Development and Evaluation of Integrated Approaches for Managing of Mexican Bean Beetle, *Epilachna varivestis* Mulsant

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ABSTRACT

The Mexican bean beetle, *Epilachna varivestis* Mulsant, is a major pest of snap beans, *Phaseolus vulgaris* L. in the Central Appalachian region of the United States. To develop pertinent research objectives, background information on this pest was gathered from literature sources and personal communications with growers, extension agents and other agricultural professionals. In objective one, Mexican bean beetle preference, developmental success and plant injury were compared among three snap bean and three lima bean cultivars in field and greenhouse trials. The cultivar 'Dragon's Tongue' was the most preferred, suitable for development, and prone to injury. Growers may benefit from growing less susceptible cultivars, or by using 'Dragon's Tongue' in trap cropping or push-pull strategies. In objective two, Mexican bean beetle densities, feeding injury, and yield were compared among snap beans grown on metallized plastic (highly reflective), white plastic, black plastic, and bare soil. Metallized plastic provided the greatest level of control, and resulted in the highest yields. Managing Mexican bean beetle by growing beans on metallized plastic may be used as a stand-alone method, or in a push-pull strategy. In the final objective, the effects of snap beans grown from thiamethoxam (a neonicotinoid insecticide)-treated seeds on Mexican bean beetle were assessed in greenhouse and field experiments. Thiamethoxam-treated plants killed 40 to 50% of Mexican bean beetle adults and larvae up to 16 days after planting. In the field, thiamethoxam-treated plants mitigated Mexican bean beetle densities and damage in one out of five experiments, resulting in a yield increase. In none of the five field experiments were differences detected in predatory arthropod species between thiamethoxam and non-insecticide

treated beans. In summary, the results of this project suggest that non-chemical management methods, such as cultivar selection and planting beans on reflective mulch, can provide effective control of Mexican bean beetle. Thiamethoxam-treated seed may also provide control of this pest, but only within two to three weeks after planting; otherwise, there is typically no effect on beetles, injury or yield. This doctoral research has laid a foundation for an integrated pest management approach for Mexican bean beetle.

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GENERAL AUDIENCE ABSTRACT

Integrated pest management (IPM) is an economical and environmentally-sensible approach to pest management that considers numerous control and decision-making strategies. This dissertation examined non-chemical strategies and the use of an insecticide seed-treatment for management of Mexican bean beetle, a major pest of green beans in the Central Appalachian region of the United States. To develop pertinent research objectives, background information on this pest was gathered from literature sources and personal communications with growers, extension agents and other agricultural professionals. In objective one, Mexican bean beetle preference, developmental success and plant injury were compared among various bean cultivars in field and greenhouse trials. The cultivar 'Dragon's Tongue' was the most suitable host for Mexican bean beetle, and consequently incurred the greatest injury. Growers in high risk areas for Mexican bean beetle may benefit from growing less susceptible cultivars. In objective two, Mexican bean beetle severity and yield were compared among green beans grown on reflective metallized plastic, white plastic, black plastic, and bare soil. Metallized plastic provided the greatest level of control, and resulted in the largest yields. Our results suggest that growing beans on reflective surfaces may be an effective, chemical-free management strategy for Mexican bean beetle. In the final objective, the effects of green beans grown from thiamethoxam (a neonicotinoid insecticide)-coated seeds was examined on Mexican bean beetle severity, non-pest arthropods, and crop performance in greenhouse and field experiments. Bean plants grown from insecticide-coated seeds were highly-toxic to Mexican bean beetle for about 16 days after planting. In one out of five experiments, thiamethoxam-treated plants reduced pest

levels, resulting in increased yields. More often, Mexican bean beetles arrived after the

insecticide had disappeared from plants and there was no effect. There were no detectable

effects from the thiamethoxam treatment on non-pest arthropods in any experiment. Overall,

seed-treatments may provide occasional control of Mexican bean beetle, but non-chemical

methods may be as effective and more practical. This doctoral research project has provided a

foundation for an integrated pest management approach for Mexican bean beetle.

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I would like the thank the coauthors for their help in publishing the two manuscripts that are included in this dissertation. Chapter one, the introduction and literature review, entitled "Natural History, Ecology and Management of the Mexican Bean Beetle (Coleoptera: Coccinellidae) in the U.S." was coauthored by Drs. Thomas P. Kuhar, Galen P. Dively, Peter B. Schultz, and D. Ames Herbert. Chapter three, entitled "Reflective Polyethylene Mulch Reduces Mexican Bean Beetle (Coleoptera: Coccinellidae) Densities and Damage in Snap Beans" was coauthored by Dr. Thomas Kuhar.

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CHAPTER ONE

Introduction and Literature Review

Natural History, Ecology and Management of the Mexican Bean Beetle (Coleoptera: Coccinellidae) in the U.S.

Abstract

Mexican bean beetle, *Epilachna varivestis* Mulsant, is an invasive, phytophagous ladybeetle that has occurred in the U.S. since the late 1800s. In the 1970s, it was a major defoliating pest of soybeans in the eastern U.S., before populations mysteriously crashed. Today, the insect remains a devastating pest of *Phaseolus* species, such as common bean, *P. vulgaris*, and lima bean, *P. lunatus* in geographic locations with moderate summer temperatures and regular rainfall, such as the Mid-Atlantic and southern Appalachian Mountain regions of the United States. Larvae and adults injure plants by consuming leaf tissue, which promotes desiccation and decreases photosynthetic activity. Beetle damage can be successfully mitigated with various insecticides (both conventional and organic), or via augmentative releases of the biological control agent, *Pediobius foveolatus* (Crawford). Various cultural and mechanical management tactics also exhibit management potential; however, more research is necessary to determine specific criteria for effective implementation of these strategies. This paper will review the general biology of Mexican bean beetle, management options to mitigate crop damage, and its historical timeline as a pest in the United States.

Key words: Mexican bean beetle; *Phaseolus*; Coccinellidae; pest management; biology

Introduction

Mexican bean beetle, *Epilachna varivestis* Mulsant, is an aboveground chewing pest of many commercially grown legumes (Fabaceae). Hosts may include tepary beans (*Phaseolus acutifolius*), common beans (*Phaseolus vulgaris*), lima beans (*Phaseolus lunatus*), soybeans (*Glycine max*), alfalfa (*Medicago sativa*), beggarweed (*Desmodium incanum*) and cowpea (*Vigna unguiculata*); however, this insect survives and reproduces most successfully on bean species in the genus *Phaseolus* (Friend and Turner 1931, Bernhardt and Shepard 1978). The native range of Mexican bean beetle is thought to be in the high elevations of western Mexico and Central America, though the exact distribution is unknown (Howard and English 1924). The current range includes most of the United States and southern Canada (Marcovitch and Stanley 1930, Nicholas and Kogan 1972, Fess 2008).   Damaging populations are most common in the Mid-Atlantic and southern Appalachian Mountain regions of the United States (Nottingham and Kuhar 2013, 2014 a), due to moderate summer temperatures (high, day-time temperatures between 25 and 29.5°C [77 and 85°F]) and regular summer rainfall (Marcovitch and Stanley 1930, Sweetman 1932).

Beetles overwinter as adults and emerge in the late spring or early summer to feed and mate (Friend and Turner 1931, Howard 1941; Figure 1.1). Oviposition may occur from spring until fall (Howard and English 1924). All lifestages of this insect occur within the canopy of host plants, making this pest easy to find and identify. Larvae and adults feed primarily on the soft leaf tissue of hosts plants, resulting in leaves that appear lacy and skeletonized (Howard 1941; Figure 1.2). Larvae and adults also feed on pods as a secondary option (Howard 1924). Although past documents suggest that pod damage occurs after plants are severely defoliated (Howard and English 1924, Capinera 2001), our observations in Virginia indicate that it is fairly

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common for beetles to damage pods, even while leaf matter is available (Figure 1.3) (L. B. Nottingham, unpublished data).

Description and Life Cycle.

Adult. Like many coccinellids, Mexican bean beetle adults have a round body shape, a concealed head, and black dorsal spots. Immediately following eclosion from the pupal stage, the visible cuticle of the adult is bright yellow and often without spots. Spots generally develop within minutes or hours, and the cuticle will gradually darken to a copper color within two to three weeks (Figure 1.4; Friend and Turner 1931). Each elytron has eight black spots in three horizontal rows. Adults are 6-8 mm (0.24-0.31 in) long and 4-6 mm (0.16-0.24 in) wide; though size varies based on their diet and sex (Friend and Turner 1931). Males are usually smaller than females, and can be distinguished by a notch at the end of the last abdominal segment (Figure 1.5) (Capinera 2001). Adults can walk and fly, but often remain in one location once they have found a suitable host. Adults spend much of their time feeding and mating within the plant canopy; however, adults can fly long distances to find host plants after overwintering, disperse when populations become crowded, or when locating overwintering sites (Howard and English 1924, Auclair 1959).

Egg. Eggs are light yellow when first deposited (Figure 1.1), but darken when they are close to hatching (Capinera 2001). They are 1.2 mm (0.05 in) long and 0.6 mm (0.02 in) wide. Clusters of 30-70 eggs are deposited on the undersides of leaves to avoid direct sunlight, which increases egg mortality (Howard and English 1924, Miller 1930, Barrigossi 1997). Eggs typically hatch within seven days (Capinera 2001). Eggs can hatch sooner as temperatures increase; however, total hatch is generally reduced in warmer conditions (Howard and English 1924).

Larva. Larvae are cylindrical and soft bodied. The cuticle is yellow and covered in spines that are either black, or yellow with black tips (Figure 1.6). Mexican bean beetle larvae are stout and sluggish, compared with the larvae of predatory lady beetles. They generally remain on the undersides of leaves where they continuously feed on leaf tissue (Friend and Turner 1931). Larvae undergo four instars in approximately twenty days, growing from 1.5 mm (0.06 in) to 8.5 mm (0.33 in) long. First instars develop in four to six days; second instars in two to four days; third instars in three to five days; and fourth instars in six to ten days (Friend and Turner 1931).

Pupa. Pupae are similar in general appearance to larvae; however, at this stage, the beetle attaches to a plant by its posterior end and becomes immobile (Friend and Turner 1931). Pupae are often found aggregated on a single leaf (Howard and English 1924) in the lower half of the plant canopy (Figure 1.7). Within the first couple of days as a pupa, the spiny cuticle turns pale and recedes to the posterior end of the insect. The rest of the pupa is yellow and relatively smooth. Pupation lasts about nine days (Friend and Turner 1931, Capinera 2001).

Overwintering. Mexican bean beetle overwinters as an adult and emerges in the late spring to early summer, after 238-277 degree days, baseline temperature: 10°C [50°F]; (Fess 2008). Higher levels of precipitation during overwintering months can increase survival and emergence of overwintering adults (Douglass 1933, Auclair 1959). Beetles generally overwinter in groups, and in areas with well-draining soils and leaf cover (Howard and English 1924, Friend and Turner 1931). Well-drained leaf litter such as pine needles were shown to provide the highest quality substrate for beetle survival, rather than open fields or under crop debris (Thomas 1924).

Feeding and Injury

Adults and larvae feed on plant tissue with chewing mouthparts; however, Howard (1941) described the mechanism as being unique, and more similar to the rasping/sucking technique used by thrips. Beetles use their mandibles to scrape the leaf surface, piling plant tissue together. The mandibles then compress the dislodged tissue, extracting plant juices. Plant juices are ingested, while solid matter is discarded.

The majority of feeding injury occurs from third and fourth instars (McAvoy and Smith 1979). Beetles generally feed on the lower leaf surface while avoiding veins, creating a lacy, skeletonized appearance of the remaining leaf (Howard 1941; Figure 1.2). Foliar feeding injury results in decreased photosynthetic activity and desiccation of the plant (Peterson et al. 1998). Though beetles feed primarily on the foliage, they will also feed on pods and flowers once they become present (Howard and English 1924, Capinera 2001). Even minor pod feeding can render the fruit unmarketable, as well as increasing opportunity for plant pathogens entry (Krupke et al. 2015).

When other environmental conditions are favorable (particularly adequate rainfall), beans can easily tolerate a fair amount of pest injury (Haile et al. 1998). Snap beans and soybeans can withstand 45-80% defoliation at vegetative stages, and 20-60% at flowering and pod fill stages before yields decrease (Capinera et al. 1987, Schaafsma and Ablett 1994, Haile et al. 1998). Unfortunately, Mexican bean beetle also prevails under moist conditions. Consistent rainfall increases survivorship of all life-stages (Marcovitch and Stanley 1930, Miller 1930, Sweetman 1932, Kitayama et al. 1979, Wilson et al. 1982) as well as larval feeding (McAvoy and Smith 1979).

Because environmental conditions can affect the fitness of both pest and host, economic injury levels and treatment recommendations can be dubious, or even misleading. For instance, while beans may be capable of tolerating more foliar feeding in high precipitation years, densities of Mexican bean beetle may be higher under those conditions as well. Moreover, larger pest populations are more likely to damage pods. Also, the larger the pest population, the higher the number of individuals that will go into overwintering and emerge the following spring. During dry conditions, Mexican bean beetle populations may stay below threshold levels; however, beans will be more sensitive to desiccation from feeding injury. For these reasons, it is important to consider the weather when making pest management decisions. See section "Management" (Chemical) for thresholds and treatment recommendations.

Colonization of Host Plants. In the eastern United States, spring-planted snap beans (common beans sold for fresh market consumption) and lima beans are typically already growing when beetles emerge from overwintering, allowing beetles to move directly into these crops (Fess 2008, Gatton 2008). If preferred hosts are not initially available, emerging beetles may occupy less preferred legumes, such as kudzu, *Pueraria lobate,* alfalfa, *Medicago sativa,* cowpea, *Vigna unguiculata,* and beggarweed, *Desmodium incanum* (Howard 1924, Friend and Turner 1931, Barrigossi 1997). Overwintered adults are capable of flying long distances to find host plants (Auclair 1959). Once a host is located, females will feed for approximately 12 days before depositing their eggs (Bernhardt and Shepard 1979). Females lay eggs throughout the season, producing between 500-1200 eggs per female (Friend and Turner 1931, Sweetman 1932, Capinera 2001).

Snap beans are usually harvested in mid to late summer, at which point beetles may move into less preferred crops (often soybeans) if snap or lima beans are no longer available. In some

cases, especially in cool and wet conditions, beetles may cause economic damage to these secondary hosts (Howard and English 1924, Wilson et al. 1982).

Susceptibility and Resistance among Host Plants. Like most herbivorous insects, Mexican bean beetle experiences variable developmental and reproductive success when feeding on different host plants. Snap beans (stingless common beans) and lima beans are the most *susceptible* host species of Mexican bean beetle (tepary bean, *Phaseolus acutifolius*, is also highly susceptible; but is rarely grown commercially in the eastern United States) (Friend and Turner 1931). Plants of these species are likely to experience significant feeding damage because they provide optimal nutrition and physical habitat, while lacking strong defensive qualities. Cultivars such as 'Spartan arrow' snap bean, 'Jackson Wonder' lima, and nearly all "wax" bean cultivars (snap beans with yellow pods) are among the most susceptible to Mexican bean beetle (Campbell and Brett 1966, Raina et al. 1978).

Cultivars such as 'Regal' snap bean, 'Idaho Refugee' snap bean, 'Baby 'Fordhook'' lima bean, and 'Baby White' lima bean exhibit high levels of *resistance* to Mexican bean beetle feeding and development. When raised on these cultivars, beetles consumed less plant matter, gained less weight and laid fewer eggs, when compared with other cultivars (Campbell and Brett 1966, Raina et al. 1978). Overall, wax bean cultivars rank among the most susceptible, while lima bean cultivars often exhibit resistance to Mexican bean beetle (Campbell and Brett 1966, Raina et al. 1978). Cultivar trials in Virginia agree with similar findings, in which wax cultivars incur greater beetle densities and damage than lima beans and green snap beans (Nottingham 2014; Figure 1.8).

Host plant physiology plays an important role in their resistance and susceptibility to Mexican bean beetle. Internal sugar concentrations are known to dictate how attractive plants

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are to their attackers. Bean plants with greater concentrations of sucrose and fructose are shown

to be more attractive to Mexican bean beetle (Augustine et al. 1964). Among the legumes

(Fabaceae), *Phaseolus* beans tend to contain the highest concentration of these sugars, which

may explain, at least partially, why they are the most preferred hosts of Mexican bean beetle

(LaPidus et al. 1963, Augustine et al. 1964).

Certain physiological components of host plants can aid in defense against Mexican bean

beetle. Most beans, and many other plant groups, contain chemical compounds called

cyanogenic glycosides (Vetter 1999). Although these chemicals are sugar compounds, they play

an important role in a host plant's ability to deter insect pests, including Mexican bean beetle

(Ballhorn and Lieberei 2006). In response to pest feeding, cyanogenic glycosides are converted

into toxic hydrogen cyanide, which can either poison the attacker, or at least halt feeding

(Gleadow and Woodrow 2002, Ballhorn et al. 2009). Because cyanogenic glycosides occur at

varying concentrations in most *Phaseolus* beans, some varieties are well protected from attack,

while others remain susceptible (Nayar and Fraenkel 1963, Ballhorn and Lieberei 2006). For

instance, cyanogenic glycosides are more likely to occur at significant levels in lima beans than

snap beans, which may explain why many lima beans, especially wild varieties, exhibit

resistance to Mexican bean beetle (Viehoever 1940). Interestingly, at low concentrations, these

compounds are not poisonous, and actually stimulate Mexican bean beetle feeding (Nayar and

Fraenkel 1963).

<div align="center">**Management.**</div>

Cultural. Cultural pest management is the manipulation of an agricultural system,

without the use of chemical inputs, in order to prevent or interfere with a pest's ability to damage

the crop(s) (Ferro 2013). Examples include crop-rotation, trap cropping, using resistant plant

cultivars, and adjusting planting dates. From an integrated pest management (IPM) stand-point, cultural practices should be the first line of defense for managing insect pests (Groves 2014). Developing cultural management strategies requires a thorough understanding of the biology of pests and their hosts.

Cultural management strategies for Mexican bean beetle are understudied and deserve greater consideration. Grower surveys convey that Mexican bean beetle is a greater challenge in pesticide-free operations, and therefore, the continued pursuit of effective cultural strategies should be a top priority (Nottingham and Kuhar 2014 a).

Timed planting. Planting beans early in the spring, or late in the summer, can mitigate Mexican bean beetle damage (Howard and English 1924, Thomas 1924). This method encompasses various mechanisms. Overwintering Mexican bean beetles normally emerge early in the summer, after 238-277 degree days (baseline temperature: 10°C [50°F]) (Fess 2008), and remain active until early fall, when they begin searching for overwintering habitat. Planting bean crops as early as possible, or as late as possible, can reduce the overlap of crop development and peak beetle activity (Howard and English 1924). Also, most life-stages of Mexican bean beetle consume less foliage and develop slower at temperatures between 20 to 24°C (68 to 75°F), usually occurring in the late spring and early fall (McAvoy and Smith 1979, Fan et al. 1992).

There are two potential drawbacks to this method. First, planting beans when soil and air temperatures are still cool often results in slower germination and smaller stands (Relf and McDaniel 2015). Second, planting early or late increases the risk that crops may get exposed to frost, which can damage or kill plants (Reiter et al. 2014).

Trap Crops. Rust (1977) tested the efficacy of trap cropping for Mexican bean beetle. This study reported control in systems where snap beans were used as trap crop for adjacent

soybeans. However, there were no successful attempts to mitigate damage to snap or lima beans using trap crops. There is no evidence, to date, suggesting that trap crop methods can protect *Phaseolus* beans from Mexican bean beetle.

Resistant Crops. The work of Campbell and Brett (1966) and Raina et al. (1978) suggests that Mexican bean beetle damage may be reduced by simply growing more resistant bean cultivars, such as 'Regal' snap bean, 'Idaho Refugee' snap bean, 'Baby 'Fordhook'' lima bean, and 'Baby White' lima bean, and/or avoiding susceptible cultivars, such as 'Spartan arrow' snap bean, 'Jackson Wonder' lima bean, and nearly all "wax" bean cultivars.

Staggered Planting Dates. Mexican bean beetle management using staggered plantings of snap beans was explored by Fess (2008). This method attempts to contain beetle populations to plants from one planting group, while the rest of the crop is ignored. Though no consistent level of control was detected throughout this experiment, certain plantings in the staggered treatments did exhibit fewer Mexican bean beetle, as well as reduced damage. Staggered planting may in fact be a viable management strategy for Mexican bean beetle, but more research is needed to obtain specific implementation guidelines.

Physical/Mechanical. *Row Covers*. Fess (2008) found that polyester row covers successfully reduced abundance of Mexican bean beetle adults, larvae and pupae on snap beans in West Virginia. Many smaller farms use this method; however, some downfalls include increased labor, materials and the risk of excluding beneficial insects.

Reflective Plastic Mulch. Planting beans on plastic (polyethylene) mulches that reflect sunlight (specifically, metalized and white colored plastic) has shown potential as a cultural strategy to mitigate damage from Mexican bean beetle (Nottingham and Kuhar 2015). Mexican bean beetle adults and larvae are deterred by direct light (Howard and English 1924, Miller

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1930), and are less likely to survive when forced to remain in direct light (Howard and English 1924). Metalized (reflective silver top and black bottom) and white plastic mulches can significantly increase shortwave light intensity (300-1100nm), even on cloudy days (Ham et al. 1993). Field experiments at Virginia Tech have shown that Mexican bean beetle are less likely to colonize and deposit eggs on beans planted on metalized and white plastic mulches, compared to bare ground and black plastic (Nottingham and Kuhar 2015). Beans planted on metalized and white plastic mulches also had less foliar damage (Figure 1.9), less pod damage, and significantly greater yields than beans grown on black plastic and bare ground (Nottingham and Kuhar 2015).

Mechanical Removal. Small farms and gardens commonly use mechanical (by-hand) removal to reduce injury from Mexican bean beetle (Nottingham and Kuhar 2014 a). Because beetles complete their entire life cycle within the crop canopy, this simple strategy can adequately protect crops. The feasibility of this tactic, however, is highly reliant on the scale of the operation and the amount of labor available.

