SCBGP PROJECT PROFILE TEMPLATE

AWARD YEARS 2022 FORWARD

The State Plan should include a series of project profiles that detail the necessary information to fulfill the goals and objectives of each project. The acceptable font size for the narrative is 11 or 12 pitch with all margins at 1 inch. The following information must be included in each project profile.

PROJECT TITLE

Provide a descriptive project title in 15 words or less in the space below.

Insect-parasitic nematodes as a sustainable management tool for the Asiatic garden beetle in mint

DURATION OF PROJECT

Start Date: 9/30/2022 **End Date**: 9/29/2024

PROJECT PARTNER AND SUMMARY

Include a project summary of 250 words or less suitable for dissemination to the public. A Project Summary provides a very brief (one sentence, if possible) description of your project. A Project Summary includes:

1. *The name of the applicant organization that if awarded a grant will establish an agreement or contractual relationship with the State Department of Agriculture to lead and execute the project,*
2. *The project's purpose, deliverables, and expected outcomes and*
3. *A description of the general tasks/activities to be completed during the project period to fulfill this goal.*

FOR EXAMPLE:
The ABC University will mitigate the spread of citrus greening (Huanglongbing) by developing scientifically-based practical measures to implement in a quarantine area and disseminating results to stakeholders through grower meetings and field days.

The Asiatic garden beetle (Maladera castanea), hereafter AGB, is a small beetle that attacks more than 100 different plant species, including ornamentals, weedy species, and crop plants. Adult beetles feed on flowers and foliage, but the most severe damage is inflicted by the larval (white grub) stage, which feeds underground on plant roots for nearly 10 months of the year. Feeding damage can lead to water stress, reductions in crop growth and yield, and even plant death. Indiana mint growers have suffered significant losses to their perennial mint crop due to AGB grub damage, making this pest a serious threat to the Indiana mint industry. Limited knowledge of the success and sustainability of chemical and biological control strategies for AGB grubs in mint and potential influence of soil type on the outcome of these strategies for AGB grubs pose key decision-making challenges for mint growers that are battling this insect. Purdue University entomologists will partner with commercial mint producers in the state to grow our knowledge of this pest and its management in mint systems. The goals of this project are to: 1) evaluate the efficacy and persistence of insect-parasitic nematodes against AGB grubs in commercial mint fields, and 2) evaluate the influence of soil type and insecticide treatment on the success of insect-parasitic nematode suppression of AGB in mint using laboratory and greenhouse trials. Upon completion, these objectives will contribute to our understanding of sustainable management practices for AGB in Indiana mint.

PROJECT PURPOSE

PROVIDE THE SPECIFIC ISSUE, PROBLEM OR NEED THAT THE PROJECT WILL ADDRESS

The United States produces more than 70% of the world's supply of mint (AgHires 2017) and Indiana ranks 3rd and 4th in the nation for spearmint and peppermint oil production, respectively (USDA-NASS Census 2017). In 2021, 286,000 pounds of peppermint oil and 223,000 pounds of spearmint oil were produced in Indiana alone, placing the value of mint oil production in Indiana at ~$6.5 million (USDA-NASS Quick Stats 2021). Although mint acreage in the Midwest is not that of the Pacific Northwest states (Oregon, Washington, Idaho, and California), this high-quality oil is a valuable product that contributes significantly to the state's economy. Indiana mint growers face increasing pressure from the industry to produce mint oil more sustainably and at a price that is competitive with overseas producers. More specifically, large companies like Wrigley's and Colgate seek contracts with growers that are producing mint oil more sustainably, so they can market their own products as more sustainable and environmentally friendly.

Although Indiana mint producers are responding to this call, they are struggling with a serious insect pest that is currently only a problem in Indiana mint production: the invasive Asiatic garden beetle (AGB), whose soil-dwelling larvae feed underground on mint roots for nearly 10 months of the year. Yield losses caused by AGB grubs and costs incurred trying to manage it with little to no success (see attached letters from growers), place Indiana producers at a significant disadvantage as they seek to deliver high-quality mint oil more sustainably. More research is needed to identify the factors that predict AGB infestation and most importantly, which integrated pest management (IPM) strategies actually work to reduce crop injury when infestations of this pest occur. We now know from results of our current ISDA grant (#A337-21-SCBG-20-102), that when AGB grubs reach densities of 5 grubs/ft2 or higher, mint plants suffer enough root damage to reduce harvestable above-ground (mint foliage) growth and productivity. This preliminary damage threshold provides us with a target to aim for as we investigate IPM strategies to reduce mint damage by this pest.

Managing AGB sustainably requires that we identify both short and long-term strategies that will keep AGB grub densities below damaging levels for as long as possible in the 4 to 5-year life span of the typical commercial mint field. With the collaboration of colleagues at Cornell University, we propose a management strategy that incorporates a multi-species complex of insect-parasitic nematodes (IPNs) that have been carefully selected and reared specifically for their long-term persistence in the field as biological control agents against soil-dwelling insects. Our approach directly addresses the needs of growers and the industry to combat AGB damage, improve the longevity of control, and thereby support more sustainable and economically viable mint oil production. Key questions growers have about the use of insect-parasitic nematodes as a management strategy against AGB in mint fields include: "How effective are insect-parasitic nematodes against white grubs, like AGB?" "How long are these insect-parasitic nematodes effective against AGB grubs?" Does the use of insecticides influence the success of insect-parasitic nematodes against AGB grubs?" and "Can insect-parasitic nematodes be applied with equipment currently used to apply pesticides?" This proposal addresses key aspects of each of these grower concerns and supports extension programing to disseminate what we learn.

The culprit
AGB was first detected in New Jersey in 1921, but is now present throughout the north, southeast, and Midwest (Capinera 2001). Although this insect is perhaps best known as a turfgrass pest, adults feed on more than 100 different crop, ornamental, and weedy plant species (Capinera 2001). While adult beetles feed on foliage and flowers, major damage is caused by the larval stage (white grubs) as they feed on plant roots, causing water stress, reductions in re-growth, and in severe infestations, death of the crop. Damage typically occurs only when large numbers of grubs are present, or when crop plants are young. We can now say with a reasonable level of confidence that significant declines in mint production are likely to occur when AGB larval densities meet or exceed approximately 5/ft2 (Table 1).

Adults are 0.3 to 0.5-inch long, chestnut-brown beetles that actively fly at night, July through September, when temperatures are 70° F or higher (Capinera 2001). Egg masses are commonly laid mid-June to August, 1-2 inches

beneath the soil surface and may contain up to 20 eggs. After roughly 10 days, eggs hatch and the characteristic 'C-shaped' white grubs develop through three instars (larval phases), each larger and more ravenous than the last. The most vulnerable first instar grubs are usually present by mid-July, and by September these grubs have progressed to the largest, third instar grub phase, which overwinter 6-12 inches belowground. These same grubs become active again the following spring, pupating by June and emerging as new adults soon thereafter to start the cycle again. The grub stage lasts for nearly 10 months, actively feeding on plant roots and decaying organic matter within the top 2-3 inches of the soil column during late-spring, summer and fall (Figure 1).

Effective and Sustainable AGB management Strategies

To date, published research in a variety of systems (Brandenburg and Royals 1999; Manion et al., 2000; Pujari et al., 2017), has demonstrated that certain insecticide classes, like neonicotinoids and diamides, have strong potential against the tenacious AGB grubs. We now know from our own greenhouse studies that some of these same insecticide classes also have an impact on AGB grubs in mint, particularly their feeding behavior. When we infested potted mint plants with AGB grubs and drenched the pots with either chlorantraniliprole (diamide, labelled for mint), or imidacloprid (neonicotinoid, not currently labelled for mint) we found no difference in AGB mortality between untreated and treated mint plants over the short term. However, the root masses of mint plants treated with the diamide or neonicotinoid were 21% and 29% greater than roots of untreated mint, respectively (Figure 2). As promising as these results are, insecticides alone are unlikely to be a sustainable or economically viable tool for AGB management. However, a management strategy that successfully integrates natural enemies, like insect-parasitic nematodes (IPNs), with insecticides at strategic times (based on crop production or crop phenology) has a much greater potential to be sustainable and economically viable over the near- and long-term.

