Report to the Virginia Wine Board

Behavioral Response of Grape Root Borer Larvae to Chemical Stimuli from Grape Roots

Submitted by Chris Bergh
February 7, 2014

Objective 1: Measure the attraction of grape root borer larvae to individual compounds and blends of compounds from grape root volatiles in bioassay arenas

In 2013 we not able to isolate the quantity of root headspace volatile compounds of interest (see Appendix Fig. 1) that was needed to implement the experiments of Objective 1. A search for these compounds from several sources revealed that two of three of them were not commercially available.

General Methods (Objectives 2-4)

The soil used for bioassays of Objectives 2-4 was collected from a field next to a grape root borer infested vineyard in Virginia and spread and dried thoroughly in a greenhouse. All rocks, dried plant parts, etc. were removed and the soil was sifted. Soil columns were constructed from PVC pipes filled with the soil, which had been brought to 25% moisture content. The columns were prepared and capped to prevent evaporation about 16 hours prior to initiating assays. For Objectives 2 and 4, pipes of 2.5 cm ID were used, while pipes of 1.25 cm ID were used for Objective 3. For Objectives 2 and 4, in which straight sections of vertically or horizontally oriented pipes were used (See Appendix Figs. 2 and 3), a PVC adaptor containing a fitted piece of metal screen to prevent soil from falling out of the pipe was inserted over the bottom of each pipe. A round-bottomed PVC cap was placed over the adaptor. For Objective 2, in which only pipes with grape root pieces were used, the bottom cap contained a moist sponge plug with a circular piece of filter paper covering its top, on which grape roots pieces were presented. For Objectives 3-4, which involved comparisons of larval response in pipes with and without grape root stimuli, circular discs were cut from the sticky liner of pheromone traps and a 2 cm diam was hole cut in the middle of each. These were placed atop the filter paper on the damp sponge. Stimulus treatments were placed in the hole in the sticky circle; controls contained no stimulus.

For Objective 3, the straight section of a Y-adaptor was inserted into the bottom of a 10 cm long section of pipe (See Appendix Fig 4). Black tape was used to prevent light penetration through the exposed parts of the Y-adaptor. A section of PVC pipe (5 or 7.5 cm long) was inserted over both arms of the Y-adaptor. A PVC adaptor containing a circular piece of metal screen was placed at the end of each PVC section and the bottom cap was placed at the end of these.

Bioassays were conducted in laboratory rooms or in an environmental chamber at 25.6 ^{o}C ± 0.12 SE and 68.2% ± 0.42 RH. The stimulus sources used included two freshly collected pieces of 420-A roots or filter paper discs treated with an ethanol based extract of 420-A roots. Grape root borer eggs were obtained from females collected in vineyards and the larvae used were from those eggs. Eggs from which larvae were in the process of hatching or newly hatched larvae were used in bioassays. Eggs or larvae were transferred to small glass dishes (1 per dish). One dish was placed on the soil in each column (See Appendix Fig. 3). At intervals that varied

according to the experiment, the bottom cap was removed from each pipe and inspected for the presence of the larva.

Objective 2: Measure the movement capacity of grape root borer larvae in soil column bioassays

Vertical columns

- 15, 30, 60, 90, 120 cm long vertical columns with root pieces (n = 10/column length)
- Larval presence evaluated at 24-h intervals for 5 d
- Data analyzed using one-way ANOVA

Results

- After 24 h, there was a significant effect of vertical column length on the number of larvae recovered. Significantly fewer larvae were recovered from the bottom of 90 cm columns than the others, which differed numerically but not significantly (Fig. 1)
- Larvae were recovered from the 120 cm vertical column after ≥48 h (Fig. 1)

Horizontal columns

- 30 cm long horizontal columns (n = 15) with a hole in the middle (See Appendix Fig. 3)
- Root pieces in cap at bottom of perpendicular sections at both ends
- Larval presence in caps at both ends evaluated at 24-h intervals for 3 d

Results

- No larvae were recovered from either end of horizontal columns after 24 h. After 48 and 72 hr, respectively, the cumulative number of larvae recovered was 10 and 14, and there was no indication of differences in larval movement to either end (Fig. 2).