Biological Control. *Predators.* Mexican bean beetles are well protected from predatory organisms. In addition to the spines that adorn the larvae (Figure 1.6), larvae, pupae and adults all produce toxic, alkaloid secretions that are known to deter many arthropod predators upon contact (Happ and Eisner 1961, Eisner et al. 1986, Attygalle et al. 1993, Eisner and Meinwald 1995).

Although many arthropods are known to prey upon Mexican bean beetle, few native predators have proven effective at reducing population levels of this pest (Howard and Landis 1936). The most common predators of Mexican bean beetle include predatory stink bugs such as *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) (Waddill and Shepard 1975) and

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ladybeetles (Coleoptera: Coccinellidae). Others predators are found in the families Anthocoridae

(Hemiptera), Nabidae (Hemiptera), and Chrysopidae (Neuroptera) (Howard and Landis 1936).

In Virginia, we commonly observed ladybeetles feeding on Mexican bean beetle eggs,

while hemipteran predators, especially *Podisus maculiventris* (Say), were more likely to feed on

larvae and adults (Figure 1.10; L. B. Nottingham, unpublished data). To further explore this

predator-prey complex, numerous predator arthropods were brought to the laboratory and offered

different life-stages of Mexican bean beetle. Chewing predators, including ladybeetles and

ground beetles only fed on eggs, while ignoring other life-stages. Piercing-sucking predators,

including damsel bugs (Hemiptera: Nabidae), assassin bugs (Hemiptera: Reduviidae) and stink

bugs, readily fed upon all life-stages (L. B. Nottingham, unpublished data). These observations

suggest that piercing-sucking predators may be less sensitive to the chemical defenses of

Mexican bean beetle than chewing predators.

Parasitoids. Several native, North American tachinid flies are known to parasitize

Mexican bean beetle adults (Howard and Landis 1936). One notable species that occurs

throughout the eastern United States is *Phorocera claripennis* Macquart (Diptera: Tachinidae)

(Howard and Landis 1936). Unfortunately, these flies occur in densities that are too low to

naturally limit Mexican bean beetle populations; parasitism levels only range from one to three

percent (Howard and Landis 1936).

Landis and Howard (1940) discussed another tachinid species, *Paradexodes epilachnae*

Aldrich, which is known to parasitize Mexican bean beetle at a rate of 16 - 54 % in its native

range of central Mexico. Because of its importance in the native range, this parasitoid was

reared and released in 19 U. S. states from 1930-1935. Though the fly successfully reduced local

populations of Mexican bean beetle and dispersed, no individuals were able to survive winters in

the eastern United States (Landis and Howard 1940). This classical biological control initiative was eventually abandoned.

The most successful classical biological control method for control of Mexican bean beetle utilizes the eulophid wasp, *Pediobius foveolatus* (Crawford) (Hymenoptera: Eulophidae; Figure 1.11) (Stevens et al. 1975, Fess 2008).   This parasitoid was discovered in India, and is native to most of southern Asia and Japan (Angalet et al. 1968). Initial screenings of this wasp showed that it was unable to harm native coccinellids other than Mexican bean beetle and Squash beetle, *Epilachna borealis* (Fabricius) (Angalet et al. 1968, Schaefer et al 1983). Female wasps lay around 20 eggs in a single beetle larva, from which most will emerge as adults (Stoner 2002). Adult wasps then mate and search for more beetle larvae.

In their native range, *P. foveolatus* overwinters in host larvae (Ghani and Mohyuddin 1982) or does not overwinter at all due to the lack of a cold season. In the United States, however, *P. foveolatus* cannot survive cold winter months because all North American *Epilachna* hosts overwinter as adults, not larvae (Schaefer et al. 1983). Therefore, these wasps must be released annually in the United States in order to provide control of Mexican bean beetle (Stevens et al. 1975).

To successfully manage Mexican bean beetle using *P. foveolatus*, it is crucial to properly schedule the release. Ideally, wasps should be released at both one and two weeks after first instar beetles are discovered in beans (Stoner 2002). Accurate scouting and timing of release is necessary because wasps reproduce most successfully within third and fourth instar larvae (Angalet et al. 1968); so it is imperative that those Mexican bean beetle instars are present at wasp release. It is also important not to release wasps when it is raining or cold, as they are not well adapted to these conditions (Stoner 2002). Release wasps at a rate of 1000 wasps (or 50

mummies) per 3600 square feet of beans (Stoner 2002). Successful parasitism and emergence of

the next generation of wasps can be visibly monitored by the presence of dark-brown, dead

Mexican bean beetle larvae (mummies) with one small hole, from where adult wasps exited

(Figure 1.12) (Stoner 2002).

When considering an augmentative release of *P. foveolatus*, it is important to remember

that wasps must be ordered from a vendor, and that delivery may take anywhere from one day to

three weeks. Once the eggs of Mexican bean beetle are first discovered, the grower should

contact vendors for expected delivery times. *P. foveolatus* wasps can be purchased from the

New Jersey Department of Agriculture and other commercial insectaries.

Chemical Control. In the 1960s and 1970s, Mexican bean beetle was effectively

controlled with foliar-spray applications or in-furrow systemic applications of many of the

organophosphates, carbamates, or chlorinated hydrocarbons registered at the time (Webster and

Smith 1962, Judge et al. 1970). Today, very few of these insecticides are registered for use on

edible beans. However, numerous other insecticides including pyrethroids, neonicotinoids, and

combination insecticide products provide effective control of this pest when applied as a foliar

spray (Nault and Speese 2001, Kuhar et al. 2012). The pyrethroid bifenthrin was shown to be

more efficacious than the organophosphate acephate or the carbamates methomyl and carbaryl at

reducing beetle damage to snap bean pods in Virginia (Nault and Speese 2001). In a recent

insecticide efficacy test in which soybeans were sprayed with various insecticides in the field and

then leaves were excised and exposed to Mexican bean beetle adults, several insecticides

including the pyrethroids bifenthrin, lambda-cyhalothrin, zeta cypermethrin as well as acephate,

methomyl, and combination products containing lambda-cyhalothrin plus the neonicotinoid

thiamethoxam or beta cyfluthrin plus imidacloprid, all provided up to 90% control for 10 days

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after application (Kuhar, unpublished data). Patton et al. (2003) evaluated a number of organic

insecticides, and showed that azadirachtins, pyrethrins, and spinosad all provided significant

control of Mexican bean beetle compared to an untreated control.

The economic threshold for this pest can vary greatly depending on environmental

conditions (temperature, amount of rainfall and/or the use of irrigation), time of year, host

species or cultivar, plant maturity, etc. Guidelines for pesticide use found in state extension

publications provide current and detailed information explaining both when to spray, what to

spray, and how to safely apply these insecticides. Most current recommendations suggest

treating snap beans at 20% defoliation pre-bloom, and 10% at bloom to pod stage (Flood and

Wyman 2005, Reiter et al. 2014). Soybeans should be treated at 40% defoliation pre-bloom, and

at 15% bloom to pod stage (Krupke et al. 2015).

Historical Perspective

Native Range and Spread. Mexican bean beetle was first described by Mulsant (1850) from

specimens in Mexico. The original name was *Epilachna corrupta*, but it was later changed to

Epilachna varivestis. The native range of this beetle is thought to be a region of western Mexico

known as "The Plateau", referring to its high elevation of 1,219- 2,438 m (4,000-8,000 ft)

(Friend and Turner 1931). Unlike the hot and dry climate that is characteristic to a large portion

of Mexico, the Plateau region is more cool and moist. Daily high temperatures in the summer

average around 25°C (77°F), and summer rainfall averages 40 cm (16 in) (Marcovitch and

Stanley 1930). The exact parameters and extent of Mexican bean beetle's native range are

unknown, but it is possible that its original territory stretched across Central America and into

the Andes Mountains of South America, where there is a great diversity of *Epilachna* species

(Howard and English 1924, Gordon 1975).

The earliest records of Mexican bean beetle in the United States date back to 1864. It was first recognized as an economic pest in 1883, when severe damage to wax beans (cultivars of common beans that are yellow and stingless) was reported in Colorado (Chittenden 1919). The first sighting of this pest east of Texas was in 1918, near Birmingham, Alabama (Thomas 1924). It is assumed that beetles were transported there, from the Southwest, in cut alfalfa (Friend and Turner 1931). By the late 1920s the beetle spread north to southern Canada, and west to Michigan (Harding 1933).

Mexican bean beetle populations are currently found throughout the United States; however, economic populations are most common in the Mid-Atlantic and southern Appalachian Mountain regions (Nottingham and Kuhar 2014). This beetle is greatly limited by summer rainfall, humidity and temperature (Marcovitch and Stanley 1930, Sweetman 1932, Kitayama et al. 1979, Wilson et al. 1982, Mellors and Bassow 1983). The regions where Mexican bean beetle is most severe are characterized by summers with regular rainfall, high humidity and daily high temperatures ranging from 25 to 29.5°C (77-85°F) (Marcovitch and Stanley 1930, Sweetman 1932, Fess 2008, Nottingham and Kuhar 2014). Though this beetle is also found in the Southwest and Great Plains, populations in these regions are mostly limited to irrigated croplands, and rarely reach economic levels (Barrigossi 1997).

Fluctuations in Pest Status. Mexican bean beetle received little attention until the mid-1920s, when it became well established in all but seven states east of the Mississippi River (Tissot 1943). The subsequent torrent of scientific publications on this beetle is indicative of its breakthrough into economic importance in the 1920s and 1930s. For example, the Bibliography of American Economic Entomology lists 213 publications referencing Mexican bean beetle from the years 1930-1939 (Tissot 1943).

Friend and Turner (1931) performed damage trials on numerous legumes to determine which were most susceptible to beetle feeding, and found that only *Phaseolus* species were severely damaged. Though this study suggests that Mexican bean beetle was only economically threatening to *Phaseolus* crops, some growers from southeastern states reported Mexican bean beetle infesting and damaging soybean crops (Howard and English 1924). This was said to occur in situations where populations of Mexican bean beetle were allowed to grow very large within snap beans, then populations would migrate to adjacent soybean fields after snap beans were destroyed (Howard and English 1924). It is unclear whether it was a true threat to soybeans, or any crops other than snap bean, dry bean and lima bean, during the 1920-1940 time-period.

By the 1940s and into the 1950s, there was a substantial decrease in the number of Mexican bean beetle publications (Nichols and Kogan 1972), which suggests that there was a decline in this pest's occurrence and severity. Whether populations actually subsided is undocumented.

In the mid-1960s, the beetle once again emerged as a major pest, with economic damage reported throughout most agricultural regions of the eastern United States. This resurgence was unique, in that Mexican bean beetle was also causing widespread damage to soybean, as well as *Phaseolus* bean species. The beetle was also reported outside of its normal range, reaching the Piedmont and Coastal Plain regions of Virginia, North Carolina and South Carolina, as well as mid-western states including Indiana and Ohio (Nichols and Kogan 1972, Hallman et al. 1977, Hammond 1984, Metterhouse et al. 1989).   This pest once again became a prominent subject in research publications by the late-1960s (Nichols and Kogan 1972), and through to the mid-1980s (Nottingham and Kuhar 2014). Also, due to its conspicuous nature in the field, and suitability

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for laboratory rearing and experimentation, Mexican bean beetle gained considerable popularity among various groups of scientists as a practical research specimen (Nichols and Kogan 1972).

1979 to 1981 was the height of Mexican bean beetle pest pressure in United States history, especially for soybean (Metterhouse et al. 1989, Hudson et al. 2013; G. P. Dively, unpublished data). Nearly every soybean field in the Delmarva region of Virginia, Delaware and Maryland was severely damaged (G. P. Dively, personal communication). However, by the mid-1980s, occurrences of Mexican bean beetle declined, especially in soybean (Hudson et al. 2013). To date, Mexican bean beetle is rarely a pest of any legume species outside of the genus *Phaseolus*. Even in that host, Mexican bean beetle is generally only a concern in smaller, organic operations (Nottingham and Kuhar 2014).

Cause for Pest Decline. The cause of Mexican bean beetle's decline is still undetermined. Some resolve that classical biological control is responsible for the suppression of this pests. *Pediobius foveolatus* has been used since the 1960s to control Mexican bean beetle, and was released in mass quantities across multiple Mid-Atlantic states during the 1960s and 1970s (*P. foveolatus* is still released annually by the state of New Jersey).

Climate change is also a common theory for the decline. Compared to century averages, the weather during Mexican bean beetle's peak in the 1960s and 1970s was considerably cooler, and with more precipitation (NOAA-CAG 2014, NOAA-DATM 2014). Because cool, wet weather generally aides beetle survival, climate may have been the key factor that permitted beetles to flourish and attack the less suitable host, soybean, during this time-period. These two theories are explored below.

Parasitoid (*Pediobius foveolatus*) Releases. Beginning in 1966, *P. foveolatus*, a parasitoid wasp originally discovered in India, was imported to the United States to be tested for

potential control of Mexican bean beetle (Angalet et al. 1968). Initial testing determined that *P. foveolatus* would readily parasitize the larvae of Mexican bean beetle, while leaving native, predatory coccinellids unharmed (Angalet et al. 1968). In 1972, Maryland, then other states, began releasing these wasps to control Mexican bean beetle (Schaefer et al. 1983). USDA branches in New Jersey, Maryland, Delaware and Virginia released wasps throughout these states, focusing on areas with large soybean acreage and high Mexican bean beetle populations (Reichelderfer 1979).

Inoculative releases yielded positive results; parasitism rates of 80 to 100% of Mexican bean beetle larvae were commonly documented near release sites (Stevens et al. 1975, Barrows and Hooker 1981). However, Steven et al. (1975) also reported slow parasitoid population dispersal from these sites. Also, *P. foveolatus* cannot overwinter in the United States due to cold winters and the lack of an overwintering host (Schaefer et al. 1983). In the wasps' native territory, the weather either is conducive for year-round exposure, or wasps overwinter in their hosts, which overwinter as larvae. Because Mexican bean beetle overwinter as adults, wasps are without adequate winter refuge in the United States (Schaefer et al. 1983). Because *P. foveolatus* can neither overwinter successfully nor spread rapidly, management with this wasp requires yearly releases in more locations than is practical for widespread control of Mexican bean beetle.

By the mid-1980s, all states except New Jersey had discontinued state-run releases of *P. foveolatus* (G. P. Dively and P. Schultz, personal communication). At this time, pest pressure from Mexican bean beetle began its sharp decline as well, especially in soybean (Hudson et al. 2013; G. P. Dively, unpublished data).

The short-comings of this biological control agent make it an unlikely cause for the wide-spread decline of Mexican bean beetle in the eastern United States. None-the-less, annual

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releases of *P. foveolatus* are still carried out by the State of New Jersey, as well as many individual farmers and gardeners, and can be a very effective tool for localized management (Fess 2008, Hudson et al. 2013).

Climate Change. Climate shifts are likely a major cause of Mexican bean beetle's fluctuating severity. Like most insects, this beetle's ability to feed and thrive is heavily dependent on temperature and moisture. Numerous studies describe the relative inability of this insect to tolerate temperatures increasing beyond 30°C (80°F) with low relative moisture (Marcovitch and Stanley 1930, Miller 1930, Sweetman 1932, Kitayama et al. 1970, Wilson et al. 1982, Mellors and Bassow 1983, Mellors et al. 1984). Wilson et al. (1982) also witnessed that beetles were only able to feed and survive on soybean under temperature and humidity conditions that were considered to be optimal.

Marcovitch and Stanley (1930) developed a climatic index that accurately predicts the potential for Mexican bean beetle pest pressure for specific locations (assuming the presence of plant hosts) based on temperature and rainfall. The formula reads:

$$L \times \left(\frac{L}{2}\right) \times \left(\frac{100}{R}\right)^2$$

where L = the successive number of days above 32.2°C (90°F) and R = total rainfall (inches) from June through September. Localities that consistently produce low values (below 2000) are considered "optimal" for Mexican bean beetle. The model works on a continuous scale; so increasing index values suggest relative decreases in habitability for the beetle. For instance, Norfolk, VA produced an index level of 284 in 1925, when the area experienced severe Mexican bean beetle outbreaks in local host crops; meanwhile, locations in western Tennessee had values around 15,000, and little to no beetle pressure observed (Marcovitch and Stanley 1930).

From 1950 to 1987, the University of Maryland rated Mexican bean beetle damage in soybean crops in Maryland, Delaware and Virginia, and calculated climate index values using Marcovitch and Stanley's (1930) formula. Their findings show cool and wet weather persisting throughout the 1960s and 1970s, compared to the overall average, and that Mexican bean beetle damage to soybean was most severe during this time (Nottingham and Kuhar 2014 a, G.P. Dively, unpublished data). Historical weather data from the National Oceanic and Atmospheric Administration (NOAA) validate the University of Maryland's weather data. Temperatures across most of the eastern United States were below the century average in both the 1960s and 1970s (NOAA-DATM 2014). Furthermore, the Palmer drought severity index for Virginia depicts consistently high climatic moisture levels during most of the 1970s, when pest pressure reached all-time highs (NOAA-CAG 2014). After 1980, hotter and dryer weather returned, and Mexican bean beetle severity decreased (Nottingham and Kuhar 2014 a, G. P. Dively, unpublished data).

Concluding Remarks

Recognition of Mexican bean beetle as a significant agricultural pest has greatly decreased over the past thirty years; yet it remains a devastating pest to edible bean crops throughout large portions of the United States. Within its range, outbreaks reach economic levels on an annual basis, especially for snap bean growers who attempt to minimize, or forgo, the use of chemical insecticides (Nottingham and Kuhar 2014 a). Mexican bean beetle is easily controlled with insecticides in conventional bean production, but little effort has been made to study progressive management strategies. The authors of this manuscript hope this profile on Mexican bean beetle will provide the necessary information to aide in, and encourage, the development of an integrated pest management approach to controlling this pest.

Research Objectives

Developing agricultural IPM programs that are effective and feasible is a difficult task, partially because of how much individual systems differ. Even within a single location, seasonal changes in weather, crop cultivar, farming practices, neighboring habitats, etc. can have significant effects on pests and the performance of management tactics. The steps leading to our experimental methods were as follows: 1. determine the overall impact of this Mexican bean beetle and needs of growers; 2. gain a thorough, biological understanding of the pest(s) and host plants for a region; and 3. evaluating management strategies that are relevant and feasible, based on the information gained in steps one and two. Step one was achieved by conducting surveys of growers, extension agents and other agricultural professionals at conferences, extension events, and via online vessels, such as emailing extension agents, listservs (ECOLOG-L and ENTOMO-L), and posting on various Facebook pages. Step two involved reviewing and compiling all available literature on Mexican bean beetle and related topics into a cohesive review article (Nottingham et al. 2016, see Chapter One). Using information from the literature combined with grower interview responses, these three research objectives were developed:

1. Evaluating Mexican bean beetle host choice, developmental success, and feeding injury levels among snap and lima beans cultivars commonly grown in the eastern United States.

2. Evaluating the effects of planting snap beans on a highly reflective ('metallized') polyethylene mulch on Mexican bean beetle densies and feeding injury, densities of predatory arthropods, snap bean yield, and yield from lettuce planted into the used plastic mulch (double-crop).

3. Examining the effects of snap bean plants grown from thiamethoxam-treated seeds on toxicity to Mexican bean beetle and *Podisus Maculiventris* (a common predator of Mexican bean beetle), Mexican bean beetle densities, densities of arthropods other than Mexican bean beetle (such as predators and non-pest herbivores), and crop performance factors such as NDVI (normalized difference vegetative index) and yield.

