore (1956) suggested that the family Cercopidae is represented by the subfamilies: Aphrophorinae, Cercopinae, Clastoterpinae and Machaerotinae. Only the first three families are present in North America. The subfamily Machaerotinae, or the “tube-building” spittlebugs is not represented in North America. Cercopidae is a taxonomically diverse pest complex, comprising 7 genera (sensu Metcalf 1961): *Aeneolamia*, *Deois*, *Mahanarva*, *Notozulia*, *Prosapia* (*Tomaspis*), *Sphenorhina* and *Zulia*. According to Arnett, Jr (1993), in North America the subfamily Cercopinae, consists of two genera, *Prosapia* and *Philaneus* Fitch.

In the past, the twolined spittlebug (TLS) was described as *Cercopis bicincta* by Say in 1830. Temporarily, the species was placed in the genera *Tomaspis* and *Monecephora*. The current and valid scientific name is *Prosapia bicincta* (Say). Hamilton (1977) described 14 species of *Prosapia*, but recognized no subspecies since the geographical variation in genitalia features could not be precisely defined. Scanning electron microscopy of the morphology of the antennal sensilla in *P. bicincta* allowed for comparisons of basiconic, coeloconic, campaniform, and trichord sensilla among taxa (Liang 2001).

The genus *Prosapia* Fennah occurs in North America, with most species occurring in Mexico and Central America (Hamilton 1977). *Prosapia bicincta* (Say) has been reported from Florida to Maine (Byers 1965) and as far west as Texas and Arkansas in the United States. *Prosapia*, without a designation, closely related to *P. bicincta* (*P. nr. bicincta*) (Peck 1998) has been reported in the literature from Brazil as a pest on grasses of the genus *Brachiaria* Griseb (Lapointe et al. 1992). These grasses are grown throughout Central and South America as a part of the Centro Internacional de Agricultura Tropical (CIAT) program in Cali, Colombia.

## II. Biology of *Prosapia bicincta*

*Prosapia bicincta* (Say), the twolined spittlebug (TLS) is a recognized economic pest of turf grasses including centipede grass (*Eremochloa ophiuroides* Munro. Hack) (Beard 1973, Tashiro 1987, Potter and Braman 1991, Braman 1995, Shortman et al 2002), coastal bermudagrass, a cultivar of (*Cynodon dactylon* (L.) Pers (Byers and Wells 1966) and other bermudagrass cultivars (Taliaferro et al.1969). Damage has been reported on other grasses such as panolagrass, (*Digitaria decumbens* Stent), and St. Augustinegrass, (*Stenotaphrum secundatum* Walt.) Kuntze (Genung et al. 1954). Seashore paspalum (*Paspalum vaginatum* Swartz.), zoysiagrass (*Zoysia* Wild), and tall fescue (*Festuca arundinaceae* Schreb) are also susceptible to TLS (Shortman et al. 2002). Sweet corn, (*Zea mays* L.) sustains damage by adult twolined spittlebugs (Janes 1971). Adults are also known to cause economic damage on ornamental hollies, *Ilex opaca* L. (American holly) and *I. cornuta Burfordii* De France (Burford holly) (Pass and Reed 1965, Braman and Ruter 1997) used in the landscape trade, and on southern lawns planted with centipedegrass and bermudagrass.

TLS are polyphagous, xylem feeders as nymphs and adults, with a wide range of hosts (Weaver and King 1954, Fagan and Kuitert 1969). TLS can survive on almost any host providing a sufficient amount of fluid to meet its feeding demands (Pass and Reed 1965). Thompson (1994) reports that the family Cercopidae shows a preference for nitrogen-fixing grasses. Spittlebugs demonstrate a preferential association with nitrogen-fixing hosts that transport fixed nitrogen as amino acids and amides rather than urides (Thompson 1994). The insect feeds by piercing intercellularly with its stylets to the xylem vessels and ingesting sap (Weigert 1964, Horsfield 1978). The meadow spittlebug, *Philaenus spumarius* L. feeds from the xylem vessel at full hydraulic tension of the main transpiration stream (Malone et al. 1999). Spittlebugs have

massively developed cibarial dilator muscles, with a large and heavily reinforced precibarium that is compatible for sucking against strong tensions thought to occur in the xylem vessel (Newby 1979, Backus 1985). Adult feeding results in phytotoxemia or “frohopper burn” (Byers and Wells 1966, Taliafferro et al. 1969). Age and sex were shown to be unimportant in the ability of adults to produce phytotoxemia. (Byers and Taliferro 1967). A plant growth promoter in the salivary gland may be responsible for the damage (Cutler and Stimmann 1971), however the identity of this substance remains unknown. The toxin is injected into the xylem as the insects feed, and damage begins within 24 hours. Symptoms include stippling, streaking and browning. Necrosis of the host can also result. Recovery from spittlebug damage can occur if the plant is not severely damaged, but the recovery time is much longer than would be expected, indicating a residual effect of the phytotoxin (Meyer1993; Meyer and Root 1993; Meyer and Whitlow 1992; Karban and Strauss 1993). Nymphal feeding causes water stress and loss of biomass in forage grasses. Symptoms on turf grass include wilting of grasses, and yellowing, similar to that of adults (Weigert 1964a).

Host selection by adult spittlebugs is influenced by factors such as habitat (Pickles 1938, Weaver and King 1954, Weigert 1964b), plant morphology (Hoffman and Mc Evoy 1985, Koller and Honer1993, 1994), and plant physiology (Horsfield 1977, Thompson 1994). Nymphs are particular about feeding sites, which are constrained by size/ age class and such factors as depth from exterior to xylem elements, tissue toughness, presence of trichomes (Hoffman and Mc Evoy 1986) and plant architecture (Mc Evoy 1986). Development and survival of pasture spittlebugs on *Brachiaria decumbens* varied with different morphological characteristics (Koller and Honer 1994). Survival of nymphs was best on plants not cut for the last 4 or 15 months.

Survival is correlated with the number of shoots, but not with the fresh or dry weight of green material, or with the number of green stalks or the total number of stalks.

Basic biology of TLS has already been examined (Byers 1965). Eggs are deposited near, or below the soil line. Occasionally, they have been reported as inserted into the stem of the host plant (Fagan and Kuitert 1969). Over wintering usually occurs in the egg stage, although an occasional over wintering adult has been reported in Tift Co., Georgia (Beck and Skinner 1972). After hatching, the mobile nymphs make their way to suitable hosts and immediately begin to feed. Spittle is produced within five minutes of feeding (Fagan and Kuitert 1969; Pass and Reed 1965). Five instars are confirmed for spittlebugs in the genus *Prosapia* (Pass and Reed 1965, Fagan and Kuitert 1969). Nymphs complete their development inside the spittlemass (Weaver and King 1954). Development time varies with temperature. In the field in Clemson, South Carolina, from March 1960 to March 1962, nymphal period ranges from 34 to 60 days (Pass and Reed 1965). Peak times of adult emergence were late July and early August in Clemson, S.C (Pass and Reed 1965). While in Tift Co., Georgia, three peaks of adult emergence were observed (Byers 1965). Two peaks occurred in June and early July, and were associated with the first generation. The third peak, representing the second generation, occurred in August. Adults have been collected from March until late November with two generations per year reported (Byers 1965).

Females release a male attracting pheromone. Females first mate when they are five to nine days old (Byers 1965), and continue to mate throughout the growing season. They both mate before and after the beginning of oviposition period. Oviposition preference of two species of spittlebugs *Zulia entreriana* (Berg) *Deois flavopicta* (Stal) was studied in the laboratory. Both species reacted similarly to moisture, compaction, leaf litter, litter depth and soil particle size

(Hewitt 1985). Shoot characteristics also affect oviposition preference of Willow spittlebug *Aphrophora pectoralis* Say. Spittlebugs preferred shoots >20cm long and shoot length up to 40cm for oviposition. However, egg parasitism did not affect oviposition preference, because egg parasitism is not a function of shoot size (Nozawa and Ohgushi 2002). Egg laying occurs when the female is 7 days old, and the average oviposition period is 14 days (Fagan and Kuitert 1969).

Byers (1965) studied the induction and termination of diapause at Tifton, Georgia. He concluded that soil temperature (9.5-28° C) as well as moisture (45-100%) affects diapause and hatching, but that photoperiod (0L: 24D vs 14L: 10D) has little or no effect. He also determined that eggs laid in the field were not initially in diapause, and if moisture conditions were right, such as those found in spring and summer, eggs would hatch. If conditions were too dry, eggs would diapause, or exhibit “long photoperiodism” and would not hatch despite environmental conditions. Koller and Honer (1993) found correlations between climatic factors and diapause of eggs of two species of pasture spittlebugs. The best correlation was found for photoperiod, followed by rainfall and total evaporation.

TLS experiments usually start with field collection of specimens; trapping techniques were investigated by Beck and Skinner (1972). They found that black light traps captured significantly greater number of adults. High traps (157-274 cm above ground) collected almost four times as many males as females, but as trap height was lowered to 15.2 cm above ground, ratios of males to females were more equal, and the number of TLS captured increased. Timing of trapping was also investigated and peak of flight activity occurred between 9 and 10 PM (Beck and Skinner 1972). Nilakhe et al. (1987) investigated the influence of collection method and collection time on survival, sexual proportion and number of spittlebug captured.

They concluded that spittlebugs collected with a sweep-net and transferred with an aspirator tended to live shorter than those transferred using a container lined with cotton. Sweeping at 5 p.m.-6 p.m. than at 8 a.m.-9 a.m. or midday-1 p.m captured more adults. The sweep-net method captured most adults and sweeping did not affect adult survival. However, sweep-net method tended to underestimate the proportion of females (Nilakhe et al. 1987). Population survey of adult spittlebugs, *Zulia entreriana* (Berg) *Deois flavopicta* (Stal) between 0600 h and 1900 h showed higher adult activity in the morning and late afternoon.

Rearing techniques have been greatly improved (McWilliam and Cook 1975, Lapointe et al. 1989). Mc William and Cook (1975) placed TLS eggs in the advanced egg spot stage in vermiculite substrate of pots of pearl millet, *Pennisetum americanum* (L.) K. Schum., close to the crown of the plants. Each 15.2 cm pot was infested with about thirty- five eggs. A 16 h photoperiod was maintained to prevent diapause. Hatching occurred about two days later. Approximately one-week post hatch, spittlemasses were observed, and adults emerged in about seven weeks. Adults were removed after emergence, and placed on different pots of millet for the 10-day pre-oviposition period. They were then transferred to oviposition cages constructed from cardboard ice cream containers, cut down to 10.2 cm, with cellophane tops and mesh bottoms secured by lid rims. Ovipositing females were fed on leaf bouquets composed of millet, *Sorghum bicolor* (L.) Moench, sugarcane, *Saccharum officinarum* L., Corn, *Zea mays* L. and rye *Secale cereale* L., with their proximal end(s) wrapped in Cellucotton® and placed into a vial containing 5% (by vol) corn syrup in water solution. The cotton acted as a wick to allow for feeding by the spittlebugs, and to seal the vial. Moistened Cellucotton® wrapped around a toothpick were satisfactory oviposition sites. Each cage contained six oviposition sites and twelve food bouquets. These were replaced every two days. About one hundred females were

supported in each cage. Egg recovery was accomplished by removing the Cellucotton® from the toothpick, adding enough water to make a pulp, and with air bubbling through a separatory funnel, collecting and draining the eggs that had accumulated on the bottom of the funnel. The eggs were then placed on moist filter paper and incubated.

Lapointe et al. (1989) reported spittlebug-rearing techniques different from McWilliams and Cook (1975) method. Oviposition cage was made of wood and covered with nylon mesh; the bottom was fitted with a movable drawer to allow for the insertion of trays of oviposition substrate. The substrate consists of soil imprinted with a reticulate pattern to increase oviposition and obtain a more uniform distribution of eggs. Trays were removed and replaced to obtain eggs of a particular age. When ready to hatch, the eggs were placed at the base of a potted plant. The plastic pots were covered with an aluminum foil to increase humidity around the base of the plant, and to encourage superficial rooting of the plants due to reduce light levels. In this way, adults of known age can be collected, used for experimental purposes or introduced to oviposition chambers.

Sotelo et al. (1988) reported rearing spittlebug nymphs of two species *Zulia colombiana* and *Aneneolamia reducta*. He concluded that *Brachiara* sp. planted in pots and covered with an aluminum sheet was the most effective method for rearing nymphs. Nymphs migrate to the base of the stem when they complete their development.

An interesting aspect of the insect's ecology is the production of its characteristic spittlemass during the nymphal stages. According to Rakitov (2002) there is evidence of homology in the structure and function of the Malphigian tubules of juvenile cicadas and spittlebug nymphs. The Malphigian tubules are involved in the secretion of the froth; proximal parts of the tubule contain a short smooth inflated segment, which produces granules of an acid



mucopolysaccharide. Topochemical and gel electrophoresis revealed that the spittlemass is constructed by adding hundreds of bubbles to feeding wastes that are stabilized by mucopolysaccharides, polypeptides (glycopeptides), and acid proteoglycans (Marshall 1966, Mello et al. 1987). Calcium was also detected in the structure of the spittlemass (Mello et al. 1987). The spittlemass is thought to serve as a shelter for the nymphs during development in Cercopids, but their function is yet to be proved. The conventional view is that the spittle provides protection against desiccation for the nymph (Weigert 1964a, b, Wigglesworth 1972, Kuenzi and Copel 1985), but due to the energetic cost of making and maintaining such a nest, this may be erroneous. According to Turner (1994) there is an anomalous water loss rate from the spittle nests of the pine spittlebug, *Aphrophora saratoga* Say. When the reduction of evaporation is compared with the increase in evaporation that would result from inflating a drop of anal fluid to form the spittlemass, the spittle seems to provide little net reduction of evaporative water loss. Hence, the conventional view of the spittle as providing protection to nymphs against desiccation is incorrect. Another explanation is that the spittlemass may function as an osmoregulatory device, such as a plastron would for an aquatic insect (Turner 1994). Protection from predator, parasitoids, bacterial and fungal pathogens have also been proposed (Guilbeau 1908, Weaver and King 1954, Whitaker 1970, William and Ananthasubramaium 1989).

Niche partitioning for several spittlebugs has been researched, but not for *P. bicincta*. McEvoy (1986) showed that density-dependent effects were absent regarding growth and development even when nymphs of different species shared the same axil. Axil width greater than the width of the spittlebug was a factor in host suitability.

Adults are devoid of spittle, but have camouflage coloration, jumping ability (Hamilton 1982), and warning coloration (Thompson 1973) as their defense mechanism. According to Peck

(2000), froghoppers of the New World genus *Prosapia* (Ceropidae) reflex bleed (emit a fluid from pretarsi) when disturbed. Blood or hemolymph is thought to be a mechanical deterrent in insects that reflex bleed (Happ and Eisner 1961, Wallace and Blum 1971, Benfield 1974, Blum and Sannasi 1974). Hemolymph that is sticky, viscous and rapidly coagulating poses an effective defense against arthropod predators such as ants, which cease their attack to groom. Reflex bleeding is frequently correlated with conspicuousness such as the aposematic appearance of *Prosapia*. Reflexive bleeders are also known to be repulsive to enemies by chemical components in the blood that are distasteful or noxious (Blum and Sannasi 1974, Carrel and Eisner 1974, Moore and Brown 1981, Eisner et al. 1986). The nature and origin of the precise defensive factor (pyrazines or others) in *Prosapia* remains undetermined. Examination of reflexive bleeding in *Prosapia* sp. in predator challenges showed no clear evidence of mechanical or chemical deterrence (Peck 2000). The lack of evidence for mechanical and chemical deterrence leads to the suggestion that reflex bleeding in froghoppers function in part as a startle stimulus (Sargent 1990), comprised of two components: physical discharge of blood and chemical warning odor. Since adult spittlebugs are skilled jumpers, any deterrent need only cause the predator to hesitate momentarily for the insect to hop to safety.

Spittlebugs mostly rely on graminaceous hosts and this group is not known for defensive secondary compounds (Peck 2000). However, American hollies, the alternate hosts of adult TLS is known to contain saponins, triperpenes (West et al. 1977, Potter and Kimmerer 1986, 1989 Kreuger and Potter 1994) and phenolics (Potter and Kimmerer 1986, Gargiulo and Stiles, 1991) as predominant secondary metabolites. Hence, hollies could be the source of the defensive chemical in spittlebugs. Peck (1998) reported the exclusive use of alternate host plants by adult male spittlebugs in the Monteverde region of Costa Rica. According to the author, there is

evidence in case of *P. bicincta* for the exploitation of hosts that are taxonomically and morphologically different from grasses, however, there was no proof of male-specific use of alternate hosts per se.

