HOST PREFERENCE, SEASONAL DYNAMICS AND MANAGEMENT IN SOYBEAN OF *MEGACOPTA CRIBRARIA* (HEMIPTERA: PLATASPIDAE)

by

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(Under the Direction of G. David Buntin)

ABSTRACT

Megacopta cribraria (F.) also known as the kudzu bug, was introduced into the United States in 2009 from Asia. *M. cribraria* develops on two primary hosts, kudzu and soybean. Since its introduction *M. cribraria* has become a major pest of soybean and has increased in range from nine original counties in Georgia to 13 states. Field and greenhouse trials were conducted to evaluate the developmental host range of *M. cribraria.* Effects of planting date and maturity group on soybean yield response to injury by *M. cribraria* were also evaluated in replicated trials. How geographic region influences development of *M. cribraria* was examined in kudzu and soybean. Effects of single insecticide applications to soybean for control of mid-season *M. cribraria* were evaluated. Combined effects of *M. cribraria* and defoliating lepidopterans on yield were evaluated. Population changes by year were determined monitoring by kudzu from 2012 to 2015. Relative sampling was conducted weekly to bi-weekly depending on study and consisted of sweep samples, drop clothes, cross-vane flight intercept traps, and whole plant counts. Out of the 23 species and varieties evaluated, soybean, edamame and pigeon pea were show to be developmental hosts of *M. cribraria*. Two generations developed in early planted soybean and one developed in late planted soybean. Greater yield reductions occurred in April and May planted soybean than later plantings. Maturity group did not affect *M. cribraria* populations and yield reduction was comparable between comparisons. Geographic region has no consistent effect on development of *M. cribraria*. Comparisons of weeks at which 50% of nymph populations were present coincided with a four week period in July when a single insecticide application suppressed mid-season *M. cribraria* populations. *M. cribraria* populations decreased substantially in 2014 and 2015 compared to early years when monitored in kudzu from 2012-2015 and soybean sampled from 2011-2014. Threshold levels of *M. cribraria* and subthreshold levels of lepidopterans did not affect yield, suggesting economic thresholds for *M. cribraria* management may require re-evaluation. Studies conducted in this work provide useful information for understanding and managing *M. cribraria* as it persists in its North American range.

INDEX WORDS: *Glycine max*, *Phaseolus sp.*, kudzu bug, IPM, agriculture, cultural control, entomology, Hemiptera, Pentatomoidea, Heteroptera, Plataspidae, kudzu, *Pueraria lobata* var. *montana*, phenology, soybean looper, *Chrysodeixis includens*, green cloverworm, *Hypena scabra*, velvetbean caterpillar, *Anticarsia gemmatalis*, cabbage looper, *Tricoplusia ni*, indirect injury, insects, defoliation

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DEDICATION

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Initial discovery. In October 2009 the University of Georgia Homeowner Insect and Weed Diagnostics Laboratory in Griffin, GA received several specimens and pictures of an unknown adult heteropteran, later identified as *Megacopta cribraria* (F.) (kudzu bug) (Eger et al. 2010, Suiter et al. 2011). Several submissions from locations in Northeast Georgia reported adults aggregating on the sides of homes (Eger et al. 2010, Suiter et al. 2010). Examination of kudzu vine (*Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen and S. Almeida (Fabaceae)) yielded several adults and some late instar nymphs. Adults were also observed on vehicles in the area close to a shopping center which contributed to the spread of the insect (Eger et al. 2010). Initial identification using morphological characteristics was performed by J. E. Eger (Dow AgroSciences, Tampa, FL) and confirmed by authorities from North Dakota State University and the USDA-ARS Systematics Laboratory (Washington, DC) (Jenkins et al. 2010). Identification voucher specimens were deposited at the Florida State Collection of Arthropods (Gainesville, FL) and the United States National Museum of Natural History (Washington, DC) (Suiter et al. 2011). Genetic identification was completed by T. Jenkins (University of Georgia, Griffin) and indicated that the cytochrome oxidase subunit I (COI) extracted from genomic DNA of three individuals were identical suggesting a single female lineage. The sequences were subjected to a GenBank Blast search (GenBank #AY627332) and confirmed *M. cribraria* as the closest match (Jenkins et al. 2010). This is the first record of a member of the family Plataspidae (Hemiptera) to be recorded in the Western Hemisphere. One other plataspid, *Coptosoma xanthogramma* (White) (Beardsley J. W. Jr. 1967) is known to be established in the Hawaiian Islands.

Description. Eger et al. (2010) provides a key with diagnostic characteristics to distinguish *M. cribraria* from other members of Pentatomoidea in North America. Key characteristics that distinguish this insect from others with similar morphology in North America are two segmented tarsi and a greatly enlarged scutellum which covers the forewings and most of the abdomen. Adults of *M. cribraria* are 4-6 mm anterior to posterior and range in color from light brown to olive green with dark punctuations and greenish-brown forewings (Eger et al. 2010, Suiter et al. 2011). The scutellum is enlarged and truncated or broadly rounded and widest on the posterior fourth (Eger et al. 2010). Adults also exhibit a pseudosuture (basal callosity well defined by an impressed line) on the anterior portion of the scutellum separating the basal area from the remainder of the scutellum (Yang 1934). This character is unique among North American Pentatomoidea and may be helpful diagnostically. First instars appear reddish-brown, range from 1-1.2 mm long and remain near the egg mass for the first day or so where they ingest symbiotic bacteria (*Candidatus Ishikawaella capsulata*) from the capsule provided by the female upon oviposition (Hosokawa et al. 2007, Eger et al. 2010, Jenkins et al. 2010, Jenkins and Eaton 2011, Zhang et al. 2012). Instars 2 through 4 range in length from approximately 1.5 to 3.5, mm respectively, and are light green to light greenish-brown in coloration, hirsute with scallops around the abdomen (Zhang et al. 2012). Fifth-instar nymphs range from light green to light brown, are somewhat dorso-ventrally flattened, hirsute, oval and 4-5 mm long with prominent wing pads (Eger et al. 2010, Zhang et al. 2012). In adult male *M. cribraria* the terminal sternal segment is rounded whereas the sternite of the female is v-shaped and has a distinctive suture (Zhang et al. 2012). Egg masses are typically laid in two parallel rows and occasionally may be laid in irregular groups (Zhang et al. 2012). The number of eggs deposited by female *Megacopta* spp. varies depending on temperature and other environmental conditions and ranges from 26 to 274 (Eger et al. 2010). *Megacopta cribraria* in the U.S. were found to lay 15.64 ± 0.58 eggs per mass (*N* = 200) (Zhang et al. 2012). Egg masses are white when newly oviposited and turn off-white to pinkish soon after (Zhang et al. 2012). The operculum is round and crested with short spine like projections (Zhang et al. 2012).

Megacopta cribraria was originally described by Fabricius in 1798 as *Cimex cribrarius* but has since been transferred to the genus *Coptosoma* (Amyot 1843) and now resides in *Megacopta* (Hsiao 1977). *Megacopta cribraria* and *Megacopta punctatissima* are morphologically similar species that are capable of hybridizing (Hosokawa et al. 2007, Eger et al. 2010). *Megacopta punctatissima* is still used in Japanese literature and is distinguished from *M. cribraria* as a frequent pest of soybean whereas *M. cribraria* is not considered an important soybean pest and occurs in the southwestern Japanese islands (Hosokawa et al. 2007, Eger et al. 2010) though both species are capable of developing on soybean and kudzu. Hosokawa (2007) demonstrated that exchange of the symbiotic gut capsules of these two species confers pest status in soybean, allowing the non-pestiferous *M. cribraria* to develop better on soybean and greatly inhibited development of *M. punctatissima* in soybean (Hosokawa et al. 2007). This information indicates that pest status of these insects with this unique symbiont transmission type is primarily determined by the genotype of the symbiont and not the genotype of the insect (Hosokawa et al. 2007). Mitochondrial DNA sequences of populations from the U.S., Japan and China indicate that the two species in question are not distinct, but may have diverse genotypes and phenotypes within populations. This is supported by Bayesian phylogenetic analysis which suggests that neither clade (*M. cribraria* or *M. punctatissima*) is monophyletic (Hosokawa et al. 2014). These two species are considered conspecifics (Eger et al. 2010) and for the purpose of this review, all literature on the synonyms of these two species is included.

Distribution. The exact mode and location of the introduction of *M. cribraria* in the U.S. is unknown. However, it is accepted that given the proximity of the initial reports to the Atlanta-Hartsfield International Airport it is likely that the founder insect escaped from incoming cargo. *Megacopta cribraria* is a strong flier and adults disperse readily when populations emerge from overwintering in the spring and seek overwintering habitats in the fall. They also have a propensity to land on vehicles and light colored objects which contributes to its spread. *Megacopta cribraria* was originally found in nine counties in north east Georgia in 2009, and by 2010 its distribution had increased to 96 counties across four states. Currently it has a distribution of 620 counties across 13 states and the District of Columbia (Dec. 2015) (Gardner 2015). Prior to its establishment in the U.S., *Megacopta* spp. were reported from China, Japan, India, Indonesia, Myanmar, Pakistan, Sri Lanka, Korea, Macao, Taiwan, Thailand and Vietnam (Eger et al. 2010). Mitochondrial DNA sequences were obtained from *M. cribraria* and *M. punctatissima* specimens collected from China, Japan, South Korea, Vietnam and the southeastern United States totaling 53 individuals in all. Comparisons of mitochondrial DNA sequences (8,687 bp) showed consensus between individuals from U.S. populations and individuals from the Kyushu region of Japan. These matched the sequences used in an earlier study where the gentic baseline of *M. cribraria* in the U.S. was set (Jenkins and Eaton 2011) and it was discovered that *M. cribraria* in the southeastern region shared a single haplotype (Jenkins and Eaton 2011, Hosokawa et al. 2014). Phylogentic analysis of the mtDNA sequences placed U.S. and Kyushu region populations in the same clade (E) (Hosokawa et al. 2014). This information suggests that *M. cribraria* populations in the U.S. were introduced from a population in the Kyushu region of Japan, though it is not yet clear which specific population the U.S. population originated from (Hosokawa et al. 2014).

Development. The developmental threshold from egg to adult for *M. cribraria* is 14.25°C with a thermal constant of 849.56 DD (degree days) in laboratory conditions (Shi et al. 2014). Optimum developmental temperature for *M. cribraria* is 25°C though optimum temperature varies between instar (Shu-Sen et al. 2014). Female *M. cribraria* also displayed the greatest fecundity at this temperature and the longest oviposition period, although the pre-oviposition period was greater at 25°C (35.67 d) than at 29°C (14.67 d) (temperatures tested were 17, 21, 25, 29 and 33°C) (Shu-Sen et al. 2014). It takes approximately 6-7 days for eggs to hatch after ovipostion (Thippeswamy and Rajagopal 2005b, Shu-Sen et al. 2014). Development from egg to adult ranges from 24 to 56 days but is dependent on temperature and other environmental factors (Thippeswamy and Rajagopal 2005b, Eger et al. 2010, Shu-Sen et al. 2014). Survival of the overwintering generation is much greater at 9-10 mo. compared to the first filial generation, which survives only 1.5-3 mo in studies performed in China (Zhang 2005, Zhang et al. 2012). Shi et al. (2014) reported adult longevity of adult *M. cribraria* reared on soybean to be 75.67 d under 12:12 (L:D) photoperiod with 80% RH at the developmentally optimal temperature of 25°C. Under these ideal conditions it is estimated that *M. cribraria* populations can increase 46.5 times after a single generation (Shu-Sen et al. 2014). *Megacopta cribraria* overwinters in the adult stage in leaf litter and may be found under rocks and tree bark (Ren 1984, Suiter et al. 2010). Females are more likely to accept copulation more often when males court in aggregation than singly (Hibino 1986, Eger et al. 2010). Studies conducted by Golec and Hu (2015) reported that approximately 15% of females store sperm within the spermatheca during overwintering months. This suggests that a portion of the overwintering female population mates before entering overwintering (Golec and Hu 2015). No differences were found between stored sperm in females during the overwintering period (Sept. – Mar.) indicating that mating does not occur when *M. cribraria* is in an overwintering state (Golec and Hu 2015). Development in the ovaries also does not occur in the overwintering state and oocyte formation does not occur until the end of February prior to the onset of post winter mating and early spring feeding (Golec and Hu 2015). Golec and Hu (2015) observed eggs in late developmental stages in 54% of females mated prior to overwintering approximately 30 days after detection of the first developing oocyte in ovarioles. Developing oocytes were also detected in unmated females though at much lower levels (18%), indicating that stored sperm promotes oocyte development (Golec and Hu 2015). Before overwintering the ratio of males to females is about 1:1 but shifts to between 1:1.53 and 1:1.84 after overwintering (Zhang et al. 2012, Golec and Hu 2015).

Pest status and host range. *Megacopta cribraria* was originally reported in the U.S. as a nuisance pest in homes due to its tendency to aggregate and attraction to light colored objects (Suiter et al. 2010, Suiter et al. 2011). *Megacopta cribraria* also releases a noxious odor when disturbed and when crushed against the skin; presumably the same substance can cause an irritation at the site of contact akin to a chemical burn (Eger et al. 2010, Suiter et al. 2010, Suiter et al. 2011, Gardner et al. 2013b, Ruberson et al. 2013). This aggregative behavior occurs primarily in the spring and fall as adults disperse seeking mates, host plants and overwintering sites (Eger et al. 2010, Suiter et al. 2010, Suiter et al. 2011, Gardner et al. 2013b, Ruberson et al. 2013). Adults begin to emerge from overwintering sites as temperatures increase in early spring, often before kudzu is amply available. It is likely during this period that *M. cribraria* has been reported from a wide range of plants species and acts as a nuisance pest. Though *M. cribraria* is reported from many plants, most are likely incidental possibly providing nutrients to adults but not serving as developmental hosts. *Megacopta cribraria* has been reported from several plant species however, plants in the legume family (Fabaceae) are reported as the primary hosts. Eger et al. (2010) provides an extensive list of reported leguminous and non-leguminous plant species from several authors. Among the reported leguminous host plants of interest in the U.S. are soybean (*Glycine max* Merril), kudzu (*P. montana* var. *lobata*), lablab (*Lablab purpureus* (L.) Sweet), several *Phaseolus* spp., lespedeza (*Lespedeza* spp.) and wisteria (*Wisteria brachybotrys* Sieb. et Zucc.) (Eger et al. 2010). Non-leguminous potential host plants of concern in the U.S. are cotton (*Gossypium hirsutum* L.), sweet potato (*Ipomoea batatas* Lam.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), potato (*Solanum tuberosum* L.), sugarcane (*Saccharum officinarum* L.) and citrus (*Citrus* spp.) (Srinivasaperumal et al. 1992, Eger et al. 2010). Lovejoy and Johnson (2014) utilized molecular techniques found DNA from monocot, dicot and angiosperm plant species in the diet of adult *M. cribraria* , such as: kudzu, lespedeza. (*Lespedeza* sp), peanut (*Arachis hypogaea* (L.)), black medic (*Medicago lupulina* (L.)), white sweet clover (*Melilotus alba* (Medik.)), sorghum (*Sorghum bicolor* (L.)), tomato (*Solanum lycopersicum* (L.)), lettuce (*Lactuca sativa* (L.)), red oak (*Quercus rubra* (L.)), walnut (*Juglans* sp.), sweet gum (*Liquidambar styraciflua* (L.)) and pine (*Pinus* sp.) (Lovejoy and Johnson 2014). Though many of these plants are not hosts, adults may acquire nutrients from them occasionally.

Host preference tests were conducted by Zhang et al. (2012) to determine the ability of *M. cribraria* to develop on a suit of common forest legumes in Georgia. Out of the 12 species evaluated oviposition only occurred on soybean, kudzu, hairy lespedeza (*Lespedeza hirta* (L.)) and sericea lespedeza (*Lespedeza cuneate* (Dum. Cours.) G. Don). Development to adult only occurred on kudzu and soybean (Zhang et al. 2012). Similarly Seiter et al. (2014) performed host preference test between kudzu, soybean, cotton and Chinese wisteria (*Wisteria sinensis* (Sims) de Candolle). Ovipostion occurred preferentially on kudzu and soybean though ovipostion on wisteria was greater than on cotton (Seiter et al. 2014a) demonstrating clear preference for legume species. In these two studies the size of the cages used in each experiment were confounding factors impeding *M. cribraria's* ability to find hosts and may have impacted the results (Zhang et al. 2012, Seiter et al. 2014a).The result of no development on cotton in the study by Seiter et al. (2014) is contrary to results of Srinivasaperumal et al. (1992) who detected development of nymphs to adult in no-choice laboratory assays on cotton. These opposing results demonstrate the effect of symbiotic micro-organisms on host range. The study conducted by Srinivasaperumal et al. (1992) utilized *M. cribraria* in India where they are listed as a pest of *Sesbania grandiflora* Pers. (hummingbird tree) and *Crossandra infundibuliformis* (L.) Nees (firecracker plant). This population of *M. cribraria* are likely in a separate clade from the U.S. and Japanese conspecifics (Srinivasaperumal et al. 1992, Hosokawa et al. 2014) though there has not been a phylogenetic analysis of mtDNA of populations from the Indian Subcontinent.

Medal et al. (2013) performed no-choice host preference tests in greenhouse conditions using wild caught *M. cribraria* adults which were allowed to oviposit and nymphs to develop on a given host. Eleven agriculturally important legume species and *Citrus sinensis* (L.) Osbeck (sweet orange) were evaluated. Of the eleven species evaluated development from egg to adult (reported as mean) occurred (from most to least successful) on kudzu (89a), soybean (81a), pigeon pea (*Cajanus cajan* (L.)) (75ab), black-eyed pea (*Vigna sinensis* (L.)) (27bc), lima bean (*Phaseolus lunatus* (L.)) (5cd) and pinto bean (*Phaseolus vulgaris* (L.)) (1c) (Medal et al. 2013). *Megacopta cribraria* did not oviposit or develop on sweet orange, peanut, chickpea (*Cicer arietinum* (L.)), lentil (*Lens culinaris* Medikus), mung bean (*Vigna radiata* (L.) Wilczek) or jicama (*Pachyrhizus erosus* (L.) Urb) (Medal et al. 2013). These results suggest that pigeon pea is a developmental host in addition to kudzu and soybean. A 2005 study by Thippeswamy and Rajagopal found that nymphs were unable to survive on pigeon pea (redgram) but were able to survive on lablab and soybean in both field and greenhouse conditions (Thippeswamy and Rajagopal 2005b). *Megacopta cribraria* is frequently listed as a pest of lablab and is commonly referred to as the 'lablab bug' (Ahmad and Moizuddin 1975, Thippeswamy and Rajagopal 1998, 2005b, a). *Megacopta cribraria* populations in the U.S. were unable to complete development on lablab in replicated trials (Zhang et al. 2012, Medal et al. 2013).

The potential for *M. cribraria* as a biological control agent of kudzu, its self an invasive weed, in the U.S. was evaluated by comparing biomass of insecticide protect kudzu to kudzu subjected to injury by *M. cribraria* (Zhang et al. 2012). Kudzu biomass was shown to be reduced by 32.8%, however protected plots were selectively grazed over unprotected plots by deer in the area and actual percentages of biomass reduction could not be obtained (Zhang et al. 2012). However, the usefulness of *M. cribraria* as a biological control agent of kudzu is offset by its impact as an economic pest of soybean.

Megacopta cribraria is a phloem feeder that causes indirect damage by removing vascular fluids from the plant phloem (*F. Stubbins* unpublished data). *Megacopta cribraria* feeding injury is characterized by purplish-brown lesions occurring along the stems and is often concentrated at nodes (Thippeswamy and Rajagopal 2005b). Shoots, leaves, and young pods where heavy feeding damage occurs by high populations of *M. cribraria* have been reported to 'whither and dry' (Thippeswamy and Rajagopal 2005b). Thippeswamy and Rajagopal (2005) reported that early instar nymphs tend to concentrate and feed at tender stems and move to older portions of stems and petioles as later instars, this corroborates with anecdotal observations of the author in the southeast U.S. Yield reductions of lablab under greenhouse conditions where various concentrations of *M. cribraria* were evaluated determined that infestations of 160 per plant lead to yield reductions of 44.22% (Thippeswamy and Rajagopal 1998). Yield reductions ranging from 0.55% to 50.55% at population densities ranging from 5-82 individuals per soybean bunch (5-60 plants per hill) were detected at the field level for studies conducted in China (ZhiXing et al. 1996). Suppression of growth of the main stem occurs during the vegetative growth stage of soybean when exposed to high densities of *M. cribraria* (Kikuchi and Kobayashi 2010). The longer plants are exposed and the earlier the growth stage at which they are exposed to feeding by *M. cribraria* the greater the decrease in nodes and trifoliates produced. Kikuchi and Kobayashi (2012) demonstrated that sustained feeding of adults at a density of 40 individuals per soybean plant beginning at the V3 growth stage significantly decreased the length of the main stem. Reduction in grain yield was not quantified in this study however. Seiter et al. (2013) found soybean yield reductions of 56.6% to 59.6% occurred in caged field trials under high densities of *M. cribraria* consistent with levels detected in fields in the area. A yield loss of 0.2803 ± 0.0395 kg/ha for every *M. cribraria* insect day accumulated at high population densities (183 ± 23 individuals per plant) was determined where plants were initially infested with 25 adults (Seiter et al. 2013a). In this study seeds per pod and seed weight were significantly reduced as populations of *M. cribraria*, increased but pods per plant were not affected. As cumulative insect days increased (determined by regression analysis) seeds per pod, seed weight and yield decreased significantly whereas pods per plant and protein and oil content were not affected (Seiter et al. 2013a).

The first record of soybean in North America dates back to 1765, when a royal patent was issued to Samuel Bowen of Savannah, Georgia for making soy sauce (Hymowitz and

Shurtleff 2005). Soybean was cultivated primarily as a forage crop until first being crushed for oil and protein in response to scarcity of cottonseed (Piper and Morse 1923, Dies 1944). Since its instruction, soybean has become the second largest crop in the U.S. behind corn. Soybean acreage in the U.S. has increased from 76,610,000 to 82,429,000 from 2010 to 2015 (NASS 2015) (USDA 2015b). Soybean production in the U.S. is a $40,288,536,000 industry producing 47.5 BU/A at ~$9/BU. The U.S. leads the world in soybean production and is a net exporter globally. In 2014, 300,000 acres of soybean were planted in Georgia with harvested acres valued at $125,280,000 ($10.5/BU) (USDA 2015a). Protecting this commodity from damaging pests is a matter of national financial security.

Management. *Megacopta cribraria* is susceptible to many insecticides and efficacious treatments are commercially available. Several active ingredients in the pyrethroid class (IRAC group 3A) such as bifenthrin, lambda-cyhalothrin and zeta-cypermethrin are registered for use in soybean for *M. cribraria* control. Acephate, an organophosphate (IRAC group 1B), and clothianidin in the neonicotinoid class (IRAC group 4A) are also registered for use in soybean for *M. cribraria* control (Roberts et al. 2015). During the early stages of invasion of *M. cribraria* several insecticide applications were made per season for control. Seiter et al. (2015) were able to demonstrate that a single well timed application at the R3 or R4 growth stage based on presence of nymphs was effective at providing control equivalent to aggressively protected plots.

Host plant resistance and RNAi (RNA interference) are two alternative means of managing *M. cribraria* populations. Several of the insecticides used for control of *M. cribraria* negatively impact natural enemies and other non-target organisms which may be detrimental to the ecosystem. Plants exhibiting resistance to injury are “inherently less damaged or less infested than others under comparable environmental conditions in the field” (Painter 1958). Quantitative trait loci (QTLs) from plant introductions (PIs) have been screened and characterized. Antibiosis resistance to *M. cribraria* was characterized from PI 567336A, PI 567352B and PI 567598B (Bray 2015, Fritz 2015). Antixenosis, or non-preference resistance was characterized from narrow leaf genotypes N7103 and Vance PIs (Fritz 2015). Recent research into the area of gene silencing through RNAi has yielded some promising results for *M. cribraria* control. When compared to controls, increased mortality occurs when feeding dsRNA to *M. cribraria*. However this is only true for some of the genes tested and research is underway to confirm that toxicity is indeed related to gene silencing (Juan Luis Jurat-Fuentes, personal communication, 11 December 2015).

Natural enemies. Upon its introduction into the U.S., *M. cribraria* had no known natural enemies to keep populations in check. Egg masses were collected and monitored from several counties in Georgia in 2010 and 2011. Out of the 632 total egg masses collected during this time no parasitoids were observed emerging (Zhang et al. 2012, Ruberson et al. 2013). Two species of flies (*Strongygaster triangulifera* (Loew) and *Phasia robertsonii* (Townsend)) in the Tachinid family have been reported parasitizing adult *M. cribraria* at low levels in the southeast (Golec et al. 2013, Ruberson et al. 2013). In 2013, egg masses were detected in several locations in the southeast exhibiting discoloration. Adult parasitoids were observed emerging from these egg masses and were later identified as *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Platygastridae) (Gardner et al. 2013a). This wasp is not native to the U.S. but is found in *M. cribraria's* native range of Japan, China and the Indian subcontinent (Tayutivutikul and Yano 1990, Rajmohan and Narendran 2001). This species was under review as a biological control agent and being held in quarantine at the USDA-ARS National Biological Control Laboratory in Stoneville, MS at the time of collection (Gardner et al. 2013a). However, genetic analysis confirmed that specimens collected in the wild were not accidentally released from quarantine populations (Wayne Gardner, personal communication). Molecular gut-content analysis was performed on several generalist predators in soybean. Eight native predators were found to contain *M. cribraria* DNA in their guts, these were, *Geocoris punctipes* (Say), *Geocoris uliginosus* (Say), *Orius insidiosus* (Say), *Podisus maculiventris* (Say), *Hippodamia convergens* (Guerun-Meneville), *Zelus renardii* (Kolenati), *Oxyopes salticus* (Hentz), and *Peucetia viridans* (Hentz) and one exotic species, *Solenopsis invicta* (Buren) (Greenstone et al. 2014). Other reports of predation include *Chrysoperla rufilabris* (Burmeister) and *Euthyrhynchus floridanus* (L.) (Ruberson et al. 2013). In 2010, a single specimen of *M. cribraria* was found infected with the entomopathogenic fungus, *Beauveria bassiana* (Bals.-Criv.) Vuillemin (Ruberson et al. 2013). Reports and incidents of infection of *M. cribraria* by *B. bassiana* have increased since 2012 and is commonly detected in soybean and kudzu in the southeast (personal observation) (Seiter et al. 2014b).

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CHAPTER 2

HOST PREFERENCE OF *MEGACOPTA CRIBRARIA*, (HEMIPTERA: PLATASPIDAE) ON SELECTED EDIBLE BEANS AND SOYBEAN[1]

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[1] J. L. Blount, G. D. Buntin and A. N. Sparks Jr. 2015. *Journal of Economic Entomology*. 108: 1094-1105. Reprinted here with permission of the publisher.

Abstract

Megacopta cribraria (F.) (Hemiptera: Plataspidae) is an Old World pest of legumes in Asia. Since its 2009 discovery in Georgia, it has become an economic pest of soybeans in the southeastern United States. The objective of this study was to determine the host preference of *M. cribraria* on edible legumes that might incur economic damage from injury of this pest. From 2012 to 2013 choice, no-choice and field trials were conducted to evaluate the host suitability of several beans of commercial interest including pinto bean, lima bean, winter pea and black-eyed pea. Choice and no-choice studies were conducted under greenhouse conditions. Plants in greenhouse trials were infested with adults and egg masses collected from kudzu and soybean and monitored for approximately two weeks. Field trials were allowed to be infested by naturally occurring *M. cribraria* populations. Sweep and whole plant counts of adults, egg masses, and nymphs were used to quantify field infestations. The legume crops found to be suitable developmental hosts are soybean, edamame, and pigeon pea. Low levels of development were seen on fava bean and none on the remaining entries.

