Food-Finding Capability of Grape Root Borer (Lepidoptera: Sesiidae) Neonates in Soil Column Bioassays

Author(s): J.P. Rijal and J.C. Bergh
Source: Journal of Entomological Science, 51(1):54-68.
Published By: Georgia Entomological Society
DOI: http://dx.doi.org/10.18474/JES15-21.1
URL: http://www.bioone.org/doi/full/10.18474/JES15-21.1

Food-Finding Capability of Grape Root Borer (Lepidoptera: Sesiidae) Neonates in Soil Column Bioassays[1]

J.P. Rijal[2] and J.C. Bergh

Department of Entomology, Virginia Tech, Alson H. Smith, Jr. Agricultural Research and Extension Center, 595 Laurel Grove Road, Winchester, Virginia 22602 USA

J. Entomol. Sci. 51(1): 54–68 (January 2016)

Abstract Grape root borer, *Vitacea polistiformis* (Harris), is an economically important and potentially destructive pest of grape vines in portions of the eastern United States. Its oligophagous larvae feed on the roots of cultivated and wild *Vitis* species. In commercial vineyards, adult females oviposit on aboveground parts of vines and other vegetation in vine rows. The loosely attached eggs are thought to drop to the ground and, upon eclosion, neonates quickly burrow into the soil to search for grape roots. Although vineyard infestation by grape root borer is ultimately dependent upon larval success at finding and establishing on vine roots, little is known about larval movement capabilities in the soil. In this study, soil column bioassays were used to evaluate neonate movement in the horizontal and vertical dimensions, the influence of grape root stimuli on the rate and frequency of food-finding, and the distance over which the neonates responded to these stimuli. Grape root borer neonates moved both horizontally and vertically in the soil. In vertical columns, larvae located pieces of grape root over distances up to 120 cm, although the presence of food at the bottom of columns did not affect the frequency or rate of movement to the bottom. Larvae appeared to respond to fresh grape root pieces over a distance of 5 cm in soil. Results are discussed in relation to the utility of these soil column assays for potential future studies of grape root borer food-finding and management in vineyards.

Key Words *Vitacea polistiformis*, *Vitis*, roots, bioassay, larval movement

The grape root borer, *Vitacea polistiformis* (Harris) is indigenous to the eastern United States and has been a chronic viticultural problem in parts of this region for over 100 yr (Bergh 2012, Brooks 1907, Clark and Enns 1964, Dutcher and All 1976, Harris 1854, Pollet 1975). Larvae are oligophagous and feed on roots of plants in the Vitaceae. Grape root borer infestations in commercial vineyards originate from populations on species of wild *Vitis* that are common throughout much of the eastern grape production area (All et al. 1987, Bergh 2006, Brooks 1907, Snow et al. 1991). All commercially important *Vitis* cultivars, rootstocks, and species are considered vulnerable (Johnson et al. 1981, Rijal et al. 2014a, Wylie 1972, Wylie and Johnson 1978) and it has impacted the production of both wine and table grapes from Ohio to Florida and Missouri to Mississippi (Brooks 1918, Dutcher and

[1]Received 08 April 2015; accepted for publication 01 June 2015.
[2]Corresponding author (email: jrijal@ucdavis.edu), current address: Department of Entomology and Nematology, University of California–Davis, One Shields Ave., Davis, California 95616 USA.

All 1976, Harris et al. 1994, Johnson et al. 1981, Pollet 1975, Rijal et al. 2014a, Snow et al. 1991, Webb et al. 1992).

In commercial vineyards, female grape root borers oviposit on aboveground parts of grape vines and on vegetation in the vine rows (Brooks 1907, Clark and Enns 1964, Dutcher and All 1979a, Sorensen 1975). Eggs are thought to be easily dislodged from plant surfaces and many may drop to the soil surface (Brooks 1907, Wylie and Johnson 1978). Upon eclosion, the tiny neonates must burrow into the soil to search for roots (Brooks 1918, Clark and Enns 1964), on which they feed for periods ranging from 1 to 3 yr across different portions of the grape root borer's geographic range (reviewed in Bergh 2012).

Larval feeding can completely destroy smaller roots (Dutcher and All 1979b; Sarai 1972) and a single larva has the potential to kill a vine from girdling at the base (Dutcher and All 1976, Pollet 1975). Symptoms of grape root borer feeding on roots, such as smaller leaves, reduced shoot growth, fewer and smaller berries, and vine wilting (All et al. 1987, Sorensen 1975), are indistinguishable from those associated with some other pathological or horticultural conditions. All et al. (1987) reported that infested vines usually began to show symptoms after 5 to 10 yr of feeding and declined progressively over 3 to 5 yr. Because of the lack of diagnostic symptoms on the aboveground parts of vines, detection and management of grape root borer infestations often does not occur until vines show symptoms of significant decline (Bergh 2012).

Despite the prevalence of grape root borer infestations among vineyards in Virginia (Rijal et al. 2014a) and elsewhere, the food-finding capacity of larvae and their interactions with grape roots remain poorly understood. Brooks (1907) reported that grape root borer larvae can penetrate soil to depths of 5 to 30 cm, although he recovered one individual from roots at a depth of 80 cm. Larval food-finding is likely influenced by biotic and abiotic factors that affect their survival and movement through the soil and by their ability to detect roots. Using 27.9-cm-long plastic containers containing soil and with fresh grape root pieces (~5 cm long) at the bottom, Sarai (1972) found that the survivorship of grape root borer larvae increased with increasing soil moisture and decreased with increasing soil depth over depths of up to 25 cm. Similarly, Rijal et al. (2014a) found that soil clay-to-sand ratio and water holding capacity were the factors most strongly associated with differences in the extent of grape root borer infestation among commercial vineyards in Virginia. Using small arenas in the laboratory, Bergh et al. (2011) showed that grape root borer neonates exhibited a positive behavioral response to ethanol-based extracts of grape roots. Subsequently, Rijal et al. (2013) used modified arenas to reveal that this response was mediated by grape root volatiles and that larvae responded to root volatiles emanating from small pieces of buried grape roots.