Objective 3: Measure larval grape root borer response to grape root stimuli in a Y-tube bioassay

- 10-12, vertically oriented Y-tubes per repetition with 3 repetitions
- Fresh root vs no root or filter paper discs treated with root extract vs discs treated with ethanol. Paired stimuli presented in opposing arms of the Y.
- Larval presence at end of both arms recorded at 24 h intervals for 72 h
- Data from three repetitions were pooled and analyzed using the binomial test.

Results

- 5.0 cm arms: The number of larvae recovered from the arm with root pieces (n = 20) was significantly greater than from the control arm without roots (n = 2)
- 7.5 cm long arms: There was no significant difference in the number of larvae recovered from the arm with root pieces (n = 15) versus the control arm without roots (n = 11)
- 50 cm arms: There was not a significant difference in the larval response to arms with root extract (n = 14) vs with ethanol treated filter paper (n = 9).

Objective 4: Measure the effect of grape root stimuli on the rate and success of larval grape root borer food-finding in a vertical soil column bioassay

Effect of presence/absence of root pieces on larval recovery

- 15 cm vertical columns (n = 10/treatment with 3 repetitions)
- Presence or absence of fresh root pieces at bottom of column
- Presence of larva in bottom cap recorded at 24-h intervals for 3 d
- Data from three repetitions pooled and analyzed using a 2×2 contingency table

Results

- There was not a significant effect of the presence of root pieces on the number of larvae recovered from the bottom of 15 cm columns. Of the 30 larvae per treatment, 24 and 20 were recovered from the bottom of columns with and without root pieces, respectively.

Rate of food finding

- 15, 30, 60 cm long vertical soil columns with and without root pieces (n = 5/treatment with 3 repetitions)
- Larval presence in the bottom cap evaluated at 8-h intervals for 72 h
- Data from three repetitions pooled and analyzed using a 2×3 contingency table

Results

- Over 72 h, all larvae released were recovered from the bottom of 15 cm columns, with decreasing numbers recovered as column length increased.
- There was not a significant difference between the cumulative number of larvae recovered at the bottom of three column lengths with and without root pieces at any evaluation interval (Fig. 3)

Effect of grape stimulus in soil column on rate and success of food finding

- Y-tube bioassays (Objective 3) suggested that larvae did not respond to root extract over a distance of 5 cm but did respond grape root pieces. Therefore, grape root pieces were enclosed in a fine mesh screen and incorporated into the soil in 15 cm long vertical columns with grape root pieces in the end cap. Control columns had root pieces only in the end cap, and 6 replicates of each treatment were tested. The bottom cap was examined for larvae at 12-h intervals for 48 h.

Results

- The number of larvae recovered from the bottom cap at each 12-h interval was the same or nearly the same between the columns with and without caged root pieces incorporated into the column. Soil incorporated root pieces did not affect the rate or success of larval movement through the column.

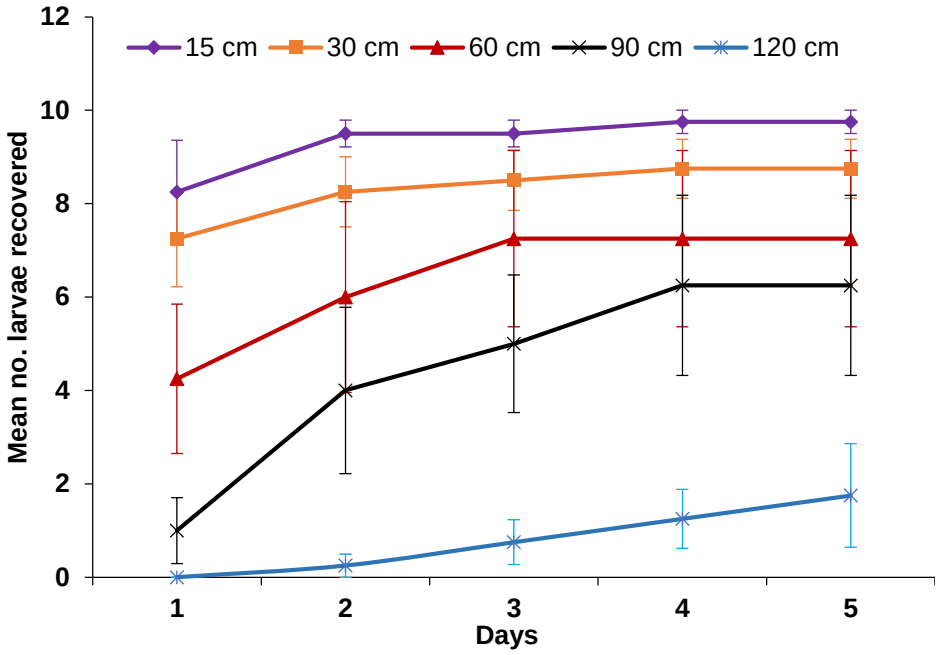

Fig. 1. Mean (±SE) number of grape root borer larvae recovered from the bottom of vertical soil columns at 24-h intervals for five days