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Figures

Figure 1.1. Mexican bean beetle overwintering adult (right) and eggs (left) on a snap bean leaf. (Photo by L. B. Nottingham)

Figure 1.2. Skeletonized snap bean leaf from Mexican bean beetle feeding. (Photo by L. B. Nottingham)

Figure 1.3. Mexican bean beetle larvae feeding on snap bean pod, despite availability of sufficient leaf matter. (Photo by L. B. Nottingham)

Figure 1.4. Mexican bean beetle adults form spots and darken with age. Youngest (far left) to oldest (far right). (Photos by L. B. Nottingham)

Figure 1.5. Sex distinction among adults. Male (on right with arrow) exhibits a small, concave notch on the posterior end of the abdomen. Notch is not present on the female (left). (Photo by L. B. Nottingham)

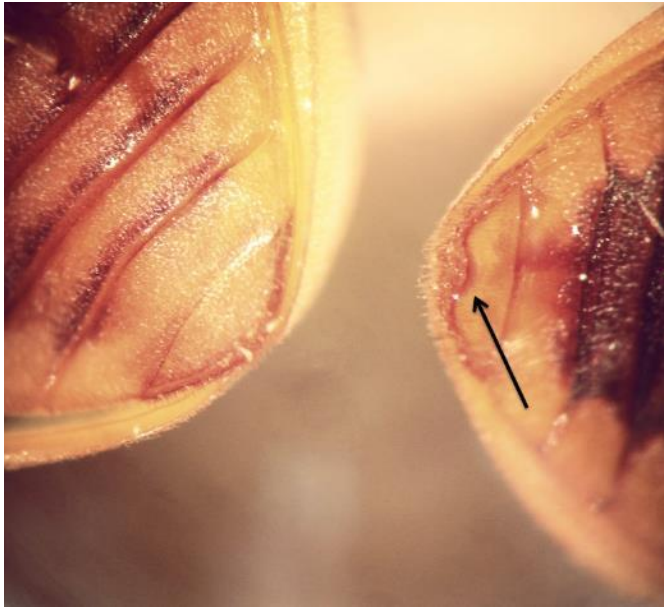**Figure 1.6.** Mexican bean beetle larva. (Photo by Taliaferro Trope)

Figure 1.7. Mexican bean beetle pupae in aggregation. (Photo by L. B. Nottingham)

Figure 1.8. Susceptible vs tolerant host beans. Wax snap bean cultivar 'Dragon's Tongue' (bottom right plot) showing more visible injury than lima bean cultivar 'Fordhook' and non-wax, snap bean cultivars (above plots). (Photo by L. B. Nottingham)

Figure 1.9. Planting bean on reflective polyethylene mulch may be an effective cultural management strategy for Mexican bean beetle. Greater levels of snap bean injury were detected on beans grown on bare ground (left) and black plastic (middle) than metallic plastic (right) and white plastic (not pictured). (Photos by L. B. Nottingham)

Figure 1.10. Predators of Mexican bean beetle. Predatory stink bug nymph (Hemiptera: Pentatomidae) (left) feeding on Mexican bean beetle adult. Ladybird beetle larva (Coleoptera: Coccinellidae) (right) feeding on Mexican bean beetle eggs. (Photos by L. B. Nottingham)

Figure 1.11. *Pediobius foveolatus* (Hymenoptera: Eulophidae) adult. An exotic, parasitoid wasp of the Mexican bean beetle. (Photo by L. B. Nottingham)
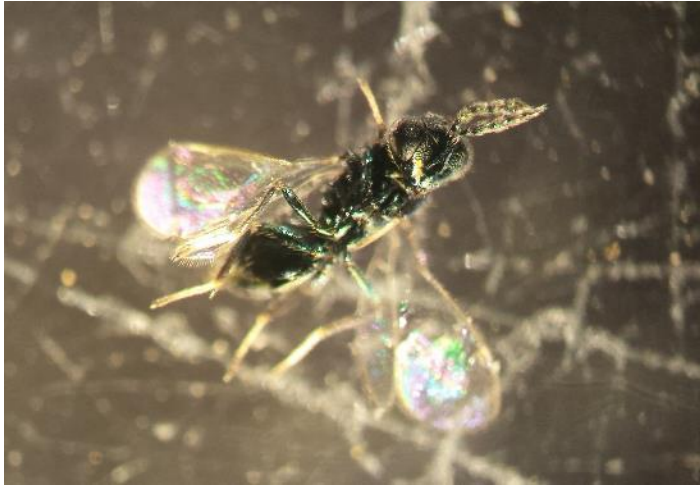

Figure 1.12. Mexican bean beetle larva, parasitized by Pediobius foveolatus. (Photo by L. B. Nottingham)

CHAPTER TWO

Evaluation of Mexican Bean Beetle Host Choice, Developmental Success, and Feeding Injury Among Snap Bean and Lima Bean Cultivars

Abstract

Mexican bean beetle, *Epilachna varivestis* Mulsant, is a serious pest of snap beans, *Phaseolus vulgaris* L., and lima beans, *Phaseolus lunatus* L., and is an occasional pest soybeans, *Glycine max* Merrill, in the United States. This insect exhibits variable preference and injury potential among different host species and cultivars. Three snap bean cultivars, three lima bean cultivars, and one soybean cultivar were evaluated for susceptibility to Mexican bean beetle via visual counts of life-stages and plant injury ratings in choice field-plot experiments, mark-release-recapture host choice field experiments, and larval development greenhouse assays. Bean cultivars were chosen based on their popularity among growers within the range of Mexican bean beetle, and because each exhibited at least one distinct morphological characteristic. Overall, the purple wax snap bean 'Dragon's Tongue', was the most susceptible cultivar. This cultivar was the most attractive to adult beetles in mark-release-recapture experiments; it produced the highest densities of late instar larvae and pupae and it sustained the highest injury levels in field experiments; and it had among the highest percentage of larvae completing their development in the greenhouse assays. This information should help inform management decisions for growers who experience damaging populations of Mexican bean beetle. Growers in areas prone to damaging infestations of Mexican bean beetle may choose to avoid planting a highly susceptible variety like 'Dragon's Tongue', be more aware of their management needs when growing a more susceptible cultivar, or attempt cultural management strategies that utilize highly attractive cultivars, such as trap-cropping and push-pull strategies.

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Introduction

Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae), is an herbivorous ladybeetle that feeds almost exclusively on legumes (Family: Fabaceae), and occasionally on plants outside this family at sub-injurious levels (Howard 1924, Howard and English 1924). Mexican bean beetle's developmental and reproductive success varies among host plant species and cultivars. Among commonly cultivated legumes, snap beans, *Phaseolus vulgaris* L., and lima beans, *Phaseolus lunatus* L., are the most suitable host species to Mexican bean beetle feeding and development, and the most susceptible to injury (Howard and English 1924, Friend and Turner 1931). Hosts plants that are less susceptible to injury include soybean, *Glycine max* Merrill, scarlet runner bean, *Phaseolus coccineus* L., cowpea or black-eyed pea, *Vigna unguiculata* Walpers, and kudzu, *Pueraria montana* Merrill (Howard and English 1924, Friend and Turner 1931, Hammond 1984). Numerous cultivars of snap beans and lima beans are available to growers; among which, some have been shown to differ in their suitability and susceptibility to Mexican bean beetle. Overall, more snap beans cultivars are susceptible to feeding injury than lima beans (Howard and English 1924, Campbell and Brett 1966); however, certain lima beans are highly susceptible while certain snap beans are not (Campbell and Brett 1966, Raina et al. 1978, Flanders 1984). Campbell and Brett (1966) and Raina et al. (1978) found that 'Spartan arrow' snap bean, 'Jackson Wonder' lima, and nearly all "wax bean" (snap beans with yellow pods) cultivars were highly susceptible to injury by Mexican bean beetle, while 'Regal' snap bean, 'Idaho Refugee' snap bean, 'Baby 'Fordhook'' lima bean, and 'Baby White' lima bean were less infested and injured. Flanders (1984) determined that 'Henderson' lima bean was highly suitable for rearing Mexican bean beetle, even compared to snap beans,

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due to its ability to promote beetle development, tolerate feeding injury, and survive longer than snap beans cultivars.

Important characteristics that affect a host plant's suitability and susceptibility to pests include attractiveness (pest preference), quality of shelter and nutrition, physiological and morphological defenses, and tolerance to injury. Examining these factors among host plants can have various functions in IPM decision making. Growers may choose species or cultivars that are less likely to incur economic injury, make better-informed treatments decisions based on crop susceptibly, or choose the best crops to use in a trap cropping strategy. Because pest and plant characteristics, as well as the availability of certain cultivars, will change over time, it is important that crop cultivars are periodically evaluated for these characteristics pertaining to suitability and susceptibility.

Very few studies examining suitability and susceptibility to Mexican bean beetle among snap bean and lima bean cultivars have been published in the last 30 years. The experiments presented in this chapter examined Mexican bean beetle attraction, developmental success and injury potential to snap beans and lima bean cultivars that are currently popular among growers in the range of Mexican bean beetle, as determined by outreach surveys (Nottingham and Kuhar 2013). Also, the chosen cultivars exhibit distinct morphological variations including: leaf color, size and texture; plant size and growth habits; and pod color and size. Although experiments were not designed to examine the effects of morphological differences on cultivar susceptibility to Mexican bean beetle, the possible effects of these characteristics are discussed.

Materials and Methods

Colonization, Development and Injury on Different Bean Cultivars - Field. Small-plot field experiments were conducted over four years (2013-2016) at Virginia Tech's Kentland

Research Farm in Whitethorne, VA (37.2013° N, -80.5656° W). For all experiments, beans were sown using a small push seeder (1001-B, Earthway Products Inc. Bristol, IN) that dropped 100 seeds per 6.1 m row. Crops were maintained according to standard commercial production practices with exception that no insecticides were applied and weeds were removed by hand.

Treatments. Treatments were individual cultivars of snap beans or lima beans, and in 2015 and 2016, also included one soybean cultivar. All seeds were untreated, and purchased at the beginning of each season. In 2013, there were four treatments (cultivars): 'Caprice' (green snap bean, *P. vulgaris*); 'Rocdor' (yellow wax snap bean, *P. vulgaris*); 'Dragon's Tongue' (purple wax snap bean, *P. vulgaris*); and 'Fordhook 242' (bush lima bean, *P. lunatus*, referred to from this point at 'Fordhook'). In 2014, there were six treatments: those tested in 2013, plus the addition of 'King of the Garden' (pole lima bean, *P. lunatus*), and 'Henderson' (dwarf bush lima bean, *P. lunatus*). In 2015 and 2016, 'Hutcheson' (soybean)*,* was added to the experiments, making seven total treatments. The soybean was added to represent a poor host, for comparison. All soybeans are expected to be of lower host quality to Mexican bean beetle than snap and lima beans (Friend and Turner 1931, McAvoy and Smith 1979, Mellors and Bassow 1983).

Plot Design. All field experiments were arranged in randomized complete blocks and were planted in mid- to late May in small field plots (two or four rows × 6.1 m long), and were naturally colonized by wild populations of Mexican bean beetle. The term "plot" represents one experimental unit.

The plot size, number of replicates, and blocking was altered among seasons due to changes in availability of land space and the increased number of treatments through the years. In 2013 and 2015, there were eight replicates per treatment, organized into four blocks (two replicates of each treatment per block); each plot was sown with 400 seeds, planted in four 6.1 m

rows. Rows within plots were separated by 45 cm of bare soil, and plots were separated by 2 m of fallow soil. In 2014 and 2016, there were four replicates per treatment, organized into four blocks (one plot of each treatment per block). Also in 2016, plots were sown with 200 seeds, in two, 6.1 meter rows.

Insect Counts and Injury Ratings. Beginning in mid-June of each year, plots were sampled either weekly or twice weekly for Mexican bean beetle life stages. In 2013, sampling was performed twice weekly from 10 June to 19 July; in 2014, sampling was performed weekly from 9 June to 7 July; and in 2015, sampling was performed weekly from 25 June to 14 July. Insect counts were not conducted in 2016. For each sample, ten arbitrarily-selected plants per plot were visually inspected for Mexican bean beetle eggs, late larvae (third and fourth instars), pupae, and adults. Egg count data from the final two weeks of experiments (last four samples in 2013, two samples in 2014, and last sample in 2015) were omitted. Eggs deposited late in experiments were omitted because they did not have enough time to contribute to (develop into) the late-instars and pupae from samples. Additionally, plants of preferred cultivars were heavily fed-upon by the final samples of experiments; making them less attractive for oviposition despite being more preferred initially.

In 2015 and 2016, Mexican bean beetle foliar feeding injury was assessed at flowering stage and pod stage. For each assessment, five plants per plot were arbitrarily-selected and individually ranked on a scale of 0 to 5 (lowest to highest injury, respectively; Table 2.2).

Larval Development on Different Bean Cultivars – Greenhouse Experiment.
Greenhouse experiments were conducted to examine the developmental success of Mexican bean beetle larvae among cultivars in a controlled setting. Developmental success was determined by percentage of beetles surviving from first instar to pupae.

Treatments. Seven cultivars were tested: 'Caprice', 'Rocdor', 'Dragon's Tongue', 'Fordhook', 'King of the Garden', 'Henderson', and 'Hutcheson'.

Design. Experiments were conducted in 2015 and 2016. Plants were grown in a peat-based growing medium (Pro-Mix HP-CC, Premier Horticulture Inc., Quakertown, PA) in 25.4 cm in diam plastic pots in a glass greenhouse at Virginia Tech in Blacksburg, VA. Plants were sown in plastic starter trays, and at first true leaf stage, two healthy plants of the same cultivar were transplanted into each individual pot. There were six replicate pots per cultivar in 2015, and five replicate pots in 2016. Pots were arranged in a completely randomized design on grated tables in the greenhouse. Plants were watered daily with overhead irrigation, and fertilized once per week with Miracle Grow, 24-6-16, All Purpose Plant Food (The Scotts Company, Marysville, OH). Temperature was set to not exceed 29.5°C (85°F).

Colony. Mexican bean beetle adults were collected from field plots and put into mesh rearing cages with scarlet runner beans, *P. coccineus*. After numerous eggs masses were deposited, adults were removed from cages, and juveniles were allowed to develop to adults. Eggs laid by the F_1 adults were collected for use in survivorship experiments.

Larval development experiments. Mexican bean beetle eggs deposited by F_1 adults from the greenhouse colony were collected and placed in 10 cm Petri dishes until hatch. When plants were between 25 and 30 days old, they were artificially infested with first instars (approximately two-days old). In 2015, five larvae were carefully placed on each potted plant using a small paint-brush. In 2016, eight first instars were used. Each plant was sampled three times throughout experiments: at the onset of second instars, fourth instars, and pupae. Total numbers of live individuals in each life stage were recorded.

Mark-Release-Recapture Host Choice Experiment. Mark-release-recapture

experiments were conducted at Virginia Tech's Kentland Research Farm in Whitethorne, VA.

Five experiments were conducted: two involving a mesh enclosure around the experimental area

to prevent beetles from escaping (cage experiment), and three without the mesh enclosure (no-

cage experiment). For each experiment an arena was established, in which all five cultivars were

arranged in a five by five row, Latin square design (five replicates per treatment in five blocks).

Each plot (one treatment replicate) was 0.6×0.6 m, and contained a cluster of nine equally

spaced bean plants (Figure 2.1). Bean plants were grown from seeds in the field.

Treatments. Treatments consisted of five bean cultivars from three species: 'Caprice'

'Rocdor' 'Dragon's Tongue' 'Fordhook' and 'Hutcheson'.

In the summers of 2013 and 2014, cage and no-cage experiments were conducted

simultaneously. An additional no-cage experiment was conducted in 2015. Cages were made

with a heavy polyethylene mesh draped over a steel frame, 3.7 (wide) $\times$ 3.7 (long) $\times$ 2.5 (tall) m,

with all sides buried and a zipper entrance. Enclosures were set up the day before beetles were

introduced, to prevent any differences in plant growth or pest occurrence among experiments.

Beetles were introduced 30 days, 25 days, and 30 days after planting in 2013, 2014, and 2015

respectively.

Mark-release-recapture methods. Mexican bean beetle pupae were collected in the field

from insecticide-free snap bean plants, and kept in cages in the greenhouse until developing into

adults. Adults were allowed to feed on scarlet runner beans in the greenhouse for one week until

their elytra hardened fully for marking. The sex of each adult was determined by the presence or

absence of a notch at the end of the eighth abdominal sternite (Sweetman 1930); only females

were used in these experiments. Each adult female was marked with one of five colors on the

right elytra, using a water-based paint pen (Sharpie USA, Downers Grove, Illinois). Each color represented the bean cultivar on which the beetle would be released. After being marked, beetles were allowed to feed for another five days, so that any beetles injured or killed by the procedure could be discarded.

On the day of release, beetles were separated by color and taken to the field. Five beetles were released into each plot (25 beetles per cultivar, 125 beetles per experimental arena). Beetles were carefully placed in the foliage of the center plant of each plot, and observed for at least one minute to make sure they did not drop to the ground or fly off plants. Beetles that dropped or fell were caught and replaced on plants if possible; but extra beetles were brought to replace those that escaped the arena while handling. After the release, plots were visually searched for marked beetles at 24, 72, 120, and 168 hours. Beetles recaptured were classified in three ways: 1. beetles recaptured within a cultivar, on which they were originally released ("remained"); 2. beetles recaptured within a cultivar, that were originally released on a different cultivar ("immigrated"); and 3. any marked beetle recaptured within a cultivar ("combined recaptures").

Statistical Analyses. All data were analyzed using JMP Pro 11 (SAS Institute Inc. 2007). Treatment (cultivar) was used as a fixed effect, and was the main independent variable in all experiments. The block factor was also a fixed effect, incorporated into field experiment models only. In the mark-release-recapture cage experiments, mean number of recaptures were pooled between 2013 and 2014, using year as the blocking factor. In all other experiments, blocking was based on physical location, described in *plot design* sections of the methods. The interaction effect between treatment and block was a fixed effect, and was only incorporated for experimental designs with replication within individual blocks. Dependent variables were

measurements of Mexican bean beetle densities for individual life-stages, plant injury ratings, proportion larval survival, and recapture numbers. Life-stage densities from small field plots, and numbers of recaptures from mark-release-recapture experiments were transformed to fit a normal distribution using the formula: $\sqrt[2]{(number\ of\ individuals) + 0.375}$ (Zar 2010). Proportion values of surviving larvae from greenhouse experiments were transformed to fit a normal distribution using the formula: $arcsine\sqrt[2]{(proportion\ surviving)}$. For all experiments, significance of treatment was determined using ANOVA, when $p < 0.05$, and significant differences among individual treatments were determined using a Students t test (Fisher's Protected LSD).

Results

Colonization, Development and Injury on Bean Cultivars - Field. *Colonization and Development.* Mean ± SEM cumulative densities of Mexican bean beetle adults, eggs, late instar larvae, and pupae per treatment (cultivar) are shown in Table 2.1. Overall, the effect of treatment was significant for densities of adults, eggs, late instars and pupae in all seasons, except for eggs in 2013, and adults in 2014.

In 2013, adult densities were significantly higher in all three snap bean cultivars ('Dragon's Tongue', 'Rocdor', and 'Caprice') than the lima bean cultivar ('Fordhook'). Significantly numbers of eggs were found in 'Fordhook' as in 'Caprice', 'Rocdor' and 'Dragon's Tongue'. However, despite the similar number of eggs found in 'Fordhook' lima and snap beans, there were fewer late larvae and pupae in 'Fordhook' than in all snap bean cultivars. Also, densities of late instar larvae were significantly higher in the two wax snap bean cultivars ('Dragon's Tongue' and 'Rocdor') than both 'Caprice' and 'Fordhook'; and densities of late larvae were significantly higher in 'Caprice' than in 'Fordhook' as well. Densities of pupae were

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also significantly higher in all snap bean cultivars than the lima bean cultivar. 'Dragon's Tongue' purple wax snap bean contained significantly more pupae than 'Caprice' as well, and had the highest numerical values, among all cultivars, for all life stages.

In 2014, two more cultivars of lima beans, 'Henderson' and 'King of the Garden', were added to the experiment to determine if developmental success of Mexican bean beetle would be low in other lima bean cultivars, as was seen in 'Fordhook' in 2013. There was a significant effect of treatment on densities of eggs, late larvae, and pupae, but not adults. As was the case in 2013, 'Dragon's Tongue' had the highest densities of all life stages among all cultivars, numerically. Mexican bean beetle laid as many or more eggs in 'Fordhook', as in the snap bean cultivars. 'King of the Garden' had as many eggs as 'Fordhook', 'Caprice' and 'Rocdor', but less than 'Dragon's Tongue'. 'Henderson' lima had as many eggs as 'Caprice', but less than all other cultivars. There were significantly fewer late larvae in 'Henderson' than all other cultivars; and 'Fordhook' and 'King of the Garden' had significantly fewer late instars than all snap bean cultivars. All three lima bean cultivars had significantly fewer pupae than the two wax snap bean cultivars, and numerically fewer pupae than all three snap bean cultivars.

In 2015, a soybean cultivar, 'Hutcheson', was added to the experiment for comparison, as soybean is known to be a low-quality host for Mexican bean beetle (Howard and English 1924, Friend and Turner 1931, Hammond 1984). Adult Mexican bean beetle densities were mostly similar among snap and lima bean cultivars, despite a significant effect of treatment, which was only due to low numbers in the soybean cultivar, 'Hutcheson'. If 'Hutcheson' is removed from the model, treatment is no longer significant (p = 0.14; F = 1.79 df = 5, 42). Densities of all life stages remained significantly lower in soybeans than all other cultivars, except for pupae, in which soybean numbers were not different from 'Fordhook' lima. 'Fordhook' lima continued to

exhibit low juvenile development, having among the highest densities of eggs, followed by the lowest densities of pupae. Significantly more eggs were found in 'Fordhook' than 'Henderson', 'King of the Garden', and 'Dragon's Tongue', and the same as in 'Caprice' and 'Rocdor'. 'Fordhook' and 'Henderson' had among the lowest pupal densities, while 'King of the Garden' and 'Dragon's Tongue' had the highest. 'Dragon's Tongue' had the highest numerical values of late instars, and significantly more pupae than all other cultivars.

Foliar Injury. Foliar injury by Mexican bean beetle among treatments is shown in Table 2.3. Significant differences in foliar injury among cultivars were detected at flowering stage, and at pod stage in both years. Differences in injury corresponded with differences in densities of late-instars of Mexican bean beetle.

'Hutcheson' soybean had significantly less feeding injury than all other beans in all samples. 'Dragon's Tongue' had significantly higher average injury levels than all other cultivars at the flowering stage in 2015. No other cultivar had significantly or numerically higher feeding injury values than 'Dragon's Tongue' in any analyses. In general, 'Dragon's Tongue' was the most injured overall; 'Caprice' was the least injured among the snap beans; 'King of the Garden' was the most injured among the lima beans and more injured than 'Caprice'; 'Fordhook' and 'Henderson' were similar, and the least injured of all *Phaseolus* cultivars; the soybean, 'Hutcheson', was the least injured overall.

Larval Development on Different Bean Cultivars – Greenhouse. In 2015, the percentage of Mexican bean beetle larvae completing their development was highest on the three snap bean cultivars ('Dragon's Tongue', 'Rocdor' and 'Caprice'), and significantly lower on all lima bean cultivars and 'Hutcheson' soybean (Table 2.4). In 2016, the percentage of larvae completing their development was similar among on the three snap bean cultivars and 'King of

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the Garden'. Development was significantly lower on 'Fordhook' than 'Caprice'. 'Henderson' lima and 'Hutcheson' soybean had significantly lower larval survival than all other cultivars, and were not different from each other (Table 2.4).

Mark-Release-Recapture Host Choice Experiment – Field. *Field Cage Plots*. Field cage plot experiments yielded the highest number of recaptures, as well as our most discernable results. Around 50% of the total number of the beetles released into cage plots (60 to 65 out of 125) were recaptured in each sample for both 2013 and 2014.

Differences in the average numbers of beetles recaptured among cultivars were significant for all three recapture types: "remained", "immigrated", and "combined" (Table 2.5). In both the 2013 and 2014 cage plot experiments, the purple wax cultivar, 'Dragon's Tongue', was the most attractive cultivar to Mexican bean beetle adults. The average number of marked-adult recaptures ("combined") in 'Dragon's Tongue' plots was significantly higher than any other cultivar; the average number of recaptured adults found in their cultivar of release ("remained") was significantly higher in 'Dragon's Tongue' than any other cultivar; and the average number of recaptured adults found in a different cultivar than their release ("immigrated") was greater in 'Dragon's Tongue' than any other cultivar. This suggests that 'Dragon's Tongue' not only retains beetles in the presence of other adequate hosts, but attracts beetles from other adequate bean cultivars. Average numbers of "remained", "immigrated" and "combined" recaptures were not different among 'Rocdor', 'Caprice', and 'Fordhook'; while 'Hutcheson' soybean had significantly fewer recaptures than all other cultivars for all three recapture types.