### III. Management Strategies of *Prosapia bicincta*

Despite a high pest status and long history in the new world, an effective and coordinated program for the management of spittlebugs does not exist. Among the challenges faced is a poor basic understanding of the biology and ecology of the species at the family level, a high diversity of insects-host-habitat associations, and Integrated Pest Management (IPM) tools are rudimentary. Attempted control of TLS has included mechanical and chemical measures. Overwintering insects were controlled by burning all refuse from the previous year in early April in Tifton, Georgia (Beck 1963). Cultural practices such as burning in *Brachiaria decumbens* Stapf pastures showed excellent reduction in the number of spittlebug eggs. Both discing and harvesting the forage also reduced the number of spittlebug nymphs and adults (Koller 1988). When pasture height was kept short, the incidence of nymphs was reduced, however, this reduced green mass and drastically reduced pasture available (Ramiro et al. 1984). Chemical and agrotechnical methods in control of spittlebug nymphs indicate the use of fire in pasture or mowing in *Cynodon dactylon* plus the application of carbaryl as recommendations (Barrientos 1985). Carbofuran when applied as a seed treatment at 350g a.i./100Kg of seeds of rice gave good protection against spittlebug, *Zulia entreriana* (Berg) *Deois flavopicta* (Stal) damage for 20 days, and for 30 days when applied as granules in furrows at the time of planting at 500g a.i./ha (Souza and Nilakhe 1985). Non-significant chemical control was reported on second-generation nymphs using granular formulations of zinophos 10%, diazinon 5%, phorate 10%, and endosulfan 4% (Beck 1963). Foliar sprays of guthion, endosulfan, endrin and DDT were the

most effective insecticides tested against nymphs. Heptachlor and endosulfan were also effective as granular formulations. Guthion, malathion, meviphos, endosulfan and carbaryl were 100% effective as foliage sprays in controlling adults. Parathion, naled and methoxychlor were slightly less effective (Pass and Reed 1965). However, residual soil samples of Endosulfan indicated that significant control of second-generation nymphs was obtained by one isomer of this chemical seventy-five days after application (Byers 1965). In the Florida Everglades, chemical control was obtained using 1.326Kg wettable Toxaphene in 380L water per 0.405ha (Mead 1962). Commercial formulation of chlorpyrifos and diazinon that were recommended for the control of spittlebugs is no longer available for homeowners use, both chemicals have been phased out by Environmental Protection Agency (EPA). Synthetic pyrethrins are the only available chemical control options for spittlebugs in urban environments. Scouting for spittlemasses and chemical control has become a routine part of landscape companies' and homeowners' maintenance procedures.

Natural enemies have been reported for the neotropical spittlebugs: parasitic mites (Acari: Erythraeidae), parasitic nematodes (Nematoda: Mermithidae), predaceous fly larvae (Diptera: Syrphidae), robber flies (Diptera: Asilidae), parasitic flies (Diptera: Pipunculidae) and fungal entomopathogens. *Zulia entreriana* (Berg) and *Deois flavopicta* (Stal) eggs and newly hatched nymphs were susceptible to attack from five species of fire ants and abiotic factors during the rainy season in Central Brazil (Hewitt and Nilakhe 1986). A predatory syrphid fly, *Salpingogaster nigra* Schiner has been reported as a specific predator of spittlebug nymphs in some regions of Brazil (Marques 1988). Predation was observed on pecan spittlebug nymphs, *Castoptera achatina* by *Deraeocoris nebulosus* (Heteroptera: Miridae) in pecan orchards. Other heteropteran predators included Pentatomidae and Reduviidae and the larvae of green lace wing

(Neuroptera: Chrysopidae). Coccinellids were also found feeding on spittlebug nymphs, but they were not confirmed as actual predators (Tedders 1995). Henderson et al. (1990) documented interaction between prairie ants and meadow spittlebugs, *Philaenus spumarius* L. Ants are predators of spittlebug nymphs and construct aphid-tents using the spittle.

Egg parasitoids in the family Aphelinidae and Mymaridae have been reported in the willow spittlebug, *Aphrophora pectoralis* (Nozawa and Ohgushi 2002). Egg parasitoids were not recorded for pecan spittlebugs, however, obvious parasitoid exit holes were found (Tedders 1995).

Leite et al. (2002) reported the occurrence of entomophthorales fungi, *Furia* sp. and *Batkoa* sp. on adult spittlebugs pests in eastern Sao Paulo state of Brazil. *Metarhizium anisopliae* has also been used as a biocontrol agent of pasture spittlebugs in Central Brazil (Fontes and Lima 1989). In separate experiments, spores of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) applied to pecan spittlebug nymphs failed to produce any infection, and there was no increased nymphal mortality (Tedders 1995).

Thompson et al. (1995) documented the fly larvae, *Cladochaeta inversa* (Walker) (Diptera: Drosophilidae) living in association with the spittlebug nymphs, *Clastoptera obtusa* (Say) in a predominantly commensal relationship rather than a parasitic association. Sympatric habitation of the spittlemasses by other dipterans *Phytomyza* sp. (Agromyzidae), *Drosophidae* sp. (Drosophilidae), and *Hippelates pusio* Loew (Chloropidae) have also been reported (Tedders 1995).

There is paucity of information regarding natural enemies of the genus *Prosapia* in the new world. Ricks and Vinson (1970) reported spittlebugs (crushed insect) among the acceptable food of two varieties of Imported fire ants, *Solenopsis saevisima richteri* Forel and *Solenopsis*

*saevisima saevisima* Smith. A recent study by Eubanks (2001) also reported red imported fire ants as significant predators of froghoppers in an agroecosystem.

The first documented biological control was a report of significant number of TLS in the crop contents of the southern meadowlark *Sturnella magna argutulla* (Bangs) (Genung and Green, Jr.1974). Adults were also observed in the webs of the garden spider, *Argiope aurantia* Lucas, and the golden silk spider, *Nephila calvipes* (L.). The reduviid, *Zelus bilobus* (Say) is also a predator of the adults. Many spittlebugs caught at the light trap had several mites attached to their legs and wings. Most of the mites were *Leptus* sp. (Trombidiformes: Errythraeidae) and one *Calvidromus transvaalensis* (Nesbitt) (Mesostigmata: Phytoseiidae).

Studies have documented the wealth of beneficial arthropods such as carabids, staphylinids, mites, spiders and ants in turfgrass habitats (Reinert 1978, Cockfield and Potter 1984, Braman and Pendley 1993a). Braman and Pendley (2002, 2003) reported *M. carolina carolina* collected in pitfall trap samples in turfgrasses and landscapes. These indigenous predators help regulate lawns, golf course and urban landscapes from pest outbreaks (Reinert 1978, Cockfield and Potter 1984, Terry et al. 1993). Seasonal activity of these predators coincides with the emergence of both generations of the TLS in turfgrass habitats (Braman and Pendley 1993a, Braman and Pendley 1993b). Hence, the presence of increased natural enemies in turfgrass can be exploited for potential biological control program of TLS.

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CHAPTER 3  
SUSCEPTIBILITY OF TWOLINED SPITTLEBUG, *PROSAPIA BICINCTA* (SAY)  
(HEMIPTERA: CERCOPIDAE) LIFE STAGES TO ENTOMOPHAGOUS  
ARTHROPODS IN TURFGRASS <sup>1</sup>

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**ABSTRACT** *Prosapia bicincta* (Say), 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: big-eyed bugs (*Geocoris uliginosus* Say, *G. punctipes* Say), red imported fire ants (*Solenopsis invincta* Buren), wolf spiders (*Lycosa* sp. Walckenaer), carabid beetles (*Harpalus pennsylvanicus* DeGeer, *Calosoma sayi* Dejean) and tiger beetles, *Megacephala carolina carolina* L. Eggs were readily consumed by generalist predators. *S. invincta* consumed 100% of the eggs offered. *H. pennsylvanicus*, 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. invincta* 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. invincta* 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 invincta*, *Lycosa* sp., *Calosoma sayi*, *Geocoris uliginosus*, *Megacephala carolina carolina*, biological control

*Prosapia bicincta* (Say), the twolined spittlebug (TLS) 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, Byers and Wells 1966, Fagan and Kuitert 1969) with a broad range of grass hosts, including centipede grass (*Eremochloa ophiuroides* Munro) Hack (Beard 1973, Vittum et al. 1999, Braman 1995, Shortman et al. 2002), coastal bermudagrass, (*Cynodon dactylon* (L.) Pers (Byers and Wells 1966, Taliaferro et al. 1996), panolagrass, (*Digitaria decumbens* Stent), and St. Augustinegrass, (*Stenotaphrum secundatum* Walt.) (Genung et al. 1954). Only adults cause economic damage on ornamental hollies, *Ilex opaca* L (American holly) and *I. cornuta Burfordii* De France (Burford holly) (Pass and Reed 1965, Braman and Ruter 1997). Nymphs and adults are also known to feed on numerous annual or perennial hosts (Pass and Reed 1965).

Two generations of spittlebugs occur in the southeast, with a possible third generation in some areas. 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 spittlemasses within five minutes of feeding (Fagan and Kuitert 1969, Pass and Reed 1965). First generation spittlebugs occur in June. Second generation adults emerge in August and continue till the end of September (Byers 1965, Pass and Reed 1965). Damage associated with the second generation is more severe.

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, bacterial and fungal pathogens (Guilbeau 1908, Whitaker 1970, William and Ananthasubramaium. 1989). Adults do not produce spittlemass, but are aposematic (black with red variations) and have the ability to reflex bleed

and jump high to evade natural enemies (Peck 1998, 2000). Reflex bleeding in *Prosapia* nr *bicincta* showed no clear evidence of mechanical or chemical deterrence to predators. The nature and origin of the precise defensive factor in the hemolymph remains undetermined.

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, Koller 1988, Ramizro et al. 1984). However, chemical control has been most successful in controlling spittlebugs in various ecosystems (Byers 1965, Pass and Reed 1965, Beck 1963).

Previous studies have reported the occurrence of natural enemies of the neotropical spittlebugs (Hewitt and Nilakhe 1986, Marques 1988) and fungal entomopathogens (Fontes and Lima 1989, Leite et al. 2002). There is paucity of information regarding natural enemies of the new world spittlebugs. Ricks and Vinson (1970) reported spittlebugs (crushed insect) among the acceptable food of two varieties of imported fire ants, *Solenopsis saevisima richteri* Forel and *Solenopsis saevisima saevisima* Smith. Predation was observed on pecan spittlebug nymphs, *Castoptera achatina* Germar by members of the family Miridae, Pentatomidae, Reduviidae and the larvae of green lace wing (Chrysopidae) (Teddars 1995). A recent study by Eubanks (2001) also reports red imported fire ants as significant predators of froghoppers in cotton ecosystems. Egg parasitoids in the family Aphelinidae and Mymaridae have been reported in the willow spittlebug, *Aphrophora pectoralis* Say (Nozawa and Ohgushi 2002).

There are no records of parasites or predators of the eggs or nymphs of *P. bicincta* (Fagan and Kuitert 1969). Numerous adult spittlebugs were found in the crop contents of the southern meadowlark bird, *Sturnella magna argutulla* (Bangs) (Genung and Green, Jr. 1974). Adults were also observed in the webs of the garden spider, *Argiope aurantia* Lucas, and the golden silk

spider, *Nephila calvipes* (L.). The reduviid, *Zelus bilobus* (Say) is also a predator of the adults. Many spittlebugs caught at light trap had several mites attached to their legs and wings. Most of the mites were *Leptus* sp. (Trombidiformes: Errythaecidae) and one *Calvidromus transvaalensis* (Nesbitt) (Mesostigmata: Phytoseiidae) (Fagan and Kuitert 1969).

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 and Pendley 1993a). These indigenous predators help regulate pest outbreaks in lawns, golf course and urban landscapes (Reinert 1978, Cockfield and Potter 1984, Potter 1993, Terry et al. 1993). Braman and Pendley (2002, 2003) reported *M. carolina carolina* collected in pitfall trap samples in turfgrasses and landscapes. Seasonal activity of these predators often coincides with the activity of one or both generations of *P. bicincta* in turfgrass habitats (Braman and Pendley 1993a, b) 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 in 800 ml mason jars ventilated with 32-mesh screens. The jars were then placed in environmental chambers (Convion, 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 110-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 dia x 21 cm h) with a camel's-hair brush and allowed to complete nymphal stage. Eggs and nymphs from the laboratory-reared colony were then used for predation studies.

Generalist predators used in the study included: big-eyed bugs (*Geocoris punctipes* Say and *G. uliginosus* Say), red imported fire ants (*Solenopsis invincta* Buren), wolf spiders (*Lycosa* sp. Walckenaer), carabid beetles (*Harpalus pennsylvanicus* De Geer, *Calosoma sayi* Dejean) and, tiger beetles (*Megacephala carolina carolina* L.). *G. uliginosus* and *G. punctipes* were collected by sweeping in centipede grass during June to September (2003-2004). *Lycosa* sp., *H. pennsylvanicus*, *C. sayi*, and *M. carolina carolina* were collected from pit-fall traps inserted into centipede grass or from light traps during June-September (2003-2004). All predators were maintained in the laboratory at room temperature, photoperiod of 15:9 (L: D) h and, fed crickets and fall armyworm, *Spodoptera frugiperda* (J. E. Smith) eggs as food.

**Egg Predation. Trial 1.** Potential predation of twolined spittlebug eggs by *G. punctipes*, *H. pennsylvanicus* and, *S. invincta* was evaluated in the laboratory. The experiments were conducted at room temperature 24-26°C and, a photoperiod of 15: 9 (L: D) h. Egg predation studies were conducted with 10-12d old twolined spittlebug eggs obtained from the laboratory-reared colony. Predators were held without food for 4 days before placing with prey item. Three, 5 or 10 eggs were placed on moist filter paper in 110-cm diam petri dishes (Pioneer Plastics, Dixon, KY) with a single predator with the exception of fire ants. Egg consumption was recorded at the end of 24 h. Controls were maintained without predators.

Laboratory evaluation of fire ant predation was similar to that described by 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 and placed in a plastic container (15-cm diameter, 6.5-cm high). The fire ant colonies were connected to 18.0 cm x 8.0 cm plastic containers (Pioneer Plastics, Dixon, KY) with 4-mm diameter clear vinyl tubing also coated with Fluon. Eggs were then placed at the base of the centipede plug and the plastic container was closed. Each fire ant colony had access to two plastic containers with the prey item. Food and water were removed from the colony during predation studies. This bioassay allowed fire ant workers to forage on spittlebugs, but prevented escape from the containers. Containers that were not connected to the fire ant colony was treated as controls.

*Trial 2.* Laboratory predation of twolined spittlebug eggs was expanded to include *G. punctipes*, *G. uliginosus*, *S. invincta*, *H. pennsylvanicus*, *C. sayi* and, *M. carolina carolina* in the second trial. The experimental design was similar to the system described for both laboratory and fire ant bioassay. Number of individuals and replicates varied for all species and life stages in the egg predation trials (Table 1).

*Trial 3.* Predation of eggs was evaluated in the greenhouse bioassay. Five 10-12d old twolined spittlebug eggs were transferred onto crowns of the centipedegrass planted in Stuewe and Sons's single cell cone-tainers (3.8 cm dia x 21 cm h) via filterpaper wedges. There were 98 single-cell containers per tray. The predators used in the greenhouse predation trials included both species of *Geocoris*, and *H. pennsylvanicus*. One predator was placed with the eggs in the cone-tainers and sealed with fiber sleeves milk-test filters (Kleentest Products, Milwaukee, WI). The milk-test filters were cut to 3 cm diam x 6 cm h and rolled down from top and then sealed



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

**Nymph Predation.** *Trial 1.* Laboratory studies to determine predation on the nymphal stage of twolined spittlebug utilized newly hatched nymphs exposed to *S. invincta* and *H. pennsylvanicus*. Experimental design was similar to the system described above, also for *S. invincta* where, eggs were replaced with nymphs. One newly hatched nymph was placed in a petri dish with a blade of centipede grass for sustenance during the experiment as a xylem source for the production of spittlemass. Nymphs were also tested without spittlemass to confirm the role of the spittlemass as a protective shield against predators. Devoid of the xylem source (grass), nymphs fail to produce the spittlemass, exposed nymphs were also challenged with the same predators in a similar experimental arena. A total of 25 replicates were obtained for both species evaluated in the nymph predation trials (Table 1).

*Trial 2.* One 2<sup>nd</sup> or 3<sup>rd</sup> instar nymphs was challenged with *S. invincta*, *H. pennsylvanicus*, *C. sayi* and, *M. carolina carolina*. Experimental arrangement was similar to the system described above, also for *S. invincta* where, instead of newly hatched nymphs, 2<sup>nd</sup> or 3<sup>rd</sup> instar nymphs were the prey offered.

**Adult Predation.** *Trial 1.* Field collected adult spittlebugs were challenged with *Lycosa* sp., and *S. invincta*. Both predators were evaluated in the similar laboratory arena that was used for egg and nymph predation trials. One adult spittlebug was placed with a single predator in the petri dish, adult mortality was recorded after a 24 h period.

*Trial 2.* Adult spittlebugs were exposed to several other predators including *Lycosa* sp., *S. invincta*, *H. pennsylvanicus*, *C. sayi* and, *M. carolina carolina*. Tests were conducted in similar experimental arenas used for egg and nymph predation. Two or 4 adult spittlebugs were placed with a single predator in the petri dish and observations were recorded after 24 h. Number of replicates varied for different predators in the adult predation bioassay.

