UTILIZING BEHAVIOR AND CHEMICAL CONTROL FOR THE MANAGEMENT OF THE COMMON ASPARAGUS BEETLE (*CRIOCERIS ASPARAGI*)

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Entomology – Master of Science

2024

ABSTRACT

The common asparagus beetle (*Crioceris asparagi)* poses a significant threat to asparagus production, particularly during the harvest season. Once laid, its eggs are difficult to remove and adults cause notable feeding damage on spears leading to economic losses for growers. Insecticides are commonly employed to manage asparagus beetle adults and eggs during harvest, aiming to prevent oviposition by killing the adults. Given the frequent harvesting of asparagus spears, insecticides with 1-day preharvest intervals are essential. My first chapter evaluated various contact synthetic and organic insecticides with 1-day preharvest intervals, testing their efficacy on asparagus spears in both field and laboratory settings. Acetamiprid and carbaryl proved effective in reducing adult and egg counts in field plots, while spinosad demonstrated comparable efficacy to carbaryl in laboratory bioassays. Pyrethrin effectively suppressed egg numbers but had no impact on adult populations. More importantly, in our bioassays we found that regardless of coverage lengths, insecticides reduced beetle numbers. Furthermore, both field and lab trials revealed efficacy was retained for lowering beetle mortality up to 48 hours. My second chapter investigated the host selection behaviors of the common asparagus beetle, revealing a preference for purple, tall, thin, and undamaged spears, with the presence of a spear tip being crucial for oviposition. Beetles mated more frequently on spears with intact tips and deposited significantly more eggs compared to those with their top parts removed. These findings provide valuable insights for developing sustainable control practices to manage the common asparagus beetle.

This thesis is dedicated to my family, partner Justin, and close friends who's love and support continually motivates me to be a better entomologist.

ACKNOWLEDGMENTS

First, I want to thank my principal investigator Zsofia Szendrei for allowing me the opportunity to work with her and the rest of the Vegetable Entomology Lab. Her mentorship and guidance throughout my master's degree will leave a lasting impact on my career as an entomologist. I would also like to thank my guidance committee members Ben Werling and Julianna Wilson whose collaboration and assistance offered great support to my thesis.

Thank you to the rest of the Vegetable Entomology Lab for their continued encouragement and friendship: Jennifer Roedel, Kayleigh Hauri, Natalie Constancio, Ray Rantz, Elizeth Cinto Mejía, Nayeli Carvajal Acosta, and DeShae Dillard. I especially want to acknowledge the research technicians who aided me in conducting research and data collection pertinent for my thesis: Tyler Reisig, Aaron Guggenheimer, Olivia Franklin, and Alex Jutila. Additionally, I would like to thank Ashley Fleser, Justin Adams, John Bakker, and Jamie Adams for their assistance. Their collaboration in my projects allowed me to better understand the asparagus industry, adding to my research and overall experience of my master's thesis. I consequently like to thank the numerous growers I had the pleasure of meeting during my thesis, their constant support promotes the need of scientific research in the agriculture industry. I also want to extend my appreciation to the Michigan State Entomology Department Chairperson, Hannah Burrack and the graduate student Advisor Heather Lenarston-Kluge for their efforts in creating a welcoming environment for all students. Thank you again to my family, friends, and partner Justin for their support throughout my educational journey. Lastly, I would like to thank my funding source: Project GREEEN for allowing me to do this important work.

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CHAPTER 1: The common asparagus beetle: An introduction to its life history, management, and industry impact

Asparagus production

Asparagus (*Asparagus officinalis* L.) is a specialty vegetable crop grown throughout the U.S. with an estimated production value of $85.2 million (AgMRC, 2021). Asparagus is one of the oldest known domesticated crops originating from Eurasia, now grown globally on all continents except for Oceania and Antarctica (Lunzy, 1980). The crop is thought to have arrived in the U.S. around 1672, with a successful establishment in New England (LeSage et al., 2008). Currently production is concentrated in Washington, New Jersey, California, and Michigan (Graper & Burrows, 2001; USDA-NASS, 2022). Of these states, Michigan is the top producer, contributing ~40% to national, annual production. Commercial production targets the fresh and processing markets, with spears sold fresh, canned, or frozen. As a perennial plant, asparagus fields are expected to produce for up to 15 years when properly managed (Falloon & Nikoloff, 1986). Throughout that time growers will interact with several species, such as the common asparagus beetle, one of the major pests of commercial asparagus.

Common asparagus beetle biology

First recorded in Michigan in 1898, the common asparagus beetle (*Crioceris asparagi* (L.), Coleoptera: Chrysomelidae) is the most challenging insect pest for Michigan asparagus farmers. The asparagus beetle is a specialized insect pest thought to have originated from the Mediterranean, coinciding with the domestication of asparagus plants (FGP Consortium 2004). After it arrived in the U.S., the asparagus beetle quickly spread to most areas where asparagus is grown (Chittenden, 1917), except for warm southern states (LeSage et al. 2008). Because of the beetles' economic impact on asparagus productivity and yields, studying asparagus beetle biology in detail is an industry priority (Adams, 2023).

Adult beetles have elongated bodies with a coloration pattern signaling unpalatability for predators (Jones, 1932; Fig. 1.1). Adults on average are ~6.5 mm in size with black-colored heads and red-orange thoraxes speckled with black. Their abdomens possess a black line along the elytra suture that is surrounded by a yellow and black patch pattern framed by maroon. Eggs of the asparagus beetle are laid throughout the summer and are ~2 mm in size. Eggs can be grey or black and are usually laid in neat rows on the spears, cladophylls, or branches of asparagus plants. Larvae emerge 3-8 days after eggs are laid, they are ~8 mm in size with small black heads and dark gray bodies. Three pairs of brown or black legs are present on the thorax with the abdomen gradually increasing in size, resembling a comma shape. The larvae feed on the plants for 10-15 days before reaching a size equivalent to an adult beetle, they then fall off the plants to pupate in the soil for 7-12 days (Drake & Harris, 1932).

Figure 1.1 (A) Asparagus beetle adult, (B) asparagus beetle chewing damage on spear, (C) asparagus beetle eggs attached to spear.

In North America, the common asparagus beetle can produce up to 3 generations in a year but 2 generations is more common (Capinera & Lilly, 1975; Dingler, 2009; Taylor & Harcourt, 1978). All life stages of the asparagus beetle can decrease the crop's market value, most notably during the harvest season. Mating and feeding begin after adults emerge from overwintering, typically in late April before harvest (Chittenden, 1898). Adults feed on the spears, while females oviposit on the upper parts of the spear throughout the harvest season (Gupta & Riley, 1967). The presence of eggs not only decreases the spears' appearance but are

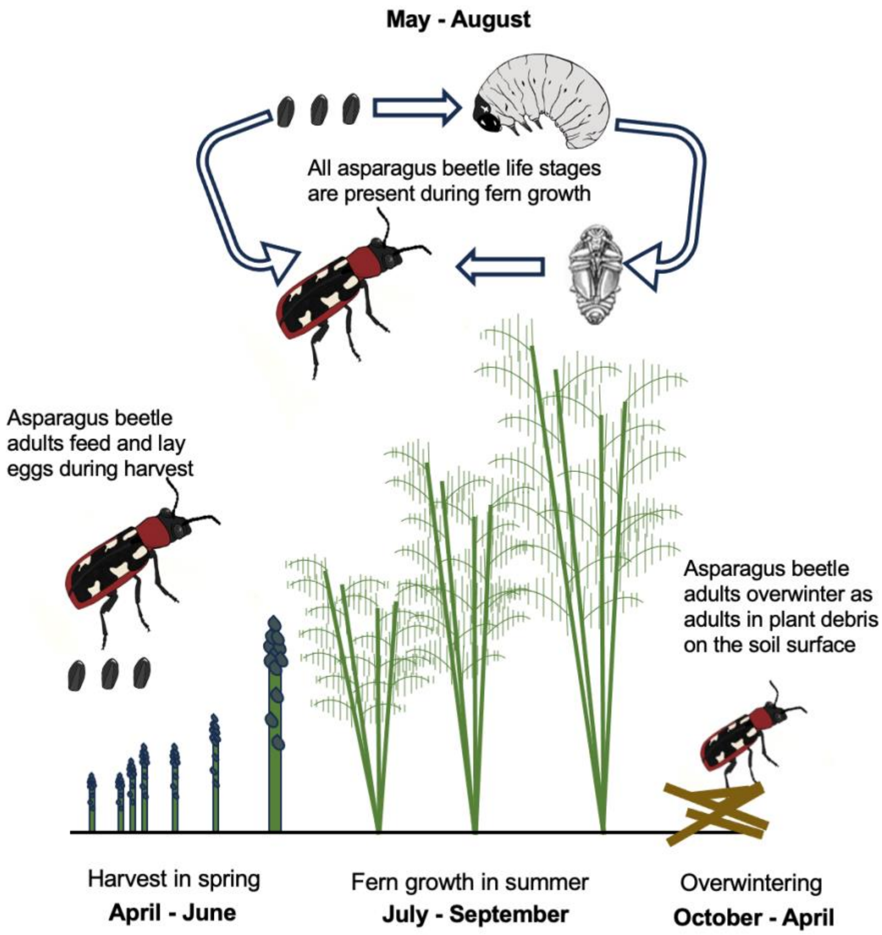

Figure 1.2 The lifecycle of the common asparagus beetle in association with the annual progress of commercial asparagus fields.

difficult to remove, requiring 8,650 times their weight to be detached (Voigt & Gorb, 2010). Post-harvest, larvae and adults consume the asparagus' axillary branches and cladophylls, decreasing the plant's photosynthetic abilities (Grafius & Hutchison, 1995). As days shorten and temperatures drop, adults initiate diapause starting in late September (Fig. 1.2).

Asparagus beetle management

Asparagus beetles are controlled using broad-spectrum insecticides such as pyrethroids, carbamates, and spinosyns (McClanahan, 1975). However, the impact of the currently registered insecticides can be variable, thus their efficacy, activity, and longevity need to be further determined. The asparagus beetle action threshold is reached when fields are infested with >5% adults or >2% eggs during harvest (Wold-Burkness et al., 2006). Control is achieved by killing beetles to reduce subsequent egg-laying. This poses a challenge as asparagus growth is rapid, growing almost 20 cm in one day and is hand-harvested daily depending on temperatures (Wilson et al., 2002). This means insecticides must have a 1-day preharvest interval (PHI) to account for rapid growth and harvesting. For this reason, there is growing interest in the use of biopesticides for insect management. Bioinsecticides differ from conventional, synthetic, insecticides because they are made from naturally derived chemical compounds. The most common of these used in vegetables are azadirachtin, spinosad, and pyrethrin (Dively et al., 2020). Previous research conducted utilizing bioinsecticides for asparagus beetles demonstrated promising results where spinosad and pyrethrin worked as effectively as the grower standard carbaryl (Zavalnitskaya & Szendrei, 2021).