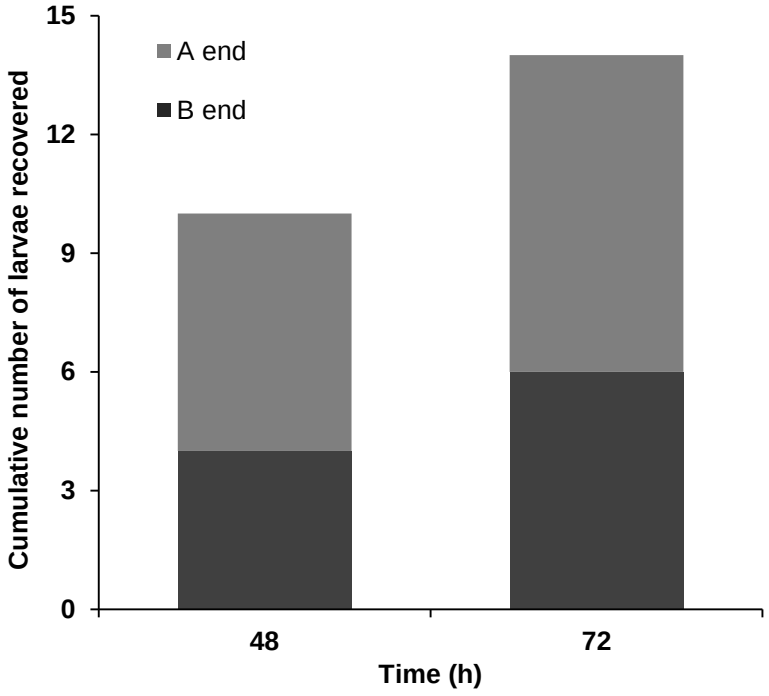

Fig. 2. Cumulative number of grape root borer larvae recovered from both ends of 30 cm horizontal soil columns at 24-h intervals (n = 15 larvae in total)

