Alternative Strategies for Managing *Megacopta cribraria* (Fabricius) (Hemiptera: Plataspidae) in Soybean

by

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Abstract

Megacopta cribraria (F.) (Hemiptera: Plataspidae) commonly known as kudzu bug was introduced from Asia into Georgia, United States in 2009. Its distribution since then has expanded to 12 other states from Arkansas to Washington D.C. *Megacopta cribraria* is a pest of soybean (*Glycine max*) Merrill, the second most planted field crop in the United States with an estimated annual market value of about $39 billion. Nymphs and adults of *M. cribraria* aggregate in large numbers on tender stems or leaves of soybean where they suck sap, resulting in significant yield loss, up to 60%. As the threat posed by this invasive insect pest increases, no effective control strategies other than chemical insecticides are currently available to help soybean farmers. This increased use of chemical insecticides is not sustainable and could result in the development of pesticide resistance.

To find alternative control strategies, this study explored the prospects of trap cropping and use of semiochemical attractants for management of *M. cribraria.* The specific objectives were to: 1) evaluate host plant preference and identify attractive trap crops for *M. cribraria*; 2) identify plant-based semiochemical attractants for *M. cribraria*; and 3) identify semiochemical attractants for the egg parasitoid, *Paratelenomus saccharalis* (Hymenoptera: Platygastridae) (Dodd). In a multiple-choice screen house preference test, *M. cribraria* showed differential attraction to phenological stages of legume cultivars tested. Soybean, *Glycine max* (L.) merr, speckled bean, *Phaseolus lunatus* L., and lima bean, *Phaseolus limensis* L., which were identified in screen house tests as attractive to *M. cribraria* at different phenological stages were

further evaluated in the field as potential trap crops at two planting dates; two weeks before main crop (May 24) and same time as main crop (June 7). Soybean trap crop planted on either dates was the most effective at intercepting *M. cribraria*.

The role of volatile organic compounds (VOCs) in host location by *M. cribraria* as well as the response of its egg parasitoid, *P. saccharalis* to host-associated odors was investigated. Female *M. cribraria* did not respond significantly to headspace volatiles of host plants in olfactometer studies. Also, in follow up multiple-choice cage experiment and olfactometer bioassays conducted in the dark, *M. cribraria* did not make a choice among the plant treatments tested. This implies that host location or preference by *M. cribraria* is either not mediated by olfactory cues or probably mediated by multiple modalities including visual and olfactory cues. In contrast, female *P. saccharalis* (egg parasitoid) showed significant attraction to host-associated odors in Y-tube olfactometer studies including damaged soybean, damaged soybean plus *M. cribraria* eggs and *M. cribraria* eggs only, suggesting that host location by the egg parasitoid is mediated by odor cues from soybean and *M. cribraria* eggs.

A new egg parasitoid was recovered from parasitized *M. cribraria* eggs collected on soybean in Auburn, AL during summer 2016. Parasitization rate ranged from 82 - 100%. The identity of this new egg parasitoid is currently unknown and voucher specimens have been sent to the Smithsonian Institute, Washington D.C. for accurate identification.

It is hoped that results from this research will enhance the economic viability of soybean and legume crop production by developing ecological-based management tools for managing *M. cribraria* in soybean.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Origin of *Megacopta cribraria* and Discovery in the United States

Megacopta cribraria (Fabricius) (Hemiptera:Plataspidae), also known as kudzu bug, globular stink bug, lablab bug or bean plataspid was found aggregating on homes and properties in Jackson Co., Georgia, United States in 2009. Further observations later traced the bugs to kudzu patches (*Puereria montana* var. *lobata* (Willdenow) Ohwi, (Fabaceae) growing within the vicinity. This was the first report of any member of the family Plataspidae in North America (Eger et al. 2010; Suiter et al. 2010b) apart from *Coptosoma xanthogramma* (White) which is established in the Hawaiian Islands (Beardsley and Fluker, 1967). Eger et al. 2010 compiled an extensive list of countries where *M. cribraria* had been reported, spanning across the continents of Asia and Australia. Gene sequence of specimens of *M. cribraria* along with their primary and secondary symbiotic gut bacteria collected from 38 counties in the states of Georgia and South Carolina lacked genetic diversity and points to a single female lineage traceable to Japan (Jenkins and Eaton, 2011).

1.2 Distribution of *Megacopta cribraria* in the United States

Since its discovery in Georgia in 2009, *M. cribraria* has been confirmed in 12 other states including Florida, Alabama, Tennessee, South Carolina, North Carolina, Virginia, Mississippi, Louisiana, Maryland, Kentucky, Delaware, Arkansas, and the District of Columbia (Suiter et al.

2010a, 2010b; Jenkins and Eaton, 2011; Gardner, 2013a). Monitoring is still ongoing and up to date information on the distribution is available at www.kudzubug.org/distribution_map.cfm

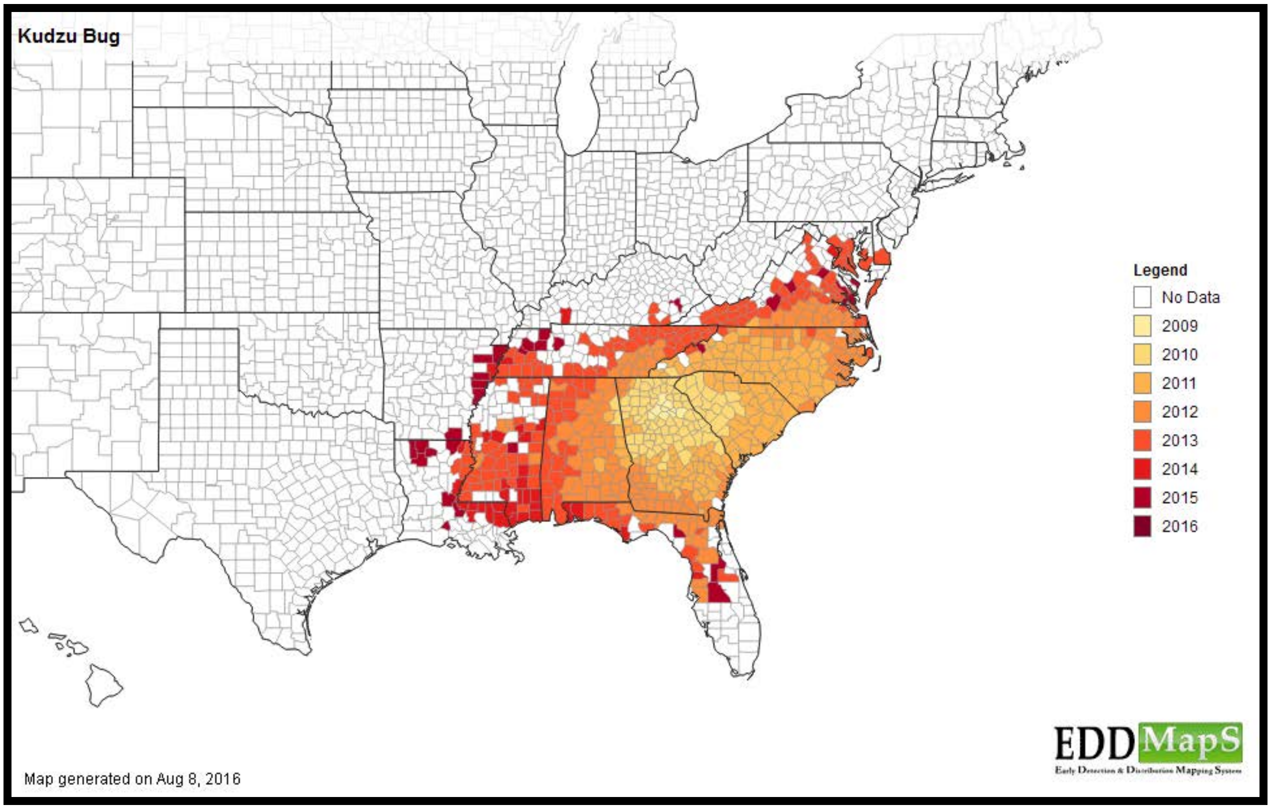

Fig. 1 Distribution of the kudzu bug, *Megacopta cribraria* in the United States from 2009 - 2016. Map compilation by Wayne A. Gardner, University of Georgia (available at http://mdkudzubug.org/kudzu-bug-distribution-)

1.3 Biology and Seasonal Activity

Adult *M. cribraria* are 4 - 6 mm long, oblong, and greenish-brown in color (Eger et al. 2010). Female *M. cribraria* lays eggs in groups of two parallel rows on the underside of leaves and apices of shoots. Endosymbiont bacteria are deposited as small brownish capsules on the underside of the egg masses. These are ingested orally by newly hatched nymphs and studies have shown that *M. cribraria* deprived of the endosymbiont bacteria show poor development

(Fukatsu and Hosokawa 2002). Nymphs undergo five instars, and depending on temperature, entire life cycle from egg to adult takes approximately six to eight weeks (Srinivasaperumal et al. 1992; Zhang et al. 2012; Del Pozo-Valdivia and Reisig, 2013). On soybean, both adults and nymphs suck sap from stems, petioles, and leaves, resulting in lower number of pods, reduced pod weight, and poor seed set (Seiter et al. 2013a). *Megacopta cribraria* completes two generations per year on both soybean and kudzu in the southeastern United States (Suiter et al. 2010; Zhang et al. 2012; Ruberson et al. 2013; Seiter et al, 2013a). In early spring, adults emerge from overwintering sites and move on to available host plants where they feed and reproduce. The first generation of adults emerges in late June, some of which leave kudzu to find new host plants such as soybean where they complete the second generation. However, greenhouse studies have shown that first generation adults are capable of developing on soybean, mung bean and lima bean, an indication that overwintering adults can bypass kudzu to feed and reproduce on early planted soybean (Del Pozo-Valdivia and Reisig, 2013; Golec et al. 2015). When temperatures drop in the Fall, the second generation adults seek warmer structures such as residential areas and behind tree bark to overwinter (Wu et al. 2006; Zhang et al, 2012).

1.4 Host Range and Preference

Megacopta cribraria has been associated with various leguminous and non-leguminous host plants in its native Asian range as well as in the United States (Srinivasaperumal et al. 1992; Eger et al. 2010, Zhang et al. 2012). Eger et al. (2010) provided a detailed compilation of leguminous and non-leguminous host plants associated with *M. cribraria* dating as far back as 1910, some of which are not necessarily reproductive hosts. A preliminary host range study by Zhang et al. (2012) indicated soybean and kudzu as the only reproductive hosts of *M. cribraria*, but also noted that females could oviposit on a wide range of other legumes without completing development on them. While Medal et al. (2013b; 2016) did not find any significant difference in the rate of oviposition of *M. cribraria* on kudzu and soybean, Zhang et al. (2012) reported a higher oviposition rate on kudzu. Apart from soybean, Golec et al. (2015) reported oviposition and development on mung bean (*Phaseolus radiatus* L.), and lima bean. Also, host preference studies by Blount et al. (2015) showed that adults and nymphs exhibit variable survival and preference among different legumes. In addition to soybean and kudzu, *M. cribraria* completed development on edamame and pigeon pea (*Cajanus cajan* L.).

1.5 Pest Status and Economic Impacts

Soybean is an economically important crop in the United States. It is the second most grown field crop after corn. The United States is the largest producer and exporter of soybean in the world and current production is about 4.2 million bushels which accounts for 50% of the world's soybean production. The current value of production is also estimated at about $39 billion (USDA - ERS, 2016). Both nymphs and adults aggregate in large numbers on tender stems and leaves where they suck sap. Earlier reports on damage caused by *M. cribraria* feeding on soybean only highlighted the impacts which include delayed growth, curled leaves, lesions and reduction in foliage density. However, a recent experiment showed a yield loss up to 60% when infested plots were compared with non-infested plots. This was evident in reduced number of seeds per pod as well as reduced weight of each seed (Seiter et al. 2013a). Yield loss of 1-50% based on bug density has also been reported (Wang et al. 1996). In addition to the severe crop loss caused to soybean, *M. cribraria* infestation also impacts international trade and commerce. For instance, the Honduran government banned all agricultural imports from Alabama, Georgia, and North and South Carolina due to detection of dead *M. cribraria* adults in a container shipment. It is also a nuisance pest and capable of causing skin irritation (Suiter et al. 2010a; Ruberson et al. 2012).

Megacopta cribaria in its native range of Japan is not a major pest of soybean (Hosokawa et al. 2007), unlike its closely related species *M. punctatissima*. Existing literature suggests there are no clearly defined morphological and genitalial differences between the two species, except for their obligate symbiotic gut bacteria (Hosokawa et al. 2007; Eger et al. 2010). The identity of the plataspid in North America is still controversial. What is accepted as *M. cribraria* has been proposed to be *M. punctatissima* in a recent review, but further research is needed (Dhammi et al. 2016).

The symbiotic gut bacteria plays a significant role in the pest status and fitness of *M. cribraria* and *M. punctatissima*, including, host choice, growth rate, body size and longevity (Fukatsu and Hosokawa 2002; Hosokawa et al. 2007). To confirm this, symbiotic gut bacteria were swapped between *M. cribraria* and *M. punctatissima*, *M. cribraria* performed better on soybean than *M. punctatissima* (Hosokawa et al. 2007; 2008). *Megacopta cribraria* collected from Georgia in 2009 had the Proteobacterium *Candidatus* Ishikawaella capsulata common to the family Plastapidae (Jenkins et al. 2010; Fukatsu and Hosokawa, 2002; Hosokawa et al. 2006).

Megacopta cribraria has been reported to feed and develop on soybeans in China, India, and Japan (Zhixing et al. 1996; Thippeswamy and Rajapogal, 2005b; Xing et al. 2006; 2008). In its new invasive range, *M. cribraria* poses a threat to soybean. The potential of *M. cribraria* becoming a major threat to soybean production in the United States has been reported (Eger et al. 2010; Zhang et al. 2012; Ruberson et al. 2012; Seiter et al. 2013b).

1.6 Control Strategies

Biological, chemical and cultural approaches are being investigated to reduce the damage and spread of *M. cribraria* in the United States. Promising natural enemies that have been identified and/or are currently under investigation include the entomopathogenic fungus *Beauveria bassiana* (Balsamo) which was reported on adult *M. cribraria* (Rubertson et al. 2012; Seiter et al. 2013a). Laboratory and field studies showed *B. bassiana* is effective against *M. cribraria* (Seiter et al. 2014a). Three adult parasitoids: *Strongygaster triangulifera* (Loew), a dipteran (Golec et al. 2013); another dipteran, *Phasia robertsonii* (Townsend) (Ruberson et al. 2012); a mermithid nematode (Stubbins et al. 2015) with less than 10% parasitization rate and an egg parasitoid, *Paratelenomus saccharalis* (Dodd) with parasitization rate as high as ≈ 95% has also been reported (Gardner et al. 2013b, Medal et al. 2015). A new egg parasitoid of *M. cribraria* belonging to the genus *Ooencyrtus* was also reported in Virginia (Dhammi et al. 2016). Evidence of predation of *M. cribraria* by a host of generalist predators through DNA analysis of gut content has also been documented and the use of soybean fields adjacent to cotton as a form of conservation biological control strategy was suggested (Greenstone et al. 2014).

Evaluations of broad spectrum insecticides like pyrethroids, neonicotenoids and organophosphates in the field as well as their residual effects on building materials for residential use have shown to be effective at controlling *M. cribraria* (Seiter et al. 2013c; 2015; Brown et al. 2015). However, cases of pyrethroid resistance in many hemipteran pests over the years as reported in the tarnished plant bug (*Lygus lineolaris* Palisot de Beauvois) (Snodgrass, 1996), Southern chinch bug, (*Blissus insularis* Barber) (Cherry and Nagata, 2005), as well as the effect on non-target organisms (Wilkinson et al. 1979; Mian and Mulla 1992) may be a limiting factor to the use of conventional insecticides.

In a study conducted by Blount et al. (2016) in which planting time was varied, early-planted soybean suffered higher *M. cribraria* infestation rate than late-planted soybean. However, late planting of soybean is counterproductive and cannot be recommended due to the downside of lower yield potential caused by environmental factors (Specht et al. 2012). Also, Fritz et al. (2016) screened different soybean cultivars for host resistant and found two cultivars that were relatively resistant to *M. cribraria* with no negative impact on yields. Studies on alternative control strategies that are environmentally sustainable are still very minimal. Other ecological-based approaches yet to be considered are trap cropping and the use of semiochemical attractants to enhance biocontrol.

1.6.1 Trap cropping

Trap crops have been defined as "plant stands grown to attract insects or other organisms like nematodes to protect target crops from pest attack, preventing the pest from reaching the crop or concentrating them in a certain part of the field where they can be economically destroyed" (Hokkanen, 1991). Trap crop systems have been developed commercially for three hemipteran pests (Shelton and Badenes-Perez, 2006). A classic example is the use of alfalfa as trap crop for managing Lygus bug, *Lygus hesperus* (Knight) in cotton (Stern et al. 1969; Godfrey and Leigh, 1994). The following key ecological aspects of *M. cribraria* suggest trap cropping will be an effective control strategy against it. 1) The bug shows preference to certain host plants for food, oviposition or development (Zhang et al. 2012; Medal et al. 2016; Seiter et al. 2014b; Blount et al. 2015). 2) migrate into the field rather than emerge from the field (Ruberson et al. 2012; Lahiri et al. 2015), 3) exhibit strong edge effect behavior– aggregate and colonize on field margins (Seiter et al. 2013b) and 4) unlike other stink bugs, *M. cribraria* shows low tendency to move from a suitable host.

1.6.2 Semiochemicals

Semiochemicals are chemical or mixture of chemicals used in communication among organisms (Law and Reigner, 1971; Nordlund and Lewis, 1976; Dicke and Sabelis, 1988). Semiochemicals such as pheromones and allelochemicals mediate intraspecific or interspecific communication respectively (Dicke and Sabelis, 1988; Vet and Dicke, 1992). Many phytophagous insects use host-specific semiochemical odors (kairomones) as cues to locate their hosts. For instance, stink bugs such as harlequin bug, *Murgantia histrionica* (Hahn), use host plant odors to find their host plants (Wallingford, 2012). Plants damaged by herbivores produce volatiles (Pare and Tumlinson 1999), these are commonly referred to as herbivore induced plant volatiles (HIPVs) (Mumm and Dicke, 2010) which is a form of defense strategy against biotic stress (Maffaei et al., 2007). HIPVs have been shown to attract predators and parasitoids to insect pests (Price at al. 1980; Sabelis et al. 1999). Such volatile-mediated tritrophic relationships have been investigated and reported extensively (Turlings et al.1993; Tumlinson et al. 1993; Takabayashi and Dicke, 1996; Hilker and Meiners, 2002; Colazza et al. 2004; Chen and Fadamiro, 2007; Ngumbi et al. 2009). Practical applications of semiochemicals in the management of insect pests have been documented (Noldus, 1984; Willson et al. 1980; Fadamiro, 2004a, 2004b).