**Statistical Analysis.** For each test, data were subjected to ANOVA using the GLM procedure of SAS (SAS institute 2001). Data were log-transformed before ANOVA to stabilize the variance. Means were separated using Fisher protected LSD test. Mean nymphal predation with and without spittlemass was compared using the PROC t-test procedure of SAS (SAS Institute 2001).

Predation of *P. bicincta* eggs, nymphs and adults was analyzed using Poisson regression, the basic distribution to describe counts (Mc Cullagh and Nelder 1989). PROC GENMOD (SAS Institute 2001) was used to perform Poisson regression. Treatment comparisons between predators were done using Likelihood ratio test for Type III analysis. Likelihood ratio statistic has a chi-square distribution, with a single parameter, its degrees of freedom. Additionally, egg density trials were analysed using a repeated measures ANOVA with correlation structure where the subject (experimental unit) was replicates x 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.* Egg predation results indicated an increase in the number of eggs consumed as the initial number of eggs increased from 3 eggs to 10 eggs per arena (Table 2). There was a significant difference among predators in the number of eggs consumed when 3

eggs were offered ( $F = 5.68$ ;  $df = 24, 2$ ;  $P = 0.006$ ). Red imported fire ants consumed 100% of the eggs provided but they were not significantly different from *H. pennsylvanicus*. *G. punctipes* consumed the least number of eggs. A similar egg consumption pattern was obtained at 5 and 10 egg densities,  $F = 7.36$ ;  $df = 24, 2$ ;  $P = 0.001$  and  $F = 5.95$ ;  $df = 24, 2$ ;  $P = 0.004$  respectively (Table 2).

*Trial 2.* There was significant predation of *P. bicincta* eggs by additional predators used in the study ( $F = 10.92$ ;  $df = 29, 5$ ;  $P < 0.0001$ ). *S. invicta* always consumed 100% of spittlebug eggs provided to them, *S. invicta*, *C. sayi* and *H. pennsylvanicus* consumed significantly more eggs than *G. uliginosus*, *G. punctipes* and *M. carolina carolina*.

Treatment comparisons using Likelihood ratio statistics ( $\chi^2$ ) for Type 3 analysis demonstrated significant differences between predators ( $\chi^2 = 36.36$ ;  $df = 5$ ;  $p < 0.0001$ ). Most of the predators had a significant effect in the general linear model: *S. invicta* ( $\chi^2 = 155.42$ ;  $df = 1$ ;  $p < 0.0001$ ), *C. sayi* ( $\chi^2 = 153.22$ ;  $df = 1$ ;  $p < 0.0001$ ), *H. pennsylvanicus* ( $\chi^2 = 294.94$ ;  $df = 1$ ;  $p < 0.0001$ ), *G. uliginosus* ( $\chi^2 = 65.67$ ;  $df = 1$ ;  $p < 0.0001$ ), *G. punctipes* ( $\chi^2 = 40.48$ ;  $df = 1$ ;  $p < 0.0001$ ), with the exception *M. carolina carolina* ( $\chi^2 = 9.61$ ;  $df = 1$ ;  $p = 1.0$ ). There were no significant differences between bigeyed bug species, *G. uliginosus* and *G. punctipes*. Carabids were not different from each other in spittlebug egg consumption. All predators except *Geocoris* sp. consumed more eggs in predation bioassay than tiger beetles (Table 3).

*Trial 3.* Time ( $\chi^2 = 21.75$ ;  $df = 2$ ;  $p < 0.0001$ ), predator ( $\chi^2 = 7.62$ ;  $df = 2$ ;  $p < 0.0221$ ) and their interaction ( $\chi^2 = 12.04$ ;  $df = 2$ ;  $p < 0.01$ ) were significant in greenhouse egg predation trials. Data show that post 24 and 48 h, there was significant differences between *G. uliginosus*, *G. punctipes*, and *H. pennsylvanicus* ( $\chi^2 = 13.36$ ;  $df = 2$ ;  $p < 0.0013$ ) and ( $\chi^2 = 7.14$ ;  $df = 2$ ;  $p <$

0.028) respectively however, there was no significant difference in egg consumption post 72 h in the greenhouse bioassay ( $\chi^2 = 4.51$ ;  $df = 2$ ;  $p < 0.105$ ) (Fig. 1).

**Nymph Predation.** *Trial 1.* Newly hatched *P. bicincta* nymphs covered in spittlemass were rarely preyed upon in the laboratory challenges ( $F = 4.38$ ;  $df = 24, 1$ ;  $P = 0.04$ ). In some cases, nymphs moved out of their spittlemass and were preyed upon by *S. invicta*. *P. bicincta* nymphs were manually removed from the spittlemass, and the exposed nymphs were challenged with predators. The nymphs are relatively immobile in the spittlemass, but once they were removed from the spittlemass they moved about considerably. We noticed significant predation of exposed nymphs by *S. invicta* and *H. pennsylvanicus* ( $F = 9.33$ ;  $df = 24, 1$ ;  $P = 0.005$ ). Likelihood ratio analysis indicated significant differences between *S. invicta* and *H. pennsylvanicus* ( $\chi^2 = 5.78$ ;  $df = 1$ ;  $p < 0.01$ ). *S. invicta* ( $\chi^2 = 6.46$ ;  $df = 1$ ;  $p < 0.01$ ), and *H. pennsylvanicus* ( $\chi^2 = 13.49$ ;  $df = 1$ ;  $p < 0.0002$ ) had significant effects in the model. Control mortality was negligible. PROC t-test indicated significant differences in the mean number of nymphs with and without spittlemass eaten by both *S. invicta* and *H. pennsylvanicus*,  $t = -4.24$ ;  $df = 48$ ;  $P = 0.0001$  and  $t = -5.98$ ;  $df = 48$ ;  $P < 0.0001$  respectively.

*Trial 2.* Second or third instar *P. bicincta* nymphs protected by the spittlemass were rarely eaten by the various predators in the laboratory challenges ( $F = 3.90$ ;  $df = 24, 3$ ;  $P = 0.01$ ). There was significant mortality of exposed *P. bicincta* nymphs caused by predators ( $F = 8.04$ ;  $df = 24, 3$ ;  $P = 0.0001$ ). *S. invicta* and *M. carolina carolina* consumed similar numbers of exposed nymphs. *C. sayi* and *H. pennsylvanicus* were also able to kill exposed nymphs, although less frequently than *S. invicta* and *M. carolina carolina* (Fig. 2). No mortality was observed in the controls. There were differences between predators in *P. bicincta* nymph predation ( $\chi^2 = 10.12$ ;  $df = 3$ ;  $p < 0.017$ ). Tiger beetles ( $\chi^2 = 3364.5$ ;  $df = 1$ ;  $p < 0.0001$ ), and red imported fire ants ( $\chi^2 =$

12.84;  $df = 1$ ;  $p < 0.0003$ ) had significant effects in the model. Both carabids had no effect in the general linear model ( $\chi^2 = 0.00$ ;  $df = 1$ ;  $p < 1.0$ ).

Comparison of the mean nymphal predation with and without spittlemass were similar to results observed in the previous experiments. All predators killed significantly fewer nymphs in spittlemass than exposed nymphs: *S. invicta* ( $t = -10.72$ ;  $df = 46$ ;  $P < 0.0001$ ), *H. pennsylvanicus* ( $t = -5.30$ ;  $df = 48$ ;  $P < 0.0001$ ), *C. sayi* ( $t = -8.72$ ;  $df = 48$ ;  $P < 0.0001$ ) and *M. carolina carolina* ( $t = -\infty$ ;  $df = 38$ ;  $P < 0.0001$ ) respectively.

**Adult Predation. Trial 1.** When provided live, first generation adult spittlebugs, wolf spiders rejected them as food. *Lycosa* sp. consumed crickets that were given as food without any hesitation. Interestingly, wolf spiders began eating adult spittlebugs from August and continued to do so till the end of the season. Hence no statistical analysis of the data was performed. *S. invicta* demonstrated 100% predation of adult *P. bicincta*.

**Trial 2.** Significant mortality of *P. bicincta* adults was observed in laboratory challenges with generalist predators ( $F = 176.37$ ;  $df = 29, 4$ ;  $P < 0.0001$ ). *S. invicta* and *M. carolina carolina* were the most effective predators of adult twolined spittlebugs. Ground beetles, *H. pennsylvanicus* and *C. sayi* also consumed adults; however, *H. pennsylvanicus* ate significantly fewer adults than *C. sayi*. *Lycosa* sp. also preyed upon adult *P. bicincta* and none of the spiders rejected adult spittlebug as they did in the previous year's experiments (Fig 3). There was negligible mortality in the controls.

Likelihood ratio statistic shows a significant effect of different predators in the general linear model ( $\chi^2 = 112.52$ ;  $df = 4$ ;  $p < 0.0001$ ). *S. invicta* ( $\chi^2 = 184.49$ ;  $df = 1$ ;  $p < 0.0001$ ), and *M. carolina carolina* ( $\chi^2 = 146.06$ ;  $df = 1$ ;  $p < 0.0001$ ) produced a significant effect in the model. Wolf spiders also imparted an effect in the model ( $\chi^2 = 8.69$ ;  $df = 1$ ;  $p < 0.0032$ ). *H.*

*pennsylvanicus* and *C. sayi* had no significant effect in the model ( $\chi^2 = 4.85$ ;  $df = 1$ ;  $p < 0.1$ ,  $\chi^2 = 2.56$ ;  $df = 1$ ;  $p < 0.03$ ) respectively. Comparisons between predators of *P. bicincta* adults are shown in table 4. *S. invicta* and *M. carolina carolina* the most efficient predators were also similar in their effect in the general linear model ( $\chi^2 = 0.00$ ;  $df = 1$ ;  $p < 1.0$ ). *C. sayi* and *H. pennsylvanicus* were not different in adult *P. bicincta* predation (Table 4).

### 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 the twolined spittlebug: eggs, exposed nymphs and adults were demonstrated under controlled laboratory and greenhouse conditions. *P. bicincta* eggs were consumed by *G. punctipes*, *G. uliginosus*, *S. invicta*, *H. pennsylvanicus*, *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). Interestingly, *G. uliginosus*, a polyphagous predator in turf (Reinert 1978, Braman et al. 2003) did not cause significant reduction in egg numbers. *G. punctipes* seemed to consume considerable numbers of eggs than *G. uliginosus* but they were not significantly different, but both species were insignificant predators compared to *S. invicta*. One explanation for reduced consumption of eggs, could be that *G. uliginosus* is smaller in size compared to *G. punctipes* which, makes it difficult for the former to penetrate the tough chorion of the spittlebug eggs.

Our results confirm previous reports of the spittlemass providing protection against predators (Guilbeau 1908, Weaver and King 1954, Whitaker 1970, William and Ananthasubramaium. 1989). However, other functions such as osmoregulatory device,

protection against parasitoids and fungal pathogens may also play a role. Exposed *P. bicincta* nymphs challenged with predators were eaten almost immediately. Nymphs surrounded by spittlemass were left untouched; predators did not seem to recognize the presence of the nymphs in the spittlemass with the exception of red imported fire ants. *S. invicta* were aggressive towards nymphs in the spittlemass and in some cases forced the nymphs out of their spittlemass and preyed upon them. Henderson et al. (1990) documented interaction between prairie ants and meadow spittlebugs, *Philaenus spumarius* L. where, ants were predators of spittlebug nymphs and constructed aphid-tents using the spittle.

*P. bicincta* adults are brightly-colored, conspicuous insects with aposematic coloration. Peck (1998, 2000) demonstrated the ability of spittlebugs of genus *Prosapia* (Ceropidae) 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 (Wallace and Blum 1971, Benfield 1994, Blum and Sannasi 1974) or chemical components in the blood that are distasteful or noxious (Blum and Sannasi 1974, Carrel and Eisner 1974, Moore and Brown 1981). 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.

According to our study, common ground-dwelling arthropods, *S. invincta*, *M. carolina carolina*, *Lycosa* sp., and *C. sayi* were significant predators of adult *P. bicincta* in the laboratory. We did however, notice extensive grooming of mouthparts and antennae in predators when they came in contact with the hemolymph of the spittlebug. Additionally, the spittlebugs have the ability to jump high and fly to avoid predators; this 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, but they cannot completely avoid being captured and eaten in the field.

Our study identified potential predators of *P. bicincta* in turf. Additional study to define and enhance their impact is needed. Conservation of rich diversity of beneficial population is essential for sustainable management system in turf (Potter 1993). Insecticidal sprays of organophosphates, and carbamates have short-term adverse affects on beneficials including ants, spiders and parasitic hymenoptera (Braman and Pendley 1993a). It is imperative to choose selective insecticides that minimize adverse affects on the beneficial populations and other non-target invertebrates in order to promote the long-term stability of the turfgrass system (Kunkel et al. 2001).



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**Table 3.1. Natural enemy complex of *P. bicincta* and the life stages attacked by the respective predator in the laboratory.**

Predator	Prey life stages			
	n	Eggs	Nymphs	Adults
Geocoridae <i>G. uliginosus</i>	20	X	-	-
<i>G. punctipes</i>	45	X	-	-
Carabidae <i>H. pennsylvanicus</i>	60	X	X	X
<i>C. sayi</i>	20	X	X	X
Cicindelidae <i>M. carolina carolina</i>	20	X	X	X
Formicidae <i>S. invicta</i>	colony	X	X	X
Arachnida <i>Lycosa</i> sp.	30	-	-	X

**Table 3.2. Mean  $\pm$  SEM of *P. bicincta* eggs consumed by predators during 24 h in a laboratory bioassay**

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

Means  $\pm$  SE followed by the same letter are not significantly different, LSD<sub>0.05</sub>



**Table 3.3. Likelihood ratio statistics for comparisons between predators consuming *P. bicincta* eggs during 24 h in a laboratory bioassay**

Predator comparisons	Estimate ( $\pm$ SE)	$\chi^2$	<i>p</i>
<i>G. uliginosus</i> vs <i>G. punctipes</i>	0.89 (0.20)	0.34	0.5614
<i>G. uliginosus</i> vs <i>H. pennsylvanicus</i>	0.57 (0.14)	15.15	<0.0001
<i>G. uliginosus</i> vs <i>C. sayi</i>	0.54 (0.18)	8.49	0.0036
<i>G. uliginosus</i> vs <i>S. invicta</i>	0.50 (0.16)	18.02	<0.0001
<i>G. uliginosus</i> vs <i>M. carolina carolina</i>	0.78 (0.25)	0.88	0.3469
<i>G. punctipes</i> vs <i>H. pennsylvanicus</i>	0.64(0.18)	5.69	0.0171
<i>G. punctipes</i> vs <i>C. sayi</i>	0.61(0.20)	5.65	0.0175
<i>G. punctipes</i> vs <i>S. invicta</i>	0.56 (0.21)	7.27	<0.007
<i>G. punctipes</i> vs <i>M. carolina carolina</i>	1.42 (0.27)	1.62	0.2036

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<i>H. pennsylvanicus</i> vs <i>C. sayi</i>	0.95 (0.17)	0.00	0.9654
<i>H. pennsylvanicus</i> vs <i>S. invicta</i>	0.88 (0.14)	0.72	0.3952
<i>H. pennsylvanicus</i> vs <i>M. carolina carolina</i>	2.21(0.23)	11.02	0.0009
<i>C. sayi</i> vs <i>S. invicta</i>	0.92 (0.18)	0.46	0.4958
<i>C. sayi</i> vs <i>M. carolina carolina</i>	2.32 (0.26)	8.55	0.0035
<i>S. invicta</i> vs <i>M. carolina carolina</i>	2.49 (0.25)	13.43	0.0002

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Likelihood ratio parameters:  $df = 1; p < 0.0001$

**Table 3.4. Likelihood ratio statistics for comparisons between predators consuming *P. bicincta* adults during 24 h in a laboratory bioassay**

Predator comparisons	Estimate ( $\pm$ SE)	$\chi^2$	<i>p</i>
<i>H. pennsylvanicus</i> vs <i>Lycosa</i> spp.	0.37 (0.28)	11.69	0.0006
<i>H. pennsylvanicus</i> vs <i>S. invicta</i>	0.14 (0.26)	53.26	<0.0001
<i>H. pennsylvanicus</i> vs <i>M. carolina carolina</i>	0.14 (0.26)	51.23	<0.0001
<i>C. sayi</i> vs <i>H. pennsylvanicus</i>	0.88 (0.33)	7.39	0.0066
<i>C. sayi</i> vs <i>S. invicta</i>	0.34 (0.22)	23.54	<0.0001
<i>C. sayi</i> vs <i>M. carolina carolina</i>	0.35 (0.22)	22.29	<0.0001
<i>C. sayi</i> vs <i>Lycosa</i> sp.	0.88 (0.24)	0.26	0.6098
<i>S. invicta</i> vs <i>M. carolina carolina</i>	1.0 (0.15)	0.00	1.000
<i>S. invicta</i> vs <i>Lycosa</i> sp.	2.57 (0.18)	27.47	<0.0001
<i>M. carolina carolina</i> vs <i>Lycosa</i> sp.	1.20 (0.19)	0.18	<0.0001