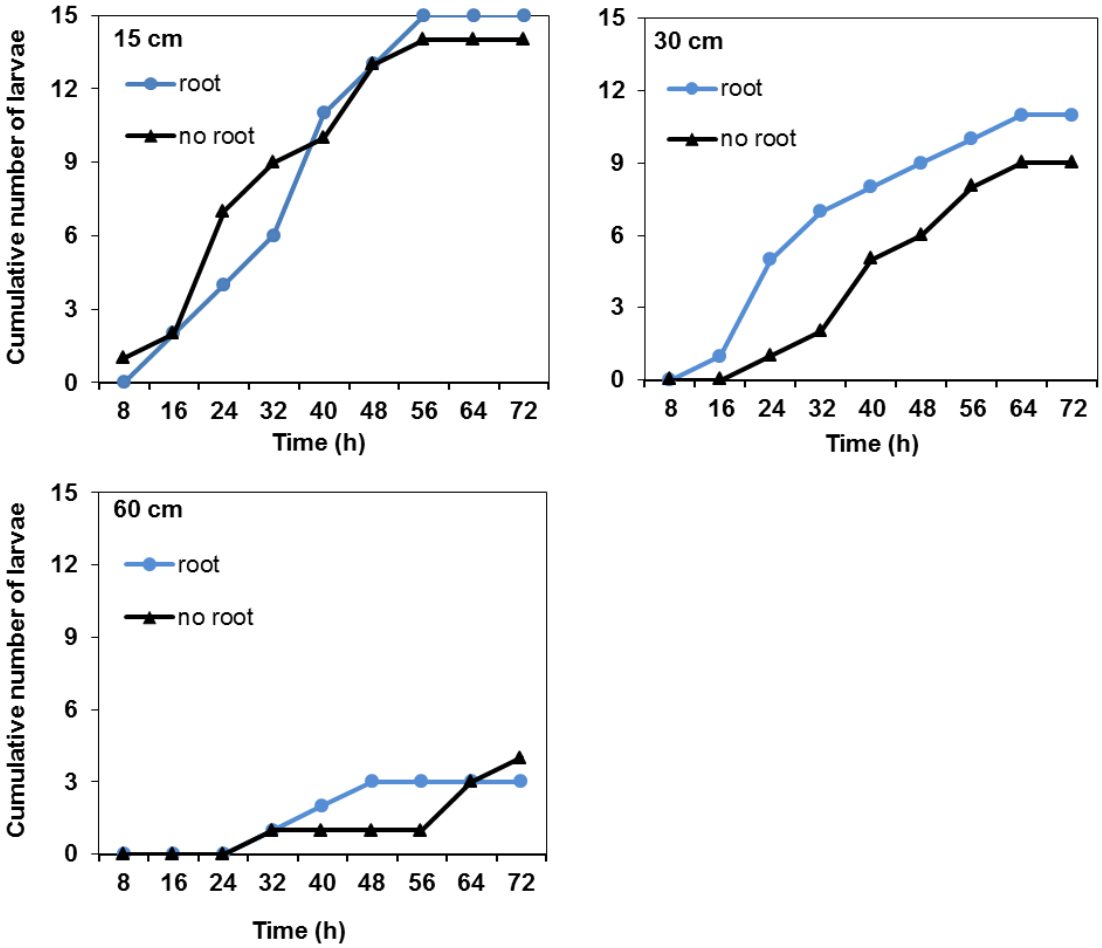

Fig. 3. Cumulative number of grape root borer larvae at the bottom of 15, 30, and 60 cm soil columns with and without root pieces at 8h intervals over 72h (n = 15 larvae per treatment)

Additional figures depicting the bioassay used are included in the Appendix, which can be obtained from David A. Robishaw, Jr., VDACS, 900 Natural Resources Dr., Suite 300, Charlottesville, VA 22903 (434-984-0573; david.robishaw@vdacs.virginia.gov

Outreach Presentations in 2013

Rijal, JP. 2013. Grape root borer infestation status in Virginia vineyards and management options. Viticulture IPM workshop, 21 March (northern Virginia)

Rijal, JP. 2013. Grape root borer infestation status in Virginia vineyards and management options. Viticulture IPM workshop, 9 April (central Virginia)

Appendix

Impact Statement

A novel and ecologically relevant bioassay method was developed to assess the behavioral response of larval grape root borer to grape root stimuli and their food-finding capabilities in the soil. Demonstration of the utility of this bioassay and the results of basic biological studies from its use provide a foundation for investigating the effects of different vineyard soils on larval grape root borer food-finding and the role of naturally-occurring nematodes in Virginia vineyard soils as biological control agents for this widely distributed pest.

Publications

A manuscript from the research presented herein is in preparation. Research by the same student that was supported by the Virginia Wine Board during his doctoral studies has been published or submitted, as follows:

Rijal, JP, A Zhang, D. Lee and JC Bergh. 2013. Behavioral response of grape root borer (Lepidoptera: Sesiidae) neonates to grape root volatiles. Environ. Entomol. 42: 1338-1347.

Rijal, JP, CC Brewster and JC Bergh. 2014. Spatial distribution of grape root borer (Lepidoptera: Sesiidae) infestations in Virginia vineyards and implications for sampling. Environ. Entomol., in press.

Rijal, JP, CC Brewster and JC Bergh. Biotic and abiotic factors associated with grape root borer (Lepidoptera: Sesiidae) infestations in commercial vineyards. Submitted to Environ. Entomol., February 2014.

Awards

In January, 2014, Mr. Jhalendra Rijal was awarded the Graduate Student Friends of IPM Award from the Southern Region IPM Center for his work with grape root borer. This prestigious award is given to one PhD student selected from a pool of nominees from Entomology departments throughout the Southern region (1 nominee per department).