Open Field Plots. Recaptures in open field plots varied from 10% to 30% per sample in 2013. Only one beetle was recaptured in 2014, and fewer than 5% per day were recaptured in

2015. Therefore, only 2013 open-field data were analyzed, and these data may still be tenuous due to low numbers.

There was a significant treatment effect for all three types of recaptures: "remained", "immigrated", and "combined total". The cultivars with the highest numbers of combined recaptures were not different among 'Dragon's Tongue', 'Rocdor', and 'Fordhook'. Combined recaptures in 'Caprice' and 'Hutcheson' soybean were the same, and both had fewer combined adult recaptures than the three other cultivars. Number of beetles recaptured which remained differed from those that were recaptured as immigrants. 'Caprice' had a relatively high number of remaining beetles, but recruited very few compared to other cultivars. On the other hand, 'Fordhook', was very attractive to beetles from other cultivars, drawing high numbers of immigrants, but had among the lowest numbers of beetles remain from the original release. 'Rocdor' and 'Dragon's Tongue' had consistently high recapture rates for both remaining and immigrating beetles, while 'Hutcheson' recaptures were low for both groups.

Discussion

The results of these experiments offer various perspectives on Mexican bean beetle host choice, larval developmental success and ability to injure certain bean cultivars. The densities of Mexican bean beetle life stages and the concomitant feeding injury in the field plots (Tables 2.1 and 2.3) served as the focal points for comparison to all other experiments. Data from field plots are more likely to be affected by extraneous factors (mainly stand density and plant size among treatments, sampling variability, weather within and across seasons, etc.), but also most accurately represent what a grower is likely to witness in a farm setting. Mark-release-recapture and greenhouse survivorship assays help to focus on possible biological factors pertaining to host attraction and developmental success, respectively, of Mexican bean beetle among host cultivars.

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Overall, the experiments presented indicate that snap bean cultivars, as a whole, tend to have higher densities of Mexican bean beetle to incur more feeding injury than lima bean cultivars, and certainly more than soybean. These results are consistent with the findings of Campbell and Brett (1966). Among all bean cultivars tested, 'Dragon's Tongue' appeared to be the most attractive to Mexican bean beetles and susceptible to their injury. To our knowledge, this study is the first to report on Mexican bean beetle and this cultivar. 'Caprice' snap bean, was notably less attractive, developmentally suitabile, and susceptibile to injury from Mexican bean beetle than 'Dragon's Tongue'. 'Rocdor' was intermediate in terms of these qualities, but was most similar to 'Caprice'. It may be of importance that 'Dragon's Tongue' was the most morpholically distinct snap bean cultivar (Table 2.6). All lima beans exhibited some level of tolorance and/or resistance to Mexican bean beetle.

Distinct, morphological characteristics of 'Dragon's Tongue' may explain why it was more susceptible to Mexican bean beetle than the other cultivars tested. 'Dragon's Tongue' has a thicker canopy and leaves that were broader, thinner, smoother, and lighter in color than other cultivars. The light, yellowish leaf color could be important for attracting Mexican bean beetles, because many lady-beetles are highly attracted to yellow plant pigments, more so than green (Mondor and Warren 2000, Adedipe and Park 2010). Susceptibility to foliar injury could be related to the fact that 'Dragon's Tongue' has smooth, thin leaves with narrow veins compared to the other cultivars. It may be easier for Mexican bean beetle, larvae in particular, to feed on these soft and thin leaves, because they are unable to consume solid leaf tissue outright. Instead, they must scrape the softest areas around the veins of leaves, dislodging tissue into a pile; which they then squeeze with their mandibles to extract and consume juices (hence the term "skeletonized" to describe heavy feeding injury) (Howard 1941). 'Dragon's Tongue' also

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produces a large and dense canopy (the largest of the snap beans, and the densest of all cultivars). As mentioned earlier, shade is likely an important factor to beetle success, due to their aversion to direct sunlight (Howard and English 1924, Miller 1930).

Among lima bean cultivars, 'King of the Garden' was probably the most suitable host for Mexican bean beetle. First, as the name suggests, these plants continued to grow throughout the season, creating a large vegetative area and shade for beetles. Shade is probably an important factor in host quality, since Mexican bean beetle is deterred by direct light, and less likely to survive if forced to remain in direct light (Howard and English 1924, Miller 1930). It is also possible that this cultivar is more nutritionally adequate for Mexican bean beetle than other lima beans, as suggested by higher injury ratings and larval densities (even higher than the snap bean, 'Caprice', in some tests). 'Henderson' and 'Fordhook' lima beans both exibited the low numbers of larvae and pupae, the lowest developmental success, and the least injury among all cultivars except the soybean, 'Hutcheson'.

Growers who regularly encounter severe populations of Mexican bean beetle may choose to avoid the cultivar 'Dragon's Tongue', due to its high level of susceptibility. However, in areas where Mexican bean beetle is regularly severe, any snap bean is likely to experience economic damage because all are at least moderately susceptible. Instead, growers may use this information to make more precise treatment decisions that cater to the cultivar grown. Since treating at economic threshold still requires some level of subjective choice, one may choose to treat more readily when growing a highly susceptible cultivar, like 'Dragon's Tongue', verse a less susceptible cultivar like 'Caprice'.

These findings may also be applicable for cultural management of Mexican bean beetle, such as in trap cropping or push-pull strategies. "Dead-end" trap crops are strategic plantings of

highly attractive hosts, which attract and consolidate most of a pest population into an area where the insects can be easily killed (Wallingford et al. 2013). Trap cops can also act as a distraction, or reservoir, to keep the pest from invading the commodity (Philips et al. 2014). Push-pull strategies are similar to trap crops; the only difference being the addition of a pest-repelling agent to the commodity zone (Gray et. al 2009). Trap cropping to manage Mexican bean beetle was attempted by Rust (1977), using snap beans as a trap for lima beans; but was unsuccessful. Different cultivar selection, such as using 'Dragons Tongue' as a trap crop, may result in better control of Mexican bean beetle. To increase the potential success of the trap crop strategy, a "push" agent could be incorporated into the cash crop zone, such as planting those beans on reflective plastic mulch (Nottingham and Kuhar 2016).

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Tables and Figures

Table 2.1. Cumulative densities (mean ± SEM) of Mexican bean beetle life stages observed on 10 plants per plot of different bean cultivars in field choice experiments conducted in Whitethorne, VA; data are summed over all samples dates within a season*.

	Cultivar	2013				2014				2015			
		Adults	Eggs	Late Instars	Pupae	Adults	Eggs	Late Instars	Pupae	Adults	Eggs	Late Instars	Pupae
Snap bean	'Dragon's Tongue'	17.19 ± 0.98 a	25.53 ± 3.36	33.67 ± 2.07 a	18.06 ± 1.22 a	13.66 ± 2.58	34.59 ± 2.96 a	15.74 ± 1.01 a	8.51 ± 0.50 a	5.29 ± 0.45 ab	15.03 ± 1.67 b	13.11 ± 0.43 a	8.06 ± 0.87 a
	'Rocdor'	16.82 ± 0.99 a	18.41 ± 2.25	31.51 ± 3.01 a	15.64 ± 1.52 ab	9.18 ± 1.00	21.41 ± 2.59 c	13.74 ± 1.61 a	6.71 ± 1.41 ab	4.59 ± 0.34 abc	16.58 ± 2.30 ab	11.83 ± 0.83 ab	6.07 ± 0.70 b
	'Caprice'	16.54 ± 0.93 a	15.03 ± 2.91	23.62 ± 1.03 b	13.44 ± 1.39 b	10.69 ± 0.88	15.97 ± 6.74 cd	13.90 ± 1.50 a	5.41 ± 1.02 bc	3.97 ± 0.77 bc	17.76 ± 2.24 ab	10.60 ± 1.40 b	5.18 ± 0.78 bc
Lima bean	'Fordhook'	14.28 ± 0.85 b	20.99 ± 3.31	15.12 ± 0.90 c	7.73 ± 0.19 c	12.37 ± 1.59	32.57 ± 3.06 ab	10.30 ± 1.00 b	3.94 ± 0.36 c	4.65 ± 0.62 abc	20.60 ± 2.28 a	7.56 ± 1.04 c	3.49 ± 0.74 cd
	'King of the Garden'	na	na	na	na	12.04 ± 1.58	24.22 ± 5.83 bc	8.73 ± 1.76 b	4.23 ± 0.76 c	5.41 ± 0.39 a	13.96 ± 1.89 b	12.07 ± 0.76 ab	6.74 ± 0.51 ab
	'Henderson'	na	na	na	na	10.67 ± 0.89	7.28 ± 3.36 d	3.79 ± 0.73 c	3.06 ± 0.00 c	3.71 ± 0.34 c	13.81 ± 1.45 b	7.40 ± 0.70 c	4.02 ± 0.56 c
Soybean	'Hutcheson'	na	na	na	na	na	na	na	na	2.05 ± 0.15 d	1.34 ± 0.12 c	2.16 ± 0.13 d	1.84 ± 0.00 d
	p value	0.036	ns	<0.0001	<0.0001	ns	0.0003	<0.0001	0.0022	0.0002	<0.0001	<0.0001	<0.0001
	F	3.63	ns	19.42	13.94	ns	9.41	16.34	6.45	5.85	13.97	21.33	9.81
	df	3, 28	ns	3, 28	3, 28	ns	5, 18	5, 18	5, 18	6, 49	6, 49	6, 49	6, 49

* In 2013, sampling was done twice weekly from 10 June to 19 July; in 2014, sampling was done weekly from 9 June to 7 July; and in 2015, sampling was done weekly from 25 June to 14 July
na = No data are displayed because the cultivar was not grown in that season.
Means within a corresponding column that do not share a similar letter are significantly different, according to Students t (Fisher's Protected LSD), $P < 0.05$.

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Table 2.2. Scale for rating Mexican bean beetle feeding injury on snap bean foliage adapted from Nottingham and Kuhar (2016).

Ordinal Rank	Definition of Injury
0	No feeding injury
1	Less than five leaves exhibit injury; no leaf with more than 10% injury
2	Most leaves exhibit injury; no leaf with above 50% injury
3	Most leaves exhibit injury; 10 or less leaves with above 50% injury
4	Most leaves exhibit injury; more than 10 leaves with above 50% injury; some green remains
5	All remaining plant tissue (if any) is brown, and plant is likely dead

Table 2.3. Foliar feeding injury ratings (mean ± SEM) based on a 1 to 5 scale (see Table 2.2) observed on different bean cultivars in field choice experiments conducted in Whitethorne, VA.

		2015		2016	
	Cultivar	**Flower Stage**	**Pod Stage**	**Flower Stage**	**Pod Stage**
Snap bean	'Dragon's Tongue'	1.98 ± 0.18 **a**	2.55 ± 0.16 **a**	3.63 ± 0.14 **a**	4.06 ± 0.54 **a**
	'Rocdor'	1.48 ± 0.15 **b**	1.70 ± 0.07 **b**	3.19 ± 0.08 **a**	3.59 ± 0.16 **ab**
	'Caprice'	1.25 ± 0.18 **bc**	1.40 ± 0.09 **b**	2.38 ± 0.24 **b**	2.97 ± 0.30 **ab**
Lima bean	'Fordhook'	1.15 ± 0.22 **bc**	1.03 ± 0.05 **c**	0.81 ± 0.25 **c**	2.81 ± 0.60 **b**
	'King of the Garden'	1.43 ± 0.22 **bc**	2.30 ± 0.18 **a**	1.97 ± 0.08 **b**	3.59 ± 0.30 **ab**
	'Henderson'	1.00 ± 0.16 **c**	1.38 ± 0.14 **b**	1.03 ± 0.30 **c**	2.66 ± 0.30 **b**
Soybean	'Hutcheson'	0.25 ± 0.13 **d**	0.10 ± 0.05 **d**	0.13 ± 0.13 **d**	0.78 ± 0.16 **c**
	p value	<0.0001	<0.0001	<0.0001	0.0005
	F	11.02	49.24	51.10	7.12
	df	6, 49	6, 49	6, 21	6, 21

Means within a corresponding column that do not share a similar letter are significantly different, according to Students t (Fisher's Protected LSD), $P < 0.05$.

Table 2.4. Percentage (mean ± SEM) of Mexican bean beetle larvae completing development from first instar to pupae on different bean cultivars in a greenhouse experiment. Values under individual dates are the average percentages (± SEM) of surviving larvae out of five (2015) and eight (2016) larvae per pot by cultivar. Values under "combined average" are the average survival percentages (± SEM) by cultivar, combined over 2015 and 2016.

Sps.	Cultivar	2015	2016	Combined Average 2015 & 2016
Snap bean	'Dragon's Tongue'	90.0 ± 4.47 **a**	62.5 ± 3.95 **ab**	77.5 ± 5.20 **a**
	'Rocdor'	76.7 ± 12.01 **a**	40.0 ± 9.19 **ab**	60.0 ± 9.38 **abc**
	'Caprice'	70.0 ± 7.83 **ab**	70.0 ± 12.87 **a**	70.0 ± 6.55 **ab**
Lima bean	'Fordhook'	46.7 ± 11.16 **bc**	37.5 ± 9.68 **b**	42.5 ± 7.28 **cd**
	'King of the Garden'	46.7 ± 11.16 **bc**	50.0 ± 13.11 **ab**	46.7 ± 11.16 **bc**
	'Henderson'	36.7 ± 12.02 **c**	10.0 ± 4.68 **c**	24.6 ± 7.81 **de**
Soybean	'Hutcheson'	30.0 ± 6.83 **c**	2.5 ± 2.50 **c**	17.5 ± 5.71 **e**
	p value	0.001	0.0002	< 0.0001
	F	5.10	7.24	7.42
	df	6, 35	6, 28	6, 70

Means within a corresponding column that do not share a similar letter are significantly different, according to Students t (Fisher's Protected LSD), $P < 0.05$.

Table 2.5. Mean (± SEM) numbers of adult female Mexican bean beetles per nine plants that remained or immigrated to different bean cultivars over four sample dates in a choice experiment conducted in a walk-in cage (a.) or in open field plots (b.) located in Whitethorne, VA.

| | Variety | a. Field Cage (2013 & 2014) | | | b. No Cage (2013) | | |
		Remained[1]	Immigrated[2]	Total Recaptures[3]	Remained[1]	Immigrated[2]	Combined Recaptures[3]
Snap bean	'Dragon's Tongue'	5.45 ± 0.33 **a**	7.72 ± 0.34 **a**	9.25 ± 0.45 **a**	3.49 ± 0.36 **ab**	5.06 ± 0.43 **a**	5.82 ± 0.23 **a**
Snap bean	'Rocdor'	3.67 ± 0.35 **b**	5.10 ± 0.27 **b**	5.93 ± 0.35 **b**	4.20 ± 0.32 **a**	4.64 ± 0.48 **a**	5.89 ± 0.37 **a**
Snap bean	'Caprice'	4.36 ± 0.33 **b**	4.87 ± 0.30 **b**	6.25 ± 0.39 **b**	3.42 ± 0.42 **abc**	2.79 ± 0.14 **b**	3.68 ± 0.43 **b**
Lima bean	'Fordhook'	4.15 ± 0.34 **b**	4.47 ± 0.40 **b**	5.71 ± 0.52 **b**	2.90 ± 0.21 **bc**	5.06 ± 0.60 **a**	5.32 ± 0.67 **a**
Soybean	'Hutcheson'	2.60 ± 0.10 **c**	2.71 ± 0.11 **c**	2.93 ± 0.12 **c**	2.56 ± 0.11 **c**	2.56 ± 0.11 **b**	2.67 ± 0.14 **b**
	p value	<0.0001	<0.0001	<0.0001	0.0123	<0.0001	<0.0001
	F	10.64	44.78	34.53	4.52	15.26	12.75
	df	4, 45	4, 45	4, 45	4, 20	4, 20	4, 20

[1] Remained: Beetles recaptured in the variety in which they were originally released.
[2] Immigrated: Beetles recaptured in a variety different than their original release.
[3] Total recaptures: Combined total of remained and immigrated beetles recaptured within a variety.
All data were square root transformed prior to analysis (total recaptures were summed prior to transformation). Transformed values are shown.
Means within a corresponding column that do not share a similar letter are significantly different, according to Students t (Fisher's Protected LSD), $P < 0.05$.

Table 2.6. Bean species, cultivars and general varietal description; relative susceptibility to Mexican bean beetle rank; and distinct visible/morphological characteristics potentially relevant to resistance or susceptibility.

Rank (least to most susceptible)	Species	Cultivar	General Description	Important Characteristics
1	*Glycine max*	'Hutcheson'	field soybean	highly pubescent leaves
2	*Phaseolus lunatus*	'Henderson'	bush, dwarf seeds	thick and waxy leaves; dark green
3	*Phaseolus lunatus*	'Fordhook' 242	bush, large seeds	widely dispersed leaves (thin canopy)
4	*Phaseolus lunatus*	'King of the Garden'	pole, large seeds	high growth rate and biomass (dense canopy)
5	*Phaseolus vulgaris*	'Caprice'	green stringless pods	wrinkled, fibrous leaves
6	*Phaseolus vulgaris*	'Rocdor'	yellow stringless pods, "wax"	wrinkled, fibrous leaves
7	*Phaseolus vulgaris*	'Dragon's Tongue'	purple and yellow streaked stringless pods, "wax"	thin, soft and smooth leaves; light green

Figure 2.1. Field plot design for Mexican bean beetle mark-release-recapture experiments conducted in large walk-in cages and in the field in Whitethorne, VA.
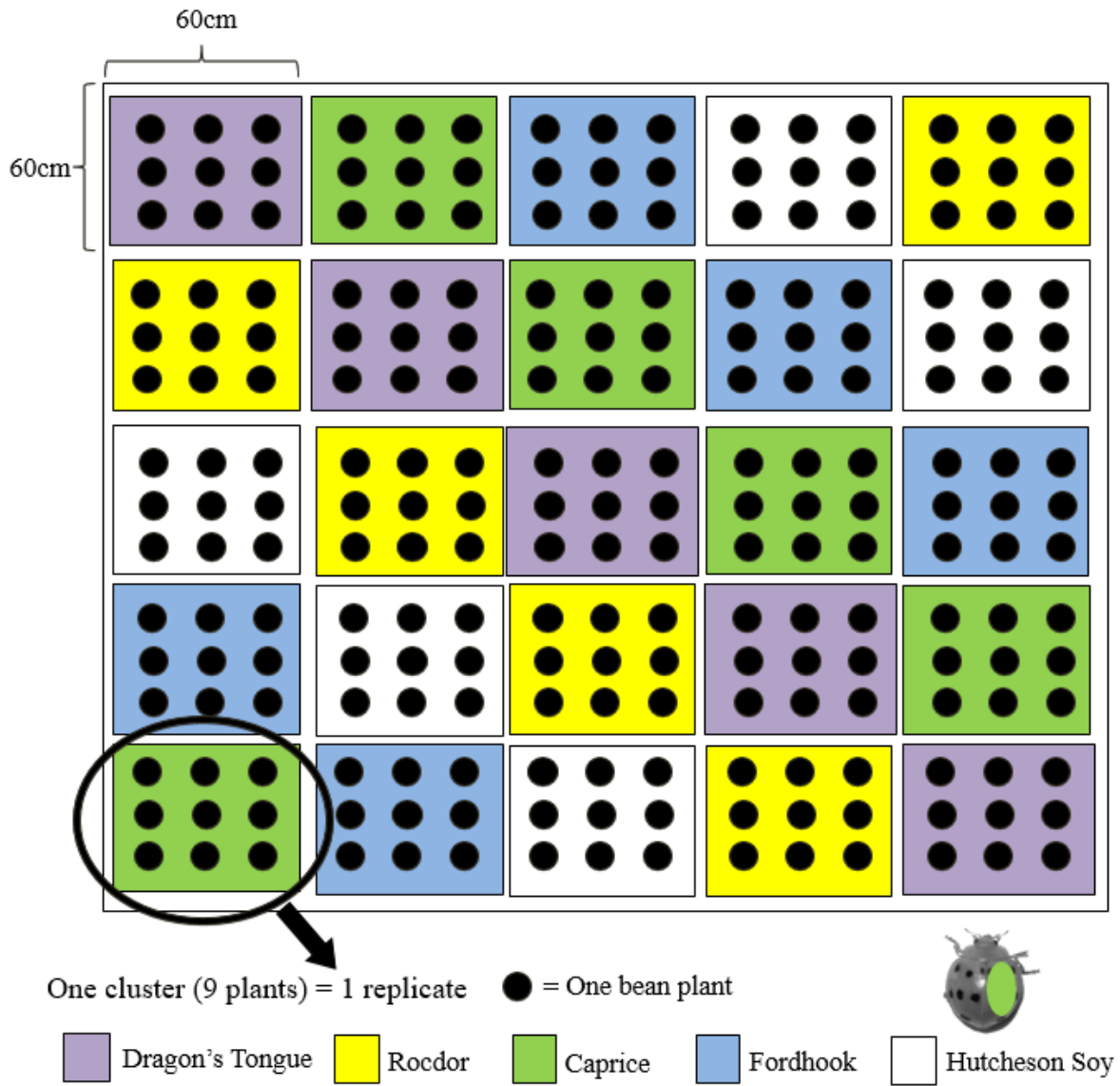
CHAPTER THREE

Reflective Polyethylene Mulch Reduces Mexican Bean Beetle (Coleoptera: Coccinellidae) Densities and Damage in Snap Beans

Abstract

Mexican bean beetle, *Epilachna varivestis* Mulsant, is a serious pest of snap beans, *Phaseolus vulgaris* L., in the eastern United States. These beetles are intolerant to direct sunlight, explaining why individuals are typically found on the undersides of leaves and in the lower portion of the plant canopy. We hypothesized that snap beans grown on reflective, agricultural polyethylene (plastic mulch) would have fewer Mexican bean beetles and less injury than those grown on black plastic or bare soil. In 2014 and 2015, beans were seeded into beds of metallized, white, and black plastic, and bare soil, in field plots near Blacksburg, VA. Mexican bean beetle density, feeding injury, predatory arthropods and snap bean yield were sampled. Reflected light intensity, temperature and humidity were monitored using data loggers. Pyranometer readings showed that reflected light intensity was highest over metallized plastic and second highest over white plastic; black plastic and bare soil were similarly low. Temperature and humidity were unaffected by treatments. Significant reductions in Mexican bean beetle densities and feeding injury were observed in both metallized and white plastic plots compared to black plastic and bare soil, with metallized plastic having the fewest Mexican bean beetle life-stages and injury. Predatory arthropod densities were not reduced by reflective plastic. Metallized plots produced the highest yields, followed by white. The results of this study suggest that growing snap beans on reflective plastic mulch can suppress the incidence and damage of Mexican bean beetle, and increase yield in snap beans.