The role of volatile organic compounds in *M. cribraria* host selection and preference as well as host location cues used by its egg parasitoid, *P. saccharalis* is not well understood, and understanding these factors may provide crucial information necessary for the development of alternative management strategies against *M. cribraria*.

1.6.3 *Paratelenomus saccharalis*, Egg parasitoid of *M. cribraria*

Paratelenomus saccharalis (Hymenoptera: Platygastridae) (Dodd) = *Asolcus minor, Archipanurus minor, Paratelenomus minor*, is a specialist egg parasitoid known to parasitize only three species of insects in the family plataspidae (*Megacopta cribraria*, *M. punctatissima* Montandon *and Brachyplatys subaeneus* Westwood) (Johnson, 1996). It has been reported only from the Eastern Hemisphere until its recent discovery in North America where it was recovered from the eggs of *M. cribraria* in 2013 (Gardner et al. 2013b; Medal et al. 2015). Gardner et al, (2013b) suggested that the parasitoid was most likely introduced into the United States from parastized eggs of *M. cribraria* from Asia. Eggs of *M. cribraria* parasitized by *P. saccharalis* have dark-grey coloration compared with unparasitized eggs. Parasitization rate is typically high, 47-95% parasitization on field collected *M. cribraria* egg has been reported in the United States (Gardner et al., 2013b). Parasitization rate ranged from 4.3 - 20% in India, (Rajmohan and Narendran, 2001; Srinivasaperum et al. 1992), 43-100% in Japan (Takasu and Hirose, 1986).

Although parasitization rate is high, *P. saccharalis* appears late in the season and has a short window period. Hence, its effectiveness can be enhanced through the use of attractants to recruit parasitoids to *M. cribraria* infested soybean fields.

1.7 Justification for Study

Soybean is the second most planted field crop in the United States with an estimated annual market value of about $39 billion (USDA-ERS, 2016). Pest management strategies have been developed for many common soybean insect pests such as caterpillars and stink bugs but not for the invasive kudzu bug which is the top yield-limiting pest of soybean in the southeastern United States (Seiter et al. 2013a). Yield loss up to 60% has been reported. The prevalent control measure has been the use of broad spectrum insecticides. These toxic chemical insecticides are harmful to beneficial insects that may help mitigate kudzu bug and other soybean pests. Soybean production cost was predicted to increase by $15–22 per hectare as a result of increased insecticide use (Ruberson et al. 2012). Pest management strategies such as trap cropping and use of semiochemicals are environmentally sustainable. Trap cropping could reduce the use of insecticides (Swezey and Daxl, 1988) and also enhances biocontrol (Stern et al. 1969).

While most host preference tests have focused on oviposition and development of *M. cribraria* on legume hosts (Medal et al. 2013, 2016; Golec et al. 2015; Blount et al. 2015), this is the first study to evaluate the preference of *M. cribraria* for legume cultivars at different phenological stages in the absence of competing herbivores in a screen house and also the first to evaluate the potentials of different legume cultivars as trap crops for managing *M. cribraria.* Since the discovery of the egg parasitoid *P. saccharalis* in 2013, most studies on the parasitoid in the United States focused only on its discovery and relative abundance (Gardner et al. 2013b; Medal et al. 2015). This is the first behavioral study both in the United States and Asia that demonstrates the response of *P. saccharalis* to host-associated odors and the implications of this in host location.

1.8 Thesis Goal and Outline

The long-term goal of this research was to develop alternative management strategies for *M*. *cribraria* in soybean production which will consequently reduce the reliance on conventional insecticides. This research aims to develop low-input management tools such as trap cropping and odor-based monitoring system for mass-trapping *M*. *cribraria* and also develop attractants to recruit *P. saccharalis* to soybean fields infested with *M*. *cribraria*. Ultimately, findings from this study will be incorporated into the development of a sustainable integrated pest management (IPM) approach for the control of *M. crabraria* in soybean production in the United States. I believe that the knowledge obtained would also be applicable in the control of future invasions by other hemipteran pests.

This study had three objectives:

1. Evaluate host plant preference and identify potential trap crops for *Megacopta cribraria.*

   Hypotheses: i. *M. cribraria* will show greater attraction to other legumes over soybean.

   ii. Trap crops will intercept migrating *M. cribraria* at field border.

2. Identify plant-based semiochemical attractants for *Megacopta cribraria.*

   Hypothesis: Host plant volatiles act as cue for host location by *M. cribraria*.

3. Identify semiochemical attractants for the egg parasitoid, *Paratelenomus saccharalis.*

   Hypothesis: Volatile organic compounds associated with soybean and *M. cribraria* act as cues for host location by *P. saccharalis*.

1.9 References Cited

Beardsley JW Jr, Fluker S (1967) *Coptosoma xanthogramma* (White), (Hemiptera: Plataspidae) a new pest of legumes in Hawaii. Proceedings of the Hawaiian Entomological Society 19:367-372

Blount JL, Buntin GD, Sparks AN (2015) Host preference of *Megacopta cribraria* (Hemiptera: Plataspidae) on selected edible beans and soybean. Journal of Economic Entomology 109: 1-12

Blount JL, Buntin GD, Roberts PM (2016) Effect of planting date and maturity group on soybean yield response to injury by *Megacopta cribraria* (Hemiptera: Plataspidae). Journal of Economic Entomology 109: 207-212

Brown SA, Kerns DL, Williams TS, Emfinger K, Jones N (2015) Evaluation of foliar insecticides for kudzu bug control in soybean. Arthropod Management Tests 40: 1

Chen L, Fadamiro HY (2007) Differential electroantennogram response of females and males of two parasitoid species to host-related green leaf volatiles and inducible compounds. Bulletin of Entomological Research 97: 515-522

Cherry R, Nagata R (2005) Development of resistance in southern chinch bugs (Hemiptera: Lygaeidae) to the insecticide bifenthrin. Florida Entomologist 88: 219-221

Colazza S, Fucarino A, Peri E, Salerno G, Conti E, Bin F (2004) Insect oviposition induces volatiles emission in herbaceous plant that attracts egg parasitoids. Journal of Experimental Biology 207: 47-53

Dhammi A, van Krestchmar JB, Ponnusamy L, Bacheler JS, Reisig DD, Herbert A, Del Pozo-Valdivia AI, Roe RM (2016) Biology, Pest Status, Microbiome and Control of Kudzu Bug (Hemiptera: Heteroptera: Plataspidae): A New Invasive Pest in the U.S. International Journal of Molecular Sciences 17: 1570-1591

Del Pozo-Valdivia AI, Reisig DD (2013) First-generation *Megacopta cribraria* (Hemiptera: Plataspidae) can develop on soybeans. Journal of Economic Entomology 106: 533-535

Department of Agriculture, Economic Research Service. Soybeans & Oil Crops. Related Data & Statistics, Updated 12 October 2016; Available online: https://www.ers.usda.gov/topics/crops/soybeans-oil-crops/related-data-statistics/ (accessed on 22 November 2016)

Dicke M, Sabelis MW (1988) How plants obtain predatory mites as bodyguards. Netherlands Journal of Zoology 3: 148-165

Eger JE Jr, Ames LM, Suiter DR, Jenkins TM, Rider DA, Halbert SE (2010) Occurrence of the Old World bug *Megacopta cribraria* (Fabricius) (Heteroptera: Plataspidae) in Georgia: a serious home invader and potential legume pest. Insecta Mundi 121: 1–11

Fadamiro HY (2004a) Monitoring the seasonal flight activity of codling moth, *Cydia pomonella*, and redbanded leafroller, *Argyrotaenia velutinana* (Lepidoptera: Tortricidae) in apple orchards using pheromone-baited traps. Environmental Entomology 33: 1711-1717

Fadamiro HY (2004b) Pest phenology and evaluation of traps and pheromone lures for monitoring flight activity of obliquebanded leafroller (Lepidoptera: Tortricidae) in Minnesota apple orchards. Journal of Economic Entomology 97: 530-538

Fritz BJ, Reisig DD, Sorenson CE, Del Pozo-Valdivia AI, Carter TE Jr (2016) Host plant resistance to *Megacopta cribraria* (Hemiptera: Plataspidae) in diverse soybean germplasm maturity groups V through VIII. Journal of Economic Entomology 1-12

Fukatsu T, Hosokawa T (2002) Capsule-transmitted gut symbiotic bacterium of the Japanese common plataspid stinkbug *Megacopta punctatissima*. Applied and Environmental Microbiology 68: 389-396

Gardner WA, Peeler HB, LaForest J, Roberts PM, Sparks AN Jr, Greene JK, Reisig DD, Suiter DR, Bachelor JS, Kidd K et al. (2013a) Confirmed distribution and occurrence of *Megacopta cribraria* (F.) (Hemiptera: Plataspidae) in the southeastern United States. Journal of Entomological Science 48: 118-127

Gardner WA, Blount JL, Golec JR, Jones WA, Hu XP, Talamas EJ, Evans RM, Dong X, Ray CH Jr, Buntin GD et al. (2013b) Discovery of *Paratelenomous saccharalis* (Dodd) (Hymenoptera: Platygastridae), an egg parasitoid of *Megacopta cribraria* F. (Hemiptera: Plataspidae) in its expanded North American range. Journal of Entomological Science 48: 355-359

Golec JR, Hu XP, Ray CH, Woodley NE (2013) *Strongygaster triangulifera* (Diptera:

Tachinidae) as a parasitoid of adults of the invasive *Megacopta cribraria* (Heteroptera: Plataspidae) in Alabama. Journal of Entomological Science 48: 352-354

Golec JR, Hu XP, Yang L, Eger JE (2015) Kudzu-deprived first-generation *Megacopta cribraria* (F.) (Heteroptera: Plataspidae) are capable of developing on alternative legume species. Journal of Agricultural and Urban Entomology 31: 52-61

Godfrey LD, Leigh TF (1994) Alfalfa harvest strategy effect on *Lygus* bug (Hemiptera: Miridae) and insect predator population density: implications for use as trap crop in cotton. Environmental Entomology 23:1106-18

Greenstone MH, Tillman PG, Hu JS (2014) Predation of the newly invasive pest *Megacopta cribraria* (Hemiptera: Plataspidae) in soybean habitats adjacent to cotton by a complex of predators. Journal of Economic Entomology 107: 947-954

Hilker M, Meiners T (2002) Induction of plant responses towards oviposition and feeding of herbivorous arthropods: a comparison. Entomologia Experimentalis et Applicata. 104: 181-192

Hokkanen HMT (1991) Trap cropping in pest management. Annual Review of Entomology 36: 119-138

Hosokawa T, Kikuchi Y, Shimada M, Fukatsu (2007) Obligate symbiont involved in pest status of host insect. Proceedings of the Royal Society of London B: Biological Science 274: 1979-1984

Hosokawa T, Kikuchi Y, Shimada M, Fukatsu (2008) Symbiont acquisition alters behavior of Stink bug nymphs. Biology Letters 4: 45-48

Johnson NF (1996) Revision of world species of *Paratelenomus saccharalis* Dodd (Hymenopteran: Scelonidae). The Canadian Entomologist 128: 273-291

Jenkins TM, Eaton TD (2011) Population genetic baseline of the first plataspid stink bug symbiosis (Hemiptera: Heteroptera: Plataspidae) reported in North America. Insects 2: 264-272

Lahiri S, Orr D, Sorenson C, Cardoza Y (2015) Overwintering refuge sites for *Megacopta cribraria* (Hemiptera: Plataspidae). Journal of Entomological Science 50: 69-73

Law JH, Regnier FE (1971) Pheromones. Annual Review of Biochemistry 40: 533-548

Maffei ME, Mithofer A, Boland W (2007) Insects feeding on plants: Rapid signals and responses preceding the induction of phytochemical release. Phytochemistry 68: 2946-2959.

Medal J, Halbert S, Smith T, Cruz AS (2013) Suitability of selected plants to the bean plataspid, *Megacopta cribraria* (Hemiptera: Plataspidae) in no-choice tests. Florida Entomologist 96: 631-633

Medal J, Cruz AS, Williams K, Fraser S, Wolaver D, Smith T, Davis BJ (2015) First record of *Paratelenomus saccharalis* (Hymenoptera: Platygastridae) on kudzu bug *Megacopta cribraria* (Heteroptera: Plataspidae) in Florida. Florida Entomologist 98: 1250-1251

Medal J, Halbert S, Cruz AS, Smith T, Davis BJ (2016) Greenhouse study to determine the host range of the kudzu bug, *Megacopta cribraria* (Heteroptera: Plataspidae). Florida Entomologist 99: 303-305

Mian L, Mulla M (1992) Effects of pyrethroid insecticides on nontarget invertebrates in aquatic ecosystems. Journal of Agricultural Entomolology 9: 73-98

Mumm R, Dicke M (2010) Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. Canadian Journal of Zoology 88: 628-667

Ngumbi E, Chen L, Fadamiro HY (2009) Comparative GC-EAD responses of a specialist (*Microplitis croceipes*) and a generalist (*Cotesia marginiventris*) parasitoid to cotton volatiles induced by two caterpillar species. Journal of Chemical Ecology 35:1009-1020

Nordlund, DA, Lewis WJ (1976) Terminology of chemical releasing stimuli in intraspecific and interspecific interactions. Journal of Chemical Ecology 2:211-220

Pare PW, Tumlinson JH (1999) Plant volatiles as a defense against insect herbivores. Plant Physiology 121: 325-331

Price PW, Bouton CE, Gross P, McPheron BA, Thompson JN, Weiss AE (1980) Interaction among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Annual Review of Ecology, Evolution, and Systematics 11:41-65

Rajmohan K, Narendran TC (2001). Parasitoid complex of *Coptosoma cribrarium* (Fabricius) (Plataspididae [sic]: Hemiptera). Insect Environment 6:163

Ruberson JR, Takasu K, Buntin GD, Eger JE, Gardner WA, Greene JK, Jenkins TM, Jones WA, Olson DM, Roberts PM et al. (2012) From Asian curiosity to eruptive American pest: *Megacopta cribraria* (Hemiptera: Plataspidae) and prospects for its biological control. Applied Entomology and Zoology 48: 3-13

Sabelis MW, van Baalen M, Bakker FM, Bruin J, Drukker B, Egas M, Janssen ARM, Lesna IK, Pels B, Van Rijn P, Scutareanu P (1999) The evolution of direct and indirect plant defense against herbivorous arthropods. In: Olff, H, Brown VK, Drent RH (Eds.), Herbivores: Between Plants and Predators. Blackwell Science, Oxford109-166

Seiter NJ, Greene JK, Reay-Jones FPF (2013a) Reduction of soybean yield components by *Megacopta cribraria* (Hemiptera: Plataspidae). Journal of Economic Entomology 106:1676-1683

Seiter NJ, Reay-Jones FPF, Greene JK (2013b) Within - field spatial distribution of *Megacopta cribraria* (Hemiptera: Plataspidae) in soybean (Fabales: Fabaceae). Environmental Entomology 42: 1363-1374

Seiter NJ, Benson E. P, Reay-Jones FPF, Greene JK, Zungoli PA (2013c) Residual efficacy of insecticides applied to exterior building material surfaces for control of nuisance infestations of *Megacopta cribraria* (Hemiptera: Plataspidae). Journal of Economic Entomology 106: 2448-2456

Seiter NJ, Grabke A, Greene JK, Kerrigan JL, Reay-Jones FPF (2014a) *Beauveria bassiana* is a pathogen of *Megacopta cribraria* (Hemiptera: Plataspidae) in South Carolina. Journal of Entomological Science 49: 326-330

Seiter NJ, Greene JK, Reay-Jones FPF (2014b) Aggregation and oviposition preferences of *Megacopta cribraria* (Hemiptera: Plataspidae) in laboratory bioassays. Journal of Entomological Science 49: 331-335

Seiter NJ, Greene JK, Reay-Jones FPF, Roberts PM, All JN (2015) Insecticidal control of *Megacopta cribraria* (Hemiptera: Plataspidae) in soybeans. Journal of Entomological Science 50: 263-283

Shelton AM, Badenes-Perez FR (2006) Concepts and applications of trap cropping in pest management. Annual Review of Entomology 51:285-308

Specht JE, Rees JM, Zoubek GL, Glewen KL, VanDeWalle BS, Schneider JW, Varner DL, Vyhnalek AR (2012). Soybean planting date: when and why. University of Nebraska-Lincoln Extension Publication EC 145: 1-7

Stubbins FL, Agudelo P, Reay-Jones FPF, Greene JK (2015) First report of a mermithid nematode infecting the invasive *Megacopta cribraria* (Hemiptera: Plataspidae) in the United States. Journal of Invertebrate Pathology 127: 35-37

Stern VM (1969) Interplanting alfalfa in cotton to control lygus bugs and other pests. In Proceedings of Tall Timbers Conference on Ecological Animal Control by Habitat Manipulation 27-28 February 1969. Tallahassee, Florida. I: 55-69

Snodgrass GL (1996) Insecticide resistance in field populations of the tarnished plant bug (Heteroptera: Miridae) in cotton in the Mississippi Delta. Journal of Economic Entomology 89: 783-790

Srinivasaperumal S, Samthitavelu P, Muthukrishnan J (1992) Host plant preference and life table of *Megacopta cribraria* (Fab.) (Hemiptera: Plataspidae). Proceedings of the Indian National Science Academy Biological Science 58: 333-340

Suiter DR, Ames LM, Eger JE Jr, Gardner A (2010a) *Megacopta cribraria* as a nuisance pest. University of Georgia, Cooperative Extension Circular 991: 2

Suiter DR, Eger JE Jr, Gardner WA, Kemerait RC, All JN, Roberts PM, Greene JK, Ames LM, Buntin GD, Jenkins TM et al (2010b) Discovery and distribution of *Megacopta cribraria* (Hemiptera: Heteroptera: Plataspidae) in northeast Georgia. Journal of Integrated Pest Management 1: 1-4

Swezey SL, Daxl RG (1988) Area-wide suppression of boll weevil populations in Nicaragua. Crop Protection 7: 168-176

Takabayashi J, Dicke M (1996) Plant-carnivore mutualism through herbivore-induced carnivore attractants. Trends in Plant Science 1:109-113

Takasu K, Hirose Y (1985) Seasonal egg parasitism of phytophagous stink bugs in a soybean field in Fukuoka. Proceedings of the Association for Plant Protection of Kyushu 31:127-131 (in Japanese with English summary)

Thippeswamy C, Rajagopal BK (2005b) Comparative biology of *Coptosoma cribraria* Fabricius on field bean, soybean, and redgram. Karnataka Journal of Agricultural Science 18: 138-140

Tumlinson JH, Lewis WJ, Vet LEM (1993) How parasitic wasps find their hosts. Scientific American 268:100-106

Turlings TCJ, Wackers FL, Vet LEM, Lewis WJ, Tumlinson JH (1993) Learning of host-finding cues by Hymenopterous parasitoids. In: Papaj DR, Lewis AC (Eds.), Insect learning: Ecological and Evolutionary Perspectives. Chapman and Hall, New York, pp. 51-78

Vet LEM, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. Annual Review of Entomology 37: 141-172

Wallingford AK (2012) Investigating host plant selection of harlequin bug, *Murgantia hisrionica* (Hahn), in order to improve a trap cropping system for its management. Ph.D. dissertation. Virginia Polytechnic Institute and State University.