To improve upon the ecological relevance of our previous laboratory-based research on food-finding by grape root borer larvae and their interactions with grape roots, here we report the development and use of soil column assays to address basic questions about their movement capabilities in the horizontal and vertical dimensions, the influence of grape root stimuli on their success and rate of food-finding, and the distance over which they respond to root stimuli in soil.

Materials and Methods

Source of insects and soil. Virgin grape root borer females sitting on the vine trunk and mated females flying or in the process of ovipositing were collected by scouting vineyards between 9 a.m. and 11 a.m. in July and August. Virgins were placed in a cage (35 × 35 × 35 cm) with male moths that had been netted while responding to a pheromone lure and observed periodically for mating. All mated females were held in screened plastic cages (14 cm diam × 12 cm deep) in shaded conditions outdoors, and their eggs were collected daily. Eggs were held in batches in tight-locking petri dishes (50 × 9 mm) (Falcon 35-1006, Becton Dickinson and Co., Franklin Lakes, NJ) in a covered plastic box in a humid growth chamber (Percival I-36LL, Percival Scientific, Perry, IA) at a 14:10-h light/dark regimen and 15°C to slow their development. As needed, batches of eggs were held at room temperature, exposed to natural light, and observed daily for larval development; larvae nearing eclosion showed dark mandibles and movement through the chorion. Larvae in the process of chewing through the chorion or that were recently eclosed (<30 min old) and crawling actively were used in these studies.

The soil used in these experiments was collected in 2008 from a grassy field next to a grape root borer–infested vineyard in Virginia and then spread and dried on a platform in a greenhouse at Virginia Tech's Alson H. Smith, Jr. Agricultural Research and Extension Center (AHSAREC), Winchester, VA. This soil was classified as dyke loam (Natural Resources Conservation Service, U.S. Department of Agriculture 2015), with a clay loam textural class, soil mass moisture of 24.3%, clay:sand ratio of 1.05, and a bulk density of 1.26 g/ml. Extraneous debris such as plant material and stones was removed by hand and the soil was sifted using a metal screen (1.5 × 1.5–mm mesh). Between uses, soil was stored in a partially covered plastic tub in a greenhouse, in which it remained thoroughly dry.

Sources of grape roots and root stimuli. The 420-A rootstock (*Vitis riparia* Michx. × *Vitis rupestris* Scheele) from vines at the AHSAREC vineyard was used. Bergh et al. (2011) showed that grape root borer neonates responded more strongly to ethanol extracts of 420-A roots than to extracts of some other *Vitis* species and rootstocks, and Rijal et al. (2013) demonstrated their attraction to headspace volatiles from 420-A roots. A shallow area around the base of vines was excavated between July and August and exposed roots were pruned and held in a covered plastic box lined with damp paper towels. Fresh root pieces were used within 1 h of collection for bioassays and for preparation of root extract.

Root extract preparation followed Bergh et al. (2011). Briefly, 5 g of root pieces (2 to 3 cm long, 2 to 3 mm diam) were eluted for 30 min in 50 ml of ethanol in a glass jar, after which the ethanol was filtered into a second glass jar with a Teflon-lined cap. The extract was stored at 0°C until being concentrated to ~2 ml in an evaporator using an ~35°C water bath and then returned to storage at 0°C.

Vertical soil columns. Vertical soil columns were created using polyvinyl chloride (PVC) pipes (2.54 cm inner diam [ID]) (Charlotte Pipe and Foundry, Charlotte, NC) filled with soil at 25% (w:w) moisture content. Soil moisture between 15% and 30% appears to be optimal for survival of grape root borer larvae (Rijal et al. 2014a, Sarai 1972). Columns were prepared 16 to 18 h prior to each experiment by thoroughly mixing distilled water into weighed batches of dry soil to standardize

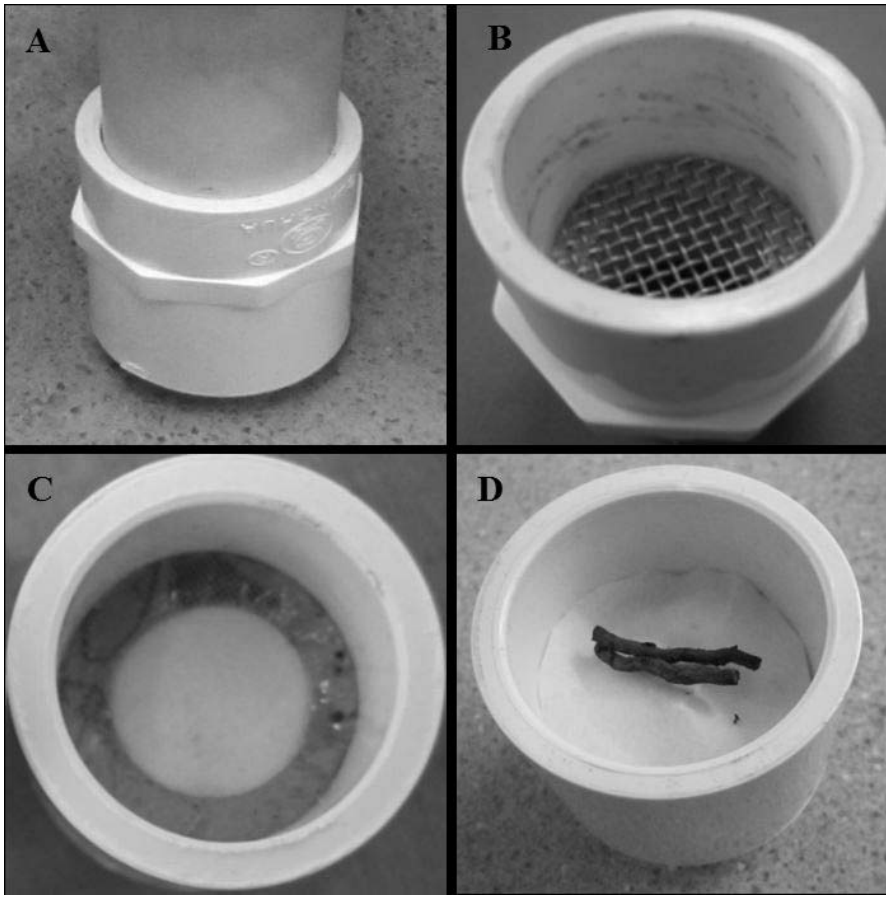

Fig. 1. Components of soil column assay: (A) PVC adaptor attached to the bottom of a PVC pipe, (B) PVC adaptor with metal screen to hold soil in place, (C) PVC bottom cap with sticky disc atop a damp sponge plug, and (D) PVC bottom cap with filter paper and grape root pieces atop a damp sponge plug.

