Assessing the Impact of Naturally-Occurring Entomopathogenic Nematodes on Grape Root Borer Infestations in Virginia Vineyards: Final Report

Submitted by Chris Bergh
July 20, 2015

Objective 1. To determine the presence and species of native, soil-dwelling entomopathogenic nematodes in Virginia vineyards

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Protocols: Twelve vineyards in northern and central Virginia were selected. At each, 1 block of ≥6-year-old vines was chosen; these were roughly equally split between Chardonnay and Cabernet Franc and had not been managed specifically for grape root borer (GRB). In May and early June, a grid of 46 – 50 sample vines was created in each block. To obtain independent samples (Rijal et al. 2014), vines in the grid were spaced at about 10 m' apart and occurred in a zig-zag pattern across 3-4 consecutive rows, according to row spacing. Ten sample vines in each sample row were marked and the vine grid encompassed between 9 and 13 rows, depending on row spacing. In June, one 10" long soil core was taken from the drive row next to each sample vine. To account for the spatial segregation of different species of entomopathogenic nematodes (EPNs) in the soil, each core was immediately separated into the top 2" and the bottom 8" sections, which were placed, respectively, in 4 oz and 8 oz plastic lidded cups. In the laboratory, 5 and 10 wax moth larvae, respectively, were placed in the 4 oz and 8 oz cups. The cups were capped and held in a dark room at ambient temperature for 5-7 days, after which the larvae were evaluated for mortality and evidence of EPN infection. EPN infection was suggested based on the color and consistency of the larval cadaver.

Cadavers showing evidence of EPN infection were placed in "White" traps (White 1927), consisting of a piece of damp filter paper in a covered Petri dish, and these stored under the conditions described previously for another 5-7 days, after which the presence of "infective juvenile" (IJ) nematodes emerging from each cadaver was assessed.

To confirm that the IJ's recovered were the cause of larval wax moth mortality (known as Koch's postulate), IJs from each dish were placed in autoclaved soil with 5 new wax moth larvae and held as described previously. Upon inspection several days later, infected cadavers were again White trapped and the IJs produced were stored in 95% ethanol for later species identification.

Results: Based only on larval color change, wax moth larvae showed evidence of possible EPN infection in all 12 vineyards (Fig. 1A,B). Cadavers showed the range of color changes anticipated, including beige, gray, red, and orange, suggesting the presence of EPNs from different nematode families and species. In addition, some cups from all vineyards showed cadavers of ≥2 colors (Fig. 2), suggesting the presence two or more species or families of EPNs in the same soil sample.

In 11 of the 12 vineyards, some vines showed evidence of EPN infection from both the top 2" (4 oz cups) and bottom 8" of soil (8 oz cups), suggesting the presence of different EPN species and families that segregate within the soil profile, although the percentage of vines showing this result varied substantially among the vineyards (Fig. 3).

When the colored cadavers were White trapped, we found that the majority of them showed infection by fungi at the time of evaluation and did not produce IJs, which precluded confirmation of EPN infection. However, some cadavers did produce of IJs following White trapping and there were essentially equal numbers of cups with larvae exposed to the top 2" of soil core in 4 oz cups (n = 28) and to the bottom 8" of core in 8 oz cups (n = 27) from which IJs were recovered (Fig. 4). Among these larvae, there were again examples of all anticipated color changes (Fig. 5).

