ENVIRONMENTAL AND BEHAVIORAL FACTORS ASSOCIATED WITH THE INFESTATION OF VINEYARDS BY LARVAE OF GRAPE ROOT BORER

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Environmental and behavioral factors associated with the infestation of vineyards by larvae of grape root borer

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ABSTRACT

Grape root borer, *Vitacea polistiformis* (Harris), is an oligophagous pest of grapevines in the eastern USA. Neonates must burrow into the soil to find grape roots. In Virginia, larvae feed on roots for ~2 years, then pupate just beneath the soil surface. Emerging adults leave an empty pupal exuviae at the soil surface around the vine base. There was no relationship between weekly captures in pheromone traps and pupal exuviae counts, indicating that exuviae sampling is most appropriate to assess infestations. Exuviae sampling in Virginia vineyards revealed infestations that ranged from light to very heavy. Eighteen biotic and abiotic variables were measured and used in analyses that assessed their relative contributions to differences in exuviae density. Water holding capacity and clay/sand ratio were most strongly associated with pupal exuviae density; these variables were used to develop a model for predicting the extent of infestation of individual vineyards. The spatial distribution of pupal exuviae was characterized using non-spatial and geospatial techniques. Although the non-spatial method (Taylor’s Power Law) indicated that exuviae showed an aggregated distribution in all blocks, spatial methods (variograms, SADIE) revealed aggregated distributions only in blocks with $\geq 0.5$ pupal exuviae per vine. Independent pupal exuviae samples for population assessment in vineyards can be achieved using sampling points separated by $>8.8$ m. Combined results from geospatial analyses and the temporal distribution of pupal exuviae within years enabled the development of a practical and quantitative sampling protocol. Bioassays used to measure the behavioral response of larvae to host stimuli revealed that neonates were attracted to grape root volatiles. In soil column bioassays, larvae moved vertically and horizontally over distances of up to 120 cm and apparently perceived the presence of grape roots from a distance of 5 cm in soil. Results are discussed in relation to their potential implications for monitoring and managing grape root borer.
DEDICATION

To my parents for opening my eyes to the world
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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Taxonomy and Geographic Distribution of Grape Root Borer. Grape root borer, *Vitacea polistiformis* (Harris), is a clear-wing moth (Lepidoptera: Sesiidae) that, as its common name suggests, uses roots of grape, *Vitis* spp., as larval food. In appearance, adult grape root borers resemble *Polistes* wasps, from which its species name was derived (Harris 1854, Brooks 1907). Adults are dark to lustrous brown with orange and yellow bands on the abdomen (Brooks 1907, Sorensen 1975) (Fig. 1.1 A-C). Males are characterized by two pairs of orange colored tufts of scales on the posterior end of the abdomen (Harris 1854, Duckworth and Eichlin 1977) (Fig. 1.1 B). The species was first placed in the family Aegeriidae and named *Aegeria polistiformis* by Harris (1854), based on a holotype collected from an infested vineyard in Albemarle, North Carolina by F. J. Kron. Walsh (1868) described the morphological appearance of adults and larvae in general and its damage to vineyards in North Carolina and the mid-western states of the U.S.A. Brooks (1907) and Brimley (1938) subsequently placed the species in the *Memythrus* and *Paranthrene* genera, respectively. Engelhardt (1946) placed grape root borer in a new genus, *Vitacea*, along with three other species (*V. admiranda* (Hy. Edwards), *V. cupressi* (Hy. Edwards), and *V. scepsiformis* (Hy. Edwards), whose hosts are in the plant family, *Vitaceae*. Duckworth and Eichlin (1977) renamed the family from Aegeriidae to Sesiidae. Taft et al. (1991) summarized the common characteristics of 38 Sesiidae species that occur in the north-central United States, including wing-length, host plant, period of flight activity, and sex pheromone, and reported that *V. polistiformis* is the only species of *Vitacea* in this geographic region. Based on records of grape root borer presence and activity using sex pheromone traps and larval damage to vineyards, its geographic range has been reported to extend from Vermont to Florida and Minnesota to Texas in the west (Brooks 1918, Pollet 1975, Snow et al. 1991, Taft et al. 1991).

Life History of Grape Root Borer. Upon completing their development on vine roots, grape root borer larvae move to the top ~5 cm of soil to pupate within cocoons made of soil, frass, and silk (Pollet 1975, Dutcher and All 1979a). Pupae are 19 mm long and 5.4 mm wide on an average and vary in color from yellowish brown to dark brown (Bambara and Neunzig 1977). Olien et al. (1993) reported that pupal development was completed in 30 - 45 days and Dutcher and All (1979a) observed an average of 29 and 33 days for males and females, respectively, at
25°C. Adult emergence is initiated when the pupa uses spiral body movements to push halfway out of the cocoon and to reach the soil surface (Dutcher and All 1979a), and daily peaks of emergence occur between 9:00 and 11:00 AM (Brooks 1918, Pearson 1992). Following adult emergence, the copper colored pupal exuviae is left protruding from or lying on the soil surface around the vine base (Pollet 1975, Dutcher and All 1979a) (Fig. 2.1 E & F). A 1:1 adult sex ratio has been recorded (Dutcher and All 1978b, Townsend and Micinski 1981).

After a brief period of wing expansion and drying that typically occurs on the lower vine trunk, newly eclosed female moths walk up to the vine canopy. Release of sex pheromone begins in the afternoon (Snow et al. 1987, Pearson 1992), mating typically occurs within 30 min of the onset of pheromone release (Dutcher and All 1979a), and copulation extends over about four hours (Clark and Enns 1964, Dutcher and All 1978a) (Fig. 1.1 C). Oviposition may begin soon after mating or on the following day (Brooks 1907, Clark and Enns 1964, Dutcher and All 1979a). Females deposit ~350-500 eggs on the trunk and leaves of grapevines and vegetation in the vine row over 7-8 days (Brooks 1907, Sorensen 1975, Dutcher and All 1979a), and are thought to lay ~50% of their egg complement during the first 1-2 days after mating (Clark and Enns 1964, Dutcher and All 1979a). Brooks (1907) observed a female that deposited single eggs at intervals of a few inches along the vine over a distance of ~10 ft.

The dark brown, elliptical eggs (Fig. 1.2A) are 1.05-1.10 mm long and 0.70-0.75 wide and have a longitudinal groove on the ventral surface extending from the anterior to the posterior end (Brooks 1918, Bambara and Neunzig 1977). They are thought to be easily dislodged from plant surfaces and many may drop to the soil surface (Brooks 1907, Wylie and Johnson 1978). Larval eclosion occurs in 14 ± 2 SEM days at constant 30°C (Dutcher and All 1978a). The newly-hatched larvae are cream colored, ~2.4 mm long and have a brown head capsule (Bambara and Neunzig 1977) (Fig. 1.2B). Immediately after hatching, larvae burrow into the soil in search of grape roots (Brooks 1918, Clark and Enns 1964). During their search for roots, larvae often penetrate to depths of 5-20 cm and some have been found at depths of up to ~80 cm (Brooks 1907, 1918).

Dutcher and All (1978b) concluded that grape root borer shows a Type-III survivorship curve (Deevy 1947), characterized by high mortality during the egg and 1st instar stages. Young larvae are distributed uniformly throughout the root system, but tend to move toward the crown of the vine during their development (Clark and Enns 1964, Sarai 1972, Dutcher and All 1979a).
Following their initial establishment on a root, larvae eventually bore into the root cortex and create diagnostic feeding channels packed with reddish frass that increase in diameter as larvae mature (Dutcher and All 1979a) (Fig. 1.2C & D). The developmental duration of grape root borer larvae ranges from 1-3 years across its geographic range (J. R. Meyer, unpublished report), with the shortest and the longest periods in southern (e.g. Florida, Georgia) and northern regions (e.g. Ohio), respectively (reviewed in Bergh 2012). Studies have reported that larvae spend ~21-22 months inside roots (Brooks 1907, Clark and Enns 1964, Dutcher and All 1979a), although feeding may cease during the winter (Brooks 1918, All et al. 1987). Based on a study using potted vines in Florida, Webb and Mortensen (1990) reported that larvae completed development in one year. At the end of their feeding period, late instar larvae are ~29 mm long and 6 mm wide (Bambara and Neunzig 1977), although the number of larval instars of grape root borer has not been determined. The pupae are distributed around the base of vines; Clark and Enns (1964) reported that the majority of them were found within a 30 cm radius from the trunk and Dutcher and All (1978c) showed that ~90% of pupae occurred within a 35 cm radius.