IPNs, also known as entomopathogenic nematodes, are tiny beneficial roundworms that only attack and consume insects. These nematodes enter the insect through natural openings in the body and once inside, they release bacteria that eventually kill the insect, turning it into a "soup-filled" cadaver. The nematodes feed within the cadaver on the insect nutrients made available by the bacteria, they mate and reproduce, and eventually spill out of the dead insect by the thousands to find new insect hosts in the soil (Figure 3) Insect-parasitic nematodes can be purchased from a variety of commercial vendors and can be very effective against white grubs (Potter and Braman 1991). We found in our own greenhouse studies with potted-mint plants that the IPN species Heterorhabditis bacteriophora reduced AGB grub survival by 50%, while increasing mint yield by 24% and mint root mass by 18% compared to untreated mint over only a two-week period (Figure 4). Additionally, published research has demonstrated that white grubs exposed to neonicotinoid and diamde insecticides are more vulnerable to attack by IPNs, such that combined application of IPNs and insecticides kills white grubs better than applications of only IPNs or insecticides alone (Koppenhöfer et al. 2000; Koppenhöfer and Fuzy 2008).

Taken together, these results suggest significant potential for IPNs as control agents for AGB grubs; however, one of the key obstacles to working with IPNs is that strains purchased from most commercial vendors are not adapted to local climates. They have been genetically selected (unintentionally), through the mass-rearing process, to infect insect hosts quickly and emerge quickly, with detrimental impacts on their ability to persist under field conditions. As such they display little to no "residual period of infectivity." As a result, these commercial strains of IPNs must be applied repeatedly, year after year, because they are not adapted to survive or persist. Mal-adapted IPNs and the upfront costs of repeated applications of IPNs at large scales have made these beneficial predators an unacceptable pest management approach in most crops…until recently.

Research published by our colleague at Cornell University has demonstrated that IPNs sourced from the local environment and are not forced through the genetic bottle neck associated with commercial scale mass rearing. A such, they are better adapted and persist in the field for 3-4 years (much longer than commercial IPN strains!). These persistent nematodes are effective against a suite of soil-dwelling insect pests including white grubs, corn rootworm, alfalfa snout beetle, and black vine weevil (Shields and Testa 2020, Shields and Testa 2021) — some of which are also important pest during other portions of the crop rotation typically practiced by Indiana growers. Furthermore, these persistent strains of nematodes include several different IPN species that complement each other to increase attack and infection of soil-dwelling insects. Dr. Shields has agreed to collaborate with us (see letter of support) and share the unique, persistent-IPN strains his team has developed to help us address the serious AGB problem in mint more sustainably. We propose to build upon our current ISDA-funded grant (#A337-21-SCBG-20-102) and standing

collaborations with Indiana mint growers to evaluate the efficacy and longevity of persistent strains of IPNs and insecticides against AGB grubs in field and greenhouse studies. We will focus specifically on determining if persistent IPNs and insecticides, alone or together, can keep AGB grub densities below our preliminary economic threshold (5 grubs per square foot) in soil types where mint is grown and AGB is consistently a problem.

The mint production system

In Indiana, growers establish new perennial mint fields by planting stolons (underground cuttings from parent plants) in rows in the field from mid-March to late April. These first year fields are referred to as "row" mint or "baby" mint. As plants grow and establish in the second year, the field transforms into dense groups of plants commonly referred to as "meadow" mint. Mature mint is harvested once or twice each year between July and September by mowing foliage and leaving it to dry in the field, similar to hay. After drying, the leaves are collected into tubs and the valuable oil is extracted by steam distillation. Following harvest, growers typically apply pre-emergent herbicides to the soil to manage weeds the following spring. Subsequently, in late September or early October, mint plants may be plowed under to provide winter protection, though not all growers follow this practice. In Indiana, mint is typically rotated with corn and soybean on a 3 to 5-year cycle. AGB has been reported as a significant pest in young corn fields, particularly in areas with sandy soils (Tiwari et al. 2019, Krupke and Obermeyer 2018). Given that mint is commonly rotated with corn in our region, there is a high likelihood that white grubs will occur in fields destined for mint within the 3-5 year rotation schedule. Continued pressure from AGB grubs pose great risk to the economic viability of mint production, because growers make high investments to plant and establish mint crops upfront, while economic gains may not be realized for 3 or 4 years.

Objectives

Although there are several questions to address in the AGB-mint system, we propose to focus this proposal on grower-identified needs to address industry demand for more sustainable production of high-quality mint oil. Our specific objectives are to: 1) evaluate the efficacy and longevity of persistent strains of IPNs against AGB grubs in commercial mint fields, 2) evaluate the influence of soil type and insecticide treatment on the success of insect-parasitic nematode suppression of AGB in mint using laboratory and greenhouse trials, and 3) continued development of a self-sufficient extension program for AGB management in Indiana mint. By completing these objectives, we will provide mint growers with the knowledge needed to determine: A) if insect-parasitic nematodes are an effective approach for keeping AGB densities below damaging levels, B) how long protection by insect-parasitic nematodes may last against AGB grubs, and C) whether factors like soil type and application of insecticides may also impact the success and longevity (persistence) of insect-parasitic nematodes against AGB in mint.

Methodology

Objective 1: Evaluating the efficacy and longevity of persistent strains of insect-parasitic nematodes against AGB grubs in mint

Insect-parasitic nematodes (IPN's) may provide a long-term, sustainable solution to the AGB problem. Ongoing work in our laboratories demonstrates that the IPN Heterorhabditis bacteriophora (Hb) can reduce AGB larval survival by 50% while increasing mint yield by 24%, and mint root mass by 18% compared to untreated controls over a two-week period.

We propose a 2-year study wherein we will evaluate the ability of IPNs to reduce populations of damaging white grubs (Asiatic garden beetle, Japanese beetle and masked chafer) in commercial mint fields, as well as the persistence of the IPNs themselves over time. To characterize white grub densities and mint yield, we will use a field-based, yield harvest and soil-excavation approach we've developed using current ISDA support (Figure 5), whereas IPM persistence will be monitored using a well-known and effective soil baiting technique commonly used to assess IPM populations (Ferguson et al., 1995). We will provide a stipend for growers each year and work closely with them to track crop rotation history, crop age (1st year or older mint), and soil type, in order to account for sources of variation outside of our ability to manipulate experimentally. Based on infestation history and preliminary sampling, we will identify a pair of 1 acre plots in each of 4 fields (n=8), and work with growers to make a single application of IPNs to one plot in each pair shortly after summer harvest (July-August). This timing also coincides with the appearance of young white grubs in the soil, providing a readily available source of hosts for the nematodes.

White grub populations and mint yields in each of the 8 plots will be assessed during the fall (September-October) of each year. However, IPM persistence will be monitored by determining nematode populations prior to application and every three months during the growing season for the duration of the project (May, July, September). This approach will provide a series of 4 comparisons, paired by soil type, for analysis.

Assessing White Grub Populations and Mint Yield

Grub and mint samples will be collected in a grid pattern from each plot using an approach modified from Dalthorp et al. (2000) and optimized based on our experience over the last two seasons. The sampling grid will consist of a series of 32, 342 ft2 cells (18.5 × 18.5 ft) arranged to conform to the topographical constraints of the plot. The exact location of the four corners of each grid will be assigned spatial coordinates with a GPS receiver using waypoint averaging to increase the spatial precision of subsequent sampling.

One, 2.7 ft2 quadrat fashioned from ¾" PVC pipe will be placed in the center of 15, randomly selected cells within each grid, and all living mint stems and leaves will be cut at the soil surface using a pair of electric hand shears. Above-ground plant material will be collected, placed into labeled plastic bags, and transported inside a cooler to the lab where it will be weighed as a measure of yield.

Within the same 2.7 ft2 quadrats, all soil will be excavated to a depth of 4". Each 0.8 ft3 mass of soil will be broken apart and sieved through a 0.25" hardware cloth mesh to collect the grubs. The number, species, and instar of all white grubs will be recorded and the number of white grubs within each sample will be used to estimate mean grub density within each 32 cell plot. Additionally, we will calculate the proportion of cells within each grid containing white grub densities at or above the economic injury level that we have now established (5 grubs/ft2) as another way to compare nematode treated plots to untreated controls.