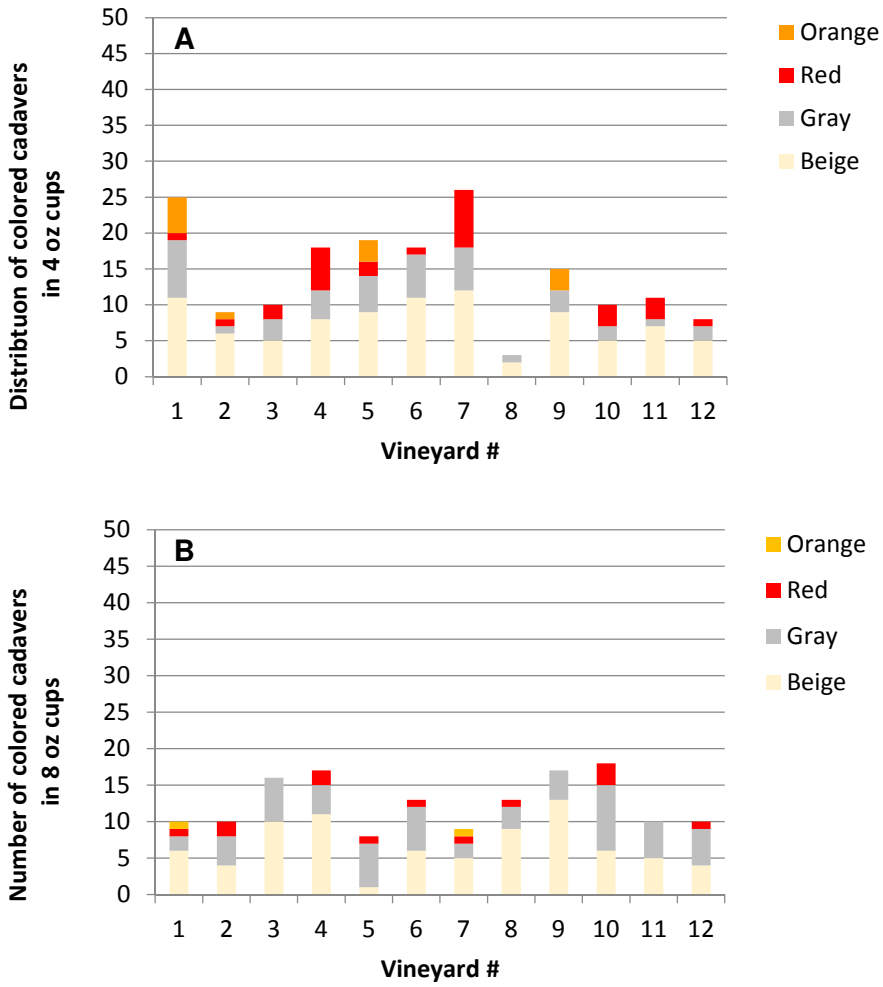

Figure 1. Distribution of wax moth larvae in A) 4 oz cups and B) 8 oz cups (n = 46-50 each) showing evidence of EPN infection based on the presence of ≥1 cadaver/cup showing one color following exposure to vineyard soil. Different cadaver colors suggest infection by different species or families of EPNs.

Since so few cadavers showing apparent infection by EPNs following White trapping were available for subsequent confirmatory bioassays, the small amount of data collected did not contribute further to the outcome and are not presented.

Objective 2: To examine the relationship between the vineyard soil characteristics and the presence of native entomopathogenic nematodes

Protocols: A second set of soil cores was collected from each vineyard block in August (1 core from each corner and 1 from the middle). These were combined, mixed and air-dried thoroughly, and then taken to Virginia Tech for analysis.

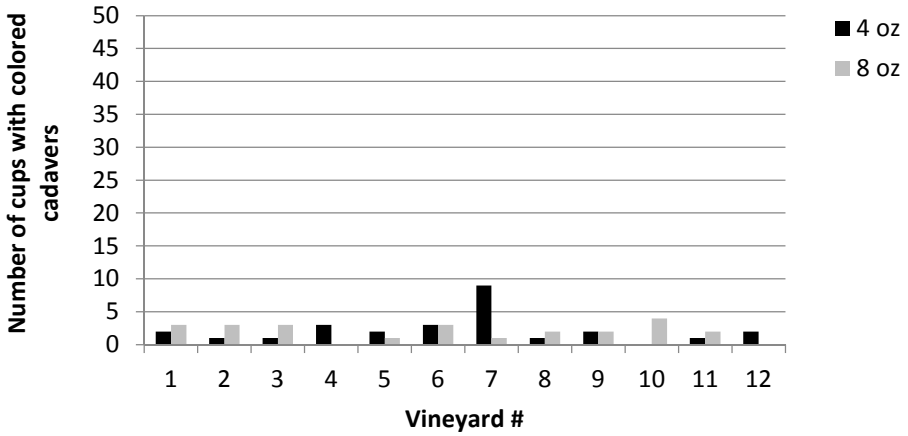

Figure 2. Number of cups (n = 46-50) with wax moth larvae showing evidence of infection by two or more species or families of EPNs, based on cadavers of two or more colors following exposure to vineyard soil.