Likelihood ratio parameters:  $df = 1$ ;  $p < 0.0001$

Fig. 3.1. Mean number of eggs consumed by predators during 72 h in a greenhouse bioassay

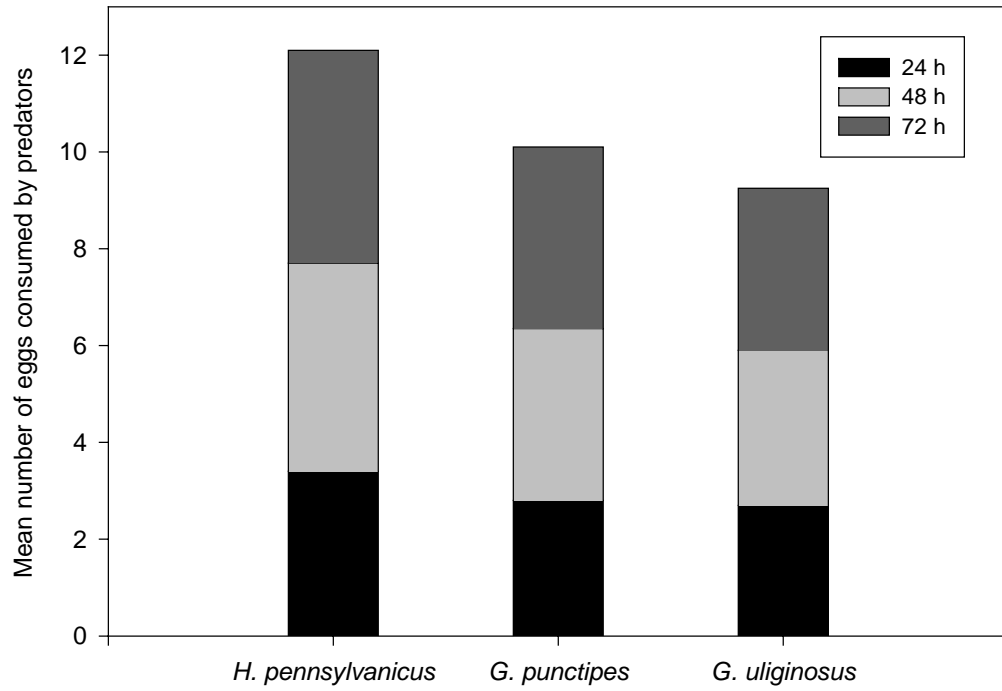


Fig.3.2. Mean number of *P. bicincta* nymphs with or without spittlemass killed by predatory insects during 24 h in the laboratory

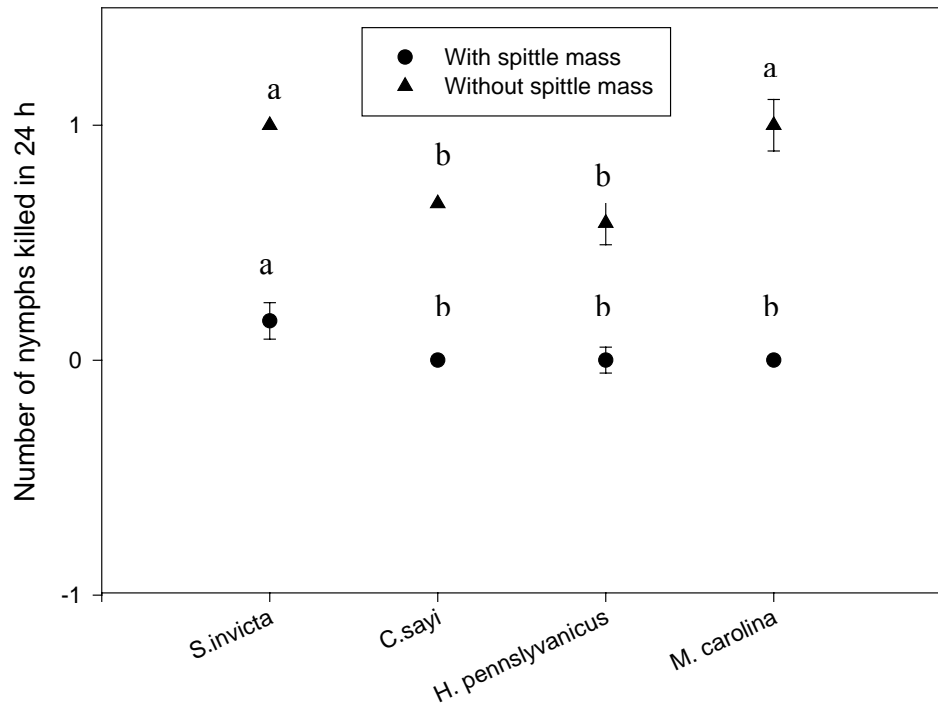
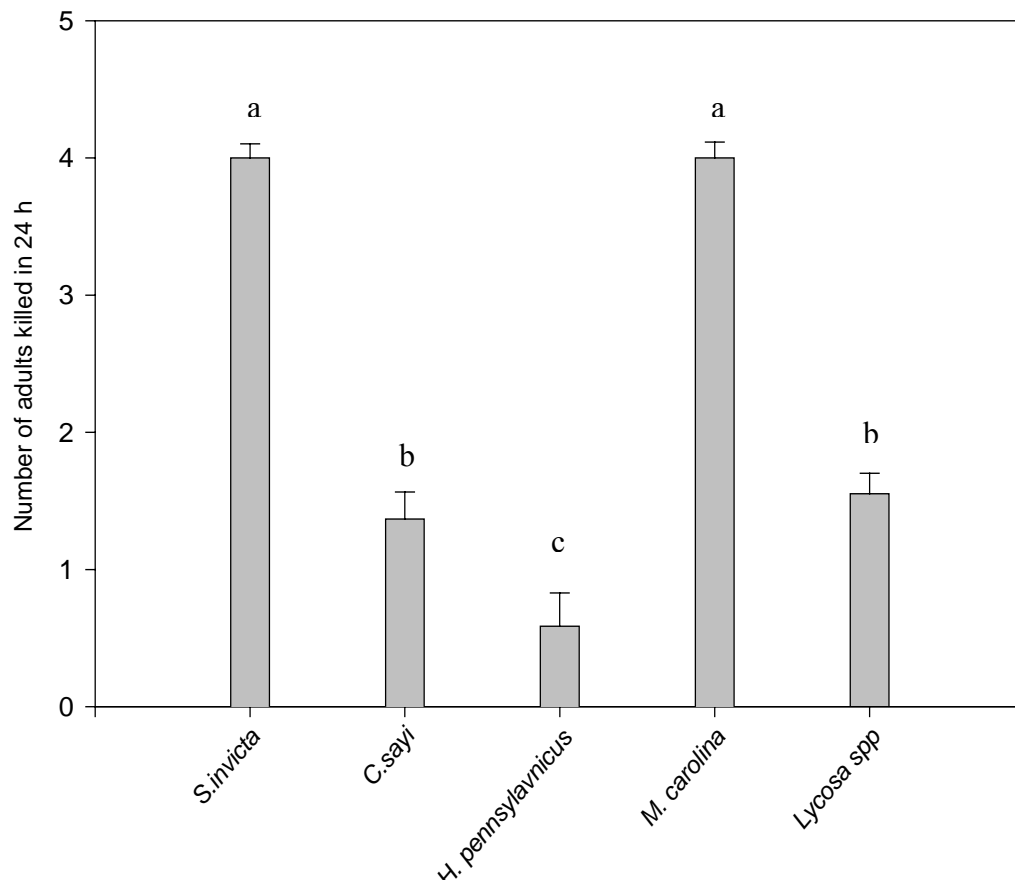


Fig. 3.3. Mean  $\pm$  SEM *P. bicincta* adults killed by entomophagous arthropods during 24 h





## CHAPTER 4

FUNCTIONAL RESPONSE OF THE TIGER BEETLE, *MEGACEPHALA CAROLINA*  
*CAROLINA* L., (COLEOPTERA: CICINDELIDAE) ON TWOLINED SPITTLEBUG  
(HEMIPTERA: CERCOPIDAE) AND FALL ARMYWORM (LEPIDOPTERA:  
NOCTUIDAE)<sup>2</sup>

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<sup>2</sup> Nachappa, P., S. K. Braman, L. P. Guillebeau, and J. N. All. 2004. To be submitted  
to Journal of Economic Entomology

**ABSTRACT** The functional response curves of the tiger beetle, *Megacephala carolina carolina* L. on various prey densities of adult twolined spittlebug, *Prosapia bicincta* (Say), and fourth instar fall armyworm larvae, *Spodoptera frugiperda* (J. E. Smith), in single-prey and two prey-systems were determined in the laboratory. In plastic cages, *M. carolina carolina* indicated a type II functional response curves for *P. bicincta* and *S. frugiperda* when both prey items were offered alone and together in the same arena. Of the total prey consumed, *M. carolina carolina* killed a significantly greater proportion of *S. frugiperda* than *P. bicincta* in the single-prey system and the two-prey system. *M. carolina carolina* killed 4.5 and 8.0 *P. bicincta* and *S. frugiperda*; and 2.0 *P. bicincta* and 5.0 *S. frugiperda* in the single-prey and two-prey system respectively. Estimates of attack coefficient,  $a$  was not significantly different for *P. bicincta* and *S. frugiperda* in the single-prey (0.07, 0.02) and two-prey system (0.04, 0.06) respectively. The handling time,  $T_h$  was significantly greater for *P. bicincta* (10.64 h) when both prey were offered together than *P. bicincta* alone (5.02 h). Estimations of attack coefficient and handling time in the single-prey system were used to predict predation rate of both prey items simultaneously. *M. carolina carolina* demonstrates no preference for either prey items.

**KEY WORDS** *Megacephala carolina carolina*, *Prosapia bicincta*, *Spodoptera frugiperda*, centipedegrass, biological contro

Managed landscapes have a rich diversity of plant materials and associated arthropod pests (Vittum et al. 1999, Potter and Braman 1991). Additionally, urban landscapes also have a wealth of beneficial arthropods including carabids, tiger beetles, staphylinids, mites, spiders and ants (Reinert 1978, Cockfield and Potter 1983,1984; Braman and Pendley 1993, Braman et al. 2000). These entomophagous invertebrates help limit pest outbreaks in urban landscapes (Reinert 1978, Cockfield and Potter 1984, Terry et al. 1993, Potter 1993).

*Prosapia bicincta* (Say), the twolined spittlebug is an injurious and widespread pest of turfgrasses and ornamentals in Southeastern United States (Beard 1973, Braman 1995, Vittum et al. 1999). Twolined spittlebug nymphs and adults are opportunistic xylem feeders and can feed on plants that provide fluids to meet its requirements (Pass and Reed 1965). Adult and nymphal feeding results in chlorosis, stippling, and phytotoxemia (Byers and Wells 1969, Taliafferro et al. 1969). There are few known natural enemies of twolined spittlebugs (Fagan and Kuitert 1969).

*Spodoptera frugiperda* (J.E. Smith), the fall armyworm is a sporadic pest of turfgrasses especially in southeastern United States and Canada (Vittum et al. 1999). The larva is the injurious stage, and has a wide variety of hosts, but preferred hosts are grasses. The larvae feed on all aboveground plant parts (Cobb 1995).

The tiger beetle, *Megacephala carolina carolina* L. is a nocturnal predatory insect found throughout southeastern United States (Graves and Pearson 1973, Pearson 1988). Tiger beetles have been captured in open areas, mud flats, lighted areas, shores of ponds, and woodland paths. Braman et al. (2002, 2003) reported *M. carolina carolina* collected in pitfall trap samples in turfgrasses and landscapes. Both larvae and adults prey on a broad range of insects, and are found between June and November. *M. carolina carolina* has been shown to be a promising

predator of *P. bicincta* adults (Nachappa et al. unpublished data). It is critical to evaluate predator response to varying prey densities in order to predict effectiveness as biological control agents in the field.

Functional response relates change in predation rates to changing prey density (Solomon 1949, Holling 1959). The type of functional response is significant as it determines the effectiveness of the natural enemies. By their shape Holling (1959) described three predator functional responses, Type I, Type II, and Type III. Type I, is linear, prey intake is proportional to prey density until satiation; Type II is a hyperbole, number of prey attacks per predator shows a negative accelerating rise to a plateau; Type III is sigmoidal, a lag rate due to learning is followed by an exponential increase in attack rate. In both Type II and Type III responses the plateau is thought to result from the joint effects of satiation and handling time. Most invertebrate predators demonstrate type II response also designated as the “invertebrate predator response”; the sigmoid curve is exhibited by both vertebrate and invertebrate predators (Riechert and Lockley 1984).

In our study, the functional response, handling time, and attack coefficient of *M. carolina carolina* on adult twolined spittlebug, *P. bicincta* and fourth instar fall armyworm, *S. frugiperda* were determined when prey items were offered alone (single-prey) or together (two-prey system) in the same arena.

## **Materials and Methods**

**Insect source.** Adult tiger beetles were collected from pitfall traps inserted in centipedegrass, (*Eremochloa ophiuroides* Munro.) Hack and fescue (*Festuca arundinaceae* Schreb) plots in Griffin, GA. Beetles were maintained in 110-cm petri dishes at room temperature, photoperiod of 14:10 (L:D) h, and fed varying prey items such as field crickets, *Gryllus rubens*

Scudder as food. Adult twolined spittlebugs were field collected from local residential areas and commercial landscapes from June to September around Griffin, GA. Spittlebugs were maintained using procedures described by Shortman et al. (2002). Adults were maintained on centipede grass in 800ml mason jars ventilated with 32-mesh screen. The jars were then placed in environmental chambers (Conviron, Manitoba, Canada) and maintained at 24°C, 75-80% RH, and a photoperiod of 15:9 (L: D) h.

Fourth instar fall armyworm larvae were obtained from a laboratory-reared colony. Fall armyworm eggs were obtained from USDA/ARS Crop protection and Management Research Unit in Tifton, GA. Neonate fall armyworms were maintained on commercial diet (Bioserve, Frenchtown, NJ) in 32-ml diet cups, and placed in environmental chambers (Percival Scientific, Perry, IA) at 24°C, 75-80% RH, and a photoperiod of 15:9 (L: D) h. One-wk old fall armyworms were used for experiments.

**Experimental design.** All experiments were conducted at room temperature and in 18.0 cm x 8.0 cm plastic cages (Pioneer Plastics, Dixon, KY), with 254.34 cm<sup>2</sup> total surface area. Centipedegrass (13.2g) was provided in the cage to simulate a more natural situation, and thus produce a functional response curve that is more field applicable. Tiger beetles were held without food for 4 d before placing in the cage with prey items. One, 3, 5, 7, and 11 adult twolined spittlebugs were placed in each cage with a single tiger beetle. Controls were maintained without the predator. After 24 h, the predator was removed from the cage and the number of live adult twolined spittlebug was determined. There were 10 replicates at all prey densities.

Laboratory reared fourth instar fall armyworms were also tested against tiger beetles using the same experimental design described above. The test was conducted using 1, 3, 5, 7, 9

11, and 15 fall armyworms in each cage with the predator. Controls were maintained without the predator. The experiment was repeated on two occasions with 10 replicates at all prey densities.

**Prey Preference.** To determine the predator functional response in the presence of two prey types, both twolined spittlebug adults and fourth instar fall armyworm larvae were placed with a single predator in the cage. Prey preference experiments were conducted under the same conditions as those described above. Tests were conducted using 1, 3, 5, 7 adult spittlebugs and similar numbers of fall armyworm in each cage, hence a total of 2, 6, 10, and 14 both of prey types. The experiment had 10 replicates at each prey density. The controls had no predator present.

**Statistical Analysis.** For each test, data were subjected to analysis of variance (ANOVA) using PROC General Linear Model (GLM) (SAS Institute 2001) to determine the number and proportion of prey killed at each density (Wells and McPherson 1999). The type of functional response was determined by performing a logistic regression of the proportion of prey killed related to their initial number present (Trexler et al. 1988). For predator functional response, it is relevant to use a logistic regression for distinguishing between type II and type III responses, which are not easily distinguished by non-linear regression that use number of prey eaten as the dependent variable (Trexler et al. 1988).

Data was fitted for both cubic and quadratic models using Maximum likelihood analysis of PROC CATMOD of SAS (SAS Institute 2001). However, both cubic and quadratic expressions were not significant; an exact fit was achieved with a linear model. The linear coefficient of the plot of the proportion of prey killed in relation to the initial prey density is negative for type II response and positive for type III response (Juliano 1993).

