Developing an Integrated Pest Management Plan for Edamame in Virginia

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Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

Entomology

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December 6th, 2022

Blacksburg, VA

Keywords: edamame, pest management, pesticides, stink bug, corn earworm

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Abstract

Edamame (*Glycine max* (L.) Merr.), also known as vegetable soybean, is primarily grown and consumed in Asia. In recent years, the demand for edamame in the United States has risen due to its health benefits as an alternative, plant-based protein. Due to the lack of domestic production, most edamame is imported from Asia. In an attempt to increase domestic production, research efforts have begun in Virginia and other regions to develop cultivars and best management practices for growing edamame in the mid-Atlantic region. Beginning in 2018, edamame trials examining breeding lines and cultivars were conducted to look at their suitability for this region. These varieties were sampled and evaluated for insect and disease complexes as well as their implications on plant yield and quality. Most of the insects and diseases that were found were very similar to pest complexes commonly found in cultivated soybeans in Virginia. However, due to edamame being marketed as a vegetable, insects and diseases that caused unsightly blemishes or damage to the pods or seeds were most concerning. Multiple insects and diseases were present but some of the most important insects and diseases we observed from 2018-2020 were pod feeding stink bug (Hemiptera: Pentatomidae), as well as the diseases like purple seed stain, *Cercospora kikuchii*, and bacterial pustule, *Xanthomonas axonopodis* pv. *glycines*. From 2019-2021 an integrated pest management study was conducted to determine best management practices for minimizing insecticide applications while applying them at thresholds to control key pests. In three growing seasons, I was able to determine that pesticides can be limited prior to flowering while insecticide inputs will need to be increased

after flowering to protect the pods from pests, specifically stink bugs. Additionally, from 2019-2021 insecticide and fungicide field trials were conducted to test different pesticides on their efficacy against pod damaging pests and diseases. The growing seasons between 2019 and 2021 resulted in varying insect and disease pressure that led to inconsistent results. However, insecticides such as cyclaniliprole and sulfoxaflor performed well compared to other treatments. Lastly in 2020-2021, corn earworm (*Helicoverpa zea* [Boddie], Lepidoptera: Noctuidae), an important soybean pest and most likely a major pest of edamame, was tested for pyrethroid susceptibility and resistance across the state using a bean-dip bioassay. Pyrethroid efficacy to control this pest across Virginia seemed to vary by location and year, however, the bean-dip bioassay proved to be a time-efficient strategy for further monitoring these populations in the coming years.

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General Audience Abstract

Edamame, also known as vegetable soybean, is primarily grown and consumed in Asia. Due to the lack of domestic production, most edamame is imported from overseas. Edamame trials were conducted in Virginia beginning in 2018, to look at production practices suitable for growing this crop in the region. Scientists observed edamame to document insect and disease complexes as well as their implications on yield and quality. Most of the insects and diseases that were found were very similar to what is known to already occur in soybeans. However, due to edamame being marketed as a vegetable, insects and diseases that left blemishes or damage to the pod were most concerning. Multiple insects and disease were present but some of the most important insects and diseases we observed were pod feeding stink bug species as well as the diseases purple seed stain and bacterial pustule. In 2019-2021 a pest management study was conducted to determine best management practices for minimizing insecticide applications while applying them at thresholds to control key pests. We were able to determine that pesticide usage can be limited prior to flowering while they will need to be increased after flowering to protect the pods. Additionally, in 2019-2021 insecticide and fungicide field trials were conducted to determine the efficacy of materials against pod damaging pests and diseases. The growing seasons between 2019 and 2021 resulted in varying insect and disease pressure, however, several insecticides with reduced ecological impacts out preformed others. Lastly in 2020-2021, corn earworm, a major pest of soybean and presumable of edamame, was tested for pyrethroid susceptibility and resistance across the state using a bean-dip bioassay. Pyrethroid efficacy

across Virginia seemed to vary by location and year but the bean dip bioassay method proved to be a time-effective strategy for monitoring the states corn earworm populations against insecticides.

Acknowledgements

I would firstly like to thank both of my advisors Dr. Tom Kuhar and Dr. Steven Rideout. Thank you both for taking me under your wings and encouraging and helping me further develop my interest for applied research and extension. I am thankful for the opportunity to have worked on this project with both of you.

I would also like to thank my committee members Dr. Alejandro Del Pozo-Valdivia, Dr. Sally Taylor, and Dr. Mark Reiter. Thank you all for the many conversations and support over the course of my time here at Virginia Tech. I not only consider you all mentors but good friends. I hope that our paths cross many times in the future.

To my parents, and siblings. Thank you all for the support and love through the years. You've always had my back and have given me great advice when I needed it.

I'd like to thank the department of entomology and all its members for providing me with a great community to develop as a graduate student. I have enjoyed all the great classes and conversations with faculty, students, and staff.

I'd like to thank the faculty and staff at the Eastern Shore Agricultural Research and Extension Center, especially Hélène Doughty, for helping me with my various research projects but also welcoming me into the stations family when I was working there in 2019.

It has been a great pleasure to have been part of the Kuhar lab, working with such an amazing group of people always led to a positive working and learning environment and I am very thankful to have had you all by my side especially when edamame harvest came around. I'd especially like to thank Kadie Britt for showing me the ropes when I first started and teaching me how to be the best graduate student I could be. Additionally, I would like to thank Kelly McIntyre, Kyle Bekelja, Sean Boyle, and Tim Bryant for not only being great colleagues and lab mates but lifelong friends.

Finally, I'd like to thank my girlfriend Erika who has supported me throughout my years in graduate school. Thank you for being by myside and loving and supporting me every single day. It has meant the world to me.

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Chapter 1: Introduction

Edamame production and its challenges in the mid-Atlantic U.S.

Edamame is large-seeded edible soybean (*Glycine max* (L.) Merr.) that is an important high protein vegetable in Asia (Schurtleff 2014). Edamame contains most of the essential amino acids found in animal proteins and are notably the most abundant plant source of protein on the planet (Velasquez & Bhathena 2007). Fresh soybean seeds are also low in lipids and most of these lipids are monounsaturated fatty acids, making edamame a highly nutritious food (Mentreddy et al. 2002). Soybeans are also one of the few natural sources of isoflavones and tocopherols (vitamin E) plus have high levels of Ca, P, K, Na, carotene, iron, vitamins B1, and B2, and ascorbic acid (Mentreddy et al. 2002). Because of the aforementioned health benefits, edamame is growing in popularity in the United States (Carneiro et al. 2021). Unlike conventional soybeans that are harvested after senescence as grain, edamame is harvested green at the R6 growth stage when the seeds have filled out the pod (Fehr & Caviness 1977). Although the United States is one of the world's largest producers of soybeans, the domestic production of edamame is miniscule in comparison. It is estimated that over 90% of edamame is imported from China and Taiwan (Roseboro 2012). This leaves a large market for edamame production in the United States.

An endeavor to develop large-seeded soybean cultivars for mechanized edamame production and improved consumer acceptance in the U.S. has been initiated by researchers at Virginia Tech partnering with soybean breeders from University of Arkansas and University of Missouri (Zhang et al. 2018). However, there are some obstacles that must be overcome before edamame can be successfully grown domestically in the United States. Some of these obstacles include developing cultivars adapted to grow in the United States. Domestic soybean cultivars do not contain the same traits as edamame cultivars from Asia because they lack the nutritional components and flavor that edamame has. Additionally, pest management strategies for insects and disease will need to be researched and developed to give edamame growers in the mid-Atlantic the proper tools to grow this crop successfully.

Although the complex of insects and diseases that attack edamame will likely be quite similar to traditional soybeans, pest management approaches will probably differ. The economic tolerance for pod injury will be considerably lower for edamame than for soybeans because edamame will be treated as a vegetable where the tolerance for blemishes to the pods will be extremely low (Wilson 2014). In addition, through decades of breeding, many high yielding soybean grain cultivars in the U.S. have tolerance and/or resistance levels to various insect pests and diseases that occur in North America; it is likely that edamame will show greater susceptibility to various pests and diseases.

Soybean in the U.S. is attacked by a wide range of insects including chewing defoliators, piercing-sucking plant sap feeders, stem feeders, and most importantly those that attack the marketable pod and seeds. Based on Reisig and Herbert (2019), in Virginia and North Carolina the chewing foliage feeders include: Lepidoptera: green cloverworm (*Plathypena scabra*); soybean looper (*Pseudoplusia includens*); velvetbean caterpillar (*Anticarsia gemmatalis*); beet armyworm (*Spodoptera exigua*); yellowstriped armyworm (*Spodoptera ornithogalli*); fall armyworm (*Spodoptera frugiperda*); saltmarsh caterpillar (*Estigmene acrea*); and silverspotted skipper (*Epargyreus clarus*); Coleoptera: Mexican bean beetle (*Epilachna varivestis*); bean leaf beetle (*Cerotoma trifurcata*); Japanese beetle (*Popillia japonica*); spotted cucumber beetle

(*Diabrotica undecimpunctata howardi*); and blister beetle (*Epicauta* spp.); Orthoptera: grasshoppers mostly (*Melanoplus* spp.).

Sucking-leaf feeding pests include potato leafhopper (*Empoasca fabae*) and soybean aphid (*Aphis glycines*). Stem feeders include three-cornered alfalfa hopper (*Spissistilus festinus*); dectes stem borer (*Dectes texanus*); grape colaspis (*Colaspis brunnea*); lesser cornstalk borer (*Elasmopalpus lignosellus*); and kudzu bug (*Megacopta cribraria*). Probably most important; however, are the insects that attack the seed and pods, and these are dominated by: corn earwom (*Helicoverpa zea*) and several species of stink bugs including (*Chinavia hilaris, Euschistus servus*, and the invasive species *Halyomorpha halys*).

Soybeans in Virginia are targeted by a wide range of diseases as well. Based on Phipps et al. 2010, soybeans in Virginia are susceptible to Downey mildew (*Peronospora manschurica*), brown spot (*Septoria glycines*), Frogeye leaf spot (*Cercospora sojina*), Cercospora blight and purple seed stain *(Cerocospora kikuchii*), target spot (*Corynespora cassiicola*), anthracnose (*Colletotrichum truncatum*), bacterial blight, (pseudemonas syringae), bacterial pustule (*Xanthomonas campestris*), charcoal rot (*Macrophomina phaseolina*), and Sclerotium blight (*Scleortium rolfsii*).

In this dissertation, I conducted research to improve our knowledge of edamame pest and disease management in Virginia. My specific objectives included:

1. To assess differences among edamame genotypes (new varieties) to insect pests and plant pathogens in Virginia.
2. To evaluate an insect pest scouting program for edamame in the mid-Atlantic.
3. To evaluate the efficacy of IPM-compatible insecticides for control of stink bugs and other heteropteran pests of edamame.

4. To evaluate the efficacy of fungicides for control of soybean diseases on edamame.

5. To assess the current susceptibility of Virginia corn earworm populations to pyrethroid insecticides using edamame bean dip bioassays.

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Chapter 2

Combining agronomic and pest studies to identify vegetable soybean genotypes suitable for commercial edamame production in the Mid-Atlantic U.S.

As published in:

Lord, N., T. Kuhar, S. Rideout, K. Sutton, A. Alford, X. Li, X. Wu, M. Reiter, H. Doughty, B. Zhang. 2021. Combining agronomic and pest studies to identify vegetable soybean genotypes suitable for commercial edamame production in the Mid-Atlantic U.S. Agricultural Sciences, 2021, 12, 738-754.

Abstract

Currently, domestic production of vegetable soybean (aka “edamame”) lags well behind consumer demand, with approximately 70% of U.S.-consumed edamame imported each year. A major barrier for growth of the U.S. edamame industry is an overall lack of varieties with adequate consumer acceptability and adaption to the U.S. climate and environment. In this study, we evaluated eleven vegetable soybean genotypes (including one commercial check) for differences in yield, pod size, and resistance to local insect, bacterial, and fungal pressures in order to identify genotypes with the greatest potential for use in commercial edamame production. Although there were variations in average pod length (42.1-53.6 mm), width (10.9-12.7 mm), and thickness (6.29-7.34 mm) among the genotypes, only pod length showed statistical significance. In addition, genotype significantly affected fresh pod yield. The prevalence of specific insect pests varied by location and year and included soybean aphid, potato leafhopper, Mexican bean beetle, as well as a complex of stink bugs and lepidopteran larvae. For each of these insect pests, significant differences were observed. Some plant diseases observed on the edamame genotypes included: downy mildew, bacterial pustule, Fusarium pod rot, Cercospora blight and purple seed stain, and damping off. In 2018, in Whitethorne, VA, soybean downy mildew was quite prevalent and disease symptoms varied considerably. Overall, genotypes V16-0524 and R15-10280 showed particularly favorable yield, and resilience to native pests compared to the commercial check, UA-Kirksey. The genotypes V16-0524 and R15-10280 showed strong potential to increase the availability of varieties that can be used for commercial edamame production in the Mid-Atlantic region.

Keywords

Edamame, Genotypes, Pests, Agronomics, Breeding

Introduction

Edamame, or immature soybean (*Glycine max* [L.] Merr.), has been a popular food in East Asia for centuries [1, 2], and is growing in popularity in the United States with around 25,000 to 30,000 tons annual consumption. The name edamame is Japanese for “stem beans” because the crop has often been marketed with the pods still on the stems [3]. Since it was first introduced to U.S. markets two decades ago, edamame has become a fixture in many sushi restaurants and salad bars nationwide and inspired many edamame or edamame-infused food products in the domestic marketplace such as freeze-dried snacks, edamame pasta, and dry roasted edamame.

Frozen edamame pods or shelled beans are also available to consumers in grocery stores year-round, which has led to increased consumption of edamame at home.

Given its unique nutritional profile, edamame is an ideal candidate to help the western world meet dietary guidelines recommended by the USDA. Fresh edamame beans contain 10 to 14% protein and are rich in essential amino acids, dietary fiber, minerals, and vitamins [4]. Studies have shown that edamame contain 50% more protein than garden peas (*P. sativum*) [5], and thus could be a better choice for people seeking a low fat, high protein food source [6]. Edamame, as a type of soybean, also contains isoflavones that have been associated with a number of potential health benefits in the human body, including increased antioxidant activity [7], cancer prevention [8], and a reduction of "bad" LDL cholesterol which contributes to cardiovascular disease [9]. This has distinguished edamame as a functional food source capable of providing consumers with many additional benefits beyond basic nutritional requirements.

The increasing availability of edamame as various commercial foods speaks for its emerging acceptance and popularity among U.S. consumers. However, with roughly 70% of edamame products consumed annually in the U.S. still being imported from overseas, U.S. growers have been largely unable to capitalize on this opportunity. The lack of domestic adoption can be attributed to many factors, primarily related to edamame's relative novelty in the U.S. Such factors include general uncertainty regarding economic potential, chemical regulations, and best management practices as well as a scarcity of commercial processing facilities. In addition, the majority of edamame varieties available to growers are introduced from other countries and expensive. Very few edamame varieties have been bred specifically for major production regions and consequently lack the yield or pest resistance to be used for commercial production [10].

In Virginia specifically, several studies have been conducted to address many of these aforementioned barriers. While there are no commercial processing facilities in the Mid-Atlantic region of the U.S. for growers to access the frozen market, a recent Virginia Tech feasibility study suggested that machine-harvested, fresh market edamame can be both a feasible and profitable enterprise for growers [11]. Strong consumer interest and high potential price premiums for fresh and locally marketed edamame were observed during a willingness to pay study conducted in Southwest Virginia (Lord, et al., unpublished data). With one of the more diverse agricultural portfolios in the country, Virginia soybean and green or snap bean producers may already be in position to add edamame into their crop rotation. This leaves development of commercially viable and pest tolerant edamame varieties as one of the final remaining market needs necessary to permit commercial production in the state and region.

From an agronomic standpoint, edamame varieties must maintain high emergence rates and fresh weight pod yield as well as tolerance to local pests and diseases [12-14]. From a marketing standpoint, large bean size (> 250 mg) and pod dimensions of fresh pods are particularly important for appearance and consumer acceptance [4,15]. Edamame pods should be considerably longer, wider, and thicker than pods of conventional grain-type soybean [16,17]. Personal communication with commercial bean growers has also indicated that for edamame, a low pubescence density (visible hairs on the pod) is preferred to make pods appear more appetizing and to reduce water accumulation on the surface of the pods following the hydro-cooling process in commercial packing houses. Lastly, to further increase profitability for producers, edamame variety development must also target reduced occurrence of pods containing a single bean, which are not generally marketable unless shelled [18]. Decades of soybean breeding in the U.S. for grain production with little interest in edamame production has resulted in limited genetic resources for breeders and edamame improvement [19]. Nevertheless, many food-grade soybean genotypes developed for tofu and soymilk possess characteristics and seed compositional traits that are essential for edamame, such as large seed size, higher sucrose, reduced antinutrient content, non-GMO designation, and enhanced digestibility. There has been a particularly long-standing focus on food-grade soybean variety development at both Virginia Tech and the University of Arkansas. In this study, we sought to evaluate the potential of food-grade soybean genotypes from both breeding programs for commercial edamame production. To do this, we collected information on bean yield, pod dimensions, and frequency of one-bean pods from 10 large-seeded soybean genotypes and one commercial variety, UA-Kirksey, to identify genotypes with the most favorable agronomic and consumer acceptance characteristics [20]. We also collected data on local pest and disease pressures and how they affected each genotype in the field in order to more holistically understand the agronomic performance of each genotype. All of the aforementioned data were analyzed over two years and two locations to identify strong candidate genotypes for edamame production in the Mid-Atlantic U.S.

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Materials & Methods

Description of Plant Materials

After a preliminary screening of twenty-three conventional genotypes to eliminate genotypes with either poor agronomic performance or bad bean appearance, ten prospective edamame genotypes and one commercial check were evaluated in this study (**Table 2.1**). The prospective edamame genotypes consisted of ten large-seeded, food-grade soybean breeding lines initially developed in Arkansas or Virginia for tofu and soymilk end-use. UA Kirksey [21], a major commercial edamame variety grown in the Mid-South region of the U.S., was included as a commercial check (standard). All genotypes in the study, including the commercial check, belonged to maturity group V.

Table 2.1. *f*-values, *df*, *p*-values, and broad-sense heritability (H^2) from the two-way ANOVA testing the treatment effect of genotype on various agronomic and pest variables among edamame genotypes evaluated in Virginia.