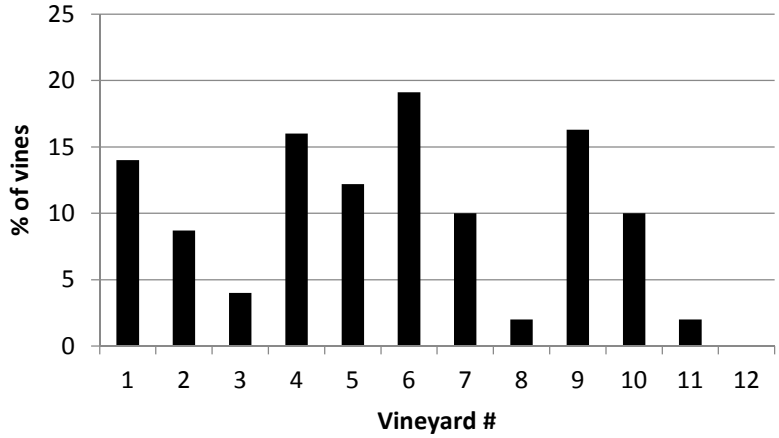

Figure 3. Percentage of vines (n = 46–50 vines) with indication of EPN infection in both 4 oz and 8 oz cups (i.e. top 2" and bottom 8" of soil core, respectively).

Results: Soil analysis at Virginia Tech was initiated in fall 2014, but affected by an unknown cause of re-wetting during storage in the soils laboratory. This was not expected to affect subsequent analyses during winter 2015, and the samples were re-dried. Unfortunately, the student responsible for completing this analysis failed to do so, despite repeated requests and reminders.

Objective 3: Correlate the intensity of entomopathogenic nematode populations in Virginia vineyards with the population density of grape root borer

Protocols: One of the 12 vineyards from which soil samples had been collected and assayed was ultimately unable to participate in the GRB pupal case monitoring, resulting in a total of 11 vineyards from which a complete data set was generated. To enable accurate sampling of GRB pupal cases, a ~1 m diam area around the base of all sample vines in each vineyard was cleaned to the soil surface using weed-trimmers and by raking in early July and these areas were kept free of new vegetation by regular hand-weeding subsequently. At weekly intervals for 5 weeks between mid-July and mid-August, GRB pupal cases were counted and removed from the cleaned area around each vine.

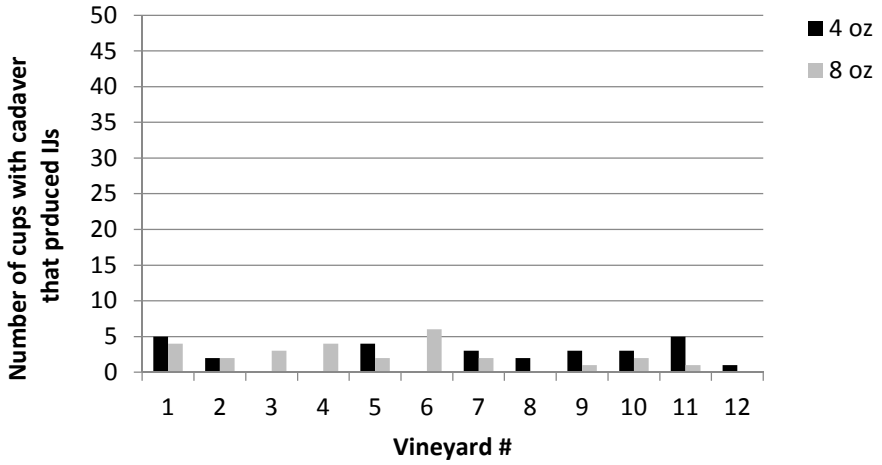

Figure 4. Number of cups (n = 46-50) that produced injective juvenile (IJ) nematodes after White trapping.