Insect host selection

In the context of ecological research, host selection is defined as the interaction by which insects choose their hosts (Ahmad, 1983; Charnov & Skinner, 1984; Moller, 2008). The process of selecting hosts is divided into two stages, host finding and host acceptance (Thorsteinson, 1960). During both stages insects utilize olfactory, gustatory, and visual stimuli for host recognition (Finch & Collier, 2000; Thorsteinson, 1960). For example, a study on the closely related spotted asparagus beetle (*Crioceris duodecimpuncata*), found that males were particularly attracted to the volatiles produced by asparagus cladophylls (Pistillo et al., 2022). Once a potential host is identified, females will assess the quality of their host for oviposition (Refsnider & Janzen, 2010). Phytophagous insects must select suitable hosts that support juvenile survival by providing adequate resources and limiting mortality through competition or abiotic stressors (Scheirs & De Bruyn, 2002). The preference-performance hypothesis states that females will select hosts that support juvenile nutritional needs to promote survival and the female's reproductive fitness (Pöykkö, 2006; Thompson, 1988). Females who select these optimal hosts for oviposition are favored by natural selection (Jaenike, 1978). While these theories have been proposed to explain insect oviposition behavior, most study systems fail to prove the correlation between female preference and offspring performance (Hufnagel et al., 2017; Rojas et al., 2018; Valladares & Lawton, 1991). Regardless, common behavioral patterns are associated with oviposition site selection. Most importantly, females select sites with a low risk of predation by natural enemies (Heisswolf et al., 2005; Higashiura, 1989; Nomikou et al., 2003). The presence of conspecifics is another important factor during oviposition site selection, as it creates competition for resources for the female's offspring (Gripenberg et al., 2010; Williams & Gilbert, 1981). The spotted asparagus beetle is found alongside the common asparagus beetle in commercial asparagus fields, yet there is no evidence of competition avoidance between the two species (Morrison & Szendrei, 2014; Schmitt, 1988). However, this behavior has been seen amongst female spotted asparagus beetle conspecifics and their offspring who select for asparagus fruit without feeding damage (Van Alphen & Boer, 1980). Similar research is needed to deduce the biology of asparagus beetle host selection. Such information is crucial to developing effective cultural control methods to improve the current management practices of the common asparagus beetle.

Thesis objectives

The goal of this thesis was to develop sustainable management measures for asparagus beetles in Michigan by investigating the efficacy of commercially available insecticides and the characteristics that influence host selection behavior.

Objective I: Assess the efficacy of insecticides for managing asparagus beetles during harvest.

Sub-objectives:

A. Evaluate the efficacy of insecticides for harvest across various coverages.

B. Asses the efficacy of insecticides for harvest over time.

Objective II: Determine asparagus beetle host selection behavior

Sub-objectives:

A. Correlate asparagus beetle host preference to asparagus characteristics.

B. Evaluate the impacts of asparagus characteristics on beetle behavior.

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CHAPTER 2: Synthetic and organic insecticides for asparagus beetle management during harvest

Introduction

Asparagus (*Asparagus officinalis*) is a vegetable crop originating from the Mediterranean, now grown worldwide (Morrison & Szendrei, 2014). In many asparagus-producing regions of the globe, including Michigan, USA, the common asparagus beetle (*Crioceris asparagi*, Coleoptera: Chrysomelidae) is a key specialist insect pest (Morrison & Szendrei, 2014). Both adults and larvae feed exclusively on the crop and large populations can build up over years in commercial fields (Morrison & Szendrei, 2014). Asparagus beetles overwinter as adults in field debris from the previous year and emergence coincides with

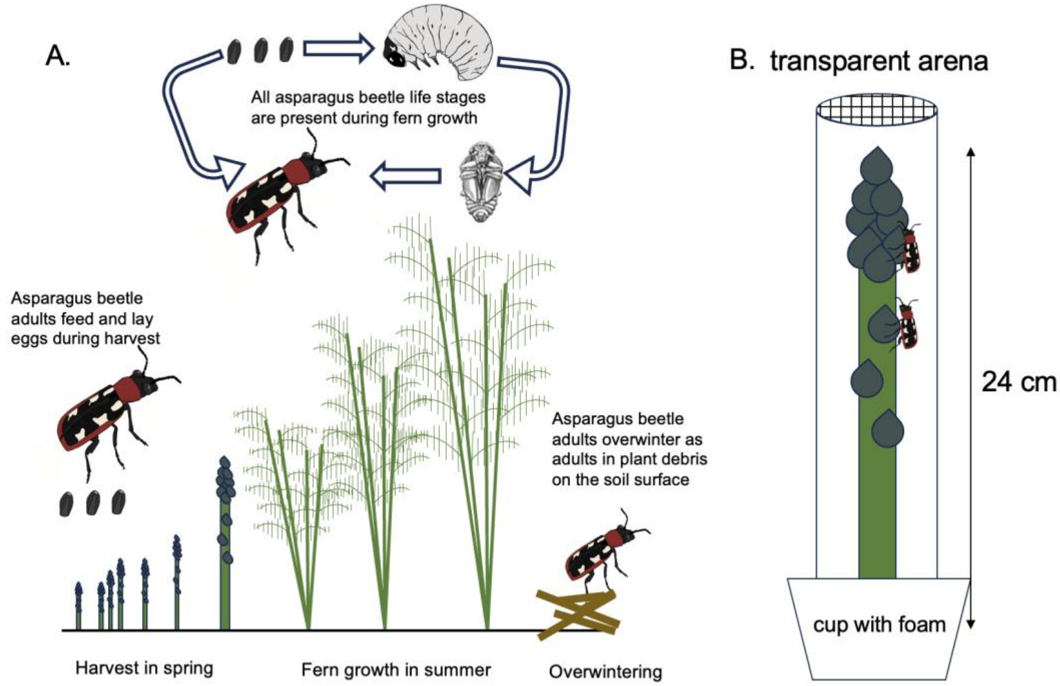

Figure 2.1 (A) The lifecycle of the common asparagus beetle in association with the annual progress of commercial asparagus fields. (B) An arena used in the insecticide bioassays was comprised of a 24 cm tall asparagus spear inserted in wet floral foam held in a plastic cup. The mating pair of asparagus beetles were kept enclosed by a transparent plastic film cage with a mesh top.

asparagus development starting in the spring (Wold-Burkness et al., 2006). During this period, adults begin to mate and feed on spears, with female beetles laying eggs soon after (Wold-Burkness et al., 2006). Eggs are glued onto asparagus spears in groups of about 3-10 at the top ~10 cm of spears (Voigt & Gorb, 2010). Overwintered adults will continue to actively feed and lay eggs on spears during the harvest period from May through June in Michigan. Since harvesting continuously removes spears, eggs are not able to develop into larvae until harvesting ends. Throughout the summer, larvae and adults will continue to feed on the asparagus fern until adults eventually overwinter in the soil in late September (Grafius & Hutchison, 1995; Morrison & Szendrei, 2014) (Fig 2.1A).

Asparagus production requires that marketable spears are completely free of beetle feeding damage and eggs. Entire loads of asparagus can be rejected even if a small percentage of spears have eggs and damage (Voigt & Gorb, 2010). Insecticides are the primary way to manage adult populations, but they cannot remove eggs, therefore the best way to reduce eggs is to stop oviposition by killing the adults. The insecticides used for asparagus beetle adult management are all contact insecticides that kill an insect when it is sprayed or encounters residues on the plant surface. Asparagus beetle adults often avoid coming into direct contact with insecticides during spray applications, often hiding when the field is disturbed by moving equipment (McClanahan, 1975). Due to frequent harvesting, insecticides used for management of asparagus beetles must be fast-acting and reapplied frequently to protect new plant tissue.

Adding to the challenges of managing this insect during harvest is the difference in the growth stages of asparagus spears. Asparagus can grow up to 20 cm a day depending on daily temperatures during the harvest season (Wilson et al., 2002). Spears are harvested when they reach ~25 cm in height, but spears of varying heights that are shorter are present in fields at any given time. Therefore, when insecticides are sprayed, the coverage will vary based on the height of the spear at the time of application. Newly emerging spears will only have their tip covered with insecticide residue, while spears that near harvesting height will have complete residue coverage. This leads to variation in insecticide coverage among spears across the field, potentially allowing beetles to find untreated plant surfaces for egg laying. However, asparagus beetles typically lay their eggs in the top ~10 cm of spears, potentially providing a unique combination of behavioral and chemical management (Martini et al., 2012; Nansen et al., 2016). Re-application will not be critical if partial coverage of spear tips is enough to provide control. It is currently unclear if insecticide residues on spear tips control asparagus beetle adults and if this leads to a reduced number of eggs. Females may exhibit behavioral resistance by avoiding the plant surfaces treated with insecticides and seeking those parts of the spears that have no residues (Nansen et al., 2016). Alternatively, they may not be able to distinguish between insecticide-treated and untreated surfaces, or the behavioral cues to lay eggs on spear tips may override insecticide avoidance. In this case, insecticide residue may only need to be on spear tips, and complete coverage of spears may not be. Understanding this phenomenon is crucial for improving asparagus beetle control with insecticides.

There are relatively few synthetic insecticides with 1-day PHIs registered during asparagus harvest. Due to the limited options and growing safety concerns for synthetic insecticides, more information regarding the efficacy of organic insecticides is needed to provide alternatives for beetle management. While organic insecticides serve as a sustainable alternative to synthetic insecticides, several problems arise when considering their use in commercial production including rapid degradation under field conditions and generally lower efficacy

(Dively et al., 2020). There is a need to explore insecticide efficacy across multiple insecticide mode of action groups to provide a wider array of options for asparagus beetle management.

We tested seven registered synthetic and organic insecticides for the management of the common asparagus beetle, focusing on those with a maximum 1-day PHI. In field plots, various insecticides were sprayed on spears. Adult and egg numbers were recorded 24 and 48 h after application. We also tested efficacy through no-choice bioassays, varying the insecticide coverage on the asparagus spear tip to determine if asparagus beetles exhibit behavioral resistance.

Materials and Methods

Field efficacy experiment

The efficacy of seven insecticides (azadirachtin, pyrethrin, spinosad, chlorantraniliprole, carbaryl, acetamiprid, Table 2.1, Appendix) and an untreated control (D.I. were tested at the Michigan Asparagus Research Station in Hart, MI. Carbaryl served as the grower standard and was tested at a half and at the maximum label rate, corresponding to variation in grower-applied rates and concerns for resistance development (see rates in Table 2.1 in Appendix). Insecticide treatments were sprayed in an experimental asparagus field (var. Sequoia, field size: 50 m x 12 m) where treatments were arranged in a randomized complete block design with 4 replications. Each plot had a single 11 m long asparagus row allowing for a 15 m^2 treatment area. Asparagus rows were sprayed (207 kPa, 234 l/ha) using a tractor-mounted sprayer with 3 nozzles (TeeJet®, Flat Fan, TP8003, Grimes, IA) spaced 45 cm apart to accommodate spraying a 135 cm wide area. After spraying, approximately 800 beetles were released into the field by randomly distributing groups of about 100 beetles throughout the field and allowing them to disperse. Beetles were collected from a commercial field in Pentwater, MI and Linden, MI. Data collection began 24 h after applying insecticides, all spears in each plot were visually surveyed and the number of beetles and eggs on spears was recorded. Data was collected at 24 h and 48 h after the spray application.