Dependent variable	Location* (year)	*f-value*	*df*	*p-value*	H^2
Pod length	W & P pooled (2018 & 2019)	2.499	10,117	0.009	0.918
Pod width	W & P pooled (2018 & 2019)	1.432	10,117	0.174	0.899
Pod thickness	W & P pooled (2018 & 2019)	0.559	10,117	0.845	NC
Pod pubescence	W (2019)	10.379	10,44	0.0001	NC
10-pod weight	W & P pooled (2018 & 2019)	3.628	10,117	0.0001	0.731
Proportion of pods with only one seed	W & P pooled (2018 & 2019	2.040	10,117	0.0003	0.858
Fresh pod yield	W & P pooled (2019)	2.286	10,65	0.0001	0.824
Soybean aphid	W (2018)	0.872	10,30	0.5686	-
Soybean aphid	P (2018)	3.484	10,30	0.0308	-
Soybean aphid	P (2019)	2.735	10,30	0.0161	-
Potato leafhopper	P (2018)	0.491	10,30	0.8615	-
Potato leafhopper	P (2019)	3.364	10,30	0.0048	-
Potato leafhopper	W (2019)	1.971	10,30	0.0737	-
Mexican bean beetle	W (2018)	0.669	10,30	0.7433	-
Mexican bean beetle	W (2019)	7.581	10,30	0.0001	-

Lepidopteran larvae	P (2018)	0.558	10,30	0.8140	-
Lepidopteran larvae	W (2018)	0.543	10,30	0.8456	-
Lepidopteran larvae	P (2019)	0.661	10,30	0.7507	-
Chewing pod damage	P (2019)	2.397	10,30	0.0314	-
Stink bug seed damage	P (2019)	1.881	10,30	0.0883	-
Stink bug seed damage	W (2019)	7.772	10,30	0.0001	-
Downy mildew	W (2018)	22.943	10,30	0.0001	-
Bacterial blight	P (2019)	2.013	10,30	0.0236	-
Diseased pods	P (2019)	10.649	10,30	0.0001	-

*Locations: W = Whitethorne, VA, P = Painter, VA

Field Plots

All ten genotypes and the commercial check were evaluated at Virginia Tech's Kentland Farm in Whitethorne, VA and the Eastern Shore Agricultural Research and Extension Center in Painter, VA in 2018 and 2019. Experimental plots were arranged in a randomized complete block design with four replications with the exception of Painter in 2018, which had two replications. Experimental units were one row plots at Whitethorne and two row plots in Painter. Painter had two row plots so that the second row could serve as seed increase. Plots in Whitethorne were 0.75 m row spacing and 5.5 m long. Plots in Painter were 0.91 m row spacing and 6.1 m long. Plots were machine planted with a cone type soybean planter in May at a seeding rate of 20 seeds/m. Two pre-emergent herbicides, metalochlor (Dual Magnum, Syngenta Crop

Protection) at 0.21 kg a.i./ha and chloransulam-methyl (First Rate, AMVAC Chemical Corp.) at 44.1 g a.i./ha were applied prior to planting. Preplant fertilizer was applied to plots if required based on soil test results. In Whitethorne, plots were entirely rainfed; no irrigation was made prior to or during planting. However, in Painter, irrigation occurred during dry periods from planting until the R4 growth stage.

Data collection

Harvest and pod assessment

Edamame plots were hand-harvested during September and October of each year when most seeds of each genotype had fully expanded in the pods at the R6 growth stage [22]. Pod dimensions, seed size, and proportion of one-seeded pods were all defined before the crop reached the R6 stage, and these traits remained constant until maturity [23]. Therefore, in order to determine suitability for commercial production, assessment of these traits was performed at the R6 or “green bean” stage when the immature seeds reach 80% to 90% pod capacity. For harvest, plants were cut at the base of their stems and placed in large garbage bags to prevent sample mixing. The edamame pods were then immediately handpicked directly from bundles to ensure quality and minimize damage. The mass of all pods per 3-m of row was recorded as kg/ ha. Pod traits including pod width (mm), pod length (mm), pod thickness (mm), number of one-bean pods, and 10-pod weight (g) were then evaluated. For pod dimensions, 10 fresh pods were randomly selected and measured for their length, width, and thickness (mm). The number of pods with one bean in each 10-pod subsample was recorded to determine the proportion of one-bean pods. 10-pod weight was averaged from the weight of 50 fresh pods. All the fresh pods were collected and weighed immediately for fresh pod weight (kilograms) and assessment of the number of single-bean pods.

Pubescence

A subsample of 10 pods per plot was assessed for pubescence using a dissecting microscope (Nikon Model SNZ-1270, Tokyo, Japan). The numbers of hairs visible per 2.4 cm^2 section in the middle of the pod was recorded. This area was used as it was found to be an optimal field of view under magnification for clear visibility of the pod hairs.

Sampling insect pests

In both years, edamame genotypes were surveyed weekly per plot and any economically-important insect pests were recorded. Based on previous research in Virginia common pests include: soybean aphid, *Aphis glycines* Matsumura, potato leafhopper *Empoasca fabae* (Harris), Mexican bean beetle, *Epilachna varivestis* Mulsant, stink bugs (*Euschistus* spp., *Chinavia hilaris* (Say), and *Halyomorpha halys* (Stål), and corn earworm *Helicoverpa zea* Boddie [24-27]. Weekly surveys occurred by visual inspection of five plants per plot from 4-leaf until the R1 growth stage. From R2 to R6, two-minute visual sampling of the entire plot occurred. Because of their abundance, soybean aphids were assessed on five leaves per plot per week. Cumulative weekly counts of the most predominant insect pests were used for analysis.

At harvest, insect damage to pods was categorized as chewing (holes) or stink bug. Chewing damage was recorded by visually assessing the exterior of the pods for any holes or scars, while stink bug feeding could only be recorded by opening the pod and observing the beans directly for feeding marks and undeveloped damaged seeds [25,27].

Sampling foliar and pod diseases

Foliar pathogens, such as bacterial blight and downy mildew, were assessed when present (downy mildew at Whitethorne in 2018 and bacterial blight at Painter in 2019). For both diseases, incidence and severity ratings were taken periodically throughout the growing season. In addition, edamame pods were assessed for disease at harvest by collecting 50 random pods per plot. Individual pods were assessed for symptoms and signs of disease infection, those showing damage were deemed unmarketable. Unmarketable pods were counted, weighed and grouped based on symptomology present. Diseased pods were grouped based on symptomology and pods were disinfected using 0.6% NaOCl for 1.5 minutes. Infected tissues from a representative pod of each symptom were cut from the disease margin and plated on acidified potato dextrose agar (APDA) to determine which pathogens were present based on treatment/plot number.

Statistical analysis

Based on a randomized complete block design, two-way analysis of variance (ANOVA) was performed using PROC GLM in JMP version 14.0 (SAS Institute Inc., Cary, NC). When possible, data were analyzed with year and location as variables, but because not all variables were collected at all locations and years or because there was insufficient replication at some locations, some variables were either pooled when appropriate and when there was no significant interaction or analyzed for specific locations and years only (**Table 2.1**). Edamame genotype and block were considered fixed effects, and means for plot yield, pod dimensions, 10-pod sample weights, proportion one-seeded pods, pod pubescence, cumulative densities of major insect pests (including aphids, leafhoppers, Mexican bean beetles, lepidopteran larvae, and stink bugs) as well as the proportion of insect-damaged pods or diseased pods were separated by Fisher's LSD when significant ($p<0.05$) or highly significant ($p<0.01$) differences were found. Prior to analysis, for each of the major pest species encountered, season total cumulative insects were calculated by multiplying the mean densities of two successive sample dates by the sampling interval (days) and totaling the insects for successive sample dates.

Results

Agronomic characteristics

Pod dimensions

Edamame pod length ranged from 40 to 51 mm among the varieties. There was a highly significant effect of genotype on pod length (**Table 2.1**), with most of the Arkansas (R) genotypes and the commercial standard UA-Kirksey having generally longer pods (47 to 51 mm) than most of the Virginia (V) genotypes (40 to 46 mm; **Table 2.2**). However, there was no significant effect of genotype in pod width or thickness (**Table 2.1**). Pod widths were quite similar ranging from 10.86 ± 0.24 to 12.16 ± 0.43 mm (**Table 2.2**). Pod thickness ranged from 6.47 ± 0.19 to 8.14 ± 0.86 mm among the genotypes.

Table 2.2. Pod dimensions and pubescence of potential edamame genotypes from Arkansas and Virginia compared with a commercial standard variety, UA-Kirksey, grown in Whitethorne, VA and Painter, VA in 2018 and 2019.

Genotype	Mean ± SEM (mm)			Pod pubescence (Hairs per 2.4 cm^2)
	Length	Width	Thickness	
R07-589	42.3 ± 1.9 cd	11.1 ± 0.3	6.9 ± 0.7	422.6 ± 49.3 fg
R14-6238	47.6 ± 2.2 abc	11.3 ± 0.4	7.0 ± 0.8	1096.0 ± 97.1 a
R14-6450	50.3 ± 2.9 a	12.2 ± 0.4	7.2 ± 0.8	629.4 ± 63.7 def
R14-16195	43.1 ± 2.2 bcd	12.1 ± 0.4	7.8 ± 0.8	869.0 ± 136.6 bc
R15-10280	47.5 ± 2.7 abc	12.0 ± 0.4	8.1 ± 0.9	340.8 ± 43.3 g
R16-5336	48.8 ± 1.9 ab	11.6 ± 0.3	6.5 ± 0.2	607.6 ± 43.8 def
UA-Kirksey	47.8 ± 1.7 abc	11.5 ± 0.4	7.2 ± 0.4	699.6 ± 32.7 cde
V10-3653	45.7 ± 1.1 abcd	11.7 ± 0.3	7.0 ± 0.3	553.0 ± 33.0 efg
V16-0524	40.4 ± 1.8 d	11.4 ± 0.3	7.6 ± 0.7	359.0 ± 22.8 g
V16-0528	42.2 ± 1.8 cd	10.9 ± 0.2	7.4 ± 0.3	984.2 ± 133.3 ab
V16-0547	42.5 ± 2.2 cd	12.0 ± 0.3	7.9 ± 0.6	819.0 ± 88.0 bcd
P <	0.0090	NS	NS	0.0001

Numbers within a columns followed by the same letter are not significantly different according to Fisher's LSD at alpha = .05.

Pubescence

There was a highly significant effect of genotype in pod pubescence (**Table 2.1**). The genotypes with the highest density of hairs on pods included R14-6283, V16-0528, R14-16195, and V16-0547; whereas, R15-10280, V16-0524, and R07-589 had the fewest hairs and significantly fewer than UA-Kirksey (**Table 2.2**).

10-pod characteristics and yield

10-pod sample weight (in grams) were pulled across locations and years to assess a robust sample. There was a highly significant effect of genotype in the average weight of ten pods (**Table 2.1**). The heaviest pods were found in the genotypes R14-6450 and R15-10280 (**Table 2.3**). One of the reasons for a lower pod weight was fewer seeds. There was a significant effect of genotype on the proportion of one-seeded pods (**Table 2.1**), with some of the genotypes such as R16-5336, V10-3653, and V16-0547 having twice as high of a proportion one-seeded pods as other genotypes like R14-6450 and UA-Kirksey (**Table 2.3**).

Because of lack of replication and significant vertebrate (deer) grazing damage to the edamame plots in 2018, yield was not analyzed, but was recorded and analyzed from the two Virginia sites in 2019. Because there was no significant interaction between variety and location in yield ($F = 0.8017$; $df = 10,65$; $P = 0.6275$), data were pooled across the two locations to assess the main effect of genotype, which was significant (**Table 2.1**). The four highest yielding genotypes were statistically the same as the commercial standard variety (UA Kirksey) and included R14-6238, V16-0524, R14-6450, and V16-0547 (**Table 2.3**).

Table 2.3. Fresh pod yield (LS Means), 10-pod sample weights, and proportion of one-seeded pods (mean ± SEM) of potential edamame genotypes from Arkansas and Virginia compared with a commercial standard variety, UA-Kirksey.

Genotype	**Fresh pod wt at harvest (kg per ha)**	**10-pod sample wt (grams)**	**Proportion one-seeded pods**
R07-589	12977.2 ± 1429.2 c	13.1 ± 0.5 bc	0.17 ± 0.04 bc
R14-6238	17964.4 ± 1378.6 a	12.9 ± 1.0 c	0.22 ± 0.05 ab
R14-6450	18324.8 ± 1289.5 a	16.4 ± 0.6 a	0.10 ± 0.03 c
R14-16195	14747.3 ± 208.5 bc	14.1 ± 1.0 bc	0.18 ± 0.03 bc
R15-10280	16898.5 ± 2042.7 ab	16.4 ± 0.7 a	0.19 ± 0.03 abc
R16-5336	15509.0 ± 1244.6 abc	12.8 ± 0.9 c	0.22 ± 0.03 ab
UA-Kirksey	17706.9 ± 529.2 ab	14.1 ± 0.5 bc	0.11 ± 0.03 c
V10-3653	15553.6 ± 1308.9 abc	13.3 ± 0.6 bc	0.29 ± 0.05 a
V16-0524	18110.9 ± 2230.3 ab	13.9 ± 0.5 bc	0.15 ± 0.04 bc
V16-0528	15639.8 ± 1030.7 abc	13.1 ± 0.6 c	0.19 ± 0.05 abc
V16-0547	17683.0 ± 2539.2 ab	15.0 ± 0.4 ab	0.22 ± 0.04 ab

P <	0.0229	0.0003	0.0351

Numbers within a columns followed by the same letter are not significantly different according to Fisher's LSD at alpha = .05.

Broad-sense heritability

Broad-sense heritability was calculated for all agronomic traits with the exception of pubescence and pod thickness due to missing data. Analysis was conducted under a genotype-environment framework, where the year-location combination was treated as a random sample in the population of the target environment in order to view year-location combinations as a single environment. Broad-sense heritability (H^2) of pod length and width was approximately 0.918 and 0.899, respectively. H^2 of 10-pod weight was 0.731, while H^2 of yield and proportion of one-bean pods was 0.824 and 0.858, respectively (**Table 2.1**).

Correlation between Agronomic traits

Pearson's correlation coefficient (*r*) and probability (*p*) values of t-test were performed by the R statistical package (version 4.0.2, https://www.r-project.org/) in order to assess correlations between agronomic traits. The results showed that pod length was negatively correlated ($p < 0.01$) with both pod width and pod thickness. Pod width was positively correlated ($p < 0.01$) with pod thickness ($r = 0.63$) and bean weight ($r = 0.63$). All of pod width, thickness, and bean weight were positively correlated ($p < 0.01$) with one bean proportion, while pod weight and yield were negatively correlated ($p < 0.01$) with one bean proportion. Yield was not significantly correlated with other traits involved in this work. Results for correlations between agronomic traits can be seen in **Table 2.4**.

Table 2.4. The correlation coefficient among fresh edamame traits.

Edamame Traits	**Pod Length (mm)**	**Pod Width (mm)**	**Pod Thickness (mm)**	**10 Pod Weight (g)**	**20 Bean Weight (g)**	**Yield (Mg/ha)**	**Proportion 1 bean**
Pod Length (mm)	NA	-0.27**	-0.43**	0.00	-0.55**	0.00	-0.26*
Pod Width (mm)	NA	NA	0.63**	0.21*	0.63**	-0.01	0.31**
Pod Thickness (mm)	NA	NA	NA	-0.30**	0.47**	-0.04	0.15
10 Pod Weight (g)	NA	NA	NA	NA	0.31*	0.10	-0.20*
20 Bean Weight (g)	NA	NA	NA	NA	NA	0.07	0.65**
Yield (Mg/ha)	NA	NA	NA	NA	NA	NA	-0.10
Proportion 1 bean	NA	NA	NA	NA	NA	NA	NA

Note: ** indicates that the correlation is significant at $p < 0.01$, and * indicates that the correlation is significant at $p < 0.05$.

Pests

Insects

Soybean aphid, *Aphis glycines* Matsumura, was an abundant pest at both Virginia locations in 2018 and in Painter in 2019. This invasive pest of soybean is native to Asia but became established in the U.S. in 2000 [28]. Although there was no significant effect of genotype on cumulative densities (recorded as aphid days) at Whitethorne in 2018 (**Table 2.1**), there was a significant genotype effect on aphids in Painter in both 2018 and 2019 (**Table 2.1**). In both years at Painter, the highest cumulative aphid densities were observed on R14-6238, V10-3653, V16-0524, V16-0528, and R16-5336; whereas, R07-589, V16-0547, and particularly, R15-10280 (220.5 +/- 225.6 in 2018, 44.0 ± 19.4 in 2019), had fewer aphids (**Table 2.5**).

Table 2.5. Cumulative insects (mean ± SEM) for two hemipteran leaf-sucking pests, soybean aphids and potato leafhoppers, sampled weekly on edamame genotypes from Arkansas and Virginia and a commercial standard variety, UA-Kirksey, grown in two locations of Virginia in 2018 and 2019[1].

	Cumulative soybean aphids per five leaves		Cumulative potato leafhopper nymphs per five plants	
Genotype	Painter, VA 2018	Painter, VA 2019	Whitethorne, VA 2019	Painter, VA 2019
R07-589	378.0 ± 152.1 de	112.0 ± 41.1 cd	85.9 ± 23.0	74.3 ± 13.0 de
R14-6238	1596.0 ± 57.9 a	304.0 ± 55.2 a	97.5 ± 39.0	162.4 ± 32.8 a
R14-6450	717.5 ± 334.4 bcde	96.0 ± 30.2 cd	166.3 ± 17.1	142.1 ± 28.0 abc
R14-16195	644.0 ± 131.1 de	142.0 ± 10.9 bcd	97.5 ± 20.7	153.0 ± 18.0 ab

R15-10280	220.5 ± 225.6 e	44.0 ± 19.4 d	70.2 ± 13.7	84.4 ± 12.1 de
R16-5336	987.0 ± 386.9 abcd	182.0 ± 46.0 abc	81.9 ± 26.6	126.4 ± 20.9 abcd
UA-Kirksey	661.5 ± 267.6 cde	261.0 ± 87.1 ab	127.3 ± 41.2	70.1 ± 6.1 e
V10-3653	1438.5 ± 64.6 ab	226.0 ± 38.3 abc	62.0 ± 20.9	90.8 ± 19.1 cde
V16-0524	1407.0 ± 285.1 abc	181.0 ± 30.3 abc	84.2 ± 9.1	106.5 ± 7.2 bcde
V16-0528	938.0 ± 190.9 abcde	184.0 ± 19.4 abc	53.5 ± 9.6	104.6 ± 15.5 bcde
V16-0547	623.0 ± 155.9 de	127.0 ± 48.0 cd	41.7 ± 15.6	57.4 ± 8.8 e
P <	0.0308	0.0161	0.073	0.0048

[1] Only data where the insect pest occurred in significant numbers are shown in the table. Numbers within a column followed by the same letter are not significantly different according to Fisher's LSD at P <0.05.