Introduction

Mexican bean beetle, *Epilachna varivestis* Mulsant, is an herbivorous lady beetle (Coleoptera: Coccinellidae) that feeds of many legumes (Fabaceae). Since 1920, when it was first discovered in the eastern United States (Howard 1924), Mexican bean beetle has achieved economic pest status primarily on snap beans, *Phaseolus vulgaris* L., lima beans, *Phaseolus lunatus* L., and to a lesser extent, soybeans, *Glycine max* (L.) Merr. (Capinera 2001, Nottingham et al. 2016). Although Mexican bean beetle is found throughout the U.S., it is most common and severe in the Mid-Atlantic and southern Appalachian Mountain regions (Nottingham et al. 2016). Adults and larvae use chewing mouthparts to dislodge tissue from leaves and pods, then compress the tissue to extract and consume plant juices (Howard 1941). Excessive feeding on leaves reduces the plant's photosynthetic potential and promotes desiccation, which can result in yield losses (Capinera et al. 1987, Nolting and Edwards 1989, Barrigossi 1997, Peterson et al. 1998). In addition, direct feeding on pods creates unsightly scars that render the crop unmarketable and more susceptible to fruit-rotting pathogens (Nottingham et al. 2016).

Many labeled insecticides provide effective control of Mexican bean beetle (Nault and Speese 2001, Patton et al. 2003, Kuhar et al. 2012, Nottingham et al. 2015), and resistance to the common groups used in vegetable production (neonicotinoids and pyrethroids) has not been documented. In the Mid-Atlantic and southern Appalachian Mountain regions of the United States, where Mexican bean beetle outbreaks are most common, snap bean production usually occurs on a small to medium-sized farm scale, and often in organic systems. Small to mid-sized producers are more likely to employ non-chemical methods for pest control, than are large scale producers (Aselage and Johnson 2009). Considering the farming culture in regions with

Mexican bean beetle, and the increasing market for organic and chemical-free produce, it is important that alternative management strategies are scientifically evaluated for this pest.

The most widely used non-chemical option for managing Mexican bean beetle is augmentative biological control, using a small parasitoid wasp, *Pediobius foveolatus* (Crawford) (Hymenoptera: Eulophidae). Inoculative releases can successfully reduce local populations of Mexican bean beetle by attacking and killing the larval stage (Angalet et al. 1968, Stevens et al. 1975, Barrows and Hooker 1981, Fess 2008). Unfortunately, these exotic parasitoids must be released annually due to their inability to overwinter in temperate climates (Stevens et al. 1975, Ghani and Mohyuddin 1982, Schaefer et al. 1983). Cool and wet weather is optimal for Mexican bean beetle, but problematic to *P. foveolatus* (Marcovitch and Stanley 1930, Auclair 1960, Stevens et al. 1975, Gale and Shepard 1978, Wilson et al. 1982, Mellors and Bassow 1983, Stoner 2002); therefor, wasps may fail to manage this pest in some seasons.

Other than biological control, few non-chemical methods exhibit adequate control of Mexican bean beetle (Capinera 2001, Fess 2008, Nottingham et al. 2016). Cultural management, which is a fundamental component of integrated pest management (IPM), uses strategic crop production practices that reduce the likelihood of damaging pest outbreaks (Zehnder et al. 2007). Two of the most common cultural strategies in agriculture, field sanitation and crop rotation, are ineffective at reducing injury from Mexican bean beetle (Capinera 2001), because adults are capable of flying over 11 km after emerging from their overwintering sites, which are usually wooded areas away from crop fields (Howard and English 1924, Thomas 1924, Friend and Turner 1931, Auclair 1960).

Developing a successful cultural management strategy often requires the diagnosis and exploitation of a specific limiting factor. Research by Miller (1930) and Howard and English

(1924) demonstrated that Mexican bean beetle is highly sensitive to direct light. Miller (1930) reported that adults and larvae became restless and absconded into shaded areas of the plant canopy when exposed to direct light. When forced to remain in direct light, mortality of eggs, larvae, pupae and adults increased (Howard and English 1924, Miller 1930). These findings probably explain why all life-stages of Mexican bean beetle are typically found on the undersides of leaves, or in the lower portion of the plant canopy (Howard 1924, Barrigossi et al. 2001, Nottingham et al. 2016).

Because Mexican bean beetle is adversely affected by direct light, it may be possible to suppress populations and injury by planting snap beans on reflective, agricultural polyethylene (plastic mulch). Plastic mulch is commonly used for weed control, manipulating soil temperature and reducing soil-water evaporation (Lament 1993). Black plastic mulches are common in vegetable agriculture, because they increase soils temperatures by absorbing short wavelength light (400-700nm); while light-reflecting mulches, such as metallized (aluminum-infused top, black bottom) and white (white top, black bottom), keep soil temperatures cool, making them more conducive for cooler weather crops (Ham et al. 1993, Lament 1993). Highly reflective mulches (those with aluminum or silver infused surfaces) have been shown to effectively mitigate injury and/or disease transmission from a wide variety of insects, including aphids (Aphididae), thrips (Thripidae, Phlaeothripidae), whiteflies (Aleyrodidae), cucumber beetles (Chrysomelidae), leafhoppers (Cicadellidae), leaf miners (Agromyzidae) and plant bugs (Miridae) (Chalfant et al. 1977, Wells et al. 1984, Scott et al. 1989, Kring and Schuster 1992, Csizinszky et al. 1995, Caldwell and Clarke 1999, Rhainds et al. 2001, Summers and Stapleton 2002a, 2002b). Wells et al. (1984) determined that, in addition to having fewer potato leafhoppers, *Empoasca fabae* (Harris) (Hemiptera: Cicadellidae), snap beans planted on

reflective mulch were larger and produced higher yields than plants grown on bare soil.

However, not all insects are controlled by this method. Stoner (1997) found that colonization

and development of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera:

Chrysomelidae), was unaffected by reflective plastic, and Schalk and Robbins (1987) witnessed

increased incidence and injury of tomato pinworm, *Keiferia lycopersicella* (Walshingham)

(Lepidoptera: Gelechiidae), and tomato fruitworm, *Helicoverpa zea* (Boddie) (Lepidoptera:

Noctuidae).

The purpose of this study was to determine if planting snap beans on metallized plastic or

white plastic would reduce Mexican bean beetle densities and concomitant damage to snap

beans. Predatory arthropods were also sampled to evaluate treatment effects on natural enemies.

Temperature, humidity, and reflected light intensity (RLI) were monitored over each treatment to

quantify differences in these environmental conditions, and to test their impact on insect

abundance.

Materials and Methods

Experimental Design. Experiments took place at Virginia Tech's Kentland Farm in

Whitethorne, VA (37.2013° N, -80.5656° W). Three experiments were conducted, one in the

summer of 2014, and two in the summer of 2015. In all experiments, 'Dragon's Tongue' snap

beans were grown on four treatments: 1. 'Metallized' plastic: aluminum-infused top and black

bottom (Sunup Reflective Films, Oceanside, CA); 2. 'White' plastic: white top and black bottom

(Berry Plastics, Evansville, IN); 3. 'Black' plastic: black top and black bottom (Berry Plastics,

Evansville, IN); and 4. Bare soil. Mulches were purchased from Harris Seeds, Rochester, NY.

Treatment and control beds were formed by a tractor-pulled plastic layer. Plastic edges were

buried by hand. One row of beans was grown per plastic bed. Adjacent beds were separated by

2 m of fallow soil. Seeding holes, 7 cm diam, were cut into beds 35 cm apart, and two bean

seeds were directly seeded per hole (two bean plants grown together will be called a "cluster").

Beans were drip irrigated twice a week from planting to harvest. Glyphosate (1.2 kg AI/ha

Roundup Ultra Max; Monsanto, St. Louis, MO) was applied between rows as needed to control

weeds. Weeds between bean plants in bare soil plots were removed by hand weekly.

2014 Experiment. Beans were planted on 17 June 2014. Plots were arranged in a

randomized complete block, with four replicates per treatment, organized into four blocks. Each

individual mulch bed was one replicate, or "plot". Each individual plot was one, 0.9 × 8 m

mulch bed, with one row of beans. Each plot contained 20 bean clusters, spaced 36cm apart.

This spacing equals one plant per 18 cm, which is slightly wider than most conventional

recommendations. Wider spacing was used to achieve stand uniformity and sampling accuracy.

Blocks were separated by no less than 100 m, in isolated locations of a diverse vegetable farm.

Blocks were not adjacent to other snap beans plantings.

2015 (Early) Experiment. Beans were planted on 18 May 2015. Plots were arranged in a

generalized, randomized complete block design, with nine replicate plots for each treatment,

organized into three blocks. Each individual plot consisted of two, adjacent 0.9 × 3 m mulch

beds, each with one row of beans per bed. Plots contained 10 bean clusters each, five per mulch

bed. Blocks were separated by 10 m of fallow soil.

2015 (Late) Experiment. Beans were planted on 29 July 2015, and spatially separated by

350 m from the 2015 (early) location. Plots were arranged in a generalized, randomized

complete block design, with eight replicate plots for each treatment, organized into two blocks.

Each individual plot consisted of four, adjacent 0.9 × 3 m mulch beds, each with one row of

beans per bed. Plots contained 20 bean clusters each, five per mulch bed. Blocks were separated

by 10 m of fallow soil.

Insect Sampling. Insect populations were sampled once per week, and samples were

taken in every plot. In each plot, 10 plant clusters were arbitrarily selected and visually

inspected. Sampling was conducted on five dates for each experiment, starting on 9 July 2014,

11 June 2015, and 18 August 2015. Mexican bean beetles were counted as eggs, early larvae

(first and second instars), late larvae (third and fourth instars), pupae, and adults. All predatory

arthropods observed were documented.

Plant Injury. *Foliar Feeding.* Mexican bean beetle feeding injury was evaluated in the

2015 experiments only, on 22 June (flowering stage) and 6 July (pod stage) for the early

experiment, and 3 September (flowering stage) and 22 September (pod stage) for the late

experiment. For each assessment, 10 plant clusters per plot were individually ranked on a scale

of 0 to 5, indicating the level of feeding injury by Mexican bean beetle (Table 3.1).

Pod Feeding. One-hundred mature pods were arbitrarily selected from each plot on 17

July 2015 for the early experiment, and 23 September 2015 for the late experiment. Each pod

was visually inspected for Mexican bean beetle feeding scars. A pod with a total combined area

of scarred tissue ≥ 1 cm² was recorded as "damaged". A pod with < 1 cm² of scarred tissue was

documented as "undamaged".

Yield. All bean pods from every plot were hand-picked at pod maturity: 15 August 2014,

15 July 2015, and 23 September 2015. Pods were weighed the same day using an Adventurer

Pro electronic scale (Ohaus Corp, Parsippany, NJ).

Reflected Light Intensity (RLI), Temperature and Humidity. RLI (µmol/m²/s),

temperature (°C), and relative humidity (%) were monitored from 2 June through 23 September

2015. Pyranometers (QSO-S PAR sensor, Decagon Devices, Pullman, WA), which measure intensity ($\mu mol/m^2 s$) of short wave-length light (400-700nm), and temperature + humidity sensors (VP-4 Humidity Temperature Sensor, Decagon Devices, Pullman, WA) were mounted to metal poles, back to back, and staked between two plants. There were four poles with sensors in total: one for each treatment. Pyranometers were mounted 10 cm above the plastic or bare soil, facing the ground; temperature + humidity sensors were mounted 15 cm above the surface, also facing the ground. Pyranometers were calibrated weekly. A white, LED light was placed flat on the surface beneath each sensor at night, and mounts were micro-adjusted until all pyranometers produced readings within five intensity units of each other. To avoid bias and create replicates, sensors were relocated to a new plot and treatments weekly. Sensors were relocated 11 times, creating 12 replicates per treatment. Each pyranometer and temp/humidity sensor was connected to a data logger (Em50G Data Logger, Decagon Devices, Pullman, WA). Data loggers recorded conditions every 10 min and remained on at all times, except when units were being remounted.

Data Analysis. All statistical tests were performed in JMP Pro 11 (SAS Institute Inc. 2007). Insect count data were analyzed by individual sample dates. Most insect count data did not fit normal distributions, either as raw data or after various transformations; therefore, non-parametric tests were used for analysis of insect densities. A Wilcoxon Rank Sum Test was used to detect significance within the model, followed by Wilcoxon Each Pair Test for multiple comparisons among treatments ($\alpha = 0.05$). Yield, foliar injury, pod damage, RLI, humidity, and temperature all exhibited normal distributions, so the following parametric methods were used for analysis. Analysis of variance (ANOVA) was used to detect overall treatment effect within the model, followed by an Each Pair, Student's t-Test for multiple comparisons among treatments ($\alpha = 0.05$). In 2014, insect counts and yield were dependent variables, analyzed

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separately. Treatment and block were independent variables, analyzed as fixed effects in both models. In 2015, foliar injury levels and % of pods damaged were additional dependent variables, analyzed independently as fixed effects. Also in 2015, the experimental design included within-block replication; so the treatment/block interaction was included in each model as a fixed effect. Per plot values for each dependent variable were calculated, and used as replicates in statistical models for all experiments.

Results and Discussion

Reflected Light Intensity (RLI), Temperature and Humidity. Temperature and humidity remained starkly similar among all treatments in 2015; however, clear and consistent trends were observed for RLI (Figure 3.1a). Weekly average RLI on metallized plastic was significantly higher than all other treatments (Figure 3.1b). White plastic RLI was significantly higher than black plastic and bare soil. Black plastic and bare soil RLIs were not different from each other.

In summary, temperature and humidity at canopy level were not affected by treatments. Metallized plastic was the most reflective treatment in terms of short wavelength light, followed by white plastic. Black plastic was not any more or less reflective than bare soil.

Insects. *Mexican bean beetle*. Treatment effects for Mexican bean beetle life-stages and instars closely coincided with the observed differences in RLI. In all three seasons, metallized plastic plots harbored lower densities of Mexican bean beetle at all, or most, life-stages compared to all other treatments. White plastic plots had the second lowest densities of Mexican bean beetle, while black plastic and bare soil plots experienced the highest beetle densities. Black plastic harbored more larvae, and bare soil harbored more adults and eggs.

Mexican bean beetle adults and eggs were found on 15 sample dates, over three seasons. Significantly fewer adults were found in metallized plastic plots than bare soil plots on seven dates, black plastic plots on six dates, and white plastic plots on six dates (Figure 3.2a). Significantly fewer eggs were found in metallized plastic plots than bare soil plots on eight sample dates, black plastic plots on four dates, and white plastic plots on two dates (Figure 3.2b). Mexican bean beetle early instars were found on 10 sample dates, and significantly fewer were found in metallized plastic plots than bare soil on two dates, black plastic plots on three dates, and white plastic plots on zero dates (Figure 3.2c). Late instar larvae were found on five sample dates, and significantly fewer were found in metallized plastic plots than bare soil plots on two dates, black plastic plots on three dates, and white plastic plots on one date (Figure 3.2d.). Pupae were found on two sample dates, and significantly fewer were found in metallized plots than bare soil plots on one date. Late instar larvae or pupae were not found in the 2015 (late) experiment, likely due to cooler evening temperatures that limited development (Sweetman 1932, Mellors and Bassow 1983). Metallized plastic plots never harbored significantly higher number of Mexican bean beetles than any other treatment, for any life-stage.

Overall, metallized plastic considerably reduced densities of Mexican bean beetle at all life-stages. White plastic exhibited a similar, but less pronounced effect. Light intensity, temperature and humidity are the most important environmental factors that limit Mexican bean beetle's potential to damage crops (Howard and English 1924, Miller 1930, Auclair 1960, Kitayama et al. 1979, Wilson et al. 1982). Because Mexican bean beetle densities decreased relative to increasing RLI, while temperature and humidity were unaffected, it is likely that RLI was responsible for the reduced beetle density.

Predatory arthropods. The predominant predatory arthropods observed in this experiment were spiders (Araneae), lady beetles, damsel bugs (Hemiptera: Nabidae), minute pirate bugs (Hemiptera: Anthocoridae), and spined soldier bug, *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae). Only spiders and spined soldier bugs were significantly affected by treatments (Table 3.2). In 2014, more spiders were found in metallized plastic than black plastic and bare soil; white plastic had more spiders than bare soil. In 2015 (early), there was, again, a significant effect of treatment on spiders, with black plastic and white plastic having higher densities than bare soil. Also in 2015 (early), more spined soldier bugs were found in black plastic than bare soil and white plastic.

Overall, the treatment effects witnessed in these experiments were marginal, and cannot be explained without a high degree of speculation. It is more worthwhile to emphasize that there was no evidence to suggest that either metallized or white plastic will reduce predator numbers compared to the conventional strategy of planting on bare soil. Simmons et al. (2010) also found reflective mulches to have no adverse effects on natural enemies of whiteflies.

Plant Injury. After the 2014 experiment, it became apparent that plots with heavily infested and injured plants early in the experiments had lower carrying capacities for beetles later in the experiments, compared to less infested plots. Therefore, density data becomes less accurate, or useful, toward the end of experiments. For example, a heavily fed upon plot may have the same, or fewer late instar Mexican bean beetles than a slightly fed upon plot; but the data suggest that there is no difference between the two. Wells et al. (1984) observed a similar phenomenon, when healthier and less injured beans on reflective mulch harbored more potato leafhoppers as the plants neared maturity. For the 2015 experiments, assessments of feeding injury by Mexican bean beetle were added to more thoroughly document pest pressure.

Foliar feeding. In the 2015 (early) experiment, there was a significant effect of treatment on Mexican bean beetle foliar injury at both flowering and pod stages. Plants in bare soil plots had the highest level of foliar injury, followed by black plastic, white plastic, and metallized plastic, respectively (22 June 2015: $F = 29.9$; $df = 3, 32$; $p < 0.0001$. 6 July 2015: $F = 20.7$; $df = 3, 32$; $p < 0.0001$. Figure 3.3a). Similar treatment effects were observed in the late experiment. At flowering stage, bare soil had the most injury, followed by black plastic, white plastic and metallized plastic, respectively ($F = 34.8$; $df = 3, 28$; $p < 0.0001$. Figure 3.3b). At pod stage, plants on metallized plastic had significantly less foliar injury than all other treatments, and injury was not different among bare soil, black plastic, and white plastic ($F = 9.3$; $df = 3, 28$; $p = 0.0003$. Figure 3.3b).

The correlation between foliar feeding injury by Mexican bean beetle and RLI was very similar to the Mexican bean beetle density results. Feeding injury decreased with increasing RLI; suggesting that increasing RLI, via reflective plastic mulch, reduced pest pressure from Mexican bean beetle. The only deviation was that black plastic experienced reduced injury compared to bare soil, though RLI between black plastic and bare soil were similar. This suggests that plastic, regardless of color, has some ability to reduce injury from Mexican bean beetle.

Our experiments demonstrated that Mexican bean beetle injury was negatively correlated to increasing light intensity from plastic mulches. Differences in beetle densities are likely responsible for decreasing injury levels in white and metallized plots, respectively. It is also possible that individual beetles feed less under higher stress from increased light intensity. Miller (1930) reported that when Mexican bean beetles were exposed to direct light, they became restless, stopped feeding, and initiated relocation behavior. This may mean that beetles spend

more time moving than feeding, when exposed to direct light. More research is needed to determine if reflective mulches may limit the feeding potential of individual beetles, in addition to suppressing their population.

Pod feeding. There was a significant effect of treatment on damaged pods in both 2015 experiments. Most notably, fewer pods were damaged by Mexican bean beetle in metallized plastic plots than bare soil plots in both 2015 experiments (Table 3.3). White plastic reduced pod damage compared to bare soil in the 2015 (early) experiment, and black plastic reduced pod damage compared to bare soil in the 2015 (late) experiment.

These results further suggest that metallized plastic mulch reduces Mexican bean beetle feeding injury on snap beans, and also show that white and black plastic exhibit a lesser potential at the same goal. Again, it is unclear whether the reductions in feeding damage were the result of lower beetle densities, reduced feeding on an individual level, or both.

Yield. There was a significant effect of treatment on yield in 2014 and 2015 (early) (Table 3.3). In 2014, plants on metallized plastic produced significantly more pods by weight (yield) than all other treatments. The average yield from metallized plastic plots was more than two times greater than white plastic and black plastic (individually), and five times greater than bare soil. White plastic and black plastic were similar, and also produced significantly higher yields than bare soil. In the 2015 (early) experiment, metallized plastic plots had significantly higher yields than all other treatments, with double the yield of bare soil plots. Yields were not different among white plastic, black plastic, or bare soil. In the 2015 (late) experiment, yields for all treatments were much lower than in the previous two experiments (94% lower than the previous seasons) due lower than normal temperatures in August and September, and consequently, these data were not analyzed.

Our experiments alone cannot define the exact mechanism(s) linked to the observed differences in snap bean yields. Reflective mulches may have affected yields indirectly, by suppressing Mexican bean beetle, or by directly influencing plant health and vigor (or a combination of both). Previous research corroborates both mechanisms. Mexican bean beetle feeding injury has been shown to reduce snap bean yield after approximately 20% defoliation (Capinera et al. 1987, Fan et al. 1993). In our study, almost all plants in bare soil plots greatly surpassed 20% defoliation, as did many plants in black plastic plots, making it very likely that feeding injury was at least partially responsible for yield differences. In addition to limiting insect pressure, higher light intensity environments have been shown to enhance snap bean vigor by increasing the dry weight of roots and shoots, leaf sugar concentrations, and density of stomata in leaves (Knecht and O'Leary 1972, Marschner and Cakmak 1989). Throughout our experiments, light intensity was significantly higher in metallized plots than all other treatments, especially bare soil and black plastic. Furthermore, reflective mulches keep soil temperatures lower than black plastic and bare soil (Ham et al. 1993, Lament 1993), which can prevent certain types of injury to snap beans, such as chlorosis, stunted growth, underdeveloped nodules, and reduced ability to fix nitrogen (Piha and Munns 1987). In a similar study to ours, Wells et al. (1984) applied insecticides in combination with reflective mulch treatments, and found that reflective mulch increased snap bean yields, regardless of insect pressure. Considering these previous studies in regard to our results, it is likely that the yield differences seen in our experiments were the result of combined direct (plant response) and indirect (pest response) mechanisms related to mulch treatments.

