

Behavioral Response of Grape Root Borer (*Lepidoptera*: Sesiidae) Neonates to Grape Root Volatiles

J. P. RIJAL,^{1,2} A. ZHANG,³ AND J. C. BERGH¹

Environ. Entomol. 42(6): 1338–1347 (2013); DOI: <http://dx.doi.org/10.1603/EN13088>

ABSTRACT Grape root borer, *Vitacea polistiformis* (Harris), is an oligophagous and potentially destructive pest of grape in commercial vineyards throughout much of the eastern United States. Larvae feed on vine roots, although little is known about their below-ground interactions with host plants. The behavioral response of groups of grape root borer neonates to stimuli from host and nonhost roots was evaluated in single and paired stimuli bioassays in which stimuli were presented in opposing wells attached to the bottom of petri dish arenas. Stimulus sources included root pieces and root headspace volatiles from 3309 and 420-A grape rootstocks (host) and apple (nonhost) and ethanol-based extracts of 3309 and 420-A roots. In single stimulus assays, significantly more larvae were recovered from wells containing grape roots, apple roots, grape extracts, and grape root volatiles than from control wells, but there was no significant response to volatiles collected from the headspace of apple roots. In paired stimuli assays, significantly more larvae were recovered from wells containing grape than apple roots. There was no difference in larval distribution between wells when 420-A and 3309 roots were presented simultaneously, although a significantly greater response to 3309 than 420-A root extract was recorded. When soil was added to the assays, significantly more larvae were recovered from wells containing grape roots than from those containing only soil, but this response was not detected in assays using buried apple roots. These results are discussed in relation to the plant–insect interactions between grape root borer larvae and their Vitaceae hosts.

KEY WORDS *Vitacea polistiformis*, *Vitis*, below-ground herbivory

Grape root borer, *Vitacea polistiformis* (Harris) (Lepidoptera: Sesiidae), is an important oligophagous pest of grape vines in portions of the eastern United States (Brooks 1907, Clark and Enns 1964, All and Dutcher 1978, Bergh 2012). Larvae feed on roots of wild and commercially important *Vitis* species and rootstocks and can cause extensive damage that in severe cases can lead to vine death (Dutcher and All 1976), as has been observed in some vineyards in Virginia (J.P.R., unpublished data). Adults are common in commercial vineyards and in forested areas with wild grape (Snow et al. 1991, Bergh et al. 2005, Bergh 2006). In vineyards, mated females deposit eggs on above-ground parts of vines, weeds, and on the soil surface in vine rows (Brooks 1907, Clark and Enns 1964, Dutcher and All 1979a). After hatching, neonates burrow through the soil to find grape roots, on which they are thought to feed for 1–3 yr, with shortest and longest larval developmental durations in the southern (e.g., Florida and Georgia) and northern (e.g., Ohio) portions of its geographic range, respectively (reviewed in Bergh 2012). Neonates may establish on any part of the root system, but tend to move toward the crown of the vine

during their development (Clark and Enns 1964, Dutcher and All 1978), creating increasingly large feeding channels in the root cortex that are packed with diagnostic reddish frass (Dutcher and All 1978). Because of the subterranean habit of larvae and no known specific symptoms of vine infestation on the above-ground plant parts, infestations often remain undetected until vine vigor and productivity decline (Dutcher and All 1979b).

The relatively indiscriminate oviposition site-selection behavior of female grape root borer (Clark and Enns 1964) differs from that of many sesiid species, which deposit eggs predominantly on specific parts of their host plants (Gentry and Wells 1982, Koehler et al. 1983, Johnson 1993, Leskey and Bergh 2005), and is likely due mainly to the below-ground source of larval food. Similarly, random egg-laying behavior has been observed in other species with root-feeding larvae, such as clover root curculio, *Sitona hispidula* F. (Herron 1953), and black vine weevil, *Otiorhynchus sulcatus* (F.) (Clark et al. 2011).

Given that vineyard infestation by grape root borer ultimately depends on neonates finding and establishing on vine roots and that, for reasons as yet unknown, infestation severity can vary widely among and within vineyards (J.P.R., unpublished data), understanding the plant–insect interactions between this pest and its wild and cultivated Vitaceae hosts will provide im-

¹ Virginia Tech, Alson H. Smith, Jr. Agricultural Research and Extension Center, 595 Laurel Grove Rd., Winchester, VA 22602.

² Corresponding author, e-mail: rijal@vt.edu.

³ USDA-ARS, Invasive Insect Biocontrol and Behavior Laboratory, BARC-W, 10300 Baltimore Ave., Beltsville, MD 20705.

portant insights that may guide research toward its sustainable management. In nature, the roots of wild *Vitis* species likely occur in proximity to those of many other plants, raising the question of whether food-finding by grape root borer neonates occurs randomly or is guided by host-specific stimuli.

Food-finding by below-ground herbivorous arthropods is mediated by host plant cues that may differ according to the degree of host specificity exhibited by each species. Plant roots produce a multitude of compounds (Uren 2000) that can impact below-ground herbivory directly or indirectly by affecting insect behavior or development (Hiltpold and Turlings 2012). Polyphagous root-feeding insects often use primary metabolites such as CO₂, sugars, and amino acids produced by roots to find food (Thorpe et al. 1947, Doane et al. 1975, Brown and Gange 1990, Bernklau et al. 2005, Reinecke et al. 2008), while recognition, finding, and acceptance of host roots by mono- and oligophagous species is often mediated by secondary metabolites (Matsumoto and Thorsteinson 1968, Jones and Coaker 1979, Soni and Finch 1979, Ross and Anderson 1992) such as esters, aldehydes, ketones, isothiocyanates, isoflavonoids, and phenolics (Johnson and Gregory 2006, Robert et al. 2012a). Studies using various bioassay approaches have shown that chemical cues from intact roots (Johnson et al. 2004), solvent-based root extracts (Kamm and Buttery 1984, Tapia et al. 2005, Bergh et al. 2011), or root volatiles (Matsumoto and Thorsteinson 1968, Jones and Coaker 1979, Soni and Finch 1979, Košťál 1992, Wenke et al. 2010, Manosalva et al. 2011) from host plants elicited positive behavioral responses by root-feeding insects. Chemically mediated interactions between root-feeding insects and their specific host plants were reviewed most recently by Johnson and Gregory (2006), Hiltpold and Turlings (2012), and Johnson and Nielsen (2012).