The annual duration of adult emergence varies with latitude, ranging from two months in the northern portions of its range to six months in central and southern Florida (Pfeiffer et al. 1990, Snow et al. 1991, Webb et al. 1992, Bergh et al. 2005, Bergh 2006, Weihman and Liburd 2007). In Virginia, grape root borer adults emerge from about late June until about early September, with peak emergence between late July and early August (Pfeiffer et al. 1990, Bergh et al. 2005, Bergh 2006). In Florida, adult activity begins between early May and early August and extends into December, with peak activity reported to vary from mid-August to early October in different parts of that State (Snow et al. 1991, Webb et al. 1992, Weihman and Liburd 2007). The onset and duration of adult emergence are affected by variations in annual temperature and rainfall (Clark and Enns 1964, Sarai 1972; Webb et al. 1992); higher temperatures can trigger an earlier onset of emergence while high rainfall can extend the duration of the emergence period.

**Pest Status of Grape Root Borer.** Vineyards in the eastern USA are often planted near forested areas containing wild grape. At least 10 species of native *Vitis* have been recorded in southeastern States (Massey 1945) and grape root borer infestations in vineyards are thought to originate from populations on wild vines (Brooks 1907, All et al. 1987, Snow et al. 1990, Bergh 2006). The earliest reports of infestations in commercial plantings were from vineyards in
Kentucky, Missouri, North Carolina, and Ohio (Harris 1854, Walsh 1868). In the 1870’s, a severe infestation was reported from Albemarle, North Carolina, involving the loss of ~5000 vines from 107 cultivars of \textit{V. vinifera} imported from France (reviewed in Brooks 1918). Pollet (1975) reported that the pest destroyed ~300-350 acres of bunch grapes, \textit{V. labrusca}, in South Carolina between 1967 and 1975, and that many growers in that State had ceased grape production as a result.

Grape root borer has caused widespread damage to wine and table grape species, including \textit{V. vinifera}, \textit{Vitis labrusca}, and \textit{V. rotundifolia} (Johnson et al. 1981, Webb and Mortensen 1990, Olien et al. 1993), and is considered to be a pest of all commercially important grape cultivars (Harris et al. 1994). Larval feeding can completely destroy younger roots (<1.5 cm diameter) (Sarai 1972) and Pollet (1975) suggested that a single larva could kill a vine if feeding resulted in girdling of the crown. Up to 25 larvae have been reported on the root system of individual vines (Brooks 1907, Clark and Enns 1964) and Rijal (unpublished) recorded 38 larvae on the exposed roots of a single vine that had been removed from a severely infested vineyard block in Virginia. Wylie and Johnson (1978) reported that larval densities were reduced on heavily damaged vine roots, and suggested that this may have been due to resource depletion or to higher numbers of natural enemies in the vineyard.

Grape growers are often unaware that larval grape root borer populations have become established in their vineyards until the cumulative effects of feeding injury over several years have become apparent (Brooks 1907, Dutcher and All 1979b). This is largely due to a chronic lack of monitoring and scouting by growers (Bergh 2012) and to a lack of distinct and diagnostic symptoms on the aboveground parts of vines that can be ascribed unequivocally to the effects of grape root borer feeding on the root system (Brooks 1918, All et al. 1987). Although indistinguishable from those that can be caused by other conditions, symptoms associated with grape root borer infestations are discolored and smaller leaves, reduced shoot growth, fewer and smaller berries, and vine wilting (Sorensen 1975, All et al. 1987), which are collectively known as ‘slow vine decline’ (All et al. 1987). All et al. (1987) reported that infested vines usually began to show symptoms after 5-10 years of feeding, and declined progressively over 3 to 5 years, although Pollet (1975) suggested that larval feeding affects vine health and berry yield from the second year of infestation. In some cases, infested vines under proper fertilization and management for more than ten years still produced a satisfactory crop (Brooks 1918).
Managing and Monitoring Grape Root Borer. Historically, cultural practices were used to manage grape root borer infestations in vineyards. Brooks (1907) suggested that soil cultivation near the vine base to expose pupae or bury them deeply during the period of pupation and adult emergence would reduce infestations in the following years. Based on a laboratory study, Sarai (1969) reported >93% mortality when pupae were buried in soil to a depth of ≥ 2.5 cm. Creating soil mounds (25-30 cm deep) around the vine base after 90% of pupation had occurred revitalized the vineyard after two years in South Carolina (Pollet 1975). Adult emergence was reduced by ~85 to 90% by creation of a 15-25 cm deep mound under vines (Sarai 1969, Wylie 1972). However, soil mounding is labor-intensive and disruptive, and in regions where larval development occurs over two years, mounds must be created and then removed each year for at least for two consecutive years. Polyethylene sheets in the vine rows have been used to create a mechanical barrier to adult emergence (Attwood and Wylie 1963), although Yonce (1995) suggested that this is not a pragmatic option for many growers.

Historically, there have been attempts to use some Vitis spp. to reduce grape root borer infestation. Walsh (1868) reported that southern fox grape, Vitis vulpina L., was potentially resistant to grape root borer and some growers used vines grafted on V. vulpina rootstock during the late 1860s. However, Engelhardt (1946) reported an infestation by V. polistiformis in an old, well-established planting of fox grape, V. labrusca, on Staten Island, New York. Although Brooks (1907) stated that V. rotundifolia was resistant to grape root borer larvae, later studies did not support that conclusion (Wylie 1972, Wylie and Johnson 1978, Johnson et al. 1981). Webb and Mortensen (1990) reported less damage by larvae on roots of cultivars having the native leatherleaf grape, Vitis shuttleworthii House, in their parentage. Besides these limited studies, there have been no recent studies evaluating the resistance of grape cultivars, rootstocks or wild Vitis species against grape root borer. Currently, there is no evidence that commercially grown grape cultivars show resistance to grape root borer.

Natural enemies of grape root borer include fungal pathogens, entomopathogenic nematodes (Dutcher and All 1978b, All et al. 1981, Saunders and All 1985, Williams et al. 2002; 2010), a parasitoid, (Brooks 1907), predatory firefly larvae, birds and other vertebrate predators (Clarke and Enns 1964, Taylor 1965, Sarai 1972, Sorensen 1975,). Although there may be scope to exploit the above-mentioned natural enemies in biological control, research thus far has focused on the effects of entomopathogenic nematodes (reviewed in Bergh 2012). The nematode,
Steinernema carpocapsae (Wiser), was effective against grape root borer larvae in laboratory studies (All et al. 1981, Saunders and All 1985), but not in greenhouse or field trials (All et al. 1981, Saunders and All 1985). Williams et al. (2002) tested several strains of nematodes in the laboratory and greenhouse and selected Heterorhabditis bacteriophora Poinar (GPS11 strain) and H. zealandica (X1 strain) for further evaluation in the field. Later, Williams et al. (2010) reported that the native nematode, H. bacteriophora, effectively reduced grape root borer infestations in the field and that it showed higher persistence in soil than the non-native, H. zealandica. Although H. bacteriophora is commercially available for use in vineyards, most grape growers are not familiar with the potential of this management tactic (Bergh 2012).

Various synthetic insecticides and fumigants for grape root borer management were evaluated in the 1970s (All and Dutcher 1977, Dutcher and All 1979a), although most of the products used are no longer registered. Currently, chlorpyrifos, an organophosphate insecticide, is the only insecticide labelled for use against grape root borer, applied as a soil drench around the base of vines as a toxic barrier to the movement of neonates to roots. The residual activity of chlorpyrifos against larvae in soil is about 4 weeks (All et al. 1985), and such applications provide better efficacy when vine rows are free of vegetation (All et al. 1987). However, due to the environmental risks posed by chlorpyrifos, the availability of this product in the long term is uncertain (Williams et al. 2002). Furthermore, its 35-day pre-harvest interval in grape is problematic for early cultivars and for use in southern states, where the harvest coincides with peak hatch of grape root borer eggs (Pritchard 2004, Bergh 2012). As well, many wine grape growers are reluctant to use chlorpyrifos in this manner, based on their perceptions of its negative impacts on soil biodiversity and associated effects on vine health, berry quality, and ultimately, wine quality (Bergh 2012). As a rescue treatment for severe infestations, 4.5 pints of Lorsban 4E or an equivalent product in 100 gallons water are applied at the rate of 2 quarts per 15 ft² of soil around the vine base (http://www.cdms.net/LDat/LD02A001.pdf).