soil moisture content and bulk density (1.20 g/ml bulk density) among pipes of a given length. A tightly fitting PVC adaptor containing a circular, fitted piece of metal screen (1.5 $\times$ 1.5–mm mesh) to hold the soil in place was placed at the bottom of each pipe (Fig. 1A, B). Soil was funneled into the pipes to within $\sim$0.5 cm of the top, and pipes of 15, 30, 60, 90, and 120 cm in length received 114, 228, 456, 654, and 912 g of soil, respectively. The top and bottom of each pipe was sealed with Parafilm (American Can Co., Neenah, WI), and the columns were stored at 5°C until assays were initiated. The larval release point in these columns was at the top of each pipe.

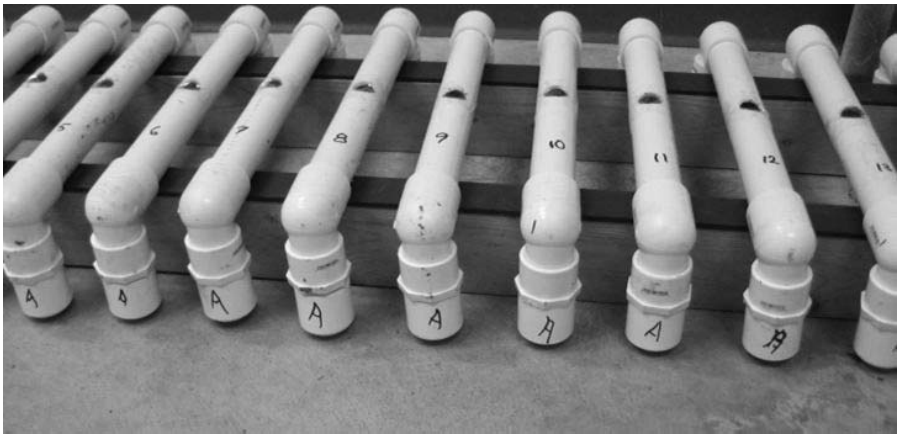

Fig. 2. Horizontal soil columns in rack, showing hole in the center for larval release.

Horizontal soil columns. Horizontal soil columns (Fig. 2) were similarly created using 30.0-cm-long PVC pipes (2.54 cm ID) filled with 228 g of soil at 20% (w:w) moisture content. The larval release point in these columns was a 2-cm-diam hole that was equidistant from both ends. When these pipes were filled with soil, the central hole was sealed with Parafilm. A PVC elbow joint was placed at both ends of the pipes (Fig. 2). PVC adaptors containing a circular, fitted piece of metal screen were placed over the elbow joints. The elbow joints and adaptors at both ends of each pipe were oriented downward and also filled with soil, creating a continuous soil column. The pipes were sealed and stored as described previously.

Y-tube soil columns. Vertically oriented Y-tube soil columns were created from PVC pipes (1.27 cm ID) and polypropylene Y adaptors (U.S. Plastic Corp., Lima, OH) (Fig. 3A, B) and used to assess the distance over which larvae responded to host stimuli. The straight section of the Y adaptor was inserted into the bottom of a 10-cm-long section of pipe, and black tape on the exposed parts of the adaptor prevented light penetration through the translucent material. A PVC pipe (5.0 or 7.5

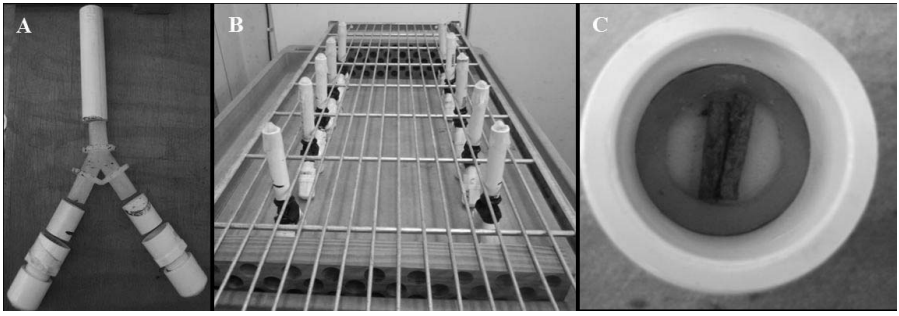

Fig. 3. Y-tube assay: (A) assembled Y-tube assay, (B) assays in frame in a controlled-environment room, (C) bottom cap with grape root pieces.

cm long) was inserted over both arms of the Y adaptor, and a PVC adaptor containing a fitted, circular piece of metal screen was placed at the end of each PVC arm. The upright section and the arms of the assembly were filled with soil, forming a continuous soil column. Y tubes with 5.0- and 7.5-cm-long arms received a total of approximately 21 and 25 g of soil, respectively. The top and both ends of each assembly were sealed with Parafilm, and they were stored as described previously. All experiments were initiated in the morning, when the majority of daily grape root borer larval eclosion was observed.