Laboratory efficacy bioassay

Plants and beetles for bioassays

Adult asparagus beetles used in the experiments were collected throughout early May to mid-June 2023 at field sites in Holt, Linden, Pentwater, and Hart, MI. Beetles were transported from the field to the laboratory where they were placed into mesh cages (122 cm x 70 cm x 70 cm; Nasco, Fort Atkinson, WI, USA) and maintained on a 16 h L: 8 h D light cycle at 25 ± 2°C in East Lansing, MI. Beetles were fed untreated asparagus spears (var. Millennium) from the Michigan State University Horticulture Farm, Holt, MI. Spears (25-30 cm) were harvested and stored in a container with water at 4 ± 2°C until fed to beetles. To feed beetles, the bottom 5 cm of spears were vertically inserted into floral foam blocks (7.1 cm x 9.6 cm x 22.3 cm, Artesia WetFōM®, Green Brick, FloraCraft®, Ludington, MI) soaked in deionized water to prevent desiccation. Fresh spears were placed in cages as needed to provide beetles with food *ad libitum*. Spears for the laboratory efficacy bioassays were also collected from the field in Holt, MI on the same day that the bioassays were set up.

Lab efficacy bioassay: half vs. full insecticide coverage on spears

Azadirachtin, azadirachtin + pyrethrin, pyrethrin, spinosad, and carbaryl were tested in this experiment at 75% of the rate indicated in Table 2.1 in the Appendix. The rate was reduced relative to the field experiment to account for potential differences between field vs. laboratory conditions. Water was used as the control treatment. For each treatment, 10 spears were fully (24 cm, 100%), or half (top 12 cm, 50%) dipped into an insecticide solution or water and then left to dry for 1 h. This was done because beetles have been observed to spend most of their time at the tip of the spears and eggs are laid at or near the tip of the spear (Szendrei Z., pers. obs.). The bottom 3 cm of spears was inserted into water-soaked floral foam cylinders (5 cm x 5 cm) held in plastic cups (59 ml) and the spears were inserted into a cylindrical transparent cage (4.5 cm diameter x 30 cm tall, transparency film, School Smart, Greenville, WI) with a mesh at the top to prevent beetles from escaping but allowing ventilation. One mating pair of asparagus beetles was placed into each arena (Fig. 2.1B). Arenas were kept in a growth chamber at (25 Cº, 16L:18D h light cycle) for 48 h. Every 24 h, we recorded the number of live and dead adults and the number of eggs.

Lab efficacy bioassay: tip insecticide coverage on spears

For this bioassay, we treated spears with either spinosad, pyrethrin, carbaryl or water. Similar methods were used as described above to treat spears and set up arenas, except the insecticide coverages were altered to full (100%), 10 cm at the top, and 5 cm at the top of the spear. This simulated varied coverage resulting from treating spears at different developmental stages in the field. Treated spears were placed into the arenas as described above with one mating pair of asparagus beetles. Arenas were arranged in a randomized block design within a growth chamber kept at the same conditions as above, for 48 hours. Arenas were surveyed after each 24 h period with the number of live/dead adults and eggs per spear recorded.

Statistical analyses

The ‘fitdist’ function in RStudio Version 2023.6.0.421 (Posit PBC, Boston, MA) was used to fit distributions of all our data using maximum likelihood estimation. The egg and adult abundance data counted on the spears for the field experiment were analyzed with a generalized mixed model with a negative binomial distribution with insecticide treatment and date as fixed factors and block as a random factor. The egg and live adult count data collected from the lab bioassay that tested half vs. full coverage were analyzed with a generalized linear model with a quasipoisson distribution using insecticide, dip depth, and date as fixed factors. The adult and egg count data from the 5 cm, 10 cm, and full coverage bioassay was analyzed with a generalized mixed effects model with a poisson distribution; tip coverage type, time point after treatment (24 h or 48 h), and insecticide- treatment was used as a fixed factor and block as a random factor. Treatment means were compared using 'emmeans' with the false discovery rate adjustment method.

Results

Field efficacy experiment

Adults

There was no significant difference between the number of adults found in plots at 24 h and 48 h ($\chi^2 = 1.64$, df = 1, $P > 0.05$). However, the insecticide treatments had significantly different numbers of beetles ($\chi^2 = 106.78$, df = 7, $P < 0.01$; Fig. 2.2A). Azadirachtin-treated plots had the highest number of adults, 6 times more than all the other treatments ($z > 4.44$, $P < 0.01$). Control, pyrethrin, spinosad, chlorantraniliprole, and carbaryl had similar numbers of adults ($z > 0.53$, $P > 0.05$) but these had 7 times more beetles on average than the acetamiprid treatment, which had the lowest numbers of adults (Fig. 2.2A).

Eggs

There was no significant difference between the two time points in the number of eggs found on spears ($\chi^2 = 0.04$, df = 1, $P > 0.05$) but the insecticide treatments had significantly different numbers of eggs ($\chi^2 = 88.97$, df = 7, $P < 0.01$; Fig. 2.2B). Azadirachtin-treated plots had the highest number of eggs, 8 times more than all the other treatments ($z > 3.09$, $P < 0.01$).

Control, pyrethrin, azadirachtin, and carbaryl at the lower rate had similar numbers of eggs (z > 0.05, P > 0.05) but these had 3 times more eggs than carbaryl at the high rate and acetamiprid (z > 0.32, P < 0.01). Carbaryl at the high rate performed similarly to acetamiprid to control eggs (z = 1.07, P > 0.05; Fig. 2.2B).

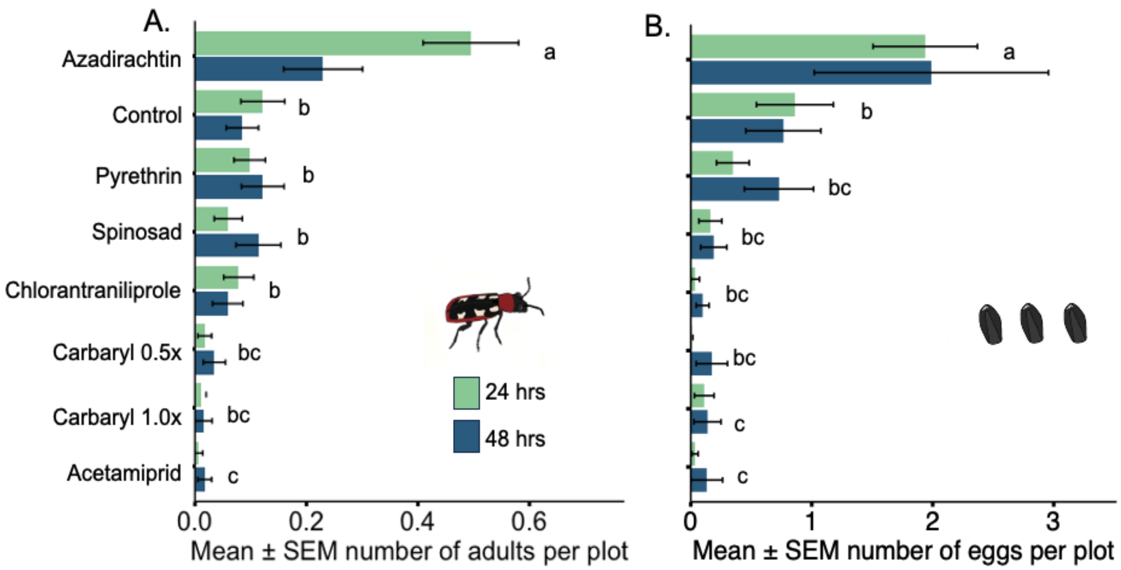

Figure 2.2. Mean ± SEM number of asparagus beetle adults (A) and eggs (B) in a field trial testing the efficacy of synthetic and organic insecticides. Asparagus spears were sprayed using a tractor-mounted sprayer and insect numbers were assessed 24 and 48 h after insecticide application. Bars with different letters are statistically different (α = 0.05).

Lab efficacy bioassay: half vs. full insecticide coverage on spears

Adults

There was no significant difference in the numbers of adults between the two time points (F = 2.90, df = 1, P > 0.05), nor the two insecticide coverage treatments (F = 0.12, df = 1, P > 0.05; Fig. 2.3A). There was a significant difference across the insecticide treatments (F = 62.49, df = 5, P < 0.01). Azadirachtin and azadirachtin/pyrethrin were not significantly different from the control (water) but these three treatments had significantly more beetles surviving than pyrethrin (z > 1.46, P < 0.01), spinosad (z > 8.46, P < 0.01), and carbaryl (z > 7.87, P < 0.01).

Pyrethrin had 10 times more beetles surviving than spinosad (z = 7.78, P < 0.01) and 4 times more than those on carbaryl-treated spears (z = 6.86, P < 0.01). Spinosad had 2.5 times fewer beetles surviving than carbaryl (z = 2.65, P < 0.01; Fig. 2.3A).

Eggs

There was a significant difference between the two time points (F = 19.04, df = 1, P < 0.01) with more eggs counted 48 h after the start of the experiment than 24 h, but the interaction between insecticide and dip treatment was not statistically significant (F = 0.52, df = 7, P > 0.05; Fig. 2.3B). There was no significant difference in egg numbers between the two coverage treatments (F = 1.20, df = 1, P > 0.05). There was a significant difference across the insecticide treatments (F = 71.62, df = 5, P < 0.01). The water control treatment had 14.6 times more eggs

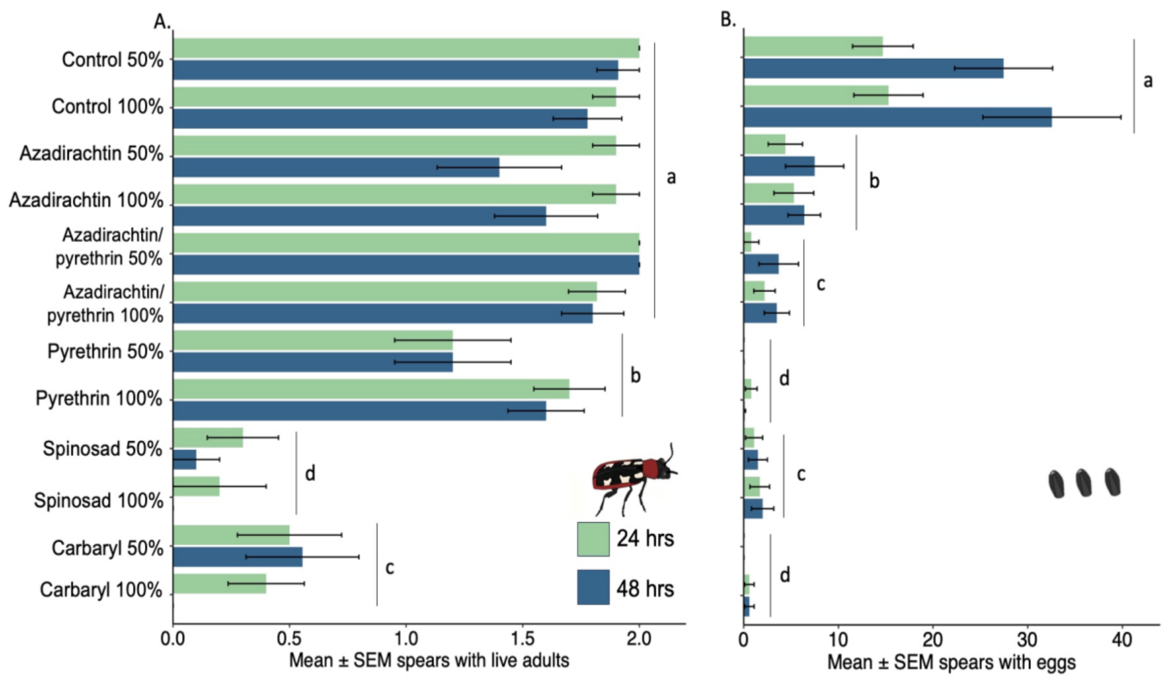

Figure 2.3 Mean ± SEM number of asparagus beetle adults (A) and eggs (B) per spear in a lab bioassay testing the efficacy of synthetic and organic insecticides. Individual asparagus spears were either dipped entirely (100%) in an insecticide solution or only the top 50% of the spear was dipped. Asparagus spears were placed in arenas with a mating beetle pair and insect numbers were assessed 24 and 48 h after insecticide application. Bars with different letters are statistically different (α = 0.05).

than the other insecticide treatments combined (z > 0.25, P < 0.01). There were 1.3 times more eggs in the azadirachtin treatment than in the other insecticide treatments (z > 2.68, P < 0.01). Azadirachtin/pyrethrin and spinosad had similar numbers of eggs, and pyrethrin and carbaryl had statistically similar numbers of eggs. Azadirachtin/pyrethrin and spinosad had 8 times more eggs than pyrethrin and carbaryl (z > 1.99, P < 0.01; Fig. 2.3B).