Potato leafhopper occurred in moderate densities in both years in Painter and in 2019 in Whitethorne. There was no significant effect of genotype on cumulative leafhoppers in 2018 in Painter, but there was in 2019 at that location (**Table 2.1**), with V16-0547, R07-589, R15-10280, and UA-Kirksey having the fewest numbers of leafhoppers (**Table 2.4**). Although there were no statistically significant differences between counts of potato leafhoppers in Whitethorne in 2019, the numeric trends were similar to those observed in Painter, with V16-0547 having the fewest leafhoppers at both locations (**Table 2.5**).

Mexican bean beetle was only present in notable numbers at the Whitethorne location. There was no significant effect of genotype on cumulative densities of that pest in 2018, but there was a highly significant effect in 2019 (**Table 2.1**) caused primarily by the susceptibility of the Arkansas genotypes, R14-6238, R14-6450, R14-16195, and R15-10280 relative to other varieties in the study (**Table 2.6**).

Table 2.6. Cumulative insects (mean ± SEM) of Mexican bean beetles observed weekly for 2-minutes and chewing or stink bug feeding pod damage at harvest on edamame genotypes from Arkansas and Virginia and a commercial standard variety, UA-Kirksey, grown in two locations of Virginia in 2018 and 2019[1].

Genotype	Cumulative Mexican bean beetles per 2-min visual sample	% of pods with chewing insect damage		% of pods with stink bug feeding injury
	Whitethorne, VA 2019	Painter, VA 2019	Whitethorne, VA 2019	Whitethorne, VA 2019
R07-589	325.5 ± 26.8 bcd	2.5 ± 1.3 ab	3.5 ± 1.8	4.0 ± 0.8 c
R14-6238	451.4 ± 86.8 ab	1.0 ± 0.5 b	2.5 ± 1.9	6.0 ± 1.9 bc
R14-6450	459.0 ± 54.0 ab	4.5 ± 1.9 a	6.5 ± 3.1	1.5 ± 1.5 c
R14-16195	567.8 ± 39.0 a	4.5 ± 0.8 a	1.5 ± 1.6	1.0 ± 1.6 c
R15-10280	556.8 ± 28.3 a	1.0 ± 0.3 b	4.5 ± 0.4 bc	6.5 ± 1.3 bc
R16-5336	262.6 ± 9.5 cd	0.5 ± 0.2 b	2.5 ± 1.4	3.0 ± 1.1 c
UA-Kirksey	360.9 ± 35.1 bc	1.0 ± 0.9 b	1.5 ± 1.0	6.0 ± 4.3 bc

V10-3653	282.0 ± 59.2 cd	2.0 ± 0.4 ab	4.0 ± 1.8	20.0 ± 4.9 a
V16-0524	328.8 ± 30.3 bcd	0.0 ± 0.3 b	2.0 ± 1.6	0.0 ± 1.1 c
V16-0528	222.5 ± 43.9 d	1.5 ± 0.5 b	3.0 ± 1.0	12.0 ± 2.9 b
V16-0547	195.2 ± 20.6 d	2.0 ± 1.3 ab	3.0 ± 1.1	20.5 ± 1.9 a
P <	0.0001	0.0314	0.7004	0.0001

[1] Only data where the insect pest occurred in significant numbers are shown in the table. Numbers within a column followed by the same letter are not significantly different according to Fisher's LSD at P <0.05.

Densities of lepidopteran larvae on edamame were generally low and were comprised of a mix of species primarily including green cloverworm, *Hypena Scabra* Fabr., corn earworm, and soybean looper, *Chrysodeixis includens* (Walker). Because of the low densities, all lepidopteran larvae were pooled together for assessment. There was no significant effect of genotype on cumulative lepidopteran larval numbers in 2018 in Painter or Whitethorne, or in Painter in 2019 (**Table 2.1**). At Whitethorne in 2019, densities of lepidopteran larvae were so low that data were not analyzed.

Lepidopteran larvae, as well as Mexican bean beetles, can chew holes in edamame pods resulting in direct damage to the marketable product. This variable was minimal (<5%) in both years and locations; however, in 2019 in Painter, there was a significant effect of genotype on chewing insect pod damage (**Table 2.1**) with the two Arkansas genotypes, R14-6450 and R14-16195 having the most chewing damage **(Table 2.6**).

A complex of stink bugs including *Euschistus servus* Say, *C. hilaris*, and *H. halys*

(Pentatomidae) occurred on the edamame during pod fill and fed upon the seeds through the pod husks. This injury showed up as malformed seeds or full seeds with conspicuous marks after shelling. This injury was recorded in 2019 only. In Painter, the percentage of pods with stink bug injured seeds averaged between 17.5 to 34.0% and there was no significant effect of genotype (**Table 2.1**). In Whitethorne in 2019, genotype had a highly significant effect on stink bug injury (**Table 2.1**). Genotypes that had the least amount of stink bug injury included V16-0524, R07-589, R14-6450, R14-16195, R16-5336 (**Table 2.6**).

Diseases

Diseases were generally low in incidence, or the actual causal agent was not or could not be confirmed. Soybean downy mildew, *Peronospora manshurica* (Naoum) Syd., is a common fungal leaf disease of soybean that occurs when weather conditions are rainy and humid, as they were at Whitethorne in 2018. The proportion of leaves exhibiting fungal infection (characteristic yellow spots or lesions on leaves) was recorded in early September at peak infection. There was a highly significant effect of genotype on the percentage of leaves with downy mildew symptoms (**Table 2.1**); UA-Kirksey exhibited 100% infected leaves, likely due to lack of adaptation specifically to the mid-Atlantic region (**Table 2.7**). Meanwhile, V16-0524 had ~ 50% infected leaves, and the remaining genotypes had little or no infection.

Table 2.7. Disease assessments comparing edamame genotypes and the commercial standard variety UA-Kirksey from trials conducted in Whitethorne (2018) and Painter (2019) in Virginia.

	Downy Mildew Incidence	Bacterial Blight Severity	Incidence of Diseased Pods

Genotype	Whitethorne, VA, 2018 (% infected leaves)	Painter, VA, 2019 (% leaf area infected)	Painter, VA, 2019 (% unmarketable pods)
R07-589	0.0 ± 0.0 c	41.0 ± 18.9 a	10.5 ± 3.3 bc
R14-6238	2.5 ± 4.2 a	16.0 ± 19.6 ab	12.3 ± 4.0 bc
R14-6450	10.0 ± 9.0 c	14.0 ± 6.2 ab	12.8 ± 5.6 bc
R14-16195	0.0 ± 0.0 c	0.8 ± 1.5 b	5.8 ± 1.3 c
R15-10280	15.0 ± 10.7 c	24.0 ± 18.0 ab	28.3 ± 3.9 a
R16-5336	0.0 ± 0.0 c	25.5 ± 15.5 ab	15.5 ± 5.3 bc
UA-Kirksey	100.0 ± 0.0 a	22.0 ± 11.9 ab	17.0 ± 8.3 abc
V10-3653	0.0 ± 0.0 c	20.8 ± 12.2 ab	14.5 ± 4.7 bc
V16-0524	52.5 ± 13.7 b	19.5 ± 22.5 ab	22.0 ± 8.3 ab
V16-0528	0.0 ± 0.0 c	2.0 ± 4.0 b	8.8 ± 5.6 c
V16-0547	0.0 ± 0.0 c	27.8 ± 20.8 ab	11.8 ± 6.2 bc

[1] Only data where the insect pest occurred in significant numbers are shown in the table. Numbers within a column followed by the same letter are not significantly different according to Fisher's LSD at $P < 0.05$.

Bacterial blight, caused by *Pseudomonas syringae* Van Hall pv. *glycinea*, was present at the Painter trial in 2019 in moderate levels on edamame foliage. Infection severity of the genotypes can be seen in **Table 2.6**. R07-589 (41.0 ± 18.9) showcased considerably higher infection severity than UA-Kirksey (22.0 ± 11.9), while genotypes R14-6450 (14.0 ± 6.2) and V16-0528 (2.0 ± 4.0) showed considerably less.

In 2019 at the Painter trial, a random sample of 50 pods/plot were assessed for being marketable or unmarketable (mostly due to disease lesions, insect lesions, or complexes between the two). Similar to bacterial blight ratings, R14-6450 (12.8 ± 5.6) and V16-0528 (8.8 ±5.6) showed a lower percentage of unmarketable pods than UA-Kirksey (17.0 ± 8.3), while R15-10280 (28.3 ± 3.9) showed a higher percentage of unmarketable pods (**Table 2.7**).

Discussion

Edamame variety trials have been conducted in the U.S. since edamame first began to be nationally recognized [29-32]. Many of these trials focused on varieties developed and imported from China, Japan, and Korea, as these varieties already contained the eating quality and pod characteristics valued by traditional consumers of edamame [6,10]. As a consequence of poor local adaptation, however, these varieties tend to yield poorly in mid-southern and southeastern production regions. A recently conducted a study by Jiang et. al. characterized 86 food-grade soybean breeding lines for commercial edamame production in Virginia and found numerous genotypes that appeared to show high yield potential [33]. These findings suggested that utilization of food-grade soybean genotypes already bred to local climatic conditions in the region may present a more immediate remedy for the poor agronomic performance of currently available edamame varieties.

Results from our study corroborated results from Jiang et. al., as many of the food-grade genotypes in this study from both Arkansas and Virginia appeared on-par with or better than the commercial check. For example, only half of the prospective edamame genotypes observed in this present study showed significantly lower yield potential than UA-Kirksey. Of the remaining five genotypes, three failed to show statistically significant differences in yield with UA-Kirksey and the other two showed even higher yield potential. In addition, many of the food-grade soybean genotypes in our study showed pod characteristics that were comparable to the commercial check. At least half of the genotypes matched or showed significantly higher pod length than UA Kirksey, while four genotypes showed comparable 10-pod weight. A wide range of pubescence densities was also observed among the genotypes with several showing lower pubescence densities than UA Kirksey which is favorable for its processability.

Studies on heritability of soybean pod characteristics are scarce in the literature, especially at the R6 stage. In the study conducted by Jiang et. al., high broad-sense heritability was observed for 100-seed weight of both fresh and dry seeds. Our study only focused on fresh pods and beans. The high broad-sense heritability for fresh pod yield ($H^2 = 0.82$) and pod length ($H^2 = 0.92$) observed in this study closely mirrors findings from Mebrahtu & Mohamed [12] who observed similarly high broad-sense heritability estimates for these same traits. We also observed relatively high broad-sense heritability estimates for other traits such as 10-pod weight (0.73) and proportion of one-bean pods (0.86), which to our knowledge have not yet been reported in the literature. The high broad-sense heritability estimates observed across agronomic traits here suggest that environmental effects did not appear to considerably impact pod weight, pod dimension traits or one-bean pod proportion. As such, these traits may be ideal breeding targets to develop commercial varieties and germplasm that more closely resemble traditional edamame and thus may benefit from increased consumer acceptance.

Two genotypes in our study, V16-0524 and R15-10280, outperformed the commercial check UA-Kirksey in several key categories. Firstly, both genotypes showed consistently high yield and notably stronger adaptation, resistance to local disease and insect pest pressures, and comparable pod characteristics to the commercial check variety. V16-0524 also showed one of the lower one-bean pod proportions observed in the study and had substantially less downy mildew and stink bug damage than the commercial check. Despite its numerous agronomic advantages, however, V16-0524 had one of the shorter pod lengths observed in this study; whereas, R15-10280 showed ideal pod dimensions that closely mirrored the commercial check and even outperformed it for ten pod weight as well as downy mildew damage. Both R15-10280 and V16-0524 showed appreciably lower pubescence densities than the other genotypes in the study, including the commercial check, which is critical for consumer appeal and drying time in fresh packing operations.

Given the dynamic nature of pests from year to year, continued studies on native pest pressures, economic thresholds, and management strategies are needed to further facilitate adoption of edamame in the state. In addition, determining the mechanical or physiological resistance mechanisms that resulted in differences in pest pressure or injury among the genotypes will help guide future plant breeding endeavors.

Conclusions

The strong agronomic performance of the genotypes in this study relative to the commercial check demonstrates how food-grade soybean genotypes can be leveraged as a useful genetic resource for edamame variety development. In addition, the high broad-sense heritability estimates for yield and pod characteristics observed here suggest that there is strong potential to improve edamame product marketability through breeding selection. Two genotypes from our study, V16-0524 and R15-10280, can be immediately used to provide growers in the region with superior, commercially-viable seed inputs with strong yield potential and local pest tolerance.

Funding

This research was supported by the USDA-NIFA, Grant No. 2018-51181-28384, Accession No. 1016465, and the Virginia Agricultural Experiment Station.

Acknowledgments

The authors thank USDA-NIFA and Virginia Agricultural Council for financial support. The authors also thank Jill Pollok, James Jenrette, Daniel Wilczek, Brian Currin, Katlyn Catron, Kadie Britt, Mika Pagani, Sean Boyle, Andy Dechaine, and Kyle Bekelja, Jim Jenrette, and Muliang Peng for help with field plot maintenance and data collection, and Leandro Mozzoni for providing seeds of edamame breeding lines.

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Chapter 3

Evaluation of an insect pest scouting program for edamame in the mid-Atlantic U.S.

Introduction

Edible soybean, commonly known as edamame *Glycine max* (L.) Merr., is a high protein vegetable from Asia that is increasing in popularity in the United States (Kelley and Sánchez 2005, Yu et al. 2021). Conventional soybean is harvested as grain after senescence and is used for products like oil seed livestock feed, and feed stock for biofuels (Chen et al. 2012). Alternatively, edamame is harvested at the R6 growth stage when the seeds are still tender (Fehr and Caviness 1977) and is bred to be more flavorful than soybean for consumption as a vegetable (Wszelaki et al. 2005, Zhang et al. 2021). Although the United States is one of the largest soybean producers in the world (112 million tons in 2020), edamame production is limited (Lord et al. 2019, FAO 2020). Around 70-90% of edamame consumed in the United States is imported from Asia (Roseboro 2012, Shurtleff et al. 2014). As demand for edamame in the United States has been increasing, as much as 40% over the past two decades, efforts are being made to increase domestic production (Duppong and Hatterman-Valenti 2005, Rosenboro 2012, Lord et al. 2019, Zhang et al. 2021). However, research is needed to assess how edamame production and pest management practices may differ from those of conventional soybean systems, and to ensure future edamame production in the United States can be established successfully.

A wide range of insects feed on soybean throughout the growing season resulting in damage that can significantly limit production, if left unmanaged (Steffey 2015). While there is limited research on edamame pest complexes in the United States, current pest management programs in soybean provide a framework to expand and/or develop upon. Integrated pest management programs that promote the use of selective insecticides (Whalen et al. 2016) and pest scouting thresholds have been developed and implemented for most of the major pests of soybean. These pests include defoliators such as lepidopteran larvae, beetles, and grasshoppers as well as pod-feeding insects such as corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) and stink bugs (Hemiptera: Pentatomidae) (Jensen et al. 1977, Kogan and Herzog 1980, McPherson et al. 1987, Higley and Boethel 1994, Bueno et al. 2013, Aigner et al. 2017). While economic damage calculations and action-thresholds have been developed for soybean, edamame is a higher value vegetable with a lower tolerance for pod and seed damage than soybean.

Differences in breeding between soy and edamame cultivars present another consideration that may alter pest management tactics and needs of these crops. Many soybean cultivars in the United States have been bred to display tolerance and/or resistance levels to various North American insect pests and diseases whereas edamame cultivars tend to be bred for increased seed size and sugar content (Smith 1985, Rufener et al. 1989, Rector et al. 1999, 2000, Yu et al. 2021, Zhang et al. 2021). As a result, edamame typically displays greater susceptibility to pests than grain soybean (Menger et al. 2018). For instance, evaluations of edamame genotypes from 2018 to 2020 in Virginia revealed high levels of Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae), pest pressure (Lord et al. 2021), a pest that traditional soybean has been bred to have resistance to in the United States (Rufener et al. 1989). Similar observations were made by Menger et al. (2018) who found more soybean aphids, *Aphis glycines* Matsumura (Hemiptera: Aphididae), and potato leafhoppers, *Empoasca fabae* (Harris) (Hemiptera: Cicadellidae), on edamame varieties compared to grain soybean varieties in Minnesota. They attributed the differences to higher trichome densities in the soybean varieties.

Stink bugs were also found to be a major pest of concern for edamame in Virginia due to their feeding on the pods and seeds (Lord et al. 2021). Primary stink bug species found in bean crops in Virginia include: Southern green stink bug, *Nezara viridula* L., green stink bug, *Chinavia halaris* (Say), brown stink bug, *Euschistus servus* (Say), and the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Aigner et al. 2017, Sutton et al. 2021). Stink bug feeding can injure edamame pods resulting in reduced marketability due to blemishes on the seed. Stink bug feeding is well documented in soybeans and has the same effect on edamame. Seed damage in soybeans is caused when stink bugs insert their piercing-sucking mouth parts into the bean injecting plant digestive enzymes and extracting the plant fluids (Pfeiffer and Felton 2014). Early stages of pod development can result in aborted pods or underdeveloped flat pods. Feeding injury in later reproductive growth stages where the bean has larger developing seeds, R5 (beginning seed) and R6 (full seed), results in shriveled, deformed, or even aborted seeds, or with edamame, discolored feeding marks (Corrêa–Ferreira and Azevedo 2002, Owens et al. 2013b). For edamame, even blemished seeds can reduce the quality and marketability of the crop further increasing the pest significance of pod-feeding piercing sucking insects such as stink bugs. This research focuses on evaluating a sampling-based action-threshold program for insect pests of edamame with a particular emphasis on stink bugs. Our goal is to provide a sound approach to pest management for this emerging vegetable in the United States that minimizes insecticide use, while not sacrificing crop quality.

Materials and Methods:

Experimental plots

We conducted small field-plot edamame experiments over three field seasons (2019-2021) at two locations; Virginia Tech's Eastern Shore Agricultural Research and Extension Center (ESAREC) in Painter VA, (37.58736N, 75.82310W) and Virginia Tech's Kentland Farm in Whitethorne, VA (37.19770N, 80.57484W). Seed was provided by Virginia Tech's edamame breeding program. Seed variety was consistent between locations though a different variety was used each year (2019: R07-589; 2020: V10-3653; 2021: VT Sweet (Zhang et al. 2022). Plots were direct-seeded on 18 July and 1 July in 2019, 10 July and 12 July in 2020, and 26 June and 1 June in 2021 in Painter and Whitethorne, respectively. All trials were arranged in randomized complete block designs with four replications of three treatments. Individual plots were three rows, each 6.096 m long with 76.2-cm spacings. Seeds were spaced 15.2 cm apart within rows.