Key words: *Phaseolus vulgaris*, Pinto bean, *Glycine max, Pisum sativum,* Winter pea, *Pueraria lobata* var. *montana,* Kudzu, *Phaseolus lunatus*

Introduction

Megacopta cribraria (F.), the kudzu bug, is referred to as the globular stinkbug, bean plataspid, and lablab bug in literature from India, China and Japan (Ruberson et al. 2013). *M. cribraria* is an Old World pest with a distribution spanning Asia, Africa, and the Indian subcontinent (Ruberson et al. 2013). In 2009, adults were observed aggregating on the outside of homes near Atlanta, GA (Suiter et al. 2010). Homes in the area of the initial report were near stands of kudzu, *Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen and S. Almeida (Zhang et al. 2012). Initial collections and identification of *M. cribraria* are detailed by Eger et al. (2010). Since its invasion into the western hemisphere, where members of the family Plataspidae had not previously occurred, *M. cribraria* has become an economically important pest of soybeans (Fabaceae: *Glycine max* (L.) Merrill) with yield losses capable of exceeding 50 percent in the southeastern U.S. (Seiter et al. 2013a). The original nine county distribution in northeast Georgia has rapidly expanded to 13 states (GA, FL, AL, MS, LA, KY, TN, SC, NC, VA, DE, AR and MD) and the District of Columbia (Gardner 2014).

In its native range *M. cribraria* is reported to complete three generations per year (Tayutivutikul and Yano 1990). In the southeastern U.S., *M. cribraria* completes two generations per year on kudzu and one to two generations on soybean depending on planting date (Zhang et al. 2012) (JLB and GDB, unpublished data). *M. cribraria* completes egg to adult development in approximately four to six weeks (Ruberson et al. 2013). Adults overwinter in protected areas, under rocks, tree bark and other debris near kudzu patches and/or in proximity to soybean fields (Zhang et al. 2012, Ruberson et al. 2013). Egg masses are laid in a characteristic two parallel rows on top of small brown capsules deposited by the female at the base of eggs. The capsules contain the symbiotic gut bacteria *Candidatus Ishikawaella capsulata* (Jenkins et al. 2010). Upon emergence, neonates feed on the capsule contents obtaining nutrients and the microorganisms critical for digestion of host plants. The presence of specific symbiotic microorganisms in the capsule impacts the pest status of this insect as well as its host range and potentially its taxonomic distinction (Hosokawa et al. 2007, Hosokawa et al. 2014). There is some confusion on the taxonomic distinction between *M. cribraria*, which is not considered a key pest of soybeans in its native range, and *Megacopta punctatissima* (F.) which is considered a pest. Both species are morphologically identical and hybridize to produce fertile offspring (Eger et al. 2010). Based on genetic and morphological information linking the invasive U.S. species to those occurring in the Southwestern Islands of Japan, *M. cribraria* has been identified in the U.S. (Eger et al. 2010, Jenkins and Eaton 2011).

Soybean and kudzu are the primary hosts of *M. cribraria* in the U.S. (Eger et al. 2010, Zhang et al. 2012, Ruberson et al. 2013). However, there are several reports indicating *M. cribraria* as a pest of legume and some non-legume plants. Eger et al. (2010) listed a number of legume hosts, including lima bean (*Phaseolus lunatus* L.), soybean, pigeon pea (*Cajanus cajan* L.), lablab (*Lablab purpureus* L.), mung bean (*Phaseolus radiates* L.), azuki bean (*Vigna angularis* L.), and fava bean (*Vicia faba* L.) which were evaluated here. They also include a list of non-legumes reported as hosts of *M. cribraria*. *M. cribraria* has been reported completing development on cotton by Srinivasaperumal (1992) but there has been no indication that cotton is a host in the U.S. *Megacopta* spp. are also reported to feed and reproduce on lablab on the Indian subcontinent (Thippeswamy and Rajagopal 1998, 2005a, b, Thejaswi et al. 2008). Lablab is not grown as a crop in the U.S. but is occasionally planted in wildlife food plots (Hehman and Fulbright 1997). A study by Zhang et al. (2012) was conducted to determine the preliminary host range of *M. cribraria* on some common forest legumes in Georgia. Of the 11 entries evaluated, nymph development was seen only on kudzu and soybean. *M. cribraria* adults have been reported on several plants, legume and non-legume, though many of them are likely not developmental hosts or are incidental (Eger et al. 2010, Gardner et al. 2013, Ruberson et al. 2013). Medal et al. (2013) conducted no-choice tests of 12 plant species and concluded that soybean, kudzu, black-eyed pea (cow pea) (*Vigna sinensis* L.), lima bean, pinto bean (*Phaseolus vulgaris* L.) and pigeon pea were suitable for nymph development.

In December of 2011 and early 2012, adult *M. cribraria* cadavers were recovered from shipping containers containing poultry products in Honduras, originating from Georgia, USA (Ruberson et al. 2013). In response, the Honduran Agricultural Protection Service (SEPA; Servicio de Proteccion Agropecuaria) embargoed all cargo originating from GA, SC, NC, and AL on 12 February 2012 and held the cargo at ports until release upon thorough inspection on 1 March 2012 (Ruberson et al. 2013). Similar actions were taken on all cargo coming from the southeastern U.S. to Guatemala (Crowley 2012). These detections in international cargo raised concern of establishment of *M. cribraria* on legume crops grown in Central and South America. This, in combination with contradictory information on the true host range of *M. cribraria* prompted this study. We evaluated a series of bean species which have been indicated as hosts of *M. cribraria* in the literature and are economically important to the U.S. and Central America. The objectives of this study were to assess the suitability of edible legumes to serve as reproductive hosts for *M. cribraria* in the laboratory and open field trials.

Materials and Methods

The host plant preferences of *M. cribraria* nymphs and adults were evaluated in choice tests, no-choice tests, and field evaluations in 2012 and 2013. Choice and no-choice tests were conducted in a greenhouse at the University of Georgia Griffin Campus, Griffin, GA (N 33.264832 W -84.284088). Field trials were conducted at the Bledsoe Plant Science Farm near Griffin, GA (N 33.178455 W -84.409058) in 2012 and 2013 and in 2012 at the UGA Southeast Research and Education Center near Midville, GA (N 32.8759167 W -82.217528). Plant species and varieties evaluated varied between years and trials and are listed in Table 2.1. Plant entries were chosen based on host reports from current literature and concern for potential distribution to crops grown in Central America (Eger et al. 2012, Ruberson et al. 2013). Legume species of economic importance in both the U.S. and Central America were primarily evaluated (i.e. pinto bean, edamame, winter pea, peanut, lima bean, soybean, and snap bean etc.) along with other agronomic crops of interest.

Choice tests. Choice tests were conducted to determine host preference of both nymph and adult *M. cribraria* on selected plant entries. In all trials a maturity group V soybean variety, AGS 5831(Asgrow, Monsanto Co., St. Louis, MO), was used as a positive control. In 2012, corn was added as a non-legume negative control. A total of 17 entries (plant species and varieties) were evaluated for both nymph development and adult longevity trials in 2012 and 2013. The number and varieties of pinto bean, lima bean, snap bean (*Phaseolus vulgaris* L.) and edamame-soybean (*Glycine max* (L.) Merril) (edamame hereafter) varied from 2012 to 2013 and are indicated in tables and figures. Seeds of each plant entry were planted in 10 cm diameter pots, thinned to two plants per pot, and allowed to grow to approximately the 3-leaf stage. One pot of each entry was placed in a 70 X 70 x 137 cm white chiffon mesh cage (BioQuip 1451DCC, Compton, CA) as a randomized complete block design where cages were considered blocks. Pots were adjacent to one another from front to back and a 10 cm space was left empty between pots from left to right. In 2012 there were seven replicates of the nymph choice study and eight replicates of the adult choice study. In 2013, both studies consisted of six replicates. All replicates were carried out simultaneously for each respective trial. Trials were conducted in a greenhouse from May to August under natural light and photoperiod. Plants were watered as needed, and a shade cloth was placed over the greenhouse to reduce heat stress.

In the nymph choice tests, *M. cribraria* egg masses were collected on 15 May 2012 and 29 May 2013 from kudzu shoot tips growing wild near the UGA, Griffin campus. Immediately after being collected excess kudzu shoot vegetation surrounding the egg mass was carefully removed and one egg mass was affixed to the underside of a single leaf of each entry (one egg mass per entry) using PVA-based synthetic glue (Elmer's GlueAll, Elmer's Products Inc., Columbus, OH). Only enough kudzu vegetation was left around the egg masses for glue to sufficiently adhere to the leaf surface without impeding nymph eclosion or providing an unforeseen advantage to neonates. Observations began the day after egg masses were attached. Pots were placed adjacent to one another from front to back with the space of one 10 cm pot in between from left to right. Foliage of all entries was allowed to touch facilitating the nymphs ability to move freely from plant to plant. Nymphs per entry were counted daily for one week and then every other day up to 14 days in 2012 and up to 25 days in 2013. A longer period of time was allocated in 2013 to allow development to the penultimate or adult stages; observations were conducted only for as long as the plants were healthy. Observations for both years included recording when nymphs eclosed from egg masses on their respective plant entry and how many nymphs were found on each entry.

For *M. cribraria* adult choice tests, F1 adults (progeny of overwintering adults) were collected from a kudzu patch near the UGA Griffin Campus on 15 June 2012 and 12 July 2013 and sexed based on external morphology of the terminal abdominal segments (Eger et al. 2010). In 2012, seventeen pairs of adults (one male, female pair per entry) were released into each mesh chiffon screen cage. In 2013, twenty adult pairs were released into screen cages. One day was allowed for adults to acclimate to the cage and observations began the day after infestation (16 June 2012, 13 July 2013) and were conducted as described in the previous section. The number of adults found on each entry and the cage, which was treated as its own entry in the analysis, were recorded.

No-choice tests. No-choice tests were conducted to determine ability of *M. cribraria* to feed and develop on a potential host. Thirteen entries were evaluated in 2012 and 17 in 2013. The experimental units consisted of individually caged 15 cm diameter pots containing one entry each. Seeds were planted and allowed to grow to approximately the 3-leaf stage prior to being caged. Mesh and clear plastic 76 x 70 cm insect rearing sleeves (BioQuip 1461W, Compton, CA) were used to cage individual pots. Caged pots were arranged in a randomized complete block design in the greenhouse and blocks were defined by bench position.

In 2012, three varieties of pinto bean and the non-legume check of corn were excluded from the trial. We chose to exclude pinto bean varieties (Frijol: Negro, Tio Caneta, and Amadeus 77) which had consistently low means (<0.5) throughout the choice trials and could reasonably be considered non-host plants. Hog peanut (*Amphicarpaea bracteata*) was initially included in 2013 but only two replicates contained a representative due to poor germination and slow growth; therefore, this entry was omitted from the analysis. *M. cribraria* egg masses were not found in sufficient numbers on kudzu in 2012 and were collected from soybean leaves at the

Bledsoe Plant Science Farm near Griffin, GA, on 17 July. In 2013, egg masses were collected from kudzu near the UGA Griffin Campus on 29 May. Excess vegetation was trimmed from around the egg masses as previously described. One egg mass was then attached with glue to the underside of one leaf per cage. Observations consisted of recording when nymphs eclosed, their instar by cage (not reported) and the number of nymphs on plant entries and the cage. Observations began the day after infestation (18 July 2012, 30 May 2013) and were taken daily for a week and every other day for an additional week in 2012 and up to 29 days in 2013.

Adult no-choice tests conducted in 2012 and 2013 consisted of 13 and 19 entries respectively. Adult *M. cribraria* were collected from kudzu and gender was determined based on external morphology of the terminal abdominal segment on 17 July 2012 and 29 May 2013. Each entry was individually caged and arranged in a randomized complete block design, replicated six times. Each cage containing an entry received one pair of adults in 2012 which did not provide adequate data on survival and was increased to six pairs in 2013. The number and location (cage or plant) of live adults was recorded daily for one week and every other day up to 17 (2012) and 19 days (2013).

Field evaluation. Trials in 2012 were conducted at Bledsoe Plant Science Farm near Griffin, GA, (N 33.178455 W -84.409058) and the Southeast Research and Education Center near Midville, GA (N 32.8759167 W -82.217528). Sixteen entries were included at both locations. Plots consisting of two, 6.1 m rows with 76 cm row spacing were arranged in a randomized complete block design and replicated five times. Trials were planted on 15 and 19 June 2012 at Griffin and Midville respectively. Samples were taken at 42, 56 and 77 days after planting (DAP) at Griffin and at 35, 44, and 66 DAP at Midville. Egg masses and all nymphs were counted within 30 cm of row, at two locations per plot. Adults were enumerated by taking five sweeps per row for a total of ten sweeps per plot with a 38 cm diameter sweep net. Care was taken to insure that sections sampled for nymphs and eggs did not overlap with sections sampled for adults.

In 2013, a single trial was conducted at Bledsoe Plant Science Farm near Griffin, GA, planted on 17 May. Eighteen entries were evaluated in a randomized complete block design consisting of four 6.1 m rows with 76 cm row spacing. Four border rows of AGS 5831 soybean were planted around the perimeter of the field to mitigate possible edge effects that can be seen from infesting *M. cribraria* populations (Seiter et al. 2013b). Plots were sampled at 35, 52, 62, and 77 DAP. Egg masses and nymphs were counted on separate 30 cm sections of the two middle rows on three contiguous plants from each plot. Nymphs were subdivided into three general groups. The 1st instar nymphs aggregate around the egg mass upon eclosion and are further characterized by a brownish red coloration and small size. Nymphs of light green coloration with wing pads absent were considered 2nd to 3rd instars. Large nymphs with well-developed wing pads were categorized as 4th and 5th instars (see Zhang et al. 2012 for detailed description). Adult numbers were enumerated from ten sweeps per plot using a 38 cm diameter sweep net taken from the middle two rows. The same precautions were used as described in the previous section. Mean numbers of egg masses, 1st instar nymphs, 2nd and 3rd instar nymphs, and 4th and 5th instar nymphs for 35, 42, 56, and 77 DAP were averaged across sample dates as were adult sweep samples at 42, 56, and 77 DAP.

Data analysis. Data from all trials were analyzed using a general linear mixed models approach (PROC GLIMMIX, SAS Institute 2012). Entries were treated as a fixed effect and replicates were treated as a random effect. Where data did not converge replicate was removed from the model as a random variable to achieve the best fit. To account for over dispersion, the counts were modeled using a negative binomial distribution. Least square means were tested using pairwise t-tests with Tukey-Kramer adjustments. When a response variable for an entry had a mean of zero it was excluded from the analysis. During the first four days of the nymph choice tests little information was gathered because, either nymphs had not eclosed or they had not moved from the egg masses; therefore, response variables for those days were combined into one analysis. Similarly, data for days one and two in the 2013 nymph no-choice test were combined as they were not found to be significant when analyzed as single days. Data for the 2012 nymph no-choice test was grouped into dates one through three days, four through six days and dates seven through fourteen and analyzed individually. These groupings were selected as the best representation of the dynamic between nymphs and their potential host. Observations on days one through three and observations during the last three dates counts were taken for the adult choice and no-choice tests were combined and analyzed individually. Field data were analyzed with-in site and averaged across sample date.

Results

Choice tests. In the 2012 nymph choice test, all nymphs eclosed by the fourth day of observation (Fig. 2.1 and Supp. Table S2.1). For the first four days of observation the effect of plant entry was found to have a significant effect on the number of nymphs found on plant entries but no significant difference was detected between entries using the Tukey-Kramer test (F = 1.81; df = 16, 102; P = 0.0401). Pairwise comparisons detected differences between soybean and the entries mung bean, cardinal pinto bean, winter pea and corn; and edamame was different than corn and winter pea ($P < 0.05$). Significantly more nymphs were observed on soybean and edamame than all other entries for days seven through 14 (*P* = 0.0017 to < 0.0001) (see Supp. Table S2.1 for specific *F* and *P* values). On the last day of observations nymphs remained on soybean, edamame, and mung bean only. There was no significant difference between nymphs on soybean and edamame which had significantly more nymphs than mung bean.

In the 2013 nymph choice test, all nymphs eclosed and/or were found on every entry by the fifth day of observation (Fig. 2.1 and Supp. Table S2.2). In the first week of the study there was no significant effect of plant entry on the number of nymphs found on all entries. Plant entry had a significant effect on the number of nymphs per plant on day nine (*F* = 1.98; df = 16, 85; *P* = 0.0236) but no significant difference was found between entries using the Tukey-Kramer test. Pairwise comparisons detected differences between soybean and the entries cardenal pinto bean, lima bean, black-eyed pea and mung bean; and edamame was found to be different than deorho pinto bean, cardenal pinto bean, winter pea, fava bean, lima bean, mung bean, snap bean and pigeon pea; pigeon pea was different than winter pea, deorho pinto pea, cardenal pinto bean, lima bean, lablab and mung bean (*P* < 0.05). On day 11 more nymphs were found on soybean, edamame, pigeon pea and tropical kudzu than all other entries (*F* = 15.59; df = 11, 60; *P* < 0.0001). Nymph densities on tropical kudzu decreased to numbers significantly lower than edamame, soybean and pigeon pea by the next date. From the 13th date after eclosion to the end of the study there were significantly more nymphs on edamame, soybean and pigeon pea than all other entries (*P* < 0.0001; see Supp. Table S2.2 for *F* and *P* values for each date) (Fig. 2.1 and Supp. Table S2.2).

In the 2012 adult choice test, adults were observed on all entries during the first three days. Entry had a significant effect on adult levels during the first three days (*F* = 11.08; df = 17, 126; *P* < 0.0001) and significantly more adults were found on the cage and edamame, followed by soybean (Fig. 2.2). Mung bean and lima bean had significantly fewer adults than edamame and the cage but were not significantly different from all other entries including soybean. During the last three observation periods (days 10, 12 and 14) the greatest number of adults were found on edamame. The mean adults found on soybean for the last three sample periods was lower than edamame and greater than adult levels on all other entries except the cage (*F* = 20.50; df = 11, 84; *P* < 0.0001).

Adults were also found on all entries at various levels for the first three days of the 2013 adult choice test (Fig. 2.3). During this time the greatest numbers of adults were found on the cage followed by edamame, soybean and Fordhook lima bean (*F* = 8.37; df = 19, 100; *P* < 0.0001), no significant difference was detected between these entries. On the last three sample dates (days 10, 12 and 14) edamame had the highest level of adults but was not significantly different from adults on soybean (Fig. 2.3). Adult densities were not different between soybean and pigeon pea, and pigeon pea was not significantly different from all other entries. Of the 19 entries evaluated in this experiment no adults were found on snap bean, cardenal pinto bean, winter pea, chick pea, tropical kudzu and peanut on the last three sample dates (*F* = 13.93; df = 13, 70; *P* < 0.0001).

No-choice tests. There was no significant effect of entry on the number of nymphs per entry for the first three days after eclosion in the 2012 nymph no-choice trial (Table 2.2). Nymphs established on all entries; however by days four through six significantly more nymphs were found on edamame, soybean and azuki bean than all other entries. Nymphs on azuki bean were not different from remaining entries. During the last week of the study no nymphs remained on any entry other than soybean, edamame and winter pea. Low levels of nymphs (0.1 ± 0.1) were only detected on one of the last dates in the final week of observations on winter pea at a mean significantly lower than means found on edamame and soybean.

In the 2013 nymph no-choice trial the number of nymphs on each entry varied for the first five days (*F* = 0.97 – 3.33; df = 16, 81; *P* = 0.4956 – 0.0002) (Supp. Table S2.3). Beginning at day six nymph survival began to decline on several entries. By day 15 nymphs only remained on six entries (Fig. 2.4). Significantly more nymphs remained on soybean, edamame and pigeon pea than lablab, fava bean and tropical kudzu (*F* = 15.19; df = 5, 26.7; *P* < 0.0001). By day 21 no nymphs survived on lablab (*F* = 13.18; df = 4, 16.7; *P* < 0.0001). By the last date nymphs survived on only soybean, edamame and pigeon pea (*F* = 0.56; df = 2, 7; *P* = 0.5931). Nymphs successfully developed to adult on soybean, pigeon pea and edamame. Nymphs also completed development to adult by day 25 on a single replicate of fava bean.

Little meaningful data were obtained for the 2012 adult no-choice test (data not shown). In the 2013 adult no-choice test, survival for the first three days was variable across all entries (Fig. 2.5). The adults found on soybean (days 1-3) were significantly greater than winter pea and chick pea but were not significantly different from the remainder of the entries (*F* = 4.44; df = 18, 83.93; *P* < 0.0001). For the last three days of observation more adults remained on soybean and edamame than winter pea, Fordhook lima bean, mung bean, tropical kudzu, peanut, hog peanut and chick pea. Adult levels on entries pigeon pea, fava bean, azuki bean, black-eyed pea and pinto-frijol were not significantly different from soybean and edamame (*F* = 13.81; df = 17, 198; *P* < 0.0001). By the last three dates chick pea was the only entry to have no adults surviving. Though few significant differences were found between entries, soybean, edamame, pigeon pea and fava bean were more successful in maintaining adult survivorship than the other entries tested.

Field evaluations. In the field, *M. cribraria* adults infested all entries but were present in the greatest numbers on soybean, edamame (2012 and 2013), and pigeon pea (2013). Oviposition was greatest on edamame and soybean in both years at all locations (Table 2.3). Oviposition also occurred on pigeon pea in 2013, though there was no significant difference in the number of egg masses on pigeon pea and all other entries where little to no oviposition occurred ($F = 49.86$; df = 8, 63; $P < 0.0001$). Growth and development of nymphs was also greatest on edamame and soybean in all trials. In 2013 late instar nymphs were observed on pigeon pea but at levels significantly less than those of edamame and soybean. In the 2013 trial, with the exception of a few first instar nymphs on lablab, no nymphs were found on any entry other than soybean, edamame, and pigeon pea.

Discussion

From 2012 to 2013, a total of 23 entries were evaluated in at least one of the three experiments (choice, no-choice and field trials). In both years we confirmed that soybean and edamame are suitable hosts for *M. cribraria* in the U.S. and confirmed pigeon pea as a developmental host in 2013. Adult, nymph and egg mass levels were not as high on pigeon pea as the two varieties of *Glycine max*. Many of the plant species that were reported in literature as hosts of *M. cribraria* (Eger et al. 2010) were not found to allow extended adult survival or nymph development. No-choice host preference studies conducted in greenhouses by Medal et al. (2013) agree with our conclusion that pigeon pea is a suitable reproductive host of *M. cribraria.* The same study also reported that significantly lower numbers of nymphs developed to adult on lima and pinto bean compared to soybean, pigeon pea, and kudzu. Contrary to their findings we did not find that black-eyed pea (cowpea), lima bean, and pinto bean were suitable hosts in both years these entries were evaluated. We also found that peanut, chickpea, and mung bean are not reproductive or developmental hosts. Thippeswamy and Rajagopal (2005) reported development of *M. cribraria* (*Coptosoma cribraria* (F.), a synonym) on lablab and soybean but did not detect oviposition by caged adults on redgram (pigeon pea) (Ruberson et al. 2013). Our results show that in both years for choice, no-choice and field trials the only plants with significant survival, growth, development and oviposition of *M. cribraria* are soybean, edamame, and pigeon pea. In one replicate of the no-choice nymph test, development to adult also was observed on fava bean (*Vicia faba*). No development was seen on fava bean in any of the other studies, though fava bean has been reported as a host in Japan (Ishihara 1950, Eger et al. 2010).

Concern for crops grown in Central America such as pinto bean and winter pea, was a major impetus for performing these host preference evaluation. Although Medal et al. (2013) reported *M. cribraria* can develop on pinto bean we found little oviposition and did not observe development of nymphs on several varieties of pinto beans obtained from Guatemalan and Honduran sources. No *M. cribraria* establishment on any of the varieties of pinto bean or winter pea was seen in either year in field trials. Results obtained from these studies indicate that if *M. cribraria* becomes established in Central America it is unlikely to become a serious pest of pinto bean and winter pea. Tropical kudzu (*Pueraria phaseoloides*) is grown as a cover crop for nitrogen fixation in orchards in Central and South America (Araújo Neto et al. 2014) and is closely related to the invasive perennial kudzu vine (*Pueraria lobata* var. montana) which is a primary host of *M. cribraria*. Nymphs developed on tropical kudzu to approximately late second instar in choice and no-choice tests but survival quickly declined thereafter, suggesting it is not a suitable host. Though it seems unlikely that *M. cribraria* could develop on tropical kudzu based on our findings, it would be prudent to re-evaluate it under other conditions.

In the U.S. lima bean and snap bean are frequently grown as fresh harvest crops. *M. cribraria* does not pose a threat to yield of these crop plants based on our findings that they are not developmental hosts. However some advantage may be provided as a temporary source of nutrition for disseminating adults in the spring. With the wide range of reported hosts it is unclear what advantage adults' gain from plants that are not developmental hosts. In field trials low levels of adults were found on most entries though development was not seen on the majority. This may demonstrate how several plant species could be misconceived as hosts though no development actually occurs on them. There is some concern that adults could be a contaminant issue for these beans and other fresh harvested beans (i.e., edamame). *M. cribraria* reportedly develops in commercial fields of lima beans (*Phaseolus lunatus* var. Fordhook) in southern Georgia (A. Sparks, unpublished data). However, we were unable to duplicate this observation in replicated field and greenhouse trials with both Fordhook and Henderson varieties of lima beans. Observational findings that suggest edamame may be a more susceptible host than field soybean indicate there can be differential preference among varieties of the same host species.

While it is clear adult *M. cribraria* can be found on a wide range of plant species, we found a limited range of host plants for oviposition and development. This contradicts some previous literature covering the hosts of *M. cribraria*. The composition of the symbiotic gut capsule has a major influence on host range of *M. cribraria* (Hosokawa et al. 2007) and may be the single most important factor affecting host range. The absence of development of *M. cribraria* on lablab (which is frequently referred to as a host of *M. cribraria* in India and Asia) in greenhouse trials and field trials in both years could be linked to symbiont composition. Differences in host ranges between geographic areas can be highly dependent on the specific composition of the symbiotic gut microorganisms. The U.S. populations of *M. cribraria* are not genetically diverse, having one haplotype linked to a single introduction (Jenkins and Eaton 2011). Lack of diversity within species and symbiotic microorganisms may limit the host range of *M. cribraria* in North America as compared with its native range. Soybean remains the primary crop of concern for this insect though some incidental feeding and low levels of reproduction may occur on pigeon pea, and possibly fava bean in the southeastern U.S.