Lab efficacy bioassay: tip insecticide coverage on spears

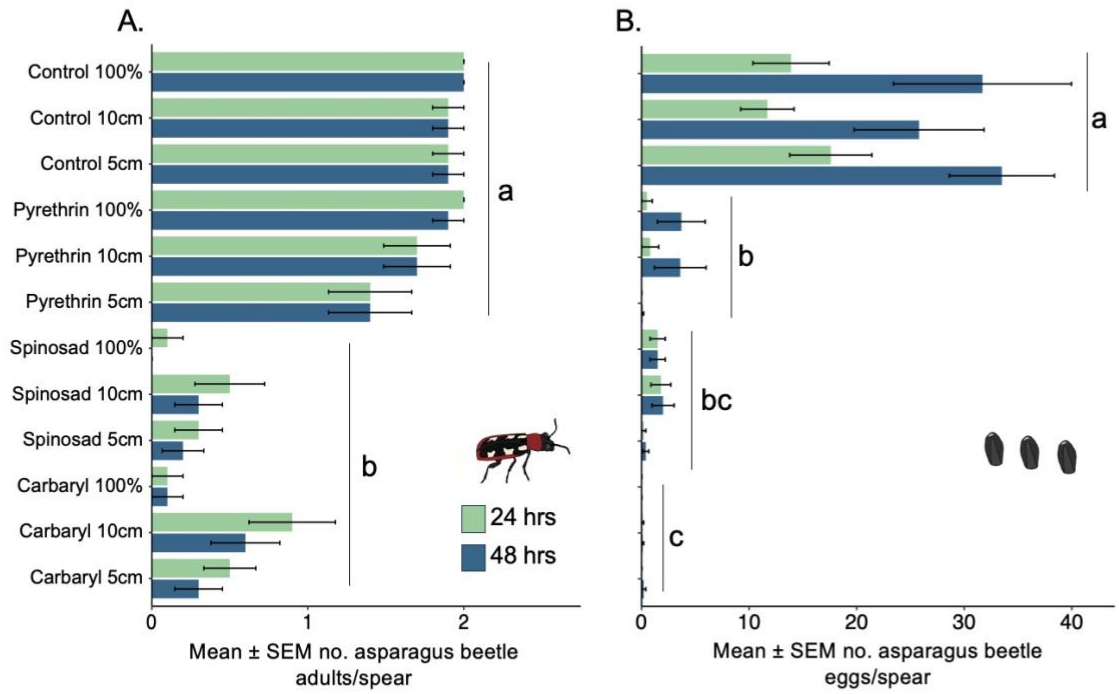

Figure 2.4 Mean ± SEM number of asparagus beetle adults (A) and eggs (B) per spear in a lab bioassay testing the efficacy of a synthetic and two organic insecticides. Asparagus spears were dipped in an insecticide solution either entirely (100%) or only the top 10 cm or 5 cm of the spears were dipped. Asparagus spears were placed in arenas with a mating asparagus beetle pair and insect numbers were assessed 24 and 48 h after insecticide application. Bars with different letters are statistically different (α = 0.05), and asterisks indicate significant differences in the number of eggs between 24 and 48 h.

Adults

There was no significant difference in the number of adults between the two time points ($\chi^2 = 0.39$, df = 1, P > 0.05) or among the tip coverage treatments ($\chi^2 = 1.67$, df = 2, P > 0.05; Fig. 2.4A). There was a significant difference in adult numbers among the insecticide treatments ($\chi^2 = 140.58$, df = 3, P < 0.01). The number of adults surviving on control (water) and pyrethrin-treated spears was not different (z = 1.02, P > 0.05); there were 6 times more beetles in these two treatments than in spinosad and carbaryl (z > 6.25, P < 0.01). Spinosad and carbaryl had only 0.3 beetles surviving on average (z = 1.74, P > 0.05; Fig. 2.4A).

Eggs

There were significantly more eggs found after 48 h than after 24 h ($\chi^2 = 201.64$, df = 1, P < 0.01; Fig. 2.4B) with a significant interaction between date and insecticide treatment ($\chi^2 = 9.06$, df = 3, P = 0.02). There were similar numbers of eggs between the two time points in the case of carbaryl and spinosad, but in the other treatments, the number of eggs increased from 24 to 48 h. There were significant differences in the numbers of eggs across insecticide treatments ($\chi^2 = 2877.32$, df = 3, P < 0.01). There were no significant differences among the tip coverage treatments ($\chi^2 = 5.75$, df = 2, P > 0.05). The number of eggs in the control treatment was 24 times higher than in the insecticide treatments (z > 11.61, P < 0.01). Pyrethrin and spinosad had similar numbers of eggs (z = 1.02, P > 0.05). The number of eggs in pyrethrin and spinosad treatments was 9 times higher than on the carbaryl-treated spears (z > 5.68, P < 0.01; Fig. 2.4B).

Discussion

In our study, we assessed the efficacy of several insecticides for managing the common asparagus beetle. We also evaluated the impact of time since insecticide application and insecticide coverage on spears. We demonstrated that effective bio- and synthetic insecticides are available to asparagus growers to manage asparagus beetles during harvest. Among the tested bioinsecticides, spinosad performed as well as the most effective synthetic insecticides for reducing both adult and egg abundance on asparagus spears. We also established that the tested insecticides performed similarly at 24 and 48 h after application in both field and laboratory trials to reduce the number of asparagus beetle adults. The number of eggs was greater in some of our lab bioassays after 48 than 24 h, likely because beetles were confined in the arenas. In addition, we proved that treating asparagus spear tips with insecticides can be as effective as treating the entire spear to reduce asparagus beetle adults and eggs.

In our field trial, acetamiprid was the only tested insecticide that significantly reduced adult and egg numbers relative to the control and performed similarly to the grower standard carbaryl. Acetamiprid is not only efficacious for reducing the number of adults and eggs on spears, but it can also reduce the percentage of eggs hatching (Kuhar et al., 2006). Most of the other tested insecticides performed similarly to the control treatment for adult management. In the case of eggs, both acetamiprid and the high-rate of carbaryl performed better than the control treatment. Carbaryl is currently used widely by asparagus producers in Michigan to control beetles during harvest and it is considered efficacious when beetle pressure is relatively low in the field. Based on our results, we found that both rates were effective at reducing the number of live adults and egg-laying across our trial settings, suggesting that resistance is not developing in this species. Chlorantraniliprole performed relatively well for controlling eggs but not for adults. The least efficacious insecticide was azadirachtin, which performed significantly worse compared to the control treatment. This could be attributed to the rate of insecticide used for the study as the manufacturer suggests adjusting the rate of azadirachtin based on spray volume (Szendrei, Z., pers. comm.). Thus, determining the most effective rate and spray volume for this product’s future viability for asparagus beetle management is necessary. Overall, the bioinsecticides used in our field study (spinosad, azadirachtin, pyrethrin, and azadirachtin/pyrethrin) had higher numbers of eggs and adults compared to the synthetic insecticides.

In the no-choice lab bioassays, we focused on comparing the efficacy of organic insecticides to the grower standard, carbaryl, and determining the performance of insecticide coverage on spear tips. First, our results showed that spears treated with spinosad or carbaryl had the lowest number of live adults compared to other treatments. Second, pyrethrin, spinosad, and carbaryl were the most efficacious treatments for lowering the number of eggs on spears. Third, we demonstrated that the various coverages of insecticides on spears retain efficacy in reducing both asparagus beetles and egg-laying. This is a significant finding indicating that spears treated with insecticides during the early growth stage may be protected as they grow, and unprotected tissue becomes exposed. This finding also implies that behavioral cues to lay eggs on spear tips are prioritized in this insect over insecticide avoidance. The number of eggs slightly increased in most lab treatments from 24 to 48 h but this was likely due to the fact that adult numbers stayed similar between the two time points, thus allowing for more oviposition in the second half of the bioassays.

We observed behavioral avoidance in pyrethrin- and chlorantraniliprole-treated spears in our bioassay, where live adult numbers on spears were similar to that in the control treatment but egg numbers were lower than the control. This indicates that adults were avoiding laying eggs on insecticide-treated spears but were not killed by them. Similarly to our results, previous studies indicated that coverage of half of the plant surface with insecticides is adequate to deter herbivore pests from feeding and oviposition, as well as alter these behaviors (Martini et al., 2012; Nansen et al., 2016). For example, a study conducted on the diamondback moth (*Plutella xylostella*) in brassica found that ovipositing females were repelled from treated leaves (Nansen et al., 2016). This insecticide avoidance or repellency is a frequently targeted mode of action especially in the case of organic insecticides (Dively et al., 2020). It is important to note that this behavioral avoidance was observed in our lab bioassays only for pyrethrin, but not in the field trial. One of the disadvantages of pyrethrin is that it breaks down in sunlight rapidly (Dively et al., 2020), and this may have been the reason for this difference between the lab and the field.

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APPENDIX

Table 2.1. Insecticides tested for asparagus beetle management in a field experiment and laboratory bioassays. (IRAC - Insecticide Resistance Action Committee mode of action classification).