Wang ZX, Wang HD, Chen GH, Zi ZG, Tong CW (1996) Occurrence and control of *Megacopta cribraria* (Fabricius) on soybean. Plant Protection 22: 7-9

Wilkinson JD, Biever KD, Ignoffo CM (1979) Synthetic pyrethroid and organophosphate insecticides against the parasitoid *Apanteles marginiventris* and the predators *Geocoris punctipes*, *Hippodamia convergens* and *Podisus maculiventris*. Journal of Economic Entomology 72: 473- 475

Willson HR, Trammel K (1980) Sex pheromone trapping for control of codling moth, Oriental fruit moth, lesser appleworm, and three tortricid leafrollers in a New York apple orchard. Journal of Economic Entomology 73: 291-295

Wu MX, Wu ZQ, Hua SM (2006) A preliminary study on some biological characters of the globular stink bug, *Megacopta cribraria*, and its two egg parasitoids. Journal of Fujian Agriculture and Forestry University (Natural Science Edition)2006: 147-150

Xing GN, Zhao TJ, Gai JY (2006) Evaluation of soybean germplasm in resistance to globular stink bug. Acta Agronomica Sinica 32: 491-496

Xing GN, Zhou B, Zhao TJ, Yu DY, Xing GH, Chen SY, Gai JY (2008) Mapping QTLs of resistance to *Megacopta cribraria* (Fabricius) in soybean. *Acta Agronomica Sinica* 34: 361-368

Zhang Y, Hanula JL, Horn (2012) The biology and preliminary host range of *Megacopta cribraria* (Heteroptera: Plataspidae) and its impact on kudzu growth. Environmental Entomology 41: 40-50

Zhixing W, Huadi W, Guihua C, Zi Z, Caiwen T (1996) Occurrence and control of *Megacopta cribraria* (Fabricius) on soybean (English abstract). Plant Protection 22: 7-9

CHAPTER 2

HOST PREFERENCE OF *MEGACOPTA CRIBRARIA* (HEMIPTERA: PLATASPIDAE) ON FOUR LEGUME CULTIVARS: IMPLICATIONS FOR TRAP CROPPING

2.1 Abstract

To investigate host preference and identify potential trap crops, the attraction of *M. cribraria* to four legume cultivars, soybean (*Glycine max* (L.) (Merr.), speckled bean (*Phaseolus lunatus* L.), lima bean (*Phaseolus limensis* L.), and Jackson wonder bean at different phenological stages, was evaluated in multiple-choice screen house experiments. *Megacopta cribraria* showed significant attraction to early vegetative and late reproductive growth stages of speckled beans and lima beans, and to the early reproductive growth stage of soybean. Jackson wonder bean was the least preferred cultivar at all growth stages, except for the late reproductive growth stage in which *M. cribraria* showed equal attraction to all four legume cultivars. Field tests were carried out to further evaluate the preferred cultivars as potential perimeter trap crops at two planting dates, two weeks before or at the same time as the main crop. Soybean trap crop planted either two week before or at the same time as the main crop was the most effective at intercepting *M. cribraria*.

2.2 Introduction

Megacopta cribraria F. (Hemiptera: Plataspidae), commonly called kudzu bug is an invasive pest of soybean in the United States. Many leguminous and non-leguminous host plants have been associated with *M. cribraria* in its native Asian range as well as in the United States (Srinivasaperumal et al. 1992; Eger et al. 2010; Zhang et al. 2012). Eger et al. (2010) provided a detailed compilation of leguminous and non-leguminous host plants associated with *M. cribraria* dating as far back as 1910, some of which are not necessarily reproductive or developmental hosts. Among the leguminous host plants listed are kudzu (*Puereria montana* var. *lobata* (Willd.) (Ohwi), soybean (*Glycine max*, Merrill), pigeon pea (*Cajanus cajan* L.), lablab bean (*Lablab purpureus* L.), mung bean (*Phaseolus radiatus* L.), lima bean (*Phaseolus lunatus* L.), and lespedeza (*Lespedeza cyrtobotrya* Miq.). The non-leguminous host plants include cotton (*Gossypium hirsutum* L.), sweet potato (*Ipomoea batatas* Lam.), wheat (*Triticum aestivum* L.), firecracker (*Crossandra infundibuliformis* L.), and jute (*Corchorus capsularis* L.). A preliminary host range study that focused mainly on forest legumes indicated soybean and kudzu as the only reproductive hosts of *M. cribraria* in southeastern United States, but also noted that females could oviposit on a wide range of other legumes without completing development on them (Zhang et al. 2012). In a no-choice bioassay of twelve legumes, complete development occurred on kudzu, soybean, pigeon pea, black-eye pea, lima bean, and pinto bean. Oviposition was observed on peanut and jicama but no development was recorded (Medal et al. 2013). While Medal et al. (2013, 2016) did not find any significant difference in the rate of oviposition of *M. cribraria* on kudzu and soybean, Zhang et al. (2012) reported a higher oviposition rate on kudzu. Host preference studies by Blount et al. (2015) showed that adults and nymphs exhibit variable survival and preference among different legumes. In addition to soybean and kudzu, *M. cribraria* completed development on edamame and pigeon pea. Also, Golec et al. (2015) reported oviposition and development on soybean, mung bean and lima bean. This contradicts the findings of Medal et al. (2013) in which no oviposition or development was observed on mung bean.

The objective of this study was to investigate host plant preference and identify potential trap crops for managing *M. cribraria* in soybean. To achieve this, the attraction of *M. cribraria* to four legume cultivars, soybean, speckled bean, lima bean and Jackson wonder bean, was evaluated at different phenological stages in a screen house (Auburn University Plant Sciences screen house facility). Legume cultivars tested in this study were chosen based on observations made on a grower's field in Lee Co. Alabama, which indicated their attractiveness to *M. cribraria*.

2.3 Materials and Methods

2.3.1 Screen house preference test

This study was conducted between May and September of 2015 in a screen house at Auburn University Plant Sciences Center. Treatments included soybean, speckled bean, lima bean and Jackson wonder bean. All four legume cultivars belong to the family Fabaceae and have synchronized phenological growth stages (i.e., same period of germination, flowering, pod, and seed development). Total developmental period for each cultivar is about 70 days. A multiple-choice bioassay was set up to determine attraction of *M. cribaria* to these select legume cultivars at four phenological growth stages (Fehr et al. 1971): early vegetative, late vegetative, early reproductive and late reproductive stages. For this study, early vegetative stage refers to plants with two to three leaf nodes, equivalent to V1-V2 stages of soybean. Late vegetative stage is characterized by four leaf nodes and above but not yet flowering, equivalent toV3-V5 stage of soybean growth stage. Early reproductive stage is signaled by the onset of flower development until about 50% of flower buds have bloomed, which corresponds to R1-R2 soybean growth stage. The appearance of pods and seed development, equivalent to R3-R5 stages of soybean growth stage indicates late vegetative stage (Fehr et al. 1971). The screen house used for the study is an open air structure in which treatment plants and insects were exposed to natural environmental conditions. A systemic non-selective herbicide containing 2% glyphosate was used to clear the weeds growing within and around the screen house three weeks before the start of experiment and manually weeded subsequently. Cages were thoroughly cleaned out after testing each phenological stage.

2.3.1.1 Legume cultivars evaluated

The following four legume cultivars commonly grown in the southeastern United States were evaluated: soybean "Pioneer P49T97R-SA2P", speckled bean (Christmas lima or speckled calico), lima bean and Jackson wonder bean. Seeds were purchased from Taleecon Farmers' Cooperative in Notalsuga, AL. Seedlings were raised in 60-well seed trays at one seed per well under controlled greenhouse conditions (26±2^{o}C and 55±5% RH). Seedlings (≈ one week old) were transplanted into pots (28 cm diameter × 25 cm high) at 5 seedlings per pot. Plants were grown with sunshine potting mixture containing 70-80% Canadian sphagnum grower grade peat moss, coarse grade perlite, coarse grade permiculite, dolomite limestone for pH adjustment, gypsum, and wetting agent (SunGro Horticulture, WA). Plants were watered ad libitum and water soluble N-P-K fertilizer mixture (20-10-20) with macronutrients (Scott-Sierra Horticultural Product Company, Marysville, OH) was added once a week after transplant. Due to the climbing nature of speckled bean and lima bean, a tomato trainer (40 cm diameter× 109 cm high) was placed over each pot to allow plant tendrils curl around it. Plants from the same batch were used for the bioassays as soon as the growth stage needed was attained. All four legume cultivars received the same handling.

2.3.1.2 *Megacopta cribraria* rearing

Adult *M. cribraria* used for this study were collected as fifth instar nymphs from kudzu *Puereria Montana* var. *lobata* patches in Auburn, AL during summer 2015 and reared on rattle snake bean *Phaseolus vulgaris* L. under controlled greenhouse conditions (25 ± 2^{o}C; 50 ± 10% RH, and a photoperiod of 14:10 L:D). This ensured that adults of known age were used for the bioassays. Nymphs were collected weekly and kept in separate cages until adult emergence. Fifty pairs of starved adults (2-5-d old) were placed in clear plastic Petri dishes (150 mm diameter × 30 mm high) before they were transferred to the screen house. Nymph identification and sex determination was based on the description by Eger et al. (2010).

2.3.1.3 Host preference tests

Multiple-choice tests. A multiple-choice bioassay was conducted in a screen house to determine the preference of adult *M. cribraria* for speckled bean, lima bean, Jackson wonder bean and soybean at four phenological stages: early vegetative, late vegetative, early reproductive and late reproductive growth stages. Cages (180 × 180 × 140 m) made with PVC pipes (2 cm diameter) frame covered with a mosquito mesh (SCS Ltd., Lake Ariel, PA) were used for the bioassay. For each phenological stage, all four treatments were tested concurrently by placing pots containing treatment plants at the corners of the cage equidistant from the center. The arrangement was replicated four times for each phenological stage and the position of the treatments was randomized within the replicates. Fifty pairs of *M. cribraria* were released in a Petri dish (150 mm diameter × 30 mm high) at the center of the cage. The number of *M. cribraria* on each treatment was counted 24 h after introducing insects and subsequently for 9 d.

Two choice tests. A two-choice bioassay between soybean and speckled bean was conducted at early and late vegetative stages. The methodology and procedures were similar to the multiple-choice tests described above.

2.3.2 Field trial

Soybean "Pioneer P49T97R-SA2P" , speckled bean and lima bean were evaluated as potential trap crops at the E.V. Smith Research Station (Shorter, Alabama) in summer of 2016 using standard agronomic practices. Seeds were purchased from Taleecon Farmers' Cooperative in Notalsuga, AL. Trap crops were planted either two weeks earlier (May, 24) or at the same time (June, 7) as the main crop. Experimental plots were arranged in randomized complete blocks with four replicates per treatment. Each plot (14 × 10 m) consisted of eight rows of soybean (Pioneer P49T97R-SA2P) as the main crop and two rows of trap crop on all four sides, such that it completely encircled the main crop (i.e., perimeter trap cropping). Seeds for the main crop were planted at a rate of 10 seeds per foot with 91 cm row spacing. The experiment was replicated four times and were separated by 4.6 m unplanted (bare ground) buffer zones. Plots were evaluated weekly by visually sampling six randomly selected plants from the soybean main crop and eight, one from each row of the trap crop for adult *M. cribraria*.

2.3.3 Data Analyses

Host preference tests. Data were not normally distributed and were therefore square-root ($\sqrt{x}$ + 0.5) transformed to attain normality (Sharma and Fadamiro 2013, Fadamiro et al. 2013). Transformed data were analyzed with repeated measures multivariate analysis of variance (MANOVA) with treatment and date as the main factors, and time as the repeated measures factor (Ott and Longnecker 2001, Norman and Streiner 2008, Frank et al. 2011) to determine significant effects of date, treatments, and date*treatment interactions. The result of MANOVA showed no significant date*treatment interaction for all phenological stages tested. Hence, the data were pooled for each treatment and analyzed again using one way analysis of variance (ANOVA). Mean comparisons were done by using the Tukey-Kramer honestly significant difference (HSD) test. Data from the two-choice bioassay were analyzed using a two-sample t-test ($P < 0.05$; JMP Pro 11.2.1, SAS Institute Inc. 2013).

Field trial. Data were analyzed with repeated measures multivariate analysis of variance (MANOVA) with treatment and date as the main factors, and time as the repeated measures factor (Ott and Longnecker 2001, Norman and Streiner 2008, Frank et al. 2011) to determine significant effects of date, treatments, and date*treatment interactions. The result of MANOVA showed no significant date*treatment interaction among trap crop treatments. Thus, data were pooled and analyzed again by ANOVA. Trap crop vs. main comparison was analyzed using a two-sample t-test. Mean comparisons were done by using the Tukey-Kramer honestly significant difference (HSD) test ($P < 0.05$; JMP Pro 11.2.1, SAS Institute Inc. 2013).

2.4 Results

2.4.1 Multiple-choice tests

Early vegetative stage. Data were first analyzed with ANOVA to determine treatment effect on each sampling date. The results showed that *M. cribaria* actively discriminated among the four host plants within 24-h after release and remained more or less on the same host plants after making the choice. Significantly greater numbers ($F = 3.78$; df = 3, 13; $P < 0.04$) of *M. cribaria* were recorded on speckled bean (10.75 ± 3.4) and lima bean (6.75 ± 3.11) than on Jackson wonder bean (2.0 ±0.91) and soybean (0.5 ±0.5) as early as 24 h after they were released in the cage. However, numbers were not significant different among the host plants on day 2 to day 8. Significantly more ($F = 10.49$; df = 3, 13; $P < 0.0011$) *M. cribaria* were recorded again on day 9 on speckled bean (6.0 ± 1.4) and lima bean (5.2 ± 1.11) than on Jackson wonder bean (0.5 ± 0.28) and soybean (0.2 ±0.2) (Fig. 1a).

Further analysis of the data using repeated measures MANOVA showed no significant date*treatment interaction (F = 0.35; df = 24, 108; P = 0.997). Thus, the data were pooled and reanalyzed using one way ANOVA to determine significant treatment effects. *Megacopta cribraria* showed preference among the treatments. Significantly greater numbers (F = 29.3680; df = 3, 137; P < 0.0001) of *M. cribraria* were found on speckled bean (12.944 ± 1.653) and lima bean (10.055 ± 1.374) than on Jackson wonder bean (1.722 ± 0.477) and soybean (0.611 ± 0.324) (Fig. 1b).

Late vegetative stage. Data were first analyzed with ANOVA to determine treatment effect on each sampling date. The results showed no significant difference in *M. cribaria* densities among the host plants on day 1 (F = 0.1192; df = 3, 13; P < 0.947). A similar trend was observed on day 2 and continued throughout the remaining observation period (Fig. 2a). Further analysis of the data using repeated measures MANOVA showed no significant effect of either treatment (F = 1.2083; df = 3, 137; P = 0.309) or date*treatment interaction (F = 0.115; df = 24, 108; P = 1.000) (Fig. 2b).

Early reproductive stage. Data were first analyzed with ANOVA to determine treatment effect on each sampling date. Results showed no significant differences in *M. cribaria* densities among the host plants on day 1 (F = 2.425; df = 3, 13; P < 0.1162). A similar trend was observed on day 2 and continued throughout the remaining observation period (Fig. 3a).

Further analysis of data using repeated measures MANOVA showed no significant date*treatment interaction (F = 0.947; df = 24, 108; P = 0.540). Thus, data were pooled and reanalyzed using one way ANOVA to determine significant treatment effects. There was a significant effect of treatment (*F* = 9.060; df = 3, 108; *P* < 0.0001). Soybean (3.083 ± 0.412) attracted significantly greater number of *M. cribraria* than the other three treatments. This is closely followed by speckled bean (2.277 ± 0.332) and Jackson wonder bean (1.250 ± 0.255). Lima bean (1.055 ± 0.248) was the least attractive to *M. cribraria* at this phenological stage (Fig. 3b).

Late reproductive stage. Data were first analyzed with ANOVA to determine treatment effect on each sampling date. The number of *M. cribaria* was not significantly different among the treatments on day 1 at late reproductive stage of the plants (*F* = 0.53; df = 3, 13; *P* < 0.6692). A similar trend was observed on day 2 up to day 7. Significant differences were observed on day 8 (*F* =3.53; df = 3, 13; *P* < 0.048) with more *M. cribaria* recorded on lima bean (19.5 ± 4.5) followed by speckled bean (8.5 ± 7.19) and soybean (6.2 ± 1.7). None was recorded on Jackson wonder bean. Similarly, significantly higher number (*F* = 4.75; df = 3, 13; *P* < 0.02) of *M. cribaria* was recorded on lima bean (22.5 ± 5.54) followed by speckled bean (7 ± 6.33) and soybean (7 ± 2.23) on day 9. None was recorded on Jackson wonder bean (Fig. 4a).

Further analysis of the data using repeated measures MANOVA showed no significant date*treatment interaction (*F* = 2.1987; df = 24, 108; *P* = 0.0911). Thus, data were pooled and reanalyzed using one way ANOVA to determine significant treatment effects. There was a significant effect of treatment (*F* = 12.8197; df = 3, 137; *P* < 0.0001). Significantly greater numbers of *M. cribraria* were found on lima bean (15.6944 ± 1.453) than either soybean (6.9167 ± 0.8714) or Jackson wonder bean (1.5833 ± 0.3931). Equal attraction was observed for lima bean (15.6944 ± 1.453) and speckled bean (12.0278 ± 2.9599). There was no significant difference in the number of *M. cribraria* attracted to speckled bean (12.0278 ± 2.9599) and soybean (6.9167 ± 0.8714), while Jackson wonder bean (1.5833 ± 0.3931) was the least preferred when compared with either lima bean (15.6944 ± 1.453) or speckled bean (12.0278 ± 2.9599) (Fig. 4b).

2.4.2 Two-choice tests

When data was analyzed by day, speckled bean was significantly more attractive to *M. cribraria* on all observation days at early vegetative growth stage. Highest significance (t_6 = 3.14, *P* = 0.019) were observed between speckled bean (22 ± 5.21) and soybean (4.5 ± 1.93) 24-h after insects were released (Fig. 5a). For the late vegetative growth stage, although soybean recorded a higher number of *M. cribraria* per day, no significant (t_6 = 0.74, *P* = 0.48) difference was observed in the attraction of *M. cribraria* to speckled bean or soybean across all days in which data was taken (Fig. 6a). When data was pooled, there was a significant preference of *M. cribraria* for speckled bean (17.1 ± 1.03) over soybean (5.1 ± 0.67), (t_{67} =9.5, *P* < 0.0001) at the early vegetative stage (Fig. 5b). However, at the late vegetative stage, significantly more *M. cribraria* preferred soybean (13.2± 1.34) over speckled bean (7.75± 1.34), (t_{67} =2.87, *P* = 0.0054) (Fig. 6b).