Assessing Nematode Persistence

IPN populations will be monitored over time using the same 15 cells selected for white grub and mint yield measurements. Three 0.75" soil cores (6" depth) will be taken at random from within each cell, placed into labeled plastic bags and immediately placed in a cooler. Once at the lab, samples will be refrigerated overnight. The next day, soil samples will be broken apart by hand and 10 waxworm larvae (Galleria mellonella) will be placed inside each bag in contact with the soil (Koppenhöfer et al. 1998). Bags will be sealed and held in the dark at room temperature for 2 days. At that time, dead waxworm larvae will be removed from the bags and placed on White's traps to confirm nematode infection as the cause of death (White 1927). Dead larvae will immediately be replaced by adding fresh larvae to the bags. This process will be repeated a total of two times for each soil sample or until no further infection is indicated. A subset of nematode infected individuals will be dissected and adult nematodes will be identified to species level microscopically. The total number of infected waxworm larvae will be used as an indirect measure of nematode population density.

Objective 2: Evaluating the influence of soil type and insecticide application on the success of insect-parasitic nematode suppression of AGB in mint

Previous research evaluating insecticides and insect-parasitic nematodes as management tools has shown that soil type (Koppenhöfer and Fuzy 2006) and insecticides (Koppenhöfer and Fuzy 2008; Koppenhöfer et al. 2000) can influence the efficacy of IPNs against soil-dwelling insects. Insect-parasitic nematodes are most effective against white grubs like AGB in coarser soil types, which allow the nematodes to move through the soil easily to search for and locate AGB prey. Insecticides, like the neonicotinoids and diamides have been shown to intoxicate or paralyze white grubs and thereby indirectly increase the success of IPNs because grubs are unable to escape or groom themselves (Koppenhöfer et al. 2000), which is a key defense against IPNs. Thus, factors like soil type and use of insecticides and IPNs (alone or together) may have implications for successful suppression of AGB grubs by persistent strains of IPNs.

We will evaluate AGB grub management in three soil types that are common across commercial mint fields in Indiana using a factorial design that includes the following treatments: 1) an application of IPNs alone, 2) insecticides alone, 3) IPNs + insecticides together, and 4) untreated soils. We will test sand, loamy sand, and silty-clay loam, using the same soil-applied, systemic insecticides that protect mint roots from AGB feeding based on our preliminary findings: diamides and neonicotinoids. These studies will be conducted in the greenhouse or laboratory over a 72-hour period

in either potted mint plants or 16-well trays so that we can isolate the independent and combined effects of soil type, IPN treatment, and insecticide treatment on AGB grub mortality.

Variation in the number of live AGB grubs found in each treatment will be examined using factorial analysis of variance (ANOVA) and treatment means will be compared to untreated controls using Fisher's least significant difference (LSD) test. This approach will allow us to determine the efficacy of insect-parasitic nematodes against AGB grubs in each soil type when they are applied alone, or in combination with soil insecticides.

Objective 3: Building out a self-sufficient extension program for AGB management in Indiana mint

We will build upon our current extension program for AGB best management practices in commercial mint by adding results and recommendations from the 2-year study we propose here. We will deliver information in three forms: (1) extension factsheets, which will include information on the life cycle and biology of insect-parasitic nematodes, how to apply them, and their management potential for AGB in mint, with or without the use of insecticides, (2) extension videos, which will include step-by-step demonstrations of nematode application and the appearance of AGB grubs that are infected with IPNs, and (3) on-site programming at annual Midwest Mint Meetings in Fair Oaks, Indiana. Factsheets will be printed in color and made available to stakeholders through Purdue County Extension Offices and at the Annual Midwest Mint Conference held in Fair Oaks, IN each year. Growers that are not able to access hard copies of these resources will have free online access to factsheets in traditional and mobile-friendly formats through Purdue's Extension Entomology website (https://extension.entm.purdue.edu/), Purdue's Extension Education Store (https://edustore.purdue.edu/), and Dr. Long's research program website (https://extension.entm.purdue.edu/longlab/). All extension videos will be professionally filmed and edited using departmental video equipment and resources available to the research team. All videos will be made available to stakeholders via a link to Purdue Extension Entomology's YouTube channel (https://www.youtube.com/user/PurdueExtensionEntm/videos). These extension materials will provide crucial support for mint growers aiming to make informed and sustainable decisions about AGB management. Programming at the annual Midwest Mint meeting will provide a venue for face-to-face delivery of best management practices coming from this research, as well as an opportunity to directly assess its impact.

PROVIDE A LISTING OF THE OBJECTIVES THAT THIS PROJECT HOPES TO ACHIEVE

Add more objectives by copying and pasting the existing listing or delete objectives that aren't necessary.

Objective 1	1) Evaluate the efficacy and longevity of unique, persistent strains of insect-parasitic nematodes against AGB grubs in commercial mint fields
Objective 2	2) Evaluate the influence of soil type and insecticide treatment on the success of AGB suppression by persistent strains of insect-parasitic nematodes in mint using laboratory and greenhouse trials
Objective 3	3) Continue development of a self-sufficient extension program for AGB management in commercial mint

PROJECT BENEFICIARIES

Estimate the number of project beneficiaries: 55

Does this project directly benefit socially disadvantaged farmers and/or underserved communities as defined in the RFA? **Yes** ☐ **No** ☑

If you selected yes, please describe how the project directly benefits socially disadvantaged farmers and/or underserved communities.

Does this project directly benefit beginning farmers as defined in the RFA? **Yes** ☐ **No** ☑

If you selected yes, please describe how the project directly benefits beginning farmers.

Does this project directly benefit veteran farmers as defined in the RFA? **Yes** ☐ **No** ☑

If you selected yes, please describe how the project directly benefits veteran farmers.

STATEMENT OF ENHANCING SPECIALTY CROPS

By checking the box to the right, I confirm that this project enhances the competitiveness of specialty crops in accordance with and defined by the Farm Bill. Further information regarding the definition of a specialty crop can be found at www.ams.usda.gov/services/grants/scbgp. ☑

List of Specialty Crops: peppermint oil, spearmint oil, spearmint herbs

CONTINUATION PROJECT INFORMATION

Does this project continue the efforts of a previously funded SCBGP project? **Yes** ☑ **No** ☐

If you have selected "yes", please address the following:

DESCRIBE HOW THIS PROJECT WILL DIFFER FROM AND BUILD ON THE PREVIOUS EFFORTS

Ongoing ISDA-funded work has allowed us to develop accurate and sensitive sampling methods to characterize and quantify relationships between AGB infestation levels and mint plant performance. As a result, we have zeroed in on a viable economic injury level - a critical AGB infestation level that results in measurable declines in the health and productivity of mint plants under field conditions. We have also identified the ability of certain insecticides and one species of insect-parasitic nematode to mitigate AGB damage.

This project will build on our on-going efforts by allowing us to test whether unique, multi-species strains of persistent insect-parasitic nematodes, both alone and in combination with insecticides, can improve AGB grub management over the long term, specifically by keeping their densities below the damage threshold we've identified as 5 grubs per square foot.

PROVIDE A SUMMARY (3 TO 5 SENTENCES) OF THE OUTCOMES OF THE PREVIOUS EFFORTS

To date, our previous efforts have yielded the following outcomes so far: 1) increased grower awareness of and ability to identify AGB grubs when found in the soil, 2) provided growers with a preliminary economic threshold for AGB grubs in commercial mint fields (a number of grubs per square foot that, once reached, causes declines in mint productivity and health), and 3) increased grower knowledge of sampling strategies that can be used to detect AGB grubs in mint fields. Taken together, these efforts have provided Indiana mint growers and stakeholders with new information that will enable them to make informed decisions about how to detect, identify, and manage AGB grubs in commercial mint.

PROVIDE LESSONS LEARNED ON POTENTIAL PROJECT IMPROVEMENTS

What was previously learned from implementing this project, including potential improvements?

We have learned that AGB grubs are the dominant white grub species found in Indiana mint fields; however, they are not the only white grubs feeding on mint roots: Japanese beetle grubs and masked chafer grubs are also present in mint fields. We've also learned that white grub densities are much higher in the fall than in the spring, which suggests that populations grow throughout the season, with increasing potential to cause feeding damage to mint roots. Thus, it may be important to take action as soon as AGB adults lay eggs in mint fields, at the end of June and beginning of July. Our evaluation of AGB grub sampling methods in mint fields over the last year suggest that rigorous, but random sampling for grubs in mint field provides the best estimates of AGB grub densities and last, but not least, we see a decrease in mint plant performance when AGB grub densities reach 5 grubs per square foot.