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Table 2.1. Plant species evaluated as suitable hosts of *Megacopta cribraria* in choice and no-choice tests in greenhouses and field evaluations, 2012 and 2013

Scientific Name	**Common Name**	**Variety**	**Year**
Arachis hypogaea	Peanut	Georgia 06G	2012, 2013
Cajanus cajan	Pigeon pea	--	2013
Cicer arietinum	Chick pea	--	2013
Glycine max	Soybean	AGS 5831 (MG5)	2012, 2013
Glycine max	Edamame-Soybean	Midori Giant	2013
Glycine max	Edamame-Soybean	Mosley' O.P. prolific	2012
Lablab purpureus	Lablab	--	2012, 2013
Phaseolus lunatus	Lima bean	Henderson	2012, 2013
Phaseolus lunatus¶	Lima bean	Fordhook	2013
Phaseolus radiatus	Mung bean	--	2012, 2013
Phaseolus vulgaris	Pinto bean	Frijol: Negro	2012, 2013
Phaseolus vulgaris	Pinto bean	Deorho	2012, 2013
Phaseolus vulgaris	Pinto bean	Cardenal	2012, 2013
Phaseolus vulgaris	Snap bean	Bronco	2012
Phaseolus vulgaris	Snap bean	Roma II	2013
Phaseolus vulgaris§	Pinto bean	Tio Caneta	2012
Phaseolus vulgaris§	Pinto bean	Carrizalita	2012
Phaseolus vulgaris§	Pinto bean	Amadeus 77	2012
Pisum sativum	Winter pea	Oregon sugar pod II	2012, 2013
Pueraria phaseoloides	Tropical kudzu	--	2013
Vicia faba	Fava bean	Windsor	2013
Vigna angularis	Azuki bean	--	2012, 2013
Vigna sinensis	Black-eyed pea	California black-eye	2012, 2013

¶ Not included in the nymph choice and no-choice tests 2013
§ Excluded from adult and nymph no-choice trials 2012

Table 2.2. Mean ± SE number of nymphs per entry by day, after eclosion in nymph no-choice test 2012

Scientific Name	**Common Name**	**Variety**	**Days 1-3**	**Days 4-6**	**Days 7-14**
Glycine max	Soybean	AGS 5831 (MG5)	49.1 ± 13.4a	39.0 ± 15.8a	37.6 ± 17.6a
Glycine max	Edamame-Soybean	Mosley' O.P. prolific	22.9 ± 6.2	16.1 ± 6.6ab	17.8 ± 8.4a
Phaseolus radiatus	Mung bean	--	27.1 ± 7.5a	4.8 ± 2.1bc	0
Phaseolus lunatus	Lima bean	Henderson	32.5 ± 8.9a	0.3 ± 0.2c	0
Lablab purpureus	Lablab	--	33.0 ± 9.1a	0.8 ± 0.5c	0
Arachis hypogaea	Peanut	Georgia 06G	33.9 ± 9.3a	0.5 ± 0.3c	0
Vigna angularis	Azuki bean	--	28.7 ± 7.9a	5.3 ± 2.3abc	0
Phaseolus vulgaris§	Pinto bean	Frijol: Negro	--	--	--
Phaseolus vulgaris	Pinto bean	Deorho	20.1 ± 5.6a	0.8 ± 0.5c	0
Phaseolus vulgaris	Pinto bean	Cardenal	25.6 ± 7.1a	4.1 ± 1.8bc	0
Vigna sinensis	Black-eyed pea	California black-eye	22.9 ± 6.4a	0	0
Pisum sativum	Winter pea	Oregon sugar pod II	43.9 ± 12.0a	1.8 ± 0.9bc	0.1 ± 0.1b
Phaseolus vulgaris	Snap bean	Roma II	30.1 ± 8.4a	3.8 ± 1.7bc	0
Phaseolus vulgaris§	Pinto bean	Tio Caneta	--	--	--
Phaseolus vulgaris	Pinto bean	Carrizalita	35.1 ± 9.6a	2.3 ± 1.1bc	0
Phaseolus vulgaris§	Pinto bean	Amadeus 77	--	--	--
		F	1.09	7.17	10.25
		P	0.3848	<0.0001	0.0016
		df	12, 57.8	11, 60	2, 15

Means with the same letters in columns are not significantly different from each other (Tukey-Kramer LSM α = 0.05). Entries with zero nymphs were excluded from the GLIMMIX analysis.
§ Entries included in choice trial but excluded in no choice trial.

Table 2.3. Field evaluations, Midville (2012) and Griffin (2012 and 2013), within site means ± SE of M. cribraria egg masses, nymphs and adults across sample date

			2012 Midville 44, and 66 DAP		**2012 Griffin 42, 56 and 77 DAP**			**2013 Griffin 35, 42, 56 and 77 DAP**		
Scientific Name	**Common Name**	**Variety**	**Adult**	**Nymphs**	**Adult**	**Egg Masses**	**Nymphs**	**Adult §**	**Egg Masses**	**Nymphs**
Glycine max	Soybean	AGS 5831 (MG5)	9.6 ± 2.6a	24.6 ± 9.0a	91.4 ± 24.1a	21.0 ± 4.7a	77.16 ± 31.9a	73.2 ± 25.2a	47.1 ± 14.3a	98.1 ± 48.2a
Glycine max	Edamame-Soybean	Midori Giant	--	--	--	--	--	76.0 ± 26.1a	54.7 ± 16.6a	80.2 ± 39.4a
Glycine max	Edamame-Soybean	Mosley'O.P. prolific	5.4 ±1.6ab	25.3 ± 9.3a	72.4 ± 19.2a	4.3 ± 1.2b	7.8 ± 3.4b	--	--	--
Cajanus cajan	Pigeon pea	--	--	--	--	--	--	38.5 ± 13.4ab	1.4 ± 0.6b	1.6 ± 0.9b
Phaseolus radiatus	Mung bean	--	0.2 ± 0.2b	0	14.7 ± 4.2b	0	0	16.5 ± 5.9abc	0.2 ± 0.1b	0
Phaseolus lunatus	Lima bean	Henderson	0	0	0.9 + 0.6b	0	0	10.2 ± 3.8bcd	0	0
Lablab purpureus	Lablab	--	0	0	10.5 ± 3.2b	0	0	9.2 ± 3.4bcd	0	0.0 ± 0.1b
Arachis hypogaea	Peanut	Georgia 06G	0	0	0.6 ± 0.5b	0	0	6.5 ± 2.5cd	0	0
Vigna angularis	Azuki bean	--	0.2 ± 0.2b	0	2.6 ± 1.6b	0	0	6.2 ± 2.4bcd	0	0
Vicia faba	Fava bean	Windsor	0	0	--	--	--	4.7 ± 1.9cd	0	0
Phaseolus vulgaris	Pinto bean	Frijol: Negro	0	0	1.6 ± 0.8b	0	0	4.5 ± 1.8cd	0	0
Phaseolus vulgaris	Pinto bean	Deorho	0	0	0.3 ± 0.3b	0	0	3.7 ± 1.5cd	0	0
Phaseolus lunatus	Lima bean	Fordhook	0	0	--	--	--	3.5 ± 1.5cd	0.2 ± 0.1b	0
Phaseolus vulgaris	Pinto bean	Cardenal	0	0	3.3 ± 1.3b	0	0	3.0 ± 1.3cd	0	0
Vigna sinensis	Black-eyed pea	California black-eye	0.2 ± 0.2b	0	2.3 ± 1.0b	0	0	3.0 ± 1.3cd	0.3 ± 0.2b	0
Pisum sativum	Winter pea	Oregon sugar pod II	0	0	0.3 ± 0.3b	0	0	1.7 ± 0.8cd	0	0
Pueria phaseoloides	Tropical kudzu	--	--	--	--	--	--	1.5 ± 0.7d	0.7 ± 0.3b	0
Phaseolus vulgaris	Snap bean	Bronco	--	--	--	--	--	1.2 ± 0.7d	0	0
Phaseolus vulgaris	Snap bean	Roma II	0.4 ± 0.2b	0	0.6 ± 0.5b	0	0	--	--	--
Cicer arietinum	Chick pea	--	--	--	--	--	--	0.2 ± 0.2d	0	0
Phaseolus vulgaris	Pinto bean	Tio Caneta	0	0	0.3 ± 0.3b	0	0	--	--	--
Phaseolus vulgaris	Pinto bean	Carrizalita	0	0	2.9 ±1.2b	0	0	--	--	--
Phaseolus vulgaris	Pinto bean	Amadeus 77	0	0	1.3 ±0.7b	0	0	--	--	--
		F	9.15	0.01	17.29	18.63	14.47	9.95	49.86	33.84
		P	<0.0001	0.9110	<0.0001	0.0015	0.0035	<0.0001	<0.0001	<0.0001
		df	5, 24	1, 11.8	15, 27.1	1, 10	1, 10	17, 54	8, 63	3, 28

Means with the same letter in columns are not significantly different (Tukey-Kramer LSM α = 0.05). Entries with zero individuals were excluded from the GLIMMIX analysis.

§ Mean number of adults for 52, 62 and 72 DAP. Whole plant counts at 35 DAP not included in analysis

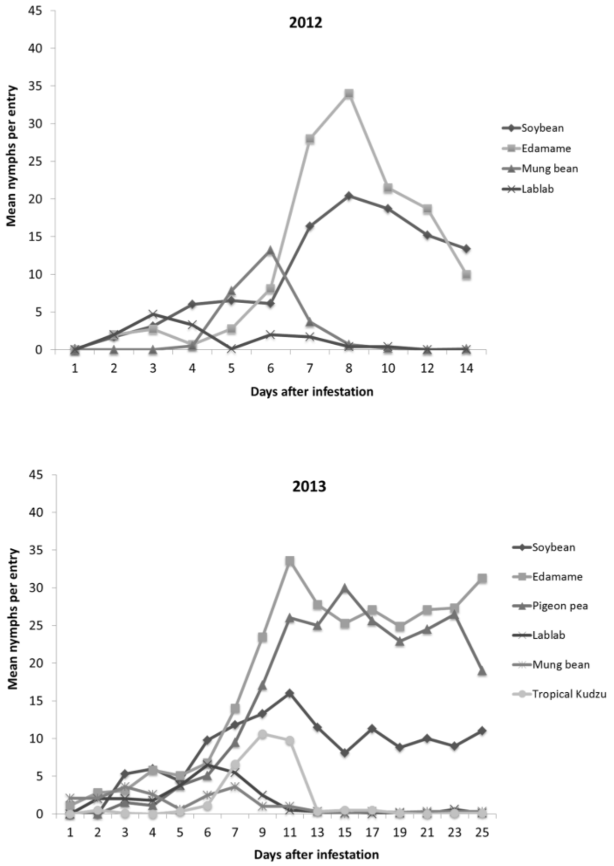

Figure 2.1. Nymph choice tests; Mean ± SE *Megacopta cribraria* nymphs per day. Entries where nymphs were present on the last day of observation are shown. (A) In 2012 nymphs remained on soybean, edamame and mung bean by day 14. No nymphs were found on lablab after day 10, the entry is included in the figure for continuity between years. (B) In 2013 nymphs remained on soybean, edamame, pigeon pea, lablab, mung bean and tropical kudzu by day 25. Standard error bars omitted for clarity of presentation (see Supp. Table S2.1 for full data set). Entries with zero adults were excluded from the GLIMMIX analysis.

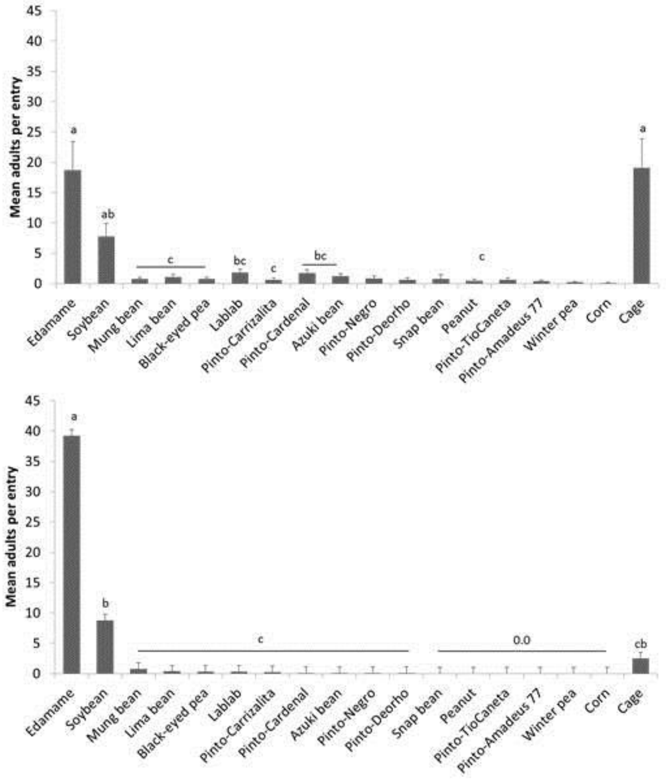

Figure 2.2. Mean ± SE *M. cribraria* adults per entry for days one through three (top), and days 10 through 14 (bottom) of adult choice test 2012. Means with the same letter are not significantly different (Tukey-Kramer LSM α = 0.05). Entries with zero adults were excluded from the GLIMMIX analysis.

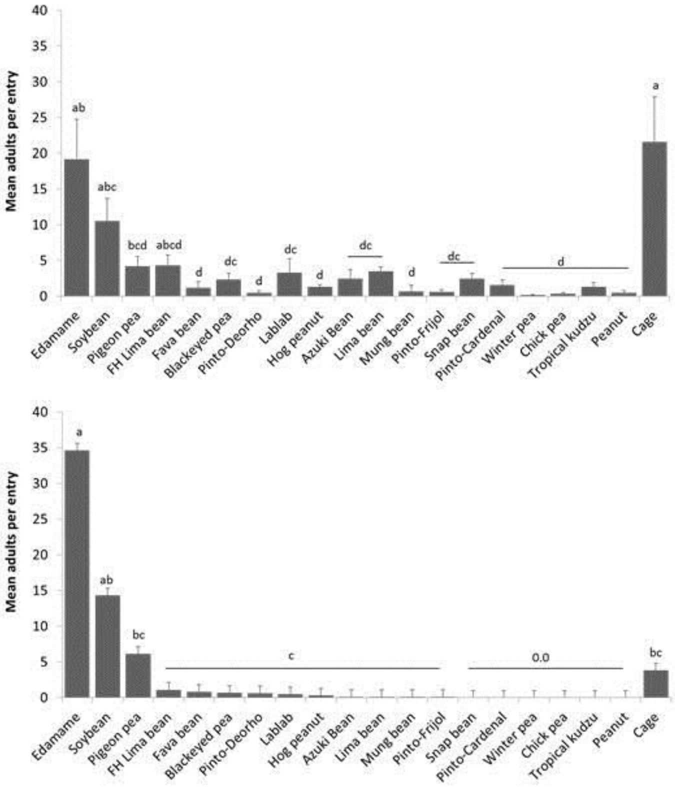

Figure 2.3. Mean ± SE *M. cribraria* adults per entry, for days one through three (top), and days 10 through 14 (bottom) of adult choice test 2013. Entries with zero adults were excluded from the GLIMMIX analysis.

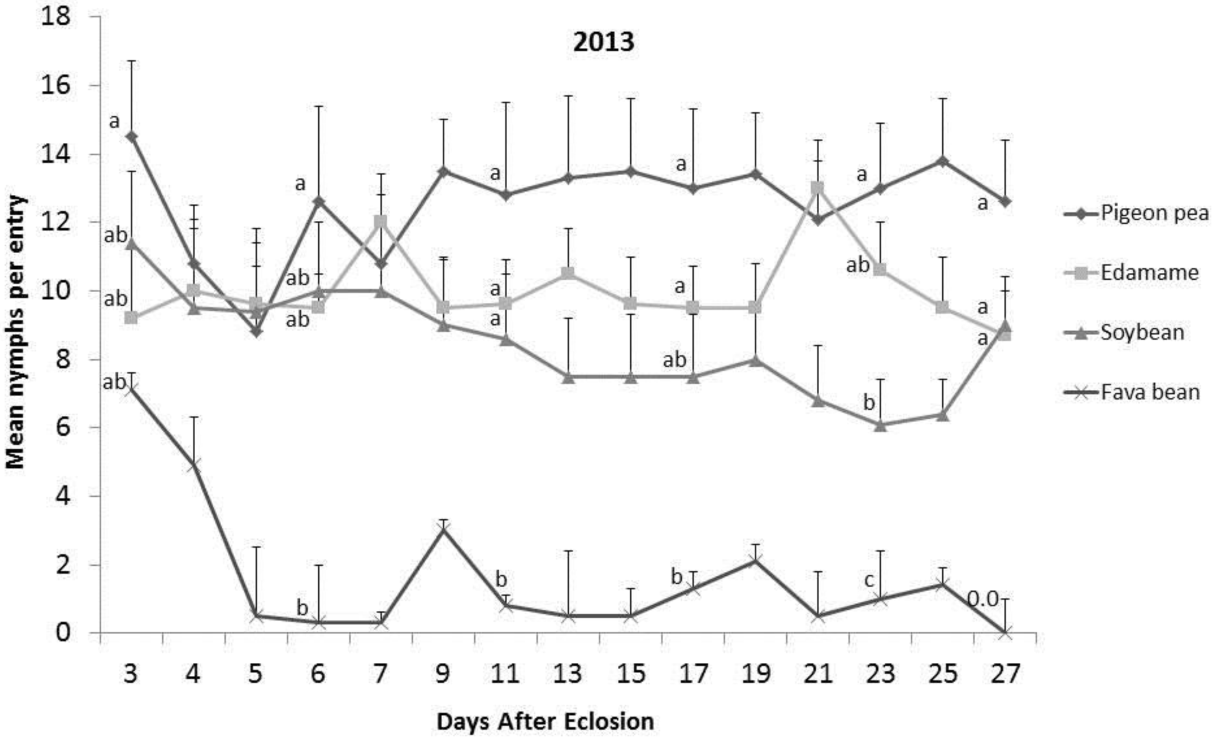

Figure 2.4. Mean ± SE *M. cribraria* nymphs in 2013 no-choice test from three days after eclosion (the first day a significant effect of entry was seen on nymph numbers) on entries where nymphs were present for the majority of the study; pigeon pea, edamame, soybean and fava bean. Means separations are given for every three sample dates after eclosion and the last day of observation. No nymphs were on fava bean after day 25, though nymphs developed to adult on this entry. Entries with zero nymphs per date were excluded from the GLIMMIX analysis (see Supp. Table S2.3 for full data set). Means with the same letter are not significantly different (Tukey-Kramer LSM α = 0.05).

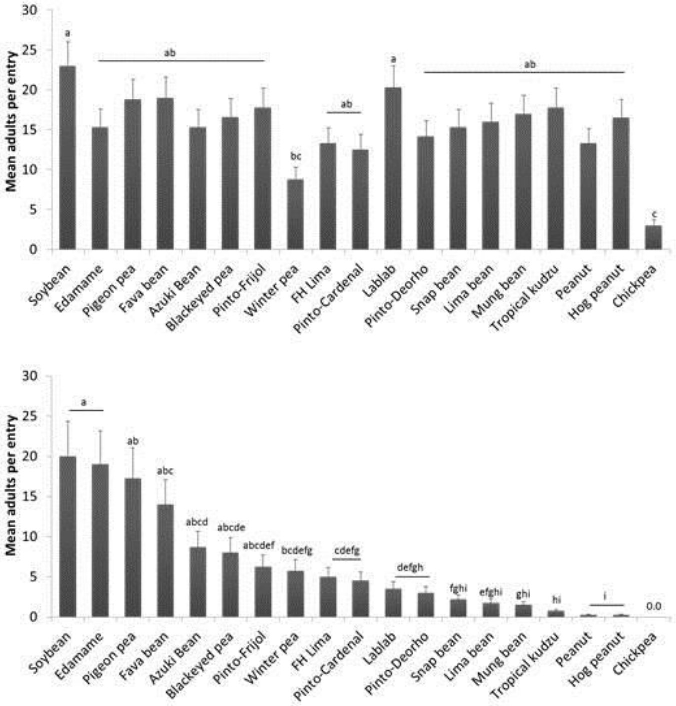

Figure 2.5. Mean ± SE *M. cribraria* adults for first three days (top) and last three dates (bottom) of the adult no-choice test 2013. Entries with zero adults were excluded from the GLIMMIX analysis. Means with the same letter are not significantly different (Tukey-Kramer LSM α = 0.05).

CHAPTER 3

EFFECT OF PLANTING DATE AND MATURITY GROUP ON SOYBEAN YIELD IN RESPONSE TO INJURY BY *MEGACOPTA CRIBRARIA*[1]

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[1] J. L. Blount, G. D. Buntin and P. M. Roberts. 2016. *Journal of Economic Entomology*. 109: 207-212. Reprinted here with permission of the publisher.

Abstract

The kudzu bug, *Megacopta cribraria* (F.), is an invasive member of the family Plataspidae originating from Asia. Since its discovery in Georgia in 2009, its distribution has increased to 13 southern and eastern states. In the U.S., *M. cribraria* is bivoltine and has two primary developmental hosts, kudzu and soybean. Here, we evaluated the yield response of soybean to *M. cribraria* feeding injury in relation to planting date and soybean maturity group. The study contained four replicated trials in Griffin, Tifton, and Midville, GA in 2012 and 2013. Four planting dates from April to July, served as the whole plot of a split plot design with maturity group five and seven soybean and insecticide (lambda-cyhalothrin) randomized within planting date. Egg masses, nymphs and adults were enumerated weekly to biweekly until soybean reached maturity. Two generations were observed in April and May plantings, but only one generation was evident in June and July soybean plantings. Insecticide protected plots had consistently higher yields than unprotected plots. Grain yield was greatest in the May planting and lowest in the July planting. Season long feeding by *M. cribraria* reduced grain yield in April, May and June plantings but not in the July planting. Maturity group and planting date had significant effects on yield components in most comparisons. This study indicated that early planted soybean are at greater risk of yield loss from *M. cribraria* injury compared to later planted soybean.

Key Words: *Glycine max*, Soybean

Introduction

Megacopta cribraria (F.) (kudzu bug) was discovered in the U.S. in 2009 aggregating on the sides of homes near kudzu (*Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen and S. Almeida) patches in northeast Georgia (Suiter et al. 2010, USDA-NRCS 2015). The North American range of *M. cribraria* has increased from nine Georgia counties in 2009 to 13 states (GA, FL, AL, MS, LA, KY, TN, SC, NC, VA, DE, AR and MD) and the District of Columbia (Gardner 2014). Since its initial entry into the U.S., *M. cribraria* has become a key pest of soybean (*Glycine max* Merrill) in the southeastern U.S. Adult *M. cribraria* disperse from overwintering sites and infest kudzu and soybean as they become available (Pozo-Valdivia and Reisig 2013). Females readily lay eggs in soybean and first instars emerge in seven to ten days completing development of five instars in about six weeks (Zhang et al. 2012, Ruberson et al. 2013). Newly emerged nymphs consume symbiotic gut capsules (*Candidatus Ishikawaella capsulata*) deposited at the base of the egg mass by the female (Jenkins et al. 2010). Nymphs stay near the egg mass for the first day or two then disperse and begin to feed along the plant stem. Injury by *M. cribraria* is characterized by purplish brown lesions occurring primarily on the stems and nodes where nymphs and adults concentrate and feeding occurs (Seiter et al. 2013a). Unlike stink bugs (Hemiptera: Pentatomidae), this sucking pest does not feed on the pod or seed of the plant but reduces yield indirectly by removing vascular fluids from the plant (Ruberson et al. 2013). *Megacopta cribraria* have been recorded aggregating in great numbers on soybean plants and increased densities are directly related to decreases in yield (Seiter et al. 2013a). *Megacopta cribraria* infestations have resulted in an average 19 percent yield reduction, with a range from 0 to 60 percent (Seiter et al. 2013a, Roberts et al. 2014). Currently, little is understood about the effect *M. cribraria* infestations have on early and double crop soybean systems (soybean planted after winter wheat harvest) in the southeastern U.S. Directly related to planting time is maturity group, which is an important consideration when selecting a soybean variety for early and double crop systems. Similarly, the impact of *M. cribraria* infestations on yield of different maturity groups commonly grown in Georgia is not well understood. Here, we determine the effect of *M. cribraria* injury on soybean yield at four different planting dates consisting of two maturity groups. *Megacopta cribraria* infestation and development was monitored in order to determine how planting date and maturity group influenced *M. cribraria* infestations.

Materials and Methods

Trials were conducted at the Coastal Plain Experiment Station at Tifton, Georgia (31.519497 N, -83.54230 W) (2012 and 2013), the Southeastern Research and Education Center, Midville, GA (32.8759167 N, -82.217528 W) (2012), and the Bledsoe Plant Science Farm near Griffin, GA (33.178455 N, -84.409058 W) (2013). Soybean maturity group and insecticide application (subplot treatments) were randomized within planting date (main plot) in a split plot design, and replicated four times at each location. Each main plot consisted of twelve 24.3 m long rows with 91 cm row spacing. Each whole plot was divided into four 12.1 m long subplots consisting of six rows. Soybean varieties in maturity group V (Asgrow AG5831) and maturity group VII (Asgrow AG7231) (Asgrow, Monsanto Co., St. Louis, MO), were planted approximately every four weeks from April to July for both years as the planting date treatment. Planting dates for each location and year are as follows: Midville 2012, 13 April, 22 May, 19 June and 11 July; Tifton 2012, 9 April, 11 May, 13 June and 10 July; Griffin 2013, 23 April, 16 May, 21 June and 15

July; Tifton 2013, 19 April, 15 May, 11 June and 15 July. All fields were prepared using conventional tillage practices. Seed was planted with a two row Monosem vacuum planter (Monosem® Inc., Edwardsville, KS) at the rate of 20 seeds per meter of row. Either pendamethalin (Prowl®, BASF Corp., Research Triangle Park, NC) or trifluralin (Treflan™, Dow AgroSciences Co., Indianapolis, IN) were pre-plant incorporated. Glyphosate (Glyfos®, Cheminova, Lemvig, Denmark) was applied post emergence at about 21 days after planting for weed control (Griffin 2013) or as needed at the Tifton and Midville (Roundup WeatherMAX®, Monsanto Co., St. Louis, MO) locations. Foliage feeding insects (*Chrysodeixis includens* (Walker), *Anticarsia gemmatalis* (Hubner), *Plathypena scabra* (F.) etc.) were managed with either flubendiamide (Belt®SC, Bayer Crop Science LP, Research Triangle Park, NC) or indoxacarb (Steward®EC, E. I. du Pont de Nemours and Co., Wilmington, DE) as needed.

Subplots with the treated designation were aggressively protected using a broad spectrum insecticide applied approximately every two weeks from a ~V4 growth stage until late September when *M. cribraria* populations began to disperse from maturing soybean. Lambda-cyhalothrin (Karate Z; Syngenta Crop Protection, LLC, Greensboro, NC) was applied at the Griffin location at a rate of 140.3 ml per hectare; thiamethoxam plus lambda-cyhalothrin (Endigo SC; Syngenta Crop Protection, LLC, Greensboro, NC) was applied at the Tifton and Midville locations at 328.9 ml per hectare.

Sampling. The two middle rows of each subplot were reserved for yield and rows two and five for sampling, thereby leaving the rows adjacent to the bordering plots untouched. Each subplot was sampled for adults, nymphs and egg masses weekly in 2012 and every 10 days in 2013. Plots were monitored for infestation from 14 days after planting until sampling began at 35 days after planting when plants were large enough to be swept. Sampling ended once plants reached the R7 developmental stage. Adults (2012, 2013) and nymphs (2012 only) were enumerated by taking 10 single row sweeps per plot with a standard 38 cm diameter sweep net. The numbers of egg masses on 25 trifoliates per plot were enumerated at the Midville and Tifton locations in 2012. Nymphs and egg masses were enumerated on three randomly selected plants within 30 cm of row at four locations per plot in 2012 and 2013 at the Griffin location and at the Tifton location in 2013.

Yield components. In 2012, yield measurements were taken from the Tifton location. Three, one row foot sections of physiologically mature plants were collected from three locations in each subplot approximately two weeks before harvest. An average of seven plants per row foot were sampled. Plant samples were collected from row two and five in order to avoid rows bordering adjacent plots and rows reserved for grain yield. Upon collection, plants were wrapped in brown packing paper and allowed to dry completely. The number of pods per plant, seeds per pod, and the weight per seed was recorded. The two center rows were harvested with a two row small plot combine (Wintersteiger Inc., Reid im Innkreis, Austria). Maturity group five and seven soybean were harvested at Tifton on 19 and 25 Oct. and 1 and 5 Nov. 2012at Midville. Both maturity groups were harvested on a single date in 2013 at the Tifton (31 Oct.) and Griffin (4 Nov.) locations. Plot seed weight and moisture content were measured and grain yield was estimated and adjusted to a standard value of 13 percent moisture for all trials. Mean yield loss within planting date was obtained by subtracting the weight of the untreated subplots from the weight of the treated subplots, then dividing the product by the weight of the treated plots and multiplying by 100.

Data analysis. Total adults, nymphs and egg masses per subplot and grain yield for all trials were combined for analysis. The data were analyzed using SAS Software’s GLIMMIX procedure (SAS/STAT software, version 9.3 ©2002-2012 SAS Institute Inc., Cary, NC, USA). Maturity group and insecticide were randomized within planting date at all locations. Location and replicate were treated as random effects. LS means are reported and were separated using pairwise t-tests, the standard separation procedure in GLIMMIX. The three subsamples of yield components in the 2012 Tifton trial were also analyzed with GLIMMIX, and replicate was treated as a random effect. Significant two-way interactions were further analyzed using the slicediff option of the LSMEANS statement. Means were separated using LSMEANS ($\alpha = 0.05$).