2.4.3 Field trial

Results showed a significant difference in the number of *M. cribaria* adults among the treatments on 14 July (*F* = 6.51; df = 6, 18; *P* = 0.0009), 21 July (*F* = 9.0217; df = 6,18; *P* = 0.0001), 27 July (*F* = 2.87; df = 6,18; *P* = 0.038), 3 August (*F* = 28.62; df = 6, 18; *P* = 0.0001), 12 August (*F* = 9.48; df = 6, 18; *P* = 0.0001), and 2 September (*F* = 4.77; df = 6, 18; *P* = 0.005). When compared among the treatments, perimeter planting of soybean at the same time as the main crop attracted significantly more *M. cribaria* on most sampling dates followed by perimeter planting of soybean two weeks prior to the main crop (Fig. 7). The number of *M. cribaria* adults on all other treatments was not significantly different from each other. When mean adult abundance was compared between trap crop and main crop, significantly fewer adults were recorded in the main crop (1.5 ± 0.35) surrounded by soybean (4.12 ± 0.90) planted either two weeks before [t $_{(63)}$ = 2.68, *P* = 0.0091] or same time as main crop [t $_{(63)}$ = 5.044, *P* = 0.0001]. These results indicate that soybean is an effective trap crop in attracting and concentrating *M. cribaria* in the field border by preventing their movement into the main crop.

2.5 Discussion

Results of the screen house preference test showed that *M. cribraria* was significantly attracted to early vegetative growth stage of speckled and lima beans than soybean and Jackson wonder bean. A two-choice preference test between soybean and speckled bean confirmed that speckled bean is preferred by *M. cribraria* at this phenological stage. *Megacopta cribraria* was also significantly attracted to early reproductive growth stage of soybean while lima bean was the preferred cultivar at late reproductive growth stage. *Megacopta cribraria* showed similar level of attraction to soybean, speckled bean, lima bean and Jackson wonder bean at the late vegetative growth stage. Jackson wonder bean was the least preferred of the four legume cultivars at all phenological stages tested. For multiple-choice preference tests, when data was analyzed by day, *M. cribraria* showed attraction to speckled bean at the early vegetative growth stage for all observation days and lima bean on day 8 and 9. Equal attraction was observed across treatments within each day at the late vegetative and early reproductive growth stages. For the two-choice preference test between soybean and speckled bean, *M. cribraria* showed greater attraction to speckled bean at early vegetative growth stage. Although, greater numbers of *M. cribraria* showed preference for soybean at late vegetative growth stage, there was no statistical difference in the attraction of *M. cribraria* to soybean and speckled bean for all observation days.

Megacopta cribraria demonstrated differential attraction to legume cultivars and actively discriminated among treatments at different phenological stages. This indicates that plant phenology plays a role in host preference of *M. cribraria*. The implications of phenology in the effectiveness of a trap crop has been demonstrated by Smyth et al. (2003) in which host plant phenology affected ovipositional preference of *Crocidolomia pavonana* (= *binotalis*) (F.), a pest of cruciferous plants. Jackai (1981) highlighted the link between cowpea *Vigna unguiculata* (Walp.) phenological stages and infestation by *Maruca testulalis* (Geyer). The attraction of *M. cribraria* to soybean at the late vegetative and early reproductive growth stages is indicative of susceptibility of soybean to *M. cribraria* at these two stages which also coincide with the stage at which *M. cribraria* was observed moving into soybean fields (Seiter et al. 2013a). This is the first study to evaluate the preference of *M. cribraria* to legume cultivars at different phenological stages in the absence of competing herbivores.

In the field trial, data collection started July 14 when first generation *M. cribraria* started migrating from kudzu into soybean. This period coincided with the late vegetative growth stage of soybean. Although populations of *M. cribraria* at this field location were generally low (< 2 per plant), lower populations were observed in midsummer (August 12) which was when 2nd generation nymphs started emerging. Soybean planted on the field border as trap crop either planted 2-wk before or same time as the main crop was the most effective at intercepting first generation *M. cribraria*. Hokkanen (1991) defined trap crop as "plant stands grown to attract insects or other organisms like nematodes to protect target crops from pest attack, preventing the pests from reaching the crop or concentrating them in a certain part of the field where they can be economically destroyed". This definition takes into account the suitability of using same species of plant as trap and main crop by varying certain modalities like seed spacing and planting time.

Megacopta cribraria showed less preference for speckled bean and lima bean in the field contrary to what was observed in the screen house tests. Plots with lima bean and speckled bean as trap crops recorded greater number of *M. cribraria* in main crop than trap crop. This can be attributed to height difference and stress factors (Fig. 9). Lima and speckled beans were much shorter than the soybean main crop they enclosed. Thus, were unable to intercept *M. cribraria* flying into the main crop. Also, the scorching effect of herbicides was more profound on the leaves of lima and speckled bean compared with soybean, which probably made the former less attractive to *M. cribraria*. However, with some adjustments, lima bean, speckled bean, and soybean could serve as suitable trap crops for managing *M. cribraria* in soybean by varying planting time to coincide with field infestation such that trap crop is available to *M. cribraria* at its most attractive phenological stage or through sequential trap crop deployment as demonstrated by Hoy et al. (2000). More studies are needed to further evaluate the prospects of these three legume cultivars as trap crops in the management of *M. cribraria* over multiple seasons in the field.

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2.7 References Cited

Blount JL, Buntin GD, Sparks AN (2015) Host preference of *Megacopta cribraria*

(Hemiptera: Plataspidae) on selected edible beans and soybean. Journal of Economic Entomology 109, 1–12

Eger JE Jr, Ames LM, Suiter DR, Jenkins TM, Rider DA, Halbert SE (2010) Occurrence of the Old World bug *Megacopta cribraria* (Fabricius) (Heteroptera: Plataspidae) in Georgia: a serious home invader and potential legume pest. Insecta Mundi 121: 1–11

Fadamiro HY, Akotsen-Mensah C, Xiao Y, Anikwe J (2013) Field evaluation of predacious mites (Acari: Phytoseiidae) for biological control of citrus red mite, *Panonychus citri* (Trombidiformes: Tetranychidae). Florida Entomologist 96: 80-91

Frank DL, Brewster CC, Leskey T, Bergh JC (2011) Factors influencing the temporal and spatial patterns of Dogwood borer (Lepidoptera: Sesiidae) infestation in newly planted apple orchards. Environmental Entomology 40: 173-183

Fehr WR, Caviness CE, Burmood DT, Pennington JS (1971) Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Science 11: 929-931

Golec JR, Hu XP, Yang L, Eger JE (2015) Kudzu-deprived first-generation *Megacopta cribraria* (F.) (Heteroptera: Plataspidae) are capable of developing on alternative legume species. Journal of Agricultural and Urban Entomology 31: 52-61

Hokkanen HMT (1991) Trap cropping in pest management. Annual Review of Entomology 36: 119-138

Hoy CW (1999) Colorado potato beetle resistance management strategies for transgenic potatoes. American Journal of Potato Research 76:215–19

Jackai LEN, Singh SR (1983) Suitability of selected leguminous plants for development of *Maruca testulalis* larvae. Entomological Experimentalis et Applicata. 34:174-78

Medal J, Halbert S, Smith T, Cruz AS (2013) Suitability of selected plants to the bean plataspid, *Megacopta cribraria* (Hemiptera: Plataspidae) in no-choice tests. Florida Entomologist 96: 631-633

Medal J, Halbert S, Cruz AS, Smith T, Davis BJ (2016) Greenhouse study to determine the host range of the kudzu bug, *Megacopta cribraria* (Heteroptera: Plataspidae). Florida Entomologist 99: 303-305

Norman GR, Streiner DL (2008) Biostatistics. The bare essentials. B.C. Decker Inc., Hamilton, Ontario, Canada.

Ott RL, Longnecker M (2001) An introduction to statistical methods and data analysis, 5th ed. Duxbury, Thompson Learning Inc., Pacific Grove, CA

Seiter NJ, Greene JK, Reay-Jones FPF (2013a) Reduction of soybean yield components by *Megacopta cribraria* (Hemiptera: Plataspidae). Journal of Economic Entomology 106:1676-1683

Sharma K, Fadamiro HY (2013) Fire ant alarm pheromone and venom alkaloids act in concert to attract parasitic phorid flies, *Pseudacteon* spp. Journal of Insect Physiology 59: 1119-1124

Smyth RR, Hoffmann MP, Shelton AM (2003) Effects of host plant phenology on oviposition preference of *Crocidolomia pavonana* (Lepidoptera: Pyralidae). Environmental Entomology 32:756-64

Srinivasaperumal S, Samthitavelu P, Muthukrishnan J (1992) Host plant preference and life table of *Megacopta cribraria* (Fab.) (Hemiptera: Plataspidae). Proceedings of the Indian National Science Academy Biological Science 58: 333-340

Zhang Y, Hanula JL, Horn (2012) The biology and preliminary host range of *Megacopta cribraria* (Heteroptera: Plataspidae) and its impact on kudzu growth. Environmental

Entomology 41: 40-50

2.8 Figure Legend

Figure 1a. Mean (± SE) number of *M. cribraria* recorded on four legume cultivars (soybean, speckled bean, lima bean, and Jackson wonder bean) at early vegetative stage in a multiple-choice screen house experiment. Fifty pairs of newly emerged and starved adult *M. cribraria* were released per test. The experiment was replicated four times. Mean number of insects across treatments within each day having no letters in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.005$)

Figure 1b. Mean (± SE) number of *M. cribraria* recorded on four legume cultivars (soybean, speckled bean, lima bean, and Jackson wonder bean) at early vegetative stage in a multiple-choice screen house experiment. Fifty pairs of newly emerged and starved adult *M. cribraria* were released per test. The experiment was replicated four times. Bars representing mean number of insects per treatment having no letters in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.005$)

Figure 2a. Mean (± SE) number of *M. cribraria* recorded on four legume cultivars (soybean, speckled bean, lima bean, and Jackson wonder bean) at late vegetative stage in a multiple-choice screen house experiment. Fifty pairs of newly emerged and starved adult *M. cribraria* were released per test. The experiment was replicated four times. Mean number of insects across treatments within each day having no letters in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.005$)

Figure 2b. Mean (± SE) number of *M. cribraria* recorded on four legume cultivars (soybean, speckled bean, lima bean, and Jackson wonder bean) at late vegetative stage in a multiple-choice screen house experiment. Fifty pairs of newly emerged and starved adult *M. cribraria* were released per test. The experiment was replicated four times. Bars representing mean number of insects per treatment having no letters in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.005$)

Figure 3a. Mean (± SE) number of *M. cribraria* recorded on four legume cultivars (soybean, speckled bean, lima bean, and Jackson wonder bean) at early reproductive stage in a multiple-choice screen house experiment. Fifty pairs of newly emerged and starved adult *M. cribraria* were released per test. The experiment was replicated four times. Mean number of insects across treatments within each day having no letters in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.005$)

Figure 3b. Mean (± SE) number of *M. cribraria* recorded on four legume cultivars (soybean, speckled bean, lima bean, and Jackson wonder bean) at early reproductive stage in a multiple-choice screen house experiment. Fifty pairs of newly emerged and starved adult *M. cribraria* were released per test. The experiment was replicated four times. Bars representing mean number of insects per treatment having no letters in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.005$)

Figure 4a. Mean (± SE) number of *M. cribraria* recorded on four legume cultivars (soybean, speckled bean, lima bean, and Jackson wonder bean) at the late reproductive stage in a multiple-choice screen house experiment. Fifty pairs of newly emerged and starved adult *M. cribraria* were released per test. The experiment was replicated four times. Mean number of insects across treatments within each day having no letters in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.005$)

Figure 4b. Mean (± SE) number of *M. cribraria* recorded on four legume cultivars (soybean, speckled bean, lima bean, and Jackson wonder bean) at the late reproductive stage in a multiple-choice screen house experiment. Fifty pairs of newly emerged and starved adult *M. cribraria* were released per test. The experiment was replicated four times. Bars representing mean number of insects per treatment having no letters in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.005$)

Figure 5a. Mean (± SE) number of *M. cribraria* recorded on two legume cultivars (soybean and speckled bean) at early vegetative stage in two-choice preference test. Fifty pairs of newly emerged and starved adult *M. cribraria* were released per test. The experiment was replicated four times. Asterisk (*) indicate significant difference in mean number of insects across treatments within each day ($P < 0.005$, Binomial test)

Figure 5b. Mean (± SE) number of *M. cribraria* recorded on two legume cultivars (soybean and speckled bean) at early vegetative stage in two-choice preference test. Fifty pairs of newly emerged and starved adult *M. cribraria* were released per test. The experiment was replicated four times. Asterisk (*) indicate significant difference ($P < 0.005$, Binomial test)

Figure 6a. Mean (± SE) number of *M. cribraria* recorded on two legume cultivars (soybean and speckled bean) at late vegetative stage in two-choice preference test. Fifty pairs of newly emerged and starved adult *M. cribraria* were released per test. The experiment was replicated four times. Mean number of *M. cribraria* attracted to soybean and speckled was not significant across all days ($P < 0.005$, Binomial test)

Figure 6b. Mean (± SE) number of *M. cribraria* recorded on two legume cultivars (soybean and speckled bean) at late vegetative stage in two-choice preference test. Fifty pairs of newly emerged and starved adult *M. cribraria* were released per test. The experiment was replicated four times. Asterisk (*) indicate significant difference ($P < 0.005$, Binomial test)

Figure 7. Mean (± SE) number of *M. cribraria* recorded on trap crops (soybean, speckled bean and lima bean) in soybean. The experiment was replicated four times. Means having no letters in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.005$)

Figure 8. Mean (± SE) number of *M. cribraria* recorded on trap crops (soybean, speckled bean and lima bean) and main crop (soybean). The experiment was replicated four times. Asterisk (*) indicate significant difference ($P < 0.005$ Two-sample t-test)

Figure 9a. Speckled bean scorched by herbicide

Figure 9b. Height difference between soybean (main crop) and speckled bean (trap crop)

Fig. 1a Early Vegetative growth stage

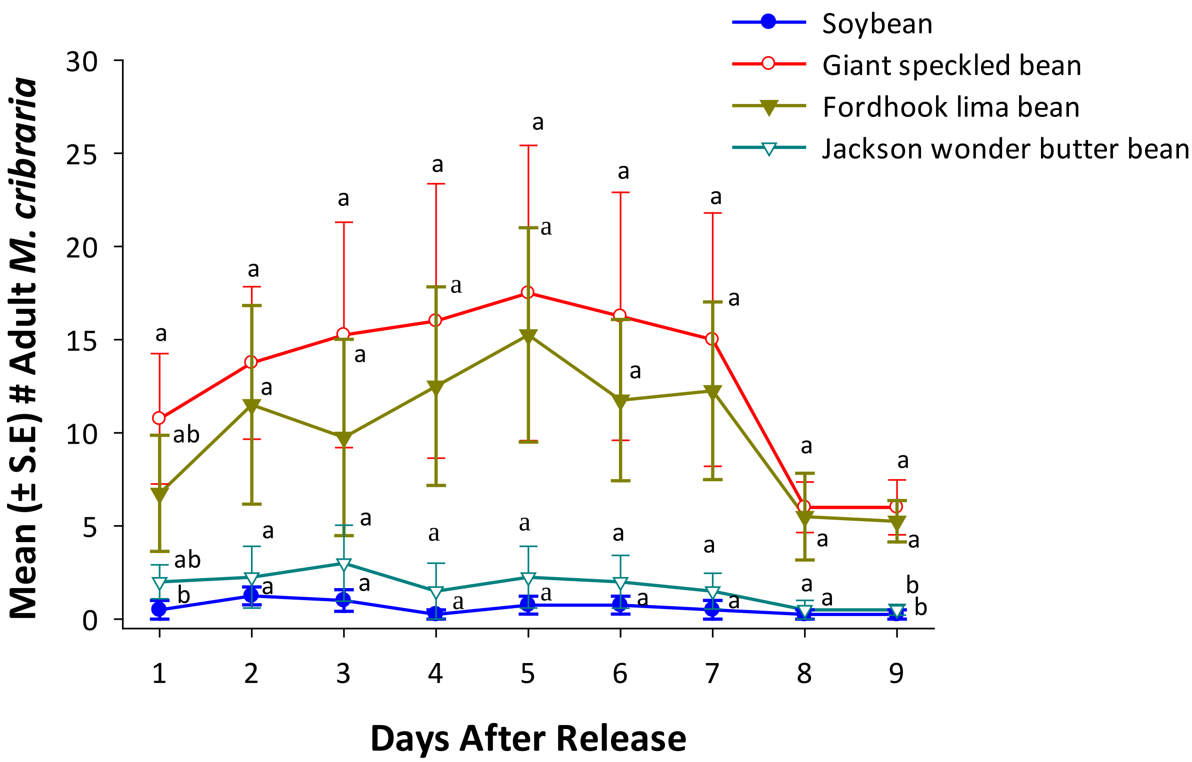
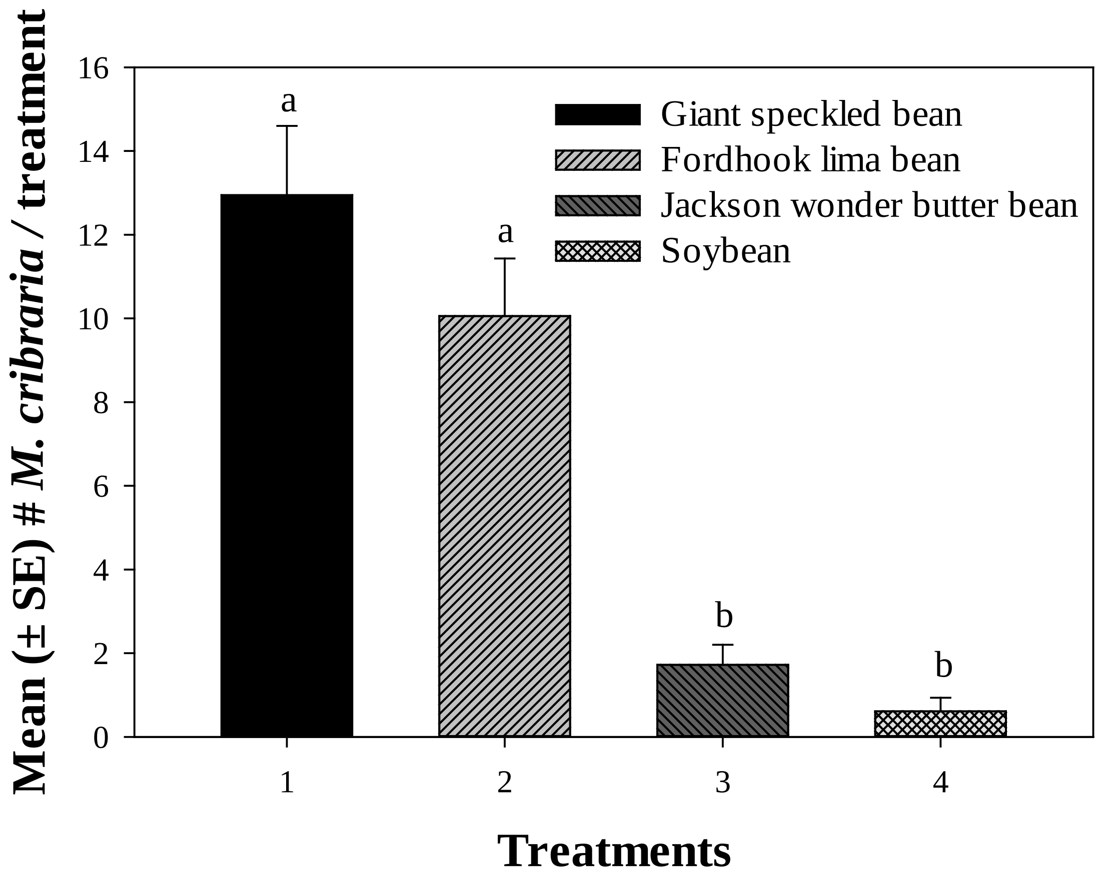