How are the lessons learned and improvements being incorporated into the project to make the ongoing project more effective and successful at meeting goals and outcomes?

We are incorporating new knowledge gained from our on-going project (knowledge of natural enemy and insecticide efficacy against AGB grubs, preliminary economic threshold of 5 AGB grubs/square foot, and optimized AGB sampling methods in mint fields) to fine-tune and improve our approach to answer grower's questions about how effective these AGB management strategies might be against AGB grubs over time when locally-adapted, persistent strains of insect-parasitic nematodes are applied post harvest, both with and without insecticides, to different soil types where mint is grown.

DESCRIBE THE LIKELIHOOD OF THE PROJECT BECOMING SELF-SUSTAINING AND NOT INDEFINITELY DEPENDENT ON GRANT FUNDS

This project has strong potential to be self-sustaining and independent of grant funds. The objectives we have listed are focused on developing research-based recommendations that will help mint growers make informed decisions about management strategies for AGB grubs, now and in the future. Without initial funding to complete this project, growers would need to invest in management strategies without knowing the likelihood or longevity of success, which is risky, especially in high-value specialty crops like mint. Once the objectives of this project are completed and the results are made available via Purdue University Extension, mint growers and stakeholders will have concrete knowledge of strategies that improve AGB grub management, as well as access to tools and information that will help them manage this serious mint pest.

OTHER SUPPORT FROM FEDERAL OR STATE GRANT PROGRAMS

The SCBGP will not fund duplicative projects. Did you submit this project to a Federal or State grant program other than the SCBGP for funding and/or is a Federal or State grant program other than the SCBGP funding the project currently?

Yes ☐ **No** ☑

IF YOUR PROJECT IS RECEIVING OR WILL POTENTIALLY RECEIVE FUNDS FROM ANOTHER FEDERAL OR STATE GRANT PROGRAM

Identify the Federal or State grant program(s).

Describe how the SCBGP project differs from or supplements the other grant program(s) efforts.

EXTERNAL PROJECT SUPPORT

Describe the specialty crop stakeholders who support this project and why (other than the applicant and organizations involved in the project).

Letters of support for this research and extension proposal are included from Kanne Farms, Wappel Farms, and Gumz Farms, the latter also representing the Indiana Mint Market and Development Research Council. These stakeholders support this project because AGB has emerged as a consistent and serious pest of perennial mint fields in Indiana, particularly fields with sandy or sandy loam soil qualities. Over the last several years, this insect has caused significant yield losses and contributed to the early termination of mint fields.
These stakeholder support this project proposal because there is limited information about the efficacy of insecticides and natural enemies, like insect-parasitic nematodes, against white grubs like AGB, and there is essentially no information available about the long-term sustainability of these strategies against AGB grubs in mint production, making it an exceptionally challenging pest to manage in this system. As a result, this insect will continue to pose a serious threat to Indiana's commercial mint industry if no efforts are made to evaluate and develop sustainable integrated pest management strategies.

EXPECTED MEASURABLE OUTCOMES

SELECT THE APPROPRIATE OUTCOME(S) AND INDICATOR(S)/SUB-INDICATOR(S)

You must choose at least one of the eight outcomes listed in the SCBGP Performance Measures, which were approved by the Office of Management and Budget (OMB) to evaluate the performance of the SCBGP on a national level.

OUTCOME MEASURE(S)

Select the outcome measure(s) that are applicable for this project from the listing below.

- ☐ **Outcome 1:** Increasing Consumption and Consumer Purchasing of Specialty Crops
- ☐ **Outcome 2**: Increasing Access to Specialty Crops and Expanding Specialty Crop Production and Distribution
- ☐ **Outcome 3**: Increase Food Safety Knowledge and Processes
- ☑ **Outcome 4**: Improve Pest and Disease Control Processes
- ☐ **Outcome 5**: Develop New Seed Varieties and Specialty Crops
- ☐ **Outcome 6**: Expand Specialty Crop Research and Development
- ☑ **Outcome 7**: Improve Environmental Sustainability of Specialty Crops

OUTCOME INDICATOR(S)

Provide at least one indicator listed in the SCBGP Performance Measures and the related quantifiable result. If you have multiple outcomes and/or indicators, repeat this for each outcome/indicator.

FOR EXAMPLE:
Outcome 1, Indicator 1.1a
Total number of consumers who gained knowledge about specialty crops, Adults 132.

Outcome 4, Indicator 1
Number of stakeholders that gained knowledge about science-based tools to combat pests and diseases [50].

Outcome 4, Indicator 2
Number of stakeholders that adopted pest and disease control best practices, technologies, or innovations [15].

Outcome 4, Indicator 5

Total number of producers/processors that enhanced or maintained pest and disease control practices [15]. Of those, the number that reported: a. Reduction in product lost to pest and diseases [15]. b. Improved crop quality [15]. c. Reduction in labor costs [15]. d. Reduction in pesticide use [15].

Outcome 7, Indicator 1
Number of stakeholders that gained knowledge about environmental sustainability best practices, tools, or technologies [50].

Outcome 7, Indicator 2
Number of stakeholders reported with an intent to adopt environmental sustainability best practices, tools, or technologies [15].

Outcome 7, Indicator 4
Number of new tools/technologies developed or enhanced to improve sustainability/ conservation or other environmental outcomes [1].

MISCELLANEOUS OUTCOME MEASURE

In the unlikely event that the outcomes and indicators above the selected outcomes are not relevant to your project, you must develop a project-specific outcome(s) and indicator(s) which will be subject to approval by AMS.

DATA COLLECTION TO REPORT ON OUTCOMES AND INDICATORS

Explain how you will collect the required data to report on the outcome and indicator in the space below.

We will conduct field, laboratory, and greenhouse trials to evaluate the efficacy and persistence of insect-parasitic nematodes, soil insecticides, and both of these strategies together against AGB and report supporting data at the end of each project period. Six months after the study's completion, we will invite growers to participate in an online Qualtrics survey to determine their perceptions of the utility and success of these pest management stragies against AGB grubs in mint. Field-based research activties (Objective 1) and laboratory/greenhouse-based research activties (Objective 2) will be conducted in Years 1 and 2 of the project period to evaluate the success of persistent insect-parasitic nematode strains, soil insecticides, and both strategies together when applied against AGB grubs in mint. This will provide novel information to mint growers and allow them to make more informed decisions about sustainable AGB grub management in mint.

We will conduct field trials to evaluate the sustainability of insect-parasitic nematodes against AGB grub populations and report supporting data at the end of each project period. Six months after the study's completion, we will invite growers to participate in an online Qualtrics survey to determine their perceptions of the sustainability of these pest management stragies against AGB grubs in mint. Field-based research activties (Objective 1) will be conducted in Years 1 and 2 of the project period to evaluate the success and longevity of persistent insect-parasitic nematode strains when applied against AGB grubs in mint. This will provide novel information to mint growers and allow them to make more informed decisions about insect-parasitic nematodes as a sustainable AGB grub management strategy in mint.

BUDGET NARRATIVE

All expenses described in this Budget Narrative must be associated with expenses that will be covered by the SCBGP. If any matching funds will be used and a description of their use is required by the State department of agriculture, the expenses to be covered with matching funds must be described separately. Applicants should review the Request for Applications section 4.7 Funding Restrictions prior to developing their budget narrative.

BUDGET SUMMARY

Expense Category	Funds Requested
Personnel	$81,952.00
Fringe Benefits	$7,860.00
Travel	$3,582.00
Equipment	$0.00
Supplies	$2,500.00
Contractual	$0.00
Other	$3,000.00
Direct Costs Sub-Total	$98,894.00
Indirect Costs	$2,966.82
Total Budget	$101,860.82

PERSONNEL

List the organization's employees whose time and effort can be specifically identified and easily and accurately traced to project activities that enhance the competitiveness of specialty crops. See the Request for Applications section 4.7.2 Allowable and Unallowable Costs and Activities, Salaries and Wages, and Presenting Direct and Indirect Costs Consistently under section 4.7.1 for further guidance.