The type II functional response was modeled using the “random-predator” equation of Rogers (1972):

$$N_e = N \{1 - \exp [a (T_h N_e - T)]\}$$

$N_e$  = number of prey killed

$N$  = number of prey available (initial number of prey)

$a$  = attack coefficient

$T_h$  = handling time

$T$  = total time prey is exposed to the predator

The parameters of the functional response, attack coefficient,  $a$ , and, handling time,  $T_h$ , were calculated using least-squares non-linear regression, using Gauss-Newton estimate method as performed by PROC NLIN of SAS (SAS Institute 2001). Mean  $\pm$  SE of the attack and handling coefficient are presented.

The estimates of attack coefficient,  $a$ , and, handling time,  $T_h$  computed for individual prey can be used in a mathematical model to describe the predation rate when both prey items are presented simultaneously under the null hypothesis that there was no preference between them (Cock 1978). The model is

$$N_{ep} = N_p \{1 - \exp [a_p (T_{hp} N_{ep} - T)]\}$$

$$N_{es} = N_s \{1 - \exp [a_s (T_{hs} N_{es} - T)]\}$$

where the variables are same as previous equation except the subscripts  $p$  and  $s$  indicate  $P$ .

*bicincta* and *S. frugiperda* respectively. Dividing the equations gives

$$N_{ep} / N_{es} = 1 - \exp (a_p T_s) N_p / 1 - \exp (a_s T_s) N_s$$

$$= \gamma N_p / N_s$$

where  $T_s$ , the time available for searching is given by  $(T_{hp} N_{ep} - T) - (T_{hs} N_{es} - T)$



$\gamma$ , prey preference.

Prey preference is dependent on number of prey eaten, handling times and the total time available in addition to search efficiencies. The prey preference term ( $\gamma$ ) cannot be estimated directly from the equation, but the equation can be used to predict actual consumption of prey and hence the ratio of  $N_{ep} / N_{es}$  when both prey are present simultaneously (Cock 1978). Any preference shown by the predator for one species or the other will indicate a statistical difference between experimental results and the model predictions (Folgar et al. 1990)

## Results and Discussion

**Proportion of Prey Killed and Search Efficiency.** Results of this study confirm previous reports that tiger beetles are voracious predators of various living arthropods (Pearson 1988). To our knowledge this is the first published report of *M. carolina carolina*, functional response to turfgrass pests. Data for total prey consumed indicates that *M. carolina carolina* killed similar numbers of *S. frugiperda* fourth instar larvae *P. bicincta* adults in 24 h when offered alone (Fig. 1). When both prey items were offered simultaneously, *M. carolina carolina* consumed significantly more *S. frugiperda* than *P. bicincta* in 24 h (Fig.1). Comparisons of proportion of prey killed at each density show that *M. carolina carolina* caused significant mortality of both *S. frugiperda* and *P. bicincta* alone (Table 1). *M. carolina carolina* killed significantly more *S. frugiperda* than *P. bicincta* in 24 h when both prey were offered together with the exception of density one (Table 1). *M. carolina carolina* killed between 0.9 - 8.3 *S. frugiperda* fourth instar larvae with,  $\approx 8.5$  at the upper asymptote. *M. carolina carolina* killed between 0.85 - 4.65 *P. bicincta* adults with,  $\approx 5.0$  at the upper asymptote. Predators that demonstrate functional response curve with a high asymptotic value are considered to have a strong functional response, i.e. it kills more prey than it needs to complete development. Based

on our results, *M. carolina carolina* shows a much stronger functional response to *S. frugiperda* than *P. bicincta*. Control mortality was insignificant with 95% survival (n = 90) of *P. bicincta* and 100% (n = 110) survival of *S. frugiperda* larvae after 24 h when prey were offered alone without *M. carolina carolina*. There was 100% survival in controls, with both prey items together without the predator.

Search efficiency is measured by the proportion of prey killed at each density. Food is one of the limiting resources for tiger beetles; the rate of food intake affects size of larvae, adults that in turn affects fecundity (Pearson and Knisley 1985). Tiger beetles locate immobile or dead prey tactilely and moving prey visually. For both *S. frugiperda* and *P. bicincta* the proportion of prey killed decreased as initial prey density increased. In prey-poor habitats, tiger beetles spends most of their time searching for scarce prey items where as, at prey- rich habitats the search efficiency is nearly zero as the predator encounters prey easily (Pearson and Knisley 1985). Search efficiency declines at a certain point as the predator is capable of capturing and handling only a finite number of prey in a given amount of time (O'Neil 1997).

**Predator Functional Response.** The type of functional response is an important characteristic as it describes the effectiveness of predators (Riechert and Lockley 1984). Most insects exhibit a type II functional response under laboratory conditions that is similar to the results of our study. In the single-prey system, the linear coefficient  $\pm$  SE of the *P. bicincta* functional response is negative and significant ( $-0.03 \pm 0.01$ ;  $t = -3.61$ ;  $P > 0.0007$ ) indicating a Type II functional response. This shows that the proportion of *P. bicincta* adults eaten decreases and levels off with increasing prey density (Fig. 2). For *S. frugiperda*, the linear coefficient  $\pm$  SE of the functional response is also negative and significant ( $-0.02 \pm 0.01$ ;  $t = -3.85$ ;  $P > 0.0003$ ) or a type II functional response where, the number of prey eaten initially rises quickly as the prey

density increase but reaches a plateau with further increase in prey density (Fig. 2). The model fit the observed data similarly for both *P. bicincta* and *S. frugiperda* with a raw  $r^2$  of 0.93 and 0.91 respectively. Besides estimates of the linear coefficient, the shape of the curve of the proportion of prey eaten at each density is also indicative of the type of functional response (Wells and McPherson 1999, Trexler et al. 1988). For both *P. bicincta* and *S. frugiperda*, the slope is negative along all parts of the curve indicates a type II response.

When equal densities of both prey items, *P. bicincta* and *S. frugiperda* were offered together in the same arena with *M. carolina carolina* the functional response could be approximated to a type II curve (Fig. 3). The estimates of the linear coefficient  $\pm$  SE are negative and significant,  $-0.05 \pm 0.02$ ;  $t = -2.15$ ;  $P = 0.03$  and  $-0.04 \pm 0.01$ ;  $t = -3.68$ ;  $P > 0.0007$  for both *P. bicincta* and *S. frugiperda* respectively. Data for total prey consumed also demonstrates a negative linear coefficient, but with the standard error that includes a zero ( $-0.02 \pm 0.00$ ;  $t = -3.33$ ;  $P = 0.002$ ). Results of both *P. bicincta* and *S. frugiperda* when placed together in the arena with the predator indicate that the predation rate decreases as the predator satiation reaches an upper limit of food consumption. This model fits the data reasonably well for *P. bicincta* type II response when both prey items were offered together in the same arena, with raw  $r^2$  of 0.71. The model fit the data for *S. frugiperda* much better with a raw  $r^2$  of 0.91. The slope of the curve for *P. bicincta* and *S. frugiperda* together and total prey consumed is negative which further supports the conclusion that *M. carolina carolina* exhibits a type II functional response in the presence of two prey items.

**Parameters of the Functional Response.** Functional response parameters attack coefficients ( $a$ ) and handling time ( $T_h$ ) are important to determine the special patterns and strategies of predators important to biological control programs. The estimates of attack

coefficients for were not significantly different for *P. bicincta* (0.07) and *S. frugiperda* (0.02) when presented alone (since the confidence intervals include zero) (Juliano 1993). This difference in attack rate could be a plausible explanation for the difference in the saturation of the functional responses for both prey types. Attack estimates obtained for *P. bicincta* and *S. frugiperda* in the two-prey system was dissimilar to the results of the single-prey systems (0.04 and 0.06, respectively).

Handling time is a combined effect of capture and consumption of prey and it varies according to the prey type (Faria et al. 2004). Some prey are easily found and captured, which is directly correlated to decrease in time and energy spent by the predator (De Clercq et al. 1998). The handling time for *P. bicincta* was 5.02 h and *S. frugiperda* 2.66 h in a single-prey system and for both *P. bicincta* and *S. frugiperda* together (10.64 and 4.41 h, respectively). It is of interest to note that handling time for *P. bicincta* is significantly greater (confidence intervals do not overlap) than *S. frugiperda* for both single-prey and two-prey systems. It has been shown that handling time is proportional to the size of the prey, the larger the prey longer the time taken to eat it (Flinn et al. 1985). Spittlebugs might represent a poor prey compared to fall armyworm as the former weighed significantly less than fall armyworms. The average weight of 10 spittlebugs was 0.33g compared to 2.07g for same number of fall armyworm. The variability observed in the handling time indicates a reverse result, which, could be due to a defense or escape response of the prey (Pastorok 1981). An increased handling time for *P. bicincta* could be associated with the defense response exhibited by spittlebugs. *P. bicincta* adults can jump away from the grasp of the predator and evade the predator where as fall armyworms are relatively immobile and are easily captured. *P. bicincta* also have the ability to reflex bleed which may cause mechanical deterrancy to the feeding by the tiger beetles. Additionally, *M.*

*carolina carolina* removes *P. bicincta* wings before consuming the insect, but fall armyworm are readily bit into and regurgitated. Hence, the time and energy spent in capturing fall armyworm is less than capturing spittlebugs.

**Prey preference.** Figures 4 and 5 show results of the observed and estimated number of prey eaten under the hypothesis that there is no preference between *P. bicincta* and *S. frugiperda*. Results strongly suggest change in the searching behaviour of *M. carolina carolina* when both prey types are present; the number of prey killed is more than the estimated for both species. However, there was no significant difference between estimated and observed number of prey killed for *S. frugiperda* ( $\chi^2 = 0.87$ ;  $df = 1$ ;  $p \Rightarrow 0.99$ ) and *P. bicincta* ( $\chi^2 = 0.89$ ;  $df = 1$ ;  $p > 0.99$ ). Switching of prey takes place when the proportion of one prey species killed changes (less to more) with increase in the proportion of that prey species (Flinn et al. 1985). The observed increase in the proportion of fall armyworms killed was not significantly different (Fig. 5). Differences in consumption of the two turfgrass pests provide information concerning its capacity to control these two pests in their common habitat. Another explanation for the different saturation responses of both prey may be attributed to the differential handling time (Faria et al. 2004). The functional response for *P. bicincta* seems to exhibit a plateau in terms of saturation as the predator spent more time consuming *P. bicincta* than *S. frugiperda*.

Results of our study indicate that *M. carolina carolina* demonstrates potential as a natural control agent of pests in landscapes and turfgrass. Biological control of turfgrass pests is appealing to management professionals and their clients because of the apparent ecological and environmental benignness of this approach. There are reports of classical, augmentation and conservation biological control being attempted in landscape settings with varying degrees of success (Raupp et al. 1992). Generalist predators have significantly reduced pest populations in

several biological control programs. The polyphagous nature of the generalists is critical for success of the biological control program in the field (Symondson et al. 2002). Tiger beetles are generalist predators that feed on a variety of prey allowing them to survive when the target prey is scarce which is a positive attribute for a biological control agent. Previous reports have shown that tiger beetles reduce insect populations in many agro ecosystems (Sastry and Appanna 1958). *M. virginica*, a tiger beetle common in southeastern United states has been shown to be a predator of mole crickets, (*Scapteriscus*) in turf and pasture (Pearson 1988). However, the indirect effects of tiger beetles on non-target arthropods, beneficials (Frick 1957) and, cannibalism among species cannot be overlooked (personal observation).

Our study was conducted in laboratory bioassays with single-prey and two-prey systems which did not allow for predictions about the effectiveness of *M. carolina carolina* for augmentative biocontrol in a realistic habitat. Field studies that incorporate variables such as host-plant resources, multiple prey, cannibalism, are necessary to further determine potential of *M. carolina carolina* in the field. However, artificial arenas are appropriate for simple comparisons of the physiological capacities of the predator to various preys. *M. carolina carolina* is a valuable ubiquitous predator and should be a target of conservation efforts. It is also possible that *M. carolina carolina* might already cause significant reduction in pest numbers before any enhancement measures have been implemented.

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**Table 4.1. Proportion of *P. bicincta* adults and *S. frugiperda* fourth instar larvae killed by *M. carolina carolina* in 24 h when prey was offered alone and together.**

Prey density	<i>P. bicincta</i>				<i>S. frugiperda</i>			
	n	Single-prey	n	Two-prey	n	Single-prey	n	Two-prey
1	10	80.0 ± 0.10ab	10	60.0 ± 0.16b	10	90.0 ± 0.10ab	10	100.0 ± 0.00a
<i>F</i> = 1.8; <i>df</i> = 9,3; <i>P</i> = 0.17								
3	10	73.3 ± 0.09ab	9	50.5 ± 0.09b	9	92.5 ± 0.07a	9	92.5 ± 0.04a
<i>F</i> = 6.74; <i>df</i> = 9,3; <i>P</i> = 0.001								
5	10	91.0 ± 0.03a	10	48.0 ± 0.11b	10	84.0 ± 0.04a	10	80.0 ± 0.07a
<i>F</i> = 8.60; <i>df</i> = 9,3; <i>P</i> = 0.004								
7	10	66.4 ± 0.04a	10	27.1 ± 0.27b	10	75.7 ± 0.04a	10	71.4 ± 0.07a
<i>F</i> = 16.16; <i>df</i> = 9,3; <i>P</i> = <0.001								
9	10	56.7 ± 0.11			10			
11	10	40.4 ± 0.03b			10	70.0 ± 0.07a		
<i>F</i> = 16.73; <i>df</i> = 9,3; <i>P</i> = 0.002								
15					10	53.0 ± 0.03		

**Table 4.2. Attack coefficients and handling time of *M. carolina carolina* when challenged with *P. bicincta* adults and *S. frugiperda* fourth instar larvae alone and together per arena.**

Prey type	n	Attack		Handling time	
		coefficient $\pm$ SE	95% CI	coefficient $\pm$ SE	95% CI
<i>P. bicincta</i> alone	50	0.07 $\pm$ 0.03	0.007-0.14	5.02 $\pm$ 0.29	4.42-5.62
<i>S. frugiperda</i> alone	69	0.02 $\pm$ 0.006	0.009-0.03	2.66 $\pm$ 0.22	2.21-3.11
<i>P. bicincta</i> with <i>S. frugiperda</i>	39	0.04 $\pm$ 0.03	-0.033-0.11	10.64 $\pm$ 1.74	7.10-14.19
<i>S. frugiperda</i> with <i>P. bicincta</i>	39	0.06 $\pm$ 0.03	-0.01-0.14	4.41 $\pm$ 0.53	3.32-5.49

Fig. 4.1. Comparison of the total prey killed by *M. carolina carolina* during 24 h in experimental arena

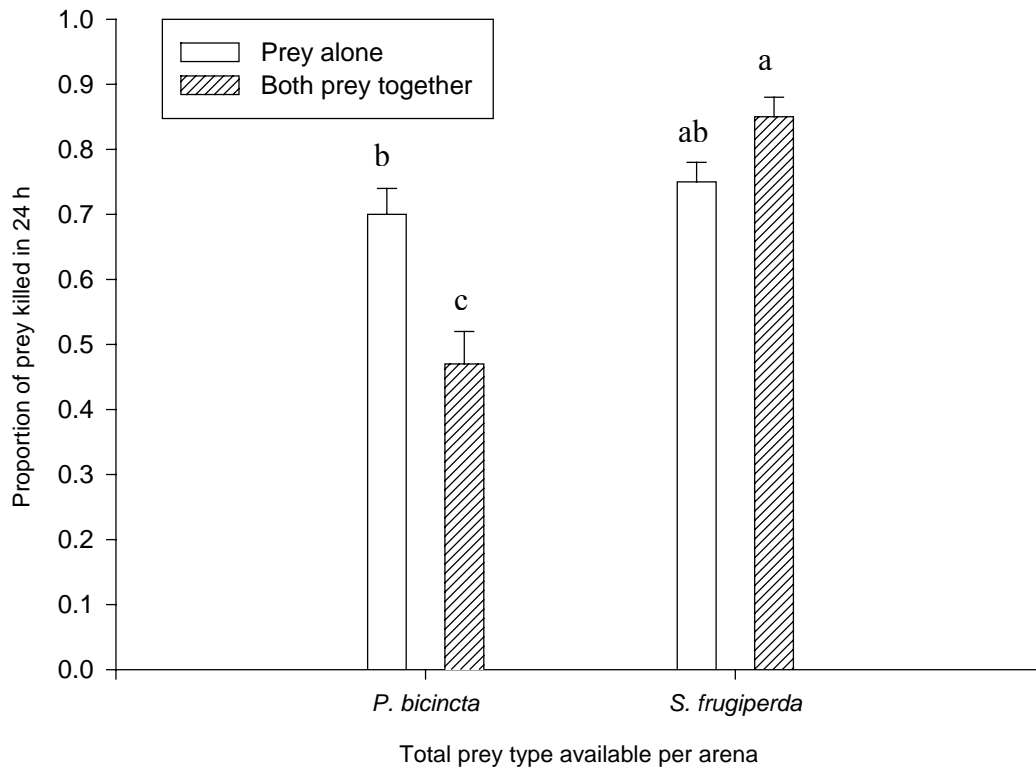


Fig. 4.2. Functional response of *M. carolina carolina* to *P. bicincta* and *S. frugiperda* when prey items were offered alone