Bergh et al. (2011) showed that recently eclosed grape root borer neonates were recorded more frequently on or near filter paper discs treated with ethanol-based grape root extracts than untreated discs or those treated with a nonhost (apple) root extract in single extract bioassays. In paired extract assays, extracts from some *Vitis* species consistently elicited a greater response than others. However, given that, 1) larval responses were evaluated in a closed system, 2) the compounds to which they were exposed were influenced by the collection method, and 3) larvae were able to contact the stimulus source and move freely and continuously within the assay arena during the exposure period, Bergh et al. (2011) were unable to differentiate among several possible behavioral responses, including attraction, arrestment, or phagostimulation. Consequently, the studies reported here were conducted to further elucidate the nature of the stimuli involved in food-finding by larval grape root borer, via examination of the response of larvae to grape root stimuli from which they were physically isolated within a ventilated assay.

Materials and Methods

Insects. Mated and virgin female grape root borer moths were collected in July and August by scouting in commercial vineyards in Virginia and from a vineyard at Virginia Tech's Alson H. Smith, Jr.-Agricultural Research and Extension Center (AHS-AREC), Winchester, VA. Scouting was conducted between 9 and 11 a.m., when the majority of adult emergence occurs (Brooks 1918, Clark and Enns 1964). Virgin and mated females were differentiated based on their behavior. Newly eclosed virgin females were typically found sitting on the lower vine trunk, whereas females collected while flying along the vine row were typically mated and usually deposited eggs in the holding containers soon after collection. Male moths were collected using sex pheromone lures. Virgin females and males were paired in wood and Plexiglas ventilated mating cages (35 cm in length by 35 cm in width by 35 cm in depth) placed in a shaded area outdoors and observed frequently for mating. Females that mated in cages and those that were mated at the time of collection were held individually in plastic containers (12 cm in depth by 14 cm in diameter) with a screened lid and water-soaked cotton ball under shaded conditions outdoors. Eggs were collected daily and kept in 50- by 9-mm tight-lock petri dishes (Falcon 35-1006, Becton Dickinson and Co., Franklin Lakes, NJ) in a covered plastic box (31 cm in length by 21.5 cm in width by 7.5 cm in depth) in a controlled environment chamber (Percival I-36LL, Percival Scientific, Perry, IA) set at a photoperiod of 14:10 (L:D) h and constant 15°C to slow egg development. Bergh (J.C.B., unpublished data) has shown that grape root borer eggs can be held at this temperature for up to 21 d without affecting percentage hatch. A water-filled tray at the bottom of the chamber maintained adequate humidity. As needed, groups of eggs were placed at room temperature under natural daylight and observed daily for larval development, which was most apparent near eclosion, when the dark mandibles could be seen. Recently eclosed (≤ 2 -h-old) actively crawling and apparently healthy larvae were used in bioassays.

Sources of Root Stimuli. The grape rootstock, 3309, is a *Vitis berlandieri* Planch \times *Vitis riparia* Michx. hybrid and was widely planted in Virginia between 1988 and 2000, while the 420-A rootstock (*V. riparia* \times *Vitis rupestris* Scheele) has been less commonly used but has commercial importance (T. Wolf, personal communication). Grape roots (≈ 1.5 -3.0 mm in diameter) from 3309 (11-yr-old) and 420-A (6-yr-old) rootstocks were collected by carefully removing the soil from around the base of vines in commercial vineyards and from a vineyard at the AHS-AREC between July and September of 2011 and 2012. Bergh et al. (2011) showed that ethanol-based extracts from both of these rootstocks elicited strong larval responses and that there was no effect of vineyard or time of collection during the growing season (July-September) on larval response. Apple roots from the M.26 rootstock were similarly collected from trees in orchards at the AHS-

AREC. Exposed roots were pruned and placed in a covered plastic box lined with damp paper towel. Within 1 h of collection, root pieces were used in bioassays or to prepare extracts.

Extract preparation followed Bergh et al. (2011). Briefly, 5 g samples of pieces of 3309 and 420-A grape root (2–3 cm in length and \approx 1.5–3.0 mm in diameter) were placed individually in 125-ml wide-mouth glass jars with Teflon-lined caps containing 50 ml histological grade ethanol (95% purity, Fisher, Pittsburgh, PA) for 30 min, then filtered in a glass funnel through Whatman No. 1 filter paper (GE Healthcare Life Sciences, Piscataway, NJ) into 60-ml amber glass jars with Teflon-lined caps. Extracts were stored at 0°C until being concentrated to \approx 2 ml in a Buchi RE 111 Rotavapor evaporator (Buchi Labortechnik AG, Flawil, Switzerland) with vacuum in a water bath at \approx 35°C. Concentrated extracts were stored at 0°C.

Headspace volatiles were collected from the roots of own-rooted 3309 and 420-A grape vines (1-yr-old) and apple trees (2-yr-old) on M.26 rootstock grown in 18.9-liter plastic pots containing 50:50 soil: peat moss in a greenhouse at the AHS-AREC. At the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, MD, two vines and two apple trees were carefully removed from the pots, and most soil was removed from the root system by gentle shaking and brushing without damaging the fine roots. The root system of each plant was enclosed in a plastic oven bag (Reynolds Oven Bags, 482 by 596 mm, Reynolds Kitchens, Richmond, VA) that was sealed tightly around base of the trunk, and the plant was held upright using a clamp and support stand on a laboratory bench. An adsorbent trap consisting of a borosilicate glass tube (15 cm in length and 0.6 cm outside diameter) containing Super Q (200 mg; Alltech Associates Inc., Deerfield, IL; Zhang et al. 1994) was connected to one side of the bag and the corner on the opposite side of the bag was cut off to allow free ventilation. Air was drawn through the bag and adsorbent trap by vacuum (\approx 1 liter/min) for 24 h at room temperature and natural light conditions. Adsorbent was eluted with dichloromethane (0.5 ml, spectrometry grade, EMD Chemicals Inc., Gibbstown, NJ) and the eluate was concentrated to \approx 200 μ l under a nitrogen stream and stored at -30°C until use.

Bioassays. Bioassays were conducted using clear, 50-by-9-mm tight-lock petri dishes (Falcon 35-1006, Becton Dickinson and Co., Franklin Lakes, NJ). Dishes were ventilated via a 12-mm-diameter hole drilled in the center of each lid that was covered with No-See-Um screen (BioQuip Products, Rancho Dominguez, CA). Each dish was divided into equal quadrants by etching on the outer surface of the bottom with a razor blade. The inner bottom surface of each was uniformly roughened using sand paper (100 medium aluminum oxide, No 5032; Ali Industries, Fairborn, OH) to facilitate neonate movement. A 12-mm-diameter hole drilled in the center of two opposing quadrants at the bottom of each dish was covered with a piece of metal screen (1- by 1-mm mesh) glued to the

outer surface. A 12-mm-diameter hole was drilled in the plastic cap of threaded glass vials (2 ml, 2.7 cm in length and 12 mm in internal diameter; Fisher, Pittsburgh, PA). The caps were glued over the screen covering each hole in the dish so that the holes in the dish and caps were aligned. Screwing a glass vial into each cap created two wells extending from the bottom of each dish, in which single or paired treatments were presented.