Product	**Company**	**Active ingredient**	**Type**	**IRAC group**	**Class**	**Rate (fl. oz/acre)**
Assail	Corteva	Acetamiprid	Conventional	4A	Neonicotinoid	--
Azera	MGK	Azadirachtin/pyrethrin	Organic	UNE	Neem/pyrethrin	2.3
Carbaryl (0.5x)	Loveland	Carbaryl	Conventional	1A	Carbamate	16
Carbaryl (1.0x)	Loveland	Carbaryl	Conventional	1A	Carbamate	32
Coragen	FMC	Chlorantraniliprole	Conventional	28	Anthranilic diamide	7
Entrust	Corteva	Spinosad	Organic	5	Spinosyn	2
Neemix	CertisBio	Azadirachtin	Organic	UNE	Neem	10
Pyganic	MGK	Pyrethrin	Organic	3A	Pyrethrin	50

CHAPTER 3: Investigating the impact of asparagus spear characteristics on asparagus beetle host selection

Introduction

A majority of chrysomelid species are mono- or oligophagous, specialized crop pests (Mitchell, 1981). Thus, the ability to perceive host plant cues is crucial to their survival. Phytophagous insect host selection occurs sequentially over two events: host locating and host acceptance (Thorsteinson, 1960). Chrysomelids are notably attracted to a range of olfactory and visual cues produced by their host plant (Fernandez & Hilker, 2007). Specialist herbivores have developed an acute sensitivity to their host plant's volatiles, which can provide important information on a plant's quality and availability (Bruce et al., 2005; Dicke & Vet, 1999). While volatiles provide long-distance information for host seeking, visual cues such as color and shape also aid host recognition (Jermy et al., 1988; Prokopy & Owens, 1978; Szentesi et al., 2002; Yang et al., 2003). Once an insect successfully identifies a host, further evaluations are made before it accepts the plant. Insects will inspect the plant's surface using their maxillary palps and tarsi, usually followed by tasting to assess the available nutrients and secondary metabolites (Chapman & Bernays, 1989; Ruedenauer et al., 2023). Overall, female insects are theorized to prefer hosts that promote offspring performance and boost reproductive fitness (Thompson, 1988). Plants that can provide limited resource competition, lack of predation, and are part of a larger host-plant patch are ideal oviposition sites (Higashiura, 1989; Root, 1973; Williams & Gilbert, 1981). Yet, the "mother knows best" approach to host selection behavior is not representative of all insects, as numerous species have demonstrated behaviors that adversely impact offspring survival (Mayhew, 2001).

The common asparagus beetle (*Crioceris asparagi*, Coleoptera: Chrysomelidae) is a pest that feeds and oviposits exclusively on asparagus (*Asparagi officinalis*; Drake and Harris, 1932;

Watts, 1938). Most notable is the presence of eggs on spears which renders them unmarketable, as the eggs cannot be removed without damaging the plant tissue (Voigt & Gorb, 2010). The current asparagus market has zero tolerance for beetle damage and eggs on spears. Thus, oviposition management is a priority for the asparagus industry. However, a current understanding of common asparagus beetle oviposition behavior is lacking. A recent study aimed to elucidate whether the spotted asparagus beetle (*Crioceris duodecipunctata*), a close relative of the common asparagus beetle, responds to chemical compounds found in asparagus volatiles. The study found that while male beetles elicit a response to the volatile blend produced by asparagus cladophylls, female beetles did not respond to the volatile cues (Pistillo et al., 2022). Additionally, we have observed female common asparagus beetles laying eggs predominantly on the top of spears, but this behavior lacks documentation. Variability in spear height, diameter, and other physical characteristics could potentially provide cues to gravid females to discern spears when selecting oviposition sites. Uncovering host preferences can allow for a thorough understanding of a species' ecology, moreover these preferences can be targeted when managing the species in agricultural settings. Improving our insights could lead to better management strategies and potentially allow breeders to develop varieties that are less desirable for oviposition.

We conducted several choice tests, a no-choice bioassay, and behavioral observations to better understand host selection by common asparagus beetles. More specifically, we aimed to determine adult preferences for different spear characteristics that vary amongst plants during the harvest season. In our trials, we sought to answer 1) What spear characteristics do asparagus beetles prefer for oviposition? 2) Are spear tips essential for host selection? 3) Are there differences in beetle behavior across host characteristics?

Materials and Methods

Insects and Plants

Adult common asparagus beetles were collected from field sites in Holt and Linden Michigan, USA from May to July 2023. After collection, beetles were kept in the laboratory in mesh cages (122 cm x 70 cm x 70 cm; Nasco, Fort Atkinson, WI, USA) on a 16 h L: 8 h D light cycle at 25 ± 2°C in East Lansing, MI. Beetles were maintained on insecticide-free asparagus spears (cv. Millennium) supplied by the Michigan State University Horticulture Farm, Holt, MI. Spears were placed into deionized-water soaked floral foam blocks (7.1 cm x 9.6 cm x 22.3 cm, Artesia WetFōM®, Green Brick, FloraCraft®, Ludington, MI) to prevent desiccation and were replaced with fresh spears *ad libitum*. Asparagus used for the laboratory choice bioassays and observation trials were collected from field sites in Holt and Hart, MI. Spears were stored in containers with water at 4 ± 2°C until the experiment was set up, but no longer than 24 h.

Laboratory choice bioassays

We investigated asparagus beetle ovipositional preference for various spear characteristics. Spears were placed vertically into individual plastic cups (59 ml) containing cylindrical floral foam soaked in deionized water (5 cm x 5 cm). Cups were placed into aluminum trays (32 cm x 8 cm, Handi-max, Wheeling, IL) 10 cm apart from one another. To provide beetles with a surface to walk on and access to the spear, cups were embedded into gardening soil (Suremix Perlite, peat perlite, Michigan Grower Products Inc., Galesburg, MI). A cylindrical transparent cover (30 cm x 27 cm, transparency film, School Smart, Greenville, WI) with a mesh at the top for ventilation was used to prevent beetles from leaving the arena (Fig. 3.1A). Arenas were placed in a randomized block design inside growth chambers at 25ºC, with a 16 h L:8 h D h light cycle. To ensure oviposition, one pair of actively mating beetles were collected from the rearing cages and placed in the center of each arena. After 24 h, the number of eggs per spear was recorded.

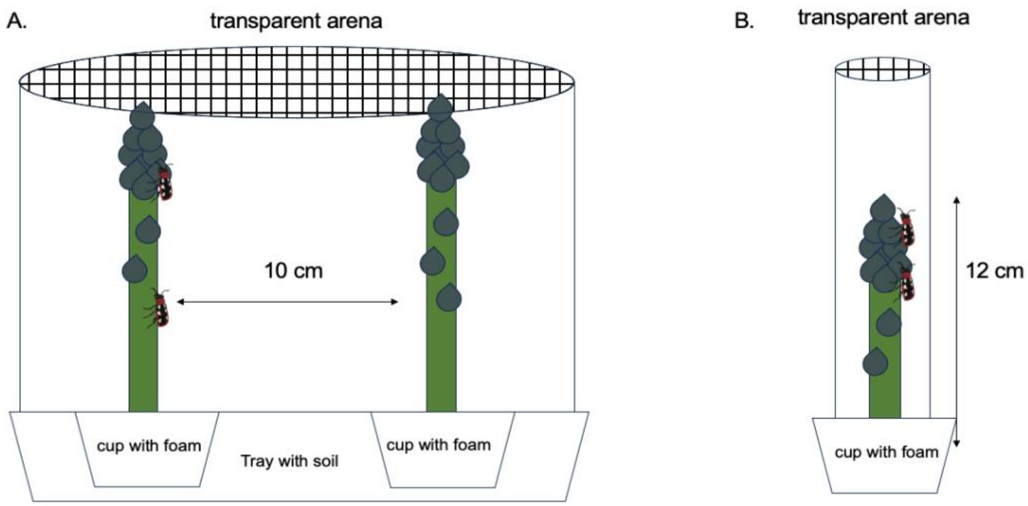

Figure 3.1 (A) An arena in the choice test bioassays was comprised of two 24 cm tall spears with contrasting characteristics, inserted in wet floral foam held in a plastic cup suspended in soil in an aluminum tray. The mating pair of asparagus beetles were kept enclosed by a transparent plastic film cage with a mesh top. (B) An arena used in the no-choice bioassays and behavioral observations was comprised of a 12 cm tall asparagus spear with or without the tip, inserted in wet floral foam held in a plastic cup. The mating pair of asparagus beetles were kept enclosed by a transparent plastic film cage with a mesh top.

Purple vs. Green

Spear color was tested to determine beetle preference by comparing a green variety (cv. Sequoia) against a purple variety (cv. Rosalie). Spears for both treatments were matched in diameter (1.5 cm) and height (24 cm) before being placed into arenas.

Tall vs. Short

Spear (cv. Millennium) height was tested to determine beetle preference by comparing tall (24 cm) and short (12 cm) spears identical in diameter (1.5 cm). Exact measurements were achieved by cutting spears from the bottom up with a razor blade, ensuring spear tips were kept intact.

Thick vs. Thin

Spear (cv. Millennium) width was tested to determine beetle preference by comparing thick spears (>1.75 cm) and thin spears (<1 cm) identical in height (24 cm). Exact measurements were achieved by cutting spears at the bottom with a razor blade, ensuring spear tips were kept intact.

No damage vs. 24 h damage

Choice tests comparing undamaged vs. previously damaged spears (cv. Millennium) were conducted to determine beetle ovipositional preference. Spears (24 cm tall, 1.5 cm diameter) used in the experiment were either previously damaged for 24 h by two common asparagus beetles or were undamaged spears. Before a new mating pair of beetles was introduced into choice test arenas, the number of eggs laid on each previously damaged spear was recorded. The number of newly laid eggs on spears was used for statistical analyses.

No damage vs. 48 h damage

Similar to the previous choice test, we compared undamaged vs. previously damaged spears to determine beetle ovipositional preference. Spears (24 cm tall, 1.5 cm, cv. Millennium) used in the experiment were either previously damaged for 48h by two common asparagus beetles or were undamaged spears. Before a new mating pair of beetles was introduced into choice test arenas, the number of eggs laid on each previously damaged spear was recorded. Only the number of newly laid eggs on spears was used for statistical analyses.

Beetle behavior experiments

No choice bioassays

No choice bioassays were conducted to determine asparagus beetle feeding and ovipositional preference of spears with and without the spear tip (cv. Millennium). We used 24 cm tall spears that were cut in half into two 12 cm pieces, one with and one without the tip. Similar to the choice bioassays, each spear piece was vertically placed into water-soaked floral foam cylinders (5 cm x 5 cm) and held in cups (59 ml). Each cup was covered by a cylindrical transparent cage (4.5 cm x 30 cm) with a mesh top to allow for ventilation and prevent beetles from escaping (Fig. 3.1B). A single mating pair of asparagus beetles was placed into each arena, and moved to growth chambers (25 ºC, 16L:18D h light cycle) for 24 h. The number of eggs on each spear was recorded after 24 h.

Behavioral observations

Behavioral observation trials were set up to compare asparagus beetle oviposition behavior on different parts of the asparagus spear. Spears (24 cm tall, cv. Millennium) were cut in half leaving two 12 cm pieces, one piece with the spear tip intact and another piece with only the bottom half of the spear. Similar to our no-choice bioassays, one mating pair was placed into individual arenas in a laboratory setting with ambient conditions and supplemental full-spectrum lighting. Observation periods were conducted at the beginning of each experiment (0 h) and at 23 h using the 'Behavye' mobile app (Fulton et al., 2023). Behaviors recorded were categorized as either an event or a state, with events being sudden actions (i.e. dropping) while states were long-lasting conditions (i.e. feeding, mating, mounting, oviposition, resting, walking). Every beetle's behavior was individually tracked within the arenas for 4 h, the duration and frequency of each behavior was recorded during this time. The amount of eggs found on each spear was recorded at 4 h, 23 h, 28 h, and 48 h marks.