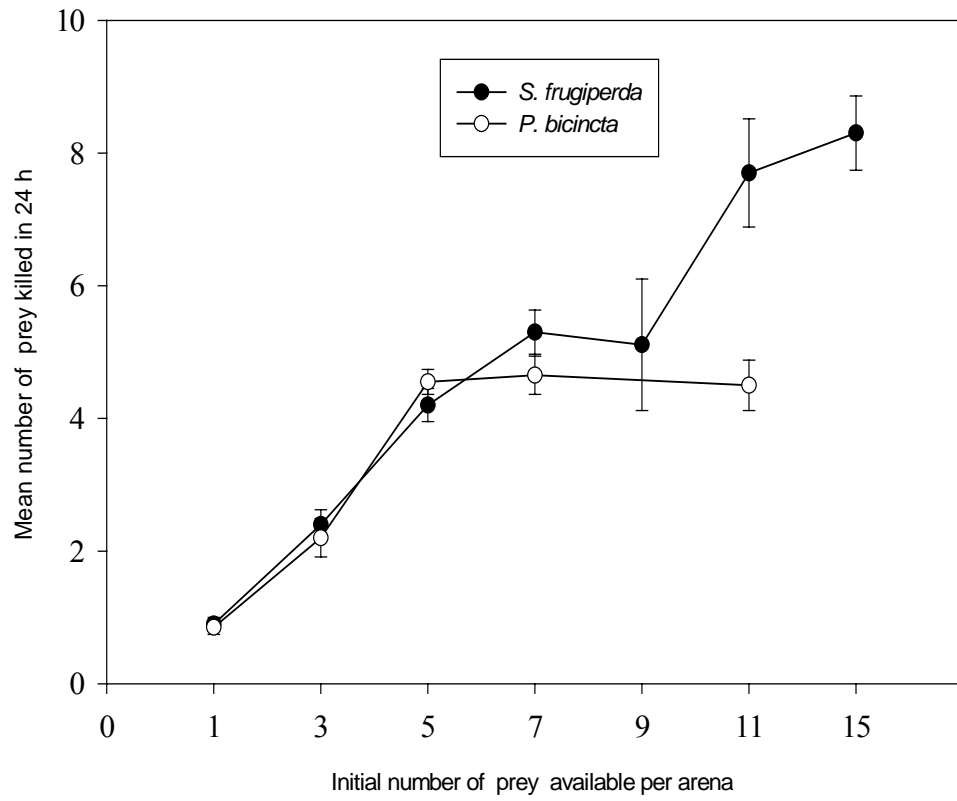


Fig. 4.3. Proportion of to *P. bicincta* and *S. frugiperda* consumed by *M. carolina carolina* when prey items were offered together in the same arena

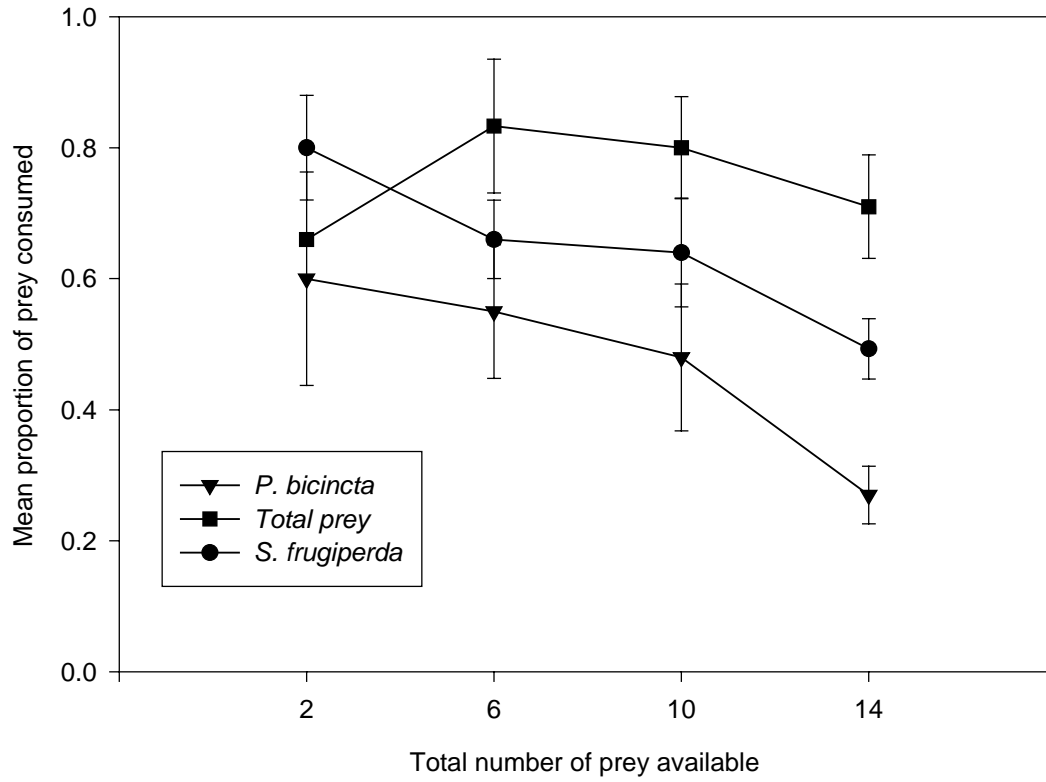


Fig. 4.4. Preference of *M. carolina carolina* for *P. bicincta* when offered simultaneously with *S. frugiperda* in the same arena. The estimated values indicate null hypothesis, no preference for either prey.

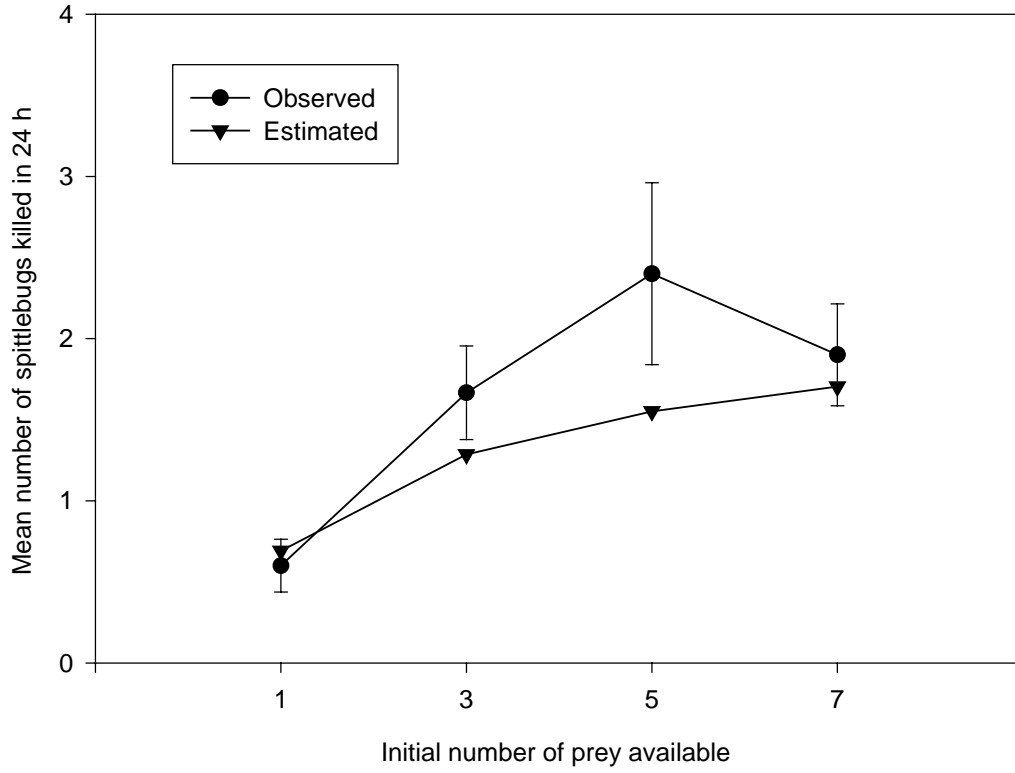
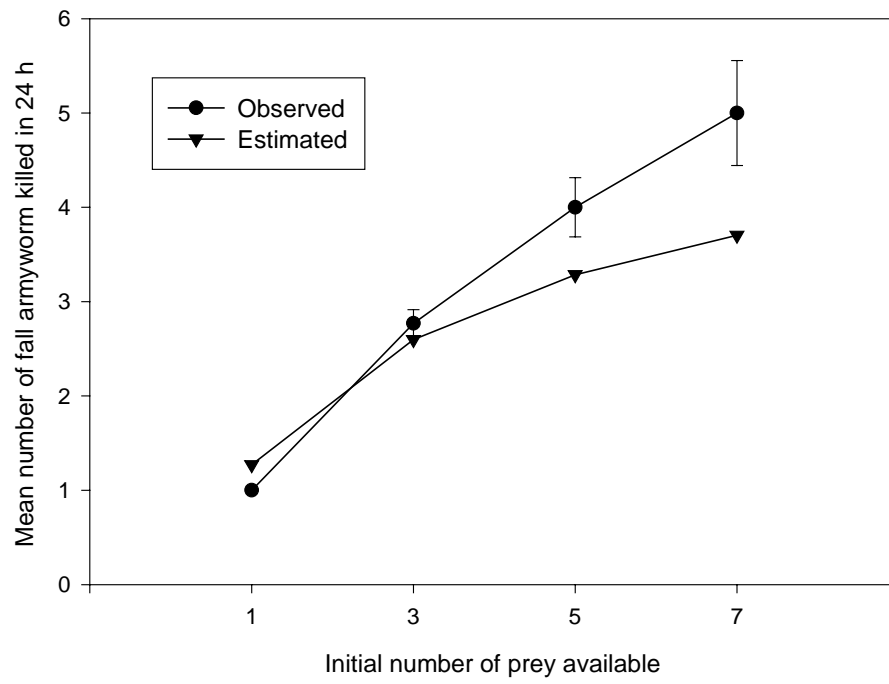


Fig. 4.5. Preference of *M. carolina carolina* for *S. frugiperda* when offered simultaneously with *P. bicincta* in the same arena. The estimated values indicate null hypothesis, no preference for either prey.



CHAPTER 5  
EVALUATION OF LOW-RISK INSECTICIDES ON TWOLINED SPITTLEBUG,  
*PROSAPIA BICINCTA* (SAY) (HEMIPTERA: CERCOPIDAE)  
IN TURF<sup>3</sup>

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<sup>3</sup> Nachappa, P., L. P. Guillebeau, J. N. All, and S. K. Braman. 2004. To be submitted to  
Journal of Entomological Science



**ABSTRACT** The effect of low-risk selective insecticides against nymphs and adults of twolined spittlebug, *Prosapia bicincta* (Say) was evaluated in the field. The two experiments, a summer trial targeting adults and a fall trial, against nymphs were conducted at different locations, but the experimental design, insecticidal treatments and, application methods were similar. Treatment plots were sprayed with neem oil, safer soaps, *Beauveria bassiana*, carbaryl, cyfluthrin, bifenthrin, acephate, imidacloprid, fipronil (replaced with dinotefuran in fall trial) or water (untreated check). Imidacloprid, fipronil and acephate provided 100% control of adults. Both synthetic pyrethroids were moderate in suppressing adult spittlebugs. Adult damage epidemiology on turfgrass was also assessed in the summer trial. Plots treated with imidacloprid, fipronil and acephate showed least damage symptomology, followed by plots sprayed with bifenthrin and cyfluthrin. Neem oil, safer soaps and, *B. bassiana* were ineffective in reducing adult spittlebug numbers or damage symptoms. Dinotefuran and acephate were the most efficient insecticides in controlling nymphs. However, imidacloprid, bifenthrin, and cyfluthrin were not as effective as they were in controlling adult spittlebugs. We also surveyed the non-target arthropod population using mini pitfall traps placed in the treatment plots in the fall trial. There were no significant differences in the non-target arthropod abundance among the treatment plots except for acephate and carbaryl treated plots.

**KEY WORDS** centipedegrass, St. Augustinegrass, beneficials, survival, homeowner

The spittlebug genus, *Prosapia* Fennah, occurs in North America, with most species found in Mexico and Central America (Hamilton 1977). *Prosapia bicincta* (Say), the twolined spittlebug is reported from Florida to Maine and as far west as Arkansas and Texas in the United States (Byers 1965). The twolined spittlebug is an injurious and wide spread pest of turf grasses and ornamentals in Southeastern United States (Vittum et al. 1999, Braman 1995). Nymphs and adults are xylem feeders and can feed on almost all plants that provide fluids to meet their requirements (Pass and Reed 1965). *P. bicincta* has a broad range of grass hosts, including centipedegrass, *Eremochloa ophiuroides* (Munro). Hack (Beard 1973, Braman 1995, Shortman et al 2002), coastal bermudagrass, a cultivar of *Cynodon dactylon* (L.) Pers (Byers and Wells 1966, Taliaferro et al. 1969), panolagrass, *Digitaria decumbens* Stent, and St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze (Genung et al. 1954). Seashore paspalum, *Paspalum vaginatum* Swartz, zoysiagrass (*Zoysia* Wild), and tall fescue *Festuca arundinaceae* Schreb (Shortman et al. 2002); as well as other graminaceous crops are also susceptible to damage. *P. bicincta* adults are also known to cause damage on ornamental hollies, *Ilex opaca* L. and *I. cornuta burfordii* De France (Pass and Reed 1965, Braman and Ruter 1997).

*P. bicincta* adult feeding results in phytotoxemia or “froghopper burn” (Byers and Wells 1966, Taliaferro et al. 1969). A plant growth promoter in the salivary gland of the spittlebug is responsible for the damage (Cutler and Stimmann 1971); however the identity of this substance remains unknown. The toxin is injected into the xylem as the insects feed, and damage begins within 24 h. Damage symptoms include stippling, streaking, browning and necrosis of the host. Recovery from spittlebug damage can occur if the plant is not severely damaged, but the recovery time is much longer than would be expected, indicating a residual effect of the

phytotoxin (Meyer1993; Meyer and Root 1993; Meyer and Whitlow 1992; Karban and Strauss 1993).

Attempted control of *P. bicincta* has included mechanical and chemical measures. Mowing lawn grasses is recommended as a mechanical control option for landscape industry. Previous studies have reported non-significant chemical control on second- generation nymphs using granular formulations of zinophos 10%, diazinon 5%, phorate 10%, and endosulfan 4% (Beck1963). Pass and Reed (1965) reported foliar sprays of guthion, endosulfan, endrin and DDT as most effective insecticides against nymphs. Guthion, malathion, meviphos, endosulfan and carbaryl were 100% effective as foliage sprays in controlling adults. Current chemical control for spittlebugs includes spraying with acephate for landscapes and golf courses and various synthetic pyrethroids for homeowners (Hudson 2004). As a result of federal mandated EPA (Environmental Protection Agency) review, acephate is no longer available for homeowner use on turf except for red imported fire ants (US EPA 1998).

Turfgrass habitat is a dynamic system with an abundance of beneficial populations such as carabids, staphylinids, mites, spiders and ants (Cockfield and Potter 1983, 1984, Braman and Pendley 1993). These predators are known to buffer pest outbreaks in lawns, golf course and urban landscapes (Reinert 1978, Potter 1993, Terry et al. 1993). Turfgrasses are pesticide and fertilizer intensive (Hull et al. 1994). As the demand for high aesthetic standards increased so do the pesticides used in turf pest management (Racke and Leslie 1993). Broad-spectrum insecticides, organophosphates and carbamates that are effective for reducing pest populations will also kill indigenous, predatory arthropods on turfgrass (Cockfield and Potter 1983, Potter 1993). It is therefore imperative to choose selective insecticides that minimize adverse affects on

the beneficial populations and other non-target invertebrates for the long-term stability of turfgrass system (Kunkel et al.2001).

We evaluated effects of low-risk selective insecticide applications on *P. bicincta* adults and nymphs. Damage assessment caused by the first generation adults after insecticide application was also evaluated as was the non-target arthropod abundance in the treatment plots.

### **Materials and Methods**

**Experimental sites.** *Summer Trial:* The study site was an established stand of centipede grass, *Eremochloa ophiuroides* (Munro). Hack and St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze at a residential lawn in Moultrie, Georgia. This site was chosen because of the high densities (>35 adults/13 m<sup>2</sup>) of adult spittlebugs in the area. The turf was fertilized 24-4-11 (N-P-K). The grass was mowed weekly to 5-cm height, and clippings were bagged and discarded.