In bioassays using freshly collected roots, two root pieces (2–3 cm in length and \approx 1.5–3.0 mm in diameter) from one plant source were presented per well. In single stimulus experiments with roots, the second well was empty while paired stimuli experiments used root pieces from different plants in opposing wells. In assays using root extracts, 1.5-cm-diameter filter paper discs (Whatman No 1) were treated with 50 μ l aliquots of root extract or ethanol and air-dried for 20 min in a fume hood before being placed in opposing wells. Single stimulus experiments compared extract- vs. solvent-treated discs in opposing wells while discs treated with extracts from two different plant sources were presented in opposing wells in paired stimuli experiments. Single stimulus bioassays using headspace volatiles from different plant sources used filter paper discs that were treated either with 10 μ l aliquots of volatiles in dichloromethane or dichloromethane alone and air-dried for 10 min before being placed in opposing wells.

Under paired dissecting microscopes and dim light, a wet, fine, natural hair brush was used to transfer neonates from the dishes in which they had emerged to the center of the bottom of each bioassay dish (i.e., intersection of the lines delineating quadrants), and the lid was placed on each. In a test in which one larva was transferred to each of 24 dishes containing pieces of grape and apple root in opposing wells, all larvae were subsequently recovered from the well with grape root, suggesting that using multiple larvae per dish should not influence the response of individuals. Consequently, five neonates per dish were used in all other bioassays. Because most larvae eclosed in the morning, all bioassays were initiated before noon. The dishes were placed in test tube racks (Nalge Nunc International Corp., Penfield, NY) so that the bottom was level and resting on the top of the rack and the glass wells were suspended below. The racks were placed in a clear covered plastic box (28 cm in length by 16.5 cm in width by 10 cm in depth) with damp paper towel lining the bottom, and the box was placed in a dark drawer of a laboratory bench for 1 h. During the bioassays, room lights were turned off and air temperature was $24.8^{\circ}\text{C} \pm 0.08$ SE.

Between bioassays, the glass vials were washed in hot water with detergent, rinsed with clean water then with 99.9% high performance liquid chromatography grade acetone (Fisher, Pittsburgh, PA) and oven-dried at 100°C for \approx 1 h. Petri dishes and caps were washed in hot water with detergent, rinsed in clean water, and air-dried for 24 h.

Bioassays Without Plant Stimuli. Before evaluating larval response to host plant stimuli, their response to

other factors that might have influenced outcomes was examined. In the first experiment, larvae were introduced to dishes with two empty wells. In a second test one well contained a 1.5-cm filter paper disc treated with 0.5 ml of distilled water and the other contained a dry disc. A third experiment involved one well with a filter paper disc that had been treated with 50 μ l of ethanol and dried in a fume hood for 20 min while the other well contained a dry untreated disc. Each treatment was replicated 10 times per day on each of two consecutive days.

Bioassays With Plant Stimuli. Single stimulus treatments included; 1) extracts of 420-A and 3309 roots, 2) fresh roots of 420-A, 3309, and apple, and 3) root volatiles from 420-A, 3309, and apple. Paired stimuli treatments included 1) 420-A or 3309 roots vs. apple roots, 2) 420-A roots vs. 3309 roots, 3) 420-A root extract vs. 3309 root extract, and 4) 420-A roots from 1-yr-old potted vines vs. apple roots from field-grown trees. All experiments except those using root volatiles were replicated 10 times per day on each of two consecutive days. Experiments using root volatiles were replicated 10 times on the first day and six to seven times on the following day.

Bioassays With Buried Roots. Soil collected from a field adjacent to a commercial vineyard in Virginia was dried thoroughly in the sun, and then sifted using a #4 sieve (4.75 mm in diameter) and hand sorted to remove large particles, rocks, and other debris, including plant material. For the bioassays, the soil was brought to a moisture content of 20% (by weight; Strnad and Bergman 1987), using distilled water. Two fresh pieces of 420-A roots were placed in one well, which was filled to the top with soil. The other well was similarly filled with soil, and the dish was filled to $\approx 2/3$ of the depth, forming a continuous soil layer from it into the wells. Five larvae were transferred to a wet filter paper disc (6 mm in diameter) that was placed on the soil surface in the center of each dish. We assumed that larval movement through soil may occur more slowly than on the dish surface and therefore evaluated the assays after 3 h. The soil in each well was poured into separate petri dishes and inspected under a microscope for larvae. If fewer than five larvae were recovered from soil in the wells, the soil in each dish was similarly inspected for larvae.

Statistical Analysis. Student *t*-tests ($\alpha = 0.05$) using SAS version 9.2 (PROC TTEST, SAS Institute Inc. Cary, NC) were used to compare the mean number of larvae recovered between the wells in each dish.

Results

Bioassays Without Plant Stimuli. Of the 300 larvae used across all tests without plant stimuli, 87% entered one of the wells during the 1-h experimental period. The distribution of larvae between empty wells (2.20 ± 0.24 SE vs. 2.15 ± 0.22 SE) was not significantly different ($t = 0.15$; $df = 38$; $P = 0.88$). Larvae were equally distributed between wells with wet (2.45 ± 0.26 SE) and dry filter paper (2.05 ± 0.19 SE; $t = -0.12$; $df = 38$; $P = 0.24$) and between wells with (1.95 ± 0.29

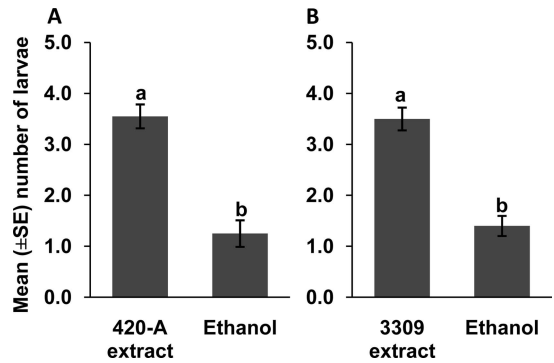


Fig. 1. Mean (\pm SE) number of *V. polistiformis* larvae in opposing bioassay wells containing dried filter paper discs treated with ethanol or ethanol-based extracts from, (A) 420-A grape roots, or (B) 3309 grape roots in single stimulus assays. Scale bars with the same letter are not significantly different at the 5% level of significance (Student's *t*-test).

