Detecting and Quantifying Populations of Two Common Nematode Enemies of Grape Root Borer (GRB) Larvae in Virginia Vineyards using qPCR

Final Report

Submitted by
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Objectives

1) To use a qPCR-based approach to determine the presence and abundance of nematode natural enemies of grape root borer larvae across Virginia vineyards that differ in soil type.
2) Confirm the virulence of these species to first instar larvae of grape root borer.

Methods: Objective 1

The fourteen vineyard cooperators recruited to participate in this study were distributed among grape production regions as follows: Northern Virginia (5 sites), Shenandoah Valley (3 sites), Central Virginia (3 sites), and Eastern Virginia (3 sites).

None of the vineyards had a recent history of management specifically for GRB. On or around June 27, 2016 paired soil core samples (1" diameter, about 8" long) were taken from 50 vines in each vineyard. One sample was from the drive row next to each vine and the other was from the vine row near the vine base. Soil samples from each vineyard were combined into 5 samples from the drive row and 5 from the vine row, each consisting of about 300g of soil. These were analyzed at Virginia Tech facilities in Blacksburg, VA. A second set of soil samples was collected from each vineyard on or around July 25, 2016 and assessed for EPN presence and abundance using qPCR.

During our first visit to each vineyard, 3 pheromone-baited plastic "bucket" traps were deployed in each block from which soil samples were collected and these were retrieved in mid-August. The GRB captured in each were counted and the mean seasonal capture per trap was calculated for each vineyard.

Soil texture was measured on a 50g subsample of soil using the hydrometer method (Gee and Bauder 1986), a method to separate the soil into its relative sand, silt, and clay fractions. One composite sample per vineyard was assessed. The composite sample was obtained by first subsampling 20g of soil from each of the 10 bagged samples from a vineyard; these subsamples were combined, thoroughly mixed, and then 50g was removed for texture analysis.

Initially, the presence of EPNs in soil samples from the June collection only was assessed by baiting approximately 50g of soil in plastic cups with 10 wax moth larvae. Containers were held at room temperature for 7 days. Larval cadavers showing evidence of infection were individually White trapped (White 1927) to confirm nematode infection. Upon emergence of nematodes from the waxworm cadaver, the nematodes were exposed to new wax moth larvae to confirm that the nematodes were EPN.

Subsequently, assessment of EPN presence and abundance from the June and July samples from each vineyard was conducted using qPCR. To prepare soil samples for qPCR, nematodes were extracted from ~ 500 mL (250g) of soil using an automatic elutriator followed by sugar centrifugation; this was

the most efficient and accurate of the various approaches we tested for EPN DNA isolation in the summer of 2016. Once the nematodes were isolated from the soil, they were digested and their DNA isolated using a PowerSoil DNA isolation kit (MoBio Laboratories Inc, Carlsbad, CA). qPCR analysis was conducted on the isolated DNA using TaqMan probes and a BioRad CFX96 qPCR system (BioRad, Hercules, CA). This approach allows calculation of both EPN presence/absence as well as estimating the number of EPN present in the soil sample. It should be noted that the second species of interest to this project, *Carpocapsae steinernemata*, was not detected using the primers used and here we report the qPCR results for *Heterorhabditis bacteriophora*, as it was the only species confirmed by qPCR.

Methods: Objective 2

From mid-July through early August, project personnel scouted vineyards at and near the Winchester research center for newly emerged GRB female moths. Despite such scouting on multiple occasions and during the period of the day when GRB emergence would be expected (early to mid-morning), we found only 1 female moth, which did not mate when caged with several males and subsequently died. Consequently, we were unable to collect the eggs required to evaluation the virulence of EPNs from vineyard soils virulence to newly hatched GRB larvae. We continue to maintain the isolated EPNs in culture, and may be able to conduct these virulence assays at some point in the future.

Results

GRB Trapping: As we have seen repeatedly over time, average total seasonal captures varied substantially and significantly among the vineyards sampled, ranging from 0.0 – 424.0 males/trap (Fig. 1). The magnitude of captures did not appear to be related to the production area; there were instances of large and smaller captures at vineyards across all four areas.