Larval response to grape roots in vertical columns. Vertical soil column**s** (15 cm long) were used to compare larval response to the presence or absence of grape root pieces at the bottom. The pipes were oriented vertically by attaching them with plastic cable ties to a supporting metal frame against a wall in a laboratory room at 23.5 ± 0.27°C SE and relative humidity 60.1 ± 2.69% SE. Circular discs (2.54 cm diam) were cut from the sticky liner of pheromone traps (Alpha Scents Inc., West Linn, OR), and a 2-cm-diam hole was cut from the center of each (Fig. 3 C). These were placed on a piece of filter paper atop a sponge plug (~1 cm long, 2.54 cm diam) dampened with distilled water in a PVC cap that fit tightly over the adaptor (Fig. 1A). The host stimulus was presented by placing two pieces of 420-A root (~2.0 mm diam, ~2.0 cm long) in the hole in the disc, and treatments were columns with and without root pieces ($n = 10$/treatment) in the cap, with three repetitions. For this experiment, eggs from which larvae were in the process of hatching were used. Eggs were transferred individually to small glass dishes (1.0 cm diam) using a fine-tipped, damp brush, and one dish was placed at the top of the soil in each column. Shortly after the experiment was initiated, larval eclosion was verified by inspecting each egg. Eggs from which the larva had not emerged were replaced. At 24-h intervals for 3 d, the bottom caps were removed and larval presence or absence was recorded. Data were analyzed using a 2×3 contingency table in SPSS (IBM SPSS Statistics, Version 21, International Business Machines Corp., Armonk, NY). The results of all statistical analyses were considered significant at $P < 0.05$.

Distance of larval movement in vertical columns. Vertical soil columns were used to examine the distance over which larvae moved to grape roots. Two pieces of 420-A roots were placed at the center of a filter paper disc atop a damp sponge plug in the PVC cap (Fig. 1D) at the bottom of each pipe. Treatments were column length (15, 30, 60, 90, and 120 cm) ($n = 10$/length), and the study was repeated four times. A single egg from which a larva was in the process of hatching was placed in a dish at the top of each column and, following verification of larval eclosion or egg replacement if necessary, the caps were inspected for larvae at 24-h intervals for 5 d. Data were transformed using square root transformation ($\sqrt{x + 0.5}$) and analyzed using one-way ANOVA and the Tukey-Kramer HSD in SPSS. This experiment was conducted in a laboratory at 26.8 ± 0.48°C SE.

Rate of larval movement in vertical columns. Vertical soil columns were used to examine the rate of larval movement in columns of different lengths (15, 30, and 60 cm) with and without root pieces ($n = 5$/treatment) with three repetitions. Root pieces were presented in caps containing a sticky disc (Fig. 1C). For this experiment, recently hatched larvae were used to avoid the potential for asynchronous eclosion. Larvae were transferred individually to a small glass dish, and one dish was placed on the soil at the top of the column. Larval presence or absence in the bottom cap was recorded at 8-h intervals for 72 h. Covariance

analysis was conducted using JMP Pro (Version 10, SAS Institute, Cary, NC) to compare regression lines, with time as the independent variable and mean cumulative number of larvae recovered from columns with or without roots as dependent variables (McDonald 2014). The slope and the *Y*-intercept of the lines depicting larval response in columns with and without roots were compared to evaluate the difference in the rate of larval recovery between two groups (root or without root) and covariate (i.e., time interval). This experiment was conducted in a walk-in controlled environment chamber at 25.8 ± 0.17°C SE and RH 69.0 ± 0.36% SE.

Larval movement in horizontal columns. Horizontal soil columns (30 cm long) were used to assess larval movement capacity in that dimension. Columns ($n = 15$) were held in place using a rectangular, notched wooden frame on the floor of a laboratory room at 23.5 ± 0.36°C SE and RH 52.5 ± 2.02% SE. Root pieces were presented in the cap (Fig. 1D) at both ends of each column. A single egg from which a larva was in the process of hatching was placed in a dish on the soil in the center hole, and the caps at both ends of each pipe were inspected for larvae at 24-h intervals for 3 d. The cumulative number of larvae recovered at column ends after 24, 48, and 72 h was summarized using descriptive statistics. The distribution of larvae between two ends after 48 and 72 h were analyzed using the exact binomial test in JMP Pro.

Larval response to grape root stimuli in Y-tube soil columns. Vertically oriented Y-tube soil columns were used to measure the distance over which larvae responded to host stimuli. Host stimulus treatments ($n = 10$–12/treatment) were presented in the PVC cap at the end of one arm of each Y-tube (Fig. 1C) and included (a) two pieces of 420-A grape roots or (b) a filter paper disc (1.5 cm diam; Whatman No. 1, GE Healthcare Life Sciences, Piscataway, NJ) treated with an ethanol extract of grape roots. Controls in the cap on the opposing arm were no root pieces or ethanol-treated disc for the root and root extract treatments, respectively. As described by Rijal et al. (2013), filter paper discs were treated with 50-μl aliquots of root extract or ethanol and air-dried for 20 min in a fume hood before being presented in the Y-tube. A single larva in a small dish was transferred to the top of the straight section of the Y-tube. Larval presence in both caps was recorded at 24-h intervals for 72 h. Treatments were randomly assigned to the Y-tube arms and the stimuli were randomly presented between two arms of the Y-tube assembly for each replicate. Each experiment was repeated three times, and pooled data were analyzed using the binomial test in SPSS.

Results

Larval response to grape roots in vertical columns. In treatments using root pieces, larvae recovered from the bottom cap were usually found feeding on the roots. When no root pieces were used, larvae were usually found on the surface of the sticky disc. The presence of root pieces did not significantly affect the cumulative number of larvae recovered from caps at the bottom of 15-cm-long vertical soil columns after 24 h ($\chi^2 = 1.104$; $P = 0.578$), 48 h ($\chi^2 = 0.784$; $P = 0.676$), or 72 h ($\chi^2 = 0.040$; $P = 0.98$) (Fig. 4). Most (73.3%) larvae were recovered from the

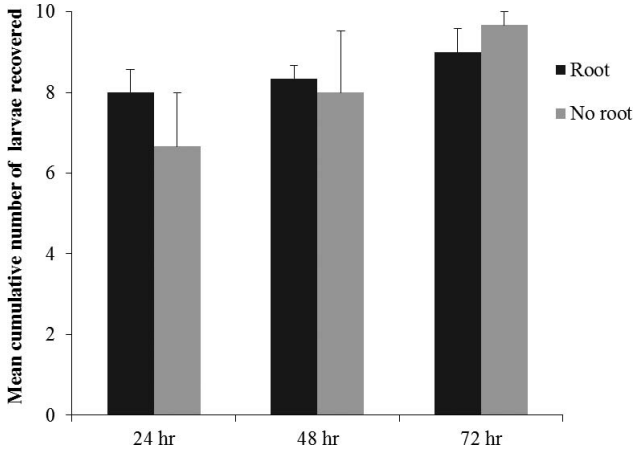

Fig. 4. Mean ± SE cumulative number of grape root borer larvae recovered at 24 h interval for 3 d from the bottom of 15-cm vertical soil columns with and without pieces of grape root in the bottom cap.

cap at the bottom of the column after 24 h, and 93.3% of larvae were recovered after 72 h.