#	Name/Title	Level of Effort (# of hours OR % FTE)	Funds Requested
1	**Graduate student, PhD Assistantship**	0.50 %	$53,835.00
2	**Senior Personnel, Dr. Elizabeth Y Long**	80	$5,237.00
3	**Undergraduate student, Undergraduate student salary**	2,560	$22,880.00

Personnel Subtotal: $81,952.00

PERSONNEL JUSTIFICATION

For each individual listed in the above table, describe the activities to be completed by name/title including approximately when activities will occur. Add more personnel by copying and pasting the existing listing or deleting personnel that aren't necessary.

Personnel 1:	Support is requested for one graduate student for 6 calendar months each year of the project. The graduate student will be responsible for planning and conducting the research described in Objectives 1 & 2 and will assist with Objective 3.
Personnel 2:	1 week of summer salary is requested each year of the project. The PI will be responsible for ensuring timely progress on all objectives and will supervise the graduate and undergraduate students.
Personnel 3:	Support for two undergraduate students at $11/hr is requested each year. The undergraduates will be supervised by the graduate student in coordination with Dr. Long and will assist with field work and data collection,

FRINGE BENEFITS

Provide the fringe benefit rates for each of the project's salaried employees described in the Personnel section that will be paid with SCBGP funds.

#	Name/Title	Fringe Benefit Rate	Funds Requested
1	Graduate student, Ph Student Assistantship	0.09 %	$4,598.00
2	Senior Personnel, Dr. Elizabety Y Long	0.27 %	$1,427.00
3	Undergraduate Students, Undegraduate student researchers	0.08 %	$1,835.00

Fringe Subtotal: $7,860.00

TRAVEL

Explain the purpose for each Trip Request. Please note that travel costs are limited to those allowed by formal organizational policy; in the case of air travel, project participants must use the lowest reasonable commercial airfares. For recipient organizations that have no formal travel policy and for-profit recipients, allowable travel costs may not exceed those established by the Federal Travel Regulation, issued by GSA, including the maximum per diem and subsistence rates prescribed in those regulations. This information is available at http://www.gsa.gov. See the Request for Applications section 4.7.2 Allowable and Unallowable Costs and Activities, Travel, and Foreign Travel for further guidance.

#	Trip Destination	Type of Expense (airfare, car rental, hotel, meals, mileage, etc.)	Unit of Measure (days, nights, miles)	# of Units	Cost per Unit	# of Travelers Claiming the Expense	Funds Requested
1	Travel to Fair Oaks mint fields	mileage	miles	2,640.0	$0.41	1	$1,082.00
2	Travel to San Pierre mint fields	mileage	miles	2,640.0	$0.41	1	$1,082.00
3	Phoenix, AZ	Airfare	flights	1.0	$600.00	1	$600.00
4	Phoenix, AZ	hotel	Nights	3.0	$150.00	1	$450.00
5	Phoenix, AZ	Meals	days	3.0	$56.00	1	$168.00
6	Phoenix, AZ	Meeting registration	fees	1.0	$200.00	1	$200.00

Travel Subtotal: $3,582.00

TRAVEL JUSTIFICATION

For each trip listed in the above table describe the purpose of this trip and how it will achieve the objectives and outcomes of the project. Be sure to include approximately when the trip will occur. Add more trips by copying and pasting the existing listing or delete trips that aren't necessary.

Trip 1 (Approximate Date of Travel):	~110 miles round-trip to Fair Oaks, Indiana. Field research: visiting ~4 commercial mint fields x 3 visits a year x 2 years.

	Midwest Mint Meeting: travel to Fair Oaks, IN in Years 1 and 2 of the project to provide research updates.
Trip 2 (Approximate Date of Travel):	~110 miles round-trip to San Pierre, Indiana. Field research: visiting ~4 commercial mint fields x 3 visits a year x 2 years
Trip 3 (Approximate Date of Travel November 10, 2024):	Round-trip airfare for the graduate student to attend the National Entomological Society of America Meeting in Phoenix, AZ in 2024 to present research.
Trip 4 (Approximate Date of Travel November 10, 2024):	Lodging costs for graduate student to attend the National Entomological Society of America Meeting in Phoenix, AZ in 2024 to present research.
Trip 5 (Approximate Date of Travel November 10, 2024):	Estimated per diem for graduate student meals at the National Entomological Society of America Meeting in Phoenix, AZ in 2024 to present research.
Trip 6 (Approximate Date of Travel November 10, 2024):	Funds to cover the cost of graduate student registration to attend the National Entomological Society of America Meeting in Phoenix, AZ in 2024.

CONFORMING WITH YOUR TRAVEL POLICY

By checking the box to the right, I confirm that my organization's established travel policies will be adhered to when completing the above-mentioned trips in accordance with 2 CFR 200.474 or 48 CFR subpart 31.2 as applicable. ☐

EQUIPMENT

Describe any special purpose equipment to be purchased or rented under the grant. "Special purpose equipment" is tangible, nonexpendable, personal property having a useful life of more than one year and an acquisition cost that equals or exceeds $5,000 per unit and is used only for research, medical, scientific, or other technical activities. See the Request for Applications section 4.7.2 Allowable and Unallowable Costs and Activities, Equipment - Special Purpose for further guidance

Rental of "general purpose equipment" must also be described in this section. Purchase of general purpose equipment is not allowable under this grant. See Request for Applications section 4.7.2 Allowable and Unallowable Costs and Activities, Equipment - General Purpose for definition, and Rental or Lease Costs of Buildings, Vehicles, Land and Equipment.

#	Item Description	Rental or Purchase	Acquire When?	Funds Requested
1	N/A			$0.00

Equipment Subtotal: $0.00

EQUIPMENT JUSTIFICATION

For each Equipment item listed in the above table describe how this equipment will be used to achieve the objectives and outcomes of the project. Add more equipment by copying and pasting the existing listing or delete equipment that isn't necessary.

Equipment 1:	N/A

SUPPLIES

List the materials, supplies, and fabricated parts costing less than $5,000 per unit and describe how they will support the purpose and goal of the proposal and enhance the competitiveness of specialty crops. See Request for Applications section 4.7.2 Allowable and Unallowable Costs and Activities, Supplies and Materials, Including Costs of Computing Devices for further information.

Item Description	Per-Unit Cost	# of Units/Pieces Purchased	Acquire When?	Funds Requested
Disposable gloves	$20.00	20.0	May 1, 2023	$400.00
Gallon ziploc bags	$15.00	12.0	May 1, 2023	$360.00
Plastic petri dishes	$30.00	10.0	May 1, 2023	$300.00
Play sand	$5.00	12.0	May 1, 2023	$60.00
Potting soil	$12.00	6.0	May 1, 2023	$72.00
Sunscreen	$12.00	8.0	May 1, 2023	$100.00
Waxworms	$5.00	200.0	May 1, 2023	$1,000.00
White laboratory filter papers	$10.00	20.0	May 1, 2023	$208.00

Supplies Subtotal: $2,500.00

SUPPLIES JUSTIFICATION

Describe the purpose of each supply listed in the table above purchased and how it is necessary for the completion of the project's objective(s) and outcome(s).

Disposable gloves: Disposable nitrile gloves for researchers to use in field and laboratory in Years 1 and 2 of the project.
Gallon ziploc bags: Ziploc bags to collect soil samples in Years 1 and 2 of the project.
Plastic petri dishes: Plastic petri dishes required to detect nematodes in infected AGB grubs and waxworms.
Play sand: Play sand to pot mint plants in laboratory and greenhouse experiments with AGB and nematodes.
Potting soil: Potting soil to mix with sand and to pot mint plants in laboratory and greenhouse experiments with AGB and nematodes.
Sunscreen: Sunscreen to protect field researchers in Years 1 and 2 of the project.
Waxworms: Living waxworms (in containers of 50) for assessing nematode persistence in Objectives 1 and 2.
White laboratory filter papers: White laboratory filter paper (packs of 25 pieces) need for whites traps to collect nematodes from infected AGB grubs and waxworms.

CONTRACTUAL/CONSULTANT

Contractual/consultant costs are the expenses associated with purchasing goods and/or procuring services performed by an individual or organization other than the applicant in the form of a procurement relationship. If there is more than one contractor or consultant, each must be described separately. (Repeat this section for each contract/consultant.)

ITEMIZED CONTRACTOR(S)/CONSULTANT(S)

Provide a list of contractors/consultants, detailing out the name, hourly/flat rate, and overall cost of the services performed. Please note that any statutory limitations on indirect costs also apply to contractors and consultants.

