Laboratory feeding responses of *Euthyrhynchus floridanus* and *Podisus maculiventris* (Hemiptera: Pentatomidae) to the kudzu bug, *Megacopta cribraria* (Hemiptera: Plataspidae)

Julio Medal^{1,*}, Mrittunjai Srivastava¹, Shweta Sharma¹, Danielle Wolaver¹, Andrew Santa Cruz¹, Eric Rohrig¹, and Amy Howe¹

Abstract

The feeding responses of the predatory stink bugs *Euthyrhynchus floridanus* (L.) and *Podisus maculiventris* Say (Hemiptera: Pentatomidae) to the kudzu bug, *Megacopta cribraria* (F.) (Hemiptera: Plataspidae), as prey were studied in the laboratory. *Euthyrhynchus floridanus* was 20 to 40% more efficient than *P. maculiventris* at feeding on *M. cribraria* adults with or without the soybean looper, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae), as alternative prey. All life stages of both predators preferred to feed on *M. cribraria* compared with *C. includens*. Each predator killed an increasingly greater number of prey as prey density increased but predation did not continue to increase when 4 to 5 *M. cribraria* were present at the same time. The maximum limit in number of prey killed was probably imposed by predator satiation in conjunction with prey defense mechanisms. Our study demonstrated that *E. floridanus* is a potential biocontrol agent for *M. cribraria*. However, more testing is necessary to determine the impact of this predator in the field.

Key Words: Florida predatory stinkbug; generalist predator; invasive species; Plataspidae

Resumen

Las repuestas alimenticias de las chinches hediondas depredadoras, *Euthyrhynchus floridanus* (L.) y *Podisus maculiventris* Say (Hemiptera: Pentatomidae) a la chinche del kudzu *Megacopta cribraria* (Fabricius) (Hemiptera: Plataspidae) como presa fueron estudiadas en laboratorio. *Euthyrhynchus floridanus* fué un 20 para 40% más eficiente que *P. maculiventris* alimentándose de *M. cribraria* adultos con o sin falso medidor *Chrysodeixix includens* (Walker) (Lepidoptera: Noctuidae) como presa alternativa. Todos los estadios de ambos depredadores prefirieron alimentarse de *M. cribraria* comparado con *C. includens*. Cada uno de los depredares consumió un mayor número de presas cuando hubo un aumento en el número de presas, pero no consumieron más cuando 4 para5 *M. cribraria* estuvieron presentes al mismo tiempo. El límite máximo en el número de presas consumidas probablemente estuvo condicionado cuando el depredador estuvo saciado combinado con los mecanismos de defensa de la presa. Nuestro estudio demostró que *E. floridanus* tiene potencial como agente de control biológico de *M. cribraria*. Sin embargo, se hacen necesario más pruebas para determinar el impacto de este depredador en el campo.

Palabras Claves: chinche depredadora de Florida; depredador generalista; especie invasora; Plataspidae

The kudzu bug, *Megacopta cribraria* (F.) (Hemiptera: Plataspidae) (Fig. 1), recently proposed as *Megacopta punctatissima* (Montandon) by Dhammi et al. (2016), initially was reported in northeastern Georgia in Oct 2009 (Eger et al. 2010; Suiter et al. 2010b). Since then, it has rapidly dispersed through Florida, Georgia, Alabama, Mississippi, Louisiana, Arkansas, Tennessee, Kentucky, South Carolina, North Carolina, Virginia, the District of Columbia, Maryland, and Delaware (Suiter et al. 2010a, 2010b; Roberts 2011; Medal et al. 2013a; Gardner 2014). Moreover, a Kudzu Bug Working Group maintains a web-based information exchange system for monitoring kudzu bug spread that provides a regularly updated map of this pest's distribution (Gardner 2014).

Kudzu bugs are related to the stink bugs (Pentatomidae) and shield bugs (Scutelleridae) and like these 2 families produces a

strong defensive odor when disturbed. *Megacopta cribraria* originally is from Asia where its preferred plant host is kudzu, *Pueraria montana* Lour (Merr.) variety *lobata* (Willd.) (Fabales: Fabaceae) (Hosokawa et al. 2014). In the continental United States, *M. cribraria* is found feeding on invasive kudzu vines (Ruberson et al. 2012; Zhang et al. 2012). This insect also is a major pest of soybean, *Glycine max* Merrill (Fabaceae), and other leguminous plants as well as various fruit trees (Li et al. 2001; Wang et al. 2004; Eger et al. 2010; Medal et al. 2013b, 2016). Specifically, *M. cribraria* has been reported causing significant feeding damage to soybean in the southern US (Greene et al. 2012; Gardner et al. 2013; Seiter et al. 2013a, 2013b; Roberts et al. 2014; Musser et al. 2015) and caged fig trees, *Ficus carica* L. (Moraceae), in Auburn, Alabama, USA (Hu

¹Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida 32611, USA; Email: medal@ufl.edu (J. M.); E-mail: Mrittunjai.shrivastava@freshofromflorida.com (M. S.); Shweta.sharma@freshfromflorida.com (S. S.); Danielle.wolaver@freshfromflorida.com (D. W.); Andrew.santacruz@freshfromflorida.com (A. S. C.); Eric.rohrig@freshfromflorida.com (E. R.); Amy.howe@freshfromflorida.com (A. H.) *Corresponding author; Email: Medal@ufl.edu



Fig. 1. Megacopta cribraria female.