Distance of larval movement in vertical columns. After 5 d, the mean cumulative number of larvae recovered from 15-, 30-, 60-, 90-, and 120-cm-long vertical soil columns was 9.75, 8.75, 7.25, 6.25, and 1.75, respectively (Fig. 5A). As in the previous experiment, most larvae moved to the bottom of 15-cm columns within the first 24 h, and the same was true for 30-cm columns. At 24 h, there was a significant effect of column length on the mean number of larvae recovered ($F = 15.81$; df = 4, 15; $P < 0.0001$), but there were no differences among 15-, 30-, and 60-cm columns (Fig. 5B). Larvae were first recovered from 120-cm columns after 2 d and only from those columns during the evaluation on Day 5 (Fig. 5A).

Rate of larval movement in vertical columns. The rate of larval movement through vertical columns with and without roots, represented by the slope of fitted regression lines for the mean cumulative number of larvae recovered at each sample point, were not significantly different for the 15-cm ($t = 1.193$; df = 14; $P = 0.253$), 30-cm ($t = 0.421$; df = 14; $P = 0.681$), or 60-cm columns ($t = 0.294$; df = 14; $P = 0.773$) (Fig. 6). The *Y*-intercept of lines for the root and no-root treatments was not significantly different for 15-cm ($t = 0.414$; df = 15; $P = 0.685$) and 60-cm ($t = -1.568$; df = 15; $P = 0.137$) columns but differed significantly for 30-cm columns ($t = -4.914$; df = 15; $P < 0.001$); mean cumulative number of larvae recovered from the 30-cm column with roots was significantly greater than from those without roots.

Larval movement in horizontal columns. No larvae were recovered from either end of horizontal columns after 24 h. After 48 and 72 h, respectively, the cumulative number of larvae recovered at either end was 10 and 14. There was no statistical difference in the cumulative number of larvae recovered from the two ends of the column after 48 h ($\chi^2 = 0.40$; $P = 0.526$) or 72 h ($\chi^2 = 0.29$; $P = 0.592$)

Larval response to grape root stimuli in Y-tube assays. In Y-tube assays with 5-cm arms, significantly more larvae were recovered from the arm with roots than

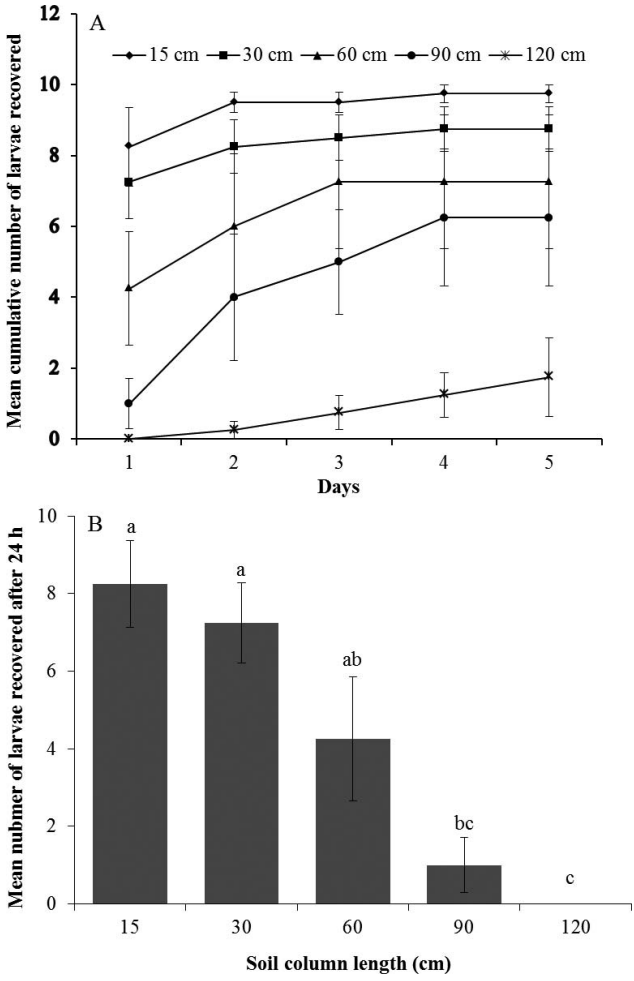

Fig. 5. Mean ± SE number of grape root borer larvae recovered from the bottom cap on vertical soil columns (A) at 24-h intervals for 5 d, (B) after 24 h. Values with the same letter are not statistically significant at 5% level of significance.

from the opposing arm ($n = 23$; $P < 0.001$) (Table 1), but this effect was not found from assays using roots in 7.5-cm arms ($n = 26$; $P = 0.279$) or from those using root extract in 5-cm arms ($n = 21$; $P = 0.095$).

