Outreach Template.

Title:
Entomopathogenic Nematodes as an Alternative Management Strategy for Grape Root Borer

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Public Abstract:

The clearwing moth, grape root borer (GRB), is a native pest that attacks the roots of both wild and cultivated grapes in the Southeast. The larvae of GRB spend nearly two years underground feeding on grape roots, subsequently damaging vines by girdling the roots. Concealed beneath the ground, injury due to GRB larvae often goes unnoticed until it is too late, leading to reduction in winter survival, fruit quality, and even vine death. The organophosphate, chlorpyrifos, is currently the only insecticide labeled for use against grape root borer, but it's 35-day pre-harvest interval in grapes can be challenging for early maturing varieties and will likely be banned within the next few years by the EPA due to its toxicity. As such, effective curative treatments for GRB are limited. Therefore, additional alternative control methods for the GRB are needed.

Thus, we worked directly with bunch grape producers and extension agents in the Southeast to implement and evaluate novel and established entomopathogenic nematodes (EPNs) as a biological control agent for GRB management. Nematodes have been shown to effectively reduce GRP infestations, but adoption of this tactic is still virtually nonexistent in the Southeast. As such, we also surveyed GA grape growers to help identify barriers to producer adoption of EPNs. At one vineyard in North GA, we evaluated the novel commercially available EPN, *Steinernema feltiae,* in comparison to the previously evaluated EPN, *Heterorhabditis bacteriophera,* and to the standard insecticide, chlorpyrifos. In addition, we compared abundance of GRB adults and pupal skins of the treated plots to a separate vineyard that was under mating disruption for GRB management.

Adult GRB moths were active from mid-July to early September, but no moths were collected throughout this period at the disrupted vineyard. Similarly, no evidence of pupae was detected in the disrupted vineyard. In the standard vineyard, numerically the ENP treated vines did as well or better than the untreated control and chlorpyrifos treated vines. Unfortunately, due to the low replication of this initial project, no statistically significant differences were found among the untreated, chlorpyrifos treated, *H. bacteriophera* treated, or *S. feltiae* treated vines. Regardless, the evidence that the EPNs may reduce GRB infestation is encouraging and warrants further research. Furthermore, 36% of surveyed growers were unaware that GRB was considered a pest and only 21% had ever heard of entomopathogenic nematodes. As such, future extension meetings and publications need to focus on educating growers about this stealthy pest and potential management tactic.

Introduction:
Bunch grapes are produced on approximately 8,000 acres throughout the Southeastern region, which is worth over $32 million annually (NASS 2015 State Agricultural Overview). A demanding management program is required to effectively control the intense insect and disease pests of grapes in the hot and humid climate of the southeastern US. One such pest is the grape root borer (GRB), *Vitacea polistiformis* (Harris) (Lepidoptera: Sesiidae). This clearwing moth attacks the roots of both wild and cultivated grapes of *Vitis* and *Muscadinia,* and as such, is a significant pest in Southeastern grape production. The life cycle of GRB is completed within 2 years, where larvae spend approximately 23 months feeding on root tissue, subsequently damaging vines by girdling the roots, thus cutting off nutrients and water to the remainder of the plant. It is estimated that a single larva feeding on the root system is able to reduce a vine's yield by 50% (Dutcher and All, 1979). Thus, several larvae within a root system can cause substantial injury to vines through the reduction in winter survival, fruit quality, and even vine death (Dutcher and All, 1979).

Damage caused by the grape root borer has resulted in enormous losses to the commercial grape industry. Since the late 1960's, grape root borer caused several counties in South Carolina to cease production of bunch grapes entirely, reducing production acreage by over 300 acres (Pollet, 1975). Damage due to GRB is still a key issue throughout the Southeast. Currently, chlorpyrifos is the only insecticide labeled for use against grape root borer, applied as a soil drench around the base of vines as a toxic barrier to the movement of neonates to roots. The residual activity of chlorpyrifos against larvae in soil is about 4 weeks (All et al. 1985), and it's 35 day pre-harvest interval in grapes can be challenging for early maturing varieties in the Southeast. Furthermore, chlorpyrifos is highly toxic and will likely be banned within the next few years by the EPA due to environmental concerns (EPA ordered to ban chlorpyrifos in 2018, but decision was appealed EPA.gov). A more environmentally friendly method for managing GRB is the use of sex pheromone-based mating disruption, which can significantly reduce vine infestation and injury due to GRB (Pfeiffer et al., 2010). While effective, the entirety of a vineyard needs to be under "disruption", which can make mating-disruption a costly method in terms of time and money. Additionally, as we encountered in 2019, grape producers many not always have access to mating-disruption, as supply can often be limited (pers. comm.). Therefore, additional alternative control methods for the GRB are needed.