Treatments

The experiment had three treatments: 1) Max spray (Weekly or bi-weekly insecticide applications from flowering until harvest); 2) IPM approach (using action-thresholds to determine insecticide applications); and 3) untreated control. For the max spray insecticide treatment, lambda-cyhalothrin (Lambda-Cy EC, 11.4% active ingredient, UPL, Inc., King of Prussia, PA) was applied at 33.62 g ai/ha beginning at V1 growth stage (Fehr and Caviness 1977) for early-season pests, then, beginning at R1 growth stage (flowering), weekly to bi-weekly, depending on insect pressure, until harvest. For the IPM treatment, insecticides were only applied if scouting action-thresholds were exceeded, and included more selective (IPM-compatible) insecticide options such as sulfoxaflor (Transform WG, Corteva Inc., Indianapolis, IN) at 54.78 g ai/ha for hemipteran pests; and chlorantraniliprole (Coragen 1.67 SC, FMC Corp., Philadelphia, PA) at 51.24 g ai/ha for lepidopteran pests. However, because narrow spectrum insecticide options are lacking for stink bugs (Kuhar and Kamminga 2017), lambda-cyhalothrin (same rate as max spray treatment) was used for stink bugs during pod development. Max spray and IPM applications were made using a three-nozzle boom CO_2-powered backpack sprayer equipped with 8003VS tips spaced 50-cm apart. Applications were made at 280.62 liters per hectare using 40 psi. Treatment dates are listed in **Table 3.8**.

Insect sampling

From the VE-V3 plant growth stages (Fehr and Caviness 1977), edamame plots were visually sampled every 7 days by counting any lepidopteran larvae as well as Mexican bean beetle adults and larvae, bean leaf beetles, *Cerotoma trifurcata* Forster (Coleoptera: Chrysomelidae), and Japanese beetles, *Popillia japonica* Newman (Coleoptera: Scarabaeidae) on five plants per plot. Then, beginning at four-leaf stage (V4-V6 [Fehr and Caviness 1977]) until harvest, counts were conducted on all plants within a two-minute timed inspection. A switch to timed visual inspection was done because inspecting five entire plants became inefficient as plants grew large. The two-minute interval was enough time to walk down the total length of each side of the one row 12 m long plot. A visual estimation of defoliation was also made weekly. In addition to the plant inspections, at each of the weekly samples, 10 random fully-formed trifoliates were excised from each plot and examined for potato leafhopper, *Empoasca fabae* Harris (Hemiptera: Cicadellidae) or soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphidae).

Hemipteran pod feedings pests, specifically stink bugs and occasionally broad-headed bugs (Family: Alydidae) increased dramatically during reproductive growth stages (R1-R6 [Fehr and Caviness 1977]). Sampling for stink bugs in field crops like soybean is traditionally done using sweep nets or beat sheets (Kogan and Pitre 1980). However, because sweeping or beating could conceivably damage edamame pods, impacting the yield and quality of edamame, we opted for a timed visual inspection of plants, which was suggested by Aigner et al. (2017) to potentially be a better stink bug sampling method because some species, such as *H. halys*, may drop to the ground and elude being caught by the sweep net. Aigner et al. (2017) also found that the thresholds for sweep nets were a 1:1 ratio with his visual sampling method so we modified soybean thresholds from Flessner and Taylor (2022) for visual sampling according to this 1:1 ratio (**Table 3.1**)

Harvest evaluation

Edamame was harvested right before senescence (R6 growth stage [Fehr and Caviness 1977]) by removing all plants from the middle 3.048 meters of the middle row of edamame in each 3-row plot. Plants were cut at their base using pruning clippers, bagged, and brought back to each research facility for processing. All pods from each plant in a plot were removed by hand, placed in a bucket, and weighed in grams using a Ohaus Ranger 7000 scale model R71MD35, Ohaus corporation USA, to determine fresh pod weight per plot. A random subsample of 50 pods was assessed for chewing damage. Each pod was then manually shelled, and seeds were examined for stink bug feeding marks or other deformities. If one bean was damaged, the entire pod was counted as damaged. Proportion stink bug damaged pods were used to calculate marketable yield.

Data Analysis:

Differences in insect densities among treatments were assessed using JMP (JMP ®, Version 2016. SAS Institute Inc., Cary, NC, 1989–2021) with significant outcomes at $\alpha \leq 0.05$. Two-way analysis of variance procedures was used to test for differences in fresh pod weight, marketable yield, damaged pods, and insect densities among treatments per sample date. If model results were $\alpha \leq 0.05$, multiple mean comparisons were conducted using a Tukey HSD test.

Results:

2019

At Whitethorne 2019, the IPM treatment was sprayed once, and the max spray treatment was sprayed four times with lambda-cyhalothrin before harvest. There was a significant treatment effect on 23 September for Alydidae per two-minute visual sample where the IPM and max spray treatment had significantly lower insect counts compared to the control ($F = 5.66$; $df = 11$; $P < 0.0416$). Additionally, there was not a significant difference between the max spray and IPM on Alydidae counts on 23 September despite the IPM treatment receiving three fewer sprays of lambda-cyhalothrin. At harvest, there was a significant treatment effect on percent piercing-sucking pod damage. The max spray and IPM treatments had significantly less pods damaged compared to the control ($F = 13.81$; $df = 2$; $P < 0.001$). There was not a significant effect of treatment on marketable yield or on chewing damage (**Table 3.2**).

In Painter 2019, the IPM treatment did not cross thresholds for any pests and was sprayed zero times. The max spray treatment was sprayed four times with lambda-cyhalothrin. There were no treatment effects on pest densities, marketable yield, or pod damage (**Table 3.3**).

2020

In Whitethorne 2020, the max spray treatment was sprayed 5 times with lambda-cyhalothrin and the IPM treatment was sprayed twice, once with lambda-cyhalothrin and once with sulfoxaflor. Although there was not a significant treatment effect on insects counts, on 16 September there was a noticeable difference in counts for stink bugs (SB). The max spray treatment had no stink bugs counted, the IPM had a total of 2.8, both of which were much lower than the control that counted 14.3. These counts were slightly above significance ($F = 4.55$; $df = 2$; $P < 0.0628$). There was not a significant effect of treatment on yield. At harvest there was a significant treatment effect on percent stink bug damaged pods, where the max spray treatment had significantly lower stink bug damaged pods compared to the IPM and Control ($F = 14.61$; $df = 2$; $P < 0.0008$). Again, there was no significance on percent chewing damaged pods or on marketable yield. (**Table 3.5**).

In Painter 2020, the IPM treatment was sprayed twice with sulfoxaflor and once with lambda-cyhalothrin. The max spray treatment was sprayed five times with lambda-cyhalothrin. Although not significant, significance was very close between treatments on insects counts for potato leafhopper (PLH) on 10 July, where the max spray treatment had fewer PLHs compared to the control and IPM ($F = 4.92$; $df = 2$; $P < 0.0544$). Stink bugs (SB) had significantly fewer insect counts in the max spray treatment and IPM treatments compared to the control on 21 September ($F = 9.32$; $df = 2$; $P < 0.0131$), and 5 October ($F = 8.75$; $df = 2$; $P < 0.0166$). Green clover worm had significantly fewer insect counts in the max spray and IPM treatments compared to the control on 09 Sep. (F = 11.74; df = 2; P < 0.0084). There was not an effect of treatment on marketable yield. At harvest there was a significant effect of treatment on piercing sucking damage, where the IPM and max spray treatments had less damaged pods compared to the control (F = 4.38; df = 2; P < 0.0399). There was no significance on chewing damaged pods or on marketable yield (**Table 3.4**.).

2021

In Whitethorne 2021, the IPM treatment was sprayed once with sulfoxaflor. The max spray treatment was sprayed five times with lambda-cyhalothrin. There was a significant treatment effect on insect counts for potato leafhoppers (PLH), where the max spray and IPM treatments had significantly fewer PLH nymphs compared to the control on 30 Jun (F = 0.0104; df = 2; P < 0.0104) and 07 Jul (F = 14.06; df = 2; P < 0.0054). Mexican bean beetles (MBB) had a significant difference of insect counts, where the control and IPM treatments had more insects compared to the max spray treatment on 17 Aug (F = 9.40; df = 2; P < 0.0142), 25 Aug (F = 0.0026; df = 2; P < 0.0026), 31 Aug (F = 5.91; df = 2; P < 0.0382), 23 Sep (F = 6.46; df = 2; P < 0.0319). At harvest, there was no difference in marketable yield, percent stink bug damaged pods, or percent chewing damaged pods among treatments (**Table 3.6**).

In Painter 2021, the max spray treatment was sprayed five times with lambda-cyhalothrin. The IPM treatment was sprayed three times, once with sulfoxaflor, once with lambda-cyhalothrin, and once with chlorantraniliprole. There was a significant treatment effect on insect counts for aphids where the IPM and max spray treatments had lower numbers compared to the control on 24 Aug (F = 4.4729; df = 2; P ≤ 0.0448), 31 Aug (F = 17.13; df = 2; P < 0.0009), and 22 Sep (F = 17.13; df = 2; P < 0.0106). There was a significant treatment effect for stink bugs

(SB) on 22 Sep, where the IPM and max spray treatments had significantly fewer insects compared to the control ($F = 33.00$; $df = 2$; $P < 0.006$). There was not a significant of treatment on yield. At harvest there was a significant effect of treatment on stink bug damaged pods where the IPM and max spray treatments had fewer damaged pods than the control ($F= 8.06$; $df = 2$; $P < 0.007$). There was a significant effect of treatment on marketable yield where the max spray treatment had significantly higher yield than compared to the control ($F = 5.53$; $df = 2$; $P < 0.0217$) Similar to previous years, there was no significant treatment effect on chewing damaged pods (**Table 3.7**).

Discussion:

The max spray treatment adequately controlled key edamame pests especially during reproductive pod development. However, The IPM treatment, which used adapted soybean thresholds, had similar control while reducing the number of insecticides being applied.

Stink bugs were the most abundant pest of edamame during this study. Their feeding resulted in obvious blemishes to the seeds, which would likely cause quality issues at market grading (USDA grades for snapbeans). These blemishes are particularly concerning since the ones on the seed are not visible while still in the pod. Similarly, in soybeans in Virginia, Owens et al. (2012) observed that stink bugs caused the most pod damage and yield loss during the R4-R6 growth stages, which are the most crucial stages for edamame. Edamame pod damage due to stink bug feeding in the control treatment averaged about 27 percent of pods observed being damaged across years and locations, with the highest damage being in Painter 2021 where 60 percent of pods were damaged by stink bugs. However, stink bug damaged pods were reduced significantly when insecticides were applied in both the IPM and max spray treatments. When insecticides were applied according to the proposed thresholds, stink bug damaged pods averaged 12.25 percent of observed pods being damaged across locations and years. The max spray treatment averaged 5.75 percent of pods being damaged by stink bugs across locations and years. The max spray treatment had the least amount of stink bug damaged pods, but it also had the highest number of insecticide applications, averaging 4.5 sprays per growing season. Comparatively, the IPM treatment averaged 1.6 sprays per season while average pod damage was only increased by 6.5 percent. Edamame pod damage due to stink bug feeding was successfully reduced by using action-based thresholds, while lowering the number of insecticides applications. There was not a significant difference in stink bug damaged pods between the max spray and IPM treatments across all six experiments except Whitethorne 2020, where the decision to spray sulfoxaflor instead of lambda-cyhalothrin on September 16 was made right before harvest. This decision is why I believe this difference occurred. Sulfoxaflor is labelled for stink bug suppression, but Sutton et al. 2020 found that in an edamame spray trial, sulfoxaflor did not significantly reduce stink bug damaged pods when compared to the untreated control. However, the pods need to be protected before harvest and an insecticide that works better on stink bugs will need to be used for edamame to be grown successfully in Virginia.

Edamame is primarily grown in small acreages in the mid-Atlantic U.S., typically on organic farms or pick your-own farming operations that have diversified crops systems. This will also probably lead to greater stink bug pest pressure as these insects prefer to move from crop to crop as plants progress through the fruiting stages (Zobel et al. 2016, Mathews et al. 2017, Formella et al. 2022). As different crop hosts are harvested on a diversified farm, edamame will receive increasing pressure towards harvest as brown marmorated stink bugs migrate onto edamame as other possible hosts are depleted.

Despite stink bugs being the most concerning pest of edamame, leading into this experiment we believed that corn earworm would be one of the most abundant and concerning pest due to its historical damage to soybean crops in the mid-Atlantic (Stinner et al. 1980). However, across the six experiments conducted across years and locations, corn earworm never had a significant sampling date nor any significant pod damage at harvest. We can only speculate as to why corn earworms were not in these trials, but I believe that planting date was a factor. Most of the trials were planted later in the growing season (June, July) which led to the maturity and harvest during late September and early October past when peak corn earworm flights occur in the mid-Atlantic which is during July and August (Herbert et al. 1991).

Besides stink bugs, there was variability in the insect pressure from other species, but the complex mostly consisted of defoliators such as Mexican bean beetles, bean leaf beetles, green clover worm, and some Japanese beetles. However, these defoliating pests, including the Mexican bean beetle, never crossed the thresholds set by the mid-Atlantic pest management guide for defoliation, showing that edamame, like soybeans, can withstand defoliating pests comfortably without pesticide use in most instances (Todd and Morgan 1972, Hammond and Pedigo 1982, Hammond 1989, Hunt et al. 1994, 1995). Early season pests such as potato leafhoppers were present in the early vegetative growth stages (VE-V3 [Fehr and Caviness 1977]) and occasionally required an insecticide application under commercial conditions. Soybean aphids were uncommon but occasionally found in the later part of the growing season but rarely required an insecticide application. The insect pressure and presence in edamame lined up similarly with an edamame variety experiment by McPherson et al. 2008. McPherseon et al. 2008, found that late maturing varieties (September and after) had much higher stink bug pressure. Corn earworm was also absent in their study. Additionally, they found that potato leaf hoppers could be a problem after planting and a very similar defoliating insect complex. The research by McPherson et al. 2008 further supports that that the integrated pest management approach conducted in this study will work in others states in the mid-Atlantic besides Virginia. In comparison, an edamame variety study in Minnesota by Menger et al. 2018 showed the key pests of edamame in their state were soybean aphids and potato leaf hoppers, showing that pest management tactics on edamame will need to be adjusted for different growing regions.

An action-based threshold approach for determining insecticide applications for key insects in edamame production resulted in fewer insecticide applications while still effectively controlling key insect pests. Edamame growers in the mid-Atlantic will now have effective methods for insect pest management. Edamame proved to present some levels of tolerance against defoliating pests, but it was very susceptible to pod feeding insects, especially stink bugs. The authors suggest scouting weekly, especially from R1 to R6. Further investigation of stink bug thresholds may be needed once official market grading standards are set specifically for edamame by the USDA.

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Table 3.1. Action thresholds used for various insect pest groups and crop developmental stages of edamame. Thresholds adapted from Flessner and Taylor (2020), except for timed visual sampling of stink bugs, which were proposed in this study following Aigner et al. (2017).

Pest	Threshold
Defoliating pests (i.e., Mexican bean beetle, green cloverworm, armyworms, soybean looper, bean leaf beetle, Japanese beetle, grasshoppers)	40% defoliation seedling, Pre-bloom 30%, Bloom and podset 15%.
Potato leafhopper	>10 PLH per 10 trifoliates
Soybean aphid	>10 aphids per trifoliate
Stink bugs	> 1 per 2.74m, or 4.5 per 6.09m plot.
Corn earworm	>1 per 4.57m or 3 per 6.09m plot.

Table 3.2 Effect of pest management treatment regime on the number of insecticide applications, marketable yield of edamame, density of alydid bugs (Hemiptera: Alydidae) on one significant sample date, and percentage of pods at harvest with seeds showing bug feeding damage from a small plot field experiment conducted in Whitethorne, VA in 2019.

Treatment	# of insecticide sprays	Marketable Yield (kg/ha)*	Alydid bugs per 2 min sample 09/23	% pods with bug damage
Untreated Control	0	1301.8 ± 285.9	4.3 ± 1.3 a	30.5 ± 3.1 a
Max Spray	4	1440.3 ± 209.6	1.0 ± 0.7 ab	5.5 ± 3.6 b
IPM	1	1742.2 ± 428.9	0.3 ± 0.3 b	4.0 ± 1.4 b
P-Value from ANOVA		NS	0.0416	0.001

*Marketable yield was calculated by subtracting total pod weight with bug damage (kg/ha) from total pod weight (kg/ha)

Means within columns followed by the same letter are not significantly different; $P < 0.05$.

Table 3.3. Effect of pest management treatment regime on the number of insecticide applications, marketable yield of edamame, and percentage of pods at harvest with seeds showing bug feeding damage from a small plot field experiment conducted in Painter, VA in 2019.

Treatment	# of insecticide sprays	Marketable Yield (kg/ha)*	% pods with bug damage
Untreated Control	0	2046.9 ± 61.4	8.8 ± 3.2
Max Spray	4	2021.8 ± 30.7	5.5 ± 2.0
IPM	0	2022.5 ± 144.6	6.0 ± 1.9
P-Value from ANOVA		NS	NS

* Marketable yield was calculated by subtracting total pod weight with bug damage (kg/ha) from total pod weight (kg/ha)

Means within columns followed by the same letter are not significantly different; P < 0.05.

Table 3.4. Effect of pest management treatment regime on the number of insecticide applications, marketable yield of edamame, density of potato leafhopper (PLH), stink bugs (SB), and green cloverworm (GCW) on five significant sample dates, and percentage of pods at harvest with seeds showing bug feeding damage from a small plot field experiment conducted in Painter, VA 2020.

			Number insects per 2 min visual sample (mean ± SE)					
Treatment	# of insecticide sprays	Marketable Yield (kg/ha)*	PLH 08/10	SB 08/23	GCW 09/09	SB 09/21	SB 10/05	% pods with bug feeding injury
Control	0	4849.2 ± 376.4	4.0 ± 1.8 a	5.8 ± 1.8 a	5.6 ± 1.3 a	6.0 ± 1.8 a	8.2 ± 2.1 a	29.5 a ± 8.4 a
Max Spray	5	5754.6 ± 224.9	0.0 ± 0.0 b	1.5 ± 0.5 b	2.0 ± 0.7 b	0.0 ± 0.0 b	1.3 ± 0.5 b	8.0 b ± 1.4 b
IPM	3	5560.4 ± 351.6	4.8 ± 1.1 a	2.8 ± 0.5 ab	0.8 ± 0.3 b	0.5 ± 0.3 b	2.0 ± 0.4 b	10.5 b ± 5.9 b
P-Value from ANOVA		NS	0.0544	0.0658	0.0084	0.0131	0.0166	P < 0.0399

* Marketable yield was calculated by subtracting total pod weight with bug damage (kg/ha) from total pod weight (kg/ha)

Means within columns followed by the same letter are not significantly different; P < 0.05.