Discussion

Using behavioral bioassays that provided conditions intended to more reasonably simulate the natural environs of grape root borer neonates than those used by Bergh et al. (2011) and Rijal et al. (2013), these experiments have further

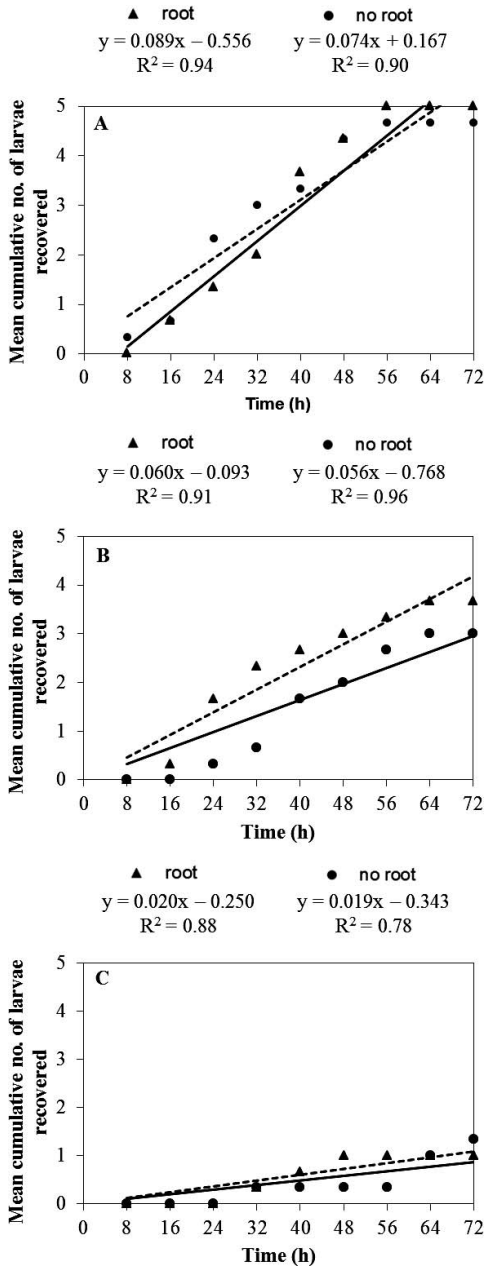

Fig. 6. Scatter plots showing fitted regression lines comparing mean cumulative number of grape root borer larvae recovered from soil columns with (dashed line with filled triangles) and without (solid line with filled circles) grape root pieces for column lengths of (A) 15 cm, (B) 30 cm, and (C) 60 cm.

Table 1. Number of grape root borer larvae recovered from Y-tube bioassays after 72 h.

	n	Host Stimulus	Control	Not Recovered*
Roots in 5-cm arms				
Repetition 1	12	10	0	2
Repetition 2	10	6	1	3
Repetition 3	10	4	2	4
Total		20a	3b	9**
Roots in 7.5-cm arms				
Repetition 1	10	5	5	0
Repetition 1	10	5	2	3
Repetition 1	10	5	4	1
Total		15a	11a	4**
Root extract in 5-cm arms				
Repetition 1	10	4	3	3
Repetition 1	10	5	3	2
Repetition 1	10	5	3	2
Total		14a	9a	7**

*Not included in analysis.
**Values with the same letter are not statistically significant at the 5% level of significance.

improved our understanding of their food-finding behavior and capacity. Vertical and horizontal soil column assays showed that they can move in both dimensions. Their success at finding roots in vertical columns was affected by column length but not by the presence of roots at the bottom. Similarly, over column lengths of up to 60 cm, their rate of movement to the bottom was not affected by the presence of roots. When larvae were released in the middle of a horizontal column, their rate of movement to roots at a distance of 15 cm from the release point appeared to be slower than their rate of movement through 15 cm vertical columns. In Y-tube assays, it appeared that stimuli associated with grape roots triggered an oriented response by larvae over a distance of about 5 cm.

Remarkably, some larvae survived for 4 to 5 d before being recovered from the bottom of 1.2 m columns. Compared with larvae of some other sesiid pests, such as Dogwood borer, *Synanthedon scitula* Harris, Sequoia pitch moth, *Synanthedon sequoiae* (Henry Edwards), or Peachtree borer, *Synanthedon exitiosa* (Say), which hatch from eggs deposited on or very near the food source (Gentry and Wells 1982, Koehler et al. 1983, Leskey and Bergh 2005), larvae of grape root borers likely expend much more energy and time searching for food. Comparison of the survivorship and longevity of food-deprived grape root borer neonates with that of

other sesiids may yield insights into grape root borer larval energetics and their adaptation to more remote food sources.

The lack of a significant influence of host stimuli on the success and rate of food-finding in vertical columns was likely due in part to the positively gravitactic behavior exhibited by grape root borer neonates; newly eclosed larvae innately and immediately burrow downward, and moved through the column regardless of the presence of the food at the bottom. The depth of grapevine roots depends on species, variety, and rootstock and on vineyard soil type and structure (Richards 1983). The majority of fine roots are at depths of 10 to 60 cm (Richards 1983), with highest density in the upper 20 cm of soil (Morlat and Jacquet 2003), while main roots (>5 mm diam) are abundant at soil depths of 18 to 80 cm, with some variation among rootstocks (Morano and Kliewer 1994). Although larvae may begin their development by feeding on the first grape root encountered, regardless of its diameter, they ultimately feed within the cortex of larger roots and mine these toward the crown of the vine. Despite the capacity of larvae to move downward over distances of up to 1.2 m under the favorable soil moisture and texture conditions provided in the assay, in vineyards the availability of smaller roots at shallower depths may sustain them initially, and their movement in vineyard soils is likely affected by soil characteristics (Rijal et al. 2014a). Their capability to move horizontally in soil also would presumably enable larvae to increase the size of the area searched, especially in response to behaviorally active cues associated with grape roots.