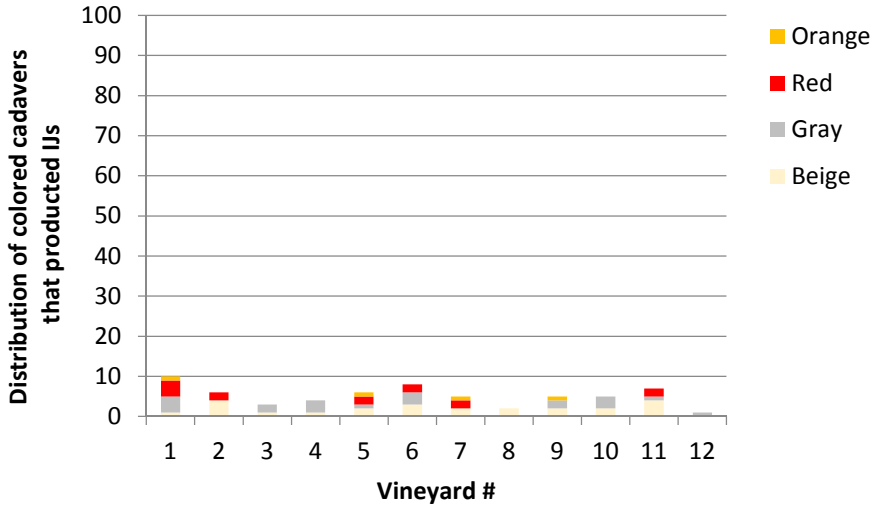

Figure 5. Distribution of wax moth larvae in 4 oz plus 8 oz cups (n = 100 cups) showing evidence of infection by EPNs that produced infective juveniles (IJs) following White trapping.

Results: GRB pupal cases were found in 10 of the 11 vineyards sampled (Fig. 6). Across all vineyards, mean total numbers of pupal cases collected per vine ranged 0.0 to 1.35 per vine (Fig. 7). Based on Rijal et al.'s (2014) conclusion that <0.1 and ≥0.1 pupal exuviae per vine represent lightly and more heavily infested vineyards, respectively, four of the blocks sampled would be considered lightly infested and the remaining 7 blocks more heavily infested.

Preliminary analyses suggested a significant positive relationship between the number of 4 oz cups (top 2" of soil core) with ≥1 EPN-infected larvae (based on IJ production after initial White trapping) and the mean number of GRB pupal cases per vine (Spearman's rank correlation = 0.7484, $P = 0.0081$, r = 0.6026) or the number of vines with ≥1 pupal case (Spearman's rank correlation = 0.7180, $P = 0.0128$, r = 0.7164). For 8 oz cups (bottom 8" of soil core) or 4 oz and 8 oz cups combined, there was not a significant correlation between the number of with ≥1 EPN-infected larvae and GRB infestation.

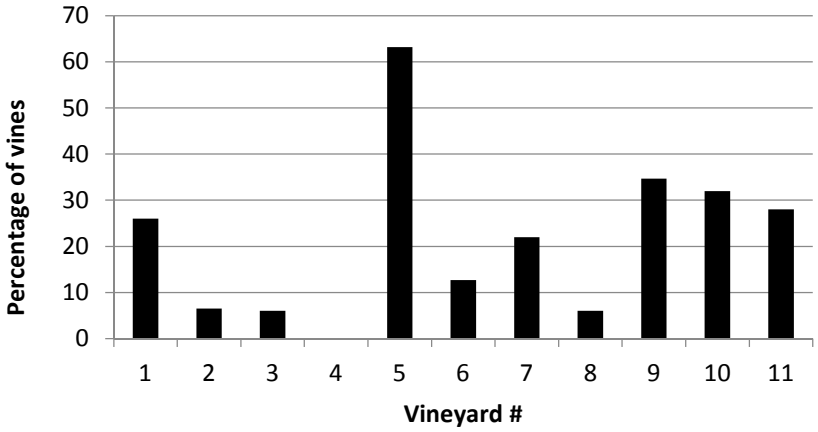

Figure 6. Percentage of vines (n = 46-50 vines) with ≥1 grape root borer pupal exuviae per vine.