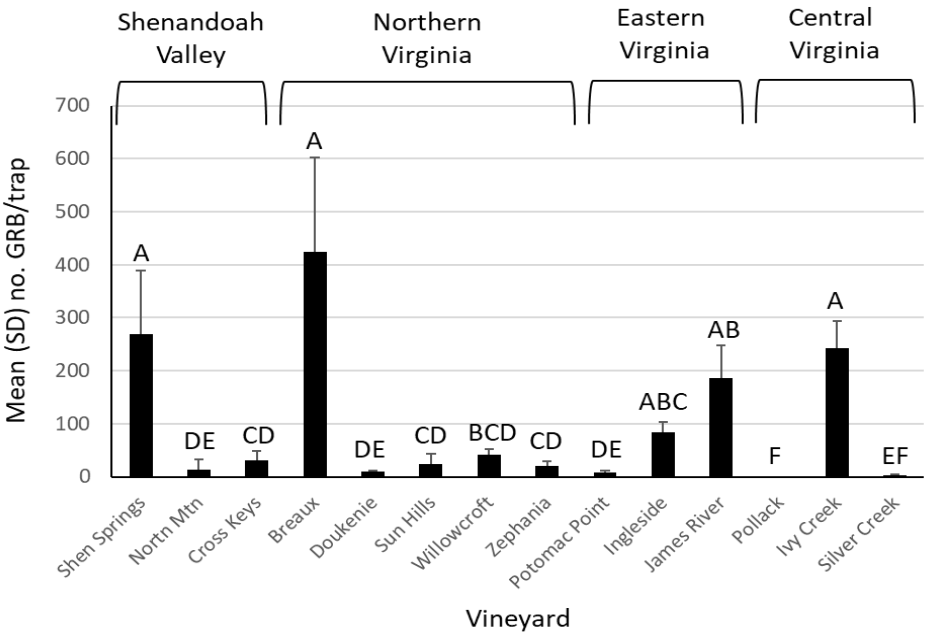

Figure 1. Mean (SD) total seasonal captures of male grape root borer in non-saturating, pheromone-baited bucket traps deployed in 14 vineyards in 4 production regions of Virginia, 2016

In a follow-up survey with our cooperating growers, we determined the total acreage planted to vines and the approximate age of the oldest vines at each farm and used this information in a post-hoc analysis of whether there was a relationship between one or both of these parameters and average total GRB captures. Neither vineyard acreage nor the age of the oldest vines were significantly correlated with captures.

Soil Analysis: As might be expected, soil texture varied across the different production regions, and across vineyards within regions. In general, sand content (one key aspect of soil texture for EPNs) was highest in the Eastern Virginia vineyards and similar, but significantly lower, elsewhere (Fig. 2).

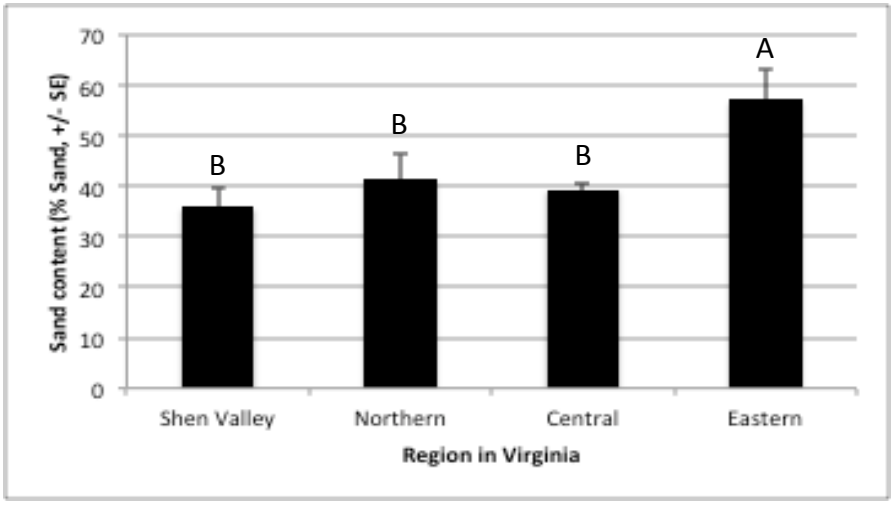

Figure 2. Mean (SE) sand content from soil samples collected from vineyards across four different production regions in Virginia, 2016

Interestingly, the sand:clay ratio showed a more variable pattern than simple sand content. The sand:clay ratios from vineyard soils were highest in Northern and Eastern Virginia, and lower in the other two regions (Fig. 3).