Many soil-dwelling insects detect and respond to chemical cues within the complex soil matrix (Rasmann et al. 2012). The distance over which insects respond to volatile host compounds depends upon the volatility of the compound, its concentration and diffusion, and the orientation mechanism of the insect (Visser 1986). Demonstration of neonate grape root borer attraction to grape root extracts (Bergh et al. 2011) and to grape root pieces and grape root headspace volatiles (Rijal et al. 2013) in small laboratory arenas suggested an important role of these stimuli in the plant-insect interactions between this oligophagous species and its Vitaceae hosts. Other studies have employed Y-tube assays to examine the behavioral response of root-feeding insects to host stimuli, but used moving air and/or substrates such as glass beads or sand (Bernklau and Bjostad 1998a, b, Boff et al. 2002, Kurtz et al. 2009). Our use of still-air conditions and characterized soil from a commercial vineyard infested by grape root borer enhanced the study's ecological relevance (Eigenbrode and Espelie 1995). The results from Y-tube experiments provided some initial indications about the distance over which grape root borer neonates can perceive and respond to grape root stimuli in the soil. Neonates appeared to respond to volatiles emanating from small grape root pieces over a distance of 5 cm but not at 7.5 cm. Over 5 cm, an ethanol-based root extract did not elicit a positive response from larvae, possibly due to differences in the presence and/or concentration of behaviorally active volatile compounds between fresh root pieces and root extract. Similar to our findings, Johnson et al. (2004) reported that clover root weevil larvae, *Sitona lepidus* Gyllenhal, differentiated host from nonhost plant roots at a distance of 6 cm in soil. In nature, it is plausible that the cumulative concentration of root volatiles within the grapevine rhizosphere may enhance grape root borer larval perception of roots and enable them to respond over longer distances than what our data suggested.

The use of these assays to examine and demonstrate the food-finding behavior and capacity of grape root borer neonates has provided baseline information upon which other research might be based. For example, systematic manipulation of soil characteristics could be used to further explore these effects on the success and rate of food-finding. As an extension of this question, the use of soil cores from commercial vineyards would further optimize the relevance of this experimental approach and may yield additional information on the suitability of different soils for larval grape root borer survivorship and establishment on roots. In combination with pupal exuviae sampling, this approach would expand upon the previous findings and conclusions of Rijal et al. (2014a), potentially strengthening our understanding of the factors underlying large differences in grape root borer larval density among commercial vineyards and/or the reasons for aggregated distributions within some vineyards (Rijal et al. 2014b). As well, use of these assays may enable controlled studies of grape root borer larval attack by soil-dwelling entomopathogenic nematodes under more natural conditions, as has been reported from experiments with other soil-dwelling insects (El-Borai et al. 2005, Kaspi et al. 2010). Finally, although purely speculative at this point, incorporating behaviorally active grape root stimuli derived from headspace collections or extracts into soil column assays may enable investigation of the potential to use these compounds to disrupt larval food-finding. Incorporation of extraneous sources of root cues into soil may increase the time and energy spent searching and the duration of exposure to soil-dwelling predators, potentially resulting in higher levels of larval mortality. Similar approaches have at least experimentally demonstrated disruption of food-finding in larvae of other root-feeding insects (Bernklau et al. 2004, Bernklau and Bjostad 2005, Dosdall et al. 2002, Koštál 1992).

Acknowledgments

This research was funded in part by the Virginia Wine Board. We thank A. Zhang and S. Abubeker for technical help with root extract preparation and A. Acebes for the help in data analysis of the horizontal soil column study.

References Cited

All, J.N., J.D. Dutcher and M.C. Saunders. 1987. Control program for the grape root borer in grape vineyards of the eastern United States. Down to Earth 43: 10–12.

Bergh, J.C. 2006. Trapping grape root borer (Lepidoptera: Sesiidae) in vineyard and non-vineyard habitats in Virginia. J. Entomol. Sci. 41: 253–256.

Bergh, J.C. 2012. Grape root borer, Pp. 383–402. *In* Bostanian, N.J., C. Vincent and R. Isaacs (eds.), Arthropod Management in Vineyards. Springer, Dordrecht, Netherlands.

Bergh, J.C., A. Zhang, J.R. Mayer and D. Kim. 2011. Response of grape root borer (Lepidoptera: Sesiidae) neonates to root extracts from Vitaceae species and rootstocks. Environ. Entomol. 40: 880–888.

Bernklau, E.J. and L.B. Bjostad. 1998a. Behavioral responses of first-instar western corn rootworm (Coleoptera: Chrysomelidae) to carbon dioxide in a glass bead bioassay. J. Econ. Entomol. 91: 444–456.

Bernklau, E.J. and L.B. Bjostad. 1998b. Reinvestigation of host location by western corn rootworm larvae (Coleoptera: Chrysomelidae): CO_2 is the only volatile attractant. J. Econ. Entomol. 91: 1331–1340.

Bernklau, E.J. and L.B. Bjostad. 2005. Insecticide enhancement with feeding stimulants in corn for western corn rootworm larvae (Coleoptera: Chrysomelidae). J. Econ. Entomol. 98: 1150–1156.

Bernklau, E.J., E.A. Fromm and L.B. Bjostad. 2004. Disruption of host location of western corn rootworm larvae (Coleoptera: Chrysomelidae) with carbon dioxide. J. Econ. Entomol. 97: 330–339.

Boff, M.I.C., R.H.W.M. Van Tol and P.H. Smits. 2002. Behavioral response of *Heterorhabditis megidis* towards plant roots and insect larvae. Biocontrol 47: 67–83.

Brooks, F.E. 1907. The grapevine root borer. West Va. Agric. Exp. Stn. Bull. 110: 20–30.

Brooks, F.E. 1918. Grape root borer. U.S. Dep. Agric. Bull. 730: 21–28.

Clark, G.N. and W.R. Enns. 1964. Life history studies of the grape root borer (Lepidoptera: Aegeriidae) in Missouri. J. Kans. Entomol. Soc. 37: 56–63.

Dosdall, L.M., R.C. Yang and P.M. Conway. 2002. Do applications of sulfur or sulfate influence infestations of root maggots (*Delia* spp.) (Diptera: Anthomyiidae) in canola? Can. J. Plant Sci. 82: 599–610.

Dutcher, J.D. and J.N. All. 1976. Beware the grape root borer. J. Am. Fruit Grower 96: 18–19.

Dutcher, J.D. and J.N. All. 1979a. Biology and control of the grape root borer in Concord grape vineyards. Ga. Agric. Exp. Stn. Res. Bull. 232: 1–18.

Dutcher, J.D. and J.N. All. 1979b. Damage impact of larval feeding by the grape root borer in a commercial Concord grape vineyard. J. Econ. Entomol. 72: 159–161.