Implications. Our experiments demonstrated that growing snap beans on the highly reflective plastic mulch, 'metallized' (Sunup Reflective Films, Oceanside, CA), reduced the

severity of Mexican bean beetle and increase yields, relative to treatments that were decreasingly reflective. In determining the implications of these findings, it is important to consider that there are various types of reflective mulches, with different constructions and levels of reflectivity. Although our findings suggest that other highly reflective plastic mulches will produce control of Mexican bean beetle and increased yields, this claim cannot be substantiated without further testing of other reflective mulch types. Future studies should also consider the planting density of snap bean on reflective mulch, so that this method can be optimized for commercial scale production. In our experiments, plants were widely spaced in clusters, to facilitate accurate data collection; however, commercial recommendations suggest closer spacing of snap beans. Lastly, this method should be evaluated in more locations. Alternate locations will have different climates and insect populations, which may alter the effects of reflective plastic mulches.

Assuming that our results can be applied to other reflective mulch types, growing strategies, and locations, reflective mulch serve as a practical IPM tool for snap beans producers. An important component of IPM involves using methods that prevent, or reduce potential for pest population growth (especially those that do not require chemical applications). Although foliar and pod injury was not entirely avoided with metallized mulch in our experiments; a substantial overall increase in clean marketable pods was achieved without the use of any other control tactic. Another element of a strong IPM strategy is multi-functionality. Planting snap beans on metallized mulch has been shown to control other snap bean pests, such as potato leaf hopper and thrips, while also providing an effective barrier against weeds.

A likely challenge in the adoption of this method by growers will be the up-front cost of metallized plastic mulch. The current price of metallized plastic ranges from 10 to 35% more

than black plastic. This monetary cost may be offset directly by the reduction is insecticides, herbicides, and time spent applying pesticides, as well as increases in overall yield, fewer insect-damaged pods, and cleaner pods (due to the barrier between pods and soil). To increase profits for each mulch application, growers may consider employing a double crop strategy, in which a second round of beans or an alternate crop is directly planted into the plastic after the first crop is harvested. For example, after the 2015 (early) experiment was harvested, bean plants were pulled and 20 lettuce plants were transplanted directly into each treatment bed (40 total lettuce plants per plot). Not only did lettuce grow adequately in the reused mulch, we witnessed 60% higher yields in metallized plots than bare soil plots, and 25% higher yields than in black plastic plots (L.N., unpublished). Numerous other crops have shown similar benefits from reflective mulches (specific details are discussed in the Introduction section), and may be successful in a double-crop planting strategy.

Due to the small scale of these experiments, the findings of this study should be considered at the proof-of-concept level until further research is completed. Future studies should incorporate planting schemes that more closely represent a commercial growing scenario. Growing snap beans on metallized should be initiated on a trial basis, with careful monitoring of pests, the environment, and economic factors.

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Table 3.1. Scale for rating Mexican bean beetle feeding injury on snap bean foliage.

Ordinal Rank	Definition of Injury
0	No feeding injury
1	Less than five leaves exhibit injury; no leaf with more than 10% injury
2	Most leaves exhibit injury; no leaf with above 50% injury
3	Most leaves exhibit injury; 10 or less leaves with above 50% injury
4	Most leaves exhibit injury; more than 10 leaves with above 50% injury; some green remains
5	All remaining plant tissue (if any) is brown, and plant is likely dead

Table 3.2. Mean cumulative densities (± SEM) of predatory arthropods from 2014 and 2015 (early)[a] observed on 10 snap bean plant clusters per plot over five sample dates, grown on different plastic mulch treatments and bare soil.

	Spiders	Lady Beetles [b]	Damsel Bugs	Minute Pirate Bugs	Spined Soldier Bugs
2014					
Bare Soil	2.3 ± 0.6**c**	5.0 ± 2.3	1.3 ± 0.8	0.3 ± 0.0	0.0 ± 0.0
Black Plastic	2.8 ± 0.5**bc**	5.0 ± 0.4	2.0 ± 0.8	0.0 ± 0.0	0.0 ± 0.0
White Plastic	7.5 ± 2.9**ab**	8.8 ± 2.8	0.5 ± 0.3	0.8 ± 0.3	0.5 ± 0.0
Metallized Plastic	7.3 ± 1.7**a**	5.3 ± 1.7	1.3 ± 0.8	0.5 ± 0.0	0.0 ± 0.0
Statistics	$\chi^2_{3,\,12} = 10.5$ $p = 0.0148$	ns	ns	ns	ns
2015 (early)					
Bare Soil	2.0 ± 0.4**b**	1.1 ± 0.5	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1**b**
Black Plastic	4.0 ± 0.7**a**	1.4 ± 0.4	0.3 ± 0.2	0.1 ± 0.1	0.8 ± 0.3**a**
White Plastic	4.9 ± 0.9**a**	0.6 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0**b**
Metallized Plastic	2.6 ± 0.5**ab**	0.7 ± 0.3	0.3 ± 0.2	0.0 ± 0.0	0.2 ± 0.1**ab**
Statistics	$\chi^2_{3,\,32} = 8.8$ $p = 0.0315$	ns	ns	ns	$\chi^2_{3,\,32} = 8.8$ $p = 0.0320$

Values within a column not sharing letters are different according to a Wilcoxon each pair test ($p < 0.05$).
[a] Predators numbers from the 2015 (late) exp were too low for analysis.
[b] 90% of lady beetles were *Coleomegilla maculata* (De Geer). Other species included *Hippodamia convergens* Guérin-Méneville, *Coccinella septempunctata* L., and *Harmonia axyridis* (Pallas).

Table 3.3. Mean snap bean yield (± SEM) from 2014, 2015 (early) and 2015 (late), and mean no. of pods damaged by Mexican bean beetle (± SEM) from 2015 (early) and 2015 (late), harvested from 10 plant clusters in four replicates per treatment in 2014, nine replicates in 2015 (early) and eight replicates in 2015 (late).

Treatment	2014	2015 (early)		2015 (late)	
	Yield (g) per 10 plants	Yield (g) per 10 plants	% Damaged Pods[1]	Yield (g) per 10 plants	% Damaged Pods[1]
Bare Soil	527.5 ± 379.5**c**	1023.6 ± 168.1**b**	67.0 ± 4.1**a**	60.6 ± 7.9	33.8 ± 3.3**a**
Black Plastic	1121.7 ± 714.7**b**	1121.0 ± 213.1**b**	51.1 ± 3.2**b**	74.9 ± 6.9	27.1 ± 2.4**ab**
White Plastic	1263.1 ± 683.9**b**	1372.9 ± 150.7**b**	41.1 ± 5.0**bc**	104.6 ± 6.8	29.8 ± 2.8**a**
Metallized Plastic	2699.4 ± 1110.7**a**	2115.1 ± 302.1**a**	39.0 ± 4.2**c**	82.1 ± 8.2	20.0 ± 3.6**b**
Statistics	$F_{3, 12} = 12.3$ $p = 0.0015$	$F_{3, 32} = 5.0$ $P = 0.0077$	$F_{3, 32} = 10.5$ $P = 0.0001$	n/a[2]	$F_{3, 28} = 5.1$ $P = 0.0069$

Values within a column not sharing letters are different according to Student's t Test ($p < 0.05$).
[1]No. of damaged pods out of 100 per plot.
[2]Yield data from 2015 (late) were not analyzed due to extremely low production.

Figure 3.1. Mean reflected light intensities (RLI) on metallized mulch, white mulch, black mulch, and bare soil from readings taken every 10 min, starting at the first positive reading (sunrise) to the last positive reading (sunset); (a) daily means; (b) seven-day mean (6SEM) from 12 replicates per treatment. Values not sharing the same letter are different according to Student's t test (P<0.05).
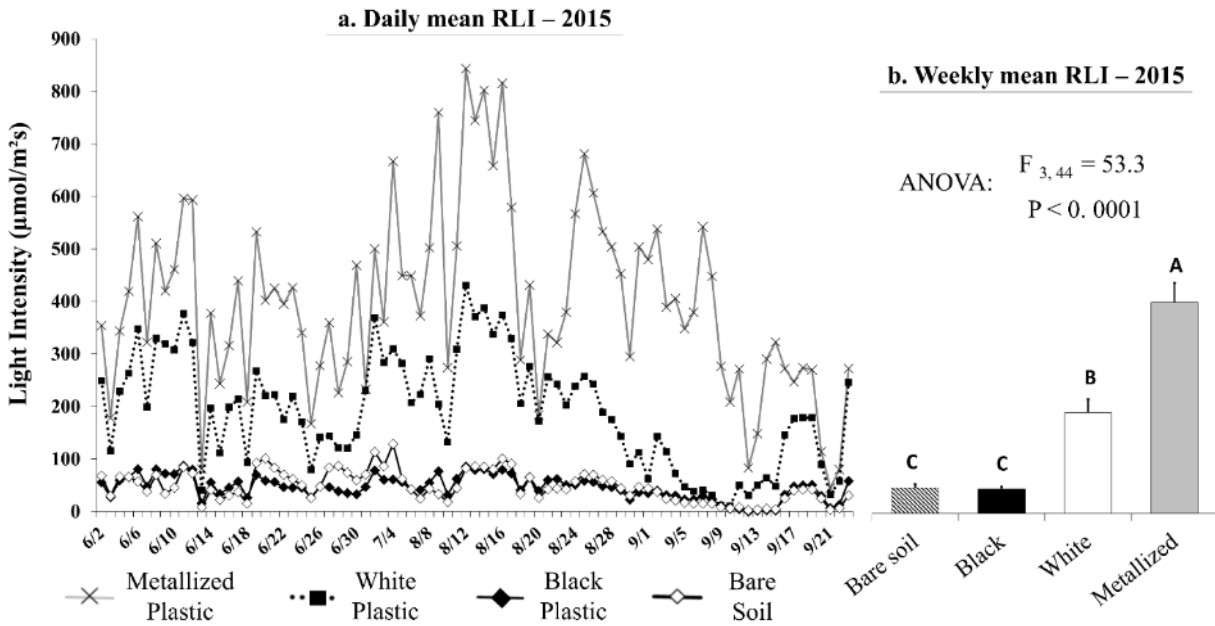
Figure 3.2. Mexican bean beetle (a) adults (first column), (b) eggs (second column), (c) early instars (third column), and (d) late instars (bottom column), observed in snap beans grown on metallized mulch, white mulch, black mulch, and bare soil. Values shown are the mean densities (6SEM) of individuals on 10 plant clusters per plot, from four replicates per treatment in 2014 (left column), nine replicates in 2015 (early) (middle column), and eight replicates in 2015 (late) (right column).
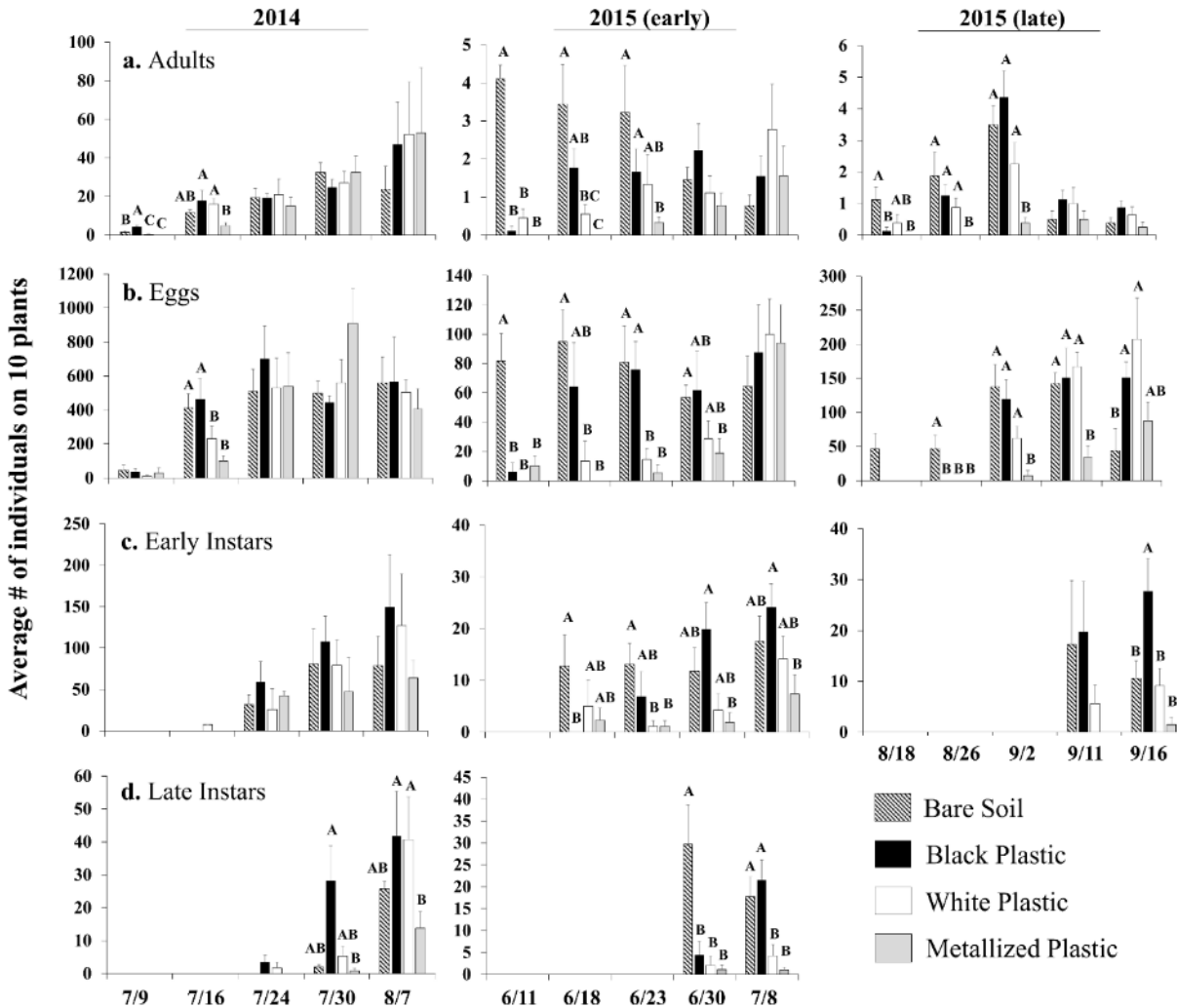
*Values within a sample date not sharing a similar letter are different according to a Wilcoxon Each Pair Test (p < 0.05)

Figure 3.3. Mexican bean beetle foliar feeding injury levels to snap bean plants grown on metallized mulch, white mulch, black mulch, or bare soil in (a) 2015 (early) and (b) 2015 (late). Individual plants were rated on a scale of 0 to 5 (Table 3.1). Values shown are the mean ratings (6SEM) of 10 plant clusters per plot, from nine replicates per treatment in 2015 (early) and eight replicates in 2015 (late).
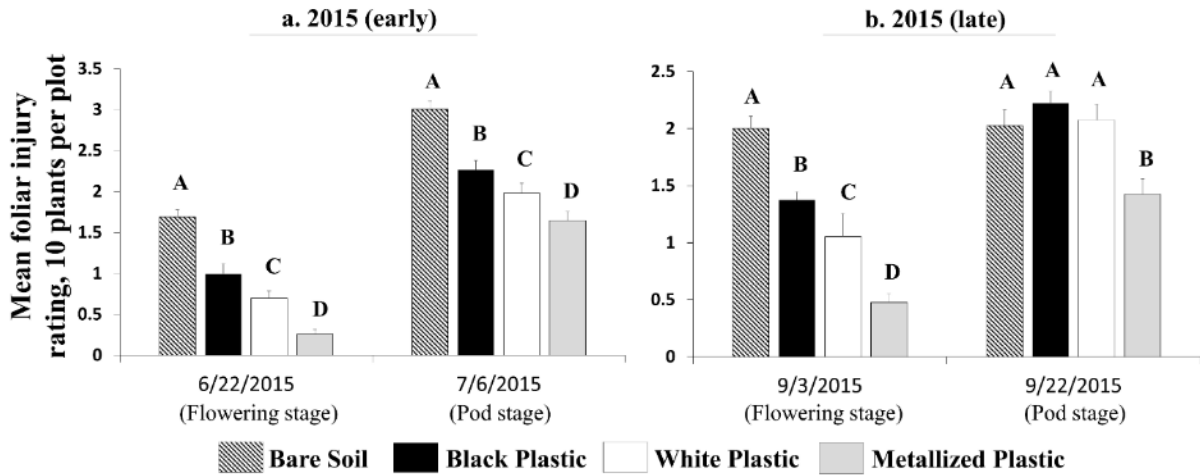
*Values within a sample date not sharing a similar letter are different according to a Student's t-Test (p < 0.05).

CHAPTER FOUR

The Effects of Thiamethoxam-treated Snap Bean Seeds on Mexican Bean Beetle, Non-target Arthropods, and Crop Performance.

Abstract

The neonicotinoid insecticide thiamethoxam is commonly applied as a seed-treatment to commercial snap beans, *Phaseolus vulgaris* L. While previous studies highlight the efficacy of this seed-treatment in snap beans, none have been conducted in agroecosystems where the predominating pest is Mexican bean beetle, *Epilachna varivestis* Mulsant. This study examined the effects of snap beans grown from thiamethoxam-treated seed on Mexican bean beetle, non-pest arthropods, and crop performance in southwestern Virginia. Greenhouse experiments were conducted to test the residual toxicity of plants grown from thiamethoxam-treated seeds to Mexican bean beetle, and its predator, *Podisus maculiventris* (Say). Seed-treatment plants were highly toxic to Mexican bean beetle up to 16 days after planting (1st true leaf to 1st trifoliate stage), moderately toxic from 16 to 24 days (1-3 trifoliate stage), and rarely toxic at 24 days and beyond. Treated plants showed no direct or secondary toxicity to *P. maculiventris*. Field plot experiments were conducted from 2013 to 2016, to measure densities of Mexican bean beetle, predatory arthropods, and non-pest herbivores, as well as crop health and yield. In four out of five field seasons, there were no effects from seed-treatments on densities of beetles, other arthropods, or crop yield. However, in 2016, planting was delayed, which resulted in Mexican bean beetle populations attacking plants at a younger developmental stage. Beans grown from thiamethoxam-treated seed had lower beetle densities and concomitant injury, resulting in significantly higher yields. Natural enemy densities were unaffected by the seed-treatment in all field experiments. In conclusion, thiamethoxam-treated seeds can provide significant control of Mexican bean beetle; however, such effects were rarely observed in our experiments.

Introduction

The use of neonicotinoid insecticides applied as a seed-coating ("seed-treatments") are prolific in commercial agriculture, due to their ease of use and efficacy against early season pests (Douglas and Tooker 2015). This method, however, is under scrutiny because of wide-scale implementation and potential non-target effects, particularly on pollinators (Douglas and Tooker 2015, 2016; Lundin et al. 2015). Neonicotinoid seed-treatments are attractive to growers for multiple reasons. Seeds are pretreated by manufacturers with lower rates of active ingredient compared to in-furrow sprays, soil drenches, and foliar applications (Taylor et al. 2001), making this method easy, relatively safe, and inexpensive to the user. Neonicotinoids move systemically throughout developing plants providing control of a broad range of insect pests, while having low mammalian toxicity (Maienfisch et al. 2001, Nault et al. 2004, Koch et al. 2005, Reisig et al. 2012). Lastly, some neonicotinoids have been shown to trigger physiological stress responses of plants, increasing plant health factors that may be unrelated to pest pressure (Ford et al. 2010, Szczepaniec et al. 2013, Afifi et al. 2015).

These benefits have arguably led to the overuse, or unjustified use, of neonicotinoid seed-treatments, by encouraging growers to use insecticides prophylactically, instead of when pests reach economic or action thresholds (Stern 1973, Philips et al. 2014, Douglas and Tooker 2015). Overuse of insecticides also has the potential to result in insecticide resistance, harm to beneficial arthropods, potential secondary pest outbreaks, and negative ecological impacts from active ingredients moving into off-target locations (Radcliffe and Hutcheson 2009, Prabhaker et al. 2011, Seagraves and Lundgren 2012). To help elucidate the situations in which insecticide seed-treatments are necessary (or not), it is important to evaluate the risks and benefits of seed-treatments under various scenarios (different crops, pest complexes, climates, geographies, etc.).

This will provide more accurate information catered to specific systems; thus, helping individual growers decide whether they should use insecticide-treated seeds from an empirical standpoint.

Thiamethoxam, a broad-spectrum neonicotinoid insecticide, is one of the most common insecticides used as a seed-treatment for snap beans, *Phaseolus vulgaris* L. Thiamethoxam seed-treatments are known to control a variety of snap bean pests, such as potato leafhopper *Empoasca fabae* (Harris) (Hemiptera: Cicadellidae), bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae) and soybean thrips, *Neohydatothrips variabilis* (Beach) (Thysanoptera: Thripidae) (Nault et al. 2004, Koch et al. 2005, Reisig et al. 2012). However, in the Appalachian Mountains of the Mid-Atlantic U.S., the primary (and sometimes the only) economic pest of snap beans is Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae) (Fess 2008, Nottingham et al. 2016). Mexican bean beetle reaches economic levels in snap beans in most years throughout this region; yet no peer-reviewed research has been conducted on the effects of thiamethoxam-treated snap beans in agroecosystems dominated by this pest. The goals of the experiments herein were to quantify the effects of growing snap beans from thiamethoxam-treated seeds on: 1) Mexican bean beetle densities and feeding injury; 2) toxicity to *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), a common predator of Mexican bean beetle; 3) densities of other arthropods (herbivores and predators); and 4) overall crop performance (stand, plant vigor and yield).