Fig. 1b Early Vegetative growth stage



2a Late Vegetative growth stage

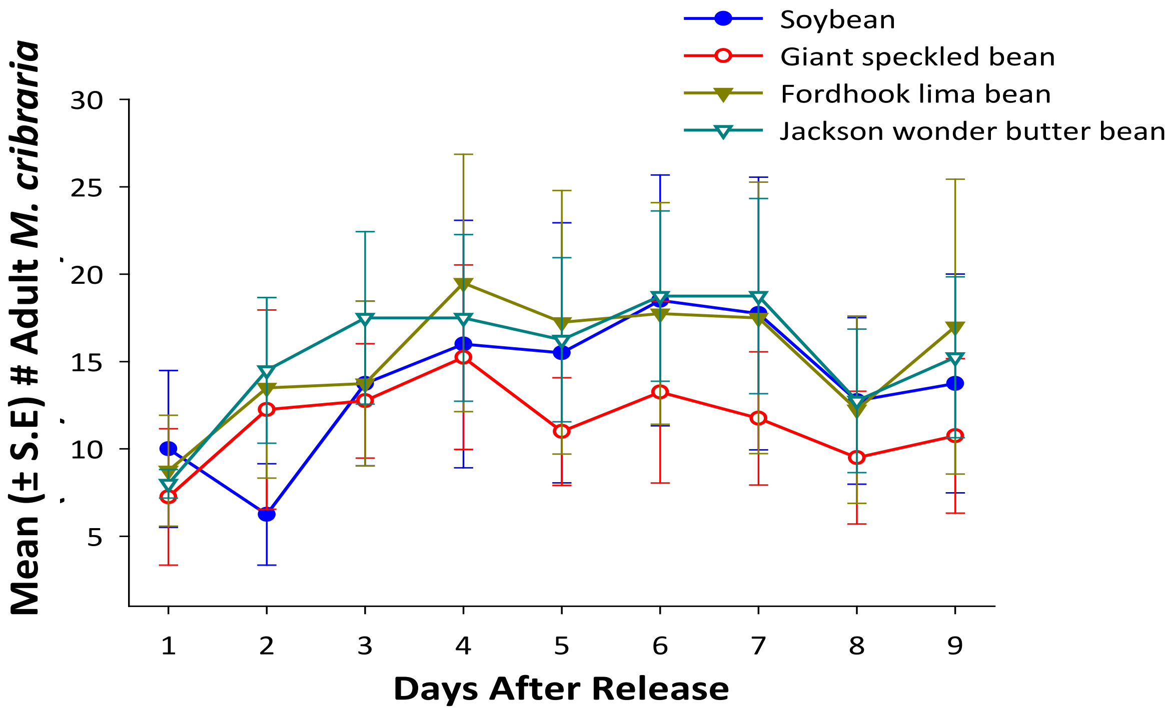


Fig. 2b Late Vegetative growth stage

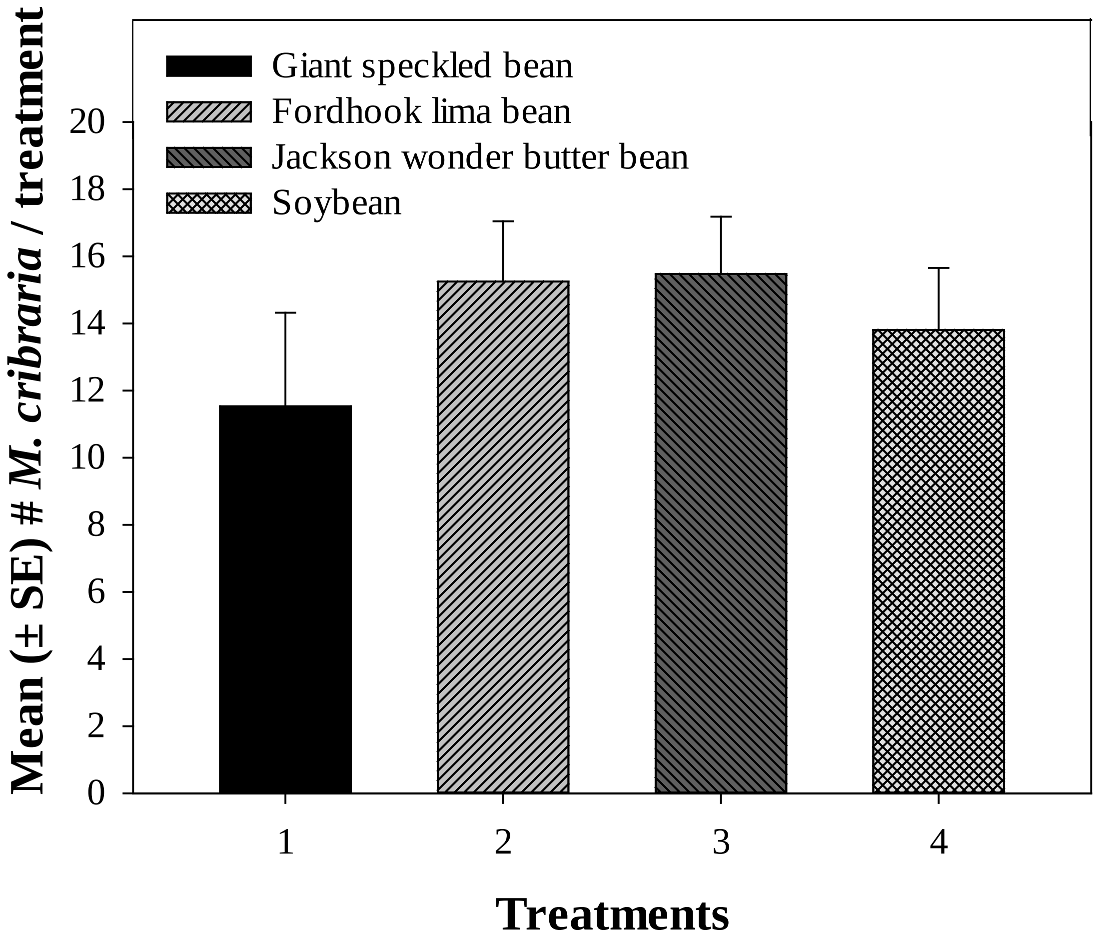


Fig. 3a Early reproductive growth stage

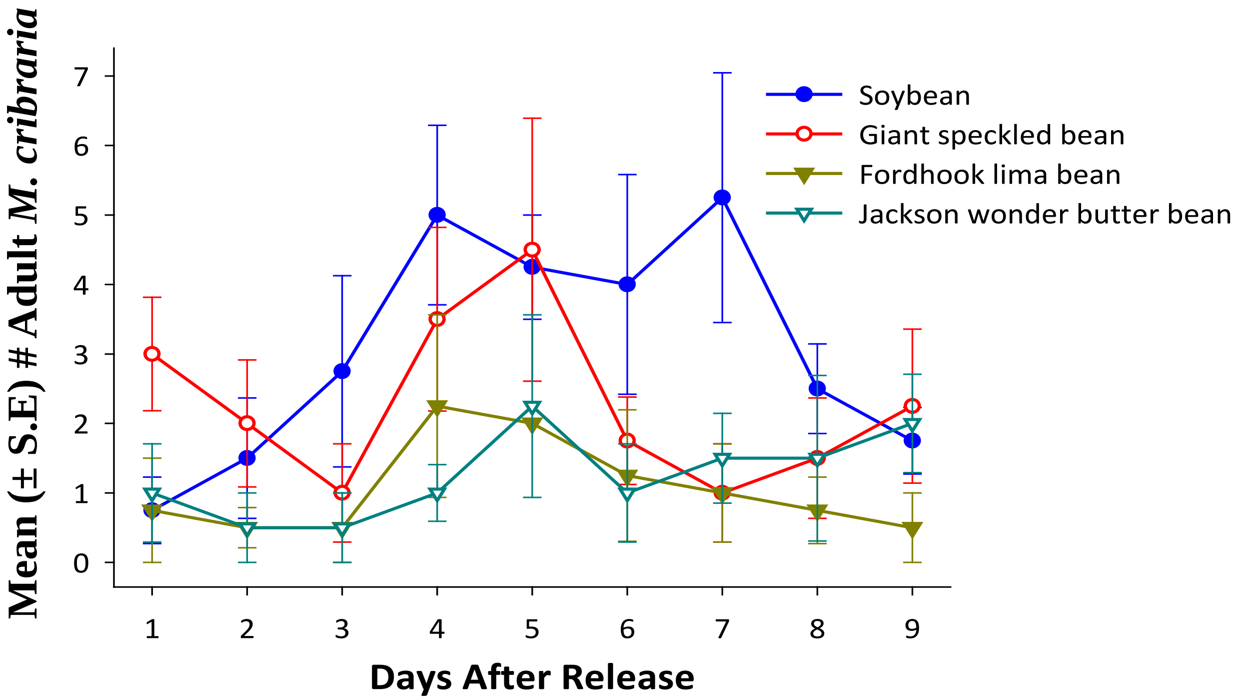


Fig. 3b Early reproductive growth stage

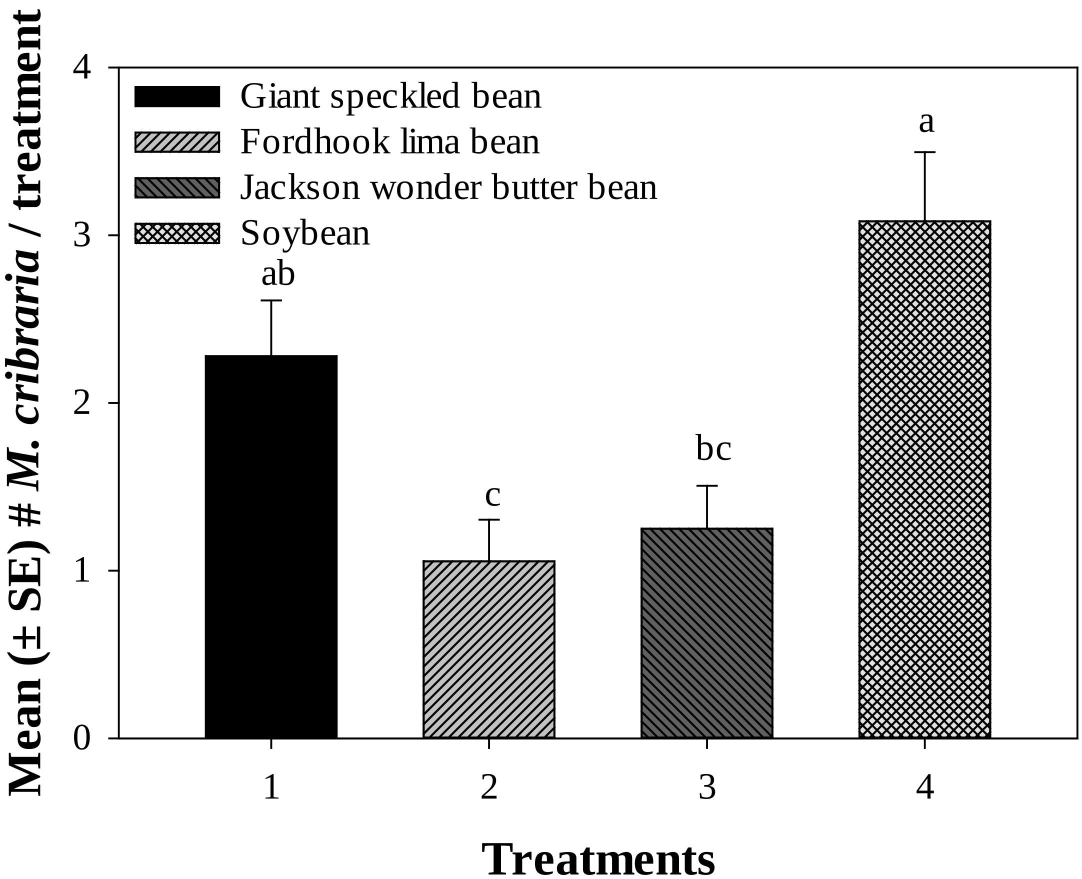


Fig. 4a Late reproductive growth stage

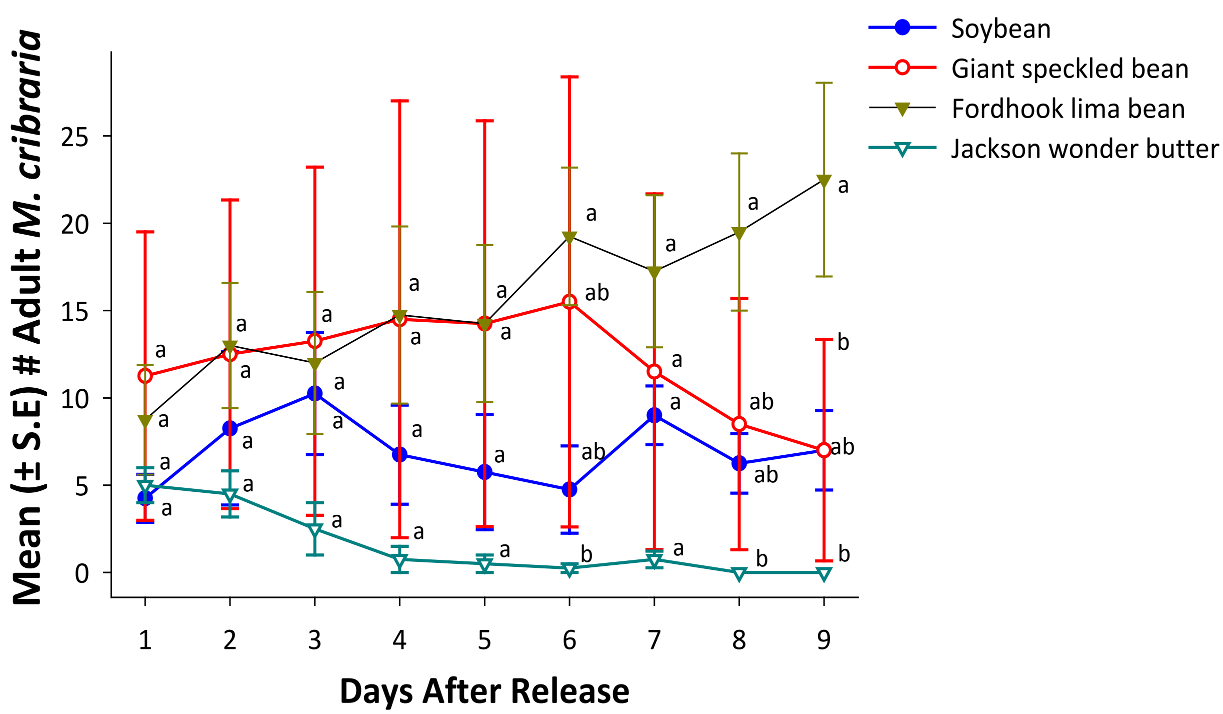


Fig. 4b Late reproductive growth stage

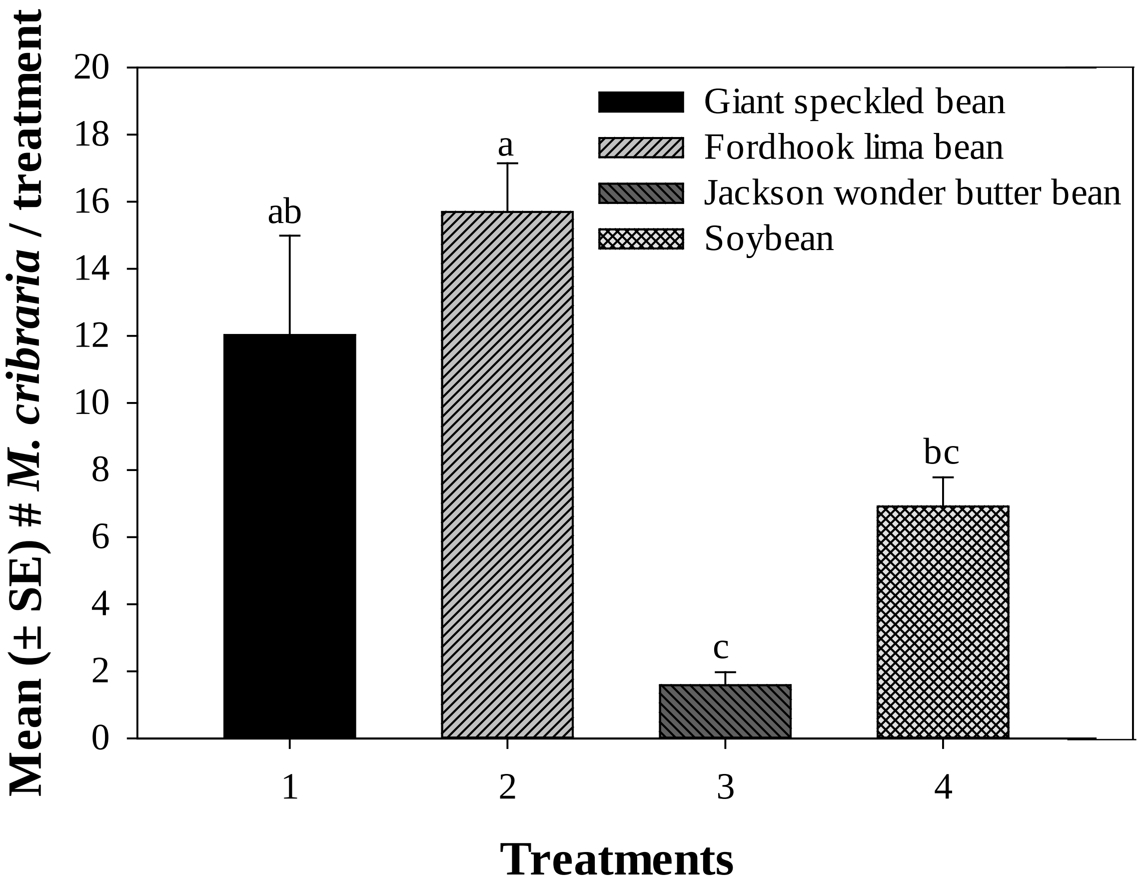


Fig. 5a Early Vegetative growth stage (Speckled Vs Soybean)