Eigenbrode, S.D. and K.E. Espelie. 1995. Effects of plant epicuticular lipids on insect herbivores. Annu. Rev. Entomol. 40: 171–194.

El-Borai, F.F, L.W. Duncan and J.F. Preston. 2005. Bionomics of a phoretic association between *Paenibacillus* sp. and the entomopathogenic nematode *Steinernema diaprepes*. J. Nematol. 37: 18–25.

Gentry, C.R. and J.M. Wells. 1982. Evidence of an oviposition stimulant for peachtree borer. J. Chem. Ecol. 8: 1125–1132.

Harris, J.P., B.J. Smith and W.C. Olien. 1994. Activity of grape root borer (Lepidopetera: Sesiidae) in southern Mississippi. J. Econ. Entomol. 87: 1058–1061.

Harris, T.W. 1854. Report on some of the diseases and insects affecting fruit trees and vines, Pp. 210–217. *In* Proceedings of Third Session Am. Pomological Soc., 13–15 September 1854; Boston, MA. Franklin Printing House, Boston, MA.

Johnson, D.T., R.L. Mayes and P.A. Gray. 1981. Status of grape root borer, *Vitacea polistiformis* (Lepidoptera: Sesiidae) management and feasibility of control by disruption of mating communication. Misc. Publ. Entomol. Soc. Am. 12: 1–7.

Johnson, S.N., P.J. Gregory, P.J. Murray, X. Zhang and I.M. Young. 2004. Host plant recognition by root feeding clover weevil, *Sitona lapidus* (Coleoptera: Curculionidae). Bull. Entomol. Res. 94: 433–439.

Kaspi, R., A. Ross, A.K. Hodson, G.N. Stevens, H.K. Kaya and E.E. Lewis. 2010. Foraging efficacy of the entomopathogenic nematode *Steinernema riobrave* in different soil types from California citrus groves. Appl. Soil Ecol. 45: 243–253.

Koehler, C.S., G.W. Frankie, W.S. Moore and V.R. Landwehr. 1983. Relationship of infestation by the sequoia pitch moth (Lepidoptera: Sesiidae) to Monterey pine injury. Environ. Entomol. 12: 979–981.

Koštál, V. 1992. Orientation behavior of newly hatched larvae of the cabbage maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae), to volatile plant metabolites. J. Insect Behav. 5: 61–70.

Kurtz, B., I. Hiltpold, T.C. Turlings, U. Kuhlmann and S. Toepfer. 2009. Comparative susceptibility of larval instars and pupae of the western corn rootworm to infection by three entomopathogenic nematodes. Biocontrol 54: 255–262.

Leskey, T.C. and J.C. Bergh. 2005. Factors promoting infestation of newly planted, non-bearing apple orchards by dogwood borer, *Synanthedon scitula* Harris (Lepidoptera: Sesiidae). J. Econ. Entomol. 98: 2121–2132.

McDonald, J.H. 2014. Handbook of Biological Statistics. 3rd ed. Sparky House Publishing, Baltimore, MD.

Morano, L. and W.M. Kliewer. 1994. Grapevine rootstocks grafted to Cabernet Sauvignon grown on a very gravelly clay loam soil in Oakville, California. Am. J. Enol. Vitic. 45: 345–348.

Morlat, R. and A. Jacquet. 2003. Grapevine root system and soil characteristics in a vineyard maintained long-term with or without interrow sward. Am. J. Enol. Vitic. 54: 1–7.

Natural Resource Conservation Service, U.S. Department of Agriculture. **2015.** Web soil survey, http://websoilsurvey.nrcs.usda.gov, Last accessed 04 April 2015.

Pollet, D.K. 1975. The grape root borer in South Carolina. Clemson Univ. Ext. Serv. Circ.

Rasmann, S., M. De Vos, C.L. Casteel, D. Tian, R. Halitschke, J.Y. Sun and G. Jander. 2012. Herbivory in the previous generation primes plants for enhanced insect resistance. Plant Physiol. 158: 854–863.

Richards, D. 1983. The grape root system. Hort. Rev. 5: 127–168.

Rijal, J.P., C.C. Brewster and J.C. Bergh. 2014a. Effects of biotic and abiotic factors on grape root borer (Lepidoptera: Sesiidae) infestations in commercial vineyards in Virginia. Environ. Entomol. 43: 1198–1208.

Rijal, J.P., C.C. Brewster and J.C. Bergh. 2014b. Spatial distribution of grape root borer (Lepidoptera: Sesiidae) infestations in Virginia vineyards and implications for sampling. Environ. Entomol. 43: 716–728.

Rijal, J.P., A. Zhang and J.C. Bergh. 2013. Behavioral response of grape root borer (Lepidoptera: Sesiidae) neonates to grape root volatiles. Environ. Entomol. 42: 1338–1347.

Sarai, D.S. 1972. Seasonal history and effect of soil moisture on mortality of newly hatched larvae of the grape root borer in southern Missouri. J. Econ. Entomol. 65: 182–184.

Snow, W.J., D.T. Johnson and J.R. Meyer. 1991. The seasonal occurrence of the grape root borer (Lepidoptera: Sesiidae) in the eastern United States. J. Entomol. Sci. 26: 157–168.

Sorensen, K.A. 1975. The grape vine root borer: Present status of research and control. Vinifera Wine Growers J. 2: 24–30.

Visser, J.H. 1986. Host odor perception in phytophagous insects. Annu. Rev. Entomol. 31: 121–144.

Webb, S.E., R.K. Sprenkel and J.L. Sharp. 1992. Seasonal flight activity of grape root borer (Lepidoptera: Sesiidae) in Florida. J. Econ. Entomol. 85: 2161–2169.

Wylie, W.D. 1972. Grape root borer control. Proc. Ann. Arkansas State Hort. Soc. 90: 71–73.

Wylie, W.D. and D.T. Johnson 1978. Summary of grape root borer and scale research. Proc. Ark. State Hort. Soc. 99: 108–110.