#	Name/Organization	Hourly Rate/Flat Rate	Funds Requested
1	N/A		$0.00

Contractual/Consultant Subtotal: $0.00

CONTRACTUAL JUSTIFICATION

Provide for each of your real or anticipated contractors listed above a description of the project activities each will accomplish to meet the objectives and outcomes of the project. Each section should also include a justification for why contractual/consultant services are to be used to meet the anticipated outcomes and objectives. Include timelines for each activity. If contractor employee and consultant hourly rates of pay exceed the salary of a GS-15 step 10 Federal employee in your area, provide a justification for the expenses. This limit does not include fringe benefits, travel, indirect costs, or other expenses. See Request for Applications section 4.7.2 Allowable and Unallowable Costs and Activities, Contractual and Consultant Costs for acceptable justifications.

Contractor/Consultant 1:	N/A

CONFORMING WITH YOUR PROCUREMENT STANDARDS

By checking the box to the right, I confirm that my organization followed the same policies and procedures used for procurements from non-federal sources, which reflect applicable State and local laws and regulations and conform to the Federal laws and standards identified in 2 CFR Part 200.317 through.326, as applicable. If the contractor(s)/consultant(s) are not already selected, my organization will follow the same requirements. ☑

OTHER

Include any expenses not covered in any of the previous budget categories. Be sure to break down costs into cost/unit. Expenses in this section include, but are not limited to, meetings and conferences, communications, rental expenses, advertisements, publication costs, and data collection.

If you budget meal costs for reasons other than meals associated with travel per diem, provide an adequate justification to support that these costs are not entertainment costs. See Request for Applications section 4.7.2 Allowable and Unallowable Costs and Activities, Meals for further guidance.

Item Description	Per-Unit Cost	Number of Units	Acquire When?	Funds Requested
Grower collaboration stipend	$325.00	3.0	September 1, 2023	$1,000.00
Grower collaboration stipend	$325.00	3.0	September 1, 2024	$1,000.00
Publication fee	$1,000.00	1.0	August 1, 2024	$1,000.00

Other Subtotal: $3,000.00

OTHER JUSTIFICATION

Describe the purpose of each item listed in the table above purchased and how it is necessary for the completion of the project's objective(s) and outcome(s).

Grower collaboration stipend: A stipend, based on $325/acre value of mint oil, to as a thank you and support to offset potential yield losses they experience as a result of working with us in Year 1.

Grower collaboration stipend: A stipend, based on $325/acre value of mint oil, to as a thank you and support to offset potential yield losses they experience as a result of working with us in Year 2.
Publication fee: Funds are requested to support the costs of publishing 1 research article in an open-access scientific journal.

INDIRECT COSTS

The indirect cost rate must not exceed 8 percent of any project's budget. Indirect costs are any costs that are incurred for common or joint objectives that therefore, cannot be readily identified with an individual project, program, or organizational activity. They generally include facilities operation and maintenance costs, depreciation, and administrative expenses. See Request for Applications section 4.7.1 Limit on Administrative Costs and Presenting Direct and Indirect Costs Consistently for further guidance.

Indirect Cost Rate	Funds Requested
3.00 %	$2,966.82

Indirect Subtotal: $2,966.82

PROGRAM INCOME

Program income is gross income—earned by a recipient or subrecipient under a grant—directly generated by the grant-supported activity or earned only because of the grant agreement during the grant period of performance. Program income includes, but is not limited to, income from fees for services performed; the sale of commodities or items fabricated under an award (this includes items sold at cost if the cost of producing the item was funded in whole or partially with grant funds); registration fees for conferences, etc.

Source/Nature of Program Income	Description of how you will reinvest the program income into the project to enhance the competitiveness of specialty crops	Estimated Income
N/A		$0.00

Program Income Total: $0.00

Table 1. Outputs from linear regression analyses characterizing the relationship between Asiatic garden beetle (AGB) grub density and mint plant performance in three Indiana commercial mint fields during the spring and fall of 2021. Importantly, no significant relationships were discernable until AGB grub densities reached nearly 5.0/ft^2. These relationships included a decrease in plant height and root rating (1-5 scale) at mean AGB larval densities of 4.84 $\pm$ 0.10 grubs /ft^2 (Field 1 during the spring) and a decrease in above ground biomass (yield) at mean AGB larval densities of 5.58 $\pm$ 0.76 b grubs /ft^2 (Field 2 during the fall). Significant relationships are shown in red. N/A indicates comparisons that were not available for analysis.

	Spring 2021			**Fall 2021**		
	Field 1	**Field 2**	**Field 3**	**Field 1**	**Field 2**	**Field 3**
Mean AGB/ft^2 (±SE)	**4.84 ± 0.10**	0.05 ± 0.00	1.32 ± 0.13	1.08 ± 0.25	**5.58 ± 0.76**	4.57 ± 0.68
	Above-ground biomass (g)					
t	N/A	0.10	0.89	-0.81	**-4.08**	-0.98
p	N/A	0.921	0.379	0.424	**<0.001**	0.337
R^2	N/A	<0.01	0.02	0.02	**0.37**	0.03
	Below-ground biomass (g)					
t	N/A	0.26	0.86	0.19	0.26	0.15
p	N/A	0.793	0.396	0.849	0.799	0.880
R^2	N/A	<0.01	0.02	<0.01	<0.01	<0.01
	Plant height (cm)					
t	**-2.18**	N/A	N/A	N/A	N/A	N/A
p	**0.033**	N/A	N/A	N/A	N/A	N/A
R^2	**0.06**	N/A	N/A	N/A	N/A	N/A
	Root Rating (1-5)					
t	**6.79**	N/A	N/A	N/A	N/A	N/A
p	**<0.001**	N/A	N/A	N/A	N/A	N/A
R^2	**0.43**	N/A	N/A	N/A	N/A	N/A