Methods to monitor and assess grape root borer populations in vineyards include inspecting the roots of vines that have been removed from the soil, sex pheromone traps, and scouting for pupal exuviae. Root inspection for larvae and feeding sites can provide a reasonable indication of the current and past infestation status of individual vines and has been employed by researchers (Brooks 1918, Sarai 1972, Dutcher and All 1979b, Jubb 1982, Harris et al. 1994). However, this rather crude and destructive sampling approach is not a pragmatic option for
growers in any but extreme circumstances. Sex pheromone-baited traps are sensitive and efficient tools for monitoring the onset, peak, and duration of flight activity in vineyards (Johnson et al. 1986, Alm et al. 1989, Johnson et al. 1991, Snow et al. 1991, Webb et al. 1992, Harris et al. 1994, Bergh et al. 2005, Bergh 2006, Weihman and Liburd 2007). However, since males originating from wild vines and/or nearby vineyards or vineyard blocks are also captured, the number of moths in traps does not necessarily reflect the infestation status of individual blocks (Snow et al. 1991, Webb et al. 1992, Bergh 2006). In Appendix A, a comparison of grape root borer captures in pheromone traps and pupal exuviae sampling showed that the results from use of these methods were not related. Recording the number of pupal exuviae on the soil surface around the base of vines at regular intervals during the adult emergence period is the only unequivocal, non-destructive indicator of the infestation status of individual vines. Pupal exuviae sampling has been used to measure the effects of cultural practices on infestations (Townsend 1991), to determine the distribution of pupae around vine base (Dutcher and All 1978c), and to assess the effects of various control tactics (Johnson et al. 1991, Pearson 1992).

Following the identification of the major component of the grape root borer sex pheromone, (E,Z)-2,13 octadecadienyl acetate, [(E,Z)-2,13-ODDA] by Schwarz et al. (1983), Snow et al. (1987) subsequently showed that the addition of 1% (Z,Z)-3,13 octadecadienyl acetate [(Z,Z)-3,13-ODDA]] significantly improved captures of male moths in traps. Early assessments of the potential utility of sex pheromone-based mating disruption for grape root borer management showed a significant reduction in the number of male moths attracted to caged virgin female in mating disruption plots (Johnson et al. 1981). Mating disruption trials using the minor (Z,Z) -3, 13-ODDA, major [(E,Z) -2, 13-ODDA], or both components of the V. polistiformis sex pheromone, have resulted in significant reductions in captures in traps or infestations in commercial vineyards (Johnson et al. 1986, Johnson et al. 1991, Pearson 1992). Complete trap shutdown was reported by Weihman and Liburd (2006), who used dispensers containing 95% (E,Z)-2, 13-ODDA (95%) and (E,Z)-3, 13-octadecien-1-ol (5%). Pfeiffer et al. (2010) reported reduced pupal exuviae counts in mating-disrupted vineyard blocks in Virginia and a wax-based “Specialized Pheromone and Lure Application Technology” (SPLAT) was also effective (Sanders et al. 2011). Currently, a mating disruption formulation (Isomate-GRB) containing (E,Z)-2, 13-ODDA (99%) and (Z,Z)-3, 13-ODDA (1%) is commercially available and is the preferred management option for grape root borer management.
Biotic and Abiotic Factors Affecting the Population Density of Grape Root Borer. Anecdotally, it is known that commercial vineyards in the eastern USA vary considerably in the extent of infestation by grape root borer; some vineyards or individual vineyard blocks support large and damaging populations while others are relatively unaffected. Ultimately, infestations are dependent upon the survivorship of first instar larvae and their successful establishment on roots. Sarai (1972) concluded that the low survivorship of first instar grape root borer larvae is likely influenced by soil moisture, with dry soil causing larval desiccation and higher mortality than soil with a higher moisture content. However, Townsend’s (1991) multi-year examination of the effects of several ground cover treatments and irrigation regimens on infestations in vineyards in Missouri resulted in no differences in pupal exuviae counts or adult emergence among the treatments after two years. Hypothetically, there are many biotic and abiotic factors that may act alone or in concert to affect the susceptibility of vineyards to attack and infestation by grape root borer, including cultivar, rootstock, vine age, planting area, proximity to wild grapevines, insecticide use, ground cover, weed control, irrigation, soil texture, soil moisture, pH, organic matter, bulk density, cation exchange capacity, soil particle size ratio. Although identifying the key risk factor(s) that affect vineyard vulnerability to grape root borer should provide important baseline information toward enhanced management capabilities for it, there has not been a comprehensive assessment of the horticultural, cultural, or environmental factors associated with differences in grape root borer infestations among vineyards.

Spatial Distribution of Insect Populations. Insect populations are often spatially heterogeneous (Southwood 1978, Liebhold et al. 1991). Knowledge of the dispersion and distribution of pest insect populations is vital to understanding their population dynamics (Sweeney and Miller 1989, Sharov 1996) and to develop reliable sampling schemes (Southwood 1978, Kuno 1991) for assessing infestations and informing management decisions (Hughes and Mckinlay 1988, Sweeney and Miller 1989, Liebhold 1991, Park et al. 2006, De Luigi et al. 2011). Insect population distributions have been widely measured using traditional mean-variance based techniques (Elliott 1971, Southwood 1978, Taylor 1984, Kuno 1991, Young and Young 1998), although they lack a spatial component. Non-spatial measures that have been used for this purpose include the negative binomial model (Kuno 1983), Lloyd's mean crowding (Lloyd 1967), the index of dispersion (Fisher et al. 1922, Elliott 1971), Iwao’s patchiness regression (Iwao 1968), and Taylor’s power law (Taylor 1961, Taylor 1984). In recent years,
geostatistical methods, including variography, kriging, and Spatial Analysis for Distance Indices (SADIE) have been used more commonly (Young and Young 1990, Midgarden et al. 1993, Perry 1995, Fortin and Dale 2005), and better characterize the true spatial distribution of insect species compared with traditional, non-spatial measures. Details about the use of geostatistics and SADIE were presented by Isaaks and Srivastava (1978), Legendre and Fortin (1989), Rossi et al. (1992), Liebhold et al (1993), Perry et al. (1999), and Perry et al. (2002). Despite its importance to the development of reliable sampling plans for estimating and predicting pest densities and to making rational management decisions (Taylor 1984, Legendre and Fortin 1989, Liebhold et al. 1993, Weisz et al. 1995), the spatial distribution of grape root borer in commercial vineyards has not been investigated.

Chemical groups such as alcohols, esters, and aldehydes represent ~37% of secondary metabolites that mediate host-plant location and recognition, 80% of which are attractants (Johnson and Gregory 2006). Chemically-mediated interactions between many root feeding insects and their host plants were reviewed by Johnson and Gregory (2006), Johnson and Nielsen (2012), and Hiltpold and Turlings (2012).

Carbon dioxide is a ubiquitous and behaviorally active compound to which many soil-dwelling insects respond. Johnson and Gregory (2006) reported more than 20 studies showing carbon dioxide as an attractant for a range of root-feeding insects, including larvae of D. virgifera virgifera (Strnad et al. 1986, Hibbard and Bjostad 1988, Bernkalu and Bjostad 1998). However, since carbon dioxide does not provide a host-specific signal, it is considered a ‘response activator’ or ‘search trigger’ rather than as a ‘key attractant’ in food-finding in the edaphic environment (Johnson et al. 2006, Reinecke et al. 2008, Schröder and Hilker 2008, Hiltpold and Turlings 2012, Turlings et al. 2012).

Food-finding by soil-dwelling herbivorous insects, particularly those with a limited host range (i.e. mono- and oligophagous), is guided primarily by plant-produced semiochemicals that enable them to recognize, locate, and establish on suitable host plants (Bais et al. 2006, Johnson and Gregory 2006, Wenke et al. 2010, Johnson and Nielson 2012). Various studies 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, Soni and Finch 1979, Jones and Coaker 1979, Koštál 1992, Quiroz et al. 2005, Kamm and Buttery 1984, Tania et al. 2007, Wenke et al. 2010, Manosalva et al. 2011) from host plants elicited positive behavioral responses from root-feeding insects.