Results

There was no significant effect on egg mass densities from the planting date by insecticide interaction ($P = 0.807$), planting date by maturity group ($P = 0.725$) or insecticide by maturity group ($P = 0.984$) interaction. While none of the interactions were significant, planting date ($F = 109.35$; df = 3, 236; $P < 0.001$) did have a significant effect on the number of egg masses. The greatest number of egg masses were found in the April planting followed by the May planting with no significant difference detected between the June and July plantings (Table 3.1). Insecticide ($P = 0.329$) and maturity group ($P = 0.735$) had no effect on the number of egg masses. Egg masses collected from each planting date at all four locations indicate two generations occurred in the April planting date, whereas the May planting date had a partial first generation and full second generation present (Fig. 3.1). Only the second generation occurred in the June and July plantings (Fig. 3.1). *Megacopta cribraria* populations in Tifton and Midville in 2012 had peak oviposition in May and June with several spikes occurring through late July (Fig. 3.1A and 3.1B). Oviposition in 2013 at the Tifton location was greater than in 2012, but the second oviposition event was less detectible (Fig. 3.1C). Peak oviposition in 2013 at the Griffin location was large early in the season, but the second generation, though present, was greatly reduced and only one distinct generation occurred (Fig. 3.1D).

The planting date by maturity group interaction did not significantly impact nymph populations ($P = 0.251$). However, nymphs were affected by the interactions of planting date and insecticide ($F = 14.39$; df = 3, 236; $P < 0.001$) and insecticide by maturity group ($F = 3.95$; df = 1, 236; $P = 0.047$). Differences in the effect of insecticide on nymph populations were found between treated and untreated subplots in the April ($t_{236} = -8.14$; $P < 0.001$) and May ($t_{236} = -2.39$; $P = 0.017$) plantings but not the June and July planting dates ($P = 0.999$ to 0.824) when controlling for planting date. This is consistent with the mean nymph populations in April and May plantings, which were significantly greater than that of the June and July plantings (Table 3.1). Differences in nymph densities were found between maturity groups five and seven ($t_{236} = 0.55$; $P = 0.004$) in untreated subplots when controlling for insecticide, but there was no difference in maturity group in treated subplots ($P = 0.977$).

Planting date by maturity group ($P = 0.206$) and insecticide by maturity group ($P = 0.268$) did not have a significant effect on the adult population. The interaction of planting date and insecticide was found to have a highly significant effect on adult populations ($F = 20.20$; df = 1, 236.1; $P < 0.001$). When examining for the effect of insecticide and controlling for planting date, differences in adult densities were highly significant for April (t_{236} -9.98; $P < 0.001$) and May ($t_{236} = -7.58$; $P <0.001$) plantings, but not June and July ($P = 0.512$ to 0.072) plantings. Adult means were greater in the untreated subplots of the April planting followed by the untreated May subplots. No significant difference was detected between the treated April subplots and the untreated June and July subplots, which were not significantly different from the remaining treatment combinations. Soybean maturity group did not have a significant impact on adult counts (*P* = 0.279).

The interaction of planting date and maturity group, (*P* = 0.187) and insecticide and maturity group (*P* = 0.359) did not have a significant effect on yield. The interaction of planting date and insecticide on yield was highly significant (*F* = 16.82; df = 3, 232; *P* < 0.001). No yield differences were found between the April and May insecticide treated subplots; however, yield of the untreated subplots in the May planting were significantly greater than in the April planting (Fig. 3.2). There was no significant difference between insecticide treated and untreated subplots between the May and June plantings, but yields from treated May plantings were significantly greater than those of the June planting. There was no significant difference between the treated and untreated subplots in the July planting (*P* = 0.591). Yield of the April untreated subplots were not significantly different from yield of the treated subplots in the July planting (Fig. 3.2). Comparisons of least square means indicate yield of the treated subplots was greater than the untreated subplots in the April (t_{236} = 10.04; *P* <0.001), May (t_{236} = 6.08; *P* < 0.001), and June (t_{236} = 2.96; *P* = 0.003) plantings. The mean percentage yield loss for all studies was greatest in the April planting (50.59%) followed by the May planting (28.18%) and decreased in the June planting (16.01%) and the July planting (5.7%). Maturity group was the only main effect that was not significant in any interaction and did not have a significant effect on yield overall (*P* = 0.983).

Yield components. The planting date by insecticide interaction had no significant effect on the number of pods per plant or weight per seed (*P* = 0.940 to 0.131) (Table 2). Seeds per pod were significantly affected by the interaction of planting date and insecticide (*F* = 21.36; df = 3, 167; *P* <0.001). Differences were found in seeds per pod between insecticide treated and untreated subplots in the April (t_{167} = -10.73; *P* <0.001) and May (t_{167} = -4.55; *P* <0.001) plantings but not the June and July plantings (*P* = 0.560 to 0.184) (Table 3.2). Planting date by maturity group interactions had no effect on seeds per pod or weight per seed (*P* = 0.356 to 0.089),) though a significant effect was observed on pods per plant (*F* = 7.05; df = 3, 167; *P* = 0.001). When examining this interaction for differences in maturity group while controlling for planting date, significant differences in the number of pods per plant were found in the May, June and July plantings (t_{167} =-4.41 to -5.15; *P* <0.001) but not the April planting (*P* = 0.586). The number of seeds per pod were not significantly affected by the interaction of maturity group by insecticide (*P* = 0.562) whereas pods per plant (*F* = 11.31; df = 1, 167; *P* = 0.001) and weight per seed (*F* = 6.48; df = 1, 167; *P* = 0.011) were significantly different. Pods per plant were significantly different between maturity group when controlling for insecticide treatment in both treated (t_{167} = -7.19; *P* < 0.001) and untreated (t_{167} = -2.44; *P* = 0.015) subplots. Weight per seed differed between maturity groups in untreated subplots (t_{167} = 11.46; *P* <0.001) but not in those treated with insecticides (*P* = 0.889). Differences in weight per seed were detected between levels of insecticide in maturity group five (t_{167} = - 2.34; *P* = 0.020) soybean but not in maturity group seven (*P* = 0.212).

Planting date did not have a significant effect on weight per seed (*P* = 0.066), but was significant on the number of pods per plant (*F* = 12.24 df = 3, 9; *P* = 0.002). Significantly more pods were found per plant in the June planting than the April and May plantings which had greater mean pods per plant than the July planting (Table 3.2). However, maturity group had a significant effect on seeds per pod (*F* = 55.48; df = 1, 167; *P* <0.001). Comparisons of least square means indicate that there was a significant difference in seeds per pod between maturity group five and seven ($t_{167} = 7.45$; $P < 0.001$). Means separation indicate that the seeds per pod are greater in maturity group five (2.1) compared to maturity group seven (1.6).

Discussion

The greatest yield reductions were observed in the untreated plots of the April planting date. May plantings had the highest yield and the second highest mean percent yield loss of the four planting dates. However, there was no significant difference between the insecticide treated plots of these first two planting dates. Likewise the number of seeds per pod in the treated subplots of the April and May plantings were significantly greater than in untreated plots, though not significantly different from each other. These observations strongly suggest that the two early planting dates have similar yield potential, but received more yield reducing *M. cribraria* pressure (Table 3.1) and season long injury (Fig. 3.2). Though yield did not differ between untreated subplots in the May and June plantings, yields of the May treated subplots were significantly greater than those of the June plantings. This suggests that as planting dates are delayed after May, a corresponding reduction in yield can be reasonably expected. Early planted soybean with a longer growing season have a greater potential yield than late planted soybean with a comparatively shorter growing period (Specht et al. 2012). Insecticide control also prevent significant yield loss in the June planting but yield reductions were less than in the April and May planting. July plantings had the lowest yields overall, and no yield differences were detected between treated and untreated subplots. Further, untreated subplots of the April planting were not different from the treated subplots of the July planting. This demonstrates the potentially drastic effects between planting date extremes and season long injury by *M. cribraria*. Though *M. cribraria* infestations greatly decrease in later plantings, the yield benefit obtained from avoiding *M. cribraria* may not be sufficient to account for the agronomic benefit of planting reasonably early and making a judicious insecticide application targeting first generation *M. cribraria* nymphs. This study and Pozo-Valdivia and Reisig (2013) both show that early planted soybean is at the greatest risk of infestation from *M. cribraria*.

The greatest reductions in yield occurred in plantings which could support two complete generations of *M. cribraria* which were the April and May plantings. *M. cribraria* injury affected seeds per pod and weight per seed similar to results of Seiter et al. (2013a). Mean seeds per pod were lower in untreated plots in comparison to treated plots in all planting dates except July, where overall yield was consistently lower. Most likely the timing of *M. cribraria* injury determines which yield components are affected. It is possible that if *M. cribraria* injury occurs during flowering and pod set, pods per plant may be reduced. Only weight per seed and seeds per pod were affected by control of *M. cribraria* with insecticide in the April planting, which may be related to the timing and intensity of season long *M. cribraria* injury. Only seeds per pod were affected in the May planting suggesting that early infestation by overwintering adults and developing nymphs is the primary factor reducing yield components associated with *M. cribraria* injury. Season long feeding by developing *M. cribraria* nymphs likely contributes more to yield loss than midseason injury by adults. How *M. cribraria* infestation and timing impact different physiological stages of soybean needs to be investigated further.

Maturity group had no effect on yield response regardless of planting date. Nevertheless, maturity group had a significant effect on each of the yield components examined. When examining the effect of maturity group on pods per plant and controlling for planting date, differences were found between maturity groups in the May, June and July plantings but not in the April planting. This indicates that by planting early, the number of pods per plant in each maturity group are equivalent and maturity group effects on yield are more apparent as planting date is delayed. Maturity group seven had greater numbers of pods per plant and greater weight per seed than maturity group five overall. But maturity group five soybean had more seeds per pod overall. Though planting date and insecticide influenced how maturity group means separated, we did not explore yield responses between multiple varieties of the same maturity groups so the effect of maturity group on yield components may possibly be related to varietal differences that are not specifically linked to maturity group.

Maturity group and insecticide treatments did not affect egg mass densities, regardless of planting date. This suggests that the egg masses present in the field before insecticide treatment are not impacted and are therefore quantifiable regardless of insecticide application. The negligible effect of maturity group on egg masses suggests that there is no ovipositional preferences dependent on physiological stage of the host plant. Contrary to members of the Pentatomidae that prefer to feed on developing seeds (McPherson and McPherson 2000), the timing of *M. cribraria* infestation is likely related to host availability more than relative maturity of the host. Availability of hosts coinciding with dispersal from overwintering sites or dispersal from first generation hosts seems to have more bearing on infestation than the relative maturity of soybean. Thus, the biology of the *M. cribraria* is driven by environmental conditions and not the phenology of the host.

Megacopta cribraria is bivoltine in the U.S. and has shown fairly predictable phenological patterns since reaching economic levels in the southeast (Zhang et al. 2012, JLB unpublished data) however, population trends were somewhat differential between 2012 and 2013. *Beauveria bassiana* (Bals.-Criv.) Vuill., was observed at unusually high levels at both the Tifton and Griffin locations in 2013. An unintentionally introduced parasitoid wasp of *M.*

cribraria egg masses, *Paratelenomus saccharalis* Dodd (Hymenoptera: Platygastridae) (Gardner et al. 2013) was also observed at both locations. These factors are possible explanations for variation in populations between years, however they were not quantified in this study and no definitive conclusions can be drawn from these observations.

The significant effect of planting date on egg mass densities suggests that timing of host availability impacts adult colonization and subsequent oviposition. Timing of *M. cribraria* reproductive maturity coinciding with host availability may also contribute to this response. Later plantings of soybean, such as in double crop systems, are more likely to avoid the first generation of *M. cribraria* thereby reducing the need for an early application of an insecticide to control this pest. Much lower seasonal mean numbers of *M. cribraria* were detected in June and July plantings which are associated with much smaller insecticide control effects on yield. The currently accepted threshold for *M. cribraria* is an average of one nymph per sweep net sample when 25 consecutive sweeps are taken (Roberts et al. 2014). In this study, all fields exceeded threshold levels when applications were initiated, though this practical threshold was not a factor in applying treatments in this study. However, growers should be aware that within field populations are typically aggregated at the field edges in clusters (Seiter et al. 2013b) and scouting should be done throughout the field. Research by Roberts et al. (2013) indicates that one well timed application of a broad spectrum insecticide registered for control of *M. cribraria* and based on the threshold recommendation can help mitigate damage by the developing nymph populations. Insecticide application should be timed to occur after adults have infested a field and small nymphs have not done extensive damage. This period typically occurs after the first peak in oviposition which typically occurs from mid-May to mid-June. Though insecticide applications are effective at decreasing nymph populations in fields (Roberts et al. 2014), adults readily re-infest soybean within a few days of treatment when using a non-persistent insecticide. Adults are more likely to re-infest treated fields when applications in soybean are applied while adults are still migrating from overwintering sites or dispersing from first generation hosts such as kudzu (Roberts et al. 2014). In this study adults were consistently detected re-infesting soybean after treatment, however, we did not record the interval between treatment and re-infestation.

Early planted soybeans are at the greatest risk of infestation and yield loss associated with severe injury by *M. cribraria* in the southeastern U.S. Late planted and double crop soybeans also are likely to be infested by *M. cribraria*, but at much lower levels than early planted soybeans. Late planted soybeans stand a greater chance of avoiding the first generation of *M. cribraria* and season long injury incurred by this damaging pest. However, planting soybeans late also reduces soybean potential yield. Planting soybeans early maximizes potential yield but will require sampling and management to mitigate season long injury by *M. cribraria* in the southeastern U.S.

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Table 3.1. Mean ± SE egg masses, nymphs, and adults per sample date of four planting dates averaged across Tifton, Midville and Griffin locations from 2012 and 2013

Planting Date	**Egg masses**[a]	**Nymph**[a]	**Adult**[b]
April	198.2 ± 19.4a	817.6 ± 164.9a	802.3 ± 126.6a
May	83.51 ± 9.9b	292.7 ± 64.0b	573.1 ± 95.8b
June	7.3 ± 0.9c	23.6 ± 5.4c	220.8 ± 42.1b
July	0.2 ± 0.0c	2.4 ± 0.7c	103.8 ± 31.5b
F	109.35	26.91	12.39
P	<0.001	<0.001	<0.001
df	3, 236	3, 236	3, 236

[a] Average egg masses and nymphs per three plants at four locations per subplot.
[b] Average adults per 10 sweeps per subplot.
Means followed by the same letter by column are not significantly different ($\alpha = 0.05$).

Table 3.2. Mean ± SE yield components across maturity groups by planting date, insecticide treated versus untreated plots

Planting	Insecticide	Pods per plant		Seeds per pod		Weight per seed (mg)	
April	Untreated	33.98 ± 2.13a	B	1.23 ± 0.08b	A	0.11 ± 0.01b	AB
	Treated	35.66 ± 1.57a		2.43 ± 0.06a		0.14 ± 0.01a	
May	Untreated	35.63 ± 2.02a	B	1.74 ± 0.13b	A	0.14 ± 0.02a	A
	Treated	39.09± 1.95a		2.23 ± 0.23a		0.16 ± 0.01a	
June	Untreated	45.40± 1.53a	A	1.87 ± 0.08a	A	0.16 ± 0.02a	A
	Treated	46.72 ± 1.90a		2.02 ± 0.06a		0.13 ± 0.01a	
July	Untreated	26.62 ± 1.94a	C	1.75 ± 0.06a	A	0.10 ± 0.00a	B
	Treated	29.15 ± 2.73a		1.82 ± 0.06a		0.10 ± 0.01a	

Means with the same letter between untreated and treated plots are not significantly different upper case letters indicate differences in components by planting date ($\alpha = 0.05$).

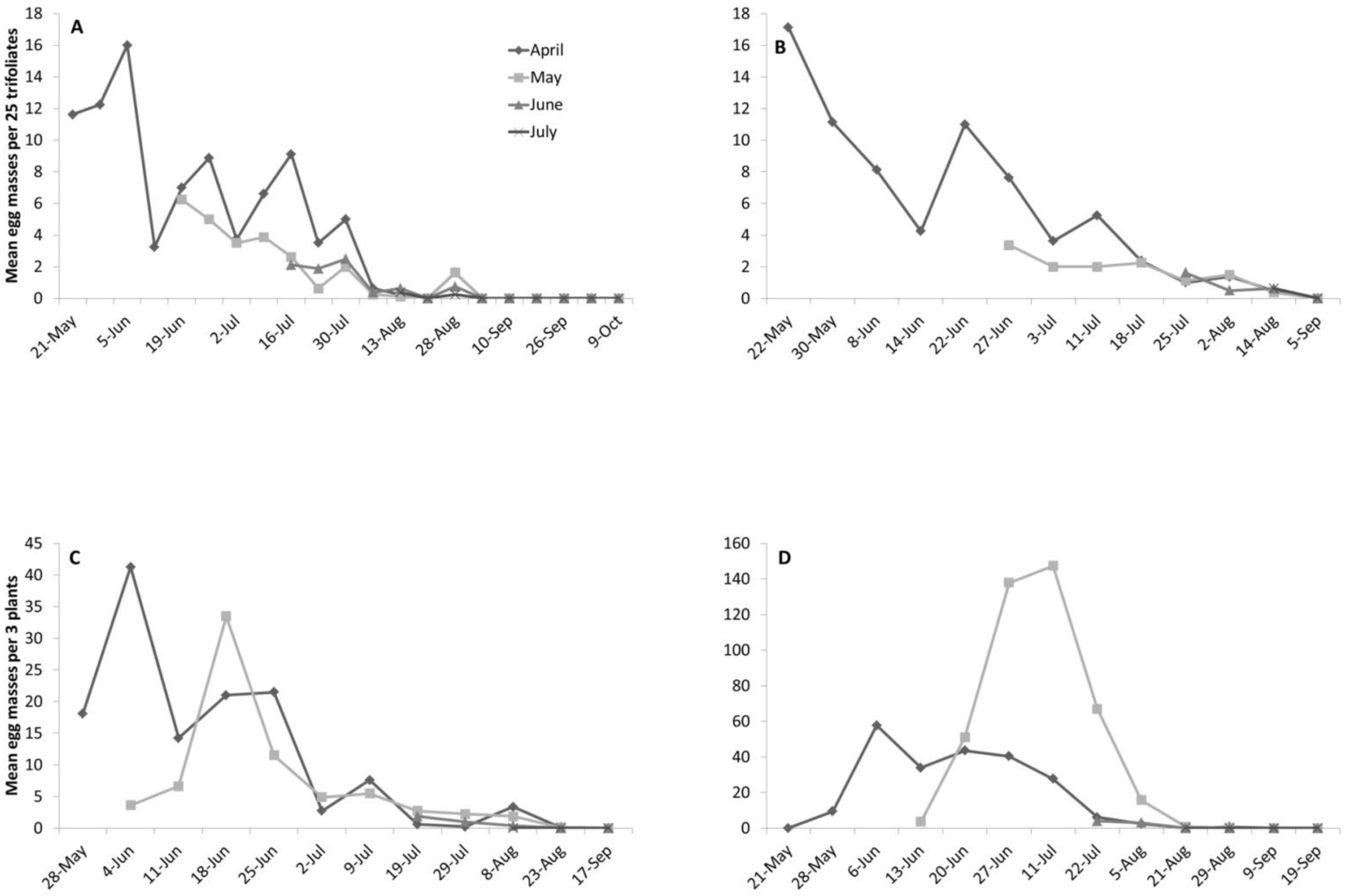

Figure 3.1. Mean egg masses from untreated subplots only by planting date by location. (A) mean egg masses per 25 trifoliates at Tifton 2012, (B) mean egg masses per 25 trifoliates at Midville 2012, (C) mean egg masses per three plants within one row foot at Tifton 2013, (D) mean egg masses per three plants within one row foot at Griffin 2013. As planting dates progress populations become less detectible and very few egg masses were detected in June and July planted soybean.

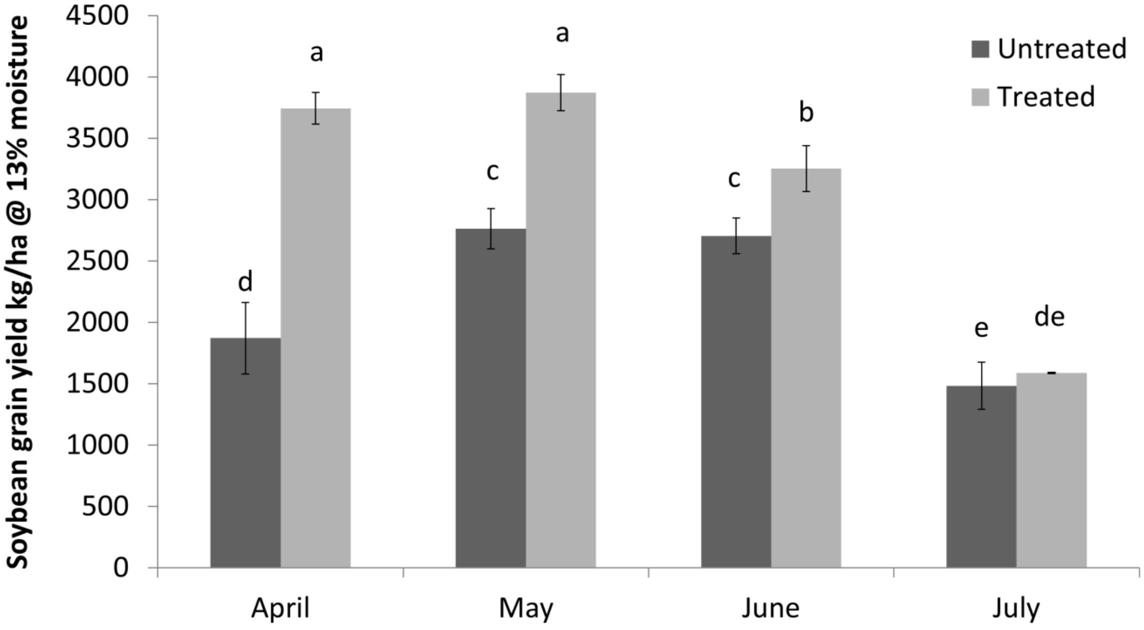

Figure 3.2. Mean ± SE yield (kg/ha) of insecticide treated and untreated plots by planting date across location, year and maturity group; all estimates are corrected for 13% moisture content. Means with the same letter are not significantly different ($\alpha = 0.05$).

CHAPTER 4

SEASONAL POPULATION DYNAMICS OF *MEGACOPTA CRIBRARIA* IN KUDZU AND SOYBEAN, AND IMPLICATION FOR INSECTICIDAL CONTROL IN SOYBEAN[1]

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[1] J. L. Blount, P. M. Roberts, M. D. Toews, W. A. Gardner, G. D. Buntin and J. N. All. To be submitted to *Journal of Economic Entomology*.

Abstract

Megacopta cribraria (F.) is an invasive species introduced from Asia in 2009 and is now prolific in the southeastern U.S. *Megacopta cribraria* develops primarily on kudzu and soybean and is an economic pest of soybean production. *M. cribraria* is bivoltine on both hosts, but depends on planting date in soybean. It is not well understood how this pest is affected by changes in geographic distribution in the U.S. or how the population has changed since its original establishment. How insecticide application timing affects field populations of *M. cribraria* also has not been well documented. In these studies, the authors seek to understand how population dynamics of *M. cribraria* vary with geographic regions in Georgia. The effect of insecticide application timing on populations throughout the growing season was also examined. From 2012 to 2013, egg masses, nymphs and adults were enumerated from kudzu and soybean environments at several locations spanning the majority of the state from south to north. Samples consisted of sweeps and flight intercept captures, which were counted weekly. Latitude and longitude were recorded for each location, which were further classified as belonging to either the Piedmont or Coastal Plain region of the state. Single spray trials were conducted from 2011 to 2014 and applications were made to soybean at intervals throughout the season. From 2012 to 2015, two kudzu patches near Griffin were monitored to detect changes population over time. Differences in populations from locations around the state were found, but no clear effect of latitude, longitude or region was observed. Insecticide applications applied in July suppressed nymph populations significantly better than treatments made earlier or later. *M. cribraria* populations declined substantially in 2014 and 2015 as compared to 2012 and 2013. These studies provide the critical information for *M. cribraria* management in soybean in the southeastern U.S.

Key words: *Glycine max, Pueraria montana,* IPM, phenology, kudzu bug

Introduction

Megacopta cribraria F. was introduced into the U.S. in 2009 and has become a pest of soybean in the southeastern U.S. (Eger et al. 2010, Suiter et al. 2010, Gardner et al. 2013b, Ruberson et al. 2013). *M. cribraria* in the U.S. has been linked to populations originating from the Kyushu Island of Japan (N 32.786058, W 130.731772) where they are reported to develop on soybean (*Glycine max* Merrill) and kudzu vine (*Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen and S. Almeida), both of which are common throughout the southeastern U.S. Upon their initial introduction large populations of *M. cribraria* were reported to build up on soybean and kudzu and have been reported on several other species of legumes (Eger et al. 2010, Jenkins and Eaton 2011). Recent research showed that soybean and kudzu are the two primary hosts in the US, although development may occur on secondary hosts such as pigeon pea (*Cajanus cajan* L.) and lima bean (*Phaseolus lunatus* L.) (Medal et al. 2013, Blount et al. 2015). *M. cribraria* is bivoltine in its new range and is capable of completing two generations per year on kudzu and on soybean, depending on planting date (Zhang et al. 2012, Blount et al. 2016). *M. cribraria* is also capable of completing development on soybean in the absence of kudzu (Pozo-Valdivia and Reisig 2013). *M. cribraria* can reduce soybean yield from 50 to 60% when season long injury is incurred (Seiter et al. 2013a, Blount et al. 2016). Though *M. cribraria* is susceptible to several insecticides, application timing is critical in suppressing populations (Seiter et al. 2015a).

The similarity in latitude between *M. cribraria's* native range and new range has likely contributed to its successful establishment and proliferation on host species in the Southeast. However, it is not well understood how the seasonal dynamics of this insect changes with latitude, longitude or geographic region as it develops on its two primary hosts in the U.S. Little information has been reported on how the population has changed since its initial discovery and how changes in populations affect soybean production.

The objective of these studies was to determine if range in location and region effects development of *M. cribraria* in soybean and kudzu and relate this information to management practices. Changes in populations from year to year are also examined to provide a clear picture of *M. cribraria's* establishment in the U.S. since its initial invasion.

Materials and Methods

Comparison of location and region. At five locations across Georgia in 2012, Elbert Co. (Elbert) (N 34.096639, W -82.852711), Athens (N 33.865778, W -83.543300), Griffin (N 33.175256, W -84.407059, Burke Co. (Burke) (N 32.8759167, W -82.217528), and Emanuel Co. (Emanuel) (N 32.498818, W -82.347193) and at two locations near Griffin and Tifton (N 31.519497, W -83.54230) in 2013, *M. cribraria* populations were sampled weekly in kudzu and soybean using sweep nets and random plant sampling. In kudzu, weekly sampling consisted of enumerating unhatched egg masses on twenty random terminal kudzu shoots (0.3 m long), and the number of small nymphs, large nymphs and adults in four sets of 10 sweeps with a standard 38-cm diameter sweep net. Nymphs were categorized as small (instars one-three) and large (instars four-five) based on presence of wing pads. Soybean sampling procedures varied by year and location but were initiated when plants were large enough to be swept, generally from approximately 35 days after planting until reaching physiological maturity (R7). Sampling was replicated four to eight times depending on location in untreated plots of a pre-existing field trial. In 2012, unhatched egg masses were enumerated from 25 randomly selected soybean trifoliates per plot at the Burke, Emanuel, Tift, and Elbert locations, whereas, the total number of egg masses found on three representative plants within one row foot at four separate randomly chosen locations within each plot were counted at the Griffin and Athens locations. Ten consecutive sweeps were performed in each plot and the total number of nymphs and adults were enumerated at all locations. Sampling in soybean was performed in Griffin and Tifton in 2013 and the total number of unhatched egg masses, small nymphs and large nymphs were enumerated from three plants selected from one row foot at four separate locations in each plot. Only adults were enumerated from ten consecutive single row sweeps in 2013.