The size of the treatment plot were 3.6 m<sup>2</sup> with 0.9 m untreated alleys. Treatments were arranged in a modified randomized complete block design with 5 replicates of each treatment including the untreated check. Treatments included wettable granules or liquid formulations of acephate (Orthene 75 S [soluble]), carbaryl (Sevin 50 WP [wetable powder]), cyfluthrin (Tempo 2E [emulsifiable]), bifenthrin (Talstar one LC [liquid concentrarte]), imidacoprid (Merit 75 WP [wetable powder]), and fipronil (Regent [granules]). In addition, formulations included neem oil extract 1.2 % (aza-direct), insecticidal soaps 49% (M-pede), and *Beauveria bassiana* (Botanigard ES [emulsifiable suspension]). The applications rates for each formulation is indicated in table 1. All formulations were applied using a solo backpack sprayer model 475 (Solo Incorporated, Newport News, VA), equipped with a fan tip nozzle that delivered at a spray pressure of 60 psi. All formulations were applied at a spray volume of 19 liter/90m<sup>2</sup>

(5gal/1000ft<sup>2</sup>). Separate spray bottles were used for each treatment, and the sprayer was thoroughly rinsed between insecticide sprays.

The plots were treated on 30 June 2004, followed by irrigation with lawn sprinklers. Efficacy of the treatments on the spittlebug population was determined 3, 7 and 21 d post-treatment (3 July, 7 July 20 July). On each date, 25 sweep-net samples were taken from each treatment plot to sample for live adult spittlebugs and beneficial arthropods. Leaf damage estimates were taken for each plot. Damage assessment was also recorded for each treatment plot on 3, 7 and 21 days. Leaf-damage assessment was recorded, using a linear scale from 0-10, with 0 being no damage and 10 being worse damage. Damage epidemiology included chlorosis, streaking, and necrosis of grass. Nymphal count was determined 21 d post-treatment, by placing a 1m x 1m, grid, divided into 9 subsections, randomly in each plot and counting the number of nymphs in each subsection.

*Fall Trial.* The test site was situated at a homeowner's lawn in Griffin, GA with an established stand of centipede grass, *Eremochloa ophiuroides* (Munro) Hack. The homeowners mowed the grass weekly to 4-5-cm height and the clippings were discarded. The experimental design was a completely randomized block design with 5 replicates. Plot size was reduced to 1.5 m x 1.5 m, as the nymphs are relatively immobile and high densities of nymphs (>20 nymphs/20.25 m<sup>2</sup>) were recorded in pretreatment assessment. Treatments were same as the summer trial, except for the addition of dinotefuran (safari 20% SG) and omission of fipronil. Insecticidal formulations, rates of application are indicated in table 3. Insecticide application method was same as the method followed in the summer trial. The plots in the fall trial were treated on 9 August 2004. Nymphal counts were recorded on 2, 5 and 10 d post-treatment by placing a 1m x 1m, grid, divided into 9 subsections, in the center of each plot and counting the

number of nymphs in only 6 subsections due to the high density of nymphs in the plots.

Observations were recorded on different days compared to adult survival because the nymphs were third and fourth instars and would emerge as adults within two weeks.

**Survey of on-site beneficial arthropods.** Non-target arthropod populations were sampled in the fall trial treatment plots using pit-fall traps similar to method used by Braman and Pendley (1993b). Pitfall traps were collected from 13 August 2004 to 23 August 2004. One 120-ml (6 cm diameter x 7 cm h) pitfall trap was placed in the center of each plot of the fall trial, a total of 50 mini pit-fall traps were setup in the entire plot area. The traps were emptied at the end of 10 d and, samples were taken back to the laboratory, sorted, counted and identified. The non-target arthropods were grouped into the class Arachnida, order Coleoptera and others.

**Statistical analysis.** For each test, data was subjected to analysis of variance (ANOVA) using PROC General Linear Procedure (GLM) procedure (SAS Institute 2001) to determine the efficacy of the insecticides on adults and nymphal survival. Damage assessments between treatments were also compared using SAS GLM procedure. Mean separation was accomplished using Fisher protected LSD test. Data concerning non-target invertebrate abundance as measured by pit-fall traps were subjected to ANOVA using PROC GLM of SAS (SAS Institute 2001). Separation of significant means were accomplished using Fisher protected LSD test. Mean  $\pm$  SE are presented for all tests.

## Results

**Effect on Adult Survival.** Based on the analysis of survival of adult *P. bicincta* post-insecticidal application, imidacloprid and fipronil were the most efficient and persistent of the insecticides, and caused significant reduction in spittlebug populations (Table 1). Although, cyfluthrin and bifenthrin are the recommended insecticides for controlling adult spittlebugs

(Hudson 2004), these were not as effective as the former insecticides in controlling adults. Neem oil, safer soaps, and *B. bassiana* provided moderate suppression of adults. The untreated check had significant survival of adult spittlebugs compared to all other treatment plots (Table 1). Acephate was consistent in suppressing spittlebugs from 3d to 21d, and was used as a treatment standard. Nymphal counts of early second-generation *P. bicincta* were low with a mean of 1.66 nymphs per treatment plot. There were no significant differences in the nymphal survival between treatments ( $F = 1.41$ ;  $df = 4, 9$ ;  $P = 0.22$ ).

**Damage Recovery.** Pretreatment damage assessments of turf were not uniform for all treatment plots, the untreated check, acephate, bifenthrin and cyfluthrin plots had significant spittlebug damage. The plots that were sprayed with imidacloprid and fipronil had less damage symptomology prior to spray application (Table 2). Three days following treatment, acephate, imidacloprid and, fipronil had significantly reduced spittlebug damage (Table 2). Plots sprayed with bifenthrin, cyfluthrin and carbaryl continued to have considerable spittlebug damage throughout the course of the experiment. The untreated check had significant damage symptoms compared to all other treatment plots throughout the test. Neem oil, safer soaps, and *B. bassiana* were not significantly different from untreated check plots with respect to spittlebug damage to the turf.

**Effects on Nymphal Survival.** *P. bicincta* nymphal survival was significant in the plot treated with safer soaps followed by cyfluthrin and bifenthrin (Table 3). Both cyfluthrin and bifenthrin were ineffective in controlling *P. bicincta* nymphs. Interestingly, imidacloprid did not reduce the number of spittlebug nymphs though it caused significant reduction in adult population in the summer trial (Table 1). Natural products such as neem oil, and *B. bassiana* offered moderate reduction in nymph numbers. Dinotefuran was most efficient in controlling spittlebug

nymphs (Table 3). Acephate was also consistent in reducing the nymphal population throughout the course of the study, and was considered a treatment standard.

**Effects on Non-Target Arthropods.** Pitfall samples yielded arthropods in the class arachnida represented by wolf spiders, *Lycosa* spp. Walcnaekar; order Coleoptera was represented by *Harpalus pennsylvanicus* DeGeer, *Scarites subterraneus* F., *Calsoma sayi* Dejean, *Aopcellus sphaericollis* (Say), *Sphenophorus venatus vestitus* Chittenden, *Cyclocephala lurida* Bland and, an unidentified species. All other arthropods including earthworms, centipedes and grasshoppers were grouped as “others”. There was no significant difference in non-target predatory spiders, Coleoptera, and other arthropod abundance (Table. 4). The untreated check had significantly more ground-dwelling beetles than acephate and carbaryl treated plots.

### Discussion

This study indicates that imidacloprid and fipronil were the most effective insecticides for adult *P. bicincta* control. In contrast, the recommended insecticides for *P. bicincta* control, cyfluthrin and bifenthrin demonstrated significant control 3 d post treatment, but was not consistent in reducing spittlebug 7 d and 21 d post-treatment. Neem oil, safer soaps, and *B. bassiana* were included in the trial for the control of spittlebug nymphs in particular, however, our data did not show any significant control of nymphs or adults. Though currently phased out by EPA for homeowners, acephate showed effective suppression of adult numbers throughout the duration of the study.

Data for pre-treatment damage assessment, showed non-uniform damage among plots. It is assumed that plots with high damage, > 8 on a scale of 10 had high densities of adult spittlebugs. Plots that were sprayed with imidacloprid and fipronil had less damage, compared to cyfluthrin and bifenthrin. One could argue about the efficiency of imidacloprid and fipronil in



controlling adults compared to other treatments. However, treatment plots sprayed with imidacloprid and fipronil saw consistent reduction in damage epidemiology from 3d to 21 d post-treatment. Neem oil, safer soaps, and *B. bassiana* were not significantly different from untreated check with respect to adult *P. bicincta* damage. The results from damage assessment in treatment plots correspond with adult survival rates in the respective plots.

Interestingly, the data from nymph trial was not strictly comparable to results of adult survival in the summer trial. Imidacloprid was not as effective in controlling nymphs compared to control observed on adult *P. bicincta* in the summer trial. Ideally, imidacloprid should have reduced nymphal densities as it is registered for controlling other ground-dwelling scarabaeid grubs, curculionid larvae, and mole crickets (Kunkel et al. 2001). One explanation for the difference in the results could be that imidacloprid was not homogenized in the spray solution in the fall trial. A simple jar-test was conducted in the laboratory to determine the solubility of imidacloprid and we noticed that the product dissolved quickly but settled at the base of the jar. The wettable powder formulation does not dissolve readily in water and requires agitation of the spray solution. We believe that the product was not uniformly distributed in the spray solution because of poor agitation of the backpack sprayer and this caused the reduced performance of the product in the fall trial. This is an important point of consideration for homeowners that commonly use backpack sprayers for pest control. Dinotefuran was used as a replacement for fipronil, it offered almost 100% control of *P. bicincta* nymphs. Dinotefuran is a new product from Valent and is not yet registered for turf. Based on our study dinotefuran seems to be a promising insecticide against spittlebug nymphs. Acephate was consistent in reducing nymph densities, although it is not registered for homeowner's lawns. The pyrethroids, bifenthrin and cyfluthrin did not offer significant suppression of nymphal population as well. A possible explanation for

inefficiency of the pyrethroids in suppressing *P. bicincta* nymphs could be that these chemicals have contact mode of action (US EPA 1987, 1988) and it may be difficult to penetrate the spittlemass surrounding the nymphs. Therefore, for effective control of spittlebugs, adults are to be targeted as they are easily exposed to the insecticides than nymphs.

It has been shown that spiders, ants, ground beetles and tiger beetles are predators on different life stages of the twolined spittlebugs (Nachappa et al. unpublished data). The insecticides used in our study had low invertebrate toxicity. Pitfall trap samples did not indicate any significant differences among treatments in non-target arthropod abundance confirming the low toxicity of the treatment insecticides. In conclusion, this study indicates that summer application of imidacloprid, bifenthrin and cyfluthrin may suppress adult spittlebug population. The nymphal stage of spittlebugs are found at the base of the plant and, is additionally protected by the spittlemass, which makes it difficult for contact insecticides to penetrate the spittlemass and kill the nymphs. Hence, adults are more susceptible to insecticidal application than nymphal stage. Dinotefuran (if it secures a label for turf) and, acephate could be sprayed for effective control of spittlebug nymphs. The insecticides used in the study did not affect non-target arthropod survival, including beneficials in turfgrass. Future research on compatibility of pesticides with beneficial arthropods is essential for sustainable integrated pest management programs in turf.

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**Table 5.1. Mean  $\pm$  SE number of adult twolined spittlebug survival post-insecticide application in summer trial (2004)**

Treatment	Formulation	Rate Kg (AI)/ha	Mean $\pm$ SE		
			3 DAT	7 DAT	21 DAT
Untreated check	-		34.4 $\pm$ 1.69a	22.4 $\pm$ 3.80a	12.4 $\pm$ 0.96a
Neem oil	1.2%	3.04	19.4 $\pm$ 3.28b	11.0 $\pm$ 3.06b	6.2 $\pm$ 1.49bcd
Insecticidal soaps	49%	7.60	27.8 $\pm$ 3.38ab	10.8 $\pm$ 2.03b	6.4 $\pm$ 1.16bcd
<i>B. bassiana</i>	ES	3.04	26.4 $\pm$ 6.29ab	11.4 $\pm$ 2.63b	7.8 $\pm$ 2.13b
Acephate	75 S	0.74	2.0 $\pm$ 0.70c	2.6 $\pm$ 0.93cd	2.4 $\pm$ 0.50d
Carbaryl	50 EC	9.14	5.2 $\pm$ 2.53c	8.2 $\pm$ 4.15bc	5.8 $\pm$ 0.20bcd
Cyfluthrin	2 E	0.5	8.4 $\pm$ 3.0c	6.0 $\pm$ 1.37bcd	6.8 $\pm$ 0.50bc
Bifenthrin	LC	3.04	6.60 $\pm$ 2.03c	7.0 $\pm$ 2.75bcd	6.8 $\pm$ 3.51bc
Imidacloprid	75 WP	0.61	3.8 $\pm$ 2.59c	1.4 $\pm$ 0.74d	2.8 $\pm$ 0.58cd
Fipronil	4 G	0.70	5.2 $\pm$ 1.24c	6.2 $\pm$ 2.47bcd	3.4 $\pm$ 1.20cd

Means  $\pm$  SE within a column followed by the same letter are not significantly different

( $P = 0.0001$ ). For 3 DAT,  $F = 15.45$ ;  $df = 9, 4$ ; For 7 DAT,  $F = 6.70$ ;

$df = 9, 4$ ; For 21 DAT,  $F = 4.07$ ;  $df = 9, 4$ .

**Table 5.2. Mean  $\pm$  SE estimate of twolined spittlebug damage on *Eremochloa* and *Stenotaphrum* based on a linear scale of 0-10 (0 = no damage, 10 = severe damage), pre and post-insecticidal application in summer trial (2004)**

Treatment	Mean $\pm$ SE			
	Pre-trt	3 DAT	7 DAT	21 DAT
Untreated check	7.24 $\pm$ 0.65ab	8.04 $\pm$ 0.53a	8.34 $\pm$ 0.15a	5.1 $\pm$ 1.47ab
Neem oil	4.82 $\pm$ 0.61cde	5.92 $\pm$ 0.32bc	5.96 $\pm$ 1.61bcd	5.9 $\pm$ 0.65a
Insecticidal soaps	5.94 $\pm$ 0.7bc	5.44 $\pm$ 0.5bcd	7.26 $\pm$ 0.41abc	4.1 $\pm$ 1.36ab
<i>B. bassiana</i>	5.78 $\pm$ 1.12bcd	6.72 $\pm$ 0.65ab	8.0 $\pm$ 0.32ab	5.32 $\pm$ 1.71a
Acephate	7.94 $\pm$ 0.27a	4.08 $\pm$ 0.36d	4.98 $\pm$ 0.46ef	4.26 $\pm$ 0.70ab
Carbaryl	7.26 $\pm$ 0.25ab	4.56 $\pm$ 0.36cd	4.28 $\pm$ 1.0d	5.10 $\pm$ 1.33ab
Cyfluthrin	7.08 $\pm$ 0.08ab	5.28 $\pm$ 0.60bcd	3.88 $\pm$ 0.97de	2.64 $\pm$ 0.57bcd
Bifenthrin	4.98 $\pm$ 0.56cde	6.22 $\pm$ 0.66b	5.74 $\pm$ 1.21dc	3.58 $\pm$ 1.08abc
Imidacloprid	3.80 $\pm$ 0.37e	4.08 $\pm$ 0.33d	1.14 $\pm$ 0.20f	1.44 $\pm$ 0.27cd
Fipronil	4.34 $\pm$ 0.46de	4.08 $\pm$ 0.48d	0.94 $\pm$ 0.05f	0.92 $\pm$ 0.26d

Means  $\pm$  SE within a column followed by the same letter are not significantly different ( $P = 0.0001$ ). For pre-treatment,  $F = 6.63$ ;  $df = 9, 4$ ;  
 3 DAT,  $F = 6.59$ ;  $df = 9, 4$ ; For 7 DAT,  $F = 12.29$ ;  $df = 9, 4$ ;  
 For 21 DAT,  $F = 3.85$ ;  $df = 9, 4$ .



**Table 5.3. Mean  $\pm$  SE number of twolined spittlebug nymph survival post-insecticide application in fall trial (2004)**

Treatment	Formulation	Rate Kg (AI)/ha	Mean $\pm$ SE		
			2 DAT	5 DAT	10 DAT
Untreated check	-		22.25 $\pm$ 7.44abc	16.5 $\pm$ 4.57abc	10.75 $\pm$ 1.49b
Dinotefuran	20% SG	4.2	1.25 $\pm$ 1.25e	0.25 $\pm$ 0.25c	0.0 $\pm$ 0.0d
Insecticidal soaps	49%	7.60	38.25 $\pm$ 11.71a	24.0 $\pm$ 6.89ab	21.25 $\pm$ 3.70a
<i>B. bassiana</i>	ES	3.04	12.75 $\pm$ 3.56cde	7.0 $\pm$ 1.73bc	3.5 $\pm$ 1.70bcd
Acephate	75 S	0.74	4.0 $\pm$ 1.47de	0.5 $\pm$ 0.50c	0.5 $\pm$ 0.47cd
Carbaryl	50 EC	9.14	19.75 $\pm$ 7.12cdb	14.75 $\pm$ 4.30abc	8.5 $\pm$ 3.84bc
Cyfluthrin	2 E	0.5	32.75 $\pm$ 7.28ab	28.75 $\pm$ 5.37a	22.0 $\pm$ 2.34a
Bifenthrin	LC	3.04	31.0 $\pm$ 10.27ab	25.0 $\pm$ 8.89ab	19.25 $\pm$ 4.26a
Imidacloprid	75 WP	0.61	28.5 $\pm$ 3.06abc	16.0 $\pm$ 3.67bc	9.75 $\pm$ 2.28b
Neem oil	1.2%	3.04	13.0 $\pm$ 3.02cde	9.75 $\pm$ 3.79abc	6.5 $\pm$ 4.29bcd

Means  $\pm$  SE within a column followed by the same letter are not significantly different ( $P = 0.001$ ). For 2 DAT,  $F = 4.15$ ;  $df = 3, 9$ ; For 5 DAT,  $F = 5.24$ ;  $df = 3, 9$ ; For 10 DAT,  $F = 8.52$ ;  $df = 3, 9$ .