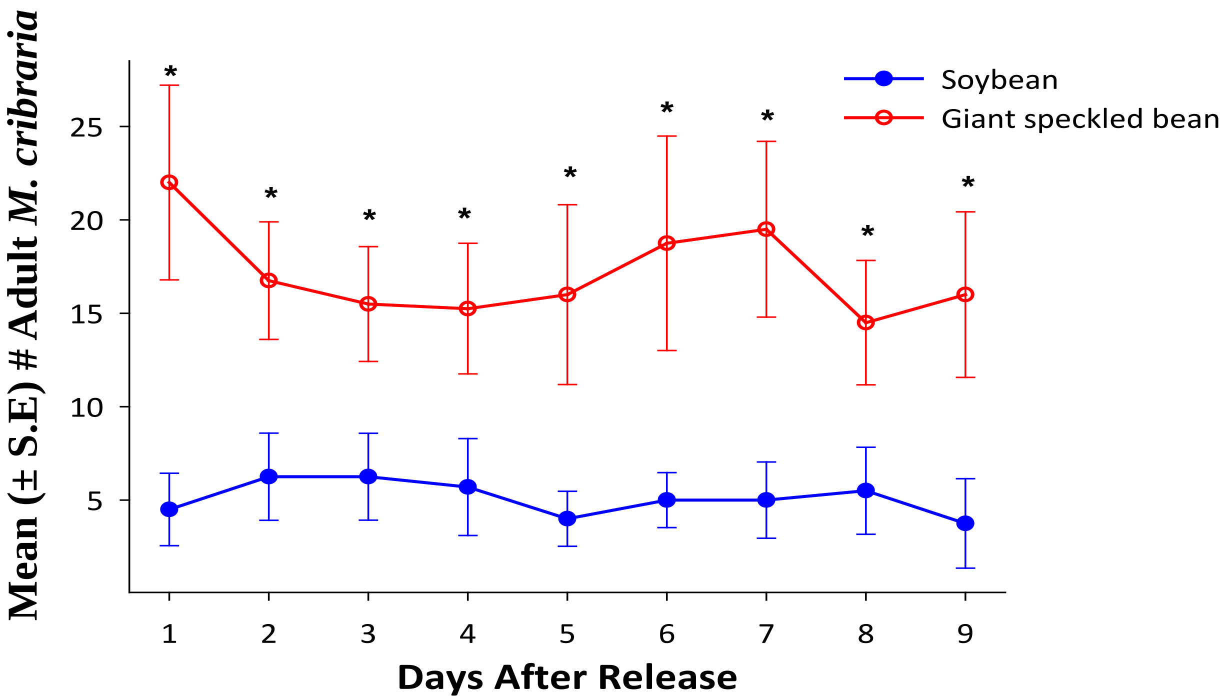


Fig. 5b Early Vegetative growth stage (Speckled Vs Soybean)

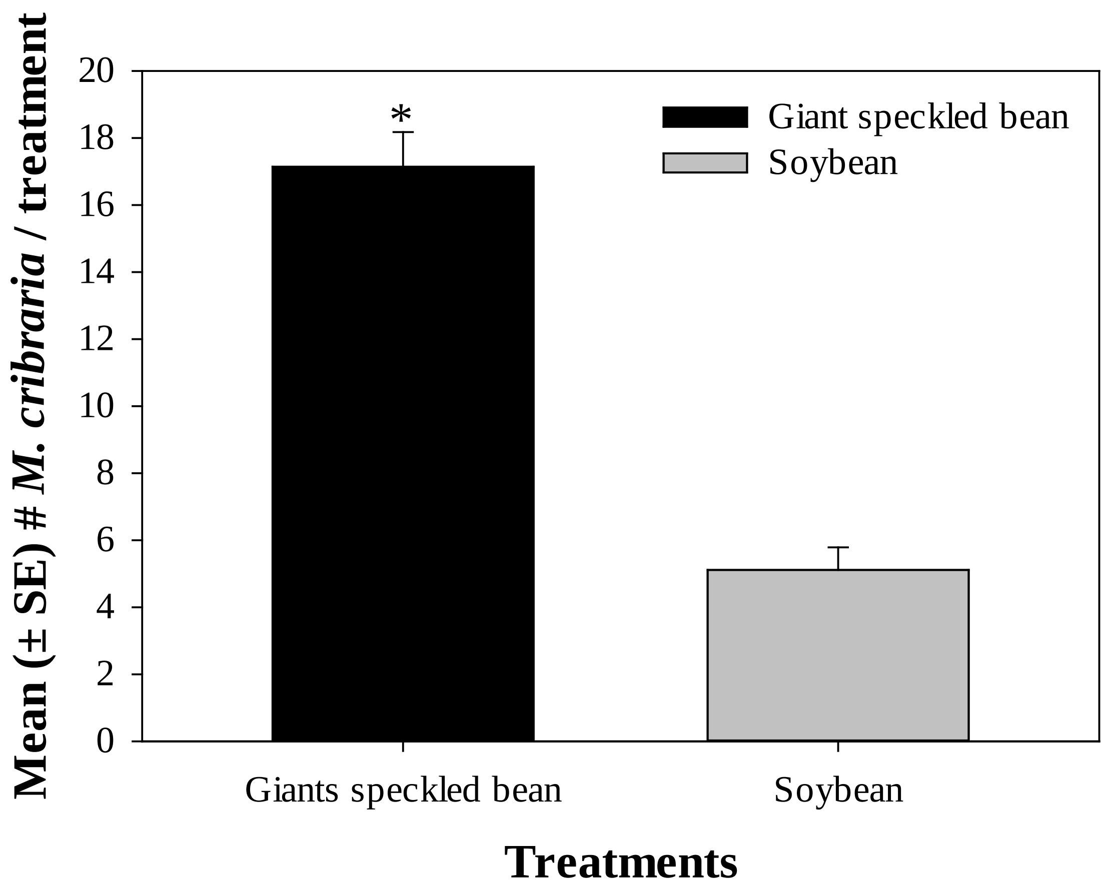


Fig. 6a Late Vegetative growth stage (Specked Vs soybean)

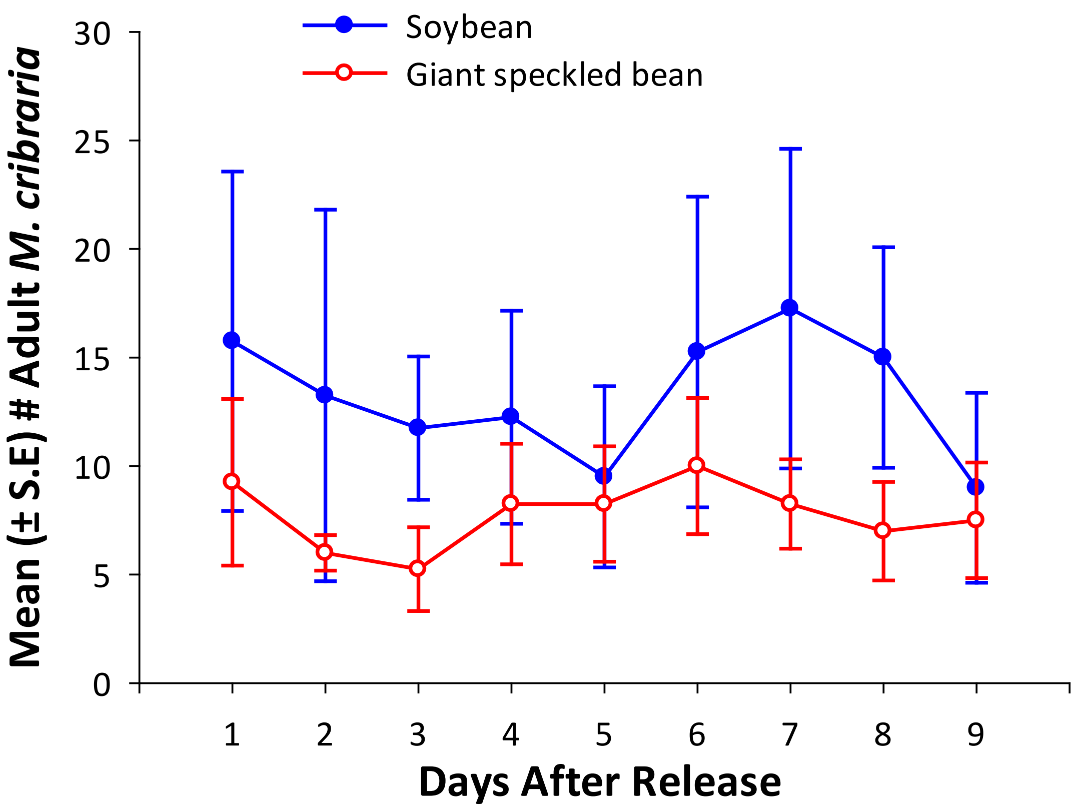


Fig. 6b Late Vegetative growth stage (Specked Vs soybean)

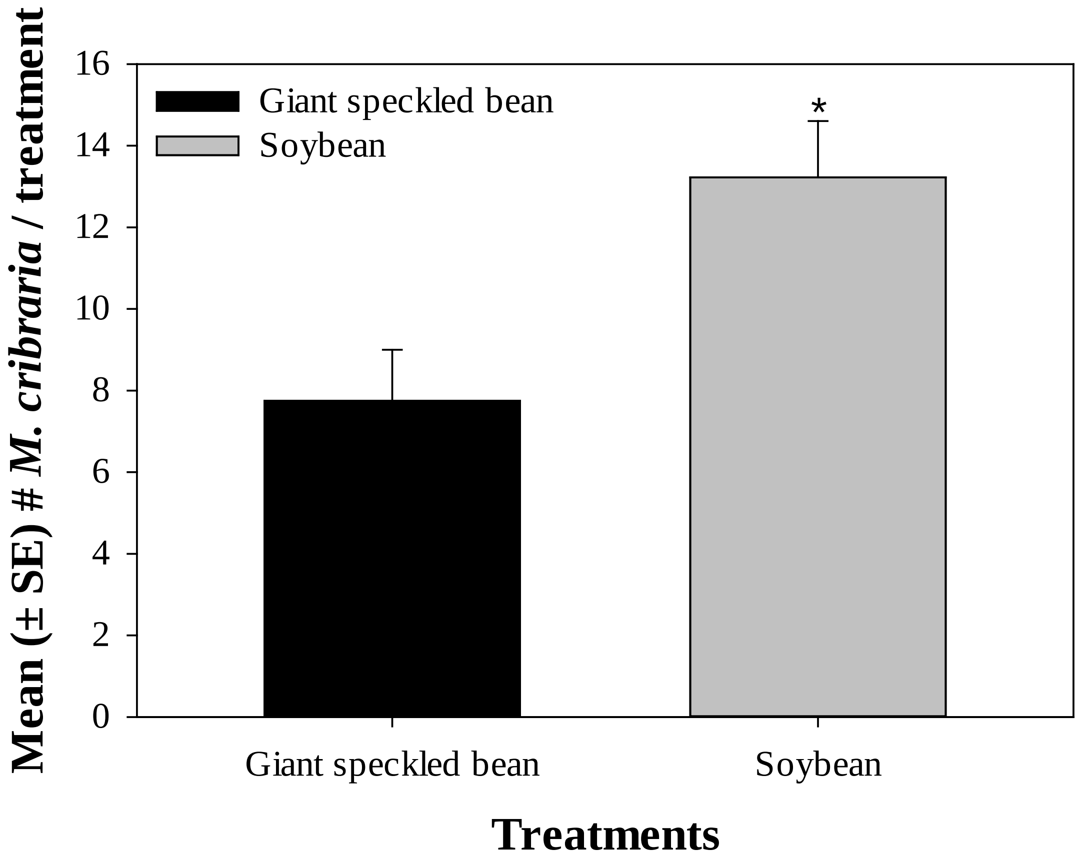


Fig. 7 Trap crop vs Trap crop comparison

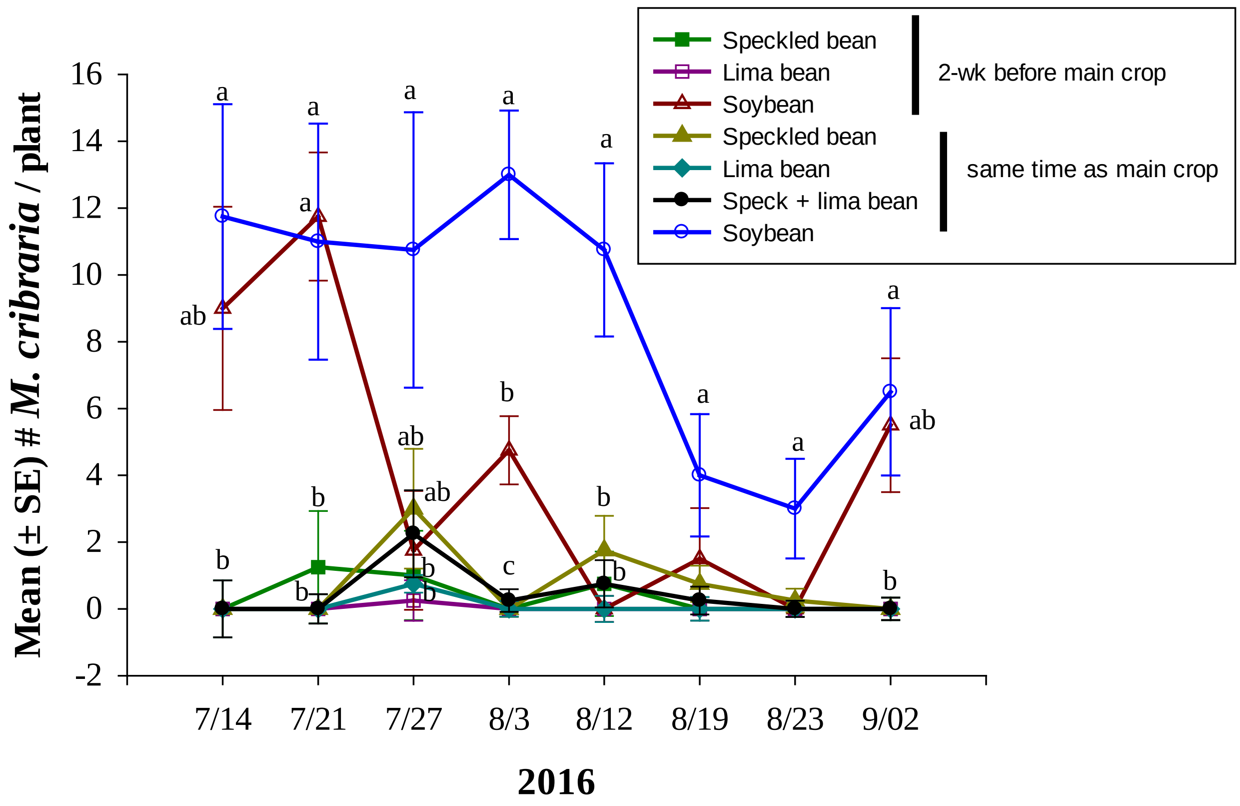


Fig. 8 Trap crop vs main crop comparison

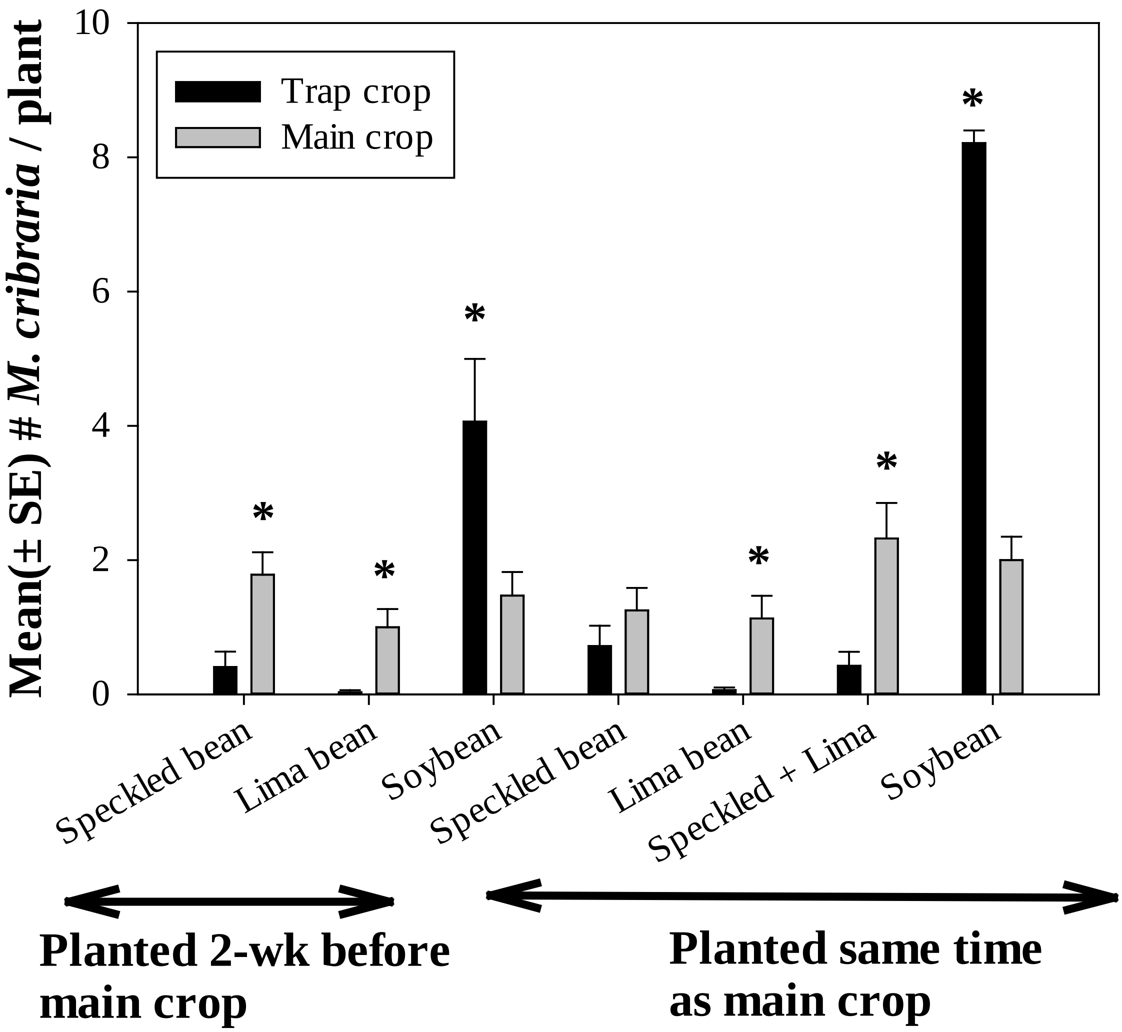
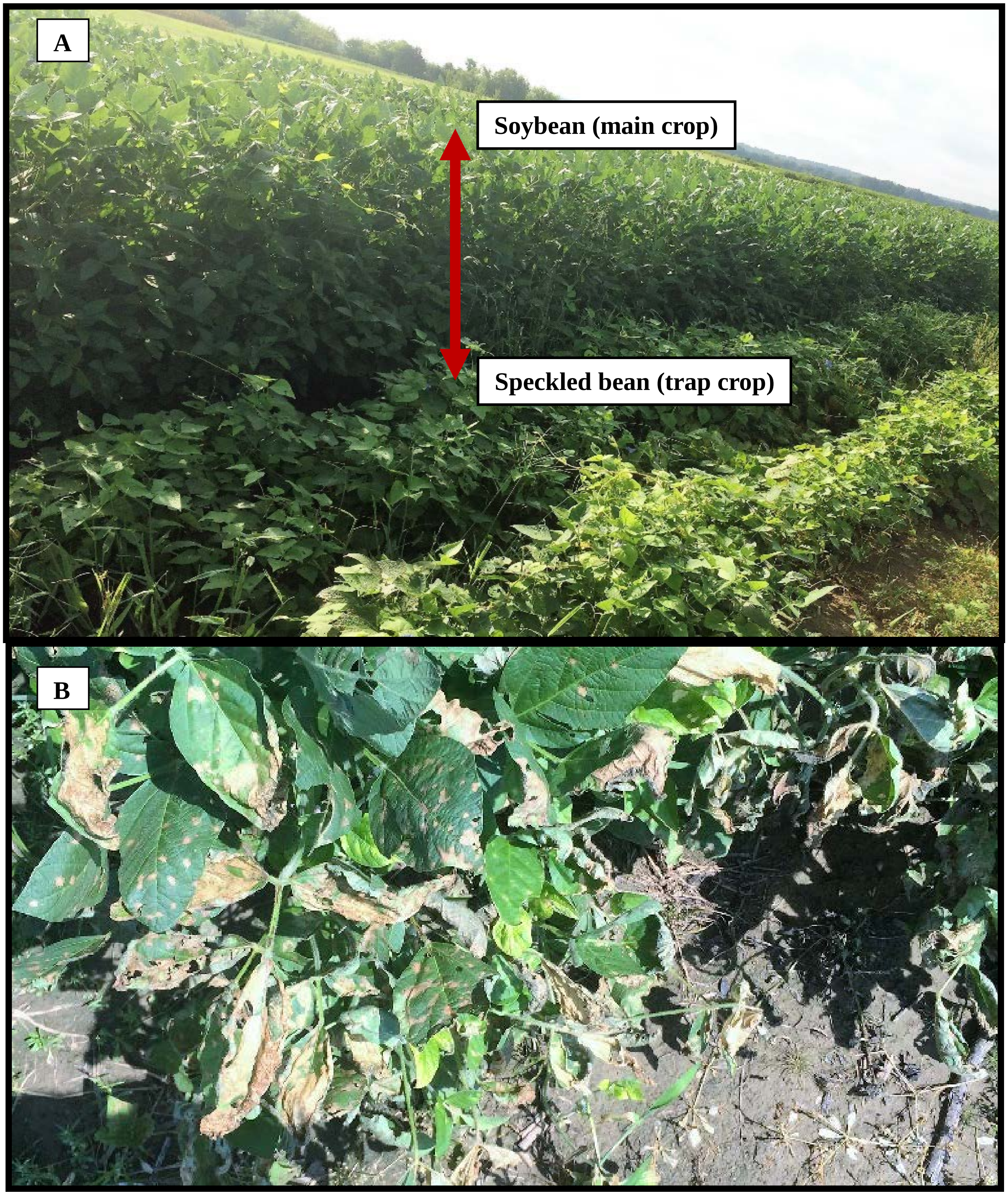