Cross-vane flight intercept traps (Horn and Hanula 2011) were positioned immediately adjacent to kudzu patches and soybean fields which were within proximity of approximately 400 m to one another in a paired arrangement. In 2012, traps were monitored at the five locations previously mentioned and at four locations (Athens, Griffin, Burke, and Toombs (N 31.983084, W -82.354556)) in 2013. Adults are attracted to light colors on upright surfaces so no pheromones were required for capture of *M. cribraria* with this method of passive collection. Traps contained soapy water to kill adults that landed on the vane and fell in the bucket; each trap was emptied and the captures counted weekly.

Sample locations were documented in decimal degrees latitude and longitude and classified as Piedmont (Elbert, Athens, and Griffin) or Coastal Plain (Burke, Emanuel, Tifton and Toombs) regions of the state. Briefly, these two regions have similar climatic conditions, but Piedmont soils are less than 1 m thick with less sand and more clay and have not developed sandy epipedons. Coastal Plain soils are typically two to eight meters thick and have a high sand content with sandy epipedons. The altitude of the Coastal Plain is also lower than the Piedmont at about ~106 m above sea level versus 213-365 m. Additionally, fewer days below freezing typically occur in the Coastal Plain (28-45) than in the Piedmont (45-55) (SERCC 2012).

Comparison of population developmental time. Estimates of population developmental time were calculated by looking at midseason populations in kudzu and soybean for studies conducted in 2012 and 2013. The week of year when 50% of the cumulative total of each life stage was reached was recorded for each location. In cases where more than one asymptote occurred, the first distinct asymptote was utilized.

Single spray efficacy. Individual efficacy evaluations were conducted to determine if a single insecticide treatment timed to correspond to adult movement and oviposition could effectively control the second generation of *M. cribraria* in soybean. Soybean was planted in Midville on 30 May 2011 (P5Y20 Pioneer seed, E. I. du Pont de Nemours and Co., Wilmington, DE), and in Tifton on 31 May 2012 (Asgrow, AG5831, Monsanto Co., St. Louis, MO), 15 May 2013 (AG7231), and on 8 May 2014 (AG6931). Response variables were the number of adults and nymphs (instars one-five) per 10 single row sweeps (20 in Midville 2011) per plot collected weekly. The experimental design was a randomized complete block design where each plot consisted of six 40 foot rows with 36 inch spacing. A broad spectrum insecticide, lambda-cyhalothrin plus thiamethoxam (Endigo ZC, Syngenta Crop Protection, LLC, Greensboro, NC) was applied at a rate of 319 ml per hectare for all trials conducted in this study. In 2011 insecticide applications were made to the same plots on at two separate occasions, 14 July (wk 29) and 24 August (wk 35), remaining plots receiving applications on 29 July (wk 31) and 9 August (wk 33). These treatments were then compared to an untreated check and a positive control where plots were aggressively protected with insecticide applications made bi-weekly beginning on 28 June (wk 27) and ending on 8 September (wk 37). In 2012, a single application was made on 30 July (wk 31) and compared to untreated plots. In 2013 treatments consisted of a single application made every other week to plots from 19 June (wk 25) until 13 September (wk

37) for a total of seven individual insecticide applications which were compared to an untreated control. In 2014, nine single insecticide applications were made to separate plots weekly beginning on 23 June (wk 26) and ending on 18 August (wk 34). The two center rows of each plot for all trials were mechanically harvested with a two row small plot combine (Almaco Co., Nevada, IA) on 17 October (Midville 2011), 16 October (Tifton 2012), 8 November (Tifton 2013), and 7 October (Tifton 2014). Yields are reported in kg/ha corrected to 13% moisture content.

Temporal change in population. Two kudzu patches were sampled weekly by the same person from 2012 to 2015 from spring to fall when kudzu was available at locations near Griffin, GA. Flight intercept traps were monitored weekly and egg masses, small and large nymphs and adults were enumerated as described in previous sections. All stages of *M. cribraria* were averaged across the two locations by Julian week during each of the four years of the study and plotted to illustrate gross differences in kudzu bug populations by year.

Data Analysis. Analyses of kudzu bug populations were made across years and locations to enable prediction over the entire study area. To enable analyses by date across years, calendar date (i.e. 06/30/14) was converted to Julian week of year (range 1 to 52) when the sample was obtained. Counts were averaged for each sample date and cumulative totals by each life stage (egg masses, nymphs, or adults) were plotted by week of year, by location. A linear regression was performed on cumulative totals of adults and nymphs in sweep samples, adults in trap samples, and the number of egg masses found on random soybean plant samples and kudzu shoots using the REG procedure in the SAS Software (SAS/STAT software, version 9.4, 2002-2015 SAS Institute Inc., Cary, NC).

Cumulative totals of nymphs and adults from single insecticide application trials were analyzed by location using the GLIMMIX procedure in SAS. Data were modeled using a Poisson distribution because preliminary analyses indicated that group variances were proportional to the means; treatment means were separated using Tukey's HSD. Treatments were arranged in at randomized complete block design at each location and block was modeled as a random effect. Yield data, corrected for 13% moisture, were also analyzed in GLIMMIX using a Gaussian distribution where block was treated as a random effect and treatment means were separated using Tukey's HSD test of significance ($\alpha = 0.05$).

The week of year when 50% of the cumulative total of egg masses, nymphs and adults was reached in kudzu and soybean for studies conducted in 2012 and 2013 was recorded. Using that number as an estimate of population developmental time, an analyses of variance was conducted in GLIMMIX using location by year as replicates of each life stage in each individual population. Similarities and differences in those responses by main effects including latitude, longitude, and region (Piedmont vs. Coastal Plain) were analyzed.

Results

Comparison of location and region. Paired comparisons of slopes for cumulative egg mass numbers in soybean in 2012 found no significant differences between the Athens and Emanuel ($F = 0.70$; df = 1, 80; $P = 0.403$) locations or the Burke and Elbert ($P = 0.629$) locations. Significant differences between slopes in egg mass numbers between locations were detected for all other paired comparisons ($P = 0.002$ to < 0.001) (Fig. 4.1A). No differences in cumulative nymph totals were found between the Athens and Burke ($P = 0.190$), or Elbert and Emanuel ($P = 0.912$) locations in soybean in 2012 though differences were found between all other locations (P

= 0.002 to < 0.001) (Fig. 4.1B). Similarly, no differences in adults were detected in 2012 when Athens and Burke (*P* = 0.651), and Elbert and Emanuel (*P* = 0.891) were compared (Fig. 4.1C). All other comparisons of locations had significantly different levels of adults collected from soybean in 2012 (*P* < 0.001). Comparisons of cumulative least square means for soybean flight intercept traps in 2012 indicated that adults captured at the Athens location were significantly greater than all other locations (*F* = 40.75 to 17.24; df = 1, 98; *P* < 0.001) (Fig. 4.2A). All other comparisons of adult trap captures between location were not significantly different (*P* = 0.986 to 0.1629). No significant difference in egg masses were found between Athens and Burke (*F* = 0.11; df = 1, 118; *P* = 0.735), Athens and Elbert (*P* = 0.909), or Burke and Elbert (*P* = 0.800) in kudzu in 2012 (Fig. 4.1A). All other comparisons of egg masses between locations were significantly different (*P* = 0.046 to < 0.001). No significant differences were found between nymphs in Burke and Elbert (*P* = 0.485), Burke and Griffin (*P* = 0.383), and Elbert and Griffin (*P* = 0.8391), whereas, all other comparisons of nymphs between locations were different (*P* = 0.005 to < 0.001) (Fig. 4.1B). Half of the comparisons for adults in kudzu in 2012 were not significantly different. Burke adult populations were not significantly different than in Elbert (*F* = 0.04; df = 1, 118; *P* = 0.844), Emanuel (*P* = 0.174), or Griffin (*P* = 0.695). Adults in Elbert also were not significantly different from Emanuel (*P* = 0.152) and Griffin (*P* = 0.431) (Fig. 4.1C). Similar to adult trap captures from soybean environments in 2012, cumulative adult means from kudzu traps at the Athens location were significantly different from all other locations (*F* = 155.85 to 404.69; df = 1, 119; *P* < 0.001) (Fig. 4.2B). Adult trap captures from Elbert were also different from those of Burke (*P* = 0.007) and Griffin (*P* < 0.001). All other comparisons of trap capture locations in kudzu for 2012 were not significantly different (*P* = 0.969 to 0.076).

Comparisons of adult trap captures from soybean in 2013 indicate that no differences occurred between the Athens and Griffin (*F* = 0.53; df = 1, 90; *P* = 0.467) locations and the Toombs and Griffin (*P* = 0.071) locations (Fig. 4.2C). Significant difference in adult trap captures were found between all other combinations of locations (*P* = 0.020 to < 0.001) for soybean in 2013 (Fig. 4.2C). For adult trap captures in kudzu in 2013, the Burke location was significantly different from all other locations (*F* = 5.52 to 21.97; df = 1, 90; *P* = 0.020 to < 0.001) (Fig. 2D). Additionally, Athens and Toombs (*P* = 0.007) were significantly different from one another while the remaining comparisons were not significantly different (*P* = 0.467 to 0.071).

When locations were classified as belonging to either the Coastal Plain (Burke and Emanuel) or Piedmont (Griffin, Athens and Elbert) regions of Georgia, and compared, no significant differences were found between cumulative egg masses (*F* = 0.25; df = 1, 86; *P* = 0.621), nymphs (*P* = 0.9075) or adults (*P* = 0.523) in soybean for 2012 (Fig. 4.1). In kudzu in 2012, differences in nymphs (*F* = 5.05; df = 1, 124; *P* = 0.026) were found between regions but no significant differences were detected in egg masses (*P* = 0.166) and adults (*P* = 0.567) (Fig. 4.3A-C). Adult trap captures between regions were significantly different in soybean (*P* = 0.017) and kudzu (*P* = 0.018) in 2012 (Fig. 4.2).

Kudzu and soybean were sampled at only the Griffin and Tifton locations in 2013. Because Griffin is located in the Piedmont region of the state and Tifton is located in the Coastal Plain, comparisons of these locations are reported below as comparisons of regions. In 2013, significant differences in egg masses (*F* = 15.10; df = 1, 29; *P* = 0.001) (Fig. 4.4A), nymphs (P < 0.001) (Fig. 4.4B), and adults (*P* < 0.001) (Fig. 4.4C) in soybean were found between regions. In 2013, no differences were detected between regions in kudzu environments for egg masses (*P* =

0.645) (Fig. 4.5A), though nymph (*F* = 58.10; df = 1, 56; *P* < 0.001) (Fig. 4.5B) and adult (*F* = 85.23; df = 1, 56; *P* < 0.001) (Fig. 4.5C) populations were significantly different. Adult trap captures in soybean were significantly different between regions in 2013 (*P* < 0.001) (Fig. 4.2A) and kudzu (*P* < 0.001) (Fig. 4.2B).

Comparison of population developmental time. The week at which 50% of the total number of each life stage accumulated at each latitude, longitude and region was compared for soybean and kudzu across 2012 and 2013. Latitude, longitude and region did not have a significant effect on the Julian week (1-52) of year that 50% of the egg masses, nymphs or adults accumulated in soybean (*F* = 0.01 to 129.29; *P* = 0.907 to 0.066) or kudzu (*F* = 0.02 to 3.07; *P* = 0.884 to 0.192). However, region had a significant effect on adults in kudzu (*F* = 14.01; df = 1, 7; *P* = 0.007) and adults reached 50% of their maximum earlier in the Coastal Plain (28.1 ± 1.5, range: 7 to 14 July) than in the Piedmont (33.8 ± 0.5, range: 11 to 18 August). There were no significant differences in the week adult populations in soybean reached 50% of their maximum between the Coastal Plain (32 ± 0.7, range: 4 to 11 August) and Piedmont (33.6 ± 1.0, range: 11 to 18 August). Egg mass populations reached 50% of their maximum within a week of each other between the Coastal Plain (22.6 ± 0.3, range: 26 May to 2 June) and Piedmont (21.9 ± 0.6, range: 17 to 25 May) regions in kudzu. Similarly, oviposition in soybean reached 50% of the maximum of the first generation during the same week (16 to 23 June) in the Coastal Plain (25 ± 2.5) and Piedmont (25.3 ± 1.8). Nymphs also reached 50% of their maximum in kudzu during the same week (23 to 30 June) in the Coastal Plain (26.7 ± 0.5) and Piedmont (26.6 ± 0.7). In soybean, nymphs reached 50% of their maximum population only slightly earlier in the Coastal Plain (27.5 ± 1.4, range: 14 to 21 July) than in the Piedmont (29.5 ± 2.0, range: 14 to 21 Jul), but these minor differences were not significant.

Single spray efficacy. Single insecticide application efficacy trials conducted in Midville in 2011 indicated that insecticides were effective at controlling nymphs (F = 324.18; df = 5, 32; $P < 0.001$) but not adults (F = 1050.90; df = 5, 22; P = < 0.001) (Supp Table S4.1). Untreated plots had significantly greater numbers of nymphs than all plots receiving insecticide applications ($P < 0.001$) (Fig. 4.6A). Aggressively protected plots had significantly fewer nymphs than all other treatments ($P < 0.001$). Treatments made on 29 July (wk 31) had significantly greater nymphs than plots receiving applications on both 14 July (wk 29) and 24 August (wk 35) ($P < 0.001$) but nymph numbers were lower than in plots treated 9 August (wk 33) ($P < 0.001$). Adults were significantly greater in the untreated plots than any other treatment ($P < 0.001$). Adult levels in the remainder of the treatments were not significantly different from each other (P = 0.239 to 0.909). Insecticide applications effected soybean yield (F = 7.16; df = 5, 15; P = 0.001) (Table 4.1). The untreated plots exhibited lower yields than all other treatments (P = 0.001 to 0.022), which were not significantly different from each other (P = 0.999 to 0.725).

In 2012, a single insecticide application was made on 30 July (wk 31) and successfully suppressed nymph (F = 484.76; df = 1, 12; $P < 0.001$) and adult populations ($P < 0.001$) (Fig. 4.6B) (Supp Table 1). Insecticide prevented significant loss of soybean yield (F = 1086.6; df = 1, 2; P = 0.001) in 2012 (Table 4.1).

In 2013, seven insecticide application dates were compared to untreated plots. The effect of insecticide was significant on nymphs (F = 26.81; df = 8, 98; $P < 0.001$) and adults (F = 6.23; df = 8, 100; $P < 0.001$) (Fig. 4.6C) (Supp Table S4.1). No significant difference was detected between the treatments with the greatest cumulative nymph populations, the untreated plots and plots sprayed on 12 August (wk 33) (P = 0.997) and 26 August (P = 0.111). No significant difference was detected in nymph populations in plots treated on 16 July (wk 29), 3 July (wk 27)

(*P* = 0.750 to 0.710) and 19 June (wk 25) (*P* = 0.321 to 0.145), all of which had the lowest nymph populations in ascending order. Untreated, 12 August, and 26 August treated plots had significantly greater numbers of nymphs than those treated on 19 June, 3 July and 16 August (*P* < 0.001). Similarly, fewer adults were found in plots treated on 16 July, which were not significantly different from plots treated on 31 July (wk 31), 3 July and 19 June (*P* = 0.999 to 0.440) (Supp Table S4.1). Significantly more adults were observed in the untreated plots and those treated on 26 August, 12 August, 19 June and 13 September compared to plots treated on 3 July, 31 July and 16 July (*P* = 0.017 to 0.001). Insecticide treatment did not significantly affect soybean yield in 2013 (*F* = 1.11; df = 8, 24; *P* = 0.3910) (Table 4.1).

In 2014, insecticide treatment had a significant impact on nymph (*F* = 10.72; df = 9, 90; *P* < 0.001) (Fig. 4.6D) and adult (*P* < 0.001) populations (Supp Table S4.1). The greatest nymph populations in 2014 were detected in plots treated on 23 June (wk 26), which were not significantly different from the untreated (*P* = 0.988) and 30 June (wk 27) (*P* = 0.113) (wk 27) treated plots. Nymph populations were lowest in plots treated on 21 July (wk 30), which were not significantly different from plots receiving insecticides on 28 July (wk 31), 4 August (wk 32), 14 July (wk 29), 7 July (wk 28) and 11 August (wk 33) (*P* = 0.999 to 0.434) (Fig. 4.6D) (Supp Table S4.1). The untreated plots accumulated significantly more adults than all other treatments (*P* < 0.001). Plots treated on 11 August had significantly fewer adults than plots treated on 21 July, and 23 June (*P* < 0.001). A significant yield response was detected between insecticides applied to plots in soybean in 2014 (*F* = 2.53; df = 9, 27; *P* = 0.0301) (Table 4.1). The untreated plots had significantly lower yields than those of the plots treated on 4 August (*P* = 0.036) and 21 July (*P* = 0.039), which were not significantly different from each other (*P* = 0.973).

Temporal change in population. Slopes of cumulative totals of egg masses, small nymphs, large nymphs, and adults were compared by year. No significant differences were detected in egg masses (Fig. 4.7A), small nymphs (Fig. 4.7B) and large nymphs (Fig. 4.7C) between 2014 and 2015 (F = 0.00 to 0.21; P = 0.995 to 0.644). Only adult populations were shown to be significantly different when 2014 and 2015 were compared (P = 0.009) (Fig 4.7D). For all other years, significant differences occurred in all life stages between all remaining combinations from 2012 to 2015 (F = 4.59 to 221.88; df = 1, 105; P = 0.034 to < 0.001) (Fig. 7A-D).

Discussion

Megacopta cribraria populations were sampled from kudzu and soybean at several locations across Georgia in 2012 and 2013 to determine if seasonal dynamics were influenced by latitude, longitude, or geographic region. In kudzu and soybean, differences in nymphs occurred among locations across both years surveyed, most notable, populations in Athens soybean and kudzu were different from all locations in 2012 except for soybean sampled at the Burke location. A similar pattern was observed for adult traps captures in both environments in 2012 with very few differences between other locations. Adult populations in Athens were greater than all other locations; however, significant differences were detected among locations in kudzu and soybean across 2012 and 2013 for at least one of the life stages examined. When examined by regions, no significant differences were found in all life stages between the Coastal Plain and Piedmont soybean populations and only nymphs were shown to be different in kudzu in 2012. In 2013, nymphs in soybeans were found to be significantly different between the two locations, representing the two regions of the state. Additionally, adults and nymphs in kudzu were significantly different between region (Griffin and Tifton) in 2013, but egg masses were not. In several instances, comparisons of *M. cribraria* collected with sweeps or flight intercept traps between locations belonging to the same region were found to be significantly different, and likewise those locations belonging to different regions were found to have no significant differences, for at least one life stage examined across years. These results suggest that though differences in locations occurred in kudzu and soybean, local *M. cribraria* populations are variable, but not predictable based on latitude, longitude or region and must be evaluated on a site specific basis.

The weeks when 50% of the cumulative total was reached, were compared to weeks when single spray trials were conducted and trends in populations were examined in response to insecticide application. It is important to note that the weeks when 50% of nymphs were present in soybean fields were not different between the Coastal Plain and Piedmont regions of Georgia in 2012. In 2013, nymphs were sampled at only two locations, which were significantly different from one another and it is likely there is only a location and not region effect being observed. This suggests that development of immatures within the area surveyed (from 31.519497 N to 34.096639 N and 84.407059 W to 82.217528 W) occurs at approximately the same time. The data suggest that populations in kudzu appear to develop one to three weeks ahead of soybean, however, previous research shows that this is highly dependent on soybean planting date (Blount et al. 2016). The average week where 50% of the population is reached in soybean in the Coastal Plain is slightly earlier than in the Piedmont, though not significantly. When a grand mean (not reported) is calculated for soybean across location, the population reaches 50% at wk 28.5 ± 1.19 (~ wk 1 Jul to wk 4 Jul). When single insecticide applications were made from week 27 (early

July) to week 31 (late July) nymph populations were suppressed when compared to earlier and later treatments. Population suppression was always observed when insecticide applications targeted developing nymph populations after oviposition (wk 25.2 ± 1.3) and adult migration had ceased. The data collected in this study indicates that for soybean growing regions in Georgia, the most effective time to target *M. cribraria* nymphs is around the middle of July, narrowing the effective treatment period to a four-week window and thereby reducing the number of insecticide applications necessary for control. Local sampling of populations in soybean is needed to refine the precise time for insecticide applications, should they be required. If treatments are made too early, populations rebound as adults continue to infest fields and oviposit. Similarly, applications occurring too late allow populations to build unchecked and season long damage is irreversible at that point. This study agrees with the conclusion of Seiter et al. (2013) that one well timed application will effectively control mid-season populations of *M. cribraria*. Positive yield responses occurred with insecticide treatment in all years except 2013. In 2014, yield of untreated plots were significantly lower than those treated on 21 July and 4 Aug in 2014. Though yield responses were not large in 2014 because infestation levels were smaller, *M. cribraria* are capable of reducing soybean yield from ~50-60% when high populations are present (Seiter et al. 2013a, Blount et al. 2016).

Megacopta cribraria populations monitored at two locations near Griffin from 2012 to 2015 served to illustrate the change in population magnitude over time. In 2012 and 2013, high populations were recorded and reflected the levels of infestation seen across the Southeast. In 2014 and 2015, populations were significantly lower than had previously been observed. Additionally, the number of *M. cribraria* collected in the single spray trials (2011 to 2014) demonstrate the decrease in populations in soybean (Fig. 4.6). A combination of factors may have contributed to this decrease in *M. cribraria* prevalence. In 2013, a parasitoid wasp was observed emerging from egg masses of *M. cribraria* collected near the University of Georgia Griffin Campus from a kudzu patch (Gardner et al. 2013a). The wasps were later identified as *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Platygastridae) and subsequently reported from the surrounding area and, Tifton and Midville, GA, as well as locations in Alabama (Gardner et al. 2013a). Several egg masses collected from soybean fields near Griffin were found to be parasitized and the second generation of nymphs in the area was significantly lower than the first generation (unpublished data *J. L. B.*). Though no reports of *P. saccharalis* parasitizing egg masses were made in 2014, several adult specimens were collected from egg masses in soybean near Tifton, GA in 2015. Additionally, reports of an entomopathogenic fungus, *Beauveria bassiana* (Bals.-Criv) Vuillemin, attacking *M. cribraria* was confirmed in Georgia in 2010 (Ruberson et al. 2013) and South Carolina in 2012 (Seiter et al. 2014b). Since then, observation of this pathogen has increased and it has become commonplace to observe *B. bassiana* sporulation on adult and nymph cadavers of *M. cribraria* in soybean in Georgia (personal observation). When this pathogen was originally observed infecting *M. cribraria* in soybean fields, it was attributed to high levels of rainfall leading to increased humidity within soybean canopies. However, the repeated appearance of it has likely had an impact on *M. cribraria* over time. Additional research is needed to quantify the impact *B. bassiana* has on *M. cribraria* populations. However, the data presented here suggest that one or both of these natural enemies have decreased the suitability of the environment for large populations of *M. cribraria* to flourish as observed prior to 2014.

Current recommended economic thresholds suggest making insecticide applications when nymphs reach one per sweep and adults are present when 25 consecutive sweeps are performed

(Roberts et al. 2015). This threshold has been effective for treating *M. cribraria* populations when they built rapidly to high numbers. In light of the lower populations that are now being observed, these thresholds need to be re-evaluated to reflect current populations and how they are impacted by natural enemies. No clear developmental differences between regions or locations occur in *M. cribraria* populations in the southeastern U.S. These data firmly establish that managers should target mid-season *M. cribraria* populations in soybean during July, but site specific scouting is needed to properly asses the need for, and timing of insecticidal control.

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Table 4.1. LS Mean (± SE) soybean yield in kg/ha for four location years in response to insecticides applied for control of *M. cribraria* on specific dates

Midville 2011		**Tifton 2012**		**Tifton 2013**		**Tifton 2014**	
Treatment	**Yield (kg/ha)**	**Treatment**	**Yield (kg/ha)**	**Treatment**	**Yield (kg/ha)**	**Treatment**	**Yield (kg/ha)**
Untreated	2457.9 ± 233.3 b	Untreated	1855.56 ± 391.3 b	Untreated	2284.7 ± 88.3 a	Untreated	2740.8 ± 365.3 b
Aggressive[a]	3381.7 ± 104.6 a	30 Jul	3230.2 ± 106.2 a	19 Jun	2884.6 ± 204.1a	23 Jun	3133.7 ± 376.5 ab
14 Jul & Aug 24	3353.5 ± 111.1 a			3 Jul	2714.8 ± 175.1 a	30 Jun	3309.4 ± 184.0 ab
29 Jul	3164.5 ± 75.7 a			16 Jul	2633.6 ± 208.6 a	7 Jul	3330.8 ± 200.5 ab
9 Aug	3434.3 ± 68.3 a			31 Jul	2763.4 ± 308.1 a	14 Jul	3600.9 ± 140.6 ab
				12 Aug	2912.1 ± 98.7 a	21 Jul	3755.3 ± 280.3 a
				26 Aug	2739.1 ± 389.0 a	28 Jul	3512.7 ± 217.7 ab
				13 Sep	2417.0 ± 228.7 a	4 Aug	3764.9 ± 133.5 a
						11 Aug	3296.3 ± 242.2 ab
						18 Aug	3702.2 ± 171.0 ab

Means with the same letter are not significantly different (Tukey LSM α = 0.05).

[a] Applications made 28 June, 14 July, 29 July, 9 August, 24 August, and 8 September.

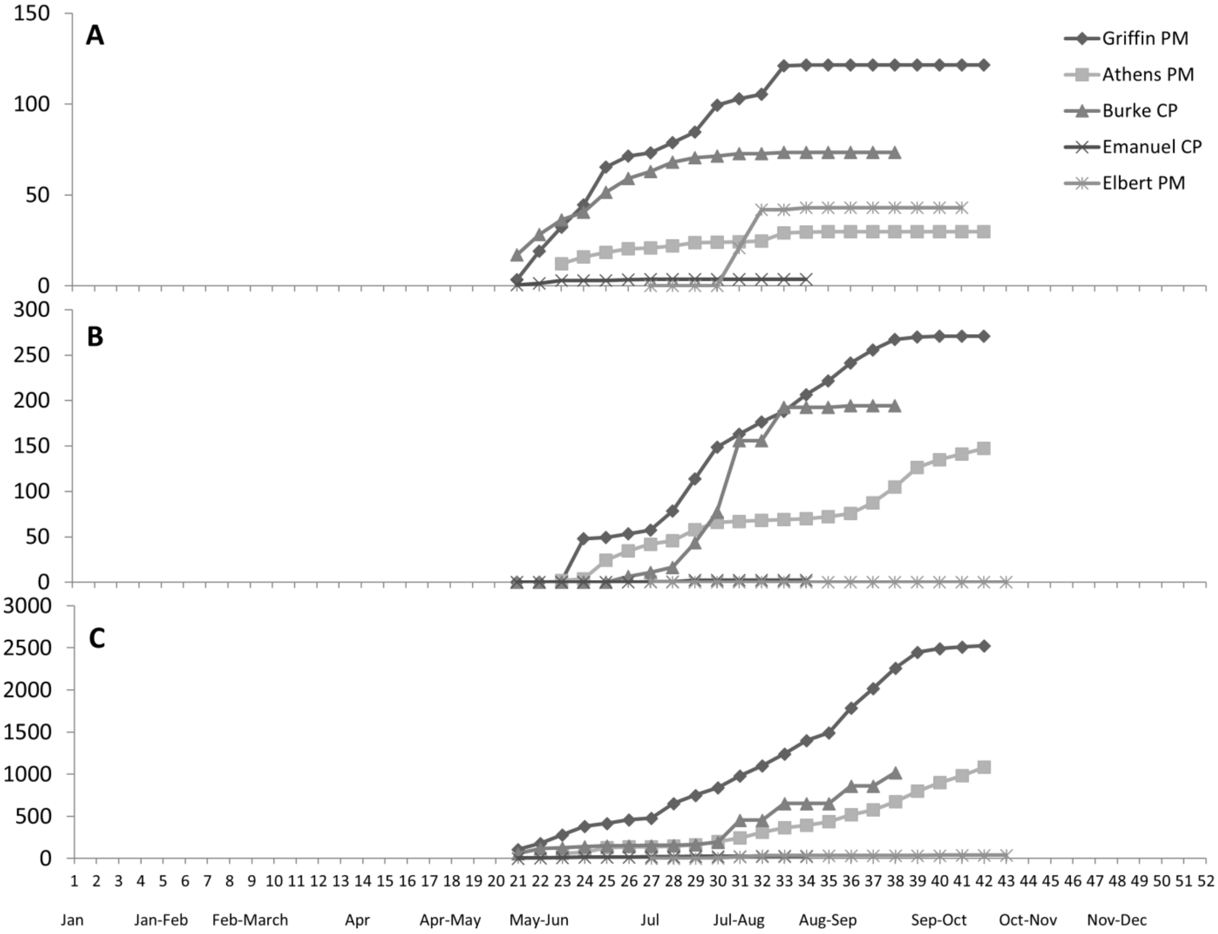

Figure 4.1 Cumulative *M. cribraria* (A) egg masses per sample, (B) nymphs and (C) adults per 10 sweeps in soybean at five locations in 2012 by Julian week and month of year. PM = Piedmont; CP = Coastal Plain.