Soil properties such as soil temperature, moisture, relative humidity, texture, and geometry of pore spaces have a significant influence on the release and movement of plant root volatiles in the soil, in which soil pore space represents 50% of total soil volume and includes two components, air and water, in approximately equal proportions (Brady and Weil 2002, Bekele et al. 2007, Hiltpold and Turlings 2008). Behaviorally active compounds released by plant roots are disseminated in gaseous or aqueous form (Hibbard and Bjostad 1988), and the diffusion of volatile secondary metabolites in soil is primarily dependent on their molecular weight, concentration gradient, and solubility, and other physico-chemical characteristics of the soil (Coleman et al. 2004, Erb and Lu 2013). However, most volatile compounds are typically
lipophilic liquids with high vapor pressure and can be released into the atmosphere or soil environment (Pichersky et al. 2006). Increasing tortuosity of soil pores can affect the behavioral response of soil-dwelling insects by either reducing the signal strength or by impeding their mobility (Villani and Wright 1990). Physical properties such as soil bulk density and soil type have pronounced effects on larval mobility (Strnad and Bergman 1987, Ellsbury et al. 1994, Pacchioli and Hower 2004).

Unlike other sesiids, such as dogwood borer and peachtree borer, which deposit their eggs predominantly on specific parts of host plant where larval feeding can occur (Gentry and Wells 1982, Koehler et al. 1983, Leskey and Bergh 2005), grape root borer females deposit eggs relatively indiscriminately on the above-ground parts of vines and other vegetation. This comparatively indiscriminate oviposition site selection behavior may be due to the below-ground larval food source. Since the native Vitis hosts of grape root borer occur in natural forest settings where the roots of many non-host species are likely co-mingled with host roots, it appears plausible that food-finding by grape root borer neonates may be guided by host-specific cues from grape roots.

Summary and Justification for Research

Grape root borer infestations in vineyards can be described as an ‘out of sight, out of mind’ issue due to the below-ground larval habitat and lack of diagnostic symptoms on the above-ground plant parts. As summarized above, problems from grape root borer are exacerbated by its widespread distribution through much of grape producing area in the eastern USA and to its use of wild species of Vitis that are common near vineyards. Importantly, there is no information about the factors that may influence the relative susceptibility of individual vineyards to infestation by grape root borer. Understanding the contribution of various biotic and abiotic factors to vineyard susceptibility may enable growers to predict and respond accordingly to the relative risk of infestation in new and established vineyard blocks. Since there has not been a comprehensive assessment of these potential risk factors, Chapter 2 describes a study that examined numerous horticultural and environmental factors of interest in relation to grape root borer pupal exuviae density among a large number of commercial vineyards. Appendix A presents data that support the appropriateness of pupal exuviae monitoring, compared with pheromone-based trapping, to assess grape root borer densities in vineyards.
Also of great importance to problems associated with grape root borer is the fact that most growers do not monitor the presence or abundance of this pest in their plantings, which too often results in economic impacts from the cumulative effects of infestation. In no small part, inadequate scouting is due largely to the fact that there has been no systematic attempt to describe the spatial distribution of grape root borer infestations in vineyards, which would enable the development of an appropriate sampling scheme. In Chapter 3, the spatial distribution of grape root borer infestations in vineyards was described using non-spatial and geospatial techniques and a quantitative sampling protocol based on these distributions was developed.

There is little known about the ecological and behavioral interactions between larval grape root borer and grape roots or about the capacity of the tiny neonates to find food in the soil environment. This is likely due in part to the widely recognized difficulties associated with research on edaphic insects. These basic aspects of grape root borer biology are fundamental to understanding how this key phase in its life history, upon which infestations ultimately depend and during which mortality is highest, influence its successful establishment on grape roots. In Chapter 4, the behavioral response of grape root borer neonates to stimuli from host and non-host roots was examined. Appendix B presents the development of a soil-based bioassay that was used to address basic questions about the food-finding behavior and capacity of neonates.

**Specific Research Objectives**

**Objective 1**: Determine the relationship between pupal exuviae sampling and pheromone trapping to assess grape root borer infestations in vineyards

**Objective 2**: Identify key risk factors associated with grape root borer infestations and develop a model to predict the likelihood of infestation

**Objective 3**: Characterize the spatial distribution of grape root borer infestations in vineyards and develop a quantitative sampling scheme

**Objective 4**: Evaluate the response of grape root borer neonates to grape root volatiles

**Objective 5**: Measure the movement capacity of grape root borer neonates in soil column bioassays
Fig. 1.1 Grape root borer adults, A) female on the trunk of a grape vine, B) newly eclosed male, C) male (right) and female (left) *in copula.*
Fig. 1.2 A) Grape root borer, A) egg, B) freshly hatched larva, C) larva feeding on root, D) larval damage on grape root showing feeding channel packed with diagnostic reddish frass, E) pupal exuviae protruding from soil at the vine base, and F) pupal exuviae lying on the soil surface.
CHAPTER 2: BIOTIC AND ABIOTIC FACTORS ASSOCIATED WITH GRAPE ROOT BORER INFESTATIONS IN COMMERCIAL VINEYARDS

Abstract
Larval grape root borer, *Vitacea polistiformis* (Harris) feeds on roots of wild *Vitis* and commercially important *Vitis* species and rootstocks in parts of the eastern USA. Grape root borer pupal exuviae sampling in Virginia vineyards from 2008 to 2012 revealed that infestation levels varied substantially among 48 vineyard blocks. Horticultural (cultivar, rootstock, vine age, planting area), cultural (insecticide use, ground cover, weed control, irrigation) and environmental data (proximity to forest, and soil composition, moisture, pH, organic matter, bulk density, and cation exchange capacity) from each block were subjected to optimal quantification using Categorical Principal Component Analysis (CATPCA). Variables with higher component loadings from the CATPCA were used as predictors and pupal exuviae density as the dependent variable in binary logistic regression. A prediction model was developed by including statistically significant variables in the logistic regression. CATPCA showed that seven vineyard factors (ground cover, soil texture, soil mass moisture, soil pH, clay/sand ratio, clay/silt ratio, sand/silt ratio) based on three selected principal components were significant for subsequent regression analysis. Binary logistic regression showed that soil mass moisture and clay/sand ratio were statistically significant factors contributing to differences in infestation among vineyard blocks. Based on these two factors, a risk prediction model for calculating the probability of grape root borer infestation in vineyards was developed. Results are discussed in relation to the practical implications of a predictive, risk assessment model for grape root borer management.

**Key words:** *Vitacea polistiformis, Vitis vinifera*, risk factors, CATPCA, logistic regression
Introduction

Grape root borer, *Vitacea polistiformis* (Harris) (Lepidoptera: Sesiidae), has been considered a pest of grapevines in parts of the eastern United States for more than 150 years (Harris 1854, Walsh 1868, Clark and Enns 1964, Pollet 1975, Dutcher and All 1976, Bergh 2012). The oligophagous larvae feed on roots of plants in the family Vitaceae, including wild *Vitis* species and commercially important *Vitis* species and rootstocks. Larval feeding creates large channels packed with reddish frass in the root cortex (Dutcher and All 1979a, Bergh 2012). A severe infestation by grape root borer can result in discolored and smaller leaves, reduced shoot growth, fewer and smaller berries, and vine wilting (Sorensen 1975, All et al. 1987). All et al. (1987) reported that vines usually began to show symptoms after 5-10 years of infestation and declined over the subsequent 3- to 5-year period. In severe cases, the cumulative effects of infestations may lead to vine death (Dutcher and All 1976). Importantly, diagnosing a grape root borer infestation based on symptoms expressed by the above-ground parts of vines is hindered by the fact that these symptoms, known collectively as ‘slow vine decline’ (All et al. 1987), also can be caused by a number of other horticultural and pathological conditions.