Table 3.5. Effect of pest management treatment regime on the number of insecticide applications, marketable yield of edamame, density of stink bugs (SB) on one significant sample date. And percentage of pods at harvest with seeds showing bug feeding damage from a small plot experiment conducted in Whitethorne, VA 2020.

Treatment	# of insecticide sprays	Marketable Yield (kg/ha)*	SB per 2 min sample 09/16	% Pods with bug damage
Control	0	4815.02 ± 988.74 a	14.3 ± 6.7 a	28.5 a ± 3.3 a
Max Spray	5	6266.61 ± 1140.64	0.0 ± 0.0 b	2.5 b ± 1.3 b
IPM	2	5165.39 ± 1000.37	2.8 ± 2.9 ab	26.0 a ± 2.9 a
P-Value from ANOVA		NS	0.0628	P < 0.0008

* Marketable yield was calculated by subtracting total pod weight with bug damage (kg/ha) from total pod weight (kg/ha)

Means within columns followed by the same letter are not significantly different; P < 0.05.

Table 3.6. Effect of pest management treatment regime on the number of insecticide applications, marketable yield of edamame density of potato leafhopper (PLH), Mexican bean beetle (MBB) on six significant sample dates, and percentage of pods at harvest with seeds showing bug feeding damage from a small plot experiment conducted in Whitethorne, VA 2021.

			Number insects per 2 min visual sample (mean ± SE)						
Treatment	# of insecticide sprays	Marketable Yield (kg/ha)*	PLH 06/30	PLH 07/07	MBB 08/17	MBB 08/25	MBB 08/31	MBB 09/23	% pod bug damage
Control	0	7296.0 ± 442.8	54.0 ± 6.4 a	9.5 ± 2.5 a	11.0 ± 1.8 a	6.5 ± 1.4 a	8.5 ± 1.2 a	5.0 ± 1.8 a	4.5 ± 0.9 a
Max Spray	5	7427.8 ± 561.0	0.0 ± 0.0 b	0.0 ± 0.0 b	0.3 ± 0.3 b	0.0 ± 0.0 b	1.0 ± 0.7 b	0.0 ± 0.0 b	5.0 ± 0.1 a
IPM	1	7908.4 ± 223.0	44.3 ± 13.1 a	0.0 ± 0.0 b	4.5 ± 1.8 ab	3.3 ± 0.5 ab	7.5 ± 2.2 ab	1.3 ± 0.9 ab	5.5 ± 0.9 a

P-Value from ANOVA		NS	0.0104	0.0054	0.0142	0.0026	0.0382	0.0319	NS

* Marketable yield was calculated by subtracting total pod weight with bug damage (kg/ha) from total pod weight (kg/ha)

Means within columns followed by the same letter are not significantly different; P < 0.05.

Table 3.7. Effect of pest management treatment regime on the number of insecticide applications, marketable yield of edamame density of soybean aphids and stink bugs (SB) on four significant sampling dates, and percentage of pods at harvest with seeds showing bug feeding damage from a small plot experiment conducted in Painter, VA 2021.

			Number insects per 2 min visual sample (mean ± SE)				
Treatment	# of insecticide sprays	Marketable Yield (kg/ha)	Aphids 08/24	Aphids 08/31	SB 09/22	Aphids 09/22	% bug pod damage
Control	0	1513.3 ± 422.5 b	41.0 ± 7.7 a	58.5 ± 13.6 a	6.5 ± 0.9 a	11.5 ± 3.3 a	60.5 ± 10.6 a
Max Spray	4	3830.2 ± 536.3 a	19.5 ± 13.2 b	1.8 ± 0.63 b	1.0 ± 0.7 b	2.0 ± 1.1 b	8.0 ± 3.4 b
IPM	3	3426.6 ± 520.7 ab	15.3 ± 5.0 b	2.5 ± 0.7 b	1.0 ± 0.4 b	2.3 ± 1.0 b	21.5 ± 5.3 b
P-Value from ANOVA		0.0217	0.0448	0.0009	0.006	0.0106	0.007

* Marketable yield was calculated by subtracting total pod weight with bug damage (kg/ha) from total pod weight (kg/ha)

Means within columns followed by the same letter are not significantly different; P < 0.05.

Table 3.8. Sprays dates for each treatment by year

Location/year	Max Spray	IPM
Whitethorne 2019	lambda-cyhalothrin: 07/19, 08/14, 09/04, 09/23	lambda-cyhalothrin: 07/19
Painter 2019	lambda-cyhalothrin: 08/03, 08/27, 09/10, 09/25	N/A
Whitethorne 2020	lambda-cyhalothrin: 08/02, 08/19, 09/02, 09/09, 09/16	lambda-cyhalothrin: 09/09, sulfoxaflor: 09/16

Painter 2020	lambda-cyhalothrin: 08/03, 08/18, 08/24, 08/31, 09/14	lambda-cyhalothrin: 09/14, sulfoxaflor: 08/18, 08/31
Whitethorne 2021	lambda-cyhalothrin: 06/23, 08/10, 08/25, 09/07, 09/23	sulfoxaflor: 06/30
Painter 2021	lambda-cyhalothrin: 08/25, 09/01, 09/16, 09/24, 10/04	lambda-cyhalothrin: 09/16, sulfoxaflor: 08/25, chlorantraniliprole: 09/24

Chapter 4: Evaluation of insecticides for control of stink bug and other heteropteran pests of edamame

Introduction

Stink bugs (Hemiptera: Pentatomidae), along with other heteropterans such as broad-headed bugs (Hemiptera: Alydidae), are serious pests of edamame in the Mid-Atlantic U.S. because they feed upon developing seeds in pods. The piercing-sucking damage by both broad-headed bugs and stink bugs is indistinguishable on the seeds and leaves undesirable feeding blemishes. Effective control of these pests during pod development is critical to ensure a high-quality edamame crop with no blemished seeds. Chemical control is usually the most effective tactic for preventing stink bug damage in many cropping systems. In soybeans, broad-spectrum insecticides such as pyrethroids, organophosphates, or carbamates are used for stink bug control. However, these insecticides are neither IPM-compatible nor the safest options for beneficial insects to be used on vegetable crops such as edamame. In this chapter, I summarized laboratory bioassays and field experiments evaluating the efficacy of some reduced risk insecticides to beneficial insects with a goal of finding alternatives and IPM-compatible options for stink bug control in edamame.

Neonicotinoids (IRAC Group 4A) such as imidacloprid, dinotefuran, and thiamethoxam, have been shown to provide effective control of stink bugs in vegetables and soybeans (Kamminga et al. 2009, Kuhar et al. 2012, Aigner et al. 2016, Kuhar and Kamminga 2017); however, their risk to pollinators and other organisms is a concern, which has led to restrictions on their use (Laycock et al. 2012, van der Sluijs et al. 2013, Fairbrother et al. 2014. In this study, I evaluated **acetamiprid**, a neonicotinoid with reduced bee toxicity (Iwasa et al. 2003); as well as **flupyradifurone** and **sulfoxaflor**, which are also nicotinic acetylcholine receptor agonists

(IRAC Group 4C and 4D, respectively), that are similar to neonicotinoids, but with reduced non-target effects (Hopwood et al. 2016, Barbosa and Michaud 2017, Naggar and Paxton 2020). These insecticides have been successful in controlling other heteropteran pests including *Lygus* spp. (Hemiptera: Miridae), harlequin bug *Murgantia histrionica* (Hahn) (Pentatomidae), and kudzu bug, *Megacopta cribraria* (Fabricius) (Plataspidae), as well as stink bugs (Pentatomidae) (Kerns et al. 2011, Wilson et al. 2015, Joseph and Bolda 2016, Kuhar and Doughty 2016b).

The broad-spectrum diamide insecticide **cyclaniliprole** may also be useful in the management of stink bugs (Kuhar and Kamminga 2017). It performed well in laboratory leaf/fruit dip bioassays conducted on brown marmorated stink bug and harlequin bug (Aigner et al. 2014). I also evaluated **GS-omega/kappa-Hxtx-Hv1a**, a novel venom-like peptide insecticide commercially available from Vestaron Inc. as Spear T. The insecticide has demonstrated minimal effects on beneficial organisms and its full pest spectrum remains unknown (Cloyd and Herrick 2017, Jeschke 2020).

Herein, I evaluated each of the aforementioned insecticides on stink bugs in laboratory edamame dip bioassays as well as field trials conducted from 2018 to 2021 in Blacksburg, VA, and one in Painter VA. Each experiment was written up and published already in the journal *Arthropod Management Tests*, a publication by the Entomological Society of America that solely focuses on insecticide and acaricide evaluations.

Experiment 1: Evaluation of Insecticides to Control Stink Bug in Edamame 2019

(As published in Arthropod Management Tests: Kemper L Sutton, Thomas P Kuhar, Steven L Rideout, Bo Zhang. 2019. Evaluation of Insecticides to Control Stink Bug in Edamame 2020. Arthropod Management Tests, Volume 45, Issue 1, 2020, tsaa045, https://doi.org/10.1093/amt/tsaa045)

The objective of this experiment was to evaluate the efficacy of insecticides on brown marmorated stink bug (BMSB) on edible soybean (edamame). Treatments include the following: untreated check; the pyrethroid bifenthrin (Bifenthrin 2E); cyclaniliprole (Harvanta 50SL); acetamiprid (Assail 30SG); Harvanta 50SL + Assail 30SG; the chordotonal organ modulator flonicamid (Beleaf 50SG) + Bifenthrin (1/10th); and flupyradifurone (Sivanto Prime).

Edamame was planted on 6 Jun at Virginia Tech Kentland Farm near Blacksburg, VA. Plots were arranged in an RCB design consisting of two row plots that were 20 ft in length spaced 3-ft apart. The experiment had seven treatments, one of which was an untreated check, with four replicates. BMSB was the predominant stink bug species observed, and bugs were assessed on 9, 19, and 23 Sep by carefully inspecting plants in each plot for 30 s and recording the numbers seen including those that flew away or dropped to the ground. Insecticide applications were made on 5 and 15 Sep using a three-nozzle boom backpack sprayer equipped with D3 tips at 40 psi. Applications were made at 10 GPA when the edamame pods were at the R4 to R5 growth stages. Immediately following the 15 Sep spray application, 5-gal paint strainer mesh bags were placed over fruiting limbs containing at least 10 pods and the bags sealed with twist ties after inserting five adult BMSB collected from nearby untreated edamame. After 4 d in the field, bags were excised from the plants and taken to the lab where insect mortality was assessed. Stink bug counts and proportion mortality data were analyzed using ANOVA. Means were separated using Fisher's protected least significant difference test ($P \leq 0.05$).

BMSB densities were relatively low on 9 Sep, but increased on 19 and 23 Sep. There was a significant treatment effect on BMSB counts on 19 Sep only, when the untreated check plots had significantly more BMSB than Bifenthrin 2E (bifenthrin), Harvanta 50SL + Assail 30SG, Sivanto Prime, Harvanta 50SL, and Beleaf 50SG + 1/10th rate Bifenthrin 2E. Survival of the caged BMSB averaged 95% in the untreated check, which was significantly higher than all insecticide treatments except Harvanta 50SL and Sivanto 50 SL. Bifenthrin 2E had the lowest survival 0.0% (**Table 4.1**). No phytotoxicity was observed.

Table 4.1 Evaluation of Insecticides to Control Stink Bug in Edamame 2019

		No. BMSB per 30 sec			Proportion survival of BMSB[a,b]
Treatment	Rate / acre	Sep 9	Sep 19	Sep 23	Sep 19
Untreated check		1.25	2.50 a	0.75	0.95 a
Bifenthrin 2E	2.1[c]	0.00	0.25 c	1.00	0.00 d
Harvanta 50SL	22.0[c]	0.00	0.75 bc	2.00	0.75 ab
Assail 30SG	3.8[d]	0.50	2.00 ab	0.75	0.35 c
Harvanta 50SL + Assail 30SG	27.2[c] + 3.8[d]	0.50	0.25 c	0.25	0.60 bc
Beleaf 50SG + Bifenthrin (1/10th)	2.8[d] + 0.21[c]	0.50	0.75 bc	0.75	0.35 c
Sivanto Prime	14.0[c]	0.25	0.50 bc	1.75	0.75 ab
P>*F*		NS	0.08	NS	0.001

Means within columns followed by the same letter are not significantly different; *P*>0.05.
[a]Proportion data were arcsine-sqrt transformed to normalize variance before analysis, although untransformed proportions are shown.
[b]After 4 d caged in the field on treated foliage and pods
[c]fl. oz product per acre.
[d]oz product (wt.) per acre

Experiment 2: Evaluation of Common, and One Novel, Insecticides to Control Stink Bug in Edamame, 2020

(as published in Arthropod Management Tests: Kemper L Sutton, Christopher McCullough, Thomas P Kuhar, Steven L Rideout, Bo Zhang. 2021. Evaluation of Common, and One Novel, Insecticides to Control Stink Bug in Edamame, 2020. Arthropod Management Tests, Volume 46, Issue 1, 2021, tsaa124, 3.2https://doi.org/10.1093/amt/tsaa124)

The objective of this experiment was to evaluate the efficacy of insecticides on stink bug species on edible soybean (edamame). Treatments include the following: untreated check; sulfoxaflor (Transform WG) at two rates; cyclaniliprole (Harvanta 50SL); flupyradifurone (Sivanto Prime); and Spear-T, which contains the novel peptide active ingredient GS-omega/kappa-Hxtx-Hv1a.

Edamame was planted at a rate of six seed per foot on 3 Jun at Virginia Tech Homefield Farm in Whitethorne, VA. Plots were arranged in a randomized complete block design consisting of one-row plots that were 20 ft in length spaced 3-ft apart. The experiment had six treatments, including an untreated check, with four replicates (**Table 4.2**). Foliar insecticide applications were made on 8 and 15 Sep using a three-nozzle boom CO2 powered backpack sprayer equipped with D3 tips at 40 psi. Applications were made at 30 GPA during the pod fill stage of plant growth. Green stink bugs and brown marmorated Stink Bug (BMSB) were the two dominant species comprising 57 and 42%, respectively, of the more than 100 stink bugs observed. Stink bugs were assessed on 14 and 21 Sep by visual samples in each plot for 1 min, recording the number and species observed. On 10 Sep, 5-gal paint strainer mesh bags were placed over plant limbs containing at least 10 pods, and bags were closed with twist ties after placing five BMSB nymphs inside. After 4 d in the field, limbs in the bag were removed from the plant, and the proportion of pods with stink bug damage was calculated. The proportion of damaged pods was assessed again from a subsample of 50 random pods per plot after plants were harvested on 21 Sep. Stink bug counts and proportion of damaged pods data were analyzed using ANOVA. Means were separated using Fisher's Protected Least Significant Difference Test ($P \leq 0.05$).

Stink bug densities were low on 14 Sep, averaging 2.6 stink bugs per 1-minute observation, but increased to 4.5 during the 21 Sep observations (**Table 4.2**). No significant treatment effect was detected for stink bug counts on 14 Sep. A significant treatment effect was detected for stink bug counts on 21 Sep. All insecticide-treated plots had fewer stink bugs than the untreated check plots. No significant treatment effects were detected for either date of pod damage assessment. The novel active ingredient, GS-omega/kappa-Hxtx-Hv1a, performed similarly to all other tested products. Overall, treatments reduced stink bug counts, but did not prevent damage to the pods on the plants (**Table 4.2**). No phytotoxicity was observed.[1]

Table 4.2 Evaluation of Common, and One Novel, Insecticides to Control Stink Bug in Edamame, 2020

		No. stink bug per 1 min		Proportion of stink bug damaged pods[a]	
Treatment	Rate / acre	Sep 14	Sep 21	Sep 14	Sep 19
Untreated control		4.75	13.0 a	0.44	0.40
Transform WG	0.75[b]	2.25	3.50 b	0.29	0.25
Transform WG	1.00[b]	2.75	2.25 b	0.38	0.25
Spear T	384.0[c]	3.50	4.25 b	0.44	0.20
Sivanto HL 400SL	10.5[c]	0.75	3.50 b	0.24	0.23
Harvanta 50SL	16.4[c]	2.00	0.75 b	0.09	0.22

P>F	NS	0.001*	NS	NS

* p < 0.05;
Means within columns followed by the same letter are not significantly different; *P*>0.05.
[a] Proportion data were arcsine-sqrt transformed to normalize variance before analysis, although untransformed proportions are shown.
[b] oz product (wt.) per acre
[c] fl. oz product per acre

Experiment 3: Evaluation of Insecticides to Control Southern Green Stink Bug in Edamame, 2020

(as submitted to Arthropod Management Tests: Kemper L Sutton, Hélène Doughty, Thomas P Kuhar, Steven S Rideout. 2021. Evaluation of Insecticides to Control Southern Green Stink Bug in Edamame, 2020. Arthropod Management Tests, Volume 46, Issue 1, 2021, tsab081, https://doi.org/10.1093/amt/tsab081)

The objective of this experiment was to evaluate the efficacy of insecticides on the southern green stink bug (SGSB) on edible soybean (edamame) through lab bioassays. Treatments include the following: untreated check; sulfoxaflor (Transform WG) at two rates; cyclaniliprole (Harvanta 50SL); flupyradifurone (Sivanto Prime); and pyrethrins (Pyganic EC).

Fourth and fifth instar SGSB nymphs were collected from edamame and soybean fields on 19 Sep, at the Eastern Shore Agricultural Research and Extension Center in Painter, VA. Five nymphs were placed in 20 cm Petri dishes with untreated field-collected edamame pods that were dipped in field rate insecticide concentrations in 500 ml of based on 40 GPA application rate. Each treatment was replicated four times. Mortality was assessed by counting the numbers of live, moribund (unable to right themselves when on their back), and dead (unresponsive to touch) nymphs per dish and were recorded at 24 h intervals for 72 h. Untreated check mortality never exceeded 30% at 96 h. Moribund nymphs that recovered were counted as alive in the following counting period. SGSB mortality included dead and moribund nymphs and was analyzed using ANOVA. Means were separated using Fisher's Protected Least Significant Difference Test (P ≤ 0.05).