SE) and without (2.25 ± 0.24 SE) ethanol-treated (and dried) discs ($t = -0.79$; $df = 38$; $P = 0.43$).

Bioassays With Plant Stimuli: Single Stimulus. Of the 750 larvae used across all single stimulus experiments, 95.6% entered one of the wells during the 1-h experimental period. Significantly more larvae were recovered from wells containing discs treated with root extracts of 420-A ($t = 6.56$; $df = 38$; $P < 0.0001$) or 3309 ($t = 7.04$; $df = 38$; $P < 0.0001$) than from wells with ethanol-treated discs (Fig. 1A and B). Significantly more larvae were found in wells containing root pieces from 420-A ($t = 8.54$; $df = 38$; $P < 0.0001$), 3309 ($t = 11.87$; $df = 38$; $P < 0.0001$), or apple ($t = 7.08$; $df = 38$; $P < 0.0001$) than in empty wells (Fig. 2A–C). Significantly more larvae were recovered from wells containing a disc treated with headspace volatiles from 420-A ($t = 8.07$; $df = 30$; $P < 0.0001$) or 3309 roots ($t = 6.18$; $df = 32$; $P < 0.0001$) than from wells containing dichloromethane-treated discs (Fig. 3A and B). There was no significant difference between wells containing discs treated with apple root volatiles or dichloromethane ($t = -0.19$; $df = 32$; $P = 0.84$; Fig. 3C).

Bioassays With Plant Stimuli: Paired Stimuli. Of the 500 larvae used across all paired stimuli experiments, 97.4% entered one of the wells during the 1-h experimental period. Significantly more larvae were found in wells containing pieces of 420-A ($t = 9.73$; $df = 38$; $P < 0.0001$) or 3309 roots ($t = 20.84$; $df = 38$; $P < 0.0001$) than in those with apple roots (Fig. 4A and B). Paired stimuli comparisons between apple roots and 420-A roots from 1-yr-old potted vines showed the same larval response ($t = 17.44$; $df = 38$; $P < 0.0001$) as was recorded when field-collected 420-A roots were used (Fig. 4C). There was no significant effect of grape rootstock on larval response from comparisons between 420-A and 3309 roots ($t = 0.13$; $df = 38$; $P = 0.89$; Fig. 5A), although significantly more larvae were found in wells containing discs treated with 3309 than 420-A root extract ($t = -3.47$; $df = 38$; $P = 0.0013$; Fig. 5B).

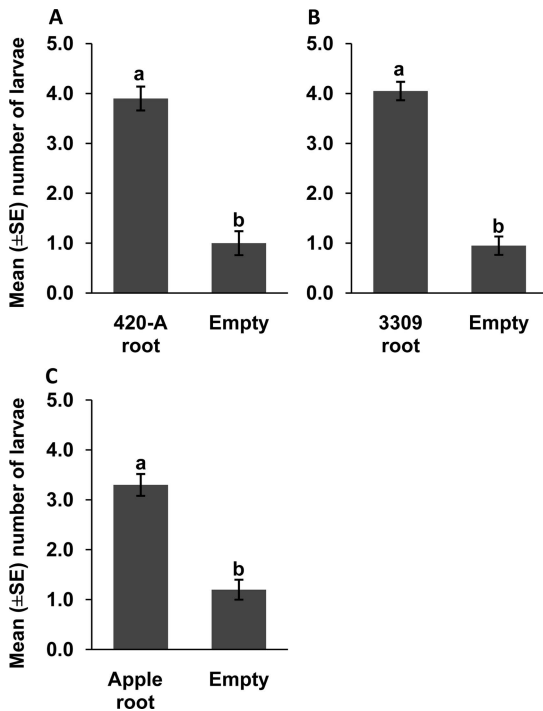


Fig. 2. Mean (\pm SE) number of *V. polistiformis* larvae in opposing bioassay wells containing root pieces from, (A) 420-A, (B) 3309, or (C) apple in single stimulus assays. Scale bars with the same letter are not significantly different at the 5% level of significance (Student's *t*-test).

Bioassays With Buried Roots. Of the 300 larvae used across all bioassays using buried roots, 86.3% entered one of the wells during the 3-h experimental period. When root pieces were buried in soil, significantly more larvae were recovered from wells containing 420-A ($t = 7.99$; $df = 38$; $P < 0.0001$) or 3309 roots ($t = 6.41$; $df = 38$; $P < 0.0001$) than from wells with soil only, but this effect was not observed with buried apple roots ($t = 0.70$; $df = 38$; $P = 0.48$; Fig. 6A–C).

Discussion

By eliminating the potential effects of visual, tactile, or contact chemoreception cues, modification of the bioassay used by Bergh et al. (2011) enabled better resolution of the behavioral response of recently eclosed grape root neonates to host and nonhost plant stimuli. Furthermore, ventilation of the assay dishes mitigated any potential effects of olfactory stimulus saturation within them. Importantly, unlike the assay used previously, which enabled larvae to move freely throughout the experiment, larvae that entered a well in the modified design were unable to leave. Consequently, a response by each larva to any stimulus that resulted in its entering a well was irreversible.

Preliminary experiments without plant cues revealed that other physical or chemical factors did not influence outcomes; larvae were equally distributed between wells in assays with both wells empty and

between wells that presumably differed in humidity level (i.e., with and without wet filter paper). Although Bergh et al. (2011) reported a significant response to filter paper discs that had been treated with ethanol and dried, this was not detected in the present studies. The high percentage of larvae recovered from wells at the end of the test period in all sets of experiments, including those without plant stimuli, was undoubtedly because of the positive geotropic behavior exhibited by grape root borer neonates that must burrow downward through soil to find food; that is, given the opportunity, larvae entered wells that enabled downward movement.