In Virginia, female grape root borers oviposit in a comparatively indiscriminate manner on above-ground parts of vines and on vegetation in vine rows in July and August (Brooks 1907, Sorensen 1975, Dutcher and All 1979a). Newly-hatched larvae enter the soil to find and establish on grape roots and vine infestation is ultimately dependent upon their success during this critical phase. In Virginia, larvae feed for about two years before pupating beneath the soil surface around the base of vines on which they developed (Clark and Enns 1964, Dutcher and All 1979a), and infested vines may support overlapping generations. Upon emerging from the soil, adult grape root borers leave a relatively large, copper-colored pupal exuviae protruding from or lying on the soil. Sampling pupal exuviae at regular intervals through the period of adult emergence is the only non-destructive and most accurate and unequivocal method by which to assess the presence of larvae on individual vines (Bergh 2012).

Anecdotal reports from Virginia and neighboring states indicate that the extent of vineyard infestation by grape root borer can vary tremendously; some sites become heavily infested and suffer significant impacts, while others are relatively unaffected. A long-standing and serious issue with grape root borer is that growers are often unaware that an infestation has developed until the effects on vine health and productivity have become apparent (Brooks 1907,
Despite these circumstances, the factors underlying differences in grape root borer density among vineyards and vineyard blocks have not been examined comprehensively or holistically.

Many biotic and abiotic factors can influence the establishment and density of below-ground insect herbivores on roots of host plants (Brown and Gange 1990, Erb and Lu 2013). Horticultural factors may include cultivar (Shapiro and Gottwald 1995), rootstock (Beavers and Hutchison 1985, Du et al. 2009), plant age (Alinia et al. 2000), or crop acreage (Nagoshi 2009). Cultural factors related to crop management, such as insecticides, irrigation, ground cover, or weed control can also influence pest density and extent of crop damage (Townsend 1991, Godfrey and Yeargan 1985, Tonhasca and Stinner 1991, Oyediran et al. 2007). Environmental variables that may affect populations of below-ground pests include the presence and proximity of wild hosts (Hoffman and Dennehy 1989), weather and associated effects on soil properties (Sarai 1972, Brown and Gange 1990), and natural enemies (Williams et al. 2010, Shapiro-Ilan et al. 2003). Soil properties that may be important to the survivorship of soil-dwelling insects include soil moisture (Johnson et al. 2010), texture (Brown and Gange 1990, Brust and House 1990), particle size (Lummus et al. 1983), pH (Li et al. 2007, Johnson et al. 2010) and organic matter content (Brust and House 1990, Villani and Wright 1990). This is particularly true for species showing a Type-III survivorship curve, characterized by high mortality during the egg and early larval stages (Hawley 1949, Marrone and Stinner 1984), as is the case for grape root borer (Dutcher and All 1978). Additionally, soil characteristics can affect the mobility of below-ground species (Strnad and Bergman 1987, Gustin and Schumacher 1989, Pacchioli and Hower 2004) and the diffusion of behaviorally active compounds from host roots (Villani and Wright 1990, Johnson and Gregory 2006).

The individual effects of some of these biotic and abiotic variables on grape root borer populations have been examined in the laboratory, semi-field, or field studies. Although rootstocks containing the Florida leatherleaf grape, *Vitis shuttleworthii* House, in their parentage were shown to confer some level of resistance to larval establishment (Webb and Mortensen 1990), the general consensus is that most commercially important rootstocks and cultivars are susceptible to this pest (Massey 1945, Wylie 1972, Wylie and Johnson 1978, Webb and Mortensen 1990, Harris et al. 1994). Bergh et al. (2011) showed that grape root borer neonates responded positively to ethanol-based extracts of roots from several native *Vitis* species and
commercial rootstocks, with indications of a stronger response to some. Subsequently, Rijal et al. (2013) revealed that volatile emissions from roots were attractive to larvae. Although Harris et al. (1994) mentioned higher infestations in older vines, All et al. (1987) reported that 1-year-old vines were infested, and Rijal (unpublished data) observed numerous instances of 3– to 5-year-old vines supporting larval populations. Townsend (1991) evaluated the effects of various cover crop and irrigation regimens on grape root borer densities and found no significant influence of those factors. The influence of rainfall and soil moisture on larval grape root borer mortality have been reported (Clark and Enns 1964, Sarai 1972); mortality increased with an increase in soil moisture and decreased with an increase in soil depth, based on a laboratory study using three soil moisture levels (0, 20, 30%) and five soil depths between 0 and 25 cm (Sarai 1972).

Despite the previous evaluations of the influence of some vineyard factors on grape root borer populations, much remains unknown about the individual or combined effects of the many variables that may influence this pest under field conditions. Identifying potential risk factors that may underlie vineyard vulnerability to grape root borer and associated differences in population densities among sites should provide important information toward understanding the relative risk from this insidious pest for individual vineyards or vineyard blocks. Here we report the results of a multi-year study comparing data from intensive pupal exuviae sampling in 50 vineyard blocks in Virginia with measurements of a broad range of biotic and abiotic variables taken from the sample sites.

**Materials and Methods**

**Site Selection and Preparation.** For the purposes of this study, we defined a vineyard as the total area planted to grapevines on each farm, regardless of cultivar or rootstock. A vineyard block was defined as a portion of a vineyard within which the vines were essentially uniform in cultivar, rootstock, vine age, and other environmental and cultural practices. At our study sites, the vineyard blocks were $1.42 \pm 0.45$ SE ha. A sampling area of $\approx 0.4$ ha within each block was created, as described below. Forty-eight blocks from 19 commercial vineyards (2 or 3 blocks per vineyard) in northern and central Virginia were used for sampling and were located in Loudoun ($n = 11$), Rappahannock ($n = 8$), Shenandoah ($n = 5$), Rockingham ($n = 3$), Albemarle ($n = 11$), Fauquier ($n = 3$), Frederick ($n = 4$) and Nelson ($n = 3$) counties. Forty-four blocks were
sampled in one year between 2008 and 2012, while four blocks were sampled in each of 3 to 4 consecutive years, using the same sample vines each year. None of the blocks selected were treated with an insecticide (i.e. chlorpyrifos) or mating disruption that specifically targeted grape root borer prior to or during the study.

Over the five years of the study, we selected blocks that represented a range of the cultural, horticultural and environmental variables of interest. Sampled blocks contained vines that were $11.5 \pm 0.82$ SE years-old (range: 2- to 23-years-old). The 11 grape cultivars represented included Chardonnay (n = 7), Vidal (n = 7), Petit Verdot (n = 5), Viognier (n = 5), Cabernet Franc (n = 8), Riesling (n = 1), Merlot (n = 2), Norton (n = 3), Cabernet Sauvignon (n = 5), Sauvignon Blanc (n = 1), and Chambourcin (n = 4). Seven rootstocks were represented, including 3309 (n = 24), 101-14 (n = 4), V. riparia Gloire (n = 4), SO4 (n = 8), 5BB (n = 3), own-rooted Norton (n = 3), and own-rooted Vidal (n = 2). The blocks varied in their proximity to forested areas, but the neither presence nor abundance of wild Vitis at the border or interior of those areas was quantified.

Per standard viticulture terminology, a panel was defined as the space between consecutive posts to which the trellis wire was attached, and typically contained 3 – 5 vines. Most sampling areas (n = 30) consisted of a rectangular grid of 80 sample vines that included the first vine in each of 10 consecutive panels within a row, in every second vine row across 16 rows. In the others (n = 18), the sampling area consisted of a grid of 39 – 118 sample vines. The sampling areas were prepared for the survey in late June or early July of each year. All vegetation and debris was removed to create a clean soil surface within a $\approx 1$ m diameter circle around the trunk of each sample vine. In blocks with weeds or grass in the vine rows, the vegetation was removed using a ‘string trimmer’, followed by raking, while only raking was required in blocks with cleaner cultivation in the rows. During the sampling period, weeds that began to grow in the cleaned area were removed by hand during the weekly visits to every vine.

**Pupal Exuviae Sampling and Measurement of Vineyard Variables.** At weekly intervals between early July and late August, the clean area around the base of each sample vine was inspected and the number of grape root borer pupal exuviae found was recorded. All exuviae found each week were removed from the site.