Fig. 1. Grape root head-space volatile collection at USDA-ARS facility, Beltsville, MD

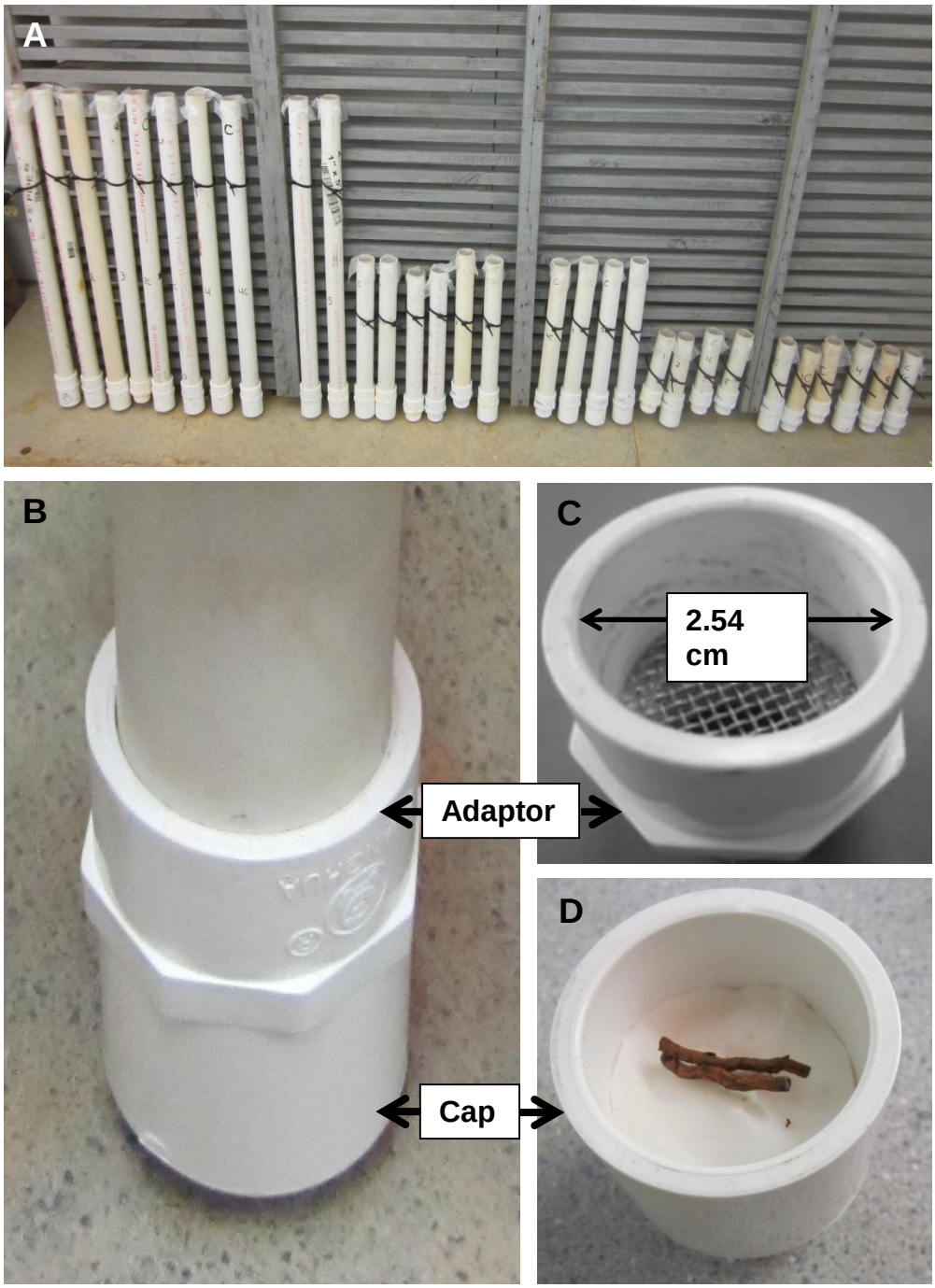

Fig. 2. Components of soil column bioassay showing: A) vertical pipes of different lengths, B) adaptor/cap assembly at the bottom of pipes, C) adaptor with metal screen, and D) bottom cap with moist filter paper and grape root pieces.