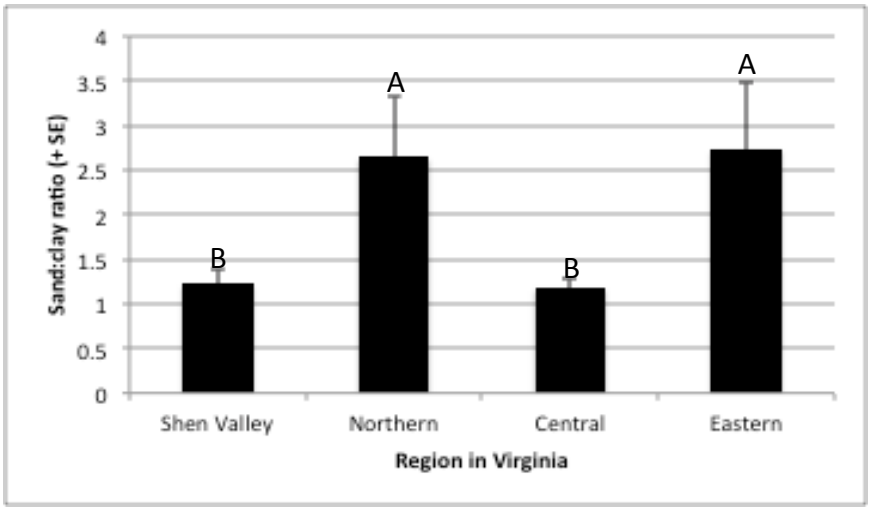

Figure 3. Mean (SE) sand:clay ratio from soil samples collected from vineyards across four production regions in Virginia, 2016

Soil Baiting: Approximately 10% of the June samples produced nematodes following baiting with waxworms. The drive row appeared to have a higher incidence of nematodes, as 70% of the positives were from the drive row and 30% from the vine row. Most of these were confirmed to be entomopathogenic nematodes by re-exposure to waxworms; others were either not parasitized initially by EPN or the infection was taken over by free-living bacterial-feeding nematodes. Five strains have been maintained in culture for eventual screening against GRB; these include 4 different local varieties of *H. bacteriophora*. Notable "hot spot" vineyards for EPN baiting (i.e. where EPNs showed up in multiple rows within the vineyard based on baiting) were Potomac Point and Doukenie, both of which had notably low GRB abundance as measured by bucket traps.

qPCR Analysis: qPCR analysis revealed different patterns in EPN abundance than soil baiting; this has been noted by prior researchers. A higher percentage of samples assayed using qPCR than by soil-baiting tested positive for EPN; 19.6% of soil samples tested in June and 52% of samples tested in July were positive for *H. bacteriophora*. Unlike the results from soil baiting, there was no difference in EPN abundance between the drive row and the vine row.

There were significant differences in *H. bacteriophora* abundance across the 14 vineyards assessed. Averaging across the June and July samples, incidence ranged from a minimum of 0% (*H. bacteriophora* never observed) in two Central Virginia vineyards, to nearly 60% of samples in one Shenandoah Valley vineyard (North Mountain) and one Eastern Virginia vineyard (James River) respectively (Fig. 4).