There was a significant treatment effect on SGSB mortality (**Table 4.3**). The untreated check, Pyganic, and Sivanto HL had significantly lower mortality when compared to the high rate of Transform and Harvanta. In addition, mortality in Pyganic and Sivanto HL was not significantly different from the untreated check at 72 h. The high rate of Transform more than doubled the mortality of the low rate of Transform. The high rate of Transform had the highest percentage of dead SGSB when compared to all other treatments at 72 h. In some treatments, moribund nymphs recovered and were then counted as alive. This is especially seen in the low rate Transform WG treatment (**Table 4.3**).

Table 4.3 % Mortality of Southern Green Stinkbugs After Insecticide Exposure

		% Mortality[a]		
Treatment	Rate/acre	24h	48h	72h
Untreated check		0.0c	15.0cd	20.0cd
Transform WG	0.75 oz	50.0b	45.0bc	40.0bc
Transform WG	2.25 oz	90.0a	85.0a	100.0a
Sivanto HL	7 fl. oz	5.0c	5.0d	10.0cd
Harvanta 50SL	16.4 fl. oz	70.0ab	65.0ab	65.0b
Pyganic	32 fl. oz	0.0c	0.0d	0.0d
P-value from Anova		<0.0001	0.0005	<0.0001

Means within columns followed by the same letter are not significantly different; *P*>0.05.
[a] Proportion mortality were dead plus moribund were arcsine-sqrt transformed to normalize variance before analysis

Experiment 4: Evaluation of Insecticides to Control Stink Bug and Broad Headed Bug in Edamame, 2021

(As published in Arthropod Management Tests: Kemper L Sutton, Daniel Wilczek, Thomas P Kuhar, Kelly McIntyre, Steven L Rideout, Bo Zhang. 2022. Evaluation of Insecticides to Control Stink Bug and Broad Headed Bug in Edamame, 2021. Arthropod Management Tests, Volume 47, Issue 1, 2022, tsac082, https://doi.org/10.1093/amt/tsac082)

The objective of this experiment was to evaluate the efficacy of insecticides on stink bug and broad headed bug (*Alydus* spp.) on edamame, *Glycine max* (L.). The latter cause very similar damage to edamame seeds as stink bugs. We compared pod damage among five insecticides, and an untreated check. Insecticide treatments include the following: bifenthrin (Sniper 2EC); acetamiprid (Assail 30SG); cyclaniliprole (Harvanta 50SL); GS-omega/kappa-Hxtx-Hv1a (Spear T); and a novel plant derived insecticide sucrose octanoate esters (Organishield).

Twelve 95-ft rows spaced 3 ft apart were planted with edamame at a rate of six seeds per ft on 17 May 2021. After emergence, rows were broken up into 20 ft plots with 5 ft alleys. Each treatment was separated by a guard row and replicated four times using a randomized complete block design (RCBD). Foliar insecticides were applied twice during pod fill growth stages (R4 and R5) on 17 and 24 September 2021. Application were made using a three-nozzle boom CO2-powered backpack sprayer equipped with 8003VS tips spaced 20 in apart. Applications were made at 30 gpa using 40 psi. On 2 October 2021, 50 edamame pods were hand harvested arbitrarily from each plot. Each pod was opened and presence/absence of piercing-sucking insect damage was recorded. If a single bean in a pod received damage, the entire pod was recoded as damaged. All data were analyzed using the analysis of variance procedures. Means were separated using Tukeys HSD test at the 0.05 level of significance. Data were square-root transformed to normalize when necessary.

Stink bug and broad headed bug damage was moderately high in the untreated check (24% of pods damaged). There was not a significant effect of treatment on percent pod damage at harvest (**Table 4.4).** However, plots sprayed with Sniper 2EC, Assail, or Harvanta 50SL had approximately half as many damaged pods than the untreated check (**Table 4.4**). No phytotoxicity was observed from any treatment.1

Table 4.4 Proportion of stink bug and broad headed bug damaged pods

		Proportion of stink bug and broad headed bug damaged pods[a]
Treatment	Rate / acre	Proportion Damaged Pods
Untreated control		24.0 a
Sniper 2EC	5.20[c]	8.5 a
Harvanta 50SL	16.40[c]	7.0 a
Assail 30SG	3.80[b]	9.0 a
Spear T	384.0[c]	15.0 a
Organishield	184.0[c]	16.5 a
P>F		0.1

* $p < 0.05$;
Means within columns followed by the same letter are not significantly different; $P>0.05$.
[a] Proportion data were arcsine-sqrt transformed to normalize variance before analysis, although untransformed proportions are shown.
[b] oz product (wt.) per acre
[c] fl. oz product per acre

Discussion

Many of the insecticides tested in this study showed some efficacy at reducing stink bug damage in edamame. In bean dip bioassays, sulfoxaflor and cyclaniliprole resulted in significant stink bug mortality. In a field experiment conducted on edamame, two foliar applications of sulfoxaflor, flupyradifurone, cyclaniliprole, and GS-omega/kappa-Hxtx-Hv1a reduced stink bug damage on seeds at harvest. In another field experiment, fewer stink bugs were observed on edamame plants treated with bifenthrin, cyclaniliprole with and without acetamiprid, flonicamid + 1/10th rate of bifenthrin, and flupyradifurone. Also, the same insecticides except cyclaniliprole and flupyradifurone resulted in significant mortality of caged stink bugs on plants after 4 days exposure.

The broad spectrum diamide cyclaniliprole has previously demonstrated activity against heteropteran pests (Aigner et al. 2015; Kuhar and Doughty 2016b). The results of my bioassays and field trials provided further evidence on the activity of this diamide on heteropteran pests in edamame. I also showed that the insecticide flupyradifurone as well as the novel peptide GS-omega/kappa-Hxtx-Hv1a reduced edamame seed damage by stink bugs. Data showed that these insecticides were also active on broad-headed bugs (Sutton et al. 2021). Sulfoxaflor is labelled for the suppression of the brown stink bug and southern green stink bug and previous studies have shown effective activity against green and brown marmorated stink bugs as well (Steckel et al. 2010; Kuhar and Doughty 2016b). This compound additionally showed some levels of effectiveness against green, southern green, and brown marmorated stink bugs in my trials and bioassays. The insecticides flupyradifurone, sulfoxaflor, and cyclaniliprole show evidence as effective chemical control options against stink bugs. More research should be conducted in the future with these insecticides to see if mixtures with other compounds or adjuvants can enhance their efficacy to provide effective reduced risk options for heteropteran pests on crops like edamame.

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Chapter 5

Evaluation of Fungicides for Control of Diseases in Edamame

Introduction

Soybeans (*Glycine max* (L.) Merr.) grown in the United States are known to be susceptible to a broad range of pathogens (Hartman et al. 1999) such as *Cercospora* spp. and *Fusarium* spp. to name a few, that can cause physiological damage to soybeans that results in damping off/yield loss (Arias et al. 2013; Price et al. 2015). Developing soybean breeding lines that have resistance to specific diseases has been a common practice for decades in U.S soybeans (Kim & Diers 2000). However, edamame is a new crop to the U.S. that has been developed from cultivars from multiple sources outside the U.S. where edamame is traditionally grown (Lord et al. 2021). Edamame historically has been bred for characteristics such as bean size and flavor (Yu et al. 2022). These cultivars are new to the U.S. and could potentially be susceptible to soybean diseases endemic to our growing areas. Not only is edamame at risk for the typical yield and quality losses associated with soybean diseases, but this crop is being marketed as a vegetable, where cosmetic damage to the pods due to disease is an important economic consideration (Wilson 2014). It is important to observe the impacts soybean diseases have on edamame grown in Virginia and to develop sound strategies to control these diseases while mitigating the increase of fungicide resistance.

In this study, a number of fungicides and bactericides were evaluated on edamame and assessed for disease incidence and yield. Fungicides, with the modes of actions according the Fungicide Resistance Action Committee (FRAC), included the following: two strobilurins (FRAC group 11) azoxystrobin and picoxystrobin, which are respiration inhibitors, that reduce the growth of fungi (Leinhous et al. 1997); four triazoles (FRAC group 3) metconazole, cyproconazole, flutriafol, propiconazole, which are cell membrane disruptors, that have been shown to be efficacious in controlling common soybean diseases like brown spot (Cruz et al. 2010); four carboxamides (FRAC group 7), fluxaoyroxad, penthiopyrad, pydiflumetofen, and inpyfluxam**,** which are respiration inhibitors , that have been shown to be efficacious in controlling several soybean diseases, including soybean rust (Reznikov et al. 2019); the phenylamide (FRAC group 4) **mefenoxam**, which is an inhibitor of RNA polymerase 1 and one of the most commonly used seed treatments (Broders et al. 2007); and finally inorganic **copper sulfate pentahydrate**, which causes non-specific denaturation of proteins (FRAC group M1). Copper is one of the oldest fungicides/bactericides but is still widely used for control of mostly bacterial diseases (Pscheidt 2022). Most of these active ingredients are mixed with one or more other active ingredients when sold as a product commercially. These premixture products were also trialed in these experiments.

The aforementioned fungicides were evaluated in field trials conducted between 2019 to 2021 in Blacksburg, VA and one in 2019 in Painter, VA. Each experiment has been written up to be published or has been published in the journal of *Plant Disease Management Reports*, a publication by the American Phytopathological Society (https://www.plantmanagementnetwork.org/pub/trial/pdmr/).

Experiment 1: Evaluation of an in-furrow applied fungicide on edamame emergence in Virginia, 2019

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Sutton, K. L., et al. 2020. Evaluation of an in-furrow applied fungicide on edamame emergence in Virginia, 2019. Plant Disease Management Report. 14:V080

A field study was conducted at Virginia Tech's Eastern Shore Agricultural Research and Extension Center in Painter, VA to evaluate an in-furrow applied fungicide at planting on emergence and yield. Bare edamame seeds of the cultivar 'R16-5336' were hand planted on 3 Jul at 6 seeds/ft at a depth of 1 in. The plots were arranged in a randomized complete block design with four replications. There were 20 ft alleys between replications and the experimental units were 6 ft wide (2 rows) x 25 ft length. Uniform 3.72SC was applied at planting in the open furrow directly over seed or over lightly covered (0.5 in) seeds at varying rates (**Table 5.1**) on 3 Jul using a CO2 backpack sprayer calibrated at 40 psi and 10 gal/A. A single nozzle boom (TeeJet flat fan 80015) was used to deliver the product. After spraying, the furrows were filled with soil and visual assessments of emergence were taken when the edamame began sprouting on 8 Jul and continued to be counted four times (8 Jul, 10 Jul, 16 Jul, 19 Jul) over 11 days. Yield was mechanically collected with an Oxbo BH100 one-row bean harvester on 14 Oct from one plot row (3 ft x 25 ft). Data was analyzed using ANOVA and Fisher's Least Significant Difference (LSD) for means of comparison (P = 0.05)

At the last assessment date (19 Jul) plants treated with Uniform 3.72SC at 0.34 fl oz/1000 row ft in-furrow (directly over seed) and Uniform 3.72SC at 0.62 fl oz/1000 row ft sprayed over lightly covered seed produced a significantly higher stand than the nontreated control (**Table 5.1**). However, these treatments did not seem to effect yield tremendously. The only significant difference observed was the 0.34 fl oz/1,000 row ft treatment significantly outperforming the 0.62 fl oz/1,000 row ft rate when seed were covered prior to application of Uniform.

Table 5.1 Experiment 1: Evaluation of an in-furrow applied fungicide on edamame emergence and Yield in Virginia, 2019

Treatment	Placement	Rate/1000 ft row	Emergence (%) 19 Jul	Yield (lb/75ft^2) 6 Nov
Nontreated Control	none	none	58.0 b*	4.838 ab
Uniform 3.72SC	Directly on Seed	0.34 fl oz	66.8 a	4.725 ab
Uniform 3.72SC	Directly on Seed	0.62 fl oz	62.8 ab	4.775 ab
Uniform 3.72SC	Covered Seed	0.34 fl oz	58.5 b	5.488 a
Uniform 3.72SC	Covered Seed	0.48 fl oz	63.5 ab	4.850 ab
Uniform 3.72SC	Covered Seed	0.62 fl oz	67.0 a	4.188 b

Column means followed by the same letter(s) are not significantly different according to Fischer's LSD test (P = 0.05)

Experiment 2: Evaluation of foliar applied fungicides at the R1 growth stage of edamame in Virginia, 2019

K.L. Sutton, S.L. Rideout, T.P. Kuhar, J.R. Pollok, J.T. Custis, Jr., B. Zhang.

A field study was conducted at Virginia Tech's Eastern Shore Agricultural Research and Extension Center in Painter, VA to evaluate the efficacy of foliar applied fungicides at the R1 growth stages of edamame (*Glycine max*) for disease control, specifically pod damaging diseases. Ten treatments replicated four times were planted and arranged into a randomized complete block design. All plots were of a single cultivar that showed susceptibility to disease and chosen from available seed. The cultivar R16-5336 was planted on 13 Jun 2019. Each plot was 25 foot in length with one row plots with guard rows at three-foot row spacing. Edamame was planted at a seeding rate of 6 seeds per row foot. After planting, at the R1 growth stage (at flowering) (23 Aug. 2019), foliar applications were made according to (**Table 5.2**) using a three-nozzle drop down boom with 8003VS tips sprayed at 40 PSI at an output of 10 GPA. Each treatment was also mixed with the surfactant Cohere at 0.25 % v/v. At harvest, R6 growth stage, edamame was mechanically harvested with an Oxbo BH100 one-row bean harvester on 11 Oct. and 100 pods per plot (50 per row) were collected randomly and assessed for disease. Any pod that had a blemish due to disease was recorded. Data was analyzed using ANOVA and Fisher's Least Significant Difference (LSD) for means of comparison ($\alpha = 0.05$)

At harvest when pods were evaluated for disease presence, there was not a significant effect of treatment on diseased pods. However, Topguard EQ did preform the best numerically, where 38% of pods showed diseased symptoms compared to the control at 53% of pods had disease **(Table 5.2)**.

Table 5.2 Evaluation of foliar applied fungicides at the R1 growth stage of edamame in Virginia, 2019

Treatment	Rate	Proportion of pods Diseased
UTC	---	0.53
Quadris	6 fl oz/a	0.53
Headline AMP	6 fl oz/a	0.49
Fontelis	15 fl oz/a	0.54
Priaxor	8 fl oz/a	0.44
Aproach Prima	6.8 fl oz/a	0.57
Topguard EQ	6 fl oz/a	0.38
Excalia	3 fl oz/a	0.49
Miravis Neo	14 fl oz/a	0.62
Priaxor	8 fl oz/a	0.59
P > F		P > 0.683

Column means followed by the same letter(s) are not significantly different according to Fisher's LSD test (P = 0.05)

Experiment 3: Evaluation of foliar applied fungicides at different reproductive growth stages of edamame in Virginia, 2020

K. L. Sutton, S. L. Rideout, T. P. Kuhar, B. Zhang

A field study was conducted at Virginia Tech's Kentland research farm in Whitethorne, VA to evaluate the efficacy of foliar applied fungicides at several different reproductive growth stages of edamame for disease control, specifically pod damaging diseases. Nine treatments replicated four times were planted and arranged into a randomized complete block design. All plots were of a single cultivar that showed susceptibility to disease and chosen from available seed. The cultivar R15-10280 was planted on 30 Jun 2020. Each plot was 20 foot in length with one row plots with guard rows. Three-foot row spacing at a seeding rate of 6 seeds per row foot. Foliar applications were made at the R1, R3 (beginning pod) or both R1 and R3 using a three-nozzle drop down boom with 8003VS tips applied 40PSI at 10 GPA total output ((**Table 5.3**)). Each treatment was also mixed with the surfactant Cohere at 0.25 % v/v. R1 (at flowering) applications were made on 28 Aug, and R3 applications were made on 10 Sep. Pods were hand harvested on 17 Oct 2020 (at the R6 growth stage) with 50 edamame pods from each plot and observed being assessed for pod diseases. Data was analyzed using ANOVA and Fisher's Least Significant Difference (LSD) for means of comparison (P = 0.05)

At pod evaluation, there were four treatments that produced significantly fewer diseased pods when compared to the control which had 86% of pods showing disease symptoms. Quadris sprayed at R3 had 54% diseased pods. Miravis Neo sprayed at R1 (at flowering) had 36.5% of pods being diseased, Quadris sprayed at R1 and R3 with MasterCop had 42.4% diseased pods, and Miravis Neo with MasterCop sprayed at R1 and R3 had 32.6% diseased pods. With the exception of Miravais Neo sprayed at R1, the Quadis and Miravis Neo sprayed both at R1 and

R3 when added with MasterCop had the best disease prevention. They both significantly decreased diseased pods when compared to the control (**Table 5.3**).

Table 5.3 Experiment 3: Evaluation of foliar applied fungicides at different reproductive growth stages of edamame in Virginia, 2020

Treatment	Rate fl oz/a	Mean Prop. Diseased pods
UTC	---	0.86 a
Quadris @ R1	6 fl oz	0.71 a
Quadris @ R3	6 fl oz	0.54 bcd
Quadris @ R1&R3	6 fl oz	0.68 ab
Miravis Neo @ R1	14 fl oz	0.365 d
Miravis Neo @ R3	14 fl oz	0.74 a
Miravis Neo at R1&R3	14 fl oz	0.64 abc
Quadris @ R1&R3 w/ MasterCop	6 + 19.21 fl oz	0.424 cd
Miravis Neo at R1&R3 w/ MasterCop	14 + 19.21 fl oz	0.326 d
P > F		P > 0.0029

Column means followed by the same letters are not significantly different according to Fisher's LSD Test (P = 0.05).

Experiment 4: Evaluation of foliar applied fungicides at different reproductive growth stages of edamame in Virginia, 2021

K. L. Sutton, S. L. Rideout, T. P. Kuhar, B. Zhang, K. McIntyre

A field study was conducted at Virginia Tech's Kentland research farm in Whitethorne, VA to evaluate the efficacy of foliar applied fungicides at several different reproductive growth stages of edamame for the control diseases, specifically pod damaging diseases. Nine treatments replicated four times were planted and arranged into a randomized complete block design. All plots were of a single cultivar that showed susceptibility to disease and chosen from available seed. The cultivar R15-10280 was planted on 17 May 2021. Each plot had one row with two guard rows. Each row was 20 foot in length with three-foot row spacing at a seeding rate of 6 seeds per row foot. After planting, at the R1 (at flowering), R3 (beginning pod) or both R1 and R3, foliar applications were made using a three-nozzle drop down boom with 8003VS tips applied at 10 GPA ((**Table 5.4**)). Each treatment was also mixed with the surfactant Cohere at 0.25 % v/v. R1 applications were made on 5 Aug, and R3 applications were made on 18 Aug. At harvest (24 Sep 2021), or at the R6 growth stage, 50 edamame pods were hand harvested from each plot and observed for pod diseases. Data was analyzed using ANOVA and Fisher's Least Significant Difference (LSD) for means of comparison (P = 0.05)

There was low disease pressure in 2021 and thus no significant difference between treatments on diseased pods were observed in this trial. However, treatments that were sprayed at both R1 and R3 growth stages coupled with the addition of MasterCop did numerically have lower numbers of diseased pods that the untreated control (**Table 5.4**).