**Table. 5.4. Mean number of non-target arthropod abundance in treatment plots (2004).**

Treatment	Mean $\pm$ SE		
	Arachnida	Coleoptera	Others
Untreated check	0.60 $\pm$ 0.40a	0.80 $\pm$ 0.20a	0.20 $\pm$ 0.20a
Dinotefuran	0.50 $\pm$ 0.40a	0.40 $\pm$ 0.25ab	0.00 $\pm$ 0.00a
Insecticidal soaps	0.60 $\pm$ 0.20a	0.20 $\pm$ 0.20ab	0.00 $\pm$ 0.00a
<i>B. bassiana</i>	0.20 $\pm$ 0.24a	0.60 $\pm$ 0.40ab	0.00 $\pm$ 0.00a
Acephate	0.00 $\pm$ 0.0a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a
Carbaryl	0.40 $\pm$ 0.24a	0.20 $\pm$ 0.20b	0.00 $\pm$ 0.00a
Cyfluthrin	0.20 $\pm$ 0.24a	0.60 $\pm$ 0.24ab	0.00 $\pm$ 0.00a
Bifenthrin	0.40 $\pm$ 0.40a	0.60 $\pm$ 0.24ab	0.00 $\pm$ 0.00a
Imidacloprid	0.00 $\pm$ 0.00a	0.60 $\pm$ 0.40ab	0.20 $\pm$ 0.20a
Neem oil	0.60 $\pm$ 0.40a	0.40 $\pm$ 0.20ab	0.00 $\pm$ 0.00a

Means  $\pm$  SE within a column followed by the same letter are not significantly different ( $P > 0.05$ ). For Arachnida,  $F = 0.83$ ;  $df = 4, 9$ ; For Coleoptera,  $F = 1.18$ ;  $df = 4, 9$ ; For others,  $F = 0.83$ ;  $df = 4, 9$ .

## CHAPTER 6

EXCLUSIVE USE OF HOLLIES AS MALE-SPECIFIC HOSTS OF TWOLINED  
SPITTLEBUG, *PROSAPIA BICINCTA* (SAY) (HEMIPTERA: CERCOPIDAE)<sup>4</sup>

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<sup>4</sup> Nachappa, P. 2004. To be submitted to Journal of Entomological Science.

**Key Words:** *Prosapia bicincta*, *Ilex opaca*, *I. cornuta burfordii*, alternate hosts

The spittlebug genus, *Prosapia* Fennah, occurs in North America, Mexico and Central America (Hamilton 1977, Can. Entomol. 109: 621-630). *Prosapia bicincta* (Say), the twolined spittlebug (TLS) is reported from Florida to Maine and as far west as Arkansas and Texas in the United States (Byers 1965, *In Georgia Agricultural Experiment Stations, University of Georgia College of Agriculture. Technical bulletin. N.S. 42: 26 pp.*). Adults and nymphs of *P. bicincta* are xylem feeders (Weigert 1964a, Am. Midl Nat. 71: 422-428; Pass and Reed 1965, J. Econ. Entomol. 58: 275-278; Byers and Wells 1966, Ann. Entomol. Soc. Amer. 59: 1067-1071) with a broad range of grass hosts, including centipedegrass (*Eremochloa ophiuroides* Munro) Hack (Beard 1973, *In Turfgrass: science and culture. Prentice-Hall, Englewood Cliffs, NJ; Vittum et al. 1999, In Turfgrass insects of United States and Canada. Second Edition, Cornell University Press, Ithaca, NY; Braman 1995, In Brandenburg, R.L., and M.G.Villani (eds.), Handbook of Turf grass Insect Pests. Entomol. Soc. Am, Lanham, MD.140 pp; Shortman et al. 2002, J. Econ. Entomol. 95(2): 478-486), coastal bermudagrass, (*Cynodon dactylon* (L.) Pers (Byers and Wells 1966, Ann. Entomol. Soc. Amer. 59: 1067-1071; Taliaferro et al.1966, Crop Sci. 9: 765-766), panolagrass, (*Digitaria decumbens* Stent), and St.Augustinegrass, (*Stenotaphrum secundatum* Walt.) (Genung et al.1954, Coop Econ. Insect Rep. 4(20): 400). Only adults cause economic damage on ornamental hollies, *Ilex opaca* L. (American holly) and *I. cornuta Burfordii* De France (Burford holly) (Pass and Reed 1965, J. Econ. Entomol. 58: 275-278; Braman and Ruter 1997, J. Environ. Hort.15: 211-214). Nymphs and adults also feed on several annual or perennial hosts (Pass and Reed 1965, J. Econ. Entomol. 58: 275-278).*

Females lay eggs in hollow stem, under leaf sheath, at the base of the soil line, and in debris (Byers1965, *In Georgia Agricultural Experiment Station, University of Georgia College*

of Agriculture. Technical bulletin. N.S. 42: 26 pp.). Eggs over winter and hatch in March-April (Beck and Skinner 1972, J. Econ. Entomol. 65: 110-114). Newly emerged nymphs make their way to a suitable host and produce spittlemass within five minutes of feeding (Fagan and Kuitert 1969, Fla. Entomol. 52: 199-206; Pass and Reed 1965, J. Econ. Entomol. 58: 275-278). First generation spittlebugs occur in June. Second generation adults emerge in August and continue till the end of September (Byers 1965, *In Georgia Agricultural Experiment Stations*, University of Georgia College of Agriculture. Technical bulletin. N.S. 42: 26 pp.). Damage associated with the second generation is more severe.

Host-plant selection by adult spittlebugs is influenced by factors such as habitat (Weaver and King 1954, Res. Bull. Ohio Agric. Exp. Station 741: 1-99; Weigert 1964, Ecol. Mongr. 34: 217-241), plant morphology (Hoffman and Mc Evoy 1985, Ecol. Entomol. 11: 415-426), and plant physiology (concentration of amino acids) (Horsfield 1977, Ecol. Entomol. 2: 259-266; Thompson 1994, Ecol. Entomol. 19: 391-394). Peck (1998, Biotropica 30: 639-644) reported the exclusive use of alternate host plants by adult male spittlebugs in the Monteverde region of Costa Rica (Table 2). According to Peck, there is evidence in case of *P. bicincta* for the exploitation of hosts that are taxonomically and morphologically different from grasses, however, there was no proof of male-specific use of alternate hosts per se. Here is reported evidence for exclusive use of ornamental hollies as male-specific hosts of adult *P. bicincta*.

#### FIELD OBSERVATIONS

Observations on host feeding of *P. bicincta* adults occurred during the months of June to August 2004, which coincides with the emergence of *P. bicincta* in Southeast United States. Adult *P. bicincta* were the only life stages observed feeding on underside of hollies leaves planted in landscapes. New terminal growth is more susceptible to spittlebug damage than older

leaves. Epidemiology includes leaves with blotchy, irregular patches, interveinal chlorosis, distortion of new growth and finally leaf defoliation (Braman and Ruter 1997, J. Environ. Hort.15: 211-214). First observations of adult *P. bicincta* on ornamental hollies were made as early as 31 May 2004. Adults were observed on two main holly species, *I. opaca* and *I. cornuta Burfordii*. The observations on *I. opaca* and *I. cornuta Burfordii* were made in a residential area with an established stand of centipede grass. Other *I. opaca* trees were located at the Research Garden, College of Agriculture and Environmental Sciences, Griffin, GA. On every occasion, only *P. bicincta* male adults were found feeding on the underside of the holly leaves. The number of males found varied from one – 14 males on a single holly (Table 1). There have been reports of early male emergence (protoandry) in many spittlebug species (Peck 1999, Environ. Entomol. 28(3): 372-386); however, *P. bicincta* males were found feeding on hollies well after two peaks of emergence (June and August).

Field observations confirm previous reports of the use ornamental hollies as alternate hosts of *P. bicincta* adults. Absence of nymphs on adult hosts could be attributed to the fact that spittlebug nymphs are less mobile than adults and only move to different locations on the stem during molts. Nymphs hatch in soil or under turf grass thatch, as high relative humidity is congenial for nymphal development. Moreover nymphal host selection is based on host characteristics such as depth from exterior to xylem elements, tissue toughness, presence of trichomes (Hoffman and Mc Evoy 1986, Ecol. Entomol. 11: 415-426) and plant architecture (Mc Evoy 1986, Ecology 67: 465-478). In order to feed on hollies, nymphs have to penetrate a hard surface tissue (holly leaves) different from their natural hosts (turf grass), suggesting an active selection of adult hosts by spittlebugs and not just casual encounter (Peck 1998, Biotropica 30: 639-644).

Females were absent on hollies but were found on centipedegrass around the base of hollies. Seasonal light-trap studies indicate the number of males exceeds that of females at greater heights. High traps (157-274 cm above ground) collected almost four times as many males as females, but as trap height was lowered to 15.2 cm above ground, ratios of males to females were more equal, (Beck and Skinner 1963, *J. Econ. Entomol.* 65: 110-114; Fagan and Kuitert 1969, *Fla. Entomol.* 52: 199-206). One explanation for the absence of females on hollies could be that they are busy laying eggs on grass or too heavy to fly well. However, adult sexes and nymphs of the pine spittlebug, *Aphrophora parallella* Say and pecan spittlebugs, *Clastoptera obtusa* Say are found feeding on tall trees.

An alternative explanation for the exclusive use of hollies as specific hosts of males is that males could gather resources for mating success, production of fertile eggs, dispersal and defense (Peck 1998, *Biotropica* 30: 639-644). Only females are known to emit male-attracting pheromone, hence the pheromone hypothesis for male is unsupported (Byers 1965, *In Georgia Agricultural Experiment Stations, University of Georgia College of Agriculture. Technical bulletin. N.S. 42: 26 pp.*). The hypothesis for defense is particularly appealing for *P. bicincta* and plants in the genus *Ilex*. *P. bicincta* is aposematic, bright-colored (red and black variations) conspicuous insects; they reflex bleed (emit a fluid from pretarsi) when disturbed (Peck 2000, *Ann. Entomol. Soc. Am.* 93:1186-1194). Reflexive bleeders have also been shown to repel enemies by chemical components in the blood that are distasteful or noxious (Blum and Sannasi 1974, *J. Insect. Physiol.* 20: 451-460; Carrel and Eisner 1974, *Sci.* 183: 755-757; Moore and Brown 1981, *Insect Biochem.* 11: 493-499; Eisner et al. 1986, *Experientia* 42: 204-207). Spittlebugs mostly rely on graminaceous hosts and this group is not known for defensive secondary compounds. However, hollies, are known to contain saponins, triterpenes (West et al.

1977, *Phytochem.* 136: 790-808; Potter and Kimmerer 1986, *Oecologia* 69: 217-224; 1989, *Oecologia* 78: 322-329; Kreuger and Potter 1994, *Am. Midl. Nat.* 132: 183-191) and phenolics (Potter and Kimmerer 1986, *Oecologia* 69: 217-224; Gargiulo and Stiles 1991, *J. Chem. Ecol.* 17: 1091-1106) as predominant secondary metabolites. Hence, hollies could be a potential source of defensive chemicals in the hemolymph of adult male spittlebugs.

There is a paucity of information on the sex ratios of spittlebugs on other alternate hosts. The evidence for male-specific use of alternate hosts is a unique phenomenon among spittlebugs (Table 2). There could be many explanations for this mechanism; the hypothesis for defense seems enticing, but it could also be the lack of opportunity by females and nymphs and the active selection by adult males towards hollies (Peck 1998, *Biotropica* 30: 639-644). Regardless, the male-limited use of hollies by spittlebugs is remarkable and its dissimilarity from other phytophagous insects validates a thorough investigation.



**Table 6.1. Sex ratios of *Prosapia bicincta* adults on alternate hosts, *I. opaca* and *I. cornuta* *Burfordii*. Observations occurred at 3 stands of *I. opaca* (1- 3) and 1 stand of *I. cornuta* *Burfordii***

Observations	<i>I. opaca</i>		<i>I. cornuta</i>	
	Sites	Males: Females	Sites	Males: Females
5 May 2004	1	6:0	4	4:0
8 June 2004	-	-	4	11:0
20 June 2004	1	2:0	-	-
7 July 2004	3	1:0	4	1:0
24 July 2004	2	14:0	-	-
5 August 2004	1	1:0	4	7:0
14 August 2004	2	4:0	-	-

**Table 2. Association of five spittlebug species with the respective primary and alternate hosts<sup>1</sup>.**

Spittlebug species	Host plant	Family	Habitat	Tissue
<i>Huaina indica</i>				
Primary host	Unknown	---	---	---
Adult host	Undetermined sp.	Solanaceae	streamside herb	stem
<i>Iphirhina quota</i>				
Primary host	<i>Piper aurtium</i>	Piperaceae	trailside treelet	stem
Male host	<i>Bourreria costaricensis</i>	Boraginaceae	forest canopy tree	trunk
<i>Prosapia</i> nr <i>bicincta</i>				
Primary host	<i>Cynodon nlemfuensis</i>	Poaceae	pasture grass	leaves
Male host	<i>I. haberi</i>	Aquifoliaceae	large tree	leaves
<i>Prosapia simulans</i>				
Primary host	<i>Cynodon nlemfuensis</i>	Poaceae	pasture grass	leaves
Adult host	<i>I. haberi</i>	Aquifoliaceae	large tree	leaves
<i>Prosapia bicincta</i>				
Primary host	<i>Cynodon dactylon</i>	Poaceae	turfgrass	leaves
Male host	<i>I. cornuta</i> , <i>I. opaca</i>	Aquifoliaceae	ornamental tree	leaves

Only *P. bicincta* is represented in North America. All other species are native to Costa Rica.

<sup>1</sup> Modified from Peck 1998.

CHAPTER 7  
CONCLUSIONS

Research indicates the presence of natural enemies of the twolined spittlebug, *P. bicincta* in turfgrass. We demonstrated predation on all life stages of the twolined spittlebug: egg, exposed nymphs and adults under controlled laboratory conditions. *P. bicincta* eggs were consumed by *G. punctipes*, *G. uliginosus*, *S. invincta*, *H. pennsylvanicus*, *C. sayi* and, *M. carolina*, with *S. invincta* being the most significant consumer of eggs. Nymphs are protected by the spittlemass from attack of predators, but exposed nymphs were susceptible to attack when, mechanically removed from their spittlemass. *S. invincta* 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. invincta* and *M. carolina carolina* killed 100% of the adult spittlebugs in laboratory bioassay. Our study reiterates the importance of naturally occurring biological control agents in reducing pest outbreaks in urban landscapes. Sound background knowledge about *P. bicincta* and its natural enemy complex is important for the development and implementation of a detailed, site-specific, biologically based pest management. Conservation of this rich diversity of natural enemies is important for sustainable management in turf.

Additionally, we demonstrated the effectiveness of the generalist tiger beetle predator, *M. carolina carolina* in reducing number of *P. bicincta* adults, and fourth instar fall armyworm larvae, *Spodoptera frugiperda* (J. E. Smith) in the laboratory. *M. carolina carolina* exhibits a type II functional response, where the number of prey attacks per predator shows a negative accelerating rise to a plateau for all prey choices. According to the research, *M. carolina carolina* exhibits potential as a biocontrol agent of pests in landscapes and turfgrass and may be an important candidate for conservation efforts in landscapes.

As a chemical control option for homeowners against *P. bicincta* nymphs and adults, various low-risk selective insecticides were evaluated in the field. Research indicates that summer application of imidacloprid, fipronil, bifenthrin and cyfluthrin may suppress adult spittlebug population. The nymphal stage of spittlebugs is buried in the thatch and, is additionally protected by the spittlemass, which makes it difficult for insecticides to penetrate the spittlemass and kill the nymphs. Hence, adults should be a priority target for insecticidal application compared to nymphs. However, dinotefuran could be sprayed for effective control of spittlebug nymphs and acephate for commercial landscapes. The insecticides used in the study did not affect non-target arthropod survival, including beneficials in turfgrass. It is imperative to choose selective insecticides that minimize adverse affects on the beneficial populations and other non-target invertebrates in order to promote the long-term stability of the turfgrass system. Hence, future research on compatibility of pesticides with beneficial arthropods is essential for sustainable integrated pest management programs in turf.