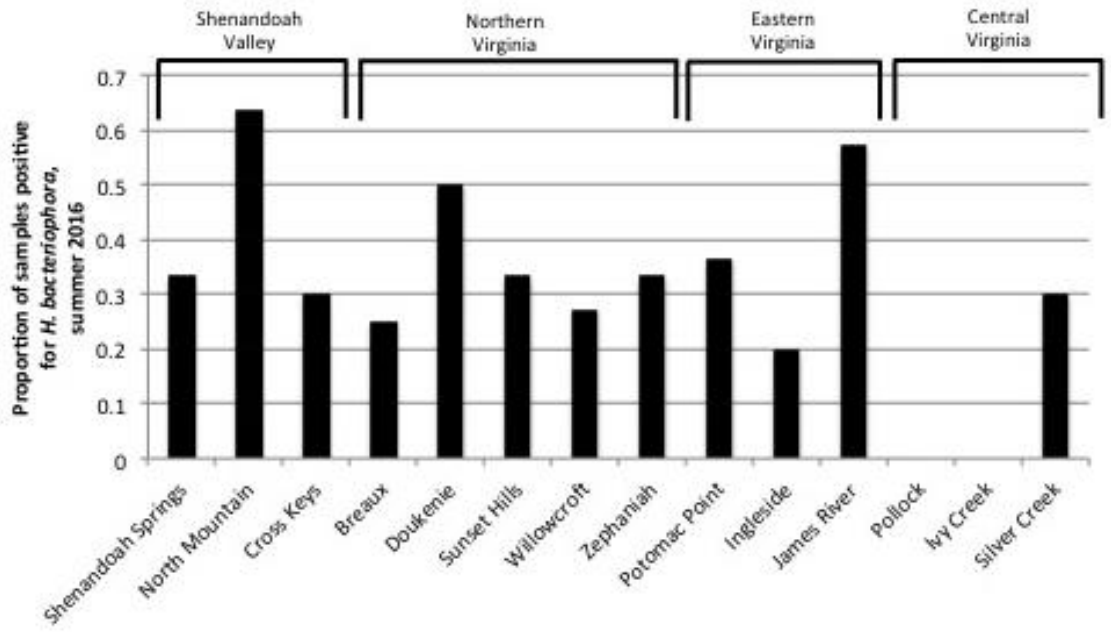

Figure 4. Proportional abundance of *H. bacteriophora* as measured in June and July, 2016

Notable "hot spot" vineyards for qPCR shared similarities to soil baiting results described above: both Doukenie and Potomac Point vineyards had multiple positives from baiting in June and a high presence of EPN from qPCR in July (100% of samples and 66.7%, respectively). Other vineyards that showed

high incidence of *H. bacteriophora* in July samples were North Mountain, Shenandoah Springs, James River, Cross Keys, and Sunset Hills.

The two variables that were the strongest predictors of GRB abundance were EPN assessments from July and vineyard soil clay content. Overall, there was a significant negative correlation between EPN abundance and GRB captures in pheromone traps (r = -0.52, Fig. 5). In general, increasing GRB captures were associated with fewer observations of *H. bacteriophora*, which has demonstrated ability to reduce GRB larval numbers both in the lab and controlled field conditions. In addition, there was a significant negative correlation between vineyard clay content and GRB captures (r = -0.31, Fig. 6); as the amount of clay in the vineyard soil increased, GRB numbers tended to decline.

We used regression techniques to assess multiple models in terms of their value to explain GRB abundance. The optimal model of GRB abundance included vineyard clay content, *H. bacteriophora* abundance as measured in July, and the interaction between the two. This model explained 60% of the variability in GRB abundance ($R^2 = 0.60$); the significant interaction term indicates that the effect of EPN on GRB depends on the clay content of the soil. Including additional variables in the model (other measures of soil texture, vineyard age, vineyard area) did not improve explanatory power.

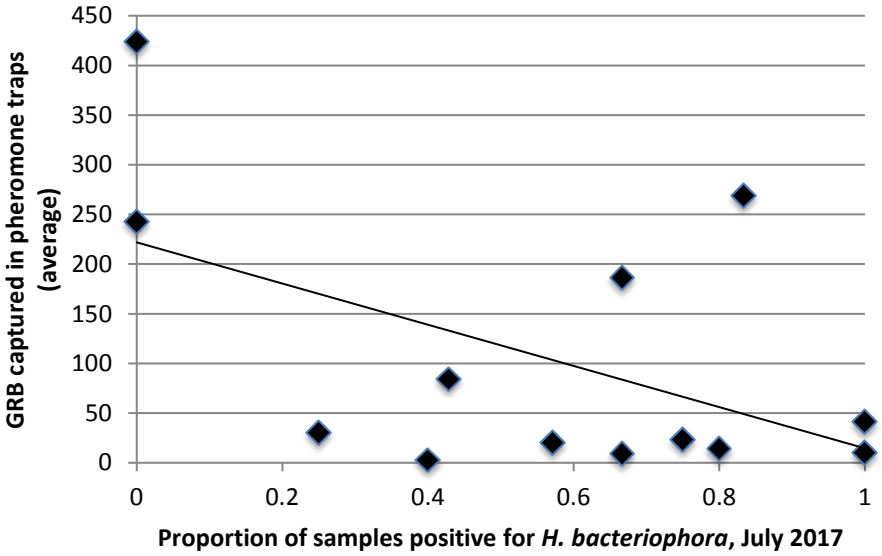

Figure 5. Comparison of *H. bacteriophora* abundance per vineyard and GRB captures