These experiments demonstrated clearly that larvae responded to volatile compounds associated with grape roots, independent of whether the cues were from fresh root pieces, root extracts, or head-space volatiles. Of interest, a significant response to apple roots was also recorded in single stimulus assays, and there are at least two possible explanations for this. First, in the absence of other cues, larvae may have responded to general root volatiles associated with the presence of plant tissue (Metcalf and Metcalf 1992, Bertin et al. 2003). Background odor can be used as a “search trigger” by specialist herbivores searching for food (Schröder and Hilker 2008) in advance of encountering specific host cues, as was shown for the clover root borer (Johnson et al. 2006, Reinecke et al. 2008). Leskey and Prokopy (2000) reported a similar behavioral response by adult plum curculio, *Conotrachelus nenuphar* (Herbst), to nonhost fruit volatiles in no-choice laboratory assays in which the outcomes were because of an all-or-nothing response by individuals, like those reported here. Second, larvae may have responded to the presence of CO₂ presumably released by actively respiring tissue from fresh apple root pieces and used this as a nonspecific plant orientation cue (Johnson and Nielsen 2012). Perception of general nonspecific plant cues such as CO₂ may elicit a behavioral shift in subterranean herbivorous larvae from random movement or immobility to a biased random movement that may ultimately lead to oriented movement on perception of host-specific cues (Johnson and Gregory 2006). Turlings et al. (2012) showed that the entomopathogenic nematode, *Heterorhabditis megidis* Poinar, Jackson & Klein, was significantly more attracted to CO₂ in combination with the induced plant volatiles, (E)- β -caryophyllene or dimethyl disulfide, compared with either CO₂ or the volatiles alone. Evidence that oligophagous insect herbivores are attracted to CO₂ (Jones and Coaker 1979, Bernklau and Bjostad 1998, Johnson and Nielsen 2012) indicates that evaluation of the behavioral response of grape root borer larvae to CO₂ is warranted.

The host-specific nature of volatile cues from grape was apparent from paired stimuli assays comparing grape roots or head-space volatiles with the corresponding stimuli from apple roots; all such comparisons resulted in a significantly greater response to grape than to apple stimuli. Johnson et al. (2004) showed similar discrimination between host and non-host roots by clover root weevil in choice tests. Leskey

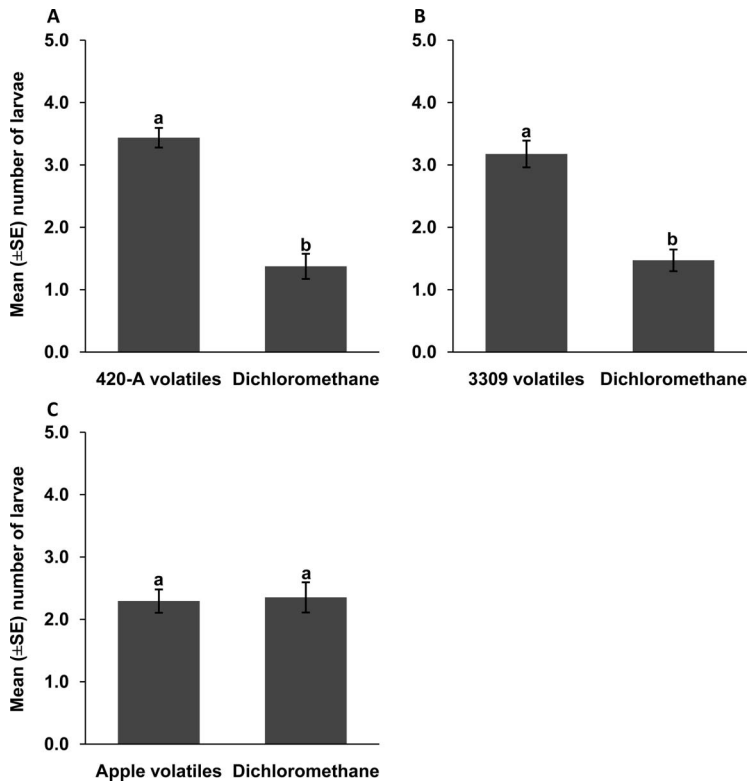


Fig. 3. Mean (\pm SE) number of *V. polistiformis* larvae in opposing bioassay wells containing dried filter paper discs treated with dichloromethane or root headspace volatiles in dichloromethane collected from roots of, (A) 420-A, (B) 3309, or (C) apple in single stimulus assays. Scale bars with the same letter are not significantly different at the 5% level of significance (Student's *t*-test).

and Prokopy (2000) reported a significantly stronger response of adult plum curculio to host than nonhost volatiles in choice test assays. The fact that 420-A roots from potted and field-grown vines elicited an essentially equal response in pairwise comparisons with apple roots was important, given that roots from potted vines were used for head-space volatile collections. Different physiological, environmental, geographic, genetic, and evolutionary factors can contribute singly or in combination to variation in plant secondary metabolite production (Figueiredo et al. 2008). Tapia et al. (2007) reported that the response of clover root borer larvae to clover root volatiles differed according to plant age; adult borers were attracted to volatiles from the roots of 1.5-yr-old red clover plants, but not to those from 2.5-yr-old plants. In our study, the response of grape root borer neonates to roots from 1-yr-old potted vines or to those from ≥ 6 -yr-old field-grown vines did not differ.

Our experiments showed no difference in larval response between 3309 and 420-A roots in a pairwise comparison, although the same comparison using root extracts resulted in a small but significantly higher response to 3309. These results differed from those of Bergh et al. (2011), who found that larval response was significantly greater to 420-A than to 3309 extracts in paired stimuli assays, raising the question of differences in the composition of root extracts from differ-

ent sources. Bergh et al. (2011) also showed a significantly greater response to root extracts from *V. riparia* 'Gloire' than to 3309 in pairwise comparisons. In that study, larvae contacting each extract-treated disc within an assay would have been exposed to a suite of compounds that likely differed widely in vapor pressure. Differences in response may have been because of different compounds or different concentrations of arresting or phagostimulatory compounds in 420-A and *V. riparia* Gloire than in 3309. Results from our comparison of 420-A and 3309 root pieces may indicate similar volatiles associated with each, although this awaits confirmation. Although not included in the studies reported here, evaluation of larval response to roots and root extract from a single rootstock in paired stimuli assays may yield additional and valuable insights.

The response of larvae in assays using root pieces buried in soil further enhanced the ecological relevance of these experiments (Eigenbrode and Espelie 1995). The significant response to grape roots indicated that the volatile cues from grape roots moved within the soil medium and were perceived by larvae. Interestingly, the significant larval response to apple roots in single stimulus assays without soil was not detected in assays with buried apple roots, conforming