Horticultural information about cultivar, rootstock, vine age, and planting area for each vineyard block was collected from the cooperating growers. The distance from each sampled
block to the nearest forested area was measured using Google Earth (Google Inc. 2013). Five perpendicular distances between the edge of each vineyard block and the nearest woodlot were measured and averaged. Information about cultural practices, including insecticide use, ground cover and weed control, and irrigation was obtained via a questionnaire to the manager of each vineyard. Since the grape root borer has a two-year developmental period in Virginia, the pupal exuviae sampled in any given year represented the generation that was initiated two years previously, and the questionnaire was designed to capture those historical data during the period between late June and early September, when grape root borer adults are active and larvae are hatching from eggs. Insecticide use included the name, rate, and frequency of insecticide applications. Information on the percentage of ground cover, including weeds and grass in the vine rows, was based on a rating of 1 (1 – 40% cover) or 2 (> 40% cover). Data on weed control were based on a qualitative scale that rated the aggressiveness of weed control practices used, where 1 = none, 2 = light, 3 = medium, and 4 = aggressive control. Information regarding the use of irrigation was based on a binary, yes or no response.

In fall, 2012, five soil samples (~25 cm long core from corners and center) from each sampled area were collected using a standard soil auger and combined to create one composite sample for each sample area, following standard protocols (Donohue 2000). Analysis of these samples for soil organic matter, pH, texture, particle size analysis, soil mass moisture, and cation exchange capacity was conducted at the Virginia Tech Soil Testing Laboratory, Blacksburg, VA. Percent soil mass moisture was estimated based on the water holding capacity of soil (at -1/3 bar FC and measured as the percentage of weight difference between wet and dry soil). In addition, one sample using a core ring sampler (5.08 cm diameter and length) was collected from each block, oven-dried at 105°C for 24 h, and weighed. The bulk density of each sample was calculated using the formula:

\[
\text{Bulk density} = \frac{\text{Mass of dry soil}}{\text{Volume of core ring}}
\]

**Statistical Analyses:** Nonlinear Principal Component Analysis (NLPCA), also known as Categorical Principal Component Analysis (CATPCA), is an alternative to traditional principal component analyses (PCA). CATPCA can be used to explore nonlinear relationships based on qualitative (i.e. nominal and ordinal variables) and quantitative data (Linting and van der Kooij
2012). The two main purposes for using CATPCA are to reduce the dimensionality of original variables into a few uncorrelated principal components (PCs) and to remove the likelihood of multicollinearity among variables for statistical validity (Howling et al. 1993, Graham 2003). Initially, the 18 variables described above were subjected to a nonlinear optimal quantification process, called optimal scaling, which transformed qualitative into quantitative variables (Linting et al. 2007). Here, the meaning of ‘optimal’ is that the transformations were optimal for the model that was fitted. Data from cultivar, rootstock, and soil texture (nominal variables), ground cover, weed control, and irrigation (ordinal variables), and vine age, planting area, proximity to forest, number of insecticide sprays, soil pH, soil bulk density, soil organic matter, soil cation exchange capacity, soil mass moisture, clay/sand ratio, clay/silt ratio, and sand/silt ratio (numeric variables) measurements were included in CATPCA analysis, conducted using SPSS (IBM Corp. 2012). The stepwise analysis process follows.

**Analysis Level Selection and Discretization.** Analysis level selection refers to the amount of freedom allowed in transforming categorical values (i.e. labels) to category quantifications. Nominal scaling, spline ordinal scaling and numeric scaling were used for the quantification process of nominal, ordinal and numeric variables, respectively, from each sampled block. The following step was ‘discretization’, which was the process by which continuous or zero values were converted to integers to satisfy the computational requirement of CATPCA (Linting and van der Kooij 2012). All nominal and ordinal variables were discretized using the ‘ranking’ option, while numeric variables were discretized using the ‘multiplying’ option. In CATPCA, only cells with missing values were excluded from analysis, rather than excluding entire rows or columns with missing values, as is the case with linear PCA. This type of passive’ treatment excludes rather than estimates those values (Linting and van der Kooij 2012). In 12 of the 48 vineyard blocks, there were one or more variables with missing values.

**Principal Components and Variable Selection.** The procedure for selecting the number of principal components in CATPCA is different from PCA, since the number of PCs (i.e. the number of dimensions) should be selected before performing the analysis and since eigen values for each PC may differ based on the number of dimensions selected. We selected three PCs using two criteria; total variance explained by selected PCs (Comrey 1973, Linting and van der Kooij 2012) and the scree plot (Fig. 2.3) (Fabrigar et al. 1999). The internal consistency (i.e. the degree of relatedness among variables) was assessed by Cronbach’s Alpha, in which a value of ≥ 0.70
indicated strong internal consistency among variables (Cortina 1993). In CATPCA, the first component accounts for the highest proportion of total variance in the data set, the second explains the majority of variance not explained by the first, and the third explains the variance not explained by first and second components. A larger component loading for a variable represents a greater contribution of that variable in the component.

Original variables that had component loadings of $\geq 0.70$ across the selected PCs, as indicated by CATPCA, were selected to determine the association of the vineyard variables with grape root borer pupal exuviae densities (i.e. mean total number of pupal exuviae per vine) (Brazzle et al. 1997). Binary logistic regression analysis was conducted, using “infestation status” (see below) as the dependent, indicator variable and the selected vineyard variables as predictors. Binary logistic regression predicts the probability of a state of a dichotomous dependent variable, based on predictor variables. Although Dutcher and All (1979b) reported an economic threshold of 0.074 larvae per vine, based on a study in Concord grape, V. labrusca, in Georgia, Bergh (2012) suggested that this may be very conservative for grape root borer on V. vinifera in Virginia. We used a cut-off of 0.10 pupal exuviae per vine to separate the sampled areas into the following two infestation status categories for binary logistic regression analysis; lightly infested ($\leq 0.10$ exuviae per vine) and heavily infested ($>0.10$ exuviae per vine). These categories were indicated by ‘0’ and ‘1’, respectively, in the analysis.

The logit of the regression model was:

$$g(x) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k,$$

The logistic regression model was,

$$\pi(x) = \frac{e^{g(x)}}{1 + e^{g(x)}}$$

where $\pi(x)$ was the conditional probability of a positive outcome (i.e. heavy infestation), ‘$k$’ was the number of independent variables (i.e. $x_1 \ldots x_k$), and $\beta$ (i.e. $\beta_1 \ldots \beta_k$) were the coefficients of the predictor variables included in the model.

The statistical significance of the regression model was tested using the Omnibus test statistic (Maroof 2012), and the fitness of the model was measured by Nagelkerke $r^2$ values
(Nagelkerke 1991), although $r^2$ values are relatively low in logistic regression compared with linear regression (Norušis 2005). The Wald statistic (at 5% significance level) was used to evaluate the effects of individual variables in the model by testing the null hypothesis that the coefficient associated with each variable is zero. The Wald value is the ratio of a parameter estimate ($\beta$) to its standard error (Texler and Travis 1993). Hosmer and Lemeshow’s Goodness of Fit Test (Hosmer and Lemeshow 2000) was conducted to quantify the level of agreement between the estimated and the observed values (Gribko et al. 1995, Wulder et al. 2006), in which $P \geq 0.05$ indicated a good fit. We used the Enter method to include independent variables into the regression, where all selected predictor variables were entered into the equation simultaneously (Maroof 2012).

**Prediction Model.** The original vineyard variables that contributed significantly to vineyard infestation status, based on the Wald statistic, were again regressed on the response variable (light or heavy infestation) using binary logistic regression to develop a final prediction model. The interpretation of a logistic regression coefficient, $\beta$, is not as straightforward as for a linear regression coefficient. Therefore, the exponential coefficient, ($e^\beta$), which is commonly known as the ‘odds ratio’ was used to interpret the outcome (Norušis 2005). The odds ratio refers to the ratio change in the odds of the event (i.e. infestation status) for a unit change in the predictor variable after taking into account all other predictors in the model (Freund and Wilson 1998, Wulder et al. 2006, Maroof 2012). Odds is defined as the ratio of the probability that an event occurs (in this case, that the block is heavily infested), divided by the probability that it does not (in this case, that the block is not infested or lightly infested) (King 2008). Binary logistic regression analyses were conducted using SPSS (IBM Corp. 2012).

**Results**

**Pupal Exuviae Sampling.** Pupal exuviae densities varied widely among the vineyard blocks, ranging from 0.0 to 6.4 exuviae per vine in a single year (Fig. 2.1). The maximum number of exuviae collected from a single vine in one year was 28. In the four blocks assessed annually over several consecutive years, the mean number of exuviae per vine declined between 2008 and 2011 in blocks A and B (Fig. 2.2). In block C, the mean number of exuviae per vine increased and then decreased during the same interval, while they remained essentially the same.
at block D from 2010 to 2012. In those blocks, the cumulative number of pupal exuviae collected from a single vine over 3 to 4 years of sampling ranged from 0 to 19.