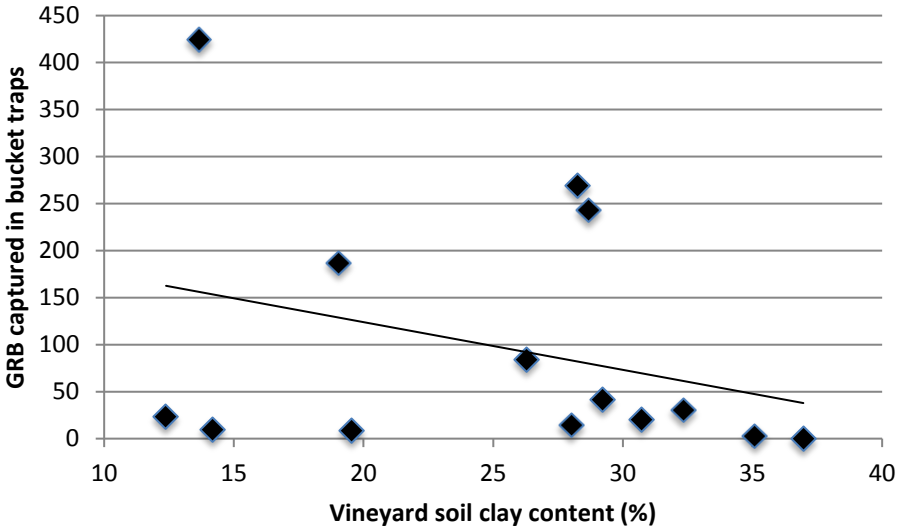

Figure 6. Comparison of vineyard clay content and GRB captures

Summary and Conclusions:

Our use of qPCR to assess the presence of EPN in vineyard soils has clearly demonstrated its sensitivity for evaluating at least one candidate biocontrol agent of GRB, *H. bacteriophora*; many more "positives" for *H. bacteriophora* resulted from use of qPCR than from traditional soil-baiting. It is encouraging that *H. bacteriophora* was detected from the majority of vineyards sampled, given that it is known to be virulent to GRB larvae. However, this was not especially surprising or novel, since this species is known to be widely distributed in nature. Yet, our finding of a negative correlation between *H. bacteriophora* abundance and GRB captures is encouraging and in some vineyards EPN are likely playing a significant role in reducing GRB abundance. However there were "outliers" that did not conform to this trend. For example, there were some vineyards at which high numbers of both GRB and *H. bacteriophora* were recorded, and the activities of EPN are clearly not the entire story across all vineyards. Our data suggest that perhaps a quarter of the variation in GRB abundance in Virginia vineyards may be explained by the abundance of *H. bacteriophora*.

This is an especially challenging system for research, given the subterranean nature of both the pest and the biocontrol agent and the fact that the pest has a 2-year generation time in our area. Sampling GRB pupal cases is by far the best approach for measuring its abundance in vineyards, but is extremely time and labor intensive, ideally requiring weekly sampling during the period of adult emergence (6-8 weeks), and not conducive to work in numerous, widely separated vineyards per season Furthermore, Bergh (2006) showed that pheromone traps for GRB placed in apple orchards near woods captured as many GRB as those in vineyards near woods, so captures of moths in traps can only be used as an approximation of GRB population density in and around vineyards and may or may not reflect the actual infestation status of the blocks sampled. Having said that, our finding that total numbers of GRB captured among blocks were not associated with the area planted to grape does provide some indication that captures reflected GRB densities within the vineyard to at least some degree.

A suggestion for future work is to investigate the effectiveness and persistence of inoculative releases of commercially available (or locally cultured) *H. bacteriophora* in vineyards in which this species is less abundant and which vary according to soil type and texture. These worms are compatible with drip or sprinkler irrigation systems and also can be applied by hand or by mechanical sprayer. Said et al. (2015)

demonstrated that applications of *H. bacteriophora* significantly reduced the presence of GRB exuviae in Florida grapes and *H. bacteriophora* efficacy against GRB has also been demonstrated in lab and greenhouse conditions (Williams et al. 2002). Applications of *H. bacteriophora* may be particularly appropriate in Virginia vineyards with high populations of GRB, using pheromone traps as a proxy for relative GRB density.

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