Statistical analyses

Statistical analyses were conducted in R (Version 4.2.1, R Core Team 2023). Chi-square tests were used to compare the total number of eggs between traits in the choice test and no-choice bioassays. The mean numbers of eggs per spear were analyzed using a two-sample t-test to determine the difference between the traits compared in the choice-test and no-choice bioassays. Chi-square tests were used to compare the number of times a spear trait was selected for oviposition in the choice test and no-choice bioassays. The egg count data from the no-choice bioassay that tested spears with and without tips were analyzed with a generalized linear model with a quasipoisson distribution using treatment, hour, and date as fixed factors. Behaviors analyzed for our study were classified as ‘states’ and included: mating, resting, oviposition, walking, feeding, and mounting. Beetle behavior frequency data was analyzed using Chi-square test to an analysis of variance for a linear mixed effects model with frequency as the dependent variable, and behavior and treatment as fixed effects. Treatment means were separated using ‘emmeans’ with a false discovery rate. Chi-square tests were run to determine differences between the frequency at which behavior occurred for either treatment. Beetle behavior duration data was analyzed using a linear mixed effects model to determine if the session of the trial influenced beetle behavior between the two treatments, behavior and treatment were fixed effects with duration as the dependent variable. A similar model was analyzed using a generalized linear model with duration as the dependent variable, behavior and treatment as fixed effects with no random effect.

Results

Laboratory choice bioassays

Purple vs. Green

There was a significant difference between purple and green spears in the total number of eggs found on spears ($\chi^2 = 8.6$, df = 1, $P < 0.005$). Purple spears had almost 2 times more eggs than green spears (Fig. 3.2A). The average number of eggs per spear between the two treatments

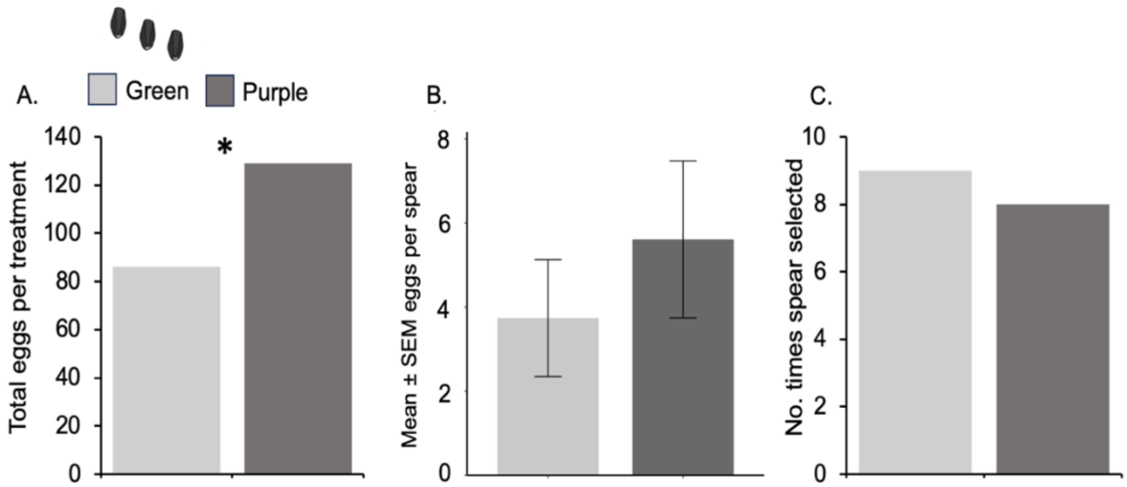

Figure 3.2 Asparagus beetle oviposition preferences between green and purple spears was examined in a choice test (A) The total number of eggs counted on all green and purple spears. (B) The mean (± SEM) number of eggs per spear on green and purple spears. (C) The number of times asparagus beetles selected a green or purple spear for oviposition. Asterisks indicate statistical differences (Chi-sq test (A, C), t-test (B), P < 0.05).

(t = -0.8, df = 40.7, P > 0.1, Fig. 3.2B) and the number of times the treatments were selected (χ^2 = 0.05, df = 1, P > 0.05, Fig. 3.2C) were statistically similar.

Tall vs. Short

There was a significant difference between tall and short spears in the total number of eggs found on spears (χ^2 = 136.9, df = 1, P < 0.01). Tall spears had 5 times more eggs than short spears (Fig. 3.3A). There was also a significant difference in the average amount of eggs per spear, with tall spears receiving more eggs on average (t = -3.9, df = 29.7, P < 0.1, Fig. 3.3B). Additionally, there was a statistically significant difference for the number of times a spear was selected for oviposition, with beetles preferring tall over short spears ($\chi^2 = 5.3$, df = 1, P < 0.05, Fig. 3.3C).

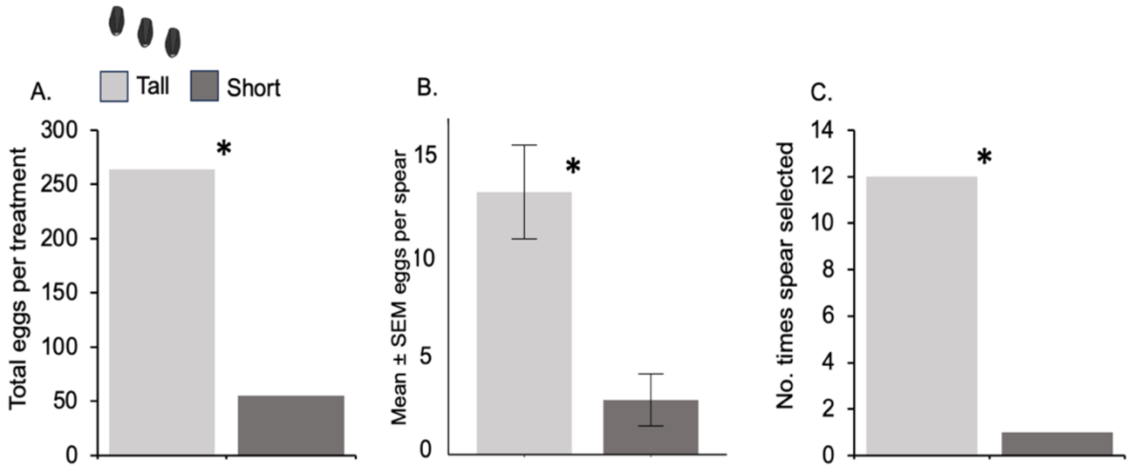

Figure 3.3 Asparagus beetle oviposition preferences between tall and short spears was examined in a choice test (A) The total number of eggs on tall and short spears. (B) The mean (± SEM) number of eggs per spear on tall and short spears. (C) The number of times asparagus beetles selected a tall or short spear for oviposition. Asterisks indicate statistical differences (Chi-sq test (A, C), t-test (B), P < 0.05).

Thick vs. Thin

There was a significant difference between thick and thin spears in the total number of eggs found on spears ($\chi^2 = 5.4$, df = 1, P < 0.05). Thin spears had almost twice as many eggs than thick spears (Fig. 3.4A). The average number of eggs per spear between treatments was similar (t = -0.8, df = 17.9, P > 0.1, Fig. 3.4B) and the number of times spears were selected for oviposition in both treatments were similar ($\chi^2 = 0$, df = 1, P = 1 Fig. 3.4C).

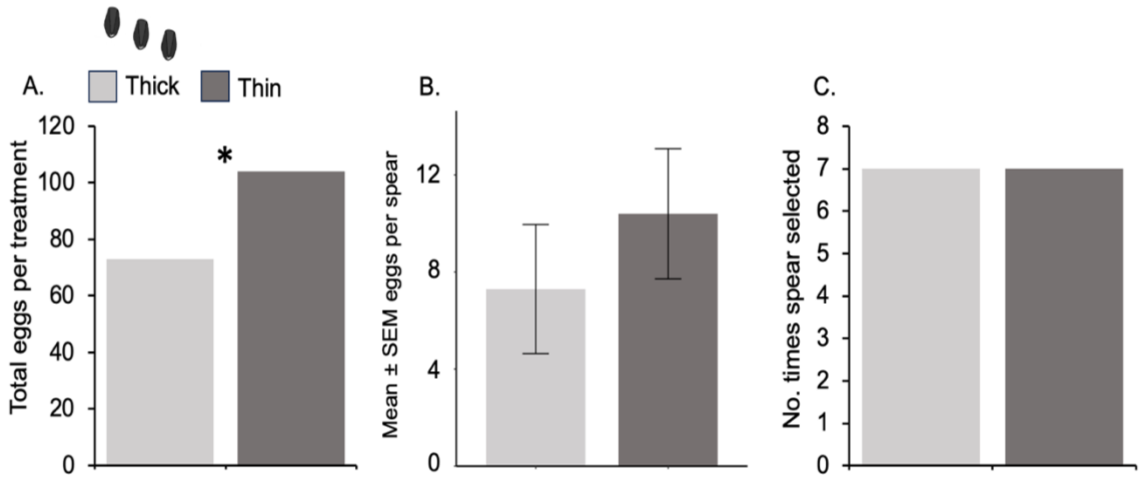

Figure 3.4 Asparagus beetle oviposition preferences between thick and thin spears was examined in a choice test (A) The total number of eggs on thick and thin spears. (B) The mean (± SEM) number of eggs per spear on thick and thin spears. (C) The number of times asparagus beetles selected either thick or thin spears for oviposition. Asterisks indicate statistical differences (Chi-sq test (A, C), t-test (B), $P < 0.05$).

No damage vs. 24 h damage

There was a significant difference in the total number of eggs found on the two treatments ($\chi^2 = 29.4$, df = 1, $P < 0.01$). Undamaged spears had almost twice as many compared to 24 h damaged spears (Fig. 3.5A). There was no significant difference between treatments in the average number of eggs per spear ($t = -1.74$, df = 34.3, $P > 0.05$, Fig. 3.5B) and number of times spears were selected ($\chi^2 = 0.04$, df = 1, $P < 0.05$, Fig. 3.5C).

No damage vs. 48 h damage

Undamaged spears had almost 5 times more eggs than 48 h damaged spears ($\chi^2 = 65.6$, df = 1, $P < 0.01$), Fig. 3.5D). Undamaged spears had a significantly higher average of eggs/spear than 48 h damaged spears ($t = -2.3$, df = 25.2, $P < 0.05$, Fig. 3.5B). There was no significant difference in the number of times spear traits were selected for oviposition ($\chi^2 = 1.7$, df = 1, $P > 0.05$, Fig. 3.5F).