& Carroll 2012). The plant host range of the kudzu bug is increasing and its distribution has expanded, due in part to dispersal by vehicles traveling to the western and northeastern United States (Medal et al. 2013a). *Megacopta cribraria* also is a nuisance, invading houses as it seeks overwintering sites (Anonymous 2010; Ruberson et al. 2012).

Generalist predatory stink bugs such as Euthyrhynchus floridanus (L.) and Podisus maculiventris Say (Hemiptera: Pentatomidae) (Figs. 2, 3), have been observed feeding on M. cribraria in green bean (Phaseolus vulgaris L. [Fabaceae]) crops in Georgia (Ruberson et al. 2012) as well as Florida kudzu fields (J. M., unpublished data). Medal et al. (2017) has studied the feeding behavior of immature male and female *E. floridanus* and *M. cribraria* with fall armyworm, *Spodoptera frugiperda* (J. F. Smith) (Lepidoptera: Noctuidae), and velvetbean caterpillar, Anticarsia gemmatalis (Hübner) (Lepidoptera: Noctuidae), as alternative preys. Preliminary results indicated that both predators have the potential for use in a biological control program for *M. cribraria* but their impact requires further evaluation. Therefore, we investigated the feeding responses of E. floridanus and P. maculiventris when exposed to M. cribraria with and without soybean looper, Chrysodeixis includens (Walker) (Lepidoptera: Noctuidae), as alternative prey. Also, we assessed the feeding effectiveness of both predators relative to M. cribraria density.

Material and Methods

INSECTS

Euthyrhynchus floridanus and *P. maculiventris* were obtained from laboratory colonies established from nymphs and adults collected in a kudzu patch in Gainesville, Alachua County, Florida, USA (29.6396°N, 82.3990°W), during the summer and fall of 2012. Prior to the start of experiments, third and fourth instar *M. cribraria* and adults were collected from the same kudzu patch and maintained in the laboratory in clear plastic containers (23 cm W × 32 cm L × 10 cm H) with moistened paper and field collected kudzu leaves and vines. Insectary conditions were set at 25 ± 3 °C, 16:8 h (L:D) photoperiod and 50 to 60% relative humidity. The alternative prey, *C. includes* (soybean looper), was field collected from wild legume plants.

EUTHYRHYNCHUS FLORIDANUS AND PODISUS MACULIV-ENTRIS PREDATION RESPONSES TO MEGACOPTA CRIBRARIA WITH AND WITHOUT ALTERNATIVE PREY

Laboratory feeding experiments were conducted exposing third to fourth instar nymphs, males and females of *E. floridanus* and *P. maculi*-



Fig. 2. Podisus maculiventris female.

ventris, to *M. cribraria* of the same instar and as adults. All insects were starved 24 h prior to predator-prey assays. *Podisus maculiventris* was chosen as a comparative predator because it is a common predatory pentatomid that can be found throughout the crop growing regions of the USA (De Clercq 2008).

Predators were placed individually in Petri dishes (14.6 cm × 2.5 cm) with a bean pod, Phaseolus vulgaris L. and moistened paper as food and moisture sources. Crumpled Kimwipes[®] (Kimberly-Clark, Roswell, Georgia, USA) were inserted in each dish to provide refugia for predators. One intermediate M. cribraria nymphal instar or an adult was provided to one third to fourth instar, or adult male, or adult female E. floridanus or P. maculiventris. In addition, a 7 to 10-d-old soybean looper was added as alternative prey to a subset of the predator-prey combinations. Larval C. includens was chosen as alternative prey because it is commonly found on kudzu plants when M. cribraria immatures and adults are present. Controls consisted of the same experimental set-up with only prey. Prey mortality in all experimental trials was recorded after 24 h. Any dead M. cribraria and soybean looper caterpillars were assumed to have been killed by a predator. Megacopta cribraria mortality data were not adjusted because of low (< 5%) mortality in controls. This study was conducted during 2013.

EUTHYRHYNCHUS FLORIDANUS AND PODISUS MACULIV-ENTRIS PREDATION RESPONSES AT DIFFERENT MEGACOPTA CRIBRARIA DENSITIES

Prior to the start of this experiment, nymphal and adult *E. floridanus* and *P. maculiventris* were maintained on mealworm larvae, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) and *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) larvae. Insects were provided with green bean pods and caged as mentioned earlier in the alternative prey experiment. Predators were starved for 24 h prior to the start of feeding tests. One to 5 (male or female) *M. cribraria* were added to each Petri dish that contained either a single third to fourth instar nymph, male or female *E. floridanus* or *P. maculiventis*. After 24 h, the number of dead prey was recorded.