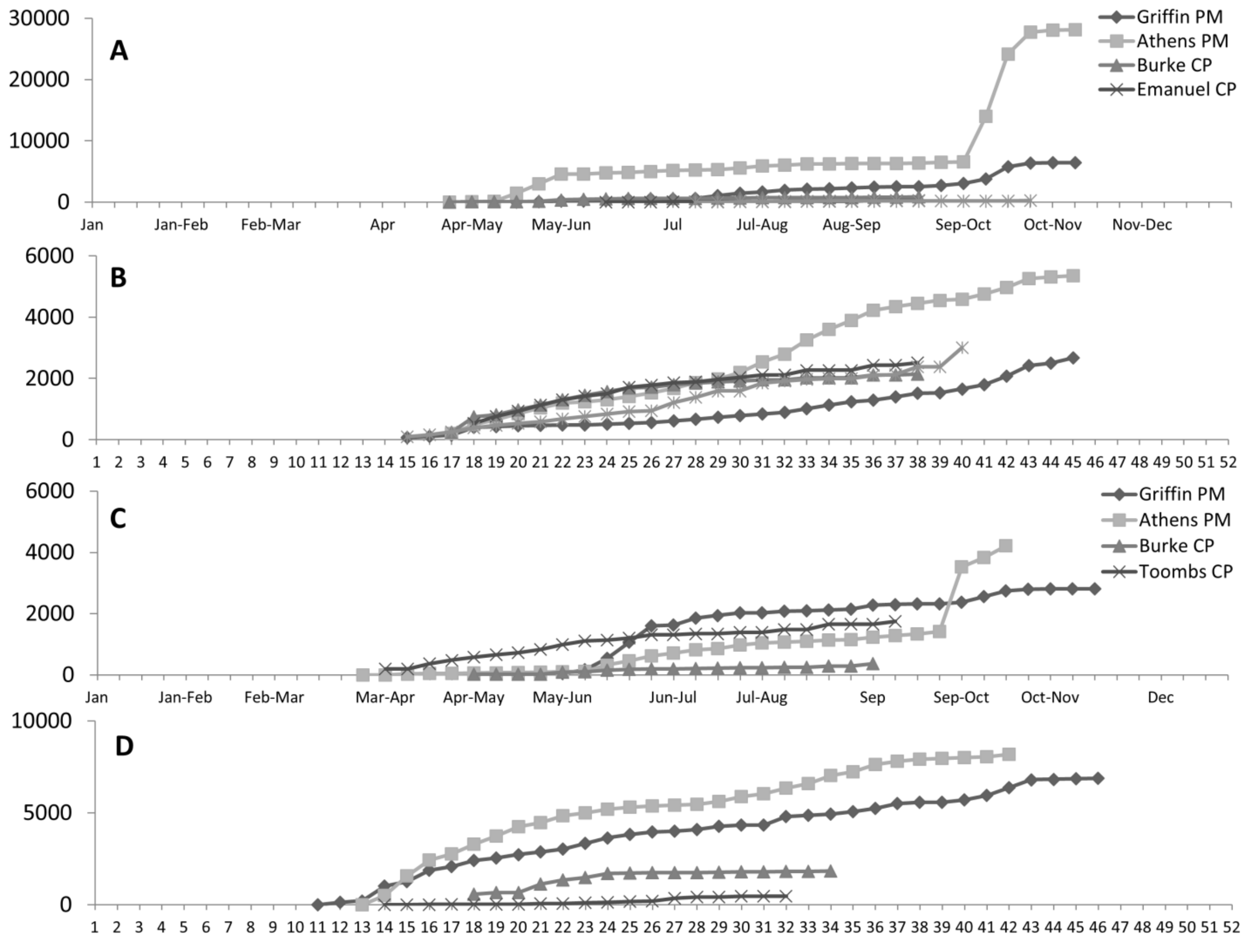

Figure 4.2 Cumulative *M. cribraria* adult trap captures in (A) soybean and (B) kudzu at five locations in 2012 by week and month of year. Cumulative *M. cribraria* adult trap captures in (C) soybean and (D) kudzu at four locations in 2013 by week and month of year. PM = Piedmont; CP = Coastal Plain.

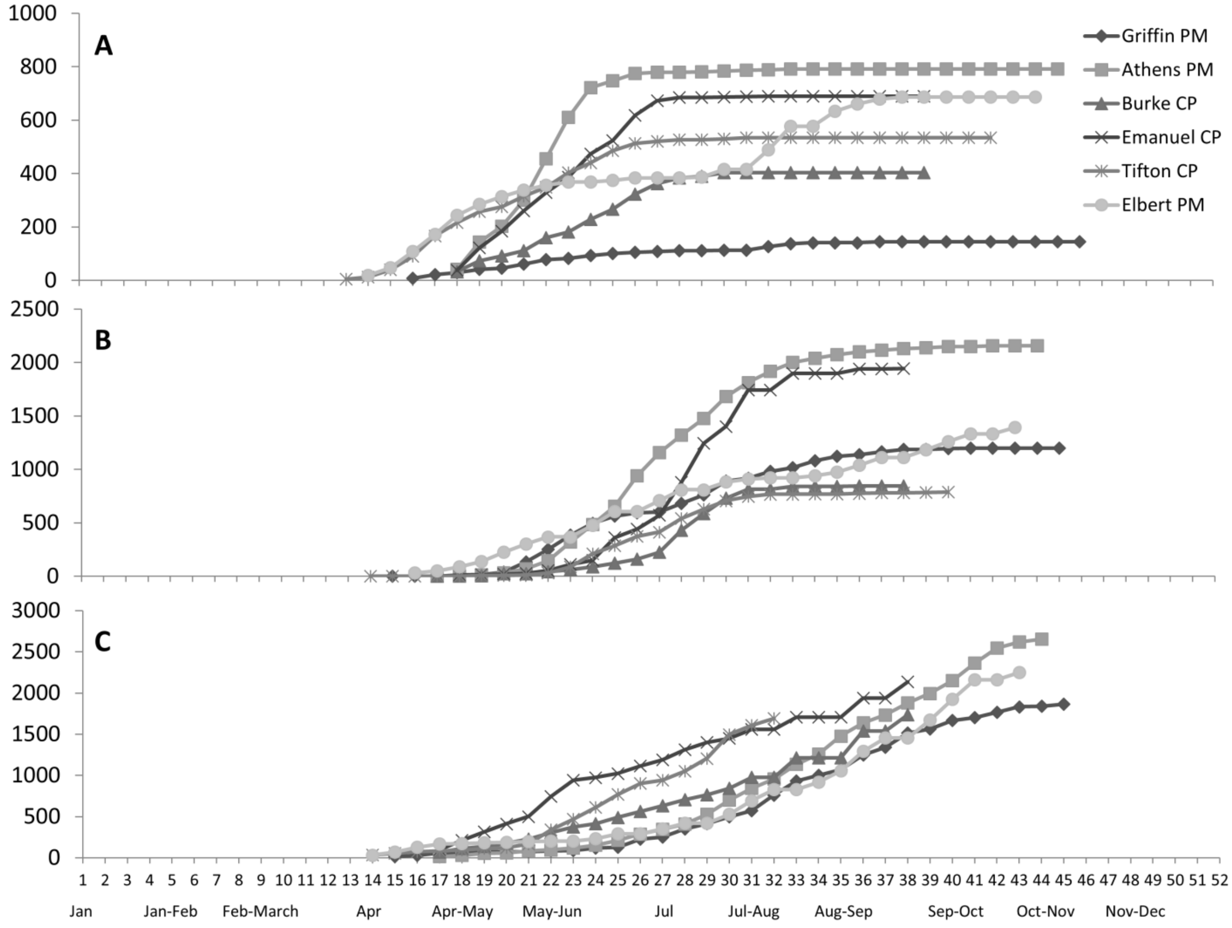

Figure 4.3. Cumulative *M. cribraria* (A) egg masses per sample, and (B) nymphs and (C) adults per 10 sweeps in kudzu at six locations in 2012 by Julian week and month of year. PM = Piedmont; CP = Coastal Plain.

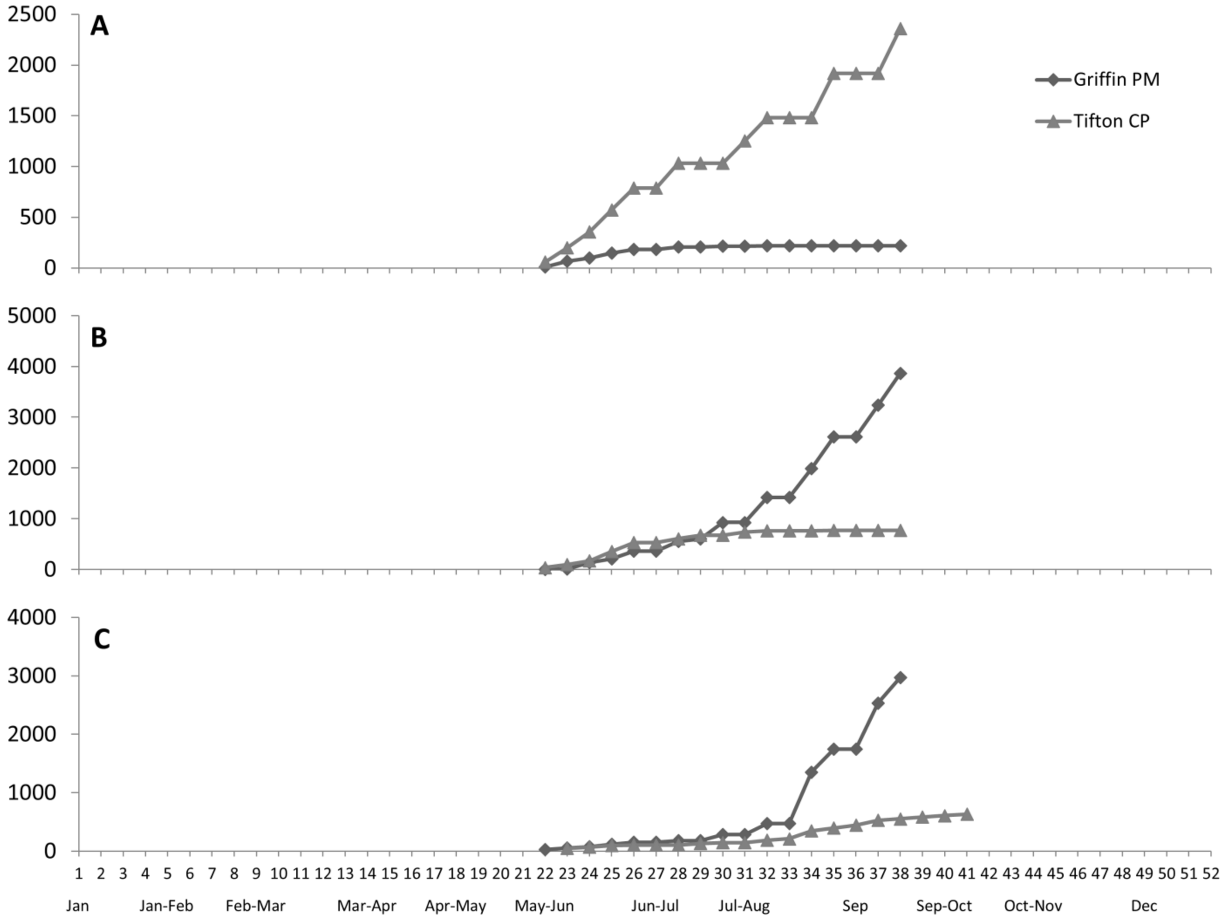

Figure 4.4. Cumulative *M. cribraria* (A) egg masses per sample, and (B) nymphs and (C) adults per 10 sweeps in soybean at two locations in 2013 by Julian week and month of year. PM = Piedmont; CP = Coastal Plain.

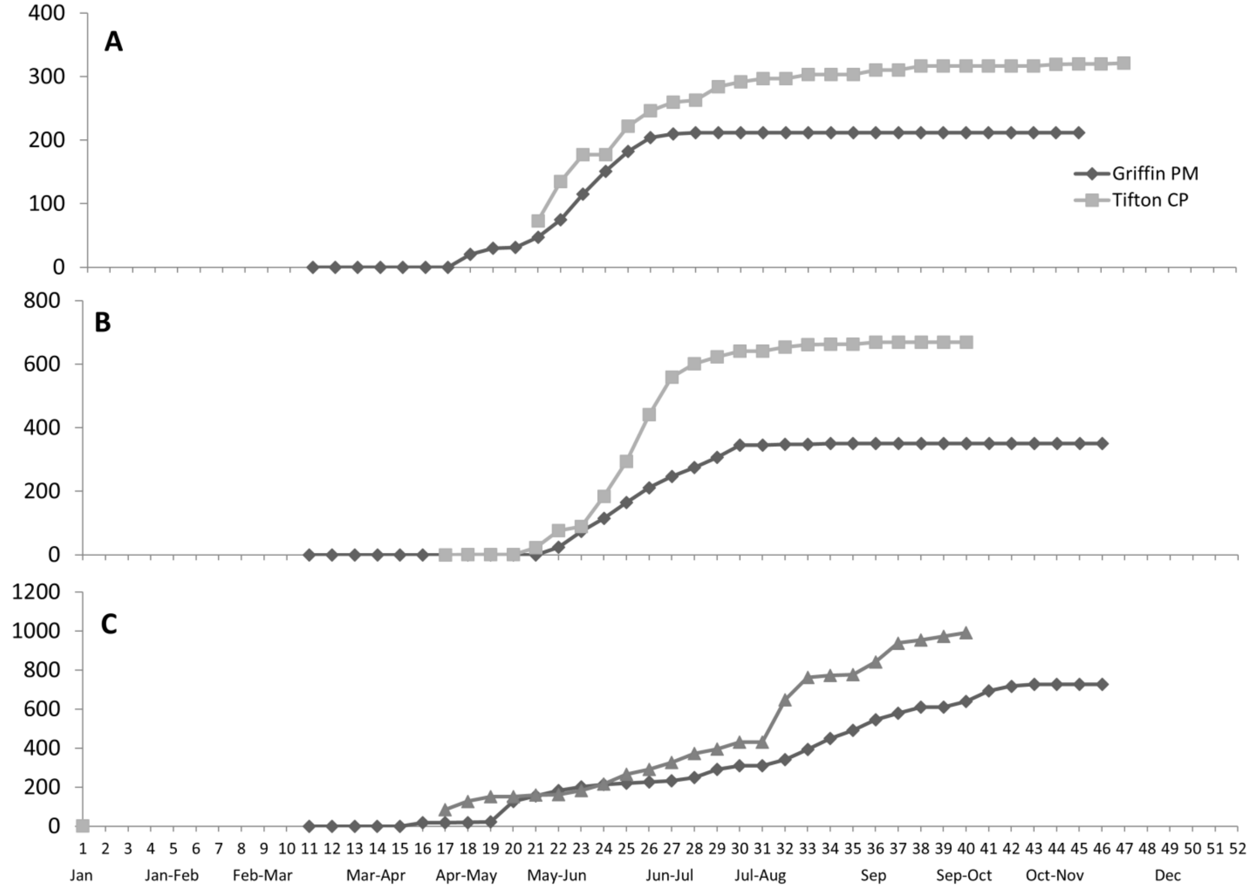

Figure 4.5. Cumulative *M. cribraria* (A) egg masses per sample, (B) nymphs and (C) adults per 10 sweeps in kudzu at two locations in 2013 by Julian week and month of year. PM = Piedmont; CP = Coastal Plain.

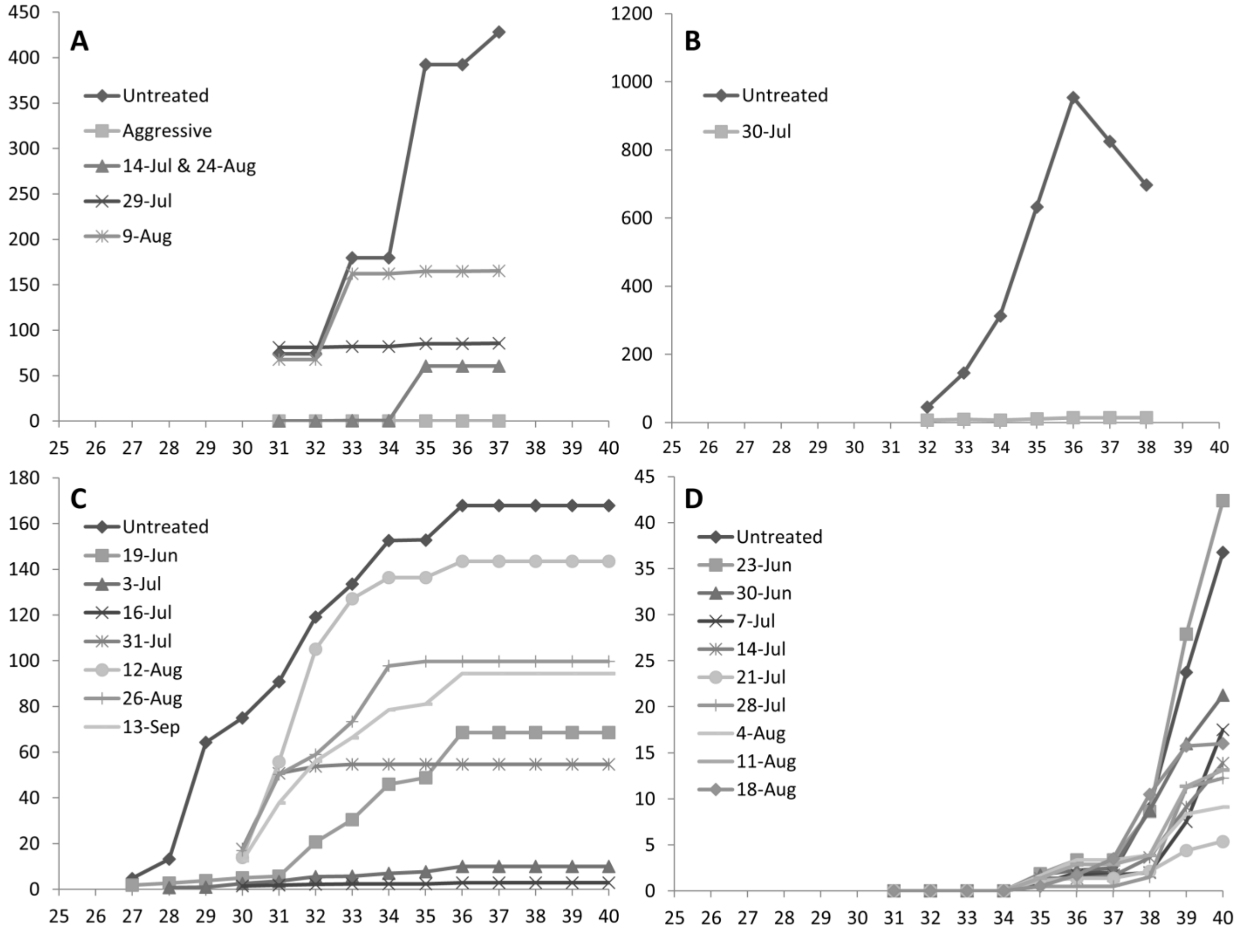

Figure 4.6. Mean cumulative *M. cribraria* nymphs detected in plots treated on dates specified at (A) Midville 2011, (B) Tifton 2012, (C) Tifton 2013 and (D) Tifton 2014, by week of year.

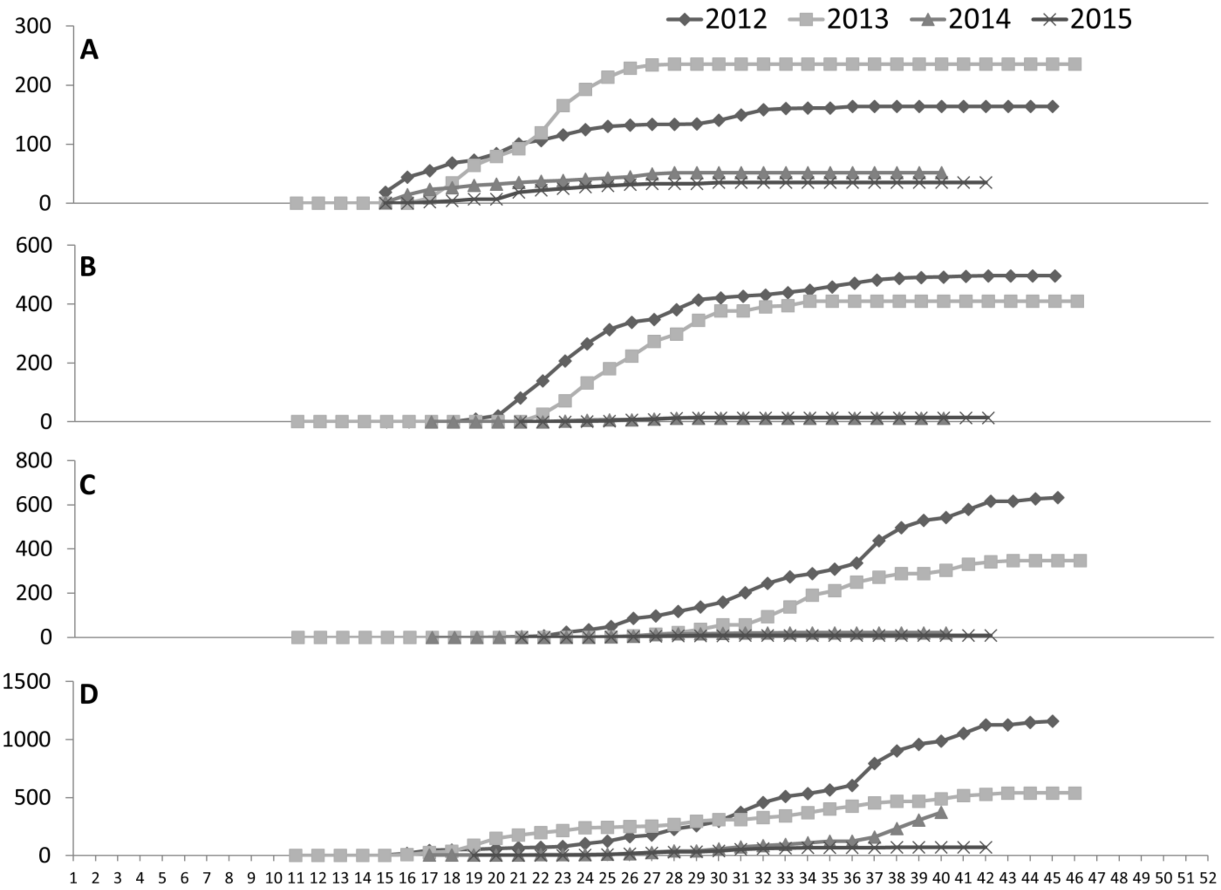

Figure 4.7. Mean cumulative *M. cribraria* (A) egg masses, (B) small nymphs (C) large nymphs and (D) adults from two locations near Griffin, GA by week of year from 2012 to 2015.

CHAPTER 5

EFFECT OF DEFOLIATING LEPIDOPTERAN AND *MEGACOPTA CRIBRARIA* INJURY ON SOYBEAN PRODUCTIVITY[1]

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[1] J. L. Blount, M. D. Toews, G. D. Buntin and P. M. Roberts. To be submitted to *Crop Protection.*

Abstract

Megacopta cribraria (F.) was unintentionally introduced into the United States in 2009 from Asia and has since become a key pest of soybean in the southeast. *Megacopta cribraria* decreases yield indirectly by removing vascular fluids from the plant phloem. The lepidopteran complex of *Chrysodiexis includes* (Walker), *Tricoplusia ni* (Hübner), *Hypena scabra* (F.), and *Anticarsia gemmatalis* (Hübner) also inflict indirect damage as defoliators. Individually, both *M. cribraria* and lepidopteran defoliators can incur economic damage in the southeastern US. It is not well understood how yield and management practices are influenced when both are present simultaneously. The objectives of this study were to determine how *M. cribraria* and the lepidopteran defoliating complex influence yield individually and combined. Caterpillars and *M. cribraria* were sampled twice monthly from soybean plots treated with four insecticide treatments including a control. Chlorantraniliprole and indoxacarb were applied for the control of defoliating lepidopterans, bifenthrin was applied for control of *M. cribraria* and a tank mix was applied to protect plots from both sets of pests. Count data were combined into three time intervals based on insecticide application timing. The effect of insecticide treatment was significant on insect counts and percent defoliation. Insect populations were insufficient to create a difference in yield between untreated plots and plots protected from *M. cribraria* and defoliating lepidopterans. *M. cribraria* reached threshold levels but defoliating lepidopterans were below recommended thresholds. A need to reevaluate economic thresholds for *M. cribraria* is apparent based on levels detected in fields and lack of yield response in this study.

Keywords: soybean looper, *Chrysodeixis includes*, cabbage looper *Tricoplusia ni*, green cloverworm, *Hypena scabra*, velvetbean caterpillar, *Anticarsia gemmatalis,* multiple pest thresholds

Introduction

Megacopta cribraria (F.), known as the kudzu bug, was unintentionally introduced into the United States in 2009 from Asia, and has since established as an important invasive pest of soybean (*Glycine max* Merril) in the southeastern U.S. (Eger et al. 2010, Suiter et al. 2010, Zhang et al. 2012, Gardner et al. 2013b, Ruberson et al. 2013). Kudzu (*Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen and S. Almeida) and soybean are the primary developmental hosts of *M. cribraria* in the U.S. (Zhang et al. 2012). Two generations are completed per year on both hosts, though completion of the first generation in soybean is planting date dependent (Pozo-Valdivia and Reisig 2013, Blount et al. 2015a). *Megacopta cribraria* reduces yield indirectly by removing vascular fluid from the phloem leading to reduced weight per seed and seeds per pod (Seiter et al. 2013a, Blount et al. 2015a). Season long feeding injury from developing *M. cribraria* nymphs can reduce yield up to 59% (Seiter et al. 2013a, Blount et al. 2015a). Defoliation from commonly occurring caterpillars such as soybean loopers (*Chrysodiexis includes* (Walker)), cabbage loopers (*Tricoplusia ni* (Hübner)), green cloverworm (*Hypena scabra* (F.)) (GCW), and velvetbean caterpillar (*Anticarsia gemmatalis* (Hübner)) (VBC) also decrease yield indirectly by removing photosynthetic leaf surface area. Defoliation can also contribute to water loss, reduced transpiration, reduced compensation to nutrient deficiencies, susceptibility to abiotic factors affecting growth, and disease susceptibility (Ostlie and Pedigo 1984, Fehr et al. 1985, Owen et al. 2013). Independent economic thresholds for both sets of damaging pests have been developed for Georgia and the southeast and previous studies have examined their impacts on yield (Fehr et al. 1981, Ostlie and Pedigo 1984, Fehr et al. 1985, Owen et al. 2013, Seiter et al. 2013a, Blount et al. 2015a). However, it is not well understood how the presence of both defoliating lepidopterans and *M. cribraria* impact yield when present together. The objective of this study was to determine how *M. cribraria* contributes to indirect yield loss associated with defoliation by the lepidopteran complex mentioned above. Conclusions from this research may be used to evaluate thresholds established for both *M. cribraria* and defoliating lepidopterans. It may also serve in increasing understanding of how soybean production and management strategies are affected by multiple guilds of pest species in a single crop.