Fig. 9



CHAPTER 3

OLFACTORY RESPONSES OF *MEGACOPTA CRIBRARIA* AND ITS EGG PARASITOID, *PARATELENOMUS SACCHARALIS* TO HOST-ASSOCIATED ODORS

3.1 Abstract

Plants release volatile organic compounds (VOCs) either constitutively or as an indirect defense strategy against herbivores damage. Many insect species use these volatiles as cues to find food and oviposition sites. These VOCs can also serve as host location cues for natural enemies of herbivorous insects such as parasitoids. The objectives of this chapter were to: i) test preferential attraction of *Megacopta cribraria* (Hemiptera: Plataspidae) to three legume cultivars including soybean, speckled bean and lima bean in four-choice olfactometer bioassays and ii) test attraction of *Paratelenomus saccharalis* (Hymenoptera: Platygastridae), egg parasitoid of *M. cribraria* to host-induced volatiles of soybean in Y-tube olfactometer bioassays.

Female *M. cribraria* did not show a clear preference for odors emitted by soybean, speckled bean, or lima bean in four-choice olfactometer bioassays. This suggests that host preference in *M. cribraria* is either not mediated by olfactory cues or probably mediated by multiple modalities including visual and olfactory cues. In contrast, female *P. saccharalis* (egg parasitoid) showed significant attraction to host-associated odor cues in Y-tube olfactometer bioassays including damaged soybean, *M. cribraria* eggs only, and damaged soybean plus *M. cribraria* eggs. This suggests that host location by the egg parasitoid is mediated by odor cues from legume host and *M. cribraria* eggs. This study has potential practical application in the

development of attractants for *P. saccharalis* to enhance its effectiveness as a biological control agent against *M. cribraria*.

3.2 Introduction

Plants routinely release constitutive volatiles which can be used as odor cues by many phytophagous insects to locate their food and oviposition sites. Similarly, herbivore damage induces the release of volatile organic compounds (VOCs) from plants (Pare and Tumlinson 1999). These volatiles are commonly referred to as herbivore-induced plant volatiles (HIPVs) (Mumm and Dicke, 2010). Natural enemies such as predators and parasitoids exploit HIPVs to locate their herbivore prey or hosts, respectively (Price at al. 1980; Sabelis et al. 1999). Such odor-mediated tritrophic interactions have been investigated and reported in several previous studies (Turlings et al. 1993; Tumlinson et al. 1993; Takabayashi and Dicke, 1996; Hilker and Meiners, 2002; Colazza et al. 2004; Fadamiro, 2004a, 2004b; Chen and Fadamiro, 2007; Ngumbi et al. 2009).

Megacopta cribraria (Hemiptera: Plataspidae) is a pest of various leguminous and non-leguminous plants in its native Asian range, as well as in the United States where it was recently introduced (Srinivasaperumal et al. 1992; Eger et al. 2010, Zhang et al. 2012). However, a preliminary host range study by Zhang et al. (2012) showed that soybean and kudzu are the only reproductive hosts of *M. cribraria*. Soybean is an economically important crop in the United States. Both nymphs and adults *M. cribraria* damage soybean by sucking sap on tender stems and leaves, causing significant yield losses (Wang et al. 1996; Seiter et al. 2013a). Currently, conventional insecticides such as pyrethroids and neonecotinoids are mostly used to control *M. cribraria* (Seiter et al. 2013c; 2015; Brown et al. 2015). More environmentally sustainable management strategies such as use of plant-based attractants and natural enemies are currently being investigated. *Paratelenomus saccharalis* (Hymenoptera: Platygastridae) is a relatively specialized egg parasitoid on *M. cribraria*. Gardner et al. (2013b) suggested that the parasitoid was most likely introduced into the United States from parasitized eggs of *M. cribraria* from Asia. Although parasitization rate is high, *P. saccharalis* appears late in the season and has a short window of activity in the field. Hence, its effectiveness can be enhanced by using attractants to recruit parasitoids to *M. cribraria*-infested soybean fields.

Little is known about the role of host plant volatiles in *M. cribraria* host selection and preference and understanding these factors may provide crucial information for the development of alternative management strategies against *M. cribraria*. In chapter II, results showed that *M. cribraria* discriminated among early vegetative stages of four potential host plants (soybean, speckled bean, lima bean and Jackson wonder bean) in multiple-choice screen house tests. Female *M. cribraria* showed a preference for speckled bean and lima bean over Jackson wonder bean in screen house tests. However, it is not clear what specific cues mediate host preference in *M. cribraria*. In this study, the attraction of *M. cribraria* to three legume cultivars including soybean, speckled bean and lima bean was tested in four-choice olfactometer bioassays to determine if odor cues mediate host plant preference in *M. cribraria*. Similarly, olfactory response of the egg parasitoid, *P. saccharalis* to host-associated odors is yet to be investigated. In this study, the attraction of *M. cribraria* to three legume cultivars including soybean, speckled bean and lima bean was tested in four-choice olfactometer bioassays to determine if odor cues mediate host plant preference in *M. cribraria*. Furthermore, the attraction of *P. saccharalis* to various host-associated odors including undamaged soybean, damaged soybean, *M. cribraria* eggs only, and damaged soybean plus *M. cribraria* eggs was tested in Y-tube olfactometer bioassays to know the odor cues potentially used by the egg parasitoid for host location.

3.3 Materials and methods

3.3.1 Insects

Parasitized eggs of *M. cribraria* were collected from kudzu *Puereria montana* var. *lobata* (Willdenow) Ohwi, (Fabaceae) and observed under laboratory conditions [25 ± 1°C, 75 ± 5% RH and 14:10 h (L: D)] until adult emergence. Emerged parasitoids identified as *P. saccharalis* were introduced into rearing cages containing *M. cribraria* adults, nymphs and eggs. *Paratelenomus saccharalis* females were allowed to parasitize the eggs under laboratory conditions. Parasitized eggs of *M. cribraria* were transferred from the rearing cage into Petri dishes and kept under controlled laboratory conditions 25 ± 1°C, 75 ± 5% RH and 14:10 h (L:D) photoperiod until parasitoid emergence. Female parasitoids (~3-d old) fed with 10% sugar water were used for the bioassays. Sugar solution was provided by filling 0.5 ml microcentrifuge tubes and threading a cotton string through a hole in the cap of the tubes. Sex was determined using antennal morphology (E. J. Talamas, Personal communication).

Adult *M. cribraria* used for this study were collected as fifth instar nymphs from kudzu *Puereria Montana* var. *lobata* patches in Auburn, AL during summer 2015 and reared on rattle snake bean *Phaseolus vulgaris* L. under controlled greenhouse conditions (25 ± 2^{o}C; 50 ± 10% RH, and a photoperiod of 14:10 L:D).

3.3.2 Treatments

Soybean (Pioneer P49T97R-SA2P) growth stage V1-V2 (early vegetative) was used for the bioassays. Seeds were purchased from Taleecon Farmers' Cooperative in Notalsuga, AL. Seedlings were raised in 60-well seed trays at one seed per well. Seedlings (≈ one week old) were transplanted into pots (12.7 cm diameter × 15 cm high) at 4 seedlings per pot and allowed to grow under controlled greenhouse conditions (26±2^{o}C and 55±5% RH). The response of *P.*

saccharalis to odors emitted by: i) undamaged soybean (i.e., plants with no form of mechanical or herbivory damage); ii) damaged soybean (i.e., plants infested with *M. cribraria* nymphs and adults). Plants were checked to ensure there are no eggs on them before use; iii) damaged soybean + *M. cribraria* eggs (N = ~100); and iv) *M. cribraria* eggs only (N = ~300). Plants used in ii) and iii) were kept in cages (60 × 60 × 60 cm) (BugDorm-1, Megaview Science Education Services Co., Ltd., Taichung, Taiwan) where feeding and / or oviposition occurred by allowing 30 nymphs and 20 pairs of adult *M. cribraria* to feed on them for 24 hours before and during the experiment.

3.3.3 Behavioral bioassays

The set-up for the Y-tube and four-choice olfactometer bioassays was similar to that previously described by Chen et al. (2012) with slight modifications. Briefly, a Y-tube olfactometer (Analytical Research Systems, Gainesville, FL) was used to test the attraction of female *P. saccharalis* to host-associated odor. The system consists of a central tube (24 mm diameter × 13.5 cm high) and two lateral arms (24 mm diameter × 5.75 cm high) which are separately connected to an extending glass tube (19 mm diameter × 1.45 cm high). There is a sieve inlayed in the extending glass tube 5.25 cm away from the connection to prevent escape of insects and to serve as an end point of each lateral arm. An air delivery system (Analytical Research Systems, Gainesville, FL) passed humidified and purified air through Teflon tubes into air tight glass chambers (22.8 cm diameter × 40.6 cm high) containing treatments. Each glass chamber was provided with an inlet at the bottom connected to the air delivery system which was in turn connected to an air source fitted with charcoal filter and an outlet at the opposite top. For Y-tube olfactometer, air was drawn at a constant rate of 200 ml/min through glass chambers containing treatments (odor sources), and into the two lateral arms of the olfactometer. Air was removed by suction via a vacuum pump connected to the central tube of the olfactometer at the rate of 400 ml/min. For the four-choice olfactometer, air was let into each of the four arms at the rate of 200 ml/min and removed at the rate of 800 ml/min. To minimize visual distraction, the olfactometers were placed inside a cardboard box (82 cm × 62 cm × 61 cm) lined with white paper, which was open on the top (for illumination) and on the front side (for observation). Illumination was provided by vertically hanging an office lamp (20 W, 250 Lux) above (~50 cm high) the olfactometer tube. One stimulus was tested each time against a control (glass chamber which contained a (12.7 diameter × 15.2 cm high) pot filled with soil wrapped with aluminum foil. An individual parasitoid was released each time into the Y-tube through the bottom of the central tube and its choice of either arm was recorded. A choice is made when an insect walked to the sieve inlayed in the arm of the olfactometer. Insects that did not make a choice after 10 minutes were recorded as “non-responders” (< 10 % for *P. saccharalis*, ~ 30% for *M. cribraria*) and excluded from statistical analysis. To exclude positional bias, the olfactometer arm was rotated 180^{o} (Y-tube), or 90^{o} (four-choice) after 4 sets of insects have been tested. Insects were used only once. There were 36 replicates per treatment. After each treatment, the olfactometer was washed with soap and water and cleaned with acetone. Bioassays were carried out between 9000 hr to 1700 hr at 26 ± 2^{o}C and 55 ± 5% RH. For eggs only, smaller glass chambers (400ml capacity, Morawo and Fadamiro, 2016) were used.

3.3.4 Data analyses

Data obtained from responses of female parasitoids to stimulus versus control in Y-tube olfactometer were analyzed using a two-sided binomial test while for the four-choice olfactometer, preference of *M. cribraria* for each odor treatment was modeled as a binary response count and treatments were compared using Logistic Regression Analysis. The model adequacy for each set of experiment was confirmed with a Likelihood Ratio (Wajnberg and Haccou 2008). Slopes were separated using Proc Logistic Contrast in SAS. All analyses were performed at $P < 0.05$; JMP Pro 11.2.1, SAS Institute Inc. 2013.

3.4 Results

Olfactory response of Megacopta cribraria. When female *M. cribraria* were simultaneously presented with a choice of soybean, speckled bean, lima bean, and control (purified air) in four-choice olfactometer, they showed no clear preference for any of the legume cultivars (χ^2 = 0.8387; *df* = 3; *P* = 0.8402; *N* = 144) (Fig. 1).

Olfactory response of Paratelenomus saccharalis. Compared to control (no plant), female *P. saccharalis* showed significantly greater attraction to all host-associated odors tested, with the exception of volatiles from undamaged soybean (Fig. 2). Parasitoids showed the greatest attraction to damaged soybean + eggs (77.78 %, *P* = 0.0012, Binomial test). Similarly, *P. saccharalis* was significantly attracted to eggs only (72.22 %, *P* = 0.0113, Binomial test) and damaged soybean (69.44 %, *P* = 0. 0288, *Binomial* test) in separate Y-tube olfactometer bioassays (Fig. 2). Compared to undamaged soybean, *P. saccharalis* also showed significantly greater attraction to damaged soybean + eggs (69.44 %, *P* = 0. 0288, *Binomial* test) (Fig. 2).

3.5 Discussion

In chapter II, results showed that *Megacopta cribraria* discriminated among four potential host plants in multiple-choice screen house tests. Female *M. cribraria* showed a preference for speckled bean and lima bean over Jackson wonder bean in screen house tests. However, it was not clear what specific cues mediated the observed preference in *M. cribraria*. In the present study, the attraction of *M. cribraria* to three legume cultivars including soybean, speckled bean and lima bean was tested in four-choice olfactometer bioassays to determine if odor cues mediated host plant preference in *M. cribraria.* The present results showed that female *M. cribraria* did not discriminate among odors emitted by the three legume cultivars, suggesting that host preference in *M. cribraria* is not mediated by olfactory cues alone. A plausible explanation is that host location or preference in *M. cribraria* is mediated by multiple modalities including olfactory and visual cues. To further investigate the importance of visual cues, follow-up experiments were set-up to test behavioral response of *M. cribraria* in darkness but during regular hours of insect activity in the day. *Megacopta cribraria* did not make a choice (no response) for any of the legume cultivars in the olfactometer when in darkness (data not shown). Similarly, *M. cribraria* did not make a choice in another multiple-choice host preference test set up in cages without light (data not shown). These results suggest that visual cues may also play a role in host location or preference in *M. cribraria*. In addition to visual cues, the possible use of tactile cues in this species also merits further investigation. Integration of inputs from multiple modalities has been reported in insect species (Brévault and Quilici, 2010; Campbell and Borden, 2006; Morehead and Feener, 2000).

In contrast to the results obtained for *M. cribraria*, female *Paratelenomus saccharalis* (egg parasitoid) showed significant attraction to host-associated odor cues in Y-tube olfactometer bioassays including damaged soybean, *M. cribraria* eggs only, and damaged soybean plus *M. cribraria* eggs. This suggests that host location by the egg parasitoid is mediated by odor cues from legume host and *M. cribraria* eggs. Furthermore, damaged soybean plus eggs elicited the greatest attraction in *P. saccharalis*. This suggests that plant volatiles induced by both adult *M. cribraria* feeding damage and egg deposition act in concert as reliable host location cues for *P. saccharalis*. Feeding activities and oviposition by herbivorous insects have been shown to induce volatiles that attract egg parasitoids (Colazza et al. 2004, 2007; Moraes et al. 2005; Mumm and

Hilker 2005; Hilker and Fatuoros 2015). For instance, a study by Colazza et al. (2004) demonstrated that the combination of herbivore damage and oviposition by *Nezara viridula* on *Vicia faba* and *Phaseolus vulgaris* elicited attraction in the egg parasitoid, *Trissolcus basalis.*

Furthermore, egg parasitoids are also known to make use of chemical cues associated with the developmental stages (nymphs and adults) of their hosts. This is known as infochemical detour strategy (Wajnberg et al. 2008). This phenomenon has been studied in some platygastridae species including *Trissolcus brochymenae* and *Trissolcus basalis* (Vinson, 1998; Vet and Dicke 1992). For instance, soybean and pigeon pea infested with nymphs of *Euschistus heros* attracted females of the egg parasitoid, *Telenomus podisi* (Moraes et al. 2005). Although eggs of herbivorous insects have small biomass and likely release relatively lower quantities of volatiles, egg odors can serve as reliable host location cues for egg parasitoids, especially at short range (Vinson 1998, Fatouros et al. 2008). However, it is more likely that constitutive or herbivore induced plant volatiles serve as background odors to enhance the detectability of egg volatiles (Shroeder and Hilker 2008; Wajnberg et al. 2008; Beyaert et al. 2010). Beyaert et al. (2010) reported that the egg parasitoid, *Closterocerus ruforum* recognizes the key resource-indicating volatile associated with eggs of its host, the pine sawfly *Diprion pini*, only when it is perceived in the context of pine volatiles (background odors). This agrees with results of the present study in which *P. saccharalis* showed the greatest attraction to damaged soybean plus *M. cribraria* eggs.

