Capstone Report

Comparing Nematode Infection Rates in Asiatic Garden Beetle Across Different Indiana Soils

Max Thornburg

Introduction

Asiatic garden beetle, Maladera formosae Brenske, (AGB) is a major pest of commercial mint in Indiana. The adult beetles emerge from the soil from late June through August and may be found in the field into October. The adults feed on many different ornamental plants such as asters, dahlias, and chrysanthemum, but are not known to feed on mint. However, the larvae feed below-ground on mint roots, root hairs and soil organic matter. AGB was first discovered in Indiana in 2007 in Elkhart County damaging corn growing in sandy soils (Goshen, 2007). Since then, it has been found in association with soybeans and commercial mint. At the moment there aren't many good management options for mint growers because mint oil buyers are placing a lot of more pressure on the growers to produce the mint more sustainably. There is also little evidence in the literature indicating that insect parasitic nematodes are useful for managing AGB outside of turfgrass. The ability of insect parasitic nematodes to serve as a useful management tool for AGB has not been examined in mint or sandy soils where mint is usually grown in Indiana. The goal of this research was to characterize the infectivity of the insect parasitic nematode Heterorhabditis bacteriophora (H.B.) against AGB and determine the if soil texture has any influence in this regard. In this experiment my hypothesis was that because of its foraging behavior, H.B. would be effective against soil-dwelling AGB larvae. I also hypothesized that the

effectiveness of H.B. would increase with increasing soil sand content. For this experiment I examined how five different soils influence nematode efficacy against AGB larvae (Table 1).

Materials & Methods

Soils for the bioassays were collected from three different production mint fields in Starke County, IN, and from the W.H. Daniel Turfgrass Research & Diagnostic Center in Tippecanoe County, IN. White play sand was purchased from a local retail store and used as a positive control. Prior to being included in the bioassay, soils were sieved to a uniform particle size by passing them through a 5.0 mm soil sieve. Soils were placed in a drying oven at 80°C overnight and dry soils were placed into sealed glass canning jars until needed.

AGB larvae were collected from the field by excavating and sieving soil from infested fields. Larvae were placed into plastic wash tubs containing a moistened mixture of play sand and peat, placed in a cooler and returned to the laboratory where they were held at 16°C until used in bioassays. Soil was sieved to collect a large amount of AGB larva needed for the experiment. Once the soil and grubs were collected the rest of the materials needed were in the lab except the nematodes which had to be ordered. Once nematodes arrived, I gathered Wells, pipettes, pens, tape, glass jars to store the soils. Petri dishes were also used for wax worm white trapping to collect fresh nematodes.

Insect Parasitic nematodes (Heterorhabditis bacteriophora) were purchased from Biobest USA (Romulus, MI) and cycled through Galleria melonella larvae to produce fresh infective juveniles for the experiment. Infective juveniles were held at in culture flasks at 16°C until needed. Soils were dried in a specialized oven prior to being used in experiments.

Five replicates of each soil type were added to the wells of a 24 well plate and a single, 3rd instar AGB larva was added to each well. I calibrated a micropipette to deliver 7 nematodes volumetrically to ensure equal doses could be administered and soil moisture was adjusted to 10% by weight after the nematodes were added. Wells were then set aside for one week at room temperature. After that time, each larva was inspected to determine if it was infected. These results were then analyzed in statstica using ANOVA and univariate analysis.

Results

Statistica was used to perform statistical analyses and generate figures portraying relationships between independent and response variables. Although the mean nematode infection rate varied from 35% (Field 1) to 65% (washed play sand) (Table 1), ANOVA indicated that infection rates did not vary significantly regardless of soil ($F=1.4$; $df=4, 20$; $P=0.28$) (Figure 1). Although soil sand content varied from 14% (DANL) to 100% (washed play sand)(Table 2), regression analysis indicated that nematode infection did not vary with soil sand content ($F=0.40$; $df=1, 23$; $P=0.53$).