A well-researched area for alternative methods of management for GRB is natural enemies, specifically entomopathogenic nematodes (EPN) (for example: All et al. 1981; Williams et al., 2002; Said et al., 2015; Jhalendra and Bergh, 2017). The nematode, *Heterorhabditis bacteriophora*, effectively reduced grape root borer infestations in the field was found to be as effective as the insecticide, chlorpyrifos (Williams et al., 2002; Said et al., 2015). Despite the success of entomopathogenic nematodes in research field trials, adoption of this control tactic on a commercial scale is still virtually nonexistent, possibly due to a lack of grower education. Thus, we worked directly with bunch grape producers and extension agents in North GA to implement and evaluate novel and established EPNs for GRB management. We will evaluate the EPN, *Steinernema feltiae,* which was isolated from GRB (All et al. 1981), is commercially available, and has been successfully used under harsh conditions in the Southeast for other insect pests, such as plum curculio (*Conotrachelus nenuphar*) in peaches (Shapiro-Ilan et al., 2011). *Steinernema feltiae* will be compared to the tested *H. bacteriophera* and the standard insecticide, chlorpyrifos. In addition to the field trials, we surveyed grape growers in GA in order to help identify barriers to producer adoption of EPNs for GRB management.

Objectives:

1.) To compare field efficacy of a novel EPN, *Steinernema feltiae*, against GRB with an established biological control agent, the EPN *Heterorhabditis bacteriophora* and the conventional insecticide chlorpyrifos.
2.) Work directly with bunch grape producers and extension agents to implement and evaluate EPN use for GRB management to help identify barriers to adoption and subsequently determine methods to encourage grower adoption of EPNs.

Description of Outreach Activity:

In order to accomplish our objectives, we initially established sites two bunch grape vineyards in Dahlonega, GA that had identified having a history of GRB infestations. Unfortunately, due to miscommunication one of the sites (Vineyard II) had used mating disruption for GRB the previous year, so that grower continued with mating disruption in 2020 and we used that site as a comparison of mating disruption with the other management options. Regardless, we worked directly with the cooperating producers at Vineyard I and Vineyard II and the local extension agent to implement and evaluate EPN use for GRB management. As a demonstration of how EPNs are applied the farm managers at the two vineyards assisted in the application of EPNs to the vines.

For Objective 1, we tested four treatments: two EPN treatments (*H. bacteriophera* and *S. feltiae,* Arbico Organics), chlorpyrifos (Lorsban 4E, Dow Agrosciences), and an untreated control. Each treatment plot consisted of a single trellis 'panel' or approximately five grape vines and was replicated four times, arranged in a Latin square design. The nematode treatments were applied on 27 May 2020 using a backpack CO_2 sprayer (Bellspray, Inc.) with a single-nozzle boom at 40 PSI (Williams et al. 2010) at a rate of approximately 2.5 million nematodes mixed with 1.89 l of water per vine. The chlorpyrifos treatment was applied on 6 July 2020 using the same sprayer set up at a rate of 23.6 ml of Lorsban 4E mixed with 1.89 l of water per vine. Solutions were applied to a circle area with a radius of approximately 0.5 m around the base of each vine. Nothing was applied to the base of the vines in the untreated control treatment plot. Disease and pest management of the of the vines were left under the growers' discretion. Adult male GRB activity was monitored throughout the study using pheromone-baited bucket traps (Great Lakes IPM; Figure 1).