Table 5.4 Evaluation of foliar applied fungicides at different reproductive growth stages of edamame in Virginia, 2021

Treatment	Rate fl oz/A	Mean Prop. Diseased Pods
UTC		0.12
Quadris @ R1	6 fl oz	0.13
Quadris @ R3	6 fl oz	0.11
Quadris @ R1&R3	6 fl oz	0.18
Miravis Neo @ R1	14 fl oz	0.19
Miravis Neo @ R3	14 fl oz	0.19
Miravis Neo @ R1&R3	14 fl oz	0.08
Quadris @ R1&R3 w/ MasterCop	6 + 19.21 fl oz	0.07
Miravis Neo at R1&R3 w/ MasterCop	14 + 19.21 fl oz	0.09
P > F		P > 0.1301

Column means followed by the same letters are not significantly different according to Fisher's LSD Test (P = 0.05).

Discussion

Many of the fungicides in these experiments demonstrated some efficacy at reducing disease pressure on edamame pods. Most edamame seed are not treated leading to many issues with emergence. The seed treatment trial in 2019 that used Uniform 3.72 SC, a product that contains a combination of azoxystrobin and mefenoxam, showed some increase in plant emergence, $P < 0.031$. However, none of the treatments exceed 67% emergence which is commercially unacceptable. Increasing edamame emergence to create a higher plant population is crucial to maximize yield potential (Zhang et al. 2013). Alternative methods to increase emergence should also be investigated as Mourtzinis et al 2019, found that soybean seed treatments do little to increase yield.

The fungicide foliar spray trial of 2019 did not result in any significant differences despite having 10 different treatments. Although, not significantly different from the untreated control, Topguard EQ, a product containing flutriafol, was the treatment with the lowest percentage of diseased pods amongst treatments having 38% of pods with diseased pods compared to the untreated control which ad 53% diseased pods. Ultimately, all treatments had unacceptable levels of diseased pods. In this experiment all treatments were sprayed at R1, which we believed was a major cause of the high percentage of diseased pods. In conclusion of the 2019 field fungicide trial, we believed that more sprays during the reproductive growth stages would be required to reduce diseased pods, especially bacterial diseases which were the most problematic. We accounted for these changes in timing of fungicide applications during the 2020 and 2021 trials.

The 2020 fungicide trial had high disease pressure. The untreated control received 86% percent of pods being damaged. There were three treatments that showed efficacy in reducing diseased pods. Miravis Neo, a product containing pydiflumetofen, azoxystrobin, and propiconazole, sprayed at R1 had 36.5% of diseased pods. Additionally, Quadris, containing azoxystrobin, combined with MasterCop, containing the inorganic copper sulfate pentahydrate, sprayed at both R1 and R3 had 42.4% diseased pods. Miravis Neo was also combined with MasterCop and sprayed at R1 and R3 and resulted in 32.6% diseased pods. Both treatments that were sprayed at the growth stages R1 and R3 with the addition of copper sulfate pentahydrate seemed the most promising for disease control.

We replicated this experiment again in 2021 and disease pressure during that year was extremely low compared to previous year. Not a single treatment exceeded 19% diseased pods and there was not a significant treatment effect. We believe this was associated with a very dry growing season.

Although the field trial of 2020 showed promising results when different modes of action were sprayed at two different reproductive growth stages, the percentages were still above an economically acceptable level and would need to decrease much further to meet the vegetable industry standards (Wilson et al. 2014). These experiments showed some efficacy in increasing edamame emergence and decreasing pod disease using different fungicides. We believe that increasing the number of applications of fungicides after R1 will be needed to protect edamame pods. Fungicide applications at R1 and R3 seemed more effective than singular applications at R1 however, we believe an additional spray after R3 will be needed. Stabilizing emergence of edamame through further fungicide testing at planting will also be crucial in maximizing yield for this crop. More research will need to be conducted in the future with these fungicides at planting and during reproductive growth to provide proper control of diseases afflicting edamame to ensure it can be grown properly in Virginia and in the region.

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Chapter 6

To assess the current susceptibility of Virginia corn earworm populations to pyrethroid insecticides using a bean dip bioassay

Introduction

Corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), is one of the most important insect pests of food and fiber crops in the U.S. (Swenson et al. 2013). In Virginia, *H. zea* causes serious economic loss to soybean, cotton, corn, sweet corn, hemp, tomato, and edible bean crops (Kuhar et al. 2006, Fleisher et al. 2007, Herbert et al. 2009, Kuhar et al. 2019, Britt et al. 2021, Dively et al. 2021). For many crops, especially those that do not have commercially-available or viable transgenic varieties that contain *Bacillus thuringiensis* proteins, pyrethroids are the most widely used insecticide class (IRAC Group 3) to control corn earworm as they are effective, quick acting, relatively inexpensive, and control a wide range of other insect pests (Hutchison et al. 2007). However, pyrethroid resistance has been identified in *H. zea* populations since the 1990s in the southern U.S. (Stadelbacher et al. 1990, Abd-Elghafar et al. 1993, Brown et al. 1998), and since the 2000s in the Midwest (Hutchison et al. 2007, Jacobson et al. 2009). Researchers have determined multiple mechanisms of pyrethroid resistance including target site mutations (Head et al. 1998, Soderlund 2008, Hopkins and Pietrantonio 2010a) as well as metabolic processes including the mixed-function oxidase system or cytochrome P450 enzymes (Sasabe et al. 2004, Yang et al. 2006).

Since the 1990s, monitoring for pyrethroid resistance in local *H. zea* populations has been an important part of pest management programs for this pest throughout the U.S. (Plapp et al. 1990, Fleischer et al. 2007, Hutchison et al. 2007, Jacobson et al. 2009). One popular approach used for resistance monitoring has been the adult vial test (AVT) method in which *H. zea* male moths caught at pheromone traps are placed in 20 ml glass scintillation vials pre-treated with a discernable concentration 5 or 10 µg of cypermethrin (Plapp et al. 1990). Moths surviving the treated vial after 24 hours are believed to be pyrethroid resistant. Although this method has been useful to show changes in pyrethroid susceptibility in populations such as in the mid-Atlantic U.S. in the mid-2000s (Fleischer et al. 2007), results have not always correlated with the efficacy of pyrethroid applications on corn earworm larval populations on crops (T. Kuhar, *unpublished data*). In addition, although cypermethrin was the pyrethroid chosen for the AVT method in Texas in the 1980s (Plapp et al. 1990) and would continue to be used until present day for pyrethroid resistance monitoring, other (more advanced) pyrethroids such as lambda-cyhalothrin, bifenthrin, cyfluthrin, beta-cyfluthrin, zeta-cypermethrin, fenpropathrin, and esfenvalerate would replace the use of cypermethrin on most crops in the U.S. by the mid-1990s. Hopkins and Pietrantonio (2010b) showed that *H. zea* resistance ratio values obtained with one pyrethroid may not be predictive of resistance ratios for other pyrethroids.

Other methods to monitor insecticide resistance in *H. zea* include serial dilution topical applications to larvae (Jacobson et al. 2009) as well as diet-overlay insecticide bioassays (Marcon et al. 1999, Kaur 2018). These methods have been extremely useful for calculating LD_{50} or LC_{50} levels, which can be used for toxicological analyses. However, these methods often require technical grade formulations of the insecticides and typically are performed on F_1 or F_2 generation larvae after being reared in the laboratory (Jacobson et al. 2009).

Herein, I evaluated a simple and quick bean dip bioassay that is grower oriented for determining the susceptibility of field-collected *H. zea* larvae to pyrethroids applied at their field application rates. The method was used to assess the susceptibility of *H. zea* larvae collected from five locations in Virginia over two years. In addition, the synergist piperonyl butoxide

(PBO), was included as a treatment with each of the pyrethroids to assess if the resistance mechanism was metabolic.

Methods

Corn earworm populations

In 2020 and 2021, 2^{nd} to 4^{th} instar corn earworm (CEW) larvae were collected from untreated sweet and field corn across five locations in Virginia to be used in laboratory bioassays. 2^{nd} and 4^{th} instar sizes were collected by visual estimates according to Capinera 2000. The five collection sites were Blacksburg, Blackstone, Abingdon, Suffolk, and Painter, VA. Larvae were collected from corn when sufficient populations had been established. In 2020, CEW larvae were collected from Blacksburg on 7-Aug., Blackstone 16-Sep., Abingdon 1-Sep., Suffolk 5-Aug., and Painter 31-Aug. In 2021, larvae were collected from Blacksburg on 3-Aug, 7-Aug, 9-Aug., and 13-Aug. Blacksburg populations were lower than 2020 and all four replications for the bioassay had to be collected on different days. Blackstone was collected 28-Jul., Abingdon 12-Aug., Suffolk 12-Aug, and Painter 14-Sep. All field collected larvae were caught during or within two weeks of the peak flight months of July and August which are the primary month for peak moth flight in Virginia (Herbert at al. 1991). In addition to the field-collected *H. zea* larval populations, a pyrethroid-susceptible laboratory colony of *H. zea* obtained from Dr. Dominic Reisig, North Carolina State University Tidewater Research Station, Plymouth NC, and was included for comparison, as another location. Larvae collected from the field were placed separately in 1oz plastic cups with some fresh corn silks as a temporary food source. Larvae were taken back to the laboratory and placed into a Percival Scientific growth chamber set to 23.0 ± 2.0 ºC until they were used for bioassays, usually within a day or two of collection

Bioassay

A bean-dip bioassay was used following Kuhar et al. (2012). There were six treatments, each with 10 larvae per treatment, replicated four times, for a total of 240 larvae per location. To avoid pseudo replication, each replication was conducted on a different day.

Treatments included the following: 1) a water control; 2) lambda-cyhalothrin (Lambda-Cy EC, 11.4% active ingredient, UPL, Inc., King of Prussia, PA) at 0.12 g ai/L; 3) pyrethrins (PyGanic Crop Protection EC 5.0 II, 5.0% active ingredient, Valent U.S.A. LLC, San Ramon CA) at 0.054 g ai/L; 4) lambda-cyhalothrin at 0.12 g ai/L combined with PBO (Exponent insecticide synergist, 91.3% piperonyl butoxide, Valent U.S.A. LLC, San Ramon CA) at 0.944 g ai/L 5) pyrethrins at 0.054 g ai/L combined with PBO at 0.944 g ai/L , and 6) PBO alone at 0.944 g ai/L.

If possible, fresh untreated edamame pods were collected from Virginia Tech's Kentland Farm in Whitethorne, VA and were used for most bioassay reps. Otherwise, frozen organic edamame was thawed and used instead when fresh pods were past their prime. Edamame pods were cut into halves or thirds (~2.5 cm square) so that each piece had one bean inside the shell. Using forceps, each bean piece was dunked into a beaker with the mixed treatments for 2 sec, and allowed for excess moisture to drain off and then placed into an empty 30 ml clear plastic souffle cup (Solo Cup Company, Lake Forest, IL) making 10 cups per treatment per rep. A single CEW larva (2^{nd} to 4^{th} instar) was placed into each cup and a lid with holes placed on top, securing the larvae inside with the treated bean piece. Mortality of CEW larvae were recorded every 24 hours for 96 hours after larva placement.

Data Analysis

Proportion CEW mortality data were analyzed using a Mixed ANOVA model in JMP Version 2016 statistical software (JMP Pro 2021). Model factors included year, location, treatment, and the interactions among these effects. If significant interactions were found, data were partitioned into separate two-way ANOVAs by main factors (such as year and location) using JMP Version 2016 statistical software (JMP Pro 2021). Means were separated using Tukey's HSD at the $\alpha < 0.05$ level of significance. Additionally, a two-way ANOVA procedure was used to test differences in proportion mortality between all treatments at each location. Means were separated using Tukey's HSD, $P \leq 0.05$.

Results

We observed that mortality of *H. zea* larvae typically did not change in the insecticide treatments from 48 hr to 72 hr, thus, we used 48 hr mortality data for all of our analyses. The mixed model was highly significant ($F = 21.59$; $df = 71$; $P < 0.0001$) with year, location, and treatment, and all of the interactions of these terms being significant factors on percentage mortality of *H. zea* larvae in the pod-dip bioassays (**Table 6.1**). Because of the significant interactions, data were summarized separately by year, treatment, and location.

Pyrethrins:

In 2020, the efficacy of pyrethrins on *H. zea* larval populations varied by location in 2020 ($F = 6.26$; $df = 11$; $P \leq 0.0001$) with high mortality (>70%) occurring in the Blacksburg and Suffolk populations, which were similar to the susceptible Plymouth colony (76.0 ± 0.03%; **Fig. 6.1**; **Table 6.2**). Mortality from pyrethrins was noticeably lower in Painter (2.0 ± 0.03%), Blackstone (48.0 ± 4.4%), and Abingdon (43.0 ± 33.9%), but only the Painter population was statistically different from the susceptible colony ($P \leq 0.0001$).

The addition of piperonyl butoxide (PBO) to pyrethrins significantly increased proportion mortality in Painter (from 2.0 ± 0.03% to 31.0 ± 6.9%) and Abingdon (from 43.0 ± 33.9% to 95 ± 5.0%, $P < 0.0001$).

In 2021, the efficacy of pyrethrins varied again by location ($F = 2.83$; df = 11; $P \leq 0.0097$) with the Abingdon population having significantly less mortality (48.0 ± 23.4%) than the susceptible colony (96.0 ± 4.7%; $P \leq 0.0083$; **Fig. 6.2**; **Table 6.3**). The addition of PBO to pyrethrins did not significantly increase the proportion mortality at any location in 2021 (**Fig. 6.2**; **Table 6.3**).

Lambda-cyhalothrin

In 2020, mortality from lambda-cyhalothrin was similar amongst all of the Virginia populations (63.0 ± 1.9 % - 82.0 ± 7.4%) and the susceptible colony (80.0 ± 0.0%) ($F = 0.92$; df = 11; $P = 0.53$) and the addition of PBO to lambda-cyhalothrin did not increase mortality at any location (**Fig. 6.3**; **Table 6.1**).

In 2021, lambda-cyhalothrin mortality was, once again, not significantly different among locations or compared to the susceptible colony ($F = 0.99$; df = 11; $P = 0.47$). The addition of PBO did not significantly increase the proportion mortality at any locations (**Fig. 6.4**; **Table 6.2**).

Piperonyl butoxide

Across all locations there was a significant effect of PBO on mortality when mixed with pyrethrins in 2020. Without PBO, mean mortality was 58%, and when PBO was added morality increased to 73%, which was significantly higher ($P < 0.0037$). Additionally, PBO as a treatment by itself did not significantly differ from the water control mortality at any location or year except for the susceptible strain (Plymouth) in 2020 which had 47 ± 0.07% mortality compared to the control at 10 ± 0.11% at 48h (**Table 6.1**), and the susceptible strain (Plymouth) in 2021, which had 31 ± 13.45% mortality compared to the control at 0 ± 0.00% (**Table 6.2**.)

Discussion

Pyrethroid resistance in *H. zea* populations has been a major concern for over 30 years in the U.S. (Plapp et al. 1990, Fleischer et al. 2007, Hutchison et al. 2007, Jacobson et al. 2009). In some regions, such as the Mid-South, pyrethroids are no longer recommended for use to control *H. zea* on agronomic crops (Crow et al. 2022), or combinations with other insecticide mode of actions are suggested (Stewart 2019). Based on our monitoring of five *H. zea* larval populations across Virginia in 2020 and 2021, there was some evidence of pyrethroid resistance (based on location), but for most of the *H. zea* populations, susceptibility to lambda-cyhalothrin or pyrethrins was not different than that of a pyrethroid-susceptible colony strain. *H. zea* larvae collected from Painter, VA in 2020 and from Abingdon, VA in 2021 had significantly lower mortality than the susceptible strain. Thus, some reduced susceptibility to pyrethroids was evident in some Virginia populations but was not widespread or consistent over time and location.

Lambda-cyhalothrin resulted in mortality of *H. zea* populations from all Virginia locations 63-82% in 2020, and 85-100% in 2021. This was a little surprising as researchers believe that the efficacy of pyrethroids against *H. zea* has declined in the Mid-Atlantic U.S. as it has in other regions (Fleischer et al. 2007, Jacobson et al. 2009, Owens et al. 2022). This further supports resistance monitoring efforts as the population genetics of *H. zea* is complicated and influenced by variable overwintering survival in regions such as the Mid-Atlantic U.S. (Hardwick 1965), climate change driven patterns in temperature and storm activity moving moths from southern regions northward in different patterns (Fleisher et al. 2007, Hutchison et al. 2007, Lawton et al. 2022), and changes in insecticide selection by growers.

Unlike lambda-cyhalothrin, the efficacy of pyrethrins varied among locations and years. In 2020, pyrethrins resulted in less than 50% mortality in three locations (Painter, Blackstone, and Abingdon). However, in 2021 only one location (Abingdon) had less than 50% mortality. Britt and Kuhar (2020) collected *H. zea* larvae from the same Blacksburg field site in 2019 and exposed them to pyrethrins in a similar hemp seed head-dip bioassay, which resulted in 97.5% mortality after 48h. In my experiment, *H. zea* populations from the same field-site in Blacksburg had 76% and 86% mortality from pyrethrins in 2020 and 2021, respectively.

Pyrethrins are a type I pyrethroid, while lambda-cyhalothrin is a type II. Both prolong the opening of sodium channels, however type II pyrethroids, like lambda-cyhalothrin, slow the deactivation of the sodium channels considerably more than type I (Nasuti et al. 2003). Pyrethrin is not used often in large production systems, but it is widely used in organic growing operations for *H. zea* control and provided a good comparison to the widely used synthetic, lambda-cyhalothrin.

Although the mechanism(s) of pyrethroid resistance was not a focus of our research in Virginia, previous studies have identified both target site mutations (Head et al. 1998, Soderlund 2008, Hopkins and Pietrantonio 2010a) as well as metabolic (enzymatic) processes (Sasabe et al. 2004, Yang et al. 2006) in *H. zea* populations. As previously mentioned, PBO is a neutral antagonist of G-coupled CB1 receptors, that inhibit the cytochrome P450 enzyme system (Feyereisen 1999, Dhopeshwarkar et al. 2011). In two of our *H. zea* populations (Painter and Abingdon, VA) in 2020, the addition of PBO significantly increased the efficacy of pyrethrins over pyrethrins alone, which suggests at least some metabolic resistance is involved.