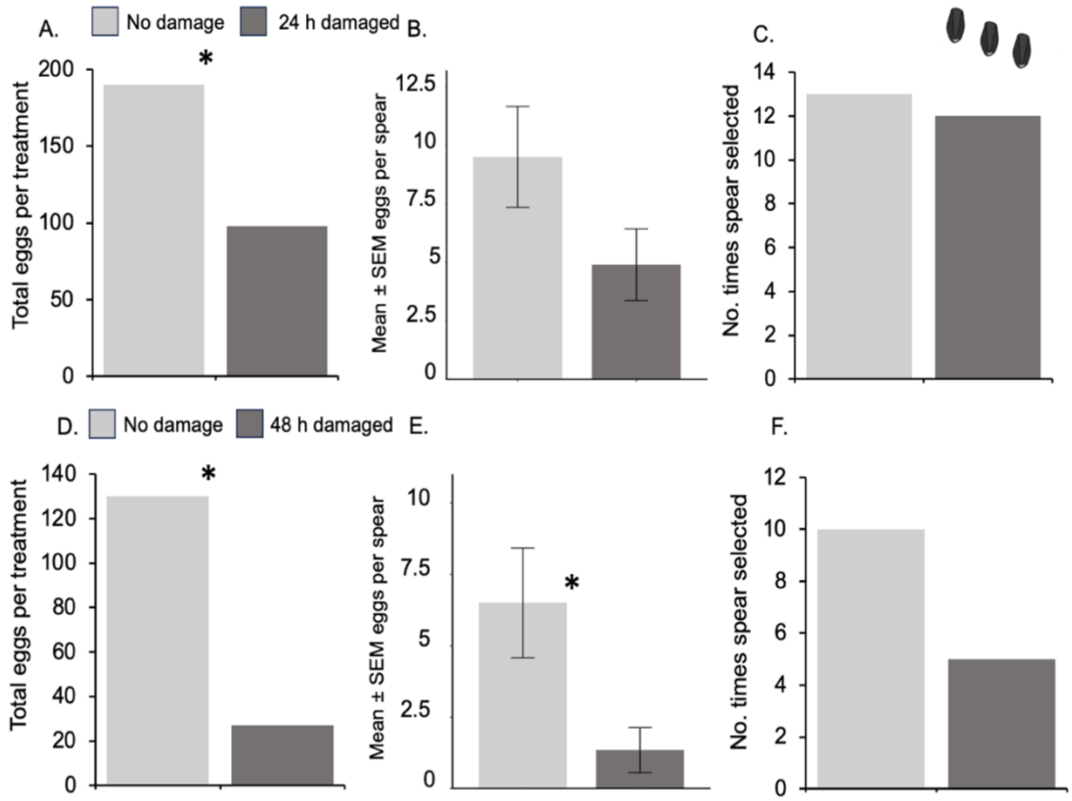

Figure 3.5 Asparagus beetle ovipositional preferences between previously conspecific damaged and undamaged spears was examined in two choice tests. (A) The total number of eggs on undamaged and spears fed on by asparagus beetles for 24 h. (B) The mean (± SEM) number of eggs per undamaged and 24 h damaged spears. (C) The number of times asparagus beetles selected either undamaged or 24 h damaged spear for oviposition. (D) The total number of eggs on undamaged and spears previously fed on by asparagus beetles for 48 h. (E) The mean (± SEM) number of eggs per undamaged and 48 h damaged spears. (F) The number of times asparagus beetles selected either undamaged or 48 h damaged spear for oviposition. Asterisks indicate statistical differences (Chi-sq test (A, C), t-test (B), $P < 0.05$).

Beetle behavior experiments

No choice bioassays

There was a significant difference between spears without tips and those with tips in the total number of eggs per spear ($\chi^2 = 129.4$, df = 1, $P < 0.01$). Tipped spears had 7 times more eggs than spears without tips (Fig. 3.6A). The average number of eggs per spear was significantly higher on tipped spears ($t = -2.9$, df = 18.8, $P < 0.05$, Fig. 3.6B). Furthermore, there was a significant difference in the number of times treatments were selected for oviposition, with tipped spears receiving 2 times more eggs than those without tips ($\chi^2 = 3.9$, df = 1, $P < 0.05$, Fig. 3.6C).

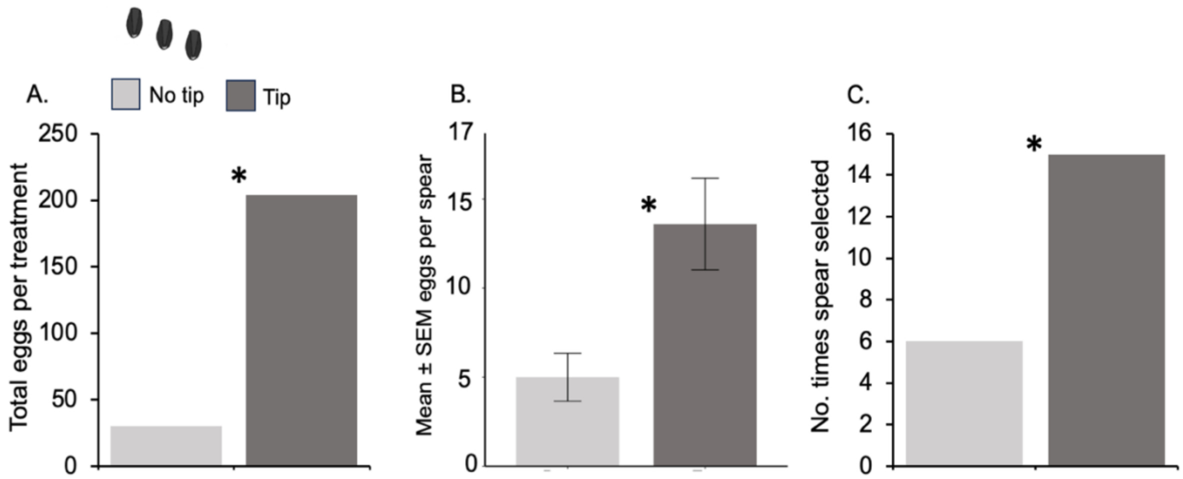

Figure 3.6 The influence of spear tips on asparagus beetle ovipositional preferences was examined through a no-choice test. (A) The total number of eggs on spears with and without tips. (B) The mean (± SEM) number of eggs per spear with and without tips. (C) The number of times asparagus beetles selected spears with or without tips for oviposition. Asterisks indicate statistical differences (Chi-sq test (A, C), t-test (B), $P < 0.05$).

Behavioral observations

There were no significant differences in the total number of eggs between spears with and without tips ($\chi^2 = 1.738e\text{-}05$, df =1, $P > 0.05$). When analyzing the interaction between treatment and time, there was no significant difference between treatments ($\chi^2 = 0.7732$, df =3, $P > 0.05$). There were significantly fewer eggs for both treatments 4 h after the start of the experiment ($\chi^2 = 0.346$, df =3, $P < .0001$), but no difference was seen in egg abundance for either treatment at the 23 h, 28 h, or 48 h marks ($\chi^2 = 2.107$, df =3, $P > 0.1$, Fig. 3.7). Spear treatment did not affect the duration of behaviors ($F = 0.405$, df = 1, $P > 0.1$), or affect the number of times behaviors occurred ($F = 0.230$, df = 1, $P > 0.1$). Of the several behaviors recorded, significant differences were only identified for mating and walking. Beetles on tipped spears had significantly more mating events recorded compared to those on untipped spears ($\chi^2 = 5.79$, df =1, P <.01, Fig. 3.8). Beetles on untipped spears were recorded walking around arenas significantly more than beetles with tipped spears ($\chi^2 = 5.49$, df =1, P <.01, Fig. 3.8).

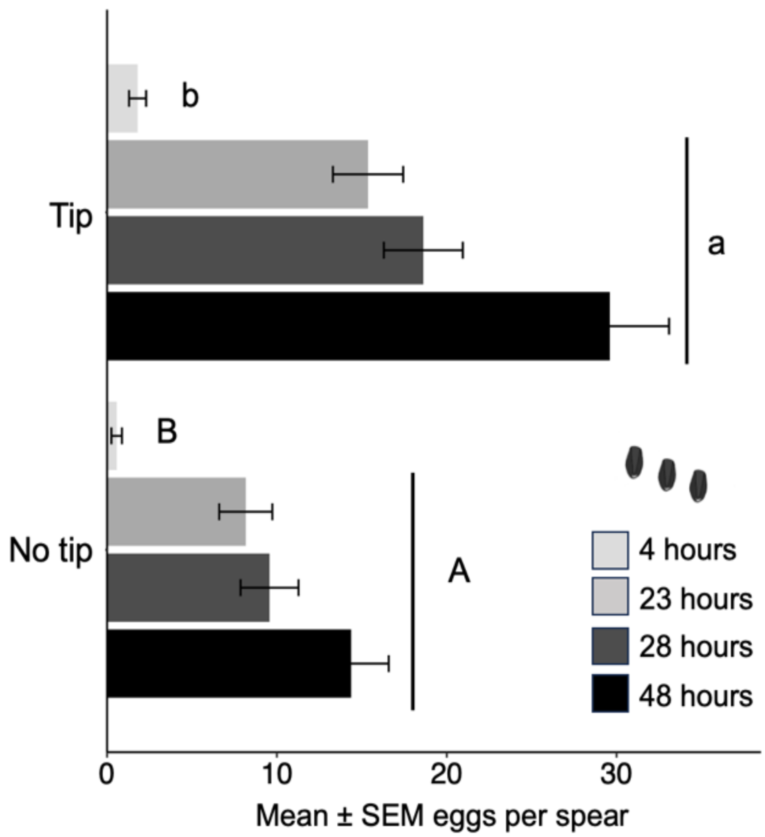

Figure 3.7 The mean (± SEM) number of eggs on spears with and without tips at 4 h, 23 h, 28 h, and 48 h time points in the behavioral observations.

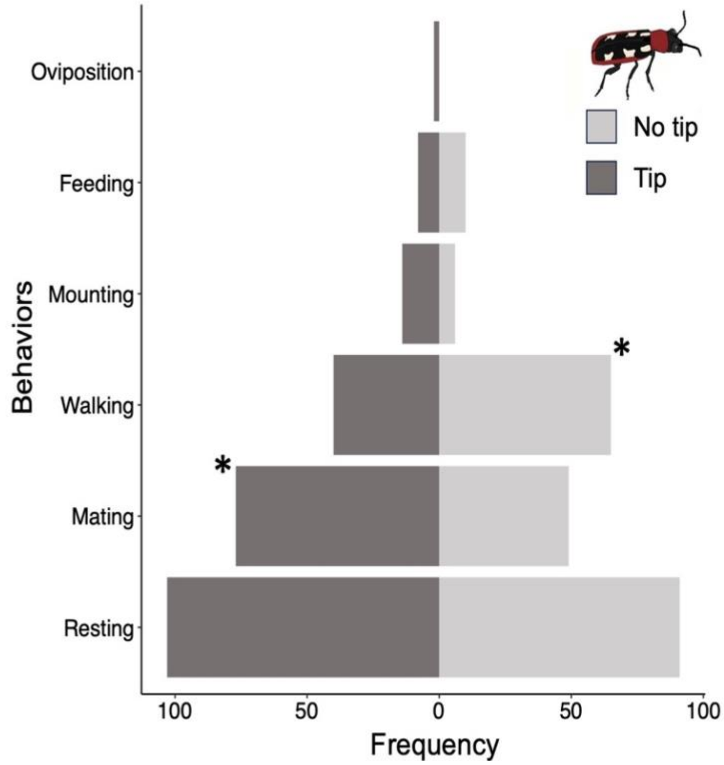

Figure 3.8 The frequency (number of times a behavior occurred) at which a beetle's behaviors were recorded on spears with or without tips during behavioral observations. Asterisks represent statistical differences in the frequency of behaviors between treatments (Chi-sq test, $P < 0.05$).

Discussion

Our study aimed to identify host selection behaviors of the common asparagus beetle. More specifically we sought to determine desirable characteristics as it relates to oviposition site selection. We determined host plant traits favorable to female beetles by conducting choice tests that examined characteristics commonly found in asparagus fields during harvest. Additionally, our no-choice bioassay and behavioral observation revealed the importance of spear tips to asparagus beetle oviposition.

