Outreach Template.

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

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Public Abstract:
The grape root borer (GRB) is a native clearwing moth 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 one of the main insecticides labeled for use against grape root borer, but with its impending ban by the EPA in 2022, effective curative treatments for GRB are limited. As such, effective curative treatments for GRB are limited. Therefore, additional alternative control methods for the GRB are needed.

Thus, in 2021 we worked directly with bunch grape producers and extension agents in North GA to implement and evaluate two entomopathogenic nematode (EPNs) species as biological control agents for GRB management. At four Georgia vineyards we evaluated two commercially available EPN species, *Steinernema feltiae,* and *Heterorhabditis bacteriophera,* the standard insecticide, chlorpyrifos, and an untreated control.

Adult GRB moths were active from mid-July to early September at all vineyards. In terms of the GRB emergence only the *H. bacteriophera* treated vines had significantly fewer exuviae than the control vines, whereas the chlorpyrifos and *S. feltiae* treated vines had marginally fewer exuviae than the control vines. As such, EPNs, particularly *H. bacteriophera*, may reduce GRB infestation, potentially better than chlorpyrifos, which is encouraging and warrants further research.

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 Southeast. One such pest is the grape root borer (GRB), *Vitacea polistiformis* (Harris) (Lepidoptera: Sesiidae) (Figure 1). This clearwing moth attacks the roots of both wild and cultivated grapes, 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 fruit quality and eventually even vine death (Dutcher and All, 1979).

Figure 1. Grape root borer adult male on grape leaf.

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). Although it often goes unnoticed until it is too late, damage due to GRB is still a key issue throughout the Southeast. Currently, chlorpyrifos is one of the only insecticides 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 larvae to the roots. Unfortunately, chlorpyrifos is highly toxic and as such, the EPA has decided to ban the use of chlorpyrifos in fruit production starting in March 2022 (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., 2010; Said et al., 2015; Jhalendra and Bergh, 2017). The nematode, *Heterorhabditis bacteriophora*, has been demonstrated to effectively reduced grape root borer infestations in the field and was found to be as effective as the insecticide, chlorpyrifos (Williams et al., 2002; Said et al., 2015). Despite the success of *H. bacteriophora* 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 evaluated 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 previously tested *H. bacteriophera* and the standard insecticide, chlorpyrifos.

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 and to help identify barriers.

Description of Outreach Activity:

In order to accomplish our objectives, we established research sites at two bunch grape vineyards in Dahlonega, GA and two in Cleveland, GA. We worked directly with the cooperating farm managers at the vineyards and two local extension agents to implement and evaluate EPN use for GRB management.

For Objective 1, we tested four treatments: two EPN treatments (*H. bacteriophera* and *S. feltiae,* reared at the USDA-ARS by Shapiro-Ilan), 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 two nematode species were preserved in a hydrophilic polymer hydrogel to reduce desiccation in the field. On 28 May 2021, 8 fl oz of hydrogel mixed at a rate of approximately 450 thousand nematode infectious juveniles (IJs) was hand applied to each vine for each respective EPN treatment and then watered with 1 liter of water per vine (Figure 2). The chlorpyrifos treatment was applied on 25 June 2021 using a backpack CO_2 sprayer (Bellspray, Inc.) with a single-nozzle boom at 40 PSI at a rate of 23.6 ml of Lorsban 4E mixed with 1.89 l of water per vine. All treatments were applied to a circle area with a radius of approximately 0.5 m around the base of each vine (Figure 2). Nothing was applied to the base of the vines in the untreated control treatment plot, but debris was still removed from around each vine. Adult male GRB activity was monitored throughout the study using pheromone-baited bucket traps (Great Lakes IPM;

Figure 3). Disease and general insect pest management of the of the vines were left under the growers' discretion.

Figure 2. Example nematodes mixed with hydrogel applied to the base of a vine.

The four management treatments were evaluated by counting the number of exuvia (pupal cases) present on the soil surface within the 0.5 m radius under each of the vines in each plot on a weekly basis from 21 June to 8 September 2021 (Figure 3). The number of exuviae per date were recorded and compared amongst the treatments using an analysis of variance and compared between means using a Student's t test (JMP Pro 15, SAS Institute Inc.).