Figure 1. Example of a bucket trap used to monitor grape root borer adult males.

The four management treatments were evaluated by counting the number of pupal cases and hibernacula present on the soil surface with a 0.5 m radius under each of the vines in each plot on a weekly basis from 6 July to 7 September 2020 (Figure 2). The number of pupal evidence (pupal cases + hibernacula) per date were recorded and compared amongst the treatments using an analysis of variance.

Figure 3. Pupal case (left) and pupal hibernacula (right) at the base of grape vines (From 2020).

To assess the persistence of the nematodes within the vineyard soils, soil samples will be collected from each of the two cooperating vineyards. Unfortunately, due to research interruption because of the 2020 pandemic, we were only able to take one soil sample post-GRB activity on 24 August 2020. At each site we used a soil-probe to collect approximately 12 cm of soil within a 0.5 m radius of each vine. We collected five soil samples per plot and the soil samples were brought back to the lab for assessment. In the lab, wax worm larvae (*Galleria mellonella*) were subjected to soil samples from each treatment replicates and incubated for approximately 1 month. Wax worm cadavers were then dissected and assessed for the presence of either *H. bacteriophora* or *S. feltiae* EPNs.

Following the field season, we discussed with the grower cooperators and surveyed other North GA grape producers to determine their perception GRB as a pest and their understanding of EPNs. We wanted to identify any barriers that may be present in terms of implementing EPNs as a management tactic for GRB.

Results or Outcome:
During the 2020 season we successfully implemented the proposed project to compare management of GRB with two species of EPNs and chlorpyrifos at a single vineyard (Vineyard I) in Dahlonega, GA. The second planned vineyard (Vineyard II) ended up using mating disruption for GRB management, but GRB adults and pupae were monitored there none-the-less. From monitoring the adult GRB activity at the Vineyard I, we determined that the moths begin flying early July and keep emerging until early September (Figure 3). Monitoring at Vineyard II resulted in no captures of GRB moths, demonstrating trap shutdown due to the mating disruption.

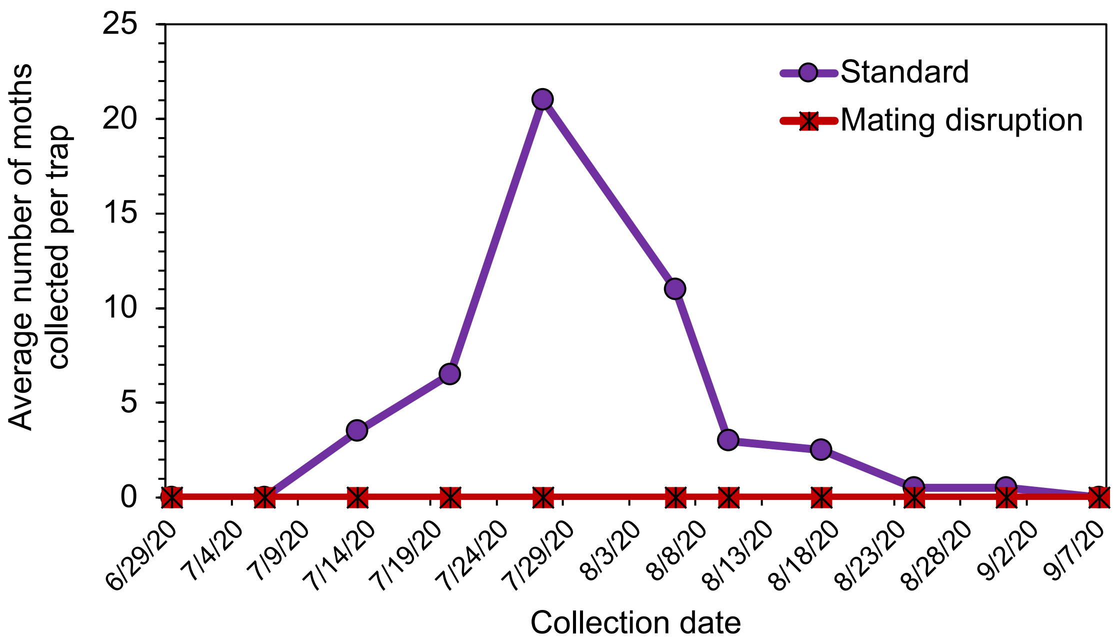

Figure 3. Activity of adult grape root borers collected in traps at Vineyard I (Standard) and Vineyard II (Mating disruption) during the 2020 field season.