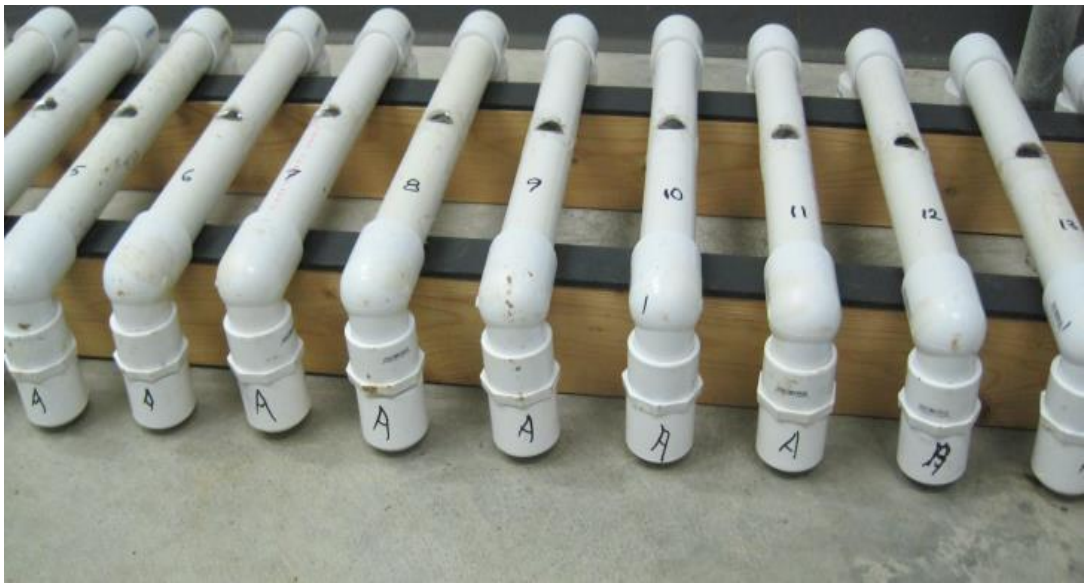

Fig. 3. Horizontal pipes with center hole for introducing grape root borer egg

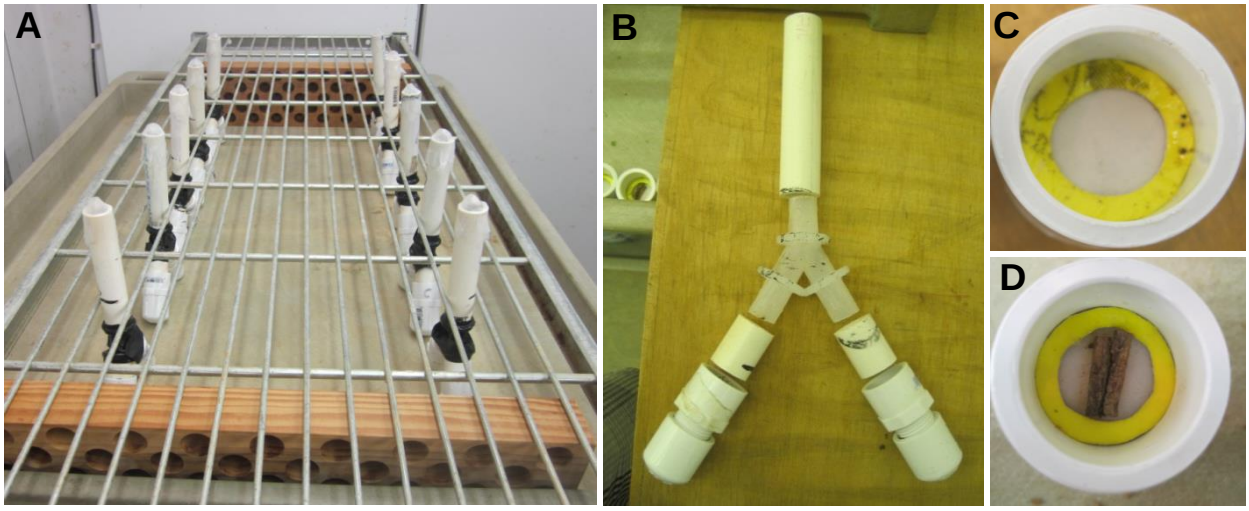

Fig. 4. Y-tube bioassay showing: A) bioassays in frame within a controlled-environment room, B) Y-tube assembly, C) bottom cap without grape root pieces, and D) bottom cap with grape root pieces.