Figure 3. An example of a bucket trap used to monitor grape root borer adult males (Left) and an exuvia (pupal case) at the base of a GA grape vine (Right).

To assess the persistence of the nematodes within the vineyard soils, soil samples were collected from each of the four cooperating vineyards. Post-GRB activity, on 8 September 2021 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 extension agents to determine their perception of 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 2021 season we successfully implemented the proposed project to compare management of GRB with two species of EPNs and chlorpyrifos at two vineyards in Dahlonega, GA and two vineyards in Cleveland, GA. From monitoring the adult GRB activity across the four vineyard sites, we determined that the moths begin flying early July and keep emerging until early September (Figure 4).

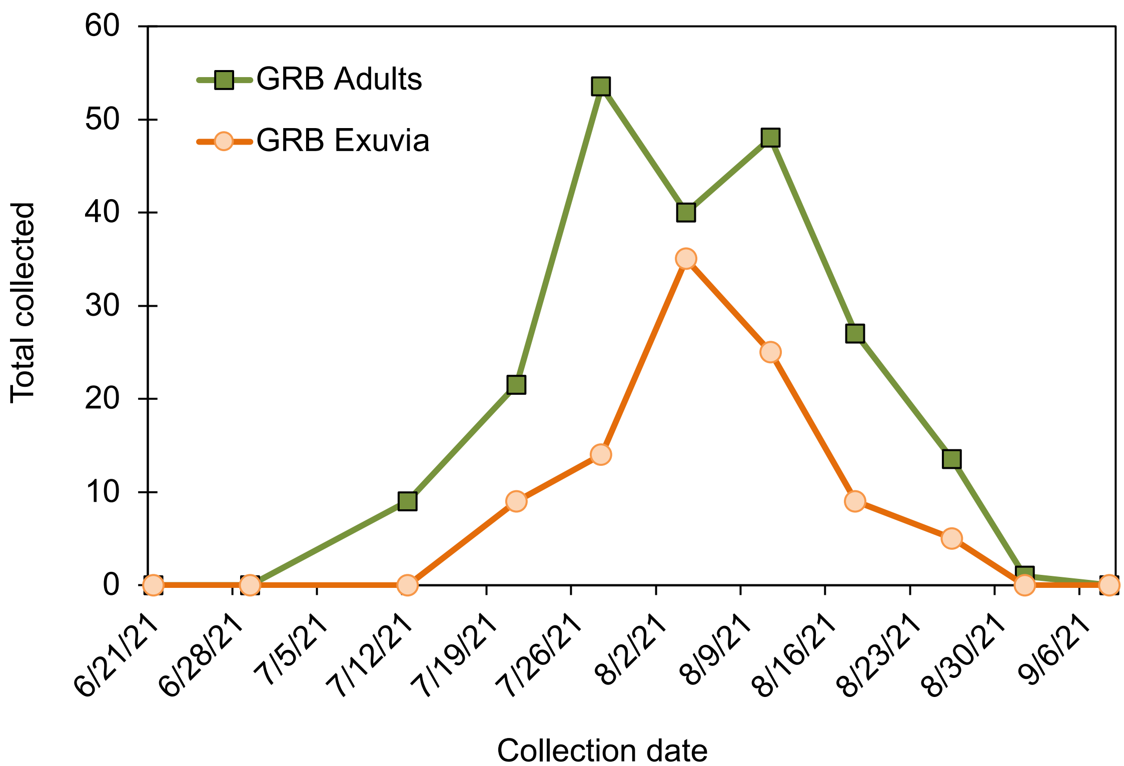

Figure 4. Activity of adult grape root borers collected in traps (green line) and exuviae (orange line) during the 2021 field season.