PBO is reported to have no pesticidal activity of its own (Conney et al. 1972), and our data showed no significant difference in mortality between PBO alone and the water control from any of the wild-collected *H. zea* populations from any of the Virginia locations or years. However, PBO alone caused some mortality (47% and 31%) in the susceptible Plymouth strain in 2020 and 2021. This lab-reared colony of *H. zea* may not have the ability to tolerate inhibition of the cytochrome P450 enzyme system without negative health effects. PBO coupled with a switch from artificial diet (Sheikh et al. 1990) to edamame for our bioassay may have contributed to mortality.

This research showed that edamame bean-dip bioassays were a quick and effective way of monitoring insecticide efficacy in field populations of *H. zea* across Virginia providing pyrethroid efficacy information within 48 h. The bioassay revealed a potential resistant population of *H. zea* from Painter, VA in 2020 showing very low to no mortality occurring in the pyrethrin dipped treatment after 48 h. Field data supported this find with several pyrethroids lacking efficacy in a sweet corn insecticide trial conducted at the same location in 2020 (Kuhar et al. 2021). That same year, our bean-dip bioassay showed high mortality with pyrethrins on *H. zea* larvae collected from Blacksburg, VA and, in the field, all pyrethroids performed well in the insecticide trial conducted on sweet corn (Kuhar et al. 2021). Although these are only anecdotal evidence, they suggest that the bean-dip bioassay may prove useful as a quick assessment tool, not only for researchers but for growers and extension agents to assess how pyrethroids may perform on local *H. zea* larval populations.

In conclusion, this experiment showed that bean-dip insecticide bioassays can be a promising tool for monitoring insecticide resistance in insects such as *H. zea*, whose pyrethroid resistance levels do not appear to be static and rather fluctuate over time and geographical location. Unlike traditional laboratory bioassays (including the adult vial test) the bean-dip bioassay can be used to give quick feedback to researchers and growers that may be experiencing immediate issues in the field and help deploy best management practices. This study also aided our current knowledge of the susceptibility of *H. zea* to pyrethroids in Virginia and additionally provided evidence of metabolic resistance in some Virginia populations. With the inevitable increase in the overwintering range of *H. zea* and increasing insecticide resistance issues, increased surveillance will be needed. The bean-dip bioassay will be an efficient tool for this due to its ease of use by testing wild larvae from field sites across the region and testing these larvae to field rate levels of currently used pyrethroids giving quick results.

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Table 6.1. Results of mixed model analysis of variance of factors influencing mortality of CEW larvae at 48 h after being placed on dipped edamame pod sections in Virginia 2021 and 2022.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Year	1	1	1.045402	46.7480	<.0001*
Location	5	5	0.727455	6.5060	<.0001*
Treatment	5	5	17.600710	157.4128	<.0001*
Year*Location*Treatment	25	25	1.211314	2.1667	0.0018*
Year*Location	5	5	0.304544	2.7237	0.0209*
Year*Treatment	5	5	9.971716	89.1825	<.0001*
Location*Treatment	25	25	1.443327	2.5817	0.0001*

***** Denotes significance at P < 0.05

Table. 6.2. Proportion mortality (mean, n = 4) of field-collected populations of *H. zea* (2nd to 4th instar) 48h after exposure to edamame beans dipped in various insecticides with or without the synergist PBO in 2020. Data within a location (column) with a letter in common are not significantly different according to Tukey's HSD at the P < 0.05 level.

Treatment	Blacksburg	Suffolk	Painter	Blackstone	Abingdon	Plymouth
UTC	0.00 c	0.00 b	0.00 c	0.00 c	0.00 b	0.10 c
Lambda-cy	0.63 b	0.67 a	0.70 a	0.82 a	0.64 ab	0.80 a
Pyganic	0.76 ab	0.72 a	0.02 c	0.48 b	0.43 ab	0.76 a
Lambda-cy + PBO	0.71 b	0.71 a	0.61 a	0.56 ab	0.63 ab	0.80 a
Pyganic + PBO	0.89 a	0.73 a	0.31 b	0.64 ab	0.95 a	0.76 a
PBO	0.15 c	0.06 b	0.16 bc	0.00 c	0.00 b	0.47 b
P-value from ANOVA	P <.0001	P <.0001	P <.0001	P <.0001	P = 0.0226	P <.0001

Data within a location (column) with a letter in common are not significantly different according to Tukey's HSD at the P < 0.05 level.

Table. 6.3. Proportion mortality (mean, n = 4) of field-collected populations of *H. zea* (2nd to 4th instar) 48h after exposure to edamame beans dipped in various insecticides with or without the synergist PBO in 2021.

Treatment	Blacksburg	Suffolk	Painter	Blackstone	Abingdon	Plymouth
UTC	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 c
Lambda-cy	0.97 a	0.90 a	0.85 a	0.92 a	0.90 a	1.0 a
Pyrethrin	0.86 a	0.925 a	0.87 a	0.90 a	0.47 ab	0.96 a
Lambda-cy + PBO	0.94 a	1.0 a	0.95 a	0.95 a	0.72 a	1.0 a
Pyrethrin + PBO	0.70 a	0.95 a	0.90 a	0.92 a	0.48 ab	1.0 a
PBO	0.05 b	0.081 b	0.07 b	0.08 b	0 b	0.31 b
P-value from ANOVA	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0013	P < 0.0001

Data within a location (column) with a letter in common are not significantly different according to Tukey's HSD at the P < 0.05 level.

Figures

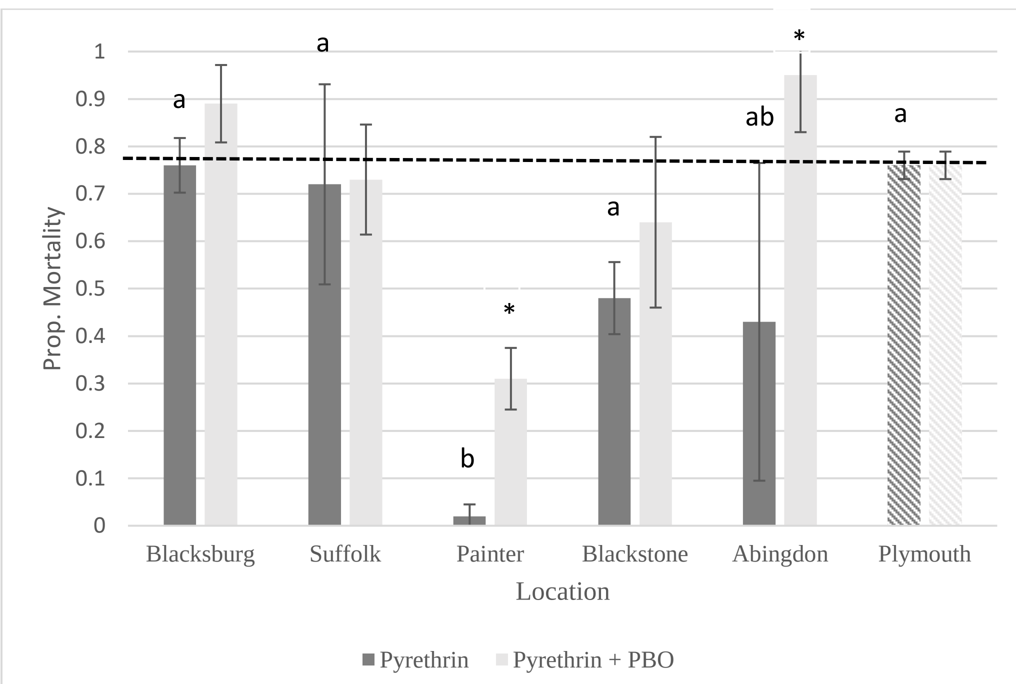

Fig. 6.1 Proportion mortality (mean ± SE) of field-collected populations of *H. zea* (2nd to 4th instar) 48h after exposure to edamame beans dipped in pyrethrins with or without PBO in 2020. Dashed line represents a pyrethroid-susceptible colony (Plymouth) for comparison. Bars with a letter in common are not significantly different according to Tukey's HSD at the P < 0.05 level.

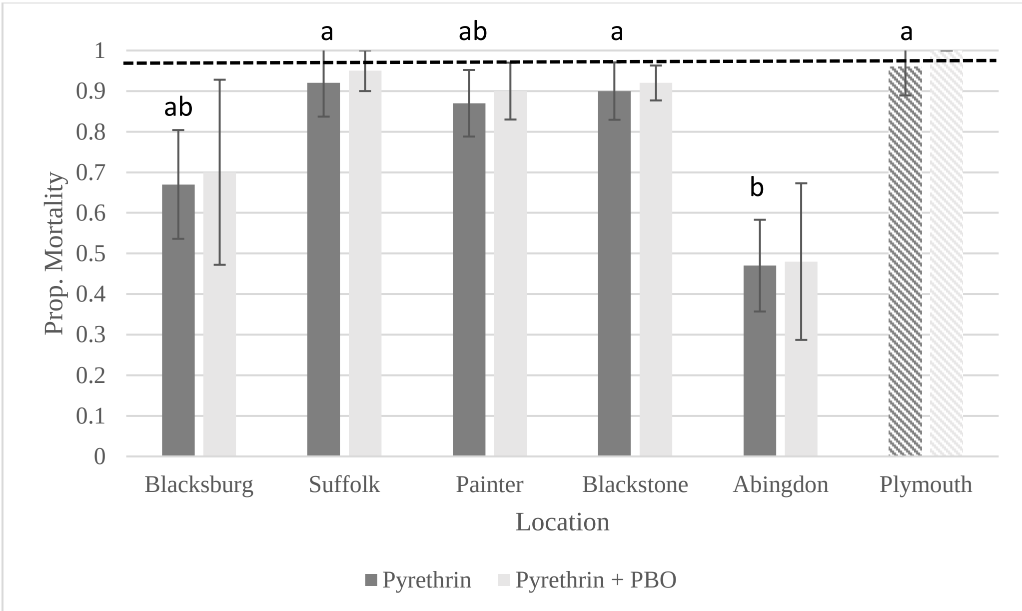

Fig. 6.2 Proportion mortality (mean ± SE) of field-collected populations of *H. zea* (2nd to 4th instar) 48h after exposure to edamame beans dipped in pyrethrins with or without PBO in 2021. Dashed line represents a pyrethroid-susceptible colony (Plymouth) for comparison. Bars with a letter in common are not significantly different according to Tukey's HSD at the P < 0.05 level.

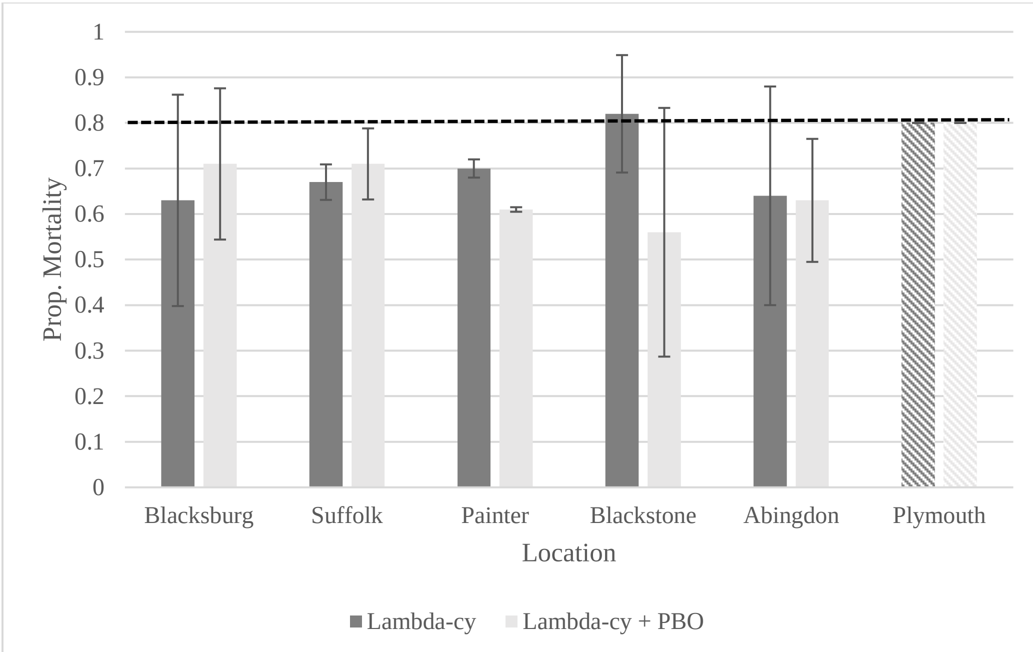

Fig. 6.3 Proportion mortality (mean ± SE) of field-collected populations of *H. zea* (2nd to 4th instar) 48h after exposure to edamame beans dipped in lambda-cyhalothrin with or without PBO in 2020. Dashed line represents a pyrethroid-susceptible colony (Plymouth) for comparison. There was no significant difference among locations or from the addition of PBO in mortality according to ANOVA, P > 0.05.

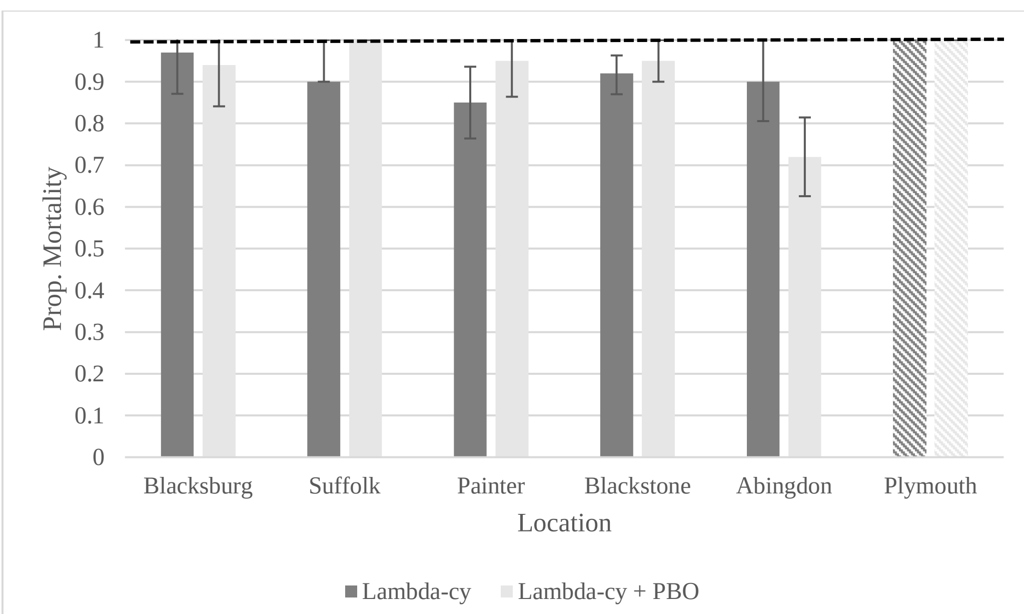

Fig. 6.4 Proportion mortality (mean ± SE) of field-collected populations of *H. zea* (2nd to 4th instar) 48h after exposure to edamame beans dipped in lambda-cyhalothrin with or without PBO in 2021. Dashed line represents a pyrethroid-susceptible colony (Plymouth) for comparison. There was no significant difference among locations or from the addition of PBO in mortality according to ANOVA, P > 0.05.

Chapter 7: Overall Conclusion

As edamame is a relatively new commodity, my research represents some of the first efforts to document insect pest and disease impacts as well as evaluate sound pest management strategies for two issues affecting the crop in the mid-Atlantic U.S. Specific outputs of this project included: 1) an assessment of edamame varieties most suitable for Virginia growing conditions based on insect and disease pressure; 2) a suggestion for a pest management program for edamame in Virginia based on economically important insects; 3) recommended insecticides for controlling economically important pests, especially stink bugs; 4) recommended fungicides for control of diseases; and 5) an efficient bioassay to monitor insecticide resistance in corn earworm populations.

At the beginning of the edamame research grant at Virginia Tech, most of the edamame varieties had some level of genetics from China. It was essential to understand the key pests and diseases of edamame to aid in developing varieties most suitable for Virginias growing conditions. Not only was evaluating edamame varieties useful for the plant breeders in the project, but it was useful by furthering research in this project by giving us insight into the key pests and diseases of this crop that would aid in steps toward a pest management program for edamame in the mid-Atlantic. Surprisingly corn earworm was a rare pest of edamame and we ultimately found pod feeding pests like stink bugs to be the most concerning due to the blemishes left behind on the pods by their feeding. Stink bug impacts on edamame were significant that field spray trials and laboratory bioassays were needed to evaluate different insecticides for their control. We found several insecticides that showed promising efficacy against stink bug feeding that included cyclaniliprole, sulfoxaflor, and bifenthrin. These trials aided in edamame being included in the mid-Atlantic pest management guide in 2022. However, more research needs to occur with timing and frequency of spraying on edamame in Virginia due to the increasing presence of southern green stink bug densities in the latter part of the growing season.

Fungicide field trials were also conducted that led to a lot of variability in the results for efficacy of tested compounds. However, the frequency of fungicide application and the use of multiple modes of actions will be necessary after flowering (R1 growth stage) as we found that in most field seasons, except for 2022 due to low incidence, that fungicide application at R1 or a fungicide application at R1 and R3 were not nearly enough to prevent fungal or bacterial growth on the edamame pods. It will also be important to further research the use of fungicides at planting as current edamame emergence levels are poor due to the lack of commercially available seed with fungicide treatments.

Despite corn earworm not being a major pest of edamame during 2018-2021 experiments, it remains the most impactful pest in Virginia of other crops. It is unlikely that corn earworm will remain absent as a major pest of edamame production in Virginia as acreage continues to increase for this crop. The bean dip bioassays were found to be a quick and efficient way to test individual location across the state for pyrethroid resistance. Within a week of collection, the experiment was run, and the results were able to be given back to growers or researchers. The bean dip bioassay methodology will be a useful tool to assess the ever-changing efficacy and resistance of corn earworm in Virginia. The efficacy of pyrethrin were notably variable between years and locations and metabolic resistance was documented in two locations in 2020. Lambda-cyhalothrin did not have resistance in either of the years, but efficacy levels across the state did change from 2020 to 2021. As climate change impacts the corn earworms capability of over wintering further north each year, the bean dip bioassay methodology will be a useful monitoring tool.

As edamame production increases across the state of Virginia and the mid-Atlantic, the research questions addressed here have created a solid foundation for pest management strategies for this crop. However, there are more targeted questions that were originated by each of these five objectives that will lead to strengthening pest management strategies of this crops as future research to establish edamame in Virginia and the mid-Atlantic continues.