In conclusion, results of the present study suggest that host location or preference in *M. cribraria* is probably mediated by multiple modalities including olfactory and visual cues, but not olfactory cues alone. However, *P. saccharalis*, egg parasitoid of *M. cribraria* uses host-associated odor cues to locate their hosts. A combination of volatiles induced by plant damage and oviposition may serve as reliable cues for foraging egg parasitoids. This study has potential practical application in the development of attractants for *P. saccharalis* to enhance its effectiveness as a biological control agent against *M. cribraria*. Future studies should investigate the possible use of visual and tactile cues by *M. cribraria* in locating plant hosts.

3.6 Acknowledgements

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3.7 References Cited

Beyaert I, Wäschke N, Scholz A, Varama M, Reinecke A, Hilker M (2010) Relevance of resource-indicating key volatiles and habitat odor for insect orientation. Animal Behavior 79:1077-1086

Brévault T, Quilici S (2010) Interaction Between Visual and Olfactory Cues During Host Finding in the Tomato Fruit Fly *Neoceratitis cyanescens*. Journal of Chemical Ecology 36:249–259

Brown SA, Kerns DL, Williams TS, Emfinger K, Jones N (2015) Evaluation of foliar insecticides for kudzu bug control in soybean. Arthropod Management Tests 40: 1

Campbell SA, Borden JH (2006) Close-range, in-flight integration of olfactory and visual information by a host seeking bark beetle. Entomologia Experimentalis et Applicata 120:91–98

Chen L, Fadamiro HY (2007) Differential electroantennogram response of females and males of two parasitoid species to host-related green leaf volatiles and inducible compounds. Bulletin of Entomological Research 97: 515-522

Chen L, Ochieng S, He X, Fadamiro HY (2012) Comparing electroantennogram and behavioral responses of two *Pseudacteon* phorid fly species to body extracts of black, red, and hybrid imported fire ants, *Solenopsis* spp. Journal of Insect Physiology 58: 1360-1367

Colazza S, Fucarino A, Peri E, Salerno G, Conti E, Bin F (2004) Insect oviposition induces volatiles emission in herbaceous plant that attracts egg parasitoids. Journal of Experimental Biology 207: 47-53

Eger JE Jr, Ames LM, Suiter DR, Jenkins TM, Rider DA, Halbert SE (2010) Occurrence of the Old World bug *Megacopta cribraria* (Fabricius) (Heteroptera: Plataspidae) in Georgia: a serious home invader and potential legume pest. Insecta Mundi 121: 1–11

Fadamiro HY (2004a) Monitoring the seasonal flight activity of codling moth, *Cydia pomonella*, and redbanded leafroller, *Argyrotaenia velutinana*(Lepidoptera: Tortricidae) in apple orchards using pheromone-baited traps. Environmental Entomology 33: 1711-1717

Fadamiro HY (2004b) Pest phenology and evaluation of traps and pheromone lures for monitoring flight activity of obliquebanded leafroller (Lepidoptera: Tortricidae) in Minnesota apple orchards. Journal of Economic Entomology 97: 530-538

Fatouros NE, Dicke M, Mumm R, Meiners T, Hilker M (2008) Foraging behavior of egg parasitoids exploiting chemical information. Behavioral Ecology 19: 677–689

Gardner WA, Blount JL, Golec JR, Jones WA, Hu XP, Talamas EJ, Evans RM, Dong X, Ray CH Jr, Buntin GD et al. (2013b) Discovery of *Paratelenomous saccharalis* (Dodd) (Hymenoptera: Platygastridae), an egg parasitoid of *Megacopta cribraria* F. (Hemiptera: Plataspidae) in its expanded North American range. Journal of Entomological Science 48: 355-359

Hilker M, Meiners T (2002) Induction of plant responses towards oviposition and feeding of herbivorous arthropods: a comparison. Entomologia Experimentalis et Applicata. 104: 181-192

Hilker M, Fatuoros NE (2015) Plant Responses to Insect Egg Deposition. Annual Review of Entomology 60: 493-515

Moraes MCB, Laumann R, Sujii ER, Pires C, Borges M (2005) Induced volatiles in soybean and pigeon pea plants artificially infested with the neotropical brown stink bug, *Euschistus heros*, and their effect on the egg parasitoid, *Telenomus podisi*. Entomologia Experimentalis et Applicata 115: 227-237

Morehead SA, Feener DH (2000) Visual and chemical cues used in host location and acceptance by a dipteran parasitoid. Journal of Insect Behavior 13: 613–625

Morawo T, Fadamiro H (2016) Identification of key plant-associated volatiles emitted by *Heliothes virescens* larvae that attract the parasitoid, *Microplitis croceipes*: Implications for parasitoid perception odor blend

Mumm R, Dicke M (2010) Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. Canadian Journal of Zoology 88: 628-667

Mumm R, Hilker M (2005) The Significance of background odor for an egg parasitoid to detect plants with host eggs. Chemical Senses 30: 337-343

Ngumbi E, Chen L, Fadamiro HY (2009) Comparative GC-EAD responses of a specialist (*Microplitis croceipes*) and a generalist (*Cotesia marginiventris*) parasitoid to cotton volatiles induced by two caterpillar species. Journal of Chemical Ecology 35:1009-1020

Pare PW, Tumlinson JH (1999) Plant volatiles as a defense against insect herbivores. Plant Physiology 121:325–331

Price PW, Bouton CE, Gross P, McPheron BA, Thompson JN, Weiss AE (1980) Interaction among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Annual Review of Ecology, Evolution, and Systematics 11:41-65

Sabelis MW, van Baalen M, Bakker FM, Bruin J, Drukker B, Egas M, Janssen ARM, Lesna IK, Pels B, Van Rijn P, Scutareanu P (1999) The evolution of direct and indirect plant defense against herbivorous arthropods. In: Olff, H, Brown VK, Drent RH (Eds.), Herbivores: Between Plants and Predators. Blackwell Science, Oxford109-166

Seiter NJ, Greene JK, Reay-Jones FPF (2013a) Reduction of soybean yield components by *Megacopta cribraria* (Hemiptera: Plataspidae). Journal of Economic Entomology 106:1676-1683

Seiter NJ, Benson E. P, Reay-Jones FPF, Greene JK, Zungoli PA (2013c) Residual efficacy of insecticides applied to exterior building material surfaces for control of nuisance infestations of *Megacopta cribraria* (Hemiptera: Plataspidae). Journal of Economic Entomology 106: 2448-2456

Seiter NJ, Greene JK, Reay-Jones FPF, Roberts PM, All JN (2015) Insecticidal control of *Megacopta cribraria* (Hemiptera: Plataspidae) in soybeans. Journal of Entomological Science 50: 263-283

Schroeder R, Hilker M (2008) The relevance of background odor in resource location by insects. Bioscience, 58: 308-316

Srinivasaperumal S, Samthitavelu P, Muthukrishnan J (1992) Host plant preference and life table of *Megacopta cribraria* (Fab.) (Hemiptera: Plataspidae). Proceedings of the Indian National Science Academy Biological Science 58: 333-340

Takabayashi J, Dicke M (1996) Plant-carnivore mutualism through herbivore-induced carnivore attractants. Trends in Plant Science 1:109-113

Tumlinson JH, Lewis WJ, Vet LEM (1993) How parasitic wasps find their hosts. Scientific American 268:100-106

Turlings TCJ, Wackers FL, Vet LEM, Lewis WJ, Tumlinson JH (1993) Learning of host-finding cues by Hymenopterous parasitoids. In: Papaj DR, Lewis AC (Eds.), Insect learning: Ecological and Evolutionary Perspectives. Chapman and Hall, New York, pp. 51-78

Vet LEM, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. Annual Review of Entomology 37: 141-172

Vinson SB (1998) The general host selection behavior of parasitoid hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. Biological Control 1:179-96

Wang ZX, Wang HD, Chen GH, Zi ZG, Tong CW (1996) Occurrence and control of *Megacopta cribraria* (Fabricius) on soybean. Plant Protection 22: 7-9

Wajnberg É, Haccou P (2008) Statistical tools for analyzing data on behavioral ecology of insect parasitoids: From Theoretical approaches to field applications (eds É. Wajnberg, C. Bernstein and J. van Alphen), Blackwell Publishing Ltd, Oxford, UK

Wajnberg É, Bernstein C, van Alphen J (2008) Behavioral ecology of insect parasitoids : from theoretical approaches to field applications. (eds É. Wajnberg, C. Bernstein and J. van Alphen), Blackwell Publishing Ltd, Oxford, UK

Zhang Y, Hanula JL, Horn (2012) The biology and preliminary host range of *Megacopta cribraria* (Heteroptera: Plataspidae) and its impact on kudzu growth. Environmental Entomology 41: 40-50

3.8 Figure legend

Figure 1. Response of female *M. cribraria* in four-choice olfactometer when presented with soybean, lima bean, speckled bean, and control (purified air). N = 31. Female *M. cribraria* did not show preference ($P > 0.05$; Logistic Regression)

Figure 2. Attraction of female *P. saccharalis* in Y-tube olfactometer when given a choice between control (purified air) versus undamaged soybean, damaged soybean, damaged soybean + *M. cribraria* eggs, or *M. cribraria* eggs only. The last bar compares headspace volatiles from undamaged plant versus damaged plant + egg mass. N = 36 wasps per test. Asterisks (*) indicate significant difference between stimulus and control ($P < 0.05$; Binomial test)

Fig. 1 Response of female *Megacopta cribraria* to host-plant VOCs in four-choice olfactometer

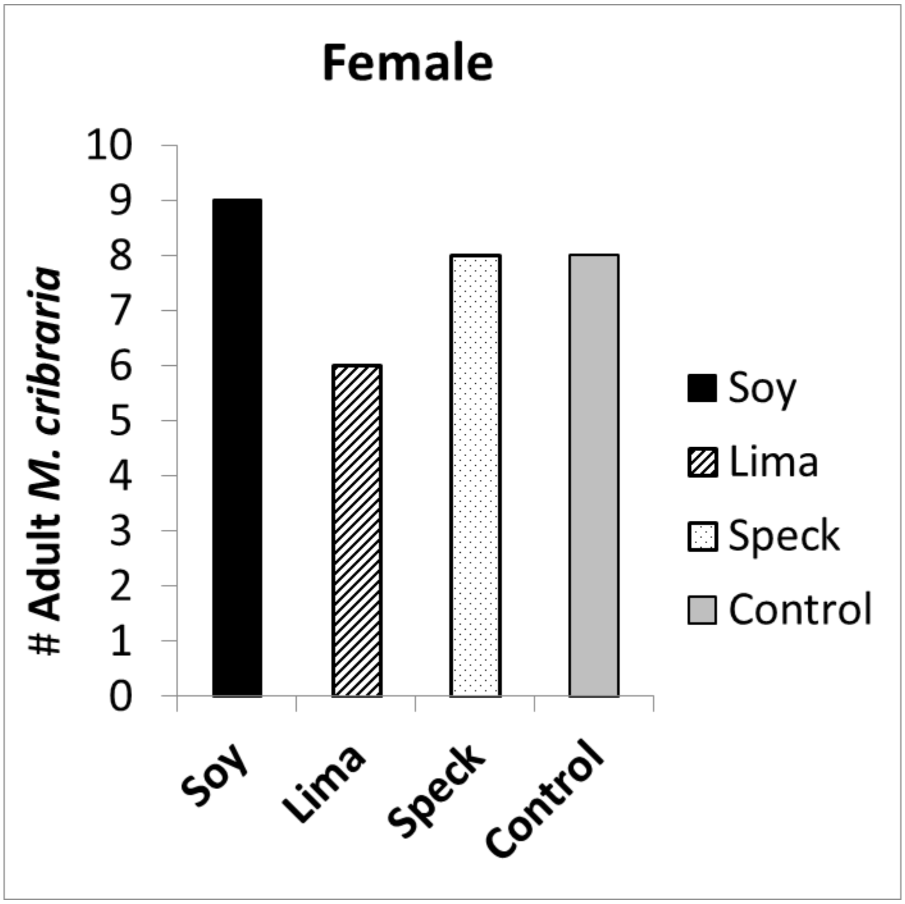
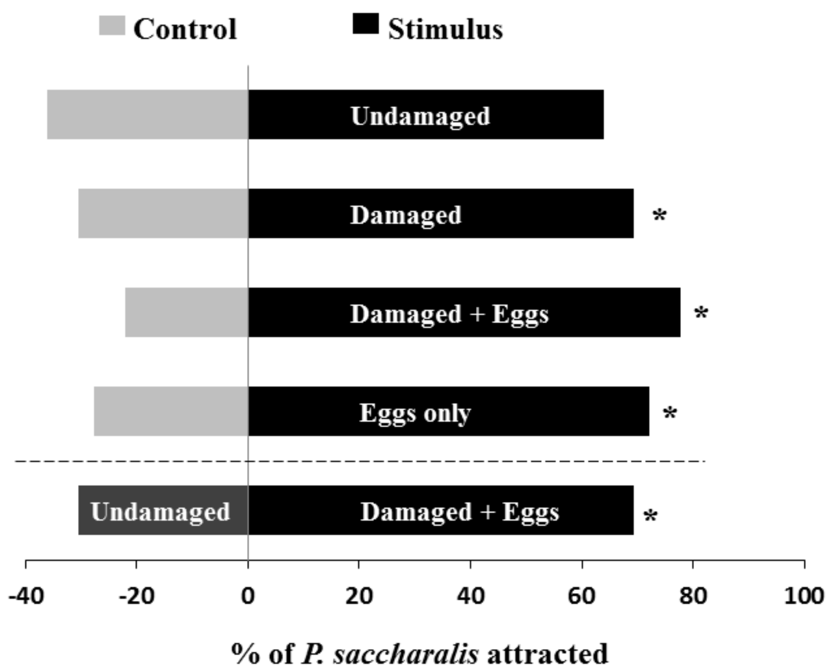


Fig. 2 Response of female *Paratelenomus saccharalis* to host-associated odors



CHAPTER 4

NEW EGG PARASITOID DISCOVERED FOR MEGACOPTA CRIBRARIA IN AUBURN, AL

A new egg parasitoid was recovered from the eggs of *Megacopta cribraria* in Auburn AL from late July until mid-September 2016. Egg masses of *Megacopta cribraria* were assumed to be parasitized by *Paratelenomus saccharalis* due to the typical grey coloration yielded a different egg parasitoid. This is not the first report of a new egg parasitoid following the accidental introduction of *M. cribraria* from Asia into the United States (Garner et al. 2013; Medal et al. 2015). More parasitized egg masses were collected from soybean fields within the Auburn University campus and kept in separate Eppendorf tubes and monitored daily for emergence. This new hymenopteran has morphological features different from *P. saccharalis*. A few of the differences are highlighted in Table 1. Egg masses observed showed parasitization rate as high as 100% with an average of ~95%. This parasitoid showed distinct sexual dimorphism in its antennal morphology. This could possibly be a new record in the United States or just a simple case of host-switching. Voucher specimens have been sent to the Smithsonian Institute, Washington D. C. for accurate identification (Fig. 1).

Table 1: Table 1. Morphological differences observed between the newly discovered egg parasitoid and *Paratelenomus saccharalis*; egg parasitoids of *Megacopta cribraria*

Morphological feature	*Paratelenomus saccharalis*; egg parasitoid of *Megacopta cribraria*	Newly discovered egg parasitoid of *Megacopta cribraria*
Antennae	Geniculate; last 4 segment of flagellomere greatly enlarged and darkened in females	Clavate; uniform color, males have a lot of setae arising from the base of flagellomeres
1st abdominal segment	Less than half the width of 3rd thoracic segment. Yellow in color	Almost as wide as 3rd thoracic segment. Black in color
Wing color	Transparent	Transparent with a green metallic sheen
Length of fringes on wings	Longer	shorter
Setae	Shorter and less dense	Longer and highly dense
Exit hole	Tip of egg; neat circular exit	Side of egg

Fig. 1 New egg parasitoid of *Megacopta cribraria* collected from soybean

4.1 References Cited

Gardner WA, Blount JL, Golec JR, Jones WA, Hu XP, Talamas EJ, Evans RM, Dong X, Ray CH Jr, Buntin GD et al. (2013b) Discovery of *Paratelenomous saccharalis* (Dodd) (Hymenoptera: Platygastridae), an egg parasitoid of *Megacopta cribraria* F. (Hemiptera: Plataspidae) in its expanded North American range. Journal of Entomological Science 48: 355-359

Medal J, Cruz AS, Williams K, Fraser S, Wolaver D, Smith T, Davis BJ (2015) First record of *Paratelenomus saccharalis* (Hymenoptera: Platygastridae) on kudzu bug *Megacopta cribraria* (Heteroptera: Plataspidae) in Florida. Florida Entomologist 98: 1250-125

CONCLUSION AND WAY FORWARD

This research evaluated the preference of *M. cribraria*, a major pest of soybean in the United States, to four legume cultivars (soybean, speckled bean, lima bean, and Jackson wonder bean) with the aim to identify attractive crops. Attractive legumes were further evaluated in the field as potential trap crops to manage *M. cribraria* in soybean. Additionally, the response of *M. cribraria* and its egg parasitoid, *Paratelenomus saccharalis* to semiochemicals was studied in olfactometers. *Megacopta cribraria* showed differential attraction to phenological stages of tested legumes in screen house studies. Of the three legumes evaluated in the field, soybean used as perimeter trap crops successfully intercepted migrating *M. cribraria* and prevented access to the main crop. *Megacopta cribraria* did not respond to odor cues from the tested legume cultivars in olfactometer studies. However, the egg parasitoid *P. saccharalis* was significantly attracted to host-associated odors, specifically, damaged soybean, damaged soybean plus *M. cribraria* eggs and *M. cribraria* eggs only. This research study demonstrates the first screen house preference study to account for the effect of plant phenology on the preference of *M. cribraria*. Also, this is the first study to demonstrate the olfactory response of *P. saccharalis* to host-associated odors in Y-tube olfactometer.

Future studies should investigate possible use of visual and tactile cues by *M. cribraria* in locating plant hosts. Biologically active components of host associated odors should be identified and exploited for the enhancement of *P. saccharalis* as a biological control agent. These can be developed as attractants for *P. saccharalis*. More field studies are needed to further evaluate the prospects of soybean, speckled bean and lima bean as trap crops in the management of *M. cribraria* over multiple seasons.

Trap cropping and use of semiochemicals are environmentally sustainable pest management tactics within the concept of IPM (integrated pest management) and have been applied extensively in many plant systems. Findings from this study will be incorporated into the development of a sustainable integrated pest management (IPM) strategy for the control of *M. crabraria* in soybean production. I believe that the knowledge obtained would also be applicable in the control of future invasions by other hemipteran pests.