In the choice tests, asparagus beetles preferred purple spears compared to green spears. There was no significant difference in the number of times colors were selected, but the total number of eggs on purple spears was significantly higher (Fig. 3.2A), suggesting that color may not be influential for asparagus beetle host selection. This finding indicates that beetles selected purple spears during the acceptance phase when gustatory and tactile cues are utilized for host selection (Dethier, 1982). It is unknown which stimuli are responsible for the beetle's preference, but the purple and green spears were of different cultivars, and purple cultivars typically have a lower fiber content compared to the green cultivar which could influence adult preference (Falloon & Andersen, 1999). Adults could be selecting purple spears as they are easier to consume for themselves and their offspring. One study distinguished preferable brassica cultivars to be used as possible trap crops for two beetle pests; the seed weevil *Ceutorhynchus assimilis* (Paykull) (Coleoptera: Curculionidae) and the pollen beetle *Brassicogethes aeneus* (Coleoptera: Nitidulidae). Particularly, the pollen beetle was attracted to the volatiles released by turnip rape which would allow for the main crop to evade beetle pests until it reached a tolerant growth stage (Cook et al., 2006). Asparagus beetle preference for spears with lower fiber content can similarly open an avenue to utilize the cultivar for trap cropping.

Spear shape may be providing fundamental long-distance cues during host finding that determine if the insect moves toward or away from the host. Our choice tests indicated that tall spears were preferred for oviposition compared to short spears. Unlike in the case of the purple and green choice test, tall spears received more eggs in total, more eggs on average, and were more often selected for oviposition (Fig. 3.3), suggesting that spear height was perceived at a distance before arrival. Conversely, the thickness of the spear seemed to play less of a role as a cue, with the two spear choices selected evenly for oviposition, but more eggs were laid on thin spears overall than thick ones (Fig. 3.4). This may indicate that there are certain spear traits that are utilized more when making host selection decisions at a distance, for example by recognizing height rather than thickness.

Interestingly in the choice test between undamaged and previously damaged spears, the preference for spears previously damaged for 24 h was only identifiable by the total number of eggs present on undamaged spears (Fig. 3.5A-B). However, in the choice test against 48 h damaged spears, beetles selected undamaged spears significantly more as shown by the total number of eggs and average number of eggs per spear (Fig. 3.5D,E). Thus, it is likely that increasing damage repelled females from oviposition. Similar studies conducted on host selection of herbivorous species have often demonstrated a preference for hosts with minimal or less damage (Wise & Weinberg, 2002). The motives for this can vary depending on host specificity; for specialists these choices are made to prevent competition for their offspring amongst conspecifics and heterospecifics (Gripenberg et al., 2010; L. C. Jones et al., 2019). This is likely the case for the common asparagus beetle, as competition for resources is usually against other asparagus beetle larvae and adults. However, it is also possible that female asparagus beetles select plants to maximize their own fitness, which in this species overlaps completely with larval food preference (Fujiyama et al., 2008; Scheirs & De Bruyn, 2002). Female beetles may also perceive the damage as a reduction in the available surface for oviposition. More specifically, herbivore-induced plant volatiles (HIPVS) emitted from the damaged spears may deter female beetles as they have been documented to attract parasitoids (Ingrao et al., 2019).

We confirmed our previous observations that spear tips are important oviposition cues for asparagus beetle. Beetles laid more eggs and created larger egg masses on spears with a tip than without when given a choice. In our no choice assay, the rate of egg accumulation over time was similar between the two types of spears indicating that once oviposition started, they committed to egg laying behavior and this pattern was consistent over time. When comparing the behaviors of beetles given either tipped or untipped spears, the frequency at which beetles were recorded to be mating was significantly higher on tipped spears (Fig. 3.8). These findings are interesting combined with the results that beetles on spears without tips were not seen ovipositing throughout the observation. These results are also supported by the no-choice bioassays, where beetles selected for spears with tips across all three parameters (Fig. 3.6A-C). This suggests that asparagus tips play an important role when female beetles are selecting hosts for oviposition. While the mechanism for this is yet unknown, spear tips may serve as both visual and tactile stimuli for female beetles. Interestingly, overall behaviors were similar on spears with and without tips, this may indicate that beetles only recognize tips when they are present. It also indicates that spear tips are not critical for mating behavior but provide an important cue, although not essential, for oviposition behavior to occur. During harvest, spears are consistently removed from the field, leaving newly emerged spears as the only resource for beetles. Since tips are the first part of the spear to emerge from the soil, this is the first plant cue beetles encounter thus making it a distinguishing characteristic beetles likely evolved to recognize.

From our study, we determined some key characteristics of asparagus spears used for asparagus beetle host selection. We determined that host selection decisions can be made both before arrival and after contacting hosts, and spear tips provide an important signal for oviposition. Like our study, previous research conducted on host preferences of crop pests utilized their findings to create management plans to target susceptible behaviors of pest ecology. Cultural methods such as trap cropping, tillage, residue management, and cultivar resistance are a few practices that can lower pest pressure in fields (Cullen & Holm, 2013; Glen, 2000; Perez et al., 2021; Sotelo et al., 2014). Due to the perennial nature of asparagus, implementing cultural control methods must rely on the available resources in a grower's field. Practices such as residue management and trap cropping using preferred spear traits are a few methods that can be feasibly executed. Utilizing such practices alongside traditional pesticide applications and biological control can help to promote integrated pest management in modern agriculture, and sustainably manage pests without relying heavily on pesticides. While our results demonstrate traits favorable to asparagus beetles, future studies should utilize this information to create field-applicable management practice.

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CHAPTER 4: Conclusions and future directions

In conclusion, this thesis provides valuable information for the management of an economically important asparagus pest, the common asparagus beetle (*Crioceris asparagi*). Previous studies on asparagus beetle management have included using alternative management methods to control asparagus beetle populations. However, these findings have gained limited application by growers, who rely primarily on insecticide applications for asparagus beetle management. While one chapter of my thesis particularly tests insecticides for management of the common asparagus beetle, my thesis as a whole attempts to create sustainable management practices for the common asparagus beetle by combining chemical and cultural control.

In Chapter 2, I sought to determine the efficacy of commercially available synthetic and organic insecticides registered for use on asparagus during the harvest season, but that are currently underutilized by growers because of uncertainty around their efficacy. My study is one of a few formal publicly available insecticide trials for the common asparagus beetle. My trials not only aimed to elucidate the efficacy of alternative conventional, synthetic insecticides but also tested various bioinsecticides that are novel for asparagus beetle management. We tested a variety of active ingredient and mode-of-actions to thoroughly compare their efficacies. In both laboratory and field trials, carbaryl represented the grower standard as it is used commonly amongst commercial asparagus growers in Michigan. Results from lab trials indicated that both bio- and alternative synthetic insecticides effectively controlled asparagus beetles. Among the tested bioinsecticides, spinosad demonstrated efficacy comparable to carbaryl in reducing both adult activity and egg abundance on treated asparagus spears. Behavioral avoidance of insecticide-treated spears was observed in lab bioassays, particularly with the bioinsecticides pyrethrin and azadirachtin/pyrethrin, suggesting a repellent effect on adult beetles. In field trials, acetamiprid was particularly effective in reducing adult and egg numbers on harvestable spears, and was comparable to the grower standard. However, the study also identified variability in insecticide performance, with chlorantraniliprole demonstrating efficacy primarily against egg-laying rather than adult beetles. Interestingly, both research settings highlighted that spears treated with insecticides retained efficacy after 48 hours in managing beetles and their oviposition, revealing that adequate coverage can significantly contribute to controlling both adult beetles and their eggs. Overall, this chapter provides valuable insights into the efficacy of insecticide used in managing the common asparagus beetle, offering a foundation for informed decision-making in pest management practices within the asparagus industry.

In Chapter 3, my research focused on deducing the common asparagus beetle's host selection preferences, specifically oviposition preference. To alleviate the heightened challenge posed by asparagus beetle eggs on growers' ability to sell spears on the market, life stage specific management practices are well-needed. However, host selection preference and oviposition behavior of the common asparagus beetle are understudied, with most research highlighting the preferences of close relatives of the species (Pistillo et al., 2022; Van Alphen & Boer, 1980). To evaluate selection preferences based on specific host characteristics, I conducted choice tests, no-choice tests, and behavioral observations of mating pairs of asparagus beetles. Choice tests examined asparagus beetle preference for spear traits commonly found during the harvest season. Results from the choice tests revealed that asparagus beetles exhibited a preference for purple spears over green ones, suggesting a potential cue for host selection during the acceptance phase, possibly influenced by factors like fiber content. Additionally, we believe spear shape played a crucial role in visual cues, as tall spears were preferred for oviposition over short ones. Interestingly, beetles showed a preference for undamaged spears over those previously damaged by other asparagus beetles, potentially indicating a behavior observed in numerous phytophagous species. In these choice tests, I further examined the influence of spear traits on oviposition decisions for the asparagus beetle. Previous observations from my asparagus beetle research revealed that asparagus beetles regularly laid eggs towards the tip of asparagus spears. For this reason, I set up no-choice bioassays and behavioral studies to determine the impact spear tips have on asparagus beetle oviposition preferences. Both studies confirmed the significance of spear tips as an oviposition cue, with beetles exhibiting a clear preference for spears with tips over those without, suggesting a role for both visual and tactile stimuli in host selection. These findings underscore the complex interplay between insect behavior and plant characteristics in pest ecology. By elucidating key characteristics influencing asparagus beetle host selection, my findings contribute to the development of integrated pest management strategies, which combine several practices to promote sustainable agriculture. Further research is needed to translate these findings into practical field management practices tailored specifically for the common asparagus beetle and the asparagus crop.

Overall, my thesis helps to build an understanding of asparagus beetle management in the context of IPM. Based on my findings, several synthetic and organic insecticides are able to effectively reduce asparagus beetles and their eggs regardless of the amount of time that has passed since application or coverage. While my findings support successful management, some of the insecticides demonstrated variable efficacy at reducing beetles and egg-laying. However, the results of my third chapter aid in providing information for the development of additional cultural management practices to target either adults or eggs. My results suggest that asparagus beetle host selection is influenced by visual and gustatory cues presented by the spear during harvest season. Favorable spears are more likely to experience high pressure from asparagus beetles and their egg laying. Horticultural strategies might be adopted that take advantage of these traits and incorporated into management programs as new cultural practices. Yet, more research is needed to uncover the underlying mechanisms for such host preferences. In combination, these findings can promote the use of alternative practices besides traditional chemical control with synthetic insecticides. Bioinsecticides and cultural practices can offer complementary support for asparagus beetle management in commercial fields. Future research should examine how to develop these findings into reasonable practices for asparagus growers that not only effectively manage beetle populations but promote sustainable agriculture.

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APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2023-14

Author and Title of thesis:
Author: Laura Marmolejo
Title: Utilizing Behavior and Chemical Control for the Management of the Common Asparagus Beetle (*Crioceris asparagi*)

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Table S. 1. Voucher specimens deposited at the Albert J. Cook Arthropod Research Collection (Michigan State University).

Family	Genus-Species	Life Stage	Quantity	Preservation
Chrysomelidae	*Crioceris asparagi*	Adult	10	pinned