Assessing the number of observed exuviae per plot, there were significant differences among the treatments (ANOVA; $F_{3,60} = 2.94$, $p = 0.04$). Only the *H. bacteriophera* treated vines had significantly fewer exuviae than the control vines, whereas the chlorpyrifos and *S. feltiae* treated vines had marginally fewer exuviae than the control vines (Figure 5). Interestingly, a preliminary study at a single vineyard in 2020 resulted in *S. feltiae* treated plots having the fewest exuviae, but due to the low replication, it was not significant compared to the control. Regardless, both years demonstrated that EPNs, particularly *H. bacteriophera*, may reduce GRB infestation, potentially better than chlorpyrifos. Unfortunately, we were unable to recover any EPNs from the soil when evaluating nematode persistence, so neither of the EPN species appear to last more than a couple of months in the soil. Additional sampling times is needed in order to better determine longevity of either species in the soil, but the current results demonstrate that the nematodes will need to be applied on an annual basis. As such, these results are still very encouraging, particularly since insecticides, such as chlorpyrifos, and mating disruption are both only preventative management strategies for GRB, but nematodes have the potential to be both preventative and curative. Additional years of research are definitely warranted, especially since GRB has a two-year life cycle, so additional affects may be uncovered in the second year.

In terms of adoption barriers, the grower cooperators were all surprised at how many of their vines were actually infected with GRB. None of the cooperators currently manage for GRB and according to a survey of GA vineyard managers, only 48% actively managing for GRB. This demonstrates that GRB may be a larger or become a larger problem for growers in north GA than currently thought. 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. Another issue may have been due to the method of EPN application. The hand application appeared to work on a small-scale trial, but growers were not convinced that such an application method would be feasible on a larger scale. As such, future projects need to evaluate additional application methods and future extension meetings and publications will focus on educating growers about this stealthy pest and potential management tactic in Southeastern grape production.

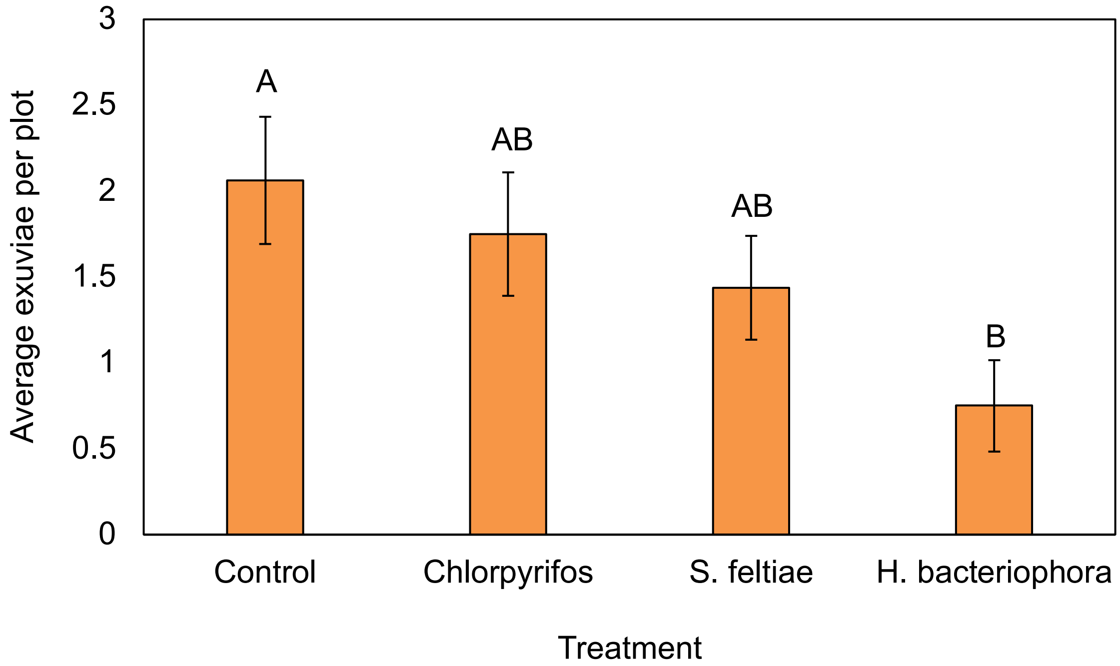

Figure 5. The season average number of exuviae observed near the base the grape vines treatment per plot. Bars with the same letter do not significantly differ (P = 0.05, Student's t).

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