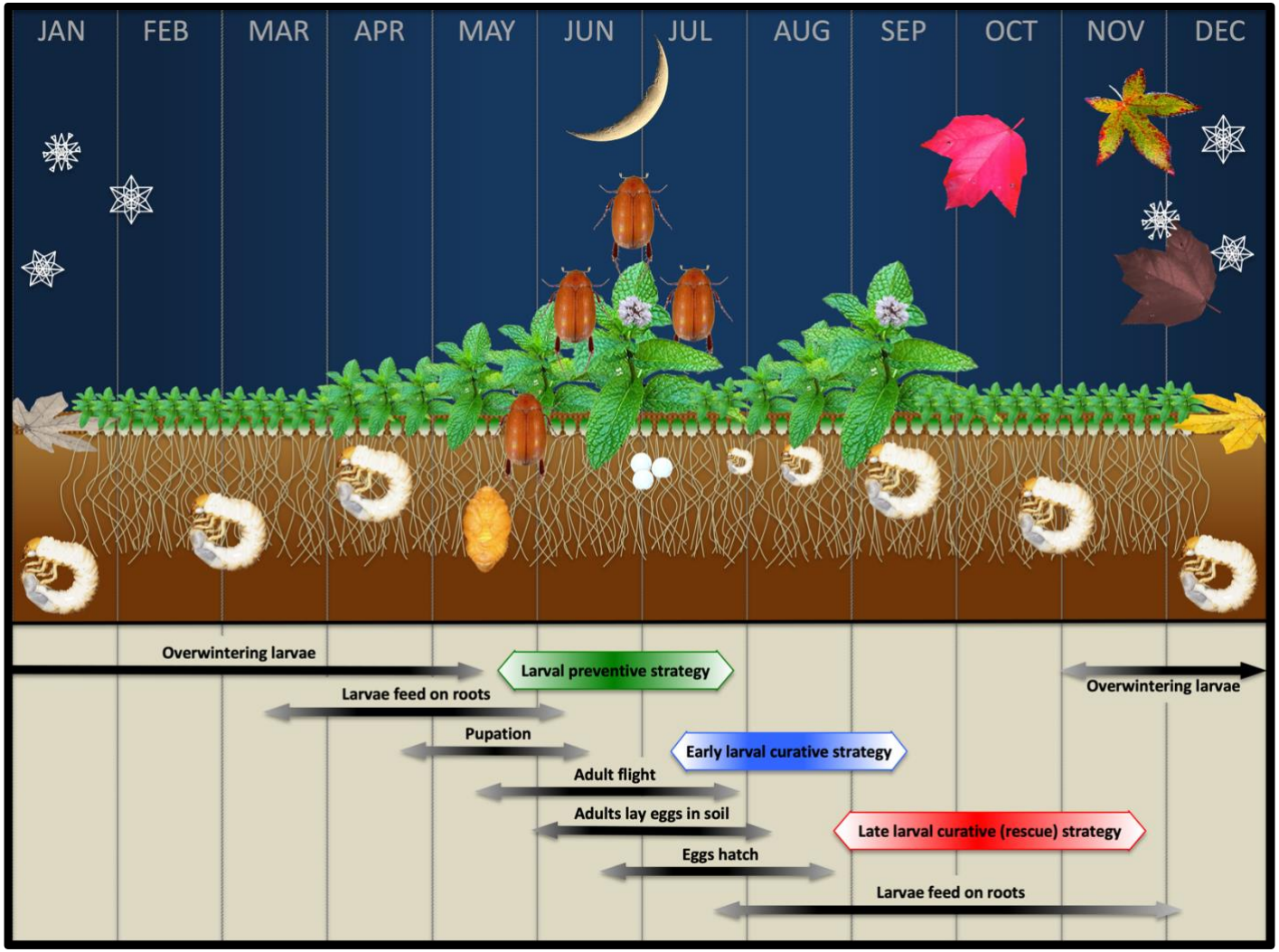

Figure 1. Life cycle schematic of the life cycle of Asiatic Garden Beetle in Indiana commercial mint production systems showing traditional insecticide use strategies (larval preventive, early larval curative, and late larval curative) for managing the damaging larval (grub) stage.

Figure 2. Examples of reduced mint root biomass resulting from Asiatic Garden Beetle larval infestations in untreated pots (control), or pots treat with Coragen (chlorantraniliprole), or Admire Pro (imidacloprid). Root masses of mint plants treated with Coragen or Admire Pro were, on average, 21% and 29% greater than roots of untreated mint, respectively.

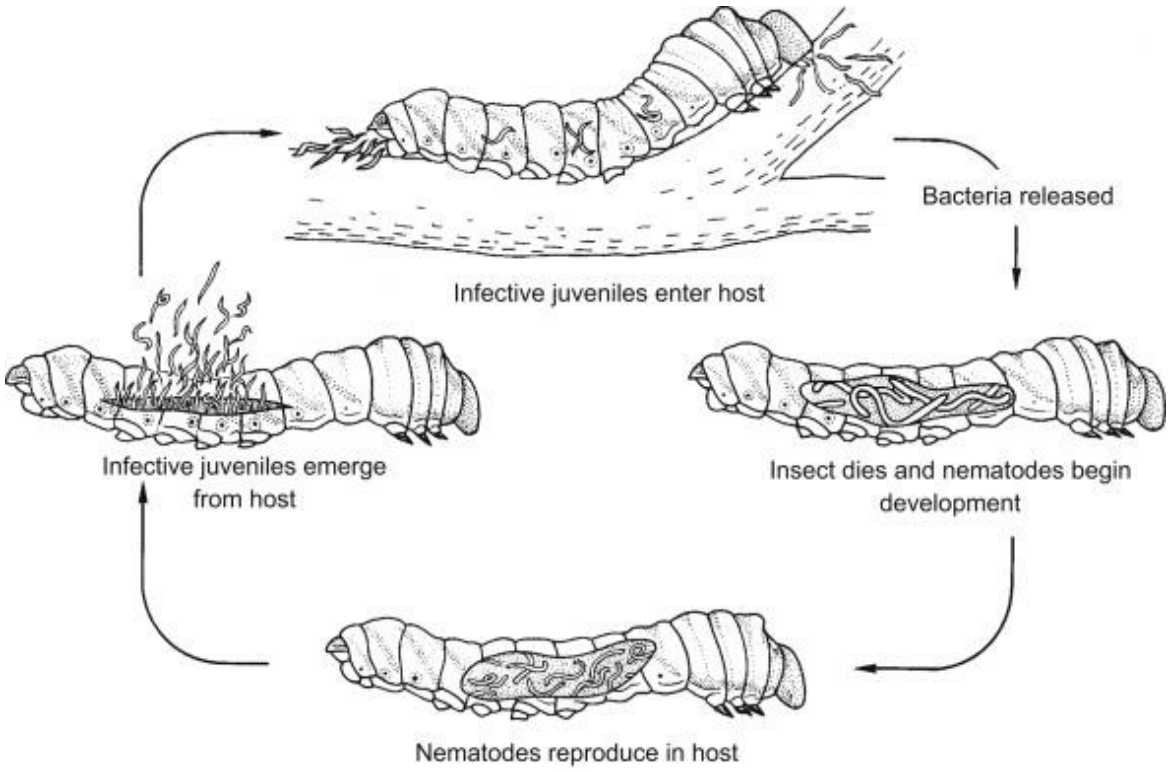

Figure 3. Generalized life cycle of a insect-parasitic nematode. nematodes enter the insect through natural openings in the body and once inside, they release bacteria that eventually kill the insect, turning it into a "soup-filled" cadaver. The nematodes feed within the cadaver on the insect nutrients made available by the bacteria, they mate and reproduce, and eventually spill out of the dead insect by the thousands to find new insect hosts in the soil. (Image courtesy of F. Garcia-del-Pino, A. Morton and D. Shapiro-Ilan, Entomopathogenic Nematodes as Biological Control Agents of Tomato Pests, In: Manual of Techniques in Invertebrate Pathology, 2nd Edition, 2012.)

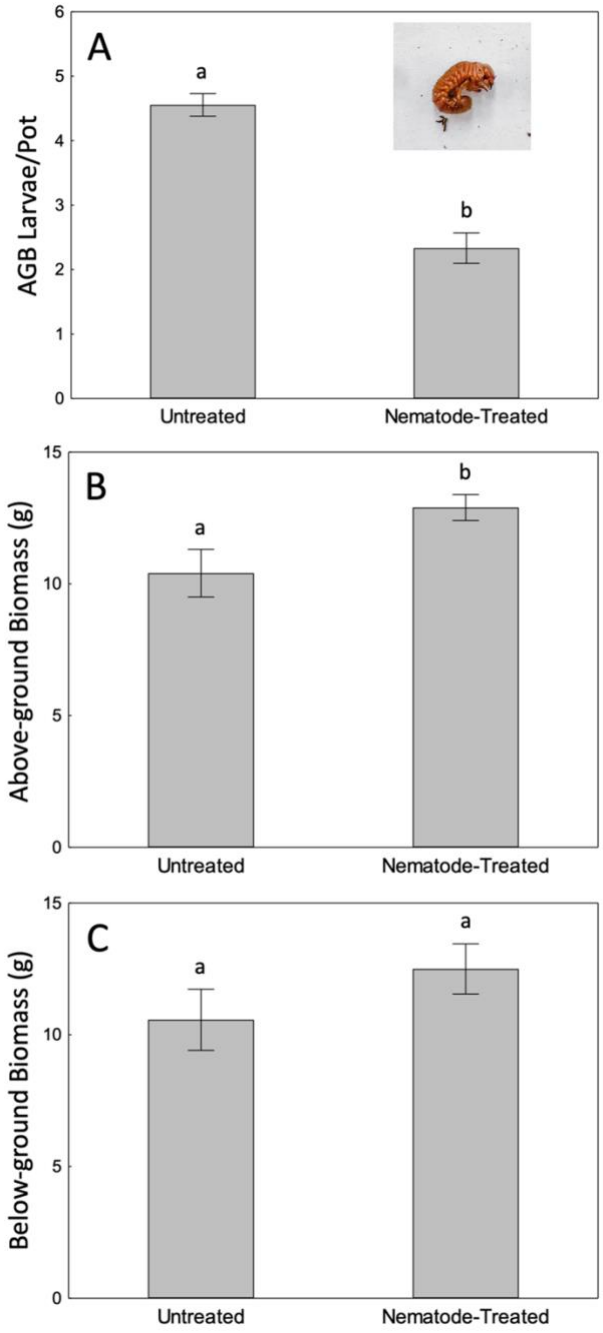

Figure 4. Asiatic Garden Beetle (AGB) larval density (A), and above-ground (B), and below-ground (C) mint plant biomass in untreated pots, or pots treated with the insect parasitic nematode *Heterorhabditis bacteriophora* (Hb). Although difference in below-ground biomass were not statistically significant, Hb reduced AGB grub survival by 50%, while increasing mint yield (above-ground biomass) by 24% and below-ground biomass (root biomass) by 18% compared to untreated mint over a relatively short (two-week) period of time. Hb-infected AGB grubs often appear reddish in color (inserted photo in figure 4A).