Similar to the adult GRB results, no pupal casings or hibernacula were detected under the vines at Vineyard II. A total of 7 pupal casings and 5 hibernacula were found across all the plots at Vineyard I. However, likely due to using only one vineyard with relatively low GRB pressure in 2020, the variation amongst the replicates at Vineyard I was high, resulting in no significant differences among the treatments (ANOVA; $F_{3,12} = 0.903$, $p = 0.468$). Regardless, no GRB pupal casings or hibernaculum were observed under the *S. feltiae* treated vines and numerically fewer were found under the *H. bacteriophora* vines (Figure 1). As such, the results from 2020 are encouraging and warrant a second year of research, particularly since GRB has a two-year life cycle, so additional affects may be uncovered in the second year.

In terms of adoption barriers, 36% of surveyed growers were unaware that GRB was considered a pest in GA and 52% of growers not currently actively managing for GRB. Of the 6 growers that have had issues with GRB in the past, there was an estimated average of over $38,000 lost due to loss in production and/or vine death. This demonstrates that where GRB is an issue in North GA, it is a very destructive and costly problem. Unfortunately, only a single grower was enthusiastically interested in using EPNs for GRB management, but this appears to be due to a lack of education. For example, only 21% of the surveyed growers had previous knowledge of EPNs. As such, future extension meetings and publications will focus on educating growers about this stealthy pest and potential management tactic in Southeastern grape production.

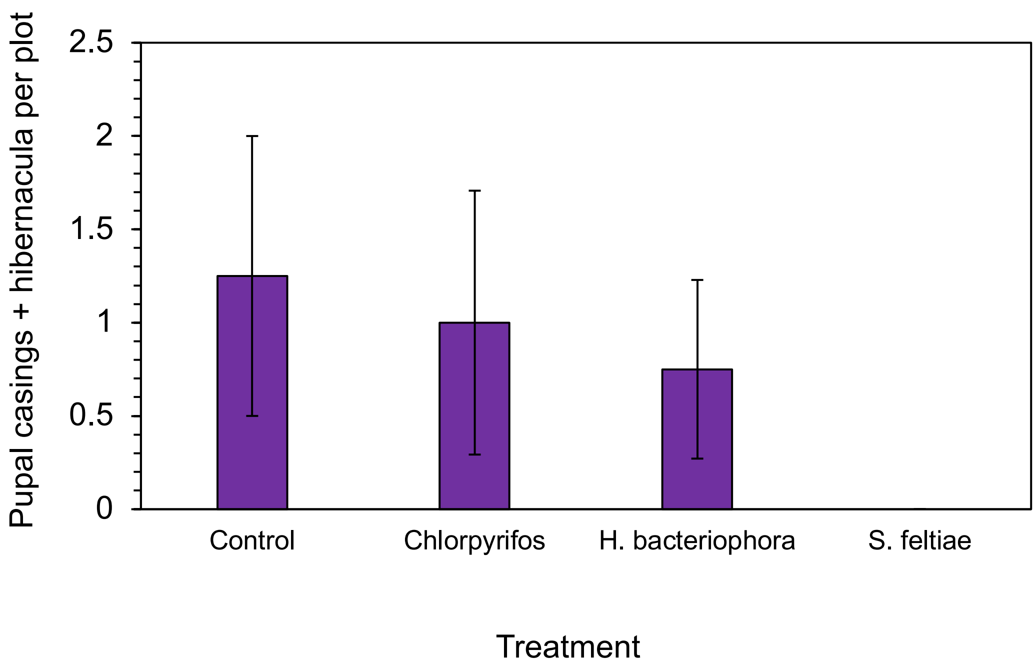

Figure 4. The season average number of pupal casings plus hibernacula observed near the bases the grape vines per plot.

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