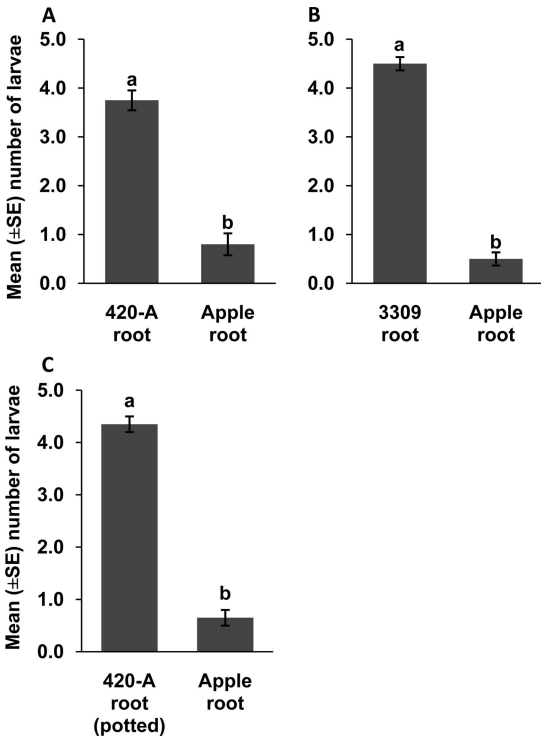


Fig. 4. Mean (\pm SE) number of *V. polistiformis* larvae in opposing bioassay wells containing root pieces from, (A) 420-A (field collected) vs. apple, (B) 3309 vs. apple, or (C) 420-A (potted vines) vs. apple in paired stimuli assays. Scale bars with the same letter are not significantly different at the 5% level of significance (Student's *t*-test).

to the results from tests using apple root head-space volatiles.

Other mono- and oligophagous insect species with root feeding stages have demonstrated behavioral responses to volatiles from host roots. Methyl eugenol

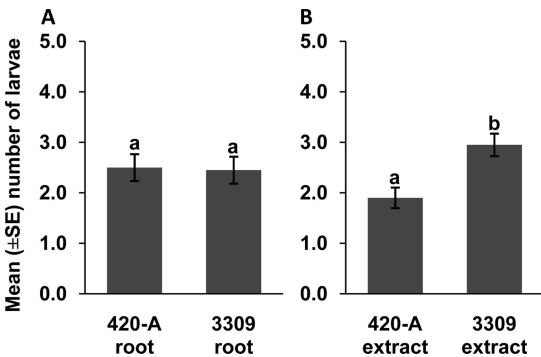


Fig. 5. Mean (\pm SE) number of *V. polistiformis* larvae in opposing bioassay wells containing, (A) root pieces from 420-A vs. 3309, or (B) dried filter paper discs treated with ethanol-based root extracts from 420-A vs. 3309 in paired stimuli assays. Scale bars with the same letter are not significantly different at the 5% level of significance (Student's *t*-test).

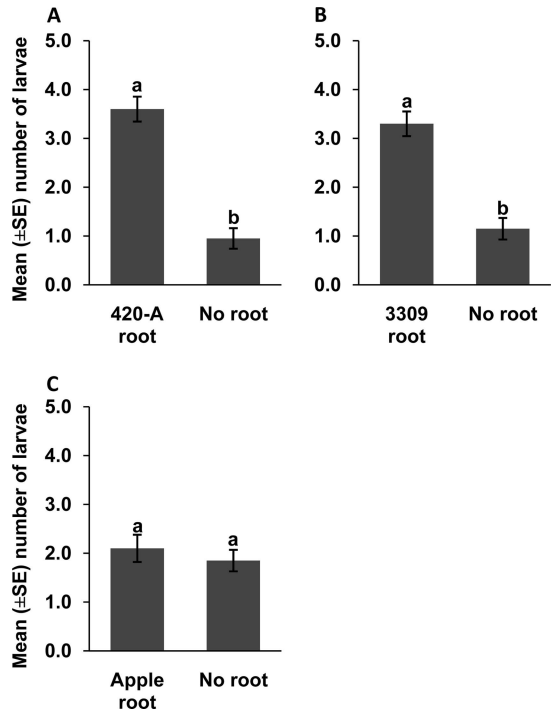


Fig. 6. Mean (\pm SE) number of *V. polistiformis* larvae in opposing bioassay wells containing root pieces from, (A) 420-A, (B) 3309, or (C) apple that were buried in soil. Scale bars with the same letter are not significantly different at the 5% level of significance (Student's *t*-test).

and sulfur compounds were attractive to carrot fly (Jones and Coaker 1977, 1979) and onion fly larvae (Matsumoto and Thorsteinson 1968, Soni and Finch 1979), respectively, and volatiles from root head-space and root extracts were attractive to clover root borer larvae (Kamm and Buttery 1984, Quiroz et al. 2005). Western corn rootworm larvae responded to CO₂ (Strnad et al. 1986, Hibbard and Bjostad 1988, Bernklau and Bjostad 1998) and also to the herbivore-induced volatiles, (E)- β -caryophyllene and ethylene (Robert et al. 2012b). A polyphagous white grub, *Costelytra zealandica* (White), used olfactory stimuli derived from rye grass roots for oriented movement toward its food (Sutherland 1972). Identification of the volatile compound(s) from grape roots that evoked the behavioral response recorded in the current study will be a critical next step toward understanding their role in the ecological relationships between grape root borer larvae and their many potential Vitaceae hosts. A preliminary comparison of the head-space volatiles from grape and apple roots (A.Z., unpublished data) has revealed grape-specific compounds that are candidates for further study.

Identification of the behaviorally active grape root volatiles will spur research on differences among commercially important rootstocks and wild *Vitis* species in the composition of root volatiles, the concentration of behaviorally active compounds, and their potential effects on host suitability to, and preference by, grape

root borer. Differences in the composition of volatiles produced by below-ground plant parts among cultivars (Guerin and Ryan 1984, Nottingham et al. 1989) have likely contributed to plant resistance to herbivory in some systems (Wang and Kays 2002). In a similar system to that reported here, two grape rootstocks, '5BB' and 'Kyoho' that differed in susceptibility to grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), showed different root volatile profiles (Du et al. 2009), although the compounds were not characterized. Although most studies over the last century have not indicated differences among *Vitis* species or rootstocks in their susceptibility to attack by grape root borer larvae (Brooks 1907, Massey 1945, Engelhardt 1946, Wylie 1972, Wylie and Johnson 1978), Webb and Mortensen (1990) reported that the native leatherleaf grape, *Vitis shuttleworthii* House, showed significantly less borer damage than other rootstocks without *V. shuttleworthii* in their parentage. An extensive and intensive survey by Rijal (J.P.R., unpublished data) has revealed that commercial vineyards in Virginia varied widely in the severity of infestation by grape root borer, leading to questions about the factors underlying this variation, including differences in susceptibility or suitability among rootstocks.