Materials and Methods

Treatments. For all field and laboratory experiments, 'Caprice' snap beans were purchased from Stokes Seeds (Buffalo, NY) at the beginning of each year. Seeds were purchased from the distributor as untreated (UT), pre-treated with thiamethoxam (Cruiser, Syngenta Crop Protection, Greensboro, NC) applied to seeds at 46.88g (a.i.)/100 kg of seed plus

a combination of fungicides (CT), or treated with fungicides only (FT). Laboratory experiments

only tested FT and CT treatments.

Lab Experiments. *Residual Toxicity to Mexican bean beetle*. In the summer of 2016,

experiments were conducted to determine the length of time after planting that thiamethoxam-

treated snap beans remained toxic to Mexican bean beetle adults and larvae. 'Caprice' snap bean

plants with the treatments CT and FT were grown in a glass green house at Virginia Tech, with

temperatures set to not exceed 29°C (85°F). Snap beans were sown in plastic growing trays until

first true leaves formed. Three plants of the same treatment were transplanted into a plastic cup

container, 12 cm diam and 20 cm tall, with 10 cm of potting soil. Mexican bean beetle adults

and larvae were collected from untreated field snap beans. Beetles were placed in 12×12 inch

mesh cages, and stored in a greenhouse for 24 h without food before being used in experiments.

For each life-stage, five beetles were placed on plants in each container using a small paintbrush,

and containers were covered with a mesh lid. There were four replicate containers per treatment

(n= 20) for each life-stage. This procedure was repeated with new plants and beetles at

incremental days after planting. Numbers of living and dead individuals were counted 72 h after

introduction and corrected mortality was calculated using the formula:

$\frac{\% \text{ mortality CT} - \% \text{ mortality FT}}{100 - \% \text{ mortality FT}} \times 100$. Four experiments were conducted using this design: two

assessing toxicity to Mexican bean beetle larvae, and two for Mexican bean beetle adults.

Direct and secondary toxicity to Podisus maculiventris (Say). In the summer of 2016,

experiments were conducted to examine the acute toxicity of snap beans grown from

thiamethoxam-treated seeds to *P. maculiventris* either directly or via ingestion of prey. This

stinkbug (family: Pentatomidae) was commonly observed feeding on most life-stages (all except

eggs) of Mexican bean beetle Virginia Tech's research farm, Kentland. Also, *P. maculiventris* is omnivorous, and known to feed on snap bean plants (O'Neil and Wiedenmann 1990).

P. maculiventris were acquired from a laboratory colony at the USDA in Beltsville, MD. Toxicity of thiamethoxam to *P. maculiventris* was evaluated in three ways: exposure to plants only, to prey only, and to plants and prey. 'Caprice' snap bean plants were grown from seeds in the same manner described in residual toxicity assays. The treatments CT and FT were used for all experiments.

Two identical experiments were conducted to evaluate direct toxicity of thiamethoxam snap beans on *P. maculiventris*. Five, fifth instar *P. maculiventris* nymphs were placed in mesh cages (30×30 cm) with snap bean plants. Twelve snap bean plants were grown in a plastic germination tray located in each cage. Experiments started when plants were 10 d old. There were four replicate cages per treatment (n = 20) for each experiment. Cages were checked every day for six days. This was enough time to allow all surviving bugs to molt into adults. The numbers of dead and living individuals were documented once all *P. maculiventris* had molted or died.

Secondary toxicity is when an individual receives a toxic dose of a substance (insecticide) by ingesting prey matter from an individual(s) that previously fed on the toxicant (Lloyd and McQueen 2000). Secondary toxicity of *P. maculiventris* via ingestion of its common prey source, Mexican bean beetle, was examined without the presence of treated plants to ensure that poisoning would not occur from ingestion of, or contact with, treated plant matter. Mexican bean beetle adults and third instar larvae were tested as the prey sources in separate experiments (one experiment for beetle adults, and one for larvae). Mexican bean beetle adults and larvae were fed FT or CT snap beans for six hours before being used in experiments. Plants used to

feed beetles were 10-13 d old (highly toxic stage for CT plants). *P. Maculiventris* nymphs were isolated without food for 48-72 h. One nymph was placed into a plastic cup container, 12 cm diam and 10 cm tall with a partially mesh lid (one replicate). Two Mexican bean beetles (either larvae or adults) were added to each container per day, for six days. One water-soaked cotton wick was added to each container and replaced daily. There were 16 replicate containers per treatment (n = 16). Cups were checked for dead *P. maculiventris* every 24 h. A mortality rating system (see Table 4.4) was developed to quantify death occurrences and the length of time to death. This scale was based on the idea that shorter times to death are more significant than longer times to death.

The final two experiments incorporated plants and prey together. One experiment used Mexican bean beetle larvae as prey, the other used adults. The design was the same as the plants-only experiments, with four cages for each treatment having 12, 10 d old plants and five, fifth instar *P. maculiventris* per cage (n=20). Each day, five new Mexican bean beetle were placed on the plants in each cage, and those from the previous day (both living and dead) were removed. Numbers of living and dead *P. maculiventris* were documented after six days.

Field Experiments. *Design.* Experiments took place at Virginia Tech's Kentland Farm in Whitethorne, VA (37.2013° N, -80.5656° W). In total, five field experiments were conducted, two in the summer 2013 (one planting in the spring, [early] and one in the summer [late]), and one per summer in 2014, 2015 and 2016. All experiments were arranged in a randomized complete block design, but the number of reps and plot size changed from year to year depending on available land-space. For all experiments, fields were prepared for planting by broadcasting 10-10-10 fertilizer at a rate of 112 kg per ha, followed by application of pre-emergence herbicides halosulfuron-methyl (Sandea 75DF, Gowan Company, Yuma, AZ) and S-

metolachlor (Dual Magnum, Syngenta, Greensboro, NC) at 55.4 mL a.i./ha and 0.59 L a.i./ha, respectively. Additional weeding was performed by hand as needed for the duration of experiments. Irrigation was supplied as needed using drip tape running alongside each row of beans.

In 2013, 2014, and 2016, snap beans were planted approximately one seed per 6.1 cm using a hand-pushed mechanical seeder (EarthWay, Bristol, IN). In 2013, there were only two treatments, UT and CT with 16 reps. Snap beans were planted on 3 May (early) and on 14 July (late). Each individual plot (one replicate) was four rows wide and 6.1 m long, rows within plots were separated by 45 cm of bare soil, and plots were separated by 2 m of bare soil. In all remaining field experiments, there were three treatments, UT, CT, and FT. In 2014, beans were planted on 8 May, each plot was eight rows wide and 7.6-m long, rows within plots were separated by 45 cm of bare soil, and plots within blocks were separated by 2 m of bare soil. In 2015, plots were planted on 18 May, and plot size and row spacing remained the same as 2014, but seeds were sown by hand at approximately 1 seed every 12.5 cm. In 2016, there were nine reps. Beans were planted on 26 May, which was later than previous years due to persistent spring precipitation. Each plot was eight rows wide and 10.7 m long; rows within plots were separated by 36 cm of bare soil and plots within blocks were separated by 2 m of bare soil.

Insect Sampling. Plots were sampled for insects using three techniques: visual plant inspections, vacuum samples, and pitfall traps. Potato leafhopper nymphs, *Empoasca fabae* (Harris), and thrips (consisting mostly of soybean thrips, *Neohydatothrips variabilis* [Beach]) were sampled by visually inspecting and counting individuals on 30 arbitrarily-selected leaves per plot. Visual inspection methods for all other insects, including Mexican bean beetle life-stages (eggs, early instars, late instar, pupae and adults), were conducted by visually inspecting

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and counting individuals on 10 arbitrarily-selected plants per plot. Visual samples were performed twice per week from 7 June through 12 July in 2013 (early) and from 2 August through 6 September in 2013 (late); and once per week from 10 June through 8 July in 2014, from 4 June through 10 July in 2015, and from 9 June through 19 July in 2016. In 2015, only potato leafhopper and thrips were sampled via visual plant inspections; all other insects were sampled using vacuuming or pitfall traps.

Vacuum samples were performed in 2014, 2015 and 2016 experiments. Samples were conducted on the same day after visual samples. Vacuum samples were conducted using a modified leaf blower (Stihl, Virginia Beach, VA), with the tube attached to the suction fan for insect collection. For each sample, 10 plants per plot were arbitrarily-selected and vacuumed for approximately two seconds per plant. A fine-mesh 3.8 liter paint strainer bag was attached to the end of the tube to catch arthropods. Bagged arthropods were immediately brought back to the laboratory and stored in a freezer before being identified.

Pitfall sampling was conducted in 2014, 2015 and 2016. Pitfall traps were made from cylindrical food storage containers (0.95 liter, 11.5 cm diam opening, W. Y. Industries, North Bergen, NJ). Containers were buried in the ground and filled with 0.4 liters of soapy water. Traps were set on Tuesdays and checked on Fridays of each week, during the same seasonal time-frame as visual and vacuum sampling. Arthropods in traps were collected and brought back to the laboratory for identification.

Stand, Plant Vigor, Yield and Pod Damage. Stand counts were conducted at first trifoliate stage for each experiment; all plants per plot were counted. Yield samples were taken at pod maturity 58-62 days after planting. Two inner rows were arbitrarily-selected from each plot and all pods were harvested by hand from half of each row. All pods collected were

weighed on an electronic scale (Adventurer Pro, Ohaus Corp, Parsippany, NJ). For each row, the

total number of plants were counted and the length of row (distance from first to last plant) was

measured. The length of row sampled for yield varied among seasons. The percentage of pods

damaged by Mexican bean beetle was determined by arbitrarily-selecting one-hundred pods per

plot, and rating each as either damaged or undamaged using methods developed by Nottingham

and Kuhar (2016). Plant vigor (foliar area) was evaluated in 2015 and 2016 only. Foliar area

measurements were obtained using a hand-held NDVI (normalized difference vegetation index)

sensor (GreenSeeker, Trimble Navigation Limited, Sunnyvale, CA) at flowering stage in 2015

and 2016. The entire foliar area of each plot was measured by walking along the side of each

row while holding the sensor at hip height.

Data Analysis. All data were analyzed using JMP Pro 11 (SAS Institute Inc. 2007).

Treatment was used as a fixed effect and was the main independent variable in all experiments.

Blocking was also a fixed effect, incorporated into field experiment models only. The

interaction effect between treatment and block was a fixed effect, and was only incorporated for

experiments with replication within blocks (2013 [early], [late], and 2015). Dependent variables

were values of arthropod densities, mortality, NDVI rating, yield, and pod damage. All

arthropod count data were transformed to fit a normal distribution using the formula:

$\sqrt[2]{(sample\ value) + 0.375}$ (Zar 2010). Treatment effects were determined using ANOVA,

when $p < 0.05$.

Results and Discussion

Greenhouse Assays. *Residual Toxicity to Mexican bean beetle*. Thirteen days after

planting, bean plants grown from thiamethoxam-treated seeds (CT) were fatally toxic to an

average of 88.9 and 95.8% of Mexican bean beetle adults and larvae, respectively (Fig. 4.1). At

16 and 20 days after planting, mortality dropped to averages of 43.5 and 41.7%, respectively, for

adults, and 53.8 and 35.4%, respectively, for larvae. At 24 days after planting, no adults were

killed, but the CT treatment resulted in 21.7% mortality of the larvae. At 26 days after planting,

only 5.2% mortality of larvae was observed in the CT. Overall, CT bean plants were highly

toxic to Mexican bean beetle from 13 to 16 days (1st true leaf to 1st trifoliate stage), moderately

toxic from 16 to 24 (1-3 trifoliate stage) days, and barely (or not) toxic at 24 days and beyond.

These assays elucidate the approximate amount of time that CT snap beans will control Mexican

bean beetle adults, and suppress larval development if adults deposit eggs without or before

ingesting a toxic dose.

    Direct and Secondary Toxicity to Podisus maculiventris. *P. maculiventris* was unaffected

by CT plants in all experiments: plants alone, prey alone, and plants and prey together (Table

4.3). Laboratory experiments were designed to create high potency exposure scenarios, by using

young plants in the first true-leaf stage. The lack of effect across three experiments (types of

exposure) suggests that these bugs may not be easily harmed by snap beans grown from

thiamethoxam-treated seeds. Nevertheless, more work should be performed in field settings, and

on sub-lethal effects such as longevity, fecundity, and offspring sex ratios.

    Field Experiments. *Insects*. The UT treatment was omitted from 2014, 2015, and 2016

statistical models, because treatment effects on insects and crop performance may have been the

result of plant health differences related to the lack of fungicides, not thiamethoxam. UT is not

present in results from 2014, 2015, and 2016.

    No treatment effects were observed in densities of any predatory arthropod group, during

any field season. For non-pest herbivore densities, the only treatment effect in any of the

experiments occurred in 2015, when thrips densities were higher in FT than CT treatments

(Table 4.1). Analyses of thrips densities at individual sample dates revealed that population

differences only occurred within the first two weeks of sampling, at first true leaf stage on 4 June

(FT = 15.89 ± 2.67, CT = 0.75 ± 0.37; p < 0.0001; F = 9.79; df = 1,14) and 1-2 trifoliate stage on

11 June (FT = 13.25 ± 2.52, CT= 4.38 ± 1.74; p = 0.0079; F = 3.18; df = 1,14). Despite these

differences, thrips numbers remained low overall (1-4 nymphs per trifoliate in the FT treatment),

and balanced among treatments by the third week of sampling (3-5 trifoliate stage).

Snap beans grown from thiamethoxam-treated seeds are known to control thrips up to

five weeks in soybean, *Glycine Max* L. (Reisig et al. 2012). Our results suggest that similar

control may occur in snap beans. Soybean thrips are generally an early-occurring herbivore in

Virginia (Reisig et al. 2012), and are an important food source to predators such as *Orius*

insidiosus (Say) (Kiman and Yeargan 1985), the most abundant predator observed in our foliar

samples (table 4.1). Lower densities of thrips could, in theory, result in lower densities of *O.*

insidiosus, given its affinity for preying on thrips. Also, previous laboratory studies have

documented lethal and sub-lethal effects to *O. insidiosus* via direct exposure to thiamethoxam-

treated plants (Prabhaker et al. 2011, Seagraves and Lundgren 2012, Gontijo et al. 2015).

Despite the seemingly high risk of thiamethoxam seed-treatments to *O. insidiosus*, especially

given their predominance in our system, no differences were observed in *O. insidiosus* densities

in any of these field experiments (Table 4.1).

Cumulative densities of Mexican bean beetle were the same among treatments from 2013

through 2015 (Table 4.1). Some differences were observed at various life-stages on individual

sample dates (Figure 4.1. a-d.); but prevailing trends were difficult to discern from individual

sample dates in most seasons, other than 2013 (late) and 2016. In the 2013 (late) experiment,

beetles colonized plants early in the crops developmental cycle due to the late planting (14 July).

Adult and egg densities were significantly higher in CT plots than FT plots during early samples (Figure 4.1. b); however, many dead adults were also found in CT plots, suggesting that young CT plants were effectively toxic. Because this experiment occurred late in the season, there was heavy immigration of Mexican bean beetle adults moving out of older bean plots on the farm. Young CT plant remained attractive to immigrating beetles because the treatment was suppressing feeding injury. Concomitant larvae were lower in CT plots than FT plots early on, demonstrating that the CT treatment was effectively toxic despite having higher adult and egg densities. In the final sample of this experiment, larval densities in CT plots were significantly higher than those in FT plots (Figure 4.1. b), resulting in no differences in season-long, cumulative larval densities (Table 4.1). The results from the 2013 (late) season provide an interesting perspective for the management potential of thiamethoxam seed-treatments and Mexican bean beetle. Under high Mexican bean beetle pressure at plant emergence, the thiamethoxam seed-treated plants seemed as though it would control infestation; however, by the end of the crop cycle, cumulative densities of all life stages balanced among treatments.

The 2016 season also yielded interesting results. Plots were sown later than in previous seasons (except 2013 [late]) due to persistent rain throughout April and May. This delay allowed Mexican bean beetles to emerge from overwintering sites prior to planting, and colonize plants as soon as the first true leaves developed. Densities of adults and eggs were significantly higher in FT plots early in the season, as were concomitant larval densities later in the season (Figure 4.1. e). Unlike in 2013 (late), cumulative larval densities were higher in FT plots than CT plots, reflecting the early suppression of adults and eggs in CT plots (Table 4.1). Cumulative adult and egg densities did not differ (Table 4.1., eggs not shown) because large numbers of ovipositing adults eventually invaded the less injured CT plants, once the insecticide diminished (Figure 4.1.

e). No differences or trends were observed among other herbivorous or predatory arthropods in 2016.

The results of these experiments exemplify how seasonal and environmental differences can impact pest population dynamics and the efficacy of management strategies. In four out of five seasons, thiamethoxam-treated snap beans had no impact on Mexican bean beetle densities or on crop yield. This is likely due to the rapidly diminishing insecticide within the plants, and the generally late occurrence of Mexican bean beetle populations in snap beans. Overwintering beetles typically colonize snap beans in late May or early June, around 238–277 degree days, baseline 10°C [50°F] (Fess 2008).   Because snap beans are generally planted from early to late April, beetles normally do not colonize until well after insecticide levels in plants are too low for impactful suppression (see section: *Residual Toxicity to Mexican bean beetle*). The long, rainy spring in 2016, which delayed us from planting until late May, demonstrated how a seemingly minor deviation can significantly impact pest pressure and management needs. In this one season alone, the thiamethoxam treatment significantly reduced Mexican bean beetle larval densities and damage, which led to increased yields (see Table 4.2). Although this season seems to depict a scenario in which planting CT seeds was justified; the 2013 (late) season also had adult beetles present at plant emergence, yet CT plants did not provide effective control. The similarities in pest occurrence and dissimilar results between these two seasons further demonstrates the narrow control potential of thiamethoxam-treated seeds in snap bean systems predominated by Mexican bean beetle.

Stand, Plant Vigor, Yield and Pod Damage. Treatment effects on factors regarding yield, pod-damage and plant health from both experiments in 2013 were not included in these results because the FT treatment was not incorporated. The lack of fungicides, instead of

thiamethoxam, in the UT treatment would be equally, if not more likely to contribute to differences in stand, plant vigor, yield and pod damage. Also, the number of plants surviving to first trifoliate stage were significantly lower in UT than CT treatments, creating a reduced stand which could confound plant health and yield data.

No treatment effects were observed in stand density, pod damage, plant vigor, or yield for 2014 and 2015 (Table 4.2). In 2016, plants in the FT plots were visibly smaller and more fed-upon by Mexican bean beetle. Stand density and percentage of pods damaged were not affected by treatment; but vigor (NDVI ratings) and yield (pods/plant and pods/m of row) were significantly higher in the CT plots than FT (Table 4.2).

In the 2016 experiment, greater yields in CT plots correspond with the lower densities of Mexican bean beetle larvae. Plants in FT plots had more injury and were noticeably stunted compared to the larger, healthier CT plants. Previous studies have shown that the presence of clothianidin, the primary metabolite of thiamethoxam, can increase a plant vigor in certain plant species, by stimulating the immune response to biotic and abiotic stressors (Ford et al. 2010, Szczepaniec et al. 2013). Although these physiological benefits may have contributed to the treatment effects observed in 2016, the lack of similar treatment effects in previous seasons with less severe beetle infestations suggests that physiological benefits were minor, if present at all.

Implications. The use of neonicotinoid seed-treatments in row crop agriculture has become more routine due to their ease of use, affordability and efficacy against numerous early season pests (Douglas and Tooker 2015). The increased use of prophylactic chemical insecticides deviates from IPM principles, as chemical application should only be implemented at specific pest levels (Stern 1973). Additionally, many studies have shown the potential for neonicotinoid seed-treatments to harm to non-target organisms such as arthropod pollinators and

predators (Prabhaker et al. 2011, Seagraves and Lundgren 2012, Lundin et al. 2015, Douglas and Tooker 2016). Although neonicotinoids have shown efficacy against many snap bean pests (Nault et al. 2004, Koch et al. 2005, Reisig et al. 2012) while using lower rates of active ingredients (Maienfisch et al. 2001), neonicotinoid seed-treatments have not been tested for efficacy or non-target effects in snap bean systems predominated by Mexican bean beetle. To reduce overuse of seed-treatments, is imperative that researches gain and distribute as much information from as many cropping systems as possible, in order to become more certain whether the use of a seed-treatment is justified.

This study assessed the benefits and potential drawbacks of thiamethoxam seed-treatments on snap beans grown in a region where Mexican bean beetle is the major pest. For four consecutive seasons over three years, it appeared that there were no benefits to planting treated snap beans at our location in the Appalachian Mountains of Virginia. But the results from the final season alone suggest that under certain circumstances, the use of thiamethoxam-treated snap bean seeds can control Mexican bean beetle and significantly increase yields. Additionally, our results did not show any negative impacts of thiamethoxam-treated seed on natural arthropod predators, or lead to secondary pest outbreaks. One could argue that using a foliar insecticide application of a pyrethroid instead of treated seeds, may be more likely to result in these undesired effects. Moreover, thiamethoxam seed-treatments have been shown to provide effective control of many other pests of snap beans in particular potato leafhopper (Nault et al. 2004), bean leaf beetle (Koch et al. 2005), and thrips (Reisig et al. 2012), which could result in pest problems in other years or locations.

While the results of this study neither overwhelmingly condemn nor justify the use of thiamethoxam-treated snap bean seed as a management tool for Mexican bean beetle, they do

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shed light on the potential and limitations of the control method. Regarding Mexican bean beetle

control, other methods may better coincide with the principles of IPM. Two methods in

particular, planting beans on reflective plastic mulch (Nottingham and Kuhar 2016, and Chapter

3 of this dissertation) and inoculative releases of the parasitoid wasp, *P. foveolatus* Crawford

(Barrows and Hooker 1981, Fess 2008), have been shown to effectively suppress Mexican bean

beetle prophylactically, and without the use of insecticides. Foliar sprays of certain narrow-

spectrum insecticides have also been shown to effectively reduce Mexican bean beetle injury

(Patton et al. 2003, Nottingham et al. 2015), and may be a better chemical control option if used

at the economic threshold (around 20% defoliated leaf area according to Capinera et al. [1987]

and Fan et al. [1993]). It is therefore more practical and cohesive with IPM principles to choose

a non-chemical, prophylactic method, followed by a foliar spray(s) if necessary.