**Identification of Risk Factors.** Using CATPCA, the 18 nominal, ordinal, and numeric variables were reduced to three Principal Components (PC) representing seven original vineyard variables, ground cover, soil texture, soil mass moisture, soil pH, clay/sand ratio, clay/silt ratio, and sand/silt ratio, which accounted for 53.65% of the total variance (Table 2.1). The CATPCA model summary resulted in a Cronbach’s Alpha of 0.949, indicating strong internal consistency among these three principal component variables. These seven variables all showed component loading values of $\geq 0.70$ (Table 2.1). Correlations among the 18 variables that were calculated using optimally quantified (i.e. transformed) values for each variable in CATPCA are shown in Table 2.2.

**Association of Key Vineyard Factors with Infestation.** The variable, soil texture, was redundant with the ratio of soil particle size and was therefore excluded. Six variables showed significant component loadings and were used for binary logistic regression analysis (Table 2.3). The regression model was significant, based on the Omnibus test statistic ($\chi^2 = 13.98; df = 6; P = 0.03$), and explained 43.1% of total variability, indicated by the Nagelkerke $r^2$ value from the regression model. Hosmer and Lemeshow’s goodness of fit test showed no significant difference ($\chi^2 = 1.77; df = 8; P = 0.987$) between observed and predicted values. Two variables, soil mass moisture and clay/sand ratio, significantly contributed to infestation status (Table 2.3).

Based on these results, soil mass moisture and clay/sand ratio were regressed against the binary infestation status categories (i.e. light and heavy). The binary logistic regression model was significant, based on the Omnibus test statistic ($\chi^2 = 13.95; df = 2; P < 0.001$), and showed a Nagelkerke $r^2$ value of 0.345. Hosmer and Lemeshow’s statistic ($\chi^2 = 15.08; df = 8; P = 0.06$) indicated a good fit of the model. Based on the Wald test on regression coefficients of individual predictors, the effect of soil mass moisture was significant ($\chi^2 = 8.46; df = 1; P = 0.004$) and negatively associated with infestation status. The clay/sand ratio was also significant ($\chi^2 = 6.54; df = 1; P = 0.011$), but positively associated with infestation status (Table 2.4). The intercept or constant of the model was significantly different from zero ($\chi^2 = 7.75; df = 1; P = 0.005$). The odds ratio ($e^\beta$) for soil mass moisture and clay/sand ratio were 0.68 and 25.63, respectively (Table 2.4).
**Prediction Model.** Based the results of regression analyses, the relationship between the significant, original vineyard variables and the probability of a vineyard block being infested by grape root borer was be calculated using the equation,

\[
\pi(x) = \frac{e^{[7.425 - 0.392(\% \text{ soil mass moisture}) + 3.244 (\text{clay to sand ratio})]}}{1 + e^{[7.425 - 0.392(\% \text{ soil mass moisture}) + 3.244 (\text{clay to sand ratio})]}}
\]

where \( \pi(x) \) is the probability of a heavy infestation in a vineyard block. The regression constant was 7.425, and -0.392 and 3.244 were the logistic regression coefficients associated with soil mass moisture percentage and soil clay/sand ratio, respectively. Fig. 2.4 shows the results of using this model to predict the probability that a vineyard block will be heavily infested by grape root borer larvae, based on the combined effects of soil mass moisture and clay/sand ratio.

**Discussion**

These results represent the most comprehensive assessment of vineyard infestation by larvae of grape root borer yet reported. Only three vineyard blocks yielded no pupal exuviae, while the rest were infested to widely varying degrees (Fig. 2.1). In the most heavily infested block, we witnessed the removal of several vines and counted between 14 and 39 live larvae per vine on just the exposed portion of the root system. Those plants were in significant decline and vine losses were occurring. However, despite the frequency of vineyard infestation that we recorded and the fact that many vines in the blocks sampled in consecutive years yielded pupal exuviae each year, most blocks did not show overt signs of problems that were of concern to the managers.

The pronounced differences in pupal exuviae density among vineyard blocks (Fig. 2.1) raises the important question of whether those that were not infested or lightly infested will become more heavily infested over time. Sampling of the same vines in several infested blocks over three to four consecutive seasons revealed instances in which annual mean pupal exuviae counts per vine decreased, increased, or remained static, which may have been due to annual differences in environmental conditions (e.g. rainfall) or other factors 2 years prior to each survey year. As well, the differences among blocks that were revealed by our sampling provided a strong basis for a comparison of putative risk factors among them.
The effects of various abiotic and biotic factors on populations of other root-feeding herbivorous insects have been reported (Strnad and Bergman 1987, Brown and Gange 1990, Villani and Wright 1990, Erb and Lu 2013), although we are not aware of another study that included as many variables as were used in the present research. None of the horticultural or cultural factors examined was significantly associated with grape root borer infestations. Of the environmental factors evaluated, there was no effect of the distance to forested area on infestation status. Using the same pupal exuviae data, I measured the spatial distribution of infestations (see Chapter 3). Despite aggregated distributions in blocks with ≥ 0.5 pupal exuviae per vine, there were no indications that populations were higher or aggregated at the edges of the sampled blocks, although Hoffman and Dennehuy (1989) reported higher infestations of grape berry moth, Paralobesia viteana (Clemens), in border rows of vineyards with wild vines nearby.

As mentioned previously, grape root borer infestations are ultimately dependent upon neonates finding and establishing on grape roots. In Chapter 4, I showed that grape root volatiles may have an important role in larval food-finding, although the present study revealed no effects of rootstock. Soil texture, moisture, porosity, particle size and compaction can affect the dispersion of behaviorally active host stimuli chemicals in the rhizosphere and also may influence the mobility and survival of root-feeding insect larvae (Villani and Wright 1990, Gregory and Johnson 2006). Very low and high soil moisture content is unfavorable for young larvae of root-feeding insects (Macdonald and Ellis 1990, Erb and Lu 2013). Sarai (1972) showed that 0, 20, and 30% soil moisture caused 100%, 65%, and 50% mortality, respectively, of grape root borer larvae seeking grape roots buried ≈ 10 cm deep in soil. We found that soil mass moisture was negatively related to infestations within the range of values measured (15 – 35% mass moisture); the four most heavily infested blocks (with > 1 pupal exuviae per vine) had a mass moisture range of 18 to 20%, while five blocks that were relatively lightly infested (mean of 0.098 pupal exuviae per vine showed soil mass moisture values of 30 to 35%. This negative association may have been due to soil pore spaces filled with water. Godfrey and Yeargan (1985) reported that 35% soil moisture content significantly reduced the survivorship of larval clover root curculio, Sitona hispidulus (F.) on alfalfa. Ladd and Buriff (1979) reported significantly higher mortality of Japanese beetle larvae, Popillia japonica Newman, in soil with 90% compared with 60% of field capacity moisture. Significant damage by wireworms in green cover crops was reported when soil moisture was below 25% of saturation (cited in Lees 1943). Soil
moisture of >30% in the study reported here may have hindered the movement of grape root borer neonates through soil (Lees 1943, Macdonald and Ellis 1990) and thus influenced their food-finding behavior (Coleman et al. 2004).

We also showed that soils with higher clay/sand ratios within the range of 0.5 – 3.0, which corresponded to finer soil types, were positively associated with grape root borer infestations. Larvae of western corn rootworm, Diabrotica virgifera virgifera LeConte moved three times faster in silty clay or loam compared with loamy sand soil (Macdonald and Ellis 1990). Similarly, Brust and House (1990) reported lower larval survival of southern corn rootworm, D. undecimpunctata howardi Barber, in soil with low clay content.

Although we did not assess the potential contribution of entomopathogenic nematodes to differences in the extent of grape root borer infestation among blocks, Williams et al. (2010) showed that at least two species of native, soil-dwelling nematodes attacked larval grape root borer and that their effectiveness varied with species, location, soil texture, and soil moisture content. Interestingly, some studies have shown an inverse relationship between soil clay content and the occurrence of entomopathogenic nematodes, while sand and silt content were positively associated with nematode occurrence (Kung et al. 1990, Koppenhöfer and Fuzy 2006, Campos-Herrera et al. 2008). Based on our results and those from the aforementioned studies, soil conditions that promote grape root borer infestations may not be optimal for entomopathogenic nematodes, suggesting that the relationships between nematode and larval grape root borer densities and soils in commercial vineyards should be addressed.