STATISTICAL ANALYSIS

The predator-prey study, with and without alternative prey, incorporated a completely randomized design with 20 replications for predatorprey interaction and a set of 10 replications for control. Percent prey mortality data for each predator life stage were analyzed using Fisher's Exact Test for Count Data (Fisher 1970). Mean prey mortality resulting from pre-



dation on developmental stages were analyzed as a 2-way factorial and compared using Tukey's multiple comparison test of means (Montgomery 2013). Means were considered statistically different at P < 0.05. The *M. cribraria* density feeding study incorporated a split-plot design with 20 replications. The feeding experiments consisted of 5 replications conducted at a time because of space and handling time limitation. Treatments consisted of male and female predators of each species (main factor) and *M. cribraria* density (split-plot factor). In both studies, data were analyzed using the statistical package 'R' (Beckerman & Petchey 2015). Differences were considered statistically significant at P < 0.05.

Results and Discussion

EUTHYRHYNCHUS FLORIDANUS AND PODISUS MACULIVENTRIS PREDATION RESPONSES TO MEGACOPTA CRIBRARIA WITH AND WITHOUT ALTERNATIVE PREY

There was a significant difference in *M. cribraria* predation rate among the *E. floridanus* developmental stages (F = 4.144; df = 8; P < 0.001) and the *P. maculiventris* developmental stages (F = 13.06; df = 8; P < 0.0001). Females and third to fourth instars of both predator species consumed on average 20 to 40% more (P = 0.023) *M. cribraria* adults or nymphs than predator males. All *E. floridanus* life stages consumed more *M. cribraria* compared with those of *P. maculiventris* (P < 0.05). There was no significant difference in the percentage of *M. cribraria* females or nymphs consumed by *P. maculiventris* adults (F = 1.650; df = 4; P = 0.051) but they were less effective feeding on *M. cribraria* males (Figs. 4, 5). Moreover, predation responses of *E. floridanus* and *P. maculiventris* males, females, and nymphs to *M. cribraria*, as the major prey, were not affected by the presence of soybean looper larvae as alternative prey and showed a general preference for feeding on *M. cribraria* (Figs. 4, 5).



Fig. 4. Mean percent predation of *Megacopta cribraria* by *Euthyrhynchus floridanus* (I) without and (II) with alternative prev^a.



Fig. 5. Mean percent predation of *Megacopta cribraria* by *Podisus maculiven*-*tris* (I) without and (II) with alternative prey^a.

EUTHYRHYNCHUS FLORIDANUS AND PODISUS MACULIV-ENTRIS PREDATION RESPONSES AT DIFFERENT MEGACOPTA CRIBRARIA DENSITIES

Prey mortality by both predators generally increased as prey density increased from 1 to 5 (Fig. 6). Interestingly, predation ceased when predators were exposed to 4 or 5 prey at one time. This result indicated that they were satiated, setting the limits for the number of prey captured and fed on by an individual predator within a 24 h period. Predation rates of E. floridanus and P. maculiventris to increasing M. cribraria densities have not been previously reported in the literature. Such information provides insights to determine the number of predators required to control a given prey density and identifies some of the limiting factors that may influence a predator's feeding response. These factors may include prey density, prey developmental stage, sex and developmental stage of the predator, search rate, availability of alternate prey or food, prey defense mechanisms, and attack techniques used by the predator. Both predators moved actively in the limited searching arena and attacked by walking quickly or running toward prey with the beak extended straight forward, and quickly inserting their stylet to subdue their quarry. The predators have been observed to lift the prey in the air with their beak while feeding. Moreover, results obtained from our study indicated that the percent of M. cribraria mortality due to male and female predators was significantly influenced by sex (F = 4.144; df = 8; P < 0.001) and prey density (R^2 values, Fig. 6). Moreover, several authors also have observed that female predators of other insect species tend to consume more prey compared with males due to their greater size and higher nutrient requirements for egg production (Tauber & Tauber 1974; Crocker et al. 1975; Medal et al. 2017).



Fig. 6. Number of prey consumed by *Podisus maculiventris* (A) and *Euthyrhynchus floridanus* (B) when exposed to increasing density of *Megacopta cribraria* adults.

In conclusion, we found that *E. floridanus* was a more effective predator of *M. cribraria* compared with *P. maculiventris* in laboratory bioassays. Similarly, Medal et al. (2015) reported that *E. floridanus* also was more efficient than *P. maculiventris* at feeding on adult citrus root weevil, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae), in no-choice laboratory tests. *Euthyrhynchus floridanus* exhibited promising potential for use in augmentative biological control programs for suppressing populations of *M. cribraria*. However, additional testing is necessary to determine whether this predator will prefer to consume *M. cribraria* under field conditions.

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