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Table 4.1. Cumulative average density ($\pm$ SEM) of individuals from arthropod groups observed on 10 plants per plot, or summed between two pitfall traps per plot. Cumulative density was calculated by summing the average density values from each plot across all sample dates in a season.

	Thiamethoxam + Fungicides (CT)	Untreated (UT)	p value	F	df
2013 (early)					
Herbivores					
Mexican bean beetle adults [VS]	12.90 ± 0.47	12.86 ± 0.50	ns		
Mexican bean beetle late instars [VS]	14.39 ± 1.32	12.82 ± 1.00	ns		
Potato leafhopper nymphs [VS]	22.61 ± 1.84	23.90 ± 2.16	ns		
Predators					
Orius insidiosus [VS]	6.55 ± 0.14	6.64 ± 0.11	ns		
Damsel bugs (Hemiptera: Nabidae) [VS]	6.49 ± 0.11	6.36 ± .10	ns		
Ladybeetles [VS]	7.375 ± 1.19	7.625 ± 1.38	ns		
2013 (late)					
Herbivores					
Mexican bean beetle adults [VS]	21.86 ± 1.38	19.66 ± 1.19	ns		
Mexican bean beetle late instars [VS]	14.70 ± 0.70	17.06 ± 1.36	ns		
Potato leafhopper nymphs [VS]	23.22 ± 1.85	24.50 ± 2.16	ns		
Predators					
Orius insidiosus [VS]	(Too few for analysis)				
Damsel bugs [VS]	(Too few for analysis)				
Ladybeetles [VS]	(Too few for analysis)				

	Thiamethoxam + Fungicides (CT)	Fungicide Only (FT)	p value	F	df
2014					
Herbivores					
Mexican bean beetle adults [VS]	11.19 ± 1.55	10.71 ± 0.88	ns		
Mexican bean beetle late instars [VS]	9.59 ± 2.04	9.19 ± 0.77	ns		
Potato leafhopper nymphs [VS]	8.45 ± 0.73	11.37 ± 1.21	ns		
Potato leafhopper adults [Vac]	24.92 ± 2.54	24.42 ± 1.62	ns		
Thrips (Thysanoptera: Thripidae) [VS]	14.35 ± 2.82	19.19 ± 3.46	ns		
Predators					
Orius insidiosus [Vac]	14.32 ± 1.35	13.82 ± 1.35	ns		
Damsel bugs [Vac]	6.62 ± 0.59	6.79 ± 0.98	ns		
Ladybeetles [Vac]	4.13 ± 0.55	4.11 ± 0.37	ns		
Ground beetles (Coleoptera: Carabidae) [P]	7.40 ± 0.44	8.14 ± 1.11	ns		

Spiders (Araneae) [Vac]	21.00 ± 5.87	20.25 ± 2.32	ns		
Spider [P]	10.84 ± 1.07	9.83 ± 1.24	ns		

2015

Herbivores

Mexican bean beetle adults [Vac]	6.15 ± 0.50	6.34 ± 0.30	ns		
Mexican bean beetle late instars [Vac]	6.35 ± 0.89	7.38 ± 0.99	ns		
Potato leafhopper nymphs [VS]	6.06 ± 0.27	5.92 ± 0.42	ns		
Potato leafhopper adults [Vac]	18.07 ± 1.12	17.59 ± 1.07	ns		
Thrips [VS]	12.04 ± 0.27	16.65 ± 0.85	0.0013*	26.47	1,15

Predators

Orius insidiosus [Vac]	3.12 ± 0.26	3.07 ± 0.21	ns		
Damsel bug [Vac]	2.84 ± 0.31	2.34 ± 0.17	ns		
Ladybeetle [Vac]	2.23 ± 0.16	2.05 ± 0.10	ns		
Ground beetle [P]	10.74 ± 0.85	10.13 ± 0.39	ns		
Spiders [Vac]	3.58 ± 0.26	3.61 ± 0.36	ns		
Spiders [P]	6.36 ± 0.41	6.42 ± 0.31	ns		

2016

Herbivores

Mexican bean beetle adults [VS]	24.90 ± 1.20	28.09 ± 1.24	ns		
Mexican bean beetle late instars [VS]	25.38 ± 3.60	63.67 ± 2.86	0.0004*	2703.0	1,4
Potato leafhopper nymphs [VS]	2.39 ± 0.07	2.52 ± 0.11	ns		
Potato leafhopper adults [Vac]	23.48 ± 1.57	25.55 ± 0.84	ns		
Thrips [VS]	2.24 ± 0.08	2.45 ± 0.07	ns		

Predators

Orius insidiosus [Vac]	6.69 ± 1.12	5.11 ± 0.38	ns		
Damsel bugs [Vac]	3.12 ± 0.11	2.83 ± 0.27	ns		
Ladybeetles [Vac]	2.58 ± 0.19	2.33 ± 0.27	ns		
Ground beetles [P]	8.59 ± 0.60	9.48 ± 0.34	ns		
Spiders [Vac]	4.04 ± 0.37	4.97 ± 0.60	ns		
Spiders [P]	8.93 ± 0.42	12.07 ± 1.03	ns		

*Values are significantly different ($p < 0.05$) according to an ANOVA.
ns = Values are not significantly different ($p < 0.05$) according to an ANOVA
[VS] Visual plant inspection sampling method
[Vac] Vacuum (reversed leaf blower) sampling method
[P] Pitfall trap sampling method

Table 4.2. Stand Counts, Plant health, Yield, and Pod damage. Average (± SEM) per plot values are shown. Sample size per plot is indicated next to dependent variable in the left column.

	Thiamethoxam + Fungicides (CT)	Fungicide Only (FT)	p value	F	df
2014					
Stand count					
First trifoliate stage, plants per plot	760.0 ± 23.1	696.0 ± 50.3	ns		
Yield					
Pod weight (g) per plant	29.10 ± 8.68	33.04 ± 9.99	ns		
Pod weight (g) per 8 m	2462.5 ± 674.0	2171.3 ± 676.5	ns		
Pod damage by Mexican bean beetle					
% damaged pods	17.50 ± 4.10	19.50 ± 6.60	ns		
2015					
Stand count					
First trifoliate stage, plants per plot	406.5 ± 7.5	402.0 ± 12.2	ns		
Plant health					
NDVI rating per plot	0.54 ± 0.01	0.53 ± 0.01	ns		
Yield					
Pod weight (g) per plant	92.48 ± 15.5	78.64 ± 8.38	ns		
Pod weight (g) per 2 m	708.25 ± 89.18	731.88 ± 77.17	ns		
Pod damage by Mexican bean beetle					
% damaged pods	12.25 ± 1.67	11.5 ± 1.92	ns		
2016					
Stand count					
First trifoliate stage, plants per plot	1004.66 ± 41.52	884.66 ± 40.08	ns		
Plant health					
NDVI rating per plot, flowering	0.79 ± 0.02	0.70 ± 0.01	0.021*	46.9	1,4
Yield					
Pod weight (g) per plant	70.18 ± 7.27	30.15 ± 3.96	0.0164*	13.59	1,4
Pod weight (g) per 12 m	4280.0 ± 252.3	2189.3 ± 284.9	0.0038*	30.53	1,4
Pod damage by Mexican bean beetle					
% damaged pods	22.00 ± 5.29	37.33 ± 4.70	ns		

*Values are statistically ($p < 0.05$) different according to an ANOVA.
ns = Values are not statistically different according to an ANOVA

Table 4.3. Mortality values (see superscript definitions) of *Podisus maculiventris* by exposure to treated plants (direct poisoning), prey that fed on treated plants (secondary poisoning), and treated plants and prey together.

Exposed to:	Thiamethoxam + Fungicides (CT)	Fungicides Only (FT)	p value
[1]Snap bean plants only	15.0 ± 5.0	15.0 ± 6.28	ns
[1]Mexican bean beetle adults and bean plants	10.0 ± 5.77	10.0 ± 5.77	ns
[1]Mexican bean beetle larvae and bean plants	10.0 ± 5.77	25.0 ± 9.57	ns
[2]Mexican bean beetle adults only	1.56 ± 0.54	0.88 ± 0.42	ns
[2]Mexican bean beetle larvae only	0.19 ± 0.19	0.0 ± 0.0	ns

[1] Five *Podisus maculiventris* individuals per replicate (n = 20). Values are the average (± SEM) percent mortality per replicate.

[2] One *Podisus maculiventris* per replicate (n = 16). Values are the average (± SEM) mortality rating (see Table 4.4) per replicate.

ns = Values are not statistically different according to an ANOVA.

Table 4.4. *Podisus maculiventris* mortality rating scale, used to quantify the occurrence and speed of a secondary poisoning event into a single value.

Podisus maculiventris **found dead at time:**	**Rating**
24 hours	6
48 hours	5
72 hours	4
96 hours	3
120 hours	2
144 hours	1
Survived	0

Figure 4.1. Residual toxicity of snap bean plants grown from thiamethoxam-treated seeds to Mexican bean beetle adults (top) and larvae (bottom). Values are the average (± SEM) corrected percent mortalities per 5 individuals at each time step, pooled from two experimental runs (n = 40).
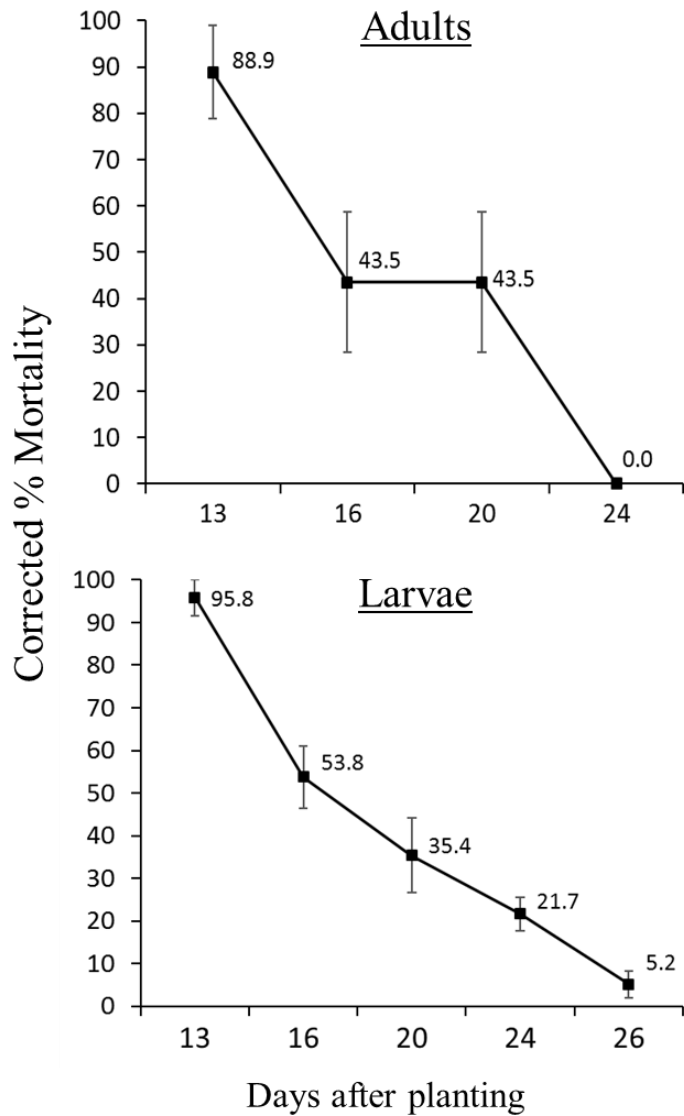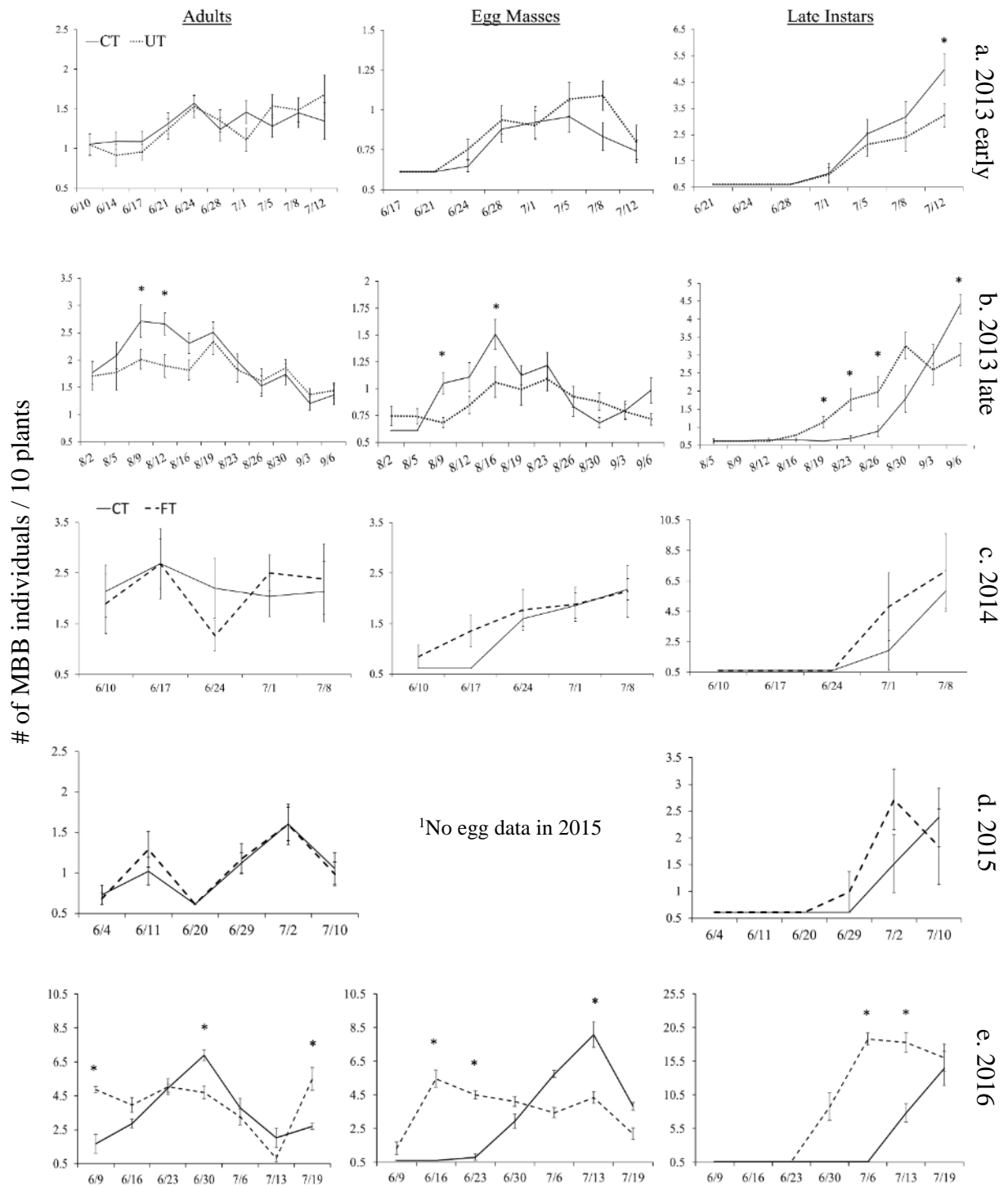
Figure 4.2. Average (± SEM) densities of Mexican bean beetle adults (left) egg masses (middle) and late instar larvae (right) per 10 plants, on individual sample dates.

*Values for this sample date are statistically different (p < 0.05) according to an ANOVA.
[1]MBB were only sampled by vacuum in 2015; eggs masses were not captured using this method. In all other years, Mexican bean beetle densities were sampled via visual plant inspections.

CHAPTER FIVE

Conclusions and Parting Remarks

In the summer of 2012, I was introduced to Mexican bean beetle at Virginia Tech's Kentland Farm, in Whitethorne, VA. Snap bean plants growing on the farm were heavily infested with all life-stages of the beetle, and exhibited severe defoliation. My advisor, Dr. Kuhar, stated that this beetle pest seemed to be present at this capacity every year at Kentland, yet he was unaware of the pest's geographic range or its overall impact on the snap and lima bean industries. In deciding to research Mexican bean beetle, the first obstacle was to explore and document the status of the pest geographically and its impact on growers.

To determine the range and severity of Mexican bean beetle, I surveyed growers across the country (primarily in the Mid-Atlantic) by attending grower conferences and outreach events, and by conducting online surveys via email listservs, extension agents, and Facebook pages. I discovered that Mexican bean beetle was mostly of little consequence to large-scale, conventional growers, for a few reasons. Mexican bean beetle no longer appears to be a pest of soybean, which it commonly attacked throughout the eastern U.S. in the 1960s and 1970s. In snap beans, the pest is relatively easy to control using one or two foliar applications of standard insecticides, mainly pyrethroids, which are frequently applied to the crop anyway. Also, the range of large-scale snap and lima bean production rarely overlaps with the range of Mexican bean beetle. In the Central Appalachian Mountains, where Mexican bean beetle is most severe, most snap and lima bean production occurs on small to mid-sized farms, which are more likely to use organic or chemical-free management practices. Snap beans on these small to mid-sized farms are often severely impacted by Mexican bean beetle, sometimes to the extent that the

entire harvest is lost. Lima beans are fed upon as well, often heavily; but most growers felt that lima beans were less severely impacted by Mexican bean beetle than snap beans.

This information guided my research objectives significantly. Overall, I established that Mexican bean beetle is a serious threat to organic growers mainly, and that it has been largely ignored in terms of management research due to its low impact in large-scale, commercial production. Because of the lack of information available on this still relevant pest, I decided to compile a thorough and up-to-date, literature review that could be made accessible to the public. After compiling this information, I published it in an open-access, peer-reviewed journal: Journal of Integrated Pest Management (Nottingham et al. 2016).

The next goal was to develop research objectives tailored to the needs of the growers most affected by Mexican bean beetle. This partially involved incorporating objectives pertaining to chemical-free management. In objective one, I screened common cultivars of snap and lima beans for susceptibility to Mexican bean beetle, in order to aide growers in cultivar selection, and to provide foundational information that could be used in cultural management strategies, such as trap cropping and push-pull. Through multiple field, greenhouse, and mark-release recapture choice experiments, I successfully identified a highly susceptible snap bean cultivar, 'Dragon's Tongue'. This cultivar would be an excellent candidate as a trap crop, or as the "pull" in a push-pull strategy.

Objective two examined a novel management strategy for Mexican bean beetle: growing beans on reflective polyethylene (plastic) mulch. Highly susceptible 'Dragon's Tongue' snap beans were grown on bare soil and three plastic mulches: 'metallized' (highly reflective), white, and black; and beetles were allowed to naturally colonize these plants. I discovered that Mexican bean beetle densities and collateral injury were significantly reduced and snap bean

yields increased in the metallized treatment compared to all other treatments, but especially compared to beans grown on bare soil. This method could be implemented as a stand-alone tactic to control Mexican bean beetle, or incorporated as a "push" agent in a push-pull strategy. I then tested a method to help growers increase their profit-to-investment ratio when using reflective plastic mulch. Planting a double-crop (a second crop directly planted into the used plastic rows) allows the grower to get double the "mileage" out of their plastic investment. I tested this strategy with lettuce as the double-crop, because lettuce is commonly grown in the fall after beans are harvested, and does well under lower soil temperatures and higher light intensity (both of which are achieved by reflective plastic mulch). Not only did the lettuce adequately grow in the previously used plastic plots, the metallized treatment produced higher yields than all other treatments, especially bare soil (a three-fold yield increase was observed in lettuce grown on metallized plastic compared to bare soil).

My third objective examined the use of an increasing common pest management strategy in snap beans, planting seeds treated with a neonicotinoid insecticide (thiamethoxam) coating. Although this method does not fit into a chemical-free management plan, conventional growers are common in the range of Mexican bean beetle. Additionally, this method has not yet been examined in systems predominated by Mexican bean beetle. Neonicotinoid seed-treatments use very low rates of the insecticide; so, if effective, they could reduce the overall insecticide load used by a grower. My goal was to determine how much, if any, benefit growers gained from this seed-treatment in the region, and if it had any negative impacts on the system, such has reducing predatory arthropods.

I measured the effects on Mexican bean beetle, other potential pests including thrips and potato leaf hopper, predatory arthropods (*P. maculiventris*, lady beetles, *Orius insidiosus*, nabids,

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etc.) and factors pertaining to overall crop performance (stand, plant vigor, and yield). The primary conclusions were that thiamethoxam seed-treatments rarely had significant effects on pests, predators, or crop performance. The most likely reason is that Mexican bean beetle and most other arthropods do not colonize beans until at least 30 days after planting, which is well after the insecticide is diminished from the plant. However, I saw that the outcome of using treated seeds is subject to change based on planting date. The only season (out of five total) yielding meaningful treatment effects was 2016; in which persistent spring rains resulted in a delayed planting. By the time beans were first emerging from the ground, Mexican bean beetle adults had completed overwintering, and colonized the young plants. The thiamethoxam treatment suppressed this infestation, while untreated plants were heavily injured and suffered significant yield losses. This suggests that thiamethoxam seed-treatments can benefit bean growers within the range of Mexican bean beetle, but only under specific conditions. While the results of this study neither overwhelmingly condemn nor justify the use of thiamethoxam seed-treatment as a management tool for Mexican bean beetle, other methods may better coincide with the principles of IPM.

In summary, the information in this dissertation should provide current and directly applicable information to snap and lima bean growers within in the range of Mexican bean beetle. Readers can draw from the background and historical perspectives in the introduction for general information on this pest's biology, ecology, and previously tested management strategies. The information in the research objectives provides ready-to-use management strategies, materials for developing new management strategies, and decision-making tools to determine when to implement strategies. Combining the key findings from each objective helps form a tangible IPM approach for Mexican bean beetle. Instead of planting snap bean seeds

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treated with thiamethoxam, snap bean growers may benefit more from growing a less preferred snap cultivar, such used 'Caprice', on reflectively plastic mulch, and planting a 'pull zone' (or 'trap crop') of a more preferred cultivar, such as 'Dragon's Tongue', on bare soil. Growers may then choose to plant a late-season double-crop, like lettuce, on the plastic to expand the gains from this investment.

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Nottingham, L. B., G. P. Dively, P. B. Schultz, D. A. Herbert, and T. P. Kuhar. 2016.
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