Figure 5. Field-based, yield harvest and soil-excavation approach we've developed for estimating Asiatic Garden beetle infestation levels and mint plant performance using current ISDA support. One, 2.7 ft^2 quadrat fashioned from ¾" PVC is placed in the center of each cells within each grid, and all living mint stems and leaves are cut at the soil surface using a pair of electric hand shears (A). Above-ground plant material is collected, placed into labeled plastic bags, and transported inside a cooler to the lab where it is weighed as a measure of yield. Within that same 2.7 ft^2 quadrat, all soil is excavated to a depth of 4" (B). Each 0.8 ft^3 mass of soil is then broken apart and sieved through a 0.25" hardware cloth mesh to collect the grubs (C).

March 7, 2022

Larry Wappel Jr
LWJR Farms LLC
166 Palomino LN
Valparaiso, IN 46383
219-863-4430
larry_wappel@yahoo.com

Dr. Elizabeth Y. Long
Dept. of Entomology
Purdue University
901 West State Street
West Lafayette, IN 47907
Office: (765) 496-1918
E-mail: eylong@purdue.edu

Dear Dr. Long,

Please accept this letter of support for your Indiana State Department of Agriculture-Specialty Crop Block Grant proposal entitled, **"Insect-parasitic nematodes as a sustainable management tool for soil-dwelling grubs, like the Asiatic garden beetle, in Commercial Mint Production**," that has the goal of evaluating the efficacy and persistence of insect-parasitic nematodes as a sustainable strategy for maintaining Asiatic garden beetle densities below economic thresholds in commercial mint production in Indiana.

Asiatic garden beetle grubs have been devastating to our mint yields for the last 6 to 8 years. We have tried everything we can to control AGB while staying on label. Nothing seems to work. Our only option is to rotate and hope. I am very excited about your work. Your passion for this project really shines through! We want to be sustainable as a farm and industry. If we can find ways to control AGB in mint, especially a non-chemical approach, it may revitalize the struggling industry.

Towards this goal, I agree to partner with you and your team in on-farm trials to evaluate the efficacy and persistence of insect-parasitic nematodes against AGB grubs in mint field trials. I understand that you will need access to my fields to monitor AGB grub densities, apply insect-parasitic nematodes, and conduct seasonal soil sampling to evaluate the persistence of insect-parasitic nematodes over time. I am happy to provide mint fields and we can work together to select the most suitable locations.

I look forward to working with you and your team on this project and hope your proposal receives favorable review.

Sincerely,
Larry Wappel Jr.

March 4, 2022
Kanne Farms – Gregg and Griffin Kanne
10502 W State Road 14
Fair Oaks, IN 47943

Dr. Elizabeth Y. Long
Dept. of Entomology
Purdue University
901 West State Street
West Lafayette, IN 47907
Office: (765) 496-1918
E-mail: eylong@purdue.edu

Dear Dr. Long,

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The possibility of using parasitic nematodes as a tool to combat the Asiatic garden beetle is very exciting. This pest has caused reduced stands and early removal or some of our mint fields. For example, we have had heavily reduced stands of peppermint showing up after the second year. Keep in mind these fields are expected to remain in mint for four years. We have had to destroy fields after three years due to thin stands and reduced yields. We have tried chemical applications with soil-applied insecticides with very little success. We currently apply insecticide when the adults are in flight with some positive results, be we need a practice that goes after the actual grub in the soil to reduce its population year after year. There could be a chemical free, sustainable practice which could help all mint growers in Indiana and the entire Midwest.

Towards this goal, I agree to partner with you and your team in on-farm trials to evaluate the efficacy and persistence of insect-parasitic nematodes against AGB grubs in mint field trials. I understand that you will need access to my fields to monitor AGB grub densities, apply insect-parasitic nematodes, and conduct seasonal soil sampling to evaluate the persistence of insect-parasitic nematodes over time.

I look forward to working with you and your team on this project and hope your proposal receives favorable review.

Sincerely,

Gregg E. Kanne
219-863-1394

Cliffton E Kanne
219-863-5726

RICHARD GUMZ FARMS, LLC
8905 South Gumz Road
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March 2, 2022

Dr. Elizabeth Y. Long
Dept. of Entomology
Purdue University
901 West State Street
West Lafayette, IN 47907
Office: (765) 496-1918
E-mail: eylong@purdue.edu

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Please accept this letter of support for your Indiana State Department of Agriculture-Specialty Crop Block Grant proposal entitled, **"Insect-parasitic nematodes as a sustainable management tool for soil-dwelling grubs, like the Asiatic garden beetle, in Commercial Mint Production**," that has the goal of evaluating the efficacy and persistence of insect-parasitic nematodes as a sustainable strategy for maintaining Asiatic garden beetle densities below economic thresholds in commercial mint production in Indiana.

Many Indiana mint growers have had issues with Asiatic garden beetle (AGB) grubs. The pest causes severe yield loss due to stand reductions in peppermint and weakens the plants which reduces oil yield and increases winter losses. Right now, there are no chemical treatments identified to control AGB in peppermint and little is known on cultural practices for control. The potential for insect-parasitic nematodes as a long-term, sustainable tool for management of this pest in commercial mint in Indiana is important for our growers. Indiana's peppermint industry is under intense pressure to reduce production costs in order to compete with foreign-produced mint oils and stay a viable industry.

I look forward to working with you and your team on this project and hope your proposal receives favorable review.

Sincerely,

Daniel E. Gumz
President, Indiana Mint Market Development and Research Council

Cornell University
College of Agriculture and Life Sciences

Elson Shields
Department of Entomology
Comstock Hall
Ithaca, NY 14853-2601
607.279.1849
Email: es28@cornell.edu

March 1, 2022

Dr. Elizabeth Y. Long
Dept. of Entomology
Purdue University
901 West State Street
West Lafayette, IN 47907
E-mail: eylong@purdue.edu

Dear Dr. Long,

Please accept this letter of support for your Indiana State Department of Agriculture-Specialty Crop Block Grant proposal entitled, **"Insect-parasitic nematodes as a sustainable management tool for soil-dwelling grubs, like the Asiatic garden beetle, in Commercial Mint Production**," that has the goal of evaluating the efficacy and persistence of insect-parasitic nematodes as a sustainable strategy for maintaining Asiatic garden beetle densities below economic thresholds in commercial mint production in Indiana.

During my 36-year career at Cornell University, my laboratory developed, and field tested the concept of using persistent native entomopathogenic nematodes as a classical biological control where a single inoculation provides multiyear soil insect suppression. We have been effective in showing efficacy and multiyear persistence in alfalfa (alfalfa snout beetle), corn (corn rootworm, wireworms) and small fruits (Black vine weevil – strawberries). We also have shown multiyear persistence in agricultural fields in 12 states ranging from the Northeastern US to Alabama and Southeast New Mexico. Several projects including a current one focused on potatoes – wireworms-Colorado potato beetle have been funded the NYS Specialty Block Grant program.

As I fade away into retirement and old age, I am very interested in transferring this exciting technology discovered in my laboratory to younger faculty so they can continue to explore it applicability in new agricultural systems and against new pests. Toward this goal, I agree to provide guidance and consultation to your research team to investigate the efficacy and persistence of this technology against AGB grubs in mint field trials. I look forward to working with you and your team on this project and hope your proposal receives favorable review.

Sincerely,

Professor - Entomology

References

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Dalthorp, D., J. Nyrop and M.G. Villani. 2000. Spatial ecology of the Japanese beetle, *Popillia japonica*. Entomol. Exp. Et Appl. 96: 129-139.

Ferguson, C. S., P. C. Schroeder, and E. J. Shields. 1995. Vertical distribution, persistence and activity of entomopathogenic nematodes (Nematoda: Heterorhabditidae and Steinernematidae) in alfalfa snout beetle (Coleoptera: Curculionidae) infested fields. Environmental Entomology 24: 149-158.

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