The response of grape root borer larvae to host-specific volatile compounds from grape roots leads to the question of whether this can be exploited in a management approach involving behavioral manipulation, as has been investigated in at least two other systems. In field and semifield experiments, food-finding by western corn rootworm larvae was disrupted by incorporating a source of CO₂ (Bernklau et al. 2004) or attractants from corn roots combined with insecticides (Hibbard et al. 1995, Bernklau and Bjostad 2005) into soil. Larval and adult cabbage root fly, *Delia radicum* L., respond to sulfur-based volatiles from brassica crops (Košťál 1992, Ross and Anderson 1992). Different sulfur application rates and formulations (granules, powder, prills, and sprays) significantly reduced the degree of root damage by larvae and egg deposition by adult cabbage and turnip root flies in canola, although results varied among sites and years (Doddall et al. 2002). Planned studies using soil columns will investigate the effects of soil-incorporated stimuli from grape roots on the rate and success of larval grape root borer food-finding and the distance over which they perceive these stimuli.

Acknowledgments

This research was funded in part by the Virginia Wine Board. We thank S. Abubeker for technical help with headspace volatile collections.

References Cited

All, J. N., and J. D. Dutcher. 1978. Current status of grape root borer infestations in Georgia: promising chemical control methods. *Ga. Agric. Res.* 19: 17–20.

- 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 N. J. Bostanian, C. Vincent, and R. Isaacs (eds.), *Arthropod Management in Vineyards*. Springer, Amsterdam, The 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.
- Bergh, J. C., D. G. Pfeiffer, and K. P. Love. 2005. Survey of grape root borer, *Vitacea polistiformis* (Harris), using pheromone traps in Virginia vineyards. *J. Entomol. Sci.* 40: 337–342.
- Bernklau, E. J., and L. B. Bjostad. 1998. Reinvestigation of host location by western corn rootworm larvae (Coleoptera: Chrysomelidae): CO₂ 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.
- Bernklau, E. J., E. A. Fromm, T. M. Judd, and L. B. Bjostad. 2005. Attraction of subterranean termites (Isoptera) to carbon dioxide. *J. Econ. Entomol.* 98: 476–484.
- Bertin, C., X. Yang, and L. A. Weston. 2003. The role of root exudates and allelochemicals in the rhizosphere. *Plant Soil* 256: 67–83.
- Brooks, F. E. 1907. The grapevine root borer. *W. Va. Agric. Exp. Stn. Bull.* 110: 20–30.
- Brooks, F. E. 1918. Grape root borer. *U.S. Dep. Agric. Bull.* 730: 21–28.
- Brown, V. K., and A. C. Gange. 1990. Insect herbivory below ground. *Adv. Eco. Res.* 20: 1–58.
- Clark, G. N., and W. R. Enns. 1964. Life history studies of the grape root borer (Lepidoptera: Aegeriidae) in Missouri. *J. Kansas Entomol. Soc.* 37: 56–63.
- Clark, K. E., S. E. Hartley, and S. N. Johnson. 2011. Does mother know best? The preference-performance hypothesis and parent-offspring conflict in aboveground-belowground herbivore life cycles. *Ecol. Entomol.* 36: 117–124.
- Doane, J. F., Y. W. Lee, N. D. Westcott, and J. Klingler. 1975. The orientation response of *Ctenicera destructor* and other wireworms (Coleoptera: Elateridae) to germinating grain and to carbon dioxide. *Can. Entomol.* 107: 1233–1251.
- Doddall, 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.
- Du, Y.-P., Q.-L. Zheng, H. Zhai, E.-S. Jiang, and Z.-Y. Wang. 2009. Selectivity of *Phylloxera viticola* Fitch (Homoptera: Phylloxeridae) to grape with different resistance and the identification of grape root volatiles. *Acta Entomol. Sin.* 52: 537–543.
- 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. 1978. Models of the distribution of subterranean stages of *Vitacea polistiformis* in Concord grape vineyards. *Environ. Entomol.* 7: 461–465.