Materials and Methods

Soybean was planted in 2015 at University of Georgia plant research farms near Tifton (Lang Research Farm) (31.519497 N, -83.54230 W) and Athens (Plant Science Farm, Bogart) (33.865519 N, -83.545302 W), Georgia. Maturity group five soybean (Asgrow AG5831 and AG5935) (Asgrow Seeds, Monsanto Co., St. Louis, MO) was planted at two separate locations at the Lang Farm near Tifton, GA. Two plantings were present in a single field, which was planted to AG5831 on 28 April (TF1) and 19 May (TF2). The second Tifton (TF3) field was planted to AG5935 on 5 May. In Athens, NK52Y2 (NorthrupKing Syngenta Seeds, LLC, Greensboro, NC) soybean was planted on 22 May. Treatments were arranged in a randomized complete block at each site with six replications at Athens and TF3 trials and four replications at the TF1 and TF2 trials. Each plot was the experimental unit at each field location. All plots were 12.19 m long by six rows wide with 91.44 cm row spacing, except the plots in Athens, which had eight rows.

Treatments consisted of insecticide applications targeted at control of defoliating lepidopterans (chlorantraniliprole and indoxacarb), *M. cribraria* (bifenthrin), and a tank mix for control of both sets of pests and an untreated control. Chlorantraniliprole (Prevathon®, E. I. du

Pont de Nemours and Co., Wilmington, DE) was applied at 48 oz (1419 ml) per hectare, and bifenthrin was applied at 14.4 oz (425 ml) per hectare. Combinations of the two insecticides at 62.4 oz (1845 ml) per hectare were made for control of both sets of pests. A four row Lee Spider high clearance spray tractor (LeeAgra Inc., Lubbock, TX) outfitted with hollow cone nozzles was used for insecticide applications. Treatments were applied at the R4 growth stage to the TF1 plots and TF3 field on 23 July and to the TF2 plots on 31 July. Applications were made at the R4 growth stage at the Athens location on 27 July. A second spray application for control of defoliating lepidopterans was made on 18 August and 26 August, respectively, at all Tifton fields and the Athens field (at a rate of 26.4 oz (780.64 ml) per hectare. The label rate of bifenthrin and a tank mix of bifenthrin and indoxacarb were applied as before.

Sampling. Sampling was performed bi-weekly at each location. Rows two and five were reserved for sampling at the Tifton locations, whereas rows two and six were alternately sampled with rows three and seven at the Athens location. Weekly observations were taken to monitor for *M. cribraria* infestations from 14 days after planting (DAP) until either 56 DAP or *M. cribraria* nymphs were detected. Sampling began 56 DAP on 23 June in TF1 and 14 July in TF2, 66 DAP (27 July 2015) in Athens and 73 DAP (17 July 2015) in the TF3 field. Sampling ceased at each location when plants reached the R7 growth stage (beginning maturity) on 15, 22, 11 and 21 September, respectively. Ten single row sweep samples were performed with a standard 38 cm diameter sweep net to enumerate adult *M. cribraria* from each plot. Egg masses and nymphs were enumerated by counting the total number present on three plants randomly selected from a one row foot area in two separate designated sample rows in each plot. Nymphs were categorized as either small or large nymphs. Small nymphs, defined as instars one through three, were characterized by the absence of wing pads. Large nymphs, fourth and fifth instars, featured wing pads apparent. Only unhatched egg masses were counted, defined by the presence of an intact operculum for all eggs within the mass.

Sampling for defoliating lepidopterans was conducted at all locations by taking two, 1-m drop cloth samples per plot (four row meters per plot). Drop cloths were placed between sample rows, and plants were vigorously shaken over the cloth from the middle. Dislodged caterpillars were identified and counted. Soybean loopers, cabbage loopers, green cloverworm (GCW), and velvet bean caterpillars (VBC) were differentiated by the number of pairs of larval prolegs. Soybean loopers and cabbage loopers are difficult to distinguish from one another in the field; therefore, these two species were counted together and grouped under “loopers.” These were the primary lepidopterans encountered and the only ones reported. Special care was taken to insure that shake samples were not performed in the same area of row as sweep samples.

Defoliation. Percent defoliation was measured at the R6 growth stage at all locations. Five randomly selected leaves were collected from each of the four center rows of each plot. Leaves were selected from the middle of the canopy to avoid older leaves which have may have been subjected to prolonged feeding and new leaves which have been exposed to less damage. The 20 leaves were spread out on a flat surface and an overall estimate of defoliation was made based on a visual guide (Koch 2016). Defoliation ratings were taken at the TF3 field on 28 August, TF1 and TF2 fields on 1 September and the Athens field on 4 September.

Harvest. The two middle rows of each plot were harvested at all locations. Eighty row feet of soybean were harvest from each plot at the Tifton location with a Wintersteiger two row, small plot combine (Wintersteiger Co., Hutchinson, KS) on 14 October. An Almaco small plot combine (ALMACO, Nevada, IA) was used to harvest 40 row feet of each plot at the Athens location on 21 October. Grain weight, test weight, and100 kernel weight were recorded for all plots, and grain yield was adjusted to 13% moisture content.

Data analysis. *Megacopta cribraria* nymphs were combined to include all instars in a single response variable. Mean loopers were analyzed and a separate response variable containing all defoliating lepidopterans was analyzed. All response variables were combined and averages were generated by time intervals based on insecticide treatment. Insect counts gathered before the first treatment of bifenthrin and chlorantraniliprole were given the designation time interval one, counts enumerated between the first and second spray (bifenthrin and indoxacarb) were designated time interval two, and the collection dates after the second treatment, were designated time interval three. A conditional model was fit to the insect count data using the quad method in the SAS Software's GLIMMIX procedure (SAS/STAT software, version 9.4 2002-2015 SAS Institute Inc., Cary, NC). At each location, treatments were arranged in a randomized complete block design (RCBD) and the multiple locations were combined into a split plot arrangement of treatments with treatments generalized across all locations; insect counts were modeled from a Poisson distribution since variances were proportional to the means (Zar 1999). In the split plot analyses, location served as the whole plot treatment factor while insecticide treatments served as subplot factors. All response variables were modeled in the GLIMMIX procedure. Yield was analyzed as a Gaussian distribution whereas defoliation was analyzed as a continuous proportion with a Beta distribution (Stroup 2015). Location and block were treated as random effects in both cases. Least square means are reported and were separated using pairwise *t-test*, the standard separation procedure in GLIMMIX. Significant two way interactions were further analyzed using the slicediff option of the LSMEANS statement. Means were separated using LSMEANS ($\alpha = 0.05$).

Results

Counts of *M. cribraria* egg masses were not significantly affected by the insecticide treatment by insecticide time interval interaction ($P = 0.960$) (Fig. 5.1A). The main effect of insecticide time interval had a significant effect on egg masses recorded across all field locations ($F = 111.76$; df = 2, 170; $P < 0.001$) (Fig. 5.1A). Significantly more egg masses were found in the first time interval followed by the second and third time intervals. The interaction of insecticide by treatment interval had a significant effect on *M. cribraria* nymphs ($F = 26.03$; df = 6, 170; $P < 0.001$; Fig. 5.1B), adults ($F = 22.78$; df = 6, 170; $P < 0.001$; Fig. 5.1C), loopers ($F = 7.48$; df = 6, 170; $P < 0.001$; Fig. 5.2A), and defoliating lepidopterans ($F = 8.60$; df = 6, 170; $P < 0.001$; Fig. 5.2B).

Though differences in insect means were found among treatments in the first time interval when interactions were significant, they are not reported in the text and assumed to be due to natural variation of pests in the field. While examining the effect of insecticide and holding treatment time interval constant, no differences in nymphs were found between the combination and *M. cribraria* (bifenthrin) ($P = 0.055$) targeted applications in the second time interval (Fig. 5.1B). All other insecticide comparisons for nymphs in the second interval were found to be significantly different. No difference was found between nymphs in only, the bifenthrin and combination treatments in the third interval ($P = 0.620$) where all other comparisons of insecticide were different (Fig. 5.1B). No differences in nymph numbers were found between interval two and three ($P = 0.416$) for the combination treatment when examining for the effects of insecticide treatment time interval and holding insecticide constant (Fig. 5.1B). Significant differences in nymphs were detected between all other combinations of treatment interval within each insecticide treatment. In the second interval no difference in adults was detected between the untreated plots and the combination (*P* = 0.076) and defoliating lepidopteran (*P* = 0.586) plots or the bifenthrin and combination (*P* = 0.581) plots when holding time interval constant (Fig. 5.1C). No difference in adult numbers were seen between the untreated and defoliating lepidopteran (*P* = 0.874) plots and bifenthrin and combination (*P* = 0.093) plots in the third interval. When examining for the effect of time interval on adults while holding insecticide treatment constant, no differences were found between time interval one and three in the untreated plots (*P* = 0.579). Similarly, no differences in adult levels were detected between intervals two and three for the bifenthrin plots (*P* = 0.883) or between interval one and three of the defoliating lepidopteran plots (*P* = 0.514) (Fig. 5.1C).

In the second time interval no differences in looper counts were found between the untreated plots and both the combination (*P* = 0.278) and bifenthrin (*P* = 0.342) plots, or the combination and defoliating lepidopteran plots (*P* = 0.172) when time interval was held constant and only insecticide was examined (Fig. 5.2A). Within the third time interval no significant difference between loopers was detected in the untreated and bifenthrin plots (*P* = 0.986). Similarly, no differences were found between the combination and defoliating lepidopteran (*P* = 0.539) plots in the third interval. When holding insecticide constant and considering the effects of treatment time interval, no differences in loopers were detected between the first and second treatment interval in the untreated (*P* = 0.642), bifenthrin (*P* = 0.319) or combination (*P* = 0.230) plots (Fig. 5.2A). When considering the effects of insecticide and holding time interval constant no differences in defoliating lepidopterans (loopers plus GCW and VBC) were found between the untreated and bifenthrin (*P* = 0.580) plots or the combination and defoliating lepidopteran protected plots (*P* = 0.165) in the second time interval (Fig. 5.2B). In the third interval no differences were found between the same treatments as in the second interval ($P = 0.711$ and $P = 0.670$ respectively).

Defoliation and yield. There was no significant effect of insecticide treatment on soybean grain yield (Table 1), test weight ($P = 0.917$) or 100 kernel weight ($P = 0.849$). However, the effect of insecticide treatment was significant on percent defoliation (Table 5.1). Untreated plots had significantly greater defoliation than all other treatments except the bifenthrin protected plots ($t_{62.33} = 1.42$; $P = 0.162$). No significant difference was detected between defoliation in the bifenthrin and combination plots ($t_{62.33} = 1.91$; $P = 0.060$) or the combination and defoliating lepidopteran protected plots ($t_{62.33} = 0.27$; $P = 0.788$) (Table 5.1).

Discussion

Because egg masses present in the field before an insecticide application is made remain in the field after the application, egg mass numbers were not significantly affected by insecticide treatment. However, timing of insecticide application did have a significant effect on egg masses. This may suggest that mobile stages of *M. cribraria* are controlled leading to a decreased number of egg masses being laid, and reduced subsequent nymph populations as a result of low incidence of adults. However, differences in egg masses were found between all combinations of insecticide treatment time interval in the untreated plots indicating that differences occurred due to seasonal dynamics of *M. cribraria* and cannot be solely attributed to insecticides. Oviposition occurs early in the season as overwintering adults disperse and infest early planted soybean fields and then again in mid to late summer once the first generation matures (Zhang et al. 2012, Seiter et al. 2013a, Seiter et al. 2013b, Blount et al. 2015a). Historically these two oviposition time periods have been from April to May and July to August in the southeastern U.S. (Yanzhuo et al. 2012, Seiter et al. 2013a, Blount et al. 2015, Seiter et al. 2015b). Peak oviposition was observed from approximately mid to late July suggesting only the second generation occurred in soybean during this study. Upon maturity, the second generation of adults moves from soybean into overwintering habitats. Seasonal dynamics also likely explains why there were no differences detected in adult populations in untreated and defoliating lepidopteran protected plots between time intervals one and three. Peak adult numbers in the fall represent completion of development of the second generation and dispersal into overwintering habitats. Control of adults and nymphs was obtained with both bifenthrin and the combination treatment with the first application and maintained with the second application. This finding agrees with previous studies that suggest one well timed application can effectively control damaging populations of *M. cribraria* (Blount et al. 2015a, Seiter et al. 2015b).

Previous collections from soybean in the southern U.S. found that >90% of loopers collected from soybeans are soybean loopers and <7% are cabbage loopers (Copping et al. 2012). It was for this reason soybean and cabbage loopers were combined into a single ‘looper’ response variables such that, approximately 84.5 % of defoliating lepidopterans were loopers, 14.5% were GCW and <1% were VBC based on season long means across location (not reported). Observed means of GCW and VBC were too low for accurate analysis and were combined with loopers for analysis. Control of loopers was successfully demonstrated with the use of combination and defoliating lepidopteran protected plots in comparison to the bifenthrin and untreated plots. However, the first insecticide application (chlorantraniliprole) did not affect significant change in mean numbers of loopers after treatment. After the second application (indoxacarb), significantly lower means of loopers were detected in plots where lepidopterans were targeted.

Differences of least square means between treatment interval one and two were found to be significant with the addition of GCW and VBC to loopers. This suggests that while the first application did not successfully reduce loopers, it did provide control of VBC and GCW, as did the second application. It is noteworthy that GCW is susceptible to pyrethroid insecticides (IRAC 3A) and it is therefore possible that bifenthrin applications had some impact on populations in those plots, but no differences in defoliating lepidopterans were detected between bifenthrin and untreated plots. There were differences in defoliating lepidopterans among all treatment intervals except for interval one and two in the untreated and bifenthrin plots. This suggests that a change in mean numbers of defoliating lepidopterans was due to insecticide treatment and not simply pest population dynamics. Populations of defoliating lepidopterans decreased as time interval progressed indicating that levels are greater during the early soybean reproductive stages (R2 to R4) than later stages (R6 to R7) regardless of insecticide treatment for trials in 2015.

Insecticide treatment had a significant effect on percent defoliation (Table 5.1). Significantly greater defoliation occurred in the untreated plots than the plots where defoliating lepidopterans were targeted. However, untreated plots and combination plots did not differ significantly from the bifenthrin plots. Though an insecticide response was seen, defoliation percentages were below threshold levels (15% after R2) for all plots (Roberts et al. 2015). The effect of insecticide on grain yield was contrary to that of defoliation and no significant differences in grain yield occurred among treatments across location (Table 5.1). No biological significance can be assumed from the separation of mean defoliation.

Though *M. cribraria* is susceptible to many insecticides (Seiter et al. 2015a), timing of application is critical in providing season long control. Application of insecticides before nymph emergence and when adults are still dispersing and could re-infest soybean may require multiple insecticide applications to control *M. cribraria*. Past management recommendations for the control of *M. cribraria* were developed when nymph populations frequently exceeded 100 individuals per row foot (Seiter et al. 2013a, Blount et al. 2015a). A recommended action threshold of an average of 25 nymphs per 25 consecutive sweep samples (one nymph per sweep) and adults present was developed for the southeastern growing region (Roberts et al. 2015). The intent of this threshold was to properly time insecticide applications and may not directly affect a damage loss relationship because nymph numbers were always well above economic injury level. In this study, mean nymph numbers per sample (six plants from 0.61 m of row) in the untreated plots were 25.1 ± 68.9 (n = 62) before treatment, 3.8 ± 8.5 (n = 68) in the second interval, and 0.6 ± 3.6 (n = 84) in the third interval, which are levels considerably lower than reported in previous years. Though, thresholds are based on sweep net samples and nymphs were enumerated using whole plant counts in this study, populations in 2015 approached the threshold level. Stubbins et al. (2014) developed a metric for beat sheet sampling corresponding to the economic threshold developed for sweep sampling, this number was 24.7 nymphs per 1.8 m of row. Because nymph populations in this study did not cause yield reductions, current threshold recommendations need to be re-evaluated for the new condition of *M. cribraria* infestations. At current soybean prices of ~$9/BU, the yield benefit of 75.92 kg/ha (1.13 BU/A) (mean grain yield not reported) obtained from application of the combination insecticide treatment may not be economically viable when factoring in cost of insecticides and application costs. *Megacopta cribraria* counts are greater near field edges (Seiter et al. 2013a). Nymph counts had a range of 0 to 484, 52, and 72 per sample for each treatment interval respectively (not reported), indicating that in-field distribution is aggregated, particularly at lower populations (Stubbins et al. 2014). When making management decisions for *M. cribraria,* scouting should be performed in the middle of the field as well as the edges to account for such distribution (Roberts et al. 2015).

Populations of *M. cribraria* have been on the decline in the southeast since 2013 due to a combination of factors likely including cold weather and natural enemies. Natural enemies such as *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Platygastridae), which can be effective at decreasing emerging *M. cribraria* nymph populations up to 62% (unpublished data) are susceptible (as adults) to insecticides registered for use in soybean for the control of *M. cribraria*. Adequate control may be provided by natural enemies when populations are relatively low decreasing the need for insecticide applications targeting *M. cribraria*.

Mean numbers of defoliating lepidopterans in untreated plots did not approach established threshold levels (six loopers per 0.3 m row) (Roberts et al. 2015) in the first (6.1 ± 4.5, n = 62) and second treatment interval (6.4 ± 4.5, n = 68), when two row meter beat sheet samples were performed. Likewise, percent defoliation did not exceed the 15% defoliation after bloom threshold, only reaching a mean of 5% ± 2.2 (n = 19). However, treatments targeting defoliating lepidopterans provided protection from defoliation greater than untreated plots.

A lack of control of loopers was observed with the first insecticide application of chlorantraniliprole. Soybean loopers are notoriously difficult to control and populations are reported to have resistance to organophosphates, carbamates, pyrethroids, organochlorines and cyclodienes (Boernel et al. 1992, Copping et al. 2012). Control can also be reduced by other factors such as poor coverage and environment; therefore, it is important to adhere to insecticide resistance management practice and rotate to a different class of chemistry to achieve control. The application of indoxacarb (IRAC class 22) in the second spray successfully decreased defoliating lepidopterans when chlorantraniliprole (IRAC class 28) did not have an effect on the population. It remains unknown if the first application was ineffective due to resistance or other reasons.

Combined indirect injury incurred by threshold levels of *M. cribraria* and sub-threshold levels of defoliating lepidopterans did not decrease soybean yield based on findings of this study. If populations of *M. cribraria* continue to be present at their current infestation levels in the southeast, management decisions may be made based on higher threshold levels than in the past when much higher numbers of *M. cribraria* infested soybean fields. More information is needed to determine thresholds based on sweep net samples in light of declining *M. cribraria* populations. Since more than one pest or complex of pests is typically present in a field at one time, more information also is needed to understand how infestations of pests with different mechanisms of injury affect soybean productivity and therefore, management practices.

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Table 5.1. Mean ± SE percent defoliation per 20 leaves collected from mid-canopy of the four center rows of each plot and soybean grain yield (kg/ha) across location in response to injury by defoliating lepidopterans and *M. cribraria.*

Insecticide Target	% Defoliation	Grain Yield (kg/ha)
Untreated	5.00 ± 0.51 a	3006.2 ± 255.3 a
M. cribraria	4.36 ± 0.56 ab	3024.6 ± 203.4 a
Defoliators	3.47 ± 0.38 c	3050.1 ± 258.5 a
Combination	3.57 ± 0.52 bc	3082.9 ± 259.2 a
F	5.83	0.05
P	0.0014	0.9848
Df	3, 62.33	3, 55.63

Means followed by the same letter are not significantly different ($\alpha = 0.05$).

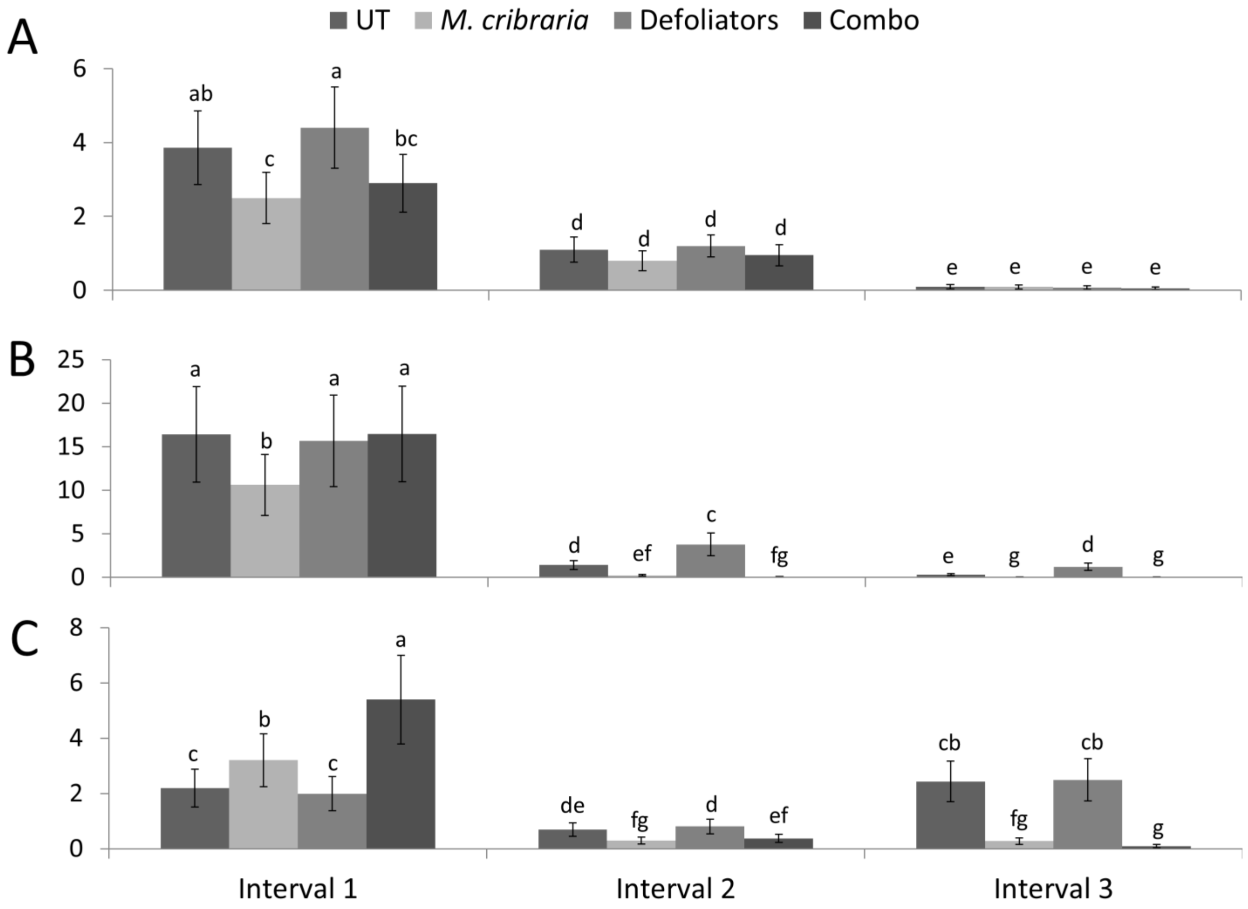

Figure 5.1. LS mean ± SE (A) *M. cribraria* egg masses and (B) nymphs per two row feet. (C) Adult *M. cribraria* per 10 single row sweeps. Means with the same letter are not significantly different (α = 0.05).

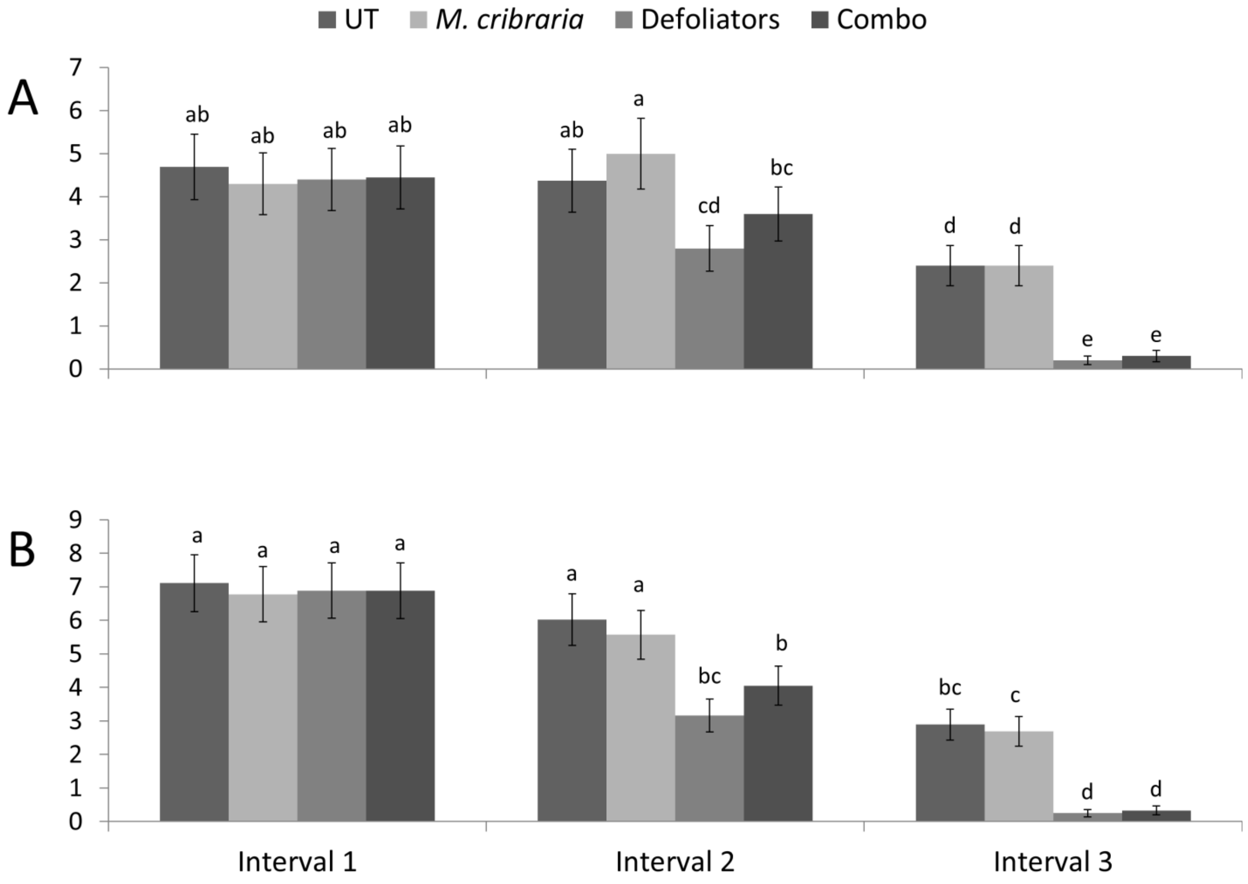

Figure 5.2. LS mean ± SE (A) loopers and (B) combined cabbage loopers, soybean loopers, GCW and VBC per two, one meter beat sheet samples per plot across location. Means with the same letter are not significantly different (α = 0.05).

CHAPTER 6

CONCLUSION

Upon the initial invasion of *Megacopta cribraria* into the U.S., no other members of the family Plataspidae (Hemiptera: Pentatomoidea) were known from the Western Hemisphere (Eger et al. 2010). *M. cribraria* have been reported from a variety of plant species spanning several families (Srinivasaperumal et al. 1992, Eger et al. 2010, Medal et al. 2013) including species of economic importance in the Southeast such as, cotton (*Gossypium hirsutum* L.), lima bean (*Phaseolus lunatus* L.), kudzu (*Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen and S. Almeida) and soybean (*Glycine max* (L.) Merrill) (Srinivasaperumal et al. 1992, Eger et al. 2010). Two generations were reported to occur in kudzu in sentinel studies performed in Georgia evaluating the life history and host preference of *M. cribraria* (Zhang et al. 2012). As a result of *M. cribraria's* ability to disseminate over large areas and their phoretic behavior (Eger et al. 2010, Suiter et al. 2010, Gardner et al. 2013b, Ruberson et al. 2013, Gardner 2015), several cadavers were discovered in agricultural commodity shipments in Honduras (Crowley 2012) and a need to elucidate the true host range for economically important crop plants became apparent. In a series of choice, no-choice, and field evaluations performed in GA in 2012 and 2013 a clear picture of the developmental hosts of *M. cribraria* was drawn. Development of nymphs from egg masses was only completed on soybean, edamame (*G. max*) and pigeon pea (*Cajanus cajan* L.). No-choice greenhouse trials conducted in Florida indicate that development of *M. cribraria* may be completed on black-eyed pea (*Vigna sinensis* L.), lima bean, and pinto bean (*Phaseolus *vulgaris* L.) (Medal et al. 2013) but these results were not duplicated in our host preference tests. The varieties of individual species evaluated among separate tests may affect development of *M. cribraria*. We were able to effectively demonstrate that soybean and kudzu are the primary and most important developmental hosts of *M. cribraria* in the U.S. though minor development may occur on alternate hosts.