Discussion

Insecticide options for controlling AGB in commercial mint are limited as buyers of mint oil place a hefty premium on mint that is grown without the use of insecticides (Morris 2011). However, the utility of biocontrol has not previously been examined in the AGB-mint system. Previous studies have examined the efficacy of various insecticides, including halofenozide, imidacloprid and clothianidin against AGB larvae in other crops, but results have been mixed (EM;, K. A. M. F). In greenhouse studies, levels of AGB larval control provided by the insecticide clothiandin varied from 62-93%. However, two species of insect parasitic nematodes

provided excellent control under these same conditions, regardless of larval instar (EM;, K. A. M. F). Although additional field evaluations will be required, the current study demonstrates that even over relatively short exposure periods (1 week), H.B. may provide moderate levels of AGB larval control (35-65%) in soils representing mint-producing areas of Indiana. Future studies should include a wider range of nematode species in tandem with insecticides in order to get a broader picture of the effectiveness of different control options.

Acknowledgments

I would like to say thank you to everyone who helped me with this experiment and got me interested in this research experience. I would first like to thank Dr. Richmond and Dr. Long for meeting with me during the summer to discuss nematodes, and the Asiatic Garden beetle problem in mint production in the state of Indiana. Without reaching out to them I would never have known that Indiana was a high mint producing state and what battle it is having with this pest at this moment in time. I would also like to thank the members of Richmond who were Gordon, Ryan, Connor, and Jack who all were very nice to talk to and very helpful during the experiment and the plot working in the fall. I really did enjoy this experience and has given me new knowledge of this pest and what we can do to possibly control and get it down to a level where it doesn't harm mint production as much.

References

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Tables and Figures

Table 1- Std Dev and Mean Nematode Soil Analysis with % of Sand in Soil

Breakdown Table of Descriptive Statistics (Sheet1 in Capstone Nematode Data for analysis with % Sand in Soil)
N=25 (No missing data in dep. var. list)

Treatment	% Infection Means	% Infection N	% Infection Std.Dev.
Field 1	0.350000	5	0.223607
Field 2	0.650000	5	0.285044
Field 3	0.400000	5	0.223607
DANL	0.450000	5	0.325960
Sand	0.650000	5	0.285044
All Grps	0.500000	25	0.279508

Figure 1- % of Infection grouped by each Separate Treatment(Field) Type

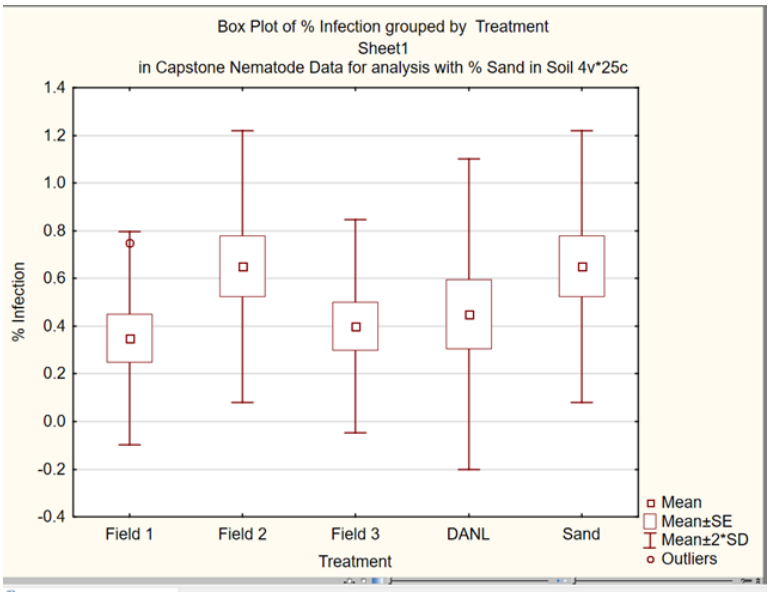

Table 2- Percentage of Sand, Silt, and Clay in Soils Examined

Field Type	Sand %	Silt %	Clay %
Field 1	84%	10%	6%
Field 2	83%	13%	4%
Field 3	87%	9%	4%
DANL	14%	56%	30%
Sand Control	100%	0%	0%

Figure 2- Scatterpolot showing the % of Infection vs. %Sand in the Soil of Each Field