- 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 (Lepidoptera: Sesiidae) 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.
- Engelhardt, G. P. 1946. The North American clearwing moths of the family Aegeriidae, pp. 222. United States National Museum Bulletin. Smithsonian Institute, Washington, D.C.
- Figueiredo, A. C., J. G. Barroso, L. G. Pedro, and J.J.C. Scheffer. 2008. Factors affecting secondary metabolite production in plants: volatile components and essential oils. *Flavour Fragr. J.* 23: 213–226.
- Gentry, C. R., and J. M. Wells. 1982. Evidence of an oviposition stimulant for peachtree borer. *J. Chem. Ecol.* 8: 1125–1132.
- Guerin, P. M., and M. F. Ryan. 1984. Relationship between root volatiles of some carrot cultivars and their resistance to the carrot fly, *Psila rosae*. *Entomol. Exp. Appl.* 36: 217–224.
- Herron, J. C. 1953. Biology of the sweet clover weevil and notes on the biology of the clover root curculio. *Ohio J. Sci.* 53: 105–112.
- Hibbard, B. E., and L. B. Bjostad. 1988. Behavioral responses of western corn rootworm larvae to volatile semiochemicals from corn seedlings. *J. Chem. Ecol.* 14: 1523–1539.
- Hibbard, B. E., F. B. Peairs, S. D. Pilcher, M. E. Schroeder, D. K. Jewett, and L. B. Bjostad. 1995. Germinating corn extracts and 6-methoxy-2-benzoxazolinone: western corn rootworm (Coleoptera: Chrysomelidae) larval attractants evaluated with soil insecticides. *J. Econ. Entomol.* 88: 716–724.
- Hiltpold, I., and T. J. Turlings. 2012. Manipulation of chemically mediated interactions in agricultural soils to enhance the control of crop pests and to improve crop yield. *J. Chem. Ecol.* 38: 641–650.
- Johnson, J. M. 1993. Flight, emergence, and oviposition patterns of the Douglas-fir pitch moth, *Synanthedon novoensis* (Hy. Edwards) (Lepidoptera: Sesiidae), in western Washington. M.S. thesis, University of Washington, Seattle, WA.
- Johnson, S. N., and P. J. Gregory. 2006. Chemically-mediated host-plant location and selection by root-feeding insects. *Physiol. Entomol.* 31: 1–13.
- Johnson, S., and U. Nielsen. 2012. Foraging in the dark—chemically mediated host plant location by belowground insect herbivores. *J. Chem. Ecol.* 38: 604–614.
- 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.
- Johnson, S. N., X. X. Zhang, J. W. Crawford, P. J. Gregory, N. J. Hix, S. C. Jarvis, P. J. Murray, and I. M. Young. 2006. Effects of carbon dioxide on the searching behaviour of the root-feeding clover weevil *Sitona lapidus* (Coleoptera: Curculionidae). *Bull. Entomol. Res.* 96: 361–366.
- Jones, O. T., and T. H. Coaker. 1977. Oriented responses of carrot fly larvae, *Psila rosae*, to plant odours, carbon dioxide and carrot root volatiles. *Physiol. Entomol.* 2: 189–197.
- Jones, O. T., and T. H. Coaker. 1979. Responses of carrot fly larvae, *Psila rosae*, to the odorous and contact-chemo-stimulatory metabolites of host and non-host plants. *Physiol. Entomol.* 4: 353–360.
- Kamm, A., and G. Buttery. 1984. Root volatile components of red clover: identification and bioassay with the clover root borer (Coleoptera: Scolytidae). *Environ. Entomol.* 13: 1427–1430.
- 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.
- Košťál, 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.
- 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.
- Leskey, T. C., and R. J. Prokopy. 2000. Sources of apple odor attractive to adult plum curculios. *J. Chem. Ecol.* 26: 639–653.
- Manosalva, L., F. Pardo, F. Perich, A. Mutis, L. Parra, F. Ortega, R. Isaacs, and A. Quiroz. 2011. Behavioral responses of clover root borer to long-chain fatty acids from young red clover (*Trifolium pratense*) roots. *Environ. Entomol.* 40: 399–404.
- Massey, A. B. 1945. Native grapes and their wildlife values. *Bull. Va. Polytec. Inst.* 38: 1–20.
- Matsumoto, Y., and A. J. Thorsteinson. 1968. Olfactory response of larvae of the onion maggot, *Hylemyia antiqua* Meigen (Diptera: Anthomyiidae) to organic sulfur compounds. *Appl. Entomol. Zool.* 3: 107–111.
- Metcalf, R. L., and E. R. Metcalf. 1992. Plant kairomones in insect ecology and control. Chapman & Hall, New York, NY.
- Nottingham, S., K.-C. Son, R. Severson, R. Arrendale, and S. Kays. 1989. Attraction of adult sweet potato weevils, *Cylas formicarius* elegantulus (Summers), (Coleoptera: Curculionidae), to sweet potato leaf and root volatiles. *J. Chem. Ecol.* 15: 1095–1106.
- Quiroz, A., F. Ortega, C. C. Ramírez, L. J. Wadhams, and K. Pinilla. 2005. Response of the beetle, *Hylastinus obscurus* Marsham (Coleoptera: Scolytidae) to red clover (*Trifolium pratense* L.) volatiles in a laboratory olfactometer. *Environ. Entomol.* 34: 690–695.
- Reinecke, A., F. Müller, and M. Hilker. 2008. Attractiveness of CO₂ released by root respiration fades on the background of root exudates. *Basic Appl. Ecol.* 9: 568–576.
- Robert, C.A.M., N. Veyrat, G. Glauser, G. Marti, G. R. Doyen, N. Villard, M.D.P. Gaillard, T. G. Köllner, D. Giron, M. Body, et al. 2012a. A specialist root herbivore exploits defensive metabolites to locate nutritious tissues. *Ecol. Lett.* 15: 55–64.
- Robert, C.A.M., M. Erb, M. Duployer, C. Zwahlen, G. R. Doyen, and T.C.J. Turlings. 2012b. Herbivore-induced plant volatiles mediate host selection by a root herbivore. *New Phytol.* 194: 1061–1069.
- Ross, K.T.A., and M. Anderson. 1992. Larval responses of three vegetable root fly pests of the genus *Delia* (Diptera: Anthomyiidae) to plant volatiles. *Bull. Entomol. Res.* 82: 393–398.
- Schröder, R., and M. Hilker. 2008. The relevance of background odor in resource location by insects: a behavioral approach. *Bioscience* 58: 308–316.
- 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.

- Soni, S. K., and S. Finch. 1979. Laboratory evaluation of sulfur-bearing chemicals as attractants for larvae of the onion fly, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae). *Bull. Entomol. Res.* 69: 291–298.
- Strnad, S. P., and M. K. Bergman. 1987. Movement of first-instar western corn rootworm (Coleoptera: Chrysomelidae) in soil. *Environ. Entomol.* 16: 975–978.
- Strnad, S. P., S. K. Bergman, and W. C. Fulton. 1986. First-instar western corn rootworm (Coleoptera: Chrysomelidae) response to carbon dioxide. *Environ. Entomol.* 15: 839–842.
- Sutherland, O.R.W. 1972. Olfactory responses of *Costelytra zealandica* (Coleoptera: Melolonthinae) larvae to grass root odours. *N.Z. J. Sci.* 15: 165–172.
- Tapia, T., F. Perich, F. Pardo, and A. Quiroz. 2005. Clover root borer, *Hylastinus obscurus* (Coleoptera: Scolytidae) has no preference for volatiles from root extracts of disease infected red clover. *Acta Agric. Scand.* 55: 158–160.
- Tapia, T., F. Perich, F. Pardo, G. Palma, and A. Quiroz. 2007. Identification of volatiles from differently aged red clover (*Trifolium pratense*) root extracts and behavioural responses of clover root borer (*Hylastinus obscurus*) (Marsham) (Coleoptera: Scolytidae) to them. *Biochem. Syst. Ecol.* 35: 61–67.
- Thorpe, W. H., A. C. Crombie, R. Hill, and J. H. Darrach. 1947. The behavior of wireworms in response to chemical stimulation. *J. Exp. Biol.* 23: 234–266.
- Turlings, T.C.J., I. Hiltbold, and S. Rasmann. 2012. The importance of root-produced volatiles as foraging cues for entomopathogenic nematodes. *Plant and Soil* 358: 51–60.
- Uren, N. C. 2000. Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants, pp. 19–40. *In* R. Pinton, Z. Varanini, and P. Nannipiero (eds.), *The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface*. Marcel Dekker, New York, NY.
- Wang, Y., and S. J. Kays. 2002. Sweetpotato volatile chemistry in relation to sweetpotato weevil (*Cylas formicarius*) behavior. *J. Am. Soc. Hort. Sci.* 127: 656–662.
- Webb, S. E., and J. A. Mortensen. 1990. Evaluation of bunch grape rootstocks and muscadine varieties for resistance to grape root borer. *Proc. Fla. State Hort. Soc.* 103: 310–313.
- Wenke, K., M. Kai, and B. Piechulla. 2010. Belowground volatiles facilitate interactions between plant roots and soil organisms. *Planta* 231: 499–506.
- Wylie, W. D. 1972. Grape root borer control. *Proc. Ann. Ark. 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.
- Zhang, A., H. Facundo, P. Robbins, C. Linn, Jr., J. Hanula, M. Villani, and W. Roelofs. 1994. Identification and synthesis of female sex pheromone of Oriental beetle, *Anomala orientalis* (Coleoptera: Scarabaeidae). *J. Chem. Ecol.* 20: 2415–2427.

Received 1 April 2013; accepted 13 August 2013.