How *M. cribraria* respond to the effects of cultural control practices such as planting date and agronomic selection of varieties for traits such as maturity group had not been evaluated in *M. cribraria's* native or new range. Additionally a need to evaluate how *M. cribraria* impacted yield when exposed to these effects arose. Though *M. cribraria* are capable of developing and reproducing in soybean in the absence of kudzu, it was unclear how the different hosts affected voltinism of the insect (Pozo-Valdivia and Reisig 2013). To evaluate these effect replicated, split-plot field trials, where maturity group and insecticide treatment were nested within planting date at several locations in Georgia were carried out. Treatment levels corresponded to maturity groups commonly used in the region (five and eight) that would still have detectible differences in maturity throughout the season were selected. Two planting dates (May and June) typical in the region, as well as a very early (April) and very late (July) planting were evaluated. A broad spectrum insecticide (lambda-cyhalothrin) was applied to plots to determine how *M. cribraria* impacted yield. The effect of injury by *M. cribraria* on yields and yield components of the maturity groups evaluated were not significant. However the effect of injury by *M. cribraria* on yield for different planting dates was significant. Soybean planted in April received the greatest reductions in yield followed by the May planting then June, with the July planting having the lowest yield overall and lowest yield loss. Two generations of *M. cribraria* were completed in April and May plantings contributing to greater season long injury than occurred in June and

July planted soybean where only the second generation of *M. cribraria* completed development. Seed weight was the primary yield component effected by removal of vascular fluids from the phloem by *M. cribraria* (*F. Stubbin*, unpublished data) which agrees with results from previous studies conducted in South Carolina (Seiter et al. 2013). Early planted soybean are at the greatest risk of yield loss by *M. cribraria,* and double crop or later planted soybean are at lower risk of yield loss.

To determine how populations of *M. cribraria* may be affected by varying geographic regions in the Southeast multiple locations were sampled in Georgia in 2012 and 2013. Egg masses, nymphs and adults were enumerated using relative sampling methods in kudzu and soybean. Linear regression analysis was performed on cumulative totals of each life stage and comparisons of locations and region were made. The two regions of interest in this study were the northern or Piedmont region, and the southern or Coastal Plain region. Similarities and differences were detected for at least one life stage between location and region. No clear trends in development between regions were apparent for either host environment. The week at which 50% of the population for each life stage was reached was estimated based on cumulative totals, and recorded. The main effects of latitude, longitude and region on these estimate were examined. Overall, the main effects were not significant on *M. cribraria* populations, suggesting that development of *M. cribraria* occurs at approximately the same rate between regions. Additionally, how single insecticide application timings affected mid-season populations in soybean was examined from 2011 to 2014. Treatments were most effective at controlling *M. cribraria* populations when applied within an approximately four week period spanning the month of July. This time period likely coincides with when adults have ceased moving into fields and ovipositing. Weeks at which 50% of the nymph population is present in soybean correspond to the four week period where insecticide treatments effectively suppress populations. These studies indicate that the need for insecticide treatments should be assessed on a site specific basis but that the most effective application date falls somewhere within the month of July.

How populations of *M. cribraria* have changed since their initial introduction into the Southeast has not been well documented. At two sites near Griffin, GA, kudzu patches were monitored weekly from 2012 to 2015 from early spring to late fall depending on availability of kudzu. Count data was averaged across the two sites, calculated as cumulative totals and combined by Julian week of the year to facilitate analysis. Differences in cumulative totals between years showed that 2014 and 2015 had significantly lower populations of *M. cribraria* than 2012 and 2013. Factors that may have contributed to declining populations are the presence of natural enemies. *Paratelenomus saccharalis* (Dodd), an egg parasitoid of plataspids was discovered in Georgia and Alabama in 2013 (Gardner et al. 2013a) and has begun to establish in the Southeast. An entomopathogenic fungus, *Beauvaria bassiana* (Bals.-Criv.) Vuillemin, has also been observed attacking *M. cribraria* nymphs and adults (Ruberson et al. 2013, Seiter et al. 2014). A combined effect of these virulent natural enemies has likely had an impact on *M. cribraria* populations though more information is needed to quantify these effects.

Megacopta cribraria causes indirect damage by removing vascular fluid from the phloem leading to reduced seed weight, overall seed quality and grain yield (Seiter et al. 2013). A complex of defoliating lepidopterans also causes indirect damage by removing photosynthetic surface material by defoliation. How the presence of these two pests effect yield when exerting injury on soybean in a single field has not been investigated in the Southeast. Primary defoliating species present in field trials conducted in 2015 composing the defoliator complex were, soybean loopers (*Chrysodiexis includes* (Walker)), cabbage loopers (*Tricoplusia ni* (Hübner)), green cloverworm (*Hypena scabra* (F.)) (GCW), and velvet bean caterpillar (*Anticarsia gemmatalis* (Hübner)) (VBC). To isolate the effects of each suite of pests, insecticides targeting *M. cribraria* (bifenthrin), and lepidopterans (chlorantraniliprole and indoxacarb) were applied individually and as a combination, and compared to an untreated check. *Megacopta cribraria* and lepidopterans were sampled bi-weekly in replicated trials in four fields in GA and means were generated based on when applications were made. Interval one consisted of samples taken before treatment with bifenthrin and chlorantraniliprole, interval two consisted of samples taken after the first application and interval three consisted of samples taken after the second application was made, which consisted of bifenthrin and indoxacarb. Data were combined across location and *M. cribraria* life stages and caterpillars were compared among time interval. Control of *M. cribraria* was obtained with bifenthrin at each application. Cabbage loopers and soybean loopers were combined into a single response variable (loopers). No differences in looper means were detected between time interval one and two though significantly fewer caterpillars were found in samples taken in time interval three than time interval two. Loopers were combined with GCW and VBC, which had means too low to analyze alone. With the addition of these two species to loopers, significant differences were found between all time intervals indicating that chlorantraniliprole effectively controlled VBC and GCW. However, populations of *M. cribraria* and defoliating lepidopterans separately and in combination were too low to have an effect on yield. Threshold levels (one nymph per sweep when 25 sweeps are performed) (Roberts et al. 2015) of *M. cribraria* were detected whereas, only sub-threshold levels of lepidopterans were detected. This information indicates that population levels like those detected in 2015 are not likely to reduce grain yield. Furthermore, threshold levels of *M. cribraria* in the U.S. may be more accurately re-evaluated based on populations currently found in the Southeast compared to previous years when substantially greater populations were encountered in soybean.

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APPENDICES

Table S2.1. Least square means ± SE of nymphs per entry by day for the nymph choice test 2012

Scientific Name	**Common Name**	**Variety**	**§Days 1-4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 10**	**Day12**	**Day 14**
Glycine max	Soybean	AG 5831 (MG5)	11.0 ± 8.8a	6.5 ± 4.4a	6.1 ± 4.2a	16.4 ± 9.1ab	20.4 ± 3.6a	18.6 ± 6.9a	15.0 ± 3.7a	13.4 ± 3.3a
Glycine max	Edamame-Soybean	Mosley' O.P. prolific	5.42 ± 4.4a	2.8 ± 1.9a	8.1 ± 5.5a	28.0 ± 15.5a	34.0 ± 5.8a	18.8 ± 6.9a	16.9 ± 4.1a	10.0 ± 2.5a
Phaseolus radiatus	Mung bean	--	0.57 ± 0.5a	7.8 ± 5.2a	13.2 ± 9.0a	3.7 ± 2.1abc	0.7 ± 0.3b	0.2 ± 0.2b	0.1 ± 0.1b	0.1 ± 0.1b
Phaseolus lunatus	Lima bean	Henderson	3.0 ± 2.4a	1.1 ± 0.8a	0	0.5 ± 0.4c	0.1 ± 0.1b	0	0	0
Lablab purpureus	Lablab	--	10.0 ± 8.0a	0.1 ± 0.1a	2.0 ± 1.4a	1.7 ± 1.0abc	0.4 ± 0.3b	0.3 ± 0.2b	0	0
Arachis hypogaea	Peanut	Georgia 06G	2.8 ± 2.3a	8.7 ± 5.8a	3.7 ± 2.5a	1.1 ± 0.7bc	0.8 ± 0.3b	0	0	0
Vigna angularia	Azuki bean	--	0.5 ± 0.5a	1.5 ± 1.1a	3.4 ± 2.4a	1.7 ± 1.0abc	0.1 ± 0.1b	0	0	0
Phaseolus vulgaris	Pinto bean	Frijol: Negro	5.8 ± 5.5a	1.2 ± 0.9a	1.7 ± 1.2a	2.7 ± 1.6abc	0.1 ± 0.1b	0.2 ± 0.1b	0	0
Phaseolus vulgaris	Pinto bean	Deorho	5.2 ± 4.3a	4.5 ± 3.0a	3.0 ± 2.1a	2.0 ± 1.2abc	0.8 ± 0.3b	0.2 ± 0.2b	0.1 ± 0.1b	0
Phaseolus vulgaris	Pinto bean	Cardenal	0.7 ± 0.6a	0.8 ± 0.6a	3.8 ± 2.6a	5.4 ± 3.1abc	0.8 ±0.3b	0.2 ± 0.1b	0	0
Vigna sinensis	Black- eyed pea	California black-eye	3.7 ± 3.0a	5.5 ± 3.7a	3.5 ± 2.5a	1.7 ± 1.0abc	0	0	0	0
Pisum sativum	Winter pea	Oregon sugar pod II	0.4 ± 0.4a	3.0 ± 2.0a	2.1 ± 1.5a	0	0	0	0	0
Phaseolus vulgaris	Snap bean	Roma II	9.0 ± 7.2a	1.4 ± 1.0a	1.1 ± 0.8a	2.0 ± 1.2abc	0.7 ± 0.3b	0.8 ± 0.4b	0.2 ± 0.2b	0
Phaseolus vulgaris	Pinto bean	Tio Caneta	7.1 ± 5.7a	1.1 ± 0.8a	2.5 ± 1.8a	3.1 ± 1.8abc	0.2 ± 0.2b	0	0	0
Phaseolus vulgaris	Pinto bean	Carrizalita	1.5 ± 1.3a	5.5 ± 3.7a	5.1 ± 3.5a	2.1 ± 1.3abc	0.8 ± 0.3b	0.6 ± 0.3b	0	0
Phaseolus vulgaris	Pinto bean	Amadeus 77	2.1 ± 1.7a	5.2 ± 3.5a	4.5 ± 3.1a	4.5 ± 2.6abc	0.5 ± 0.3b	0.2 ± 0.1b	0.5 ± 0.3b	0
Zea mays	Corn	--	0.1 ± 0.1a	1.8 ± 1.3a	2.0 ± 1.4a	1.0 ± 0.6bc	0	0	0	0
		F	1.81	1.55	0.77	2.70	27.97	16.50	19.67	9.25
		P	0.0401	0.0956	0.7075	0.0017	<0.0001	<0.0001	<0.0001	0.0017
		df	16, 102	16, 102	15, 95	15, 96	13, 84	9, 55.6	5, 36	2, 18

Means in columns with the same letter are not significantly different (Tukey-Kramer LSM α = 0.05)**.** Entries with zero nymphs were not included in the GLIMMIX analysis.

§ There were pairwise differences between means but, due to the Tukey adjustment they have the same letter grouping.

Table S2.2. Mean ± SE number of nymphs per entry by day in nymph choice test 2013

Scientific Name	Common Name	Variety	Days 1-4	Day 5	Day 6	Day7	§Day 9	Day 11	Day13	Day 15	Day 17	Day 19	Day 21	Day 23	Day 25
Glycine max	Soybean	AG 5831 (MG5)	15.5 ± 9.0a	4.3 ± 3.3a	9.8 ± 6.5a	11.8 ± 5.5a	13.3 ± 7.8a	16.0 ± 5.1a	11.5 ± 4.0a	8.1 ± 1.9b	11.3 ± 3.1a	8.8 ± 2.9a	10.0 ± 3.1a	9.1 ± 2.8a	11.0 ± 3.7a
Glycine max	Edamame-Soybean	Midori Giant	13.0 ± 12.4a	5.1 ± 3.9a	6.8 ± 4.6a	14.0 ± 6.5a	23.5 ± 13.6a	33.6 ± 10.6a	27.8 ± 9.4a	25.3 ± 5.2a	27.1 ± 7.1a	24.9 ± 7.9a	27.1 ± 8.2a	27.3 ± 7.8a	31.3 ± 10.1a
Cajanus cajan	Pigeon pea	--	2.6 ± 2.6a	3.9 ± 2.9a	5.1 ± 3.5a	9.5 ± 4.4a	17.6 ± 10.0a	26.0 ± 8.2a	25.0 ± 8.5a	30.0 ± 6.1a	25.6 ± 6.7a	22.9 ± 7.3a	24.5 ± 7.4a	26.5 ± 7.6a	19.6 ± 6.4a
Phaseolus radiatus	Mung bean	--	10.6 ± 10.1a	0.6 ± 0.5a	2.5 ± 1.7a	3.6 ± 1.8a	1.0 ± 0.7a	1.0 ± 0.5b	0.3 ± 0.2b	0	0.3 ± 0.2b	0.2 ± 0.2b	0.3 ± 0.2b	0.3 ± 0.2b	0.3 ± 0.2b
Phaseolus lunatus	Lima bean	Henderson	4.5 ± 4.3a	8.8 ± 6.6a	7.0 ± 4.7a	1.8 ± 0.9a	1.6 ± 1.0a	0.5 ± 0.3b	0.1 ± 0.1b	0	0	0	0.1 ± 0.1b	0.1 ± 0.1b	0
Lablab purpureus	Lablab	--	5.8 ± 5.6a	3.8 ± 2.9a	6.5 ± 4.3a	5.5 ± 2.6a	2.5 ± 1.5a	0.5 ± 0.3b	0.3 ± 0.2b	0.1 ± 0.1c	0	0.1 ± 0.1b	0.1 ± 0.1b	0.6 ± 0.3b	0.1 ± 0.1b
Arachis hypogaea	Peanut	Georgia 06G	2.1 ± 2.1a	9.8 ± 7.3a	11.5 ± 7.6a	2.8 ± 1.4a	3.8 ± 2.3a	0	0	0.1 ± 0.1c	0.1 ± 0.1b	0	0	0	0
Vigna angularia	Azuki bean	--	7.0 ± 6.7a	1.0 ± 0.8a	2.8 ± 1.9a	5.1 ± 2.5a	4.0 ± 2.4a	0	0.1 ± 0.1b	0	0	0	0	0	0
Vicia faba	Fava bean	Windsor	1.6 ± 1.6a	2.8 ± 2.2a	3.5 ± 2.4a	3.0 ± 1.5a	4.3 ± 2.6a	0	0	0	0	0	0	0	0
Phaseolus vulgaris	Pinto bean	Frijol: Negro	4.3 ± 4.1a	0.3 ± 0.3a	0.8 ± 0.6a	4.8 ± 2.3a	5.0 ± 3.0a	0.3 ± 0.2b	0	0	0	0	0	0	0
Phaseolus vulgaris	Pinto bean	Deorho	0	5.6 ± 4.2a	3.1 ± 2.2a	3.3 ± 1.6a	2.8 ± 1.7a	0.1 ± 0.1b	0	0	0	0	0	0	0
Phaseolus vulgaris	Pinto bean	Cardenal	9.0 ± 8.6a	2.6 ± 2.0a	6.3 ± 4.2a	4.3 ± 2.1a	2.3 ± 1.4a	0.3 ± 0.2b	0.1 ± 0.1b	0	0	0	0	0	0
Vigna sinensis	Blackeyed pea	California black-eye	7.5 ± 7.1a	5.5 ± 4.1a	14.8 ± 9.8a	8.5 ± 4.0a	1.8 ± 1.1a	0	0	0	0	0	0	0.1 ± 0.1b	0
Pisum sativum	Winter pea	Oregon sugar pod II	2.8 ± 2.7a	4.3 ± 3.3a	3.6 ± 2.5a	1.6 ± 0.9a	3.5 ± 2.1a	0	0	0	0	0	0	0	0
Pueria phaseoloides	Tropical kudzu	--	0.6 ± 0.7a	0.3 ± 0.3a	1.1 ± 0.8a	6.6 ± 3.2a	10.6 ± 6.2a	9.8 ± 3.2a	0.3 ± 0.2b	0.5 ± 0.3c	0.5 ± 0.3b	0.1 ± 0.1b	0	0	0.1 ± 0.1b
Phaseolus vulgaris	Snap bean	Roma II	1.6 ± 1.6a	6.5 ± 4.9a	4.6 ± 3.1a	4.8 ± 2.3a	4.3 ± 2.6a	0.6 ± 0.3b	0.8 ± 0.4b	0.1 ± 0.1c	0	0	0	0	0
Cicer arietinum	Chick pea	--	0.6 ± 0.7a	0.1 ± 0.2a	3.1 ± 2.2a	9.8 ± 4.6a	9.1 ± 5.4a	0.6 ± 0.3b	0	0	0	0	0	0	0
		F	0.99	1.71	1.09	1.51	1.98	15.59	14.37	19.02	16.65	14.23	13.74	16.26	13.16
		P	0.4719	0.0603	0.3738	0.1134	0.0236	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
		df	15, 80	16,85	16, 85	16, 85	16, 85	11, 60	9, 50	6, 35	5, 30	5, 30	5, 30	6, 65	5, 30

Means in columns with the same letter are not significantly different, residual df (Tukey-Kramer LSM α = 0.05). Entries with zero nymphs were excluded from the GLIMMIX analysis.

§ There were pairwise differences between means but due to the Tukey adjustment they have the same letter grouping.

Table S2.3. Mean ± SE number of nymphs per entry from days after eclosion in the no-choice nymph test 2013

Scientific Name	**Common Name**	**Variety**	**Days 1-2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 9**	**Day 11**	**Day 13**	**Day 15**	**Day 17**	**Day 19**	**Day 21**
Glycine max	Soybean	AG 5831 (MG5)	21.5 ± 3.4a	10.7 ± 2.8ab	9.3 ± 3.5a	9.8 ± 4.0a	10.0 ± 4.7ab	10.3 ± 2.9a	9.1 ± 3.1abc	8.6 ± 1.7a	7.5 ± 1.4a	7.2 ± 1.6a	6.9 ± 2.3ab	7.5 ± 1.9a	6.6 ± 1.4a
Glycine max	Edamame-Soybean	Midori Giant	18.8 ± 3.0a	8.4 ± 2.2ab	10.3 ± 3.9a	9.3 ± 3.8a	9.0 ± 4.3ab	12.0 ± 3.3a	9.5 ± 3.2ab	9.6 ± 1.8a	10.5 ± 1.8a	9.1 ± 1.9a	8.6 ± 2.8a	8.6 ± 2.2a	9.7 ± 2.0a
Cajanus cajan	Pigeon pea	--	24.3 ± 3.7a	14.5 ± 3.7a	10.8 ± 4.1a	8.8 ± 3.6ab	12.6 ± 6.0a	10.8 ± 3.0a	13.5 ± 4.5a	12.8 ± 2.3a	13.3 ± 2.2a	13.3 ± 1.9a	13.0 ± 4.1a	13.4 ± 3.2a	11.9 ± 2.3a
Phaseolus radiatus	Mung bean	--	18.3 ± 2.9a	2.6 ± 0.8bc	5.0 ± 2.0a	2.1 ± 1.0abc	2.1 ± 1.1ab	0.3 ± 0.2b	0	0	0.1 ± 0.1b	0	0	0	0
Phaseolus lunatus	Lima bean	Henderson	19.1 ± 3.1a	6.9 ± 1.9abc	2.5 ± 1.1a	1.1 ± 0.6abc	0.8 ± 0.5ab	0.8 ± 0.4b	0.6 ± 0.3bcd	0.8 ± 0.3b	0	0	0	0	0
Lablab purpureus	Lablab	--	20.0 ± 3.2a	5.6 ± 1.6abc	3.1 ± 1.3a	2.0 ± 0.9abc	1.8 ± 1.0ab	2.3 ± 0.8ab	1.8 ± 0.7bcd	1.5 ± 0.5b	1.5 ± 0.5b	0.6 ± 0.3b	0.3 ± 0.2c	0.6 ± 0.3b	0
Arachis hypogaea	Peanut	Georgia 06G	15.8 ± 2.6a	4.2 ± 1.2abc	3.5 ± 1.4a	0.5 ± 0.3bc	1.5 ± 0.8ab	0	0	0	0	0	0	0	0
Vigna angularia §	Azuki bean	--	19.4 ± 3.4a	3.1 ± 1.1bc	3.0 ± 1.4a	1.4 ± 0.8abc	0.6 ± 0.4ab	0.2 ± 0.2b	0.4 ± 0.3cd	0	0	0	0	0	0
Vicia faba	Fava bean	Windsor	20.8 ± 3.3a	6.4 ± 1.8ab	4.5 ± 1.8a	0.5 ± 0.3bc	0.3 ± 0.2b	0.3 ± 0.2b	3.0 ± 1.1abcd	0.8 ± 0.3b	0.5 ± 0.2b	0.4 ± 0.2b	1.1 ± 0.5bc	1.9 ± 0.7b	0.4 ± 0.2b
Phaseolus vulgaris	Pinto bean	Frijol: Negro	24.8 ± 3.8a	7.6 ± 2.1ab	4.3 ± 1.7a	2.8 ± 1.3abc	1.3 ± 0.7ab	0.6 ± 0.3b	0	0	0	0	0	0	0
Phaseolus vulgaris ¶	Pinto bean	Deorho	18.7 ± 3.7a	7.6 ± 2.5ab	5.7 ± 2.8a	4.2 ± 2.2abc	2.7 ± 1.7ab	0.5 ± 0.3b	0.5 ± 0.4bcd	0	0	0	0	0	0
Phaseolus vulgaris	Pinto bean	Cardenal	21.1 ± 3.3a	6.9 ± 1.9ab	3.5 ± 1.4a	2.5 ± 1.7abc	1.8 ± 1.0ab	1.1 ± 0.5b	0.1 ± 0.1d	0.1 ± 0.1b	0.1 ± 0.1b	0	0	0	0
Vigna sinensis §	Black-eyed pea	California black-eye	21.0 ± 3.6a	0.5 ± 0.3c	4.2 ± 1.8a	2.8 ± 1.4abc	2.4 ± 1.3ab	0.8 ± 0.4b	0.2 ± 0.2cd	0	0	0	0	0	0
Pisum sativum	Winter pea	Oregon sugar pod II	21.1 ± 3.3a	4.4 ± 1.3abc	3.6 ± 1.5a	0.3 ± 0.2c	0.1 ± 0.1b	0.3 ± 0.2b	0.1 ± 0.1d	0	0	0	0	0	0
Pueria phaseoloides	Tropical kudzu	--	19.4 ± 3.1a	8.1 ± 2.2ab	6.0 ± 2.3a	6.1 ± 2.6abc	5.3 ± 2.6ab	3.3 ± 1.3ab	2.8 ± 1.1abcd	0.1 ± 0.1b	1.1 ± 0.4b	0.8 ± 0.3b	0.6 ± 0.3c	0.7 ± 0.3b	0.6 ± 0.3b
Phaseolus vulgaris	Snap bean	Bronco	13.5 ± 2.3a	6.3 ± 1.8abc	4.8 ± 1.9a	4.1 ± 1.8abc	3.3 ± 1.6ab	1.1 ± 0.5b	1.1 ± 0.7bcd	0	0	0	0	0	0
Cicer arietinum	Chick pea	--	14.1 ± 2.4a	3.5 ± 1.1bc	2.1 ± 0.9a	2.1 ± 1.0abc	0	0	0	0	0	0	0	0	0
		F	0.97	3.52	1.30	3.33	3.31	9.11	6.97	14.41	15.20	15.19	10.43	13.87	13.18
		P	0.4956	0.0002	0.2155	0.0002	0.0003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0005	<0.0001	<0.0001
		df	16, 81	16, 65.1	16, 81	16, 81	15, 76	14, 71	12, 61	7, 40	7, 40	5, 26.7	5, 11.6	5, 23.0	4, 16.7

Table S2.3. Continued

Day 23	**Day 25**	**Day 27**
6.0 ± 1.2b	6.5 ± 2.1ab	9.0 ± 2.2a
10.4 ± 1.9ab	10.5 ± 3.6a	8.7 ± 1.8a
12.7 ± 2.2a	13.5 ± 3.7a	12.6 ± 2.8a
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
1.0 ± 0.4c	1.2 ± 0.6bc	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0.3 ± 0.2c	0.1 ± 0.1c	0
0	0	0
0	0	0
13.34	9.01	0.56
<0.0001	0.0014	0.5931
4, 14.9	4, 11.7	2, 7

Means with the same letters in column are not significantly different from each other (Tukey-Kramer LSM α = 0.05). Entries with zero nymphs were excluded from the GLIMMIX analysis.
¶ N=4 for *Phaseolus vulgaris* (Deorho-pinto).
§ N=5 for *Vigna sinensis* (California black-eye) and *Vigna angularis* (Azuki bean).

Table S4.1. LS Mean ± SE cumulative totals of adults and nymphs by insecticide treatment date at four location years.

Midville 2011			Tifton 2012			Tifton 2013			Tifton 2014		
Treatment	Nymph	Adults	Treatment	Nymph	Adults	Treatment	Nymph	Adults	Treatment	Nymphs	Adults
Untreated	274.4 ± 60.4a	722.1 ± 328.6a	Untreated	198.6 ± 26.1a	901.2 ± 298.3a	Untreated	117.5 ± 15.6a	240.6 ± 55.5a	Untreated	7.4 ± 4.0ab	27.8 ± 16.9a
Aggressive[a]	0.1 ± 0.0b	24.8 ± 0.4b	30 Jul	5.5 ± 0.9b	28.3 ± 5.7b	19 Jun	36.2 ± 7.7cd	131.3 ± 30.6abc	23 Jun	8.7 ± 4.6a	15.2 ± 9.7b
14 Jul & Aug 24	26.1 ± 12.1b	21.3 ± 4.8b				3 Jul	6.4 ± 0.9d	63.6 ± 11.4bc	30 Jun	5.2 ± 2.4abc	10.3 ± 3.8bc
29 Jul	83.2 ± 0.7b	20.9 ± 5.9b				16 Jul	2.4 ± 0.1c	24.4 ± 5.0c	7 Jul	3.1 ± 1.7cd	8.7 ± 3.6c
9 Aug	115.5 ± 27.7b	29.0 ± 6.0b				31 Jul	50.9 ± 3.3bc	48.8 ± 5.5bc	14 Jul	3.1 ± 1.4cd	7.8 ± 3.2c
						12 Aug	117.4 ± 13.1a	206.0 ± 35.9ab	21 Jul	1.5 ± 0.6d	14.6 ± 3.6b
						26 Aug	81.3 ± 8.5ab	233.3 ± 42.1a	28 Jul	2.6 ± 1.5cd	8.0 ± 2.6c
						13 Sep	73.0 ± 8.3bc	241.1 ± 48.3a	4 Aug	2.9 ± 1.0cd	10.5 ± 2.1bc
									11 Aug	3.5 ± 1.5cd	6.6 ± 2.6c
									18 Aug	4.8 ± 2.1bc	10.1 ± 3.1bc

Means with the same letter are not significantly different (Tukey LSM α = 0.05).

[a] Applications made 28 June, 14 July, 29 July, 9 August, 24 August, and 8 September.