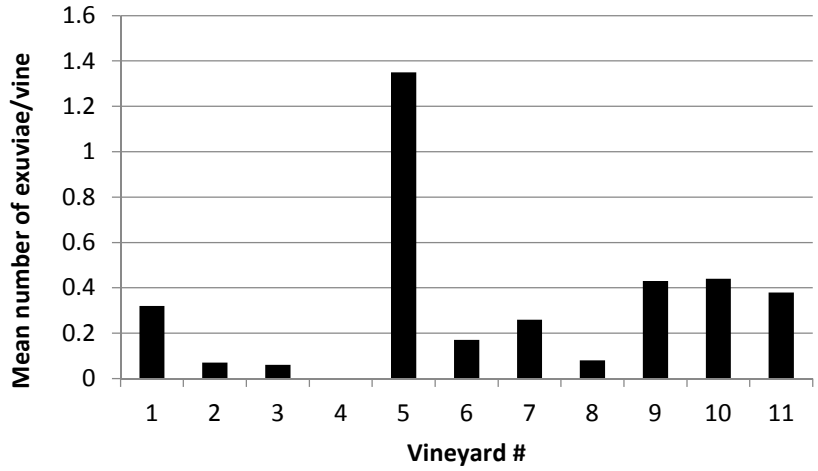

Figure 7. Mean number of grape root borer pupal exuviae per vine.

Summary: These results have again demonstrated that vineyard blocks in Virginia vary substantially in the degree of infestation by larvae of GRB. The issues associated with fungal infection during White trapping and their effects on potential confirmation of EPN infection likely weakened the potential strength of this "story". However, despite this issue, the finding that wax moth larvae showed indications of infection by a range of EPN families and species (based on color change) following their exposure to vineyard soils is very encouraging. Furthermore, EPN infection was partially confirmed for a number of larvae, based on IJ production. The soil baiting technique that we used has long been considered the "gold standard" protocol (Gruner et al. 2007) and has been widely employed. However, Gruner et al. (2007) discussed the fungal infection issue using this approach and developed a new technique that is gaining acceptance. Going forward, we propose to use this much "cleaner" method to survey for the presence and abundance of EPNs in vineyard soils, using the technique that involves inserting a centrifuge tube baited with wax moth larvae into holes in the vineyard soil. This method greatly reduces contamination of larvae from other pathogens, likely draws EPNs to the baited trap over larger distances due to the CO_2 emissions from the larvae, and has been shown to be at least as sensitive to EPN presence as baiting soil cores.

Appendix

Impact Statement

Most Virginia vineyards are infested by root-feeding larvae of the grape root borer, although the extent of infestation varies widely and growers are often unaware of a serious infestation until adverse effects on vine health have become apparent. Entomopathogenic nematodes that attack and kill pest insects, including larvae of grape root borer, were shown to co-occur with grape root borer in vineyard soils in Virginia. Although previously unstudied in Virginia vineyards, these nematodes may be an important natural cause of larval grape root borer mortality; thus, understanding their distribution is expected to yield new insights into why vineyards differ in the extent of grape root borer infestation and enable more precise predictions of vineyard susceptibility from this insidious pest.

Hi Dave,

Attached please find the Final Report for our project on entomopathogenic nematodes in Virginia vineyards. As you will see, it is nearly identical to the mid-term report submitted in January 2015. The MS student who was working on this project had some personal issues arise in fall semester and was unable to maintain a grade point average that met the minimum required during spring semester. In combination, these resulted in her resignation at the end of spring semester. I continued to support her through the end of that term so that she could write her exams and complete the soil sample analysis that we had agreed upon. To my disappointment, she failed to complete the soil analysis component of the research. All other aspects of the data are represented as well as possible. This summer I am continuing to work on the project on a more limited scale, using funds from my discretionary "field-testing" account, with the hope that our new soil baiting technique will overcome the issues of fungal contamination of the wax moth cadavers that plagued our work in 2014. I apologize for my lack of ability to provide definitive conclusions from the project, which was affected badly by the situation with the student and by the issues mentioned last summer. As you know, I declined further support for the project this year, but will continue to work on it going forward.

Please let me know if you have any questions.

Best regards - Chris

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