The risk prediction model presented here can be used to estimate the probability of a vineyard being or becoming infested by grape root borer larvae, using site-specific values for percent soil mass moisture and clay/sand ratio in the equation. The odds ratio (Table 2.4) for soil mass moisture (0.68) predicts the likelihood that a vineyard block will be highly infested, based on our criterion of >0.1 pupal exuviae per vine. With every unit increase in percent soil mass moisture, holding the clay/sand ratio constant, this likelihood is decreased 0.68 times. Similarly, with every unit increase in the clay/sand ratio, holding percentage soil mass moisture constant, the likelihood of a vineyard block being classified as highly infested is increased by more than 25 times (odds ratio = 25.63).

Given the long-standing issue with grape root borer infestations remaining unnoticed until symptoms become apparent (Brooks 1907, Dutcher and All 1979b), the ability of grape
growers to predict the likelihood that problems may arise is a key component toward the sustainable management of this pest (Bergh 2012). With further validation, the model developed here may be useful to predict the likelihood that an established vineyard block is infested by grape root borer and thereby aid its management by prompting population assessments. At new vineyard sites, the model may provide a better understanding of the relative risk from this pest and spur the vigilance that is often lacking, but necessary, to mitigate the development of damaging populations at some sites. In combination with this model, the recent development of a quantitative sampling protocol for grape root borer (see Chapter 3) and the registration of a mating disruption formulation for it, have significantly advanced our capacity to advise eastern grape growers about preventing or managing the on-going problems associated with this pest. Research on economic injury levels and action thresholds for grape root borer on different grape species and rootstocks and the role of nematodes in its biological control will further enhance the development of IPM recommendations.
Literature Cited


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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.


Google Inc. 2013. Google Earth map. Ver 7.0.3.8542 (zoom relation 1:01); Google Inc., Mountain View, CA.


Table 2.1. Mean ± SE values (for numerical) and mode (for nominal and ordinal) of 18 vineyard factors collected from 48 vineyard blocks in Virginia, and component loadings (based on variable principal normalization) obtained from Categorical Principle Component Analysis (CATPCA)

<table>
<thead>
<tr>
<th>Vineyard factors</th>
<th>Parameters</th>
<th>Principal components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Range</td>
</tr>
<tr>
<td><strong>Horticultural</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cultivar</td>
<td>Cab. Franc*</td>
<td>NA</td>
</tr>
<tr>
<td>Rootstock</td>
<td>C-3309*</td>
<td>NA</td>
</tr>
<tr>
<td>Vine age (yr.)</td>
<td>11.55 ± 0.82</td>
<td>2 – 23</td>
</tr>
<tr>
<td>Planting area (ha.)</td>
<td>1.32 ± 0.45</td>
<td>0.10 – 4.55</td>
</tr>
<tr>
<td><strong>Cultural</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of insecticide spray</td>
<td>2.33 ± 0.26</td>
<td>0 – 5</td>
</tr>
<tr>
<td>Irrigation\textsuperscript{\textdagger}</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ground cover\textsuperscript{\textdagger}</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Weed control\textsuperscript{\textdagger}</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Environmental</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance to forest (m)</td>
<td>73.13 ± 15.73</td>
<td>1.94 – 551.74</td>
</tr>
<tr>
<td>Bulk density (g/cm\textsuperscript{3})</td>
<td>1.22 ± 0.01</td>
<td>0.97 – 1.42</td>
</tr>
<tr>
<td>Mass soil moisture (%)</td>
<td>23.75 ± 0.60</td>
<td>14.63 – 35.45</td>
</tr>
<tr>
<td>Soil texture</td>
<td>Silty loam*</td>
<td>NA</td>
</tr>
<tr>
<td>Soil pH</td>
<td>6.24 ± 0.07</td>
<td>4.70 – 7.12</td>
</tr>
<tr>
<td>Soil organic matter (%)</td>
<td>3.17 ± 0.12</td>
<td>1.50 – 4.90</td>
</tr>
<tr>
<td>CEC (cmol\textsuperscript{+}/kg)</td>
<td>6.52 ± 0.24</td>
<td>3.70 – 10.60</td>
</tr>
<tr>
<td>Clay/sand ratio</td>
<td>0.84 ± 0.09</td>
<td>0.13 – 3.53</td>
</tr>
<tr>
<td>Clay/silt ratio</td>
<td>0.53 ± 0.04</td>
<td>0.19 – 1.41</td>
</tr>
<tr>
<td>Sand/silt ratio</td>
<td>0.77 ± 0.06</td>
<td>0.23 – 1.58</td>
</tr>
<tr>
<td>Eigen values</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Variance Accounted For (%)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Mode, indicating the most frequent observations. \textdagger Ranking. Irrigation: 1 = irrigated, 2 = not irrigated. Ground cover ranking: 1 = \textless 40\%, 2 = > 40\% cover around vine base. Weed control ranking: 1 = none, 2 = light, 3 = medium, 4 = aggressive. Component loadings with values \textgreater= 0.70 are shown in bold.
Table 2.2 Correlation matrix of the optimally transformed variables developed using Categorical Principal Component Analysis (CATPCA)

|     | C     | RS    | A     | PA    | I     | GC    | WC    | NI    | D     | BD    | SM    | ST    | pH    | OM    | CEC   | CDA   | CST   | SS    |
|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| C   | 1.000 | 0.270 | -0.236| -0.134| -0.028| -0.179| -0.237| 0.287 | -0.077| 0.248 | -0.319| 0.246 | 0.496 | -0.282| -0.100| -0.208| -0.122| 0.232 |
| RS  | 0.270 | 1.000 | 0.140 | -0.017| 0.101 | 0.203 | 0.013 | 0.579 | -0.021| 0.077 | -0.440| 0.028 | 0.133 | -0.163| -0.222| -0.036| 0.508 | 0.583 |
| A   | -0.236| 0.140 | 1.000 | -0.075| -0.127| 0.254 | -0.025| -0.031| -0.082| 0.098 | -0.250| 0.192 | -0.286| -0.126| -0.255| -0.160| 0.122 | 0.280 |
| PA  | -0.134| -0.017| -0.075| 1.000 | -0.048| 0.078 | 0.246 | 0.273 | 0.162 | -0.065| 0.030 | -0.178| 0.320 | 0.125 | 0.042 | 0.138 | 0.126 | -0.048 |
| I   | -0.028| 0.101 | -0.127| -0.048| 1.000 | 0.018 | -0.064| 0.199 | 0.339 | 0.046 | 0.137 | -0.034| 0.052 | -0.158| -0.098| 0.238 | 0.008 | -0.068 |
| GC  | -0.179| 0.203 | 0.254 | 0.078 | 0.018 | 1.000 | 0.085 | 0.259 | -0.040| -0.046| -0.238| -0.101| -0.065| 0.127 | -0.302| 0.195 | 0.334 | 0.138 |
| WC  | -0.237| 0.013 | -0.025| 0.246 | -0.064| 0.085 | 1.000 | 0.155 | -0.080| -0.300| -0.031| 0.038 | -0.156| -0.187| -0.061| 0.035 | -0.099| 0.016 |
| NI  | 0.287 | 0.579 | -0.031| 0.273 | 0.199 | 0.259 | 0.155 | 1.000 | -0.075| 0.024 | -0.341| -0.049| 0.168 | -0.077| -0.238| 0.042 | 0.443 | 0.466 |
| D   | -0.077| -0.021| -0.082| 0.162 | 0.339 | -0.040| -0.080| -0.075| 1.000 | -0.142| 0.099 | 0.000 | 0.059 | 0.106 | -0.104| 0.106 | 0.098 | -0.076 |
| BD  | 0.248 | 0.077 | 0.098 | -0.065| 0.046 | -0.046| -0.300| 0.024 | -0.142| 1.000 | -0.388| 0.282 | 0.222 | -0.348| -0.157| -0.280| -0.022| 0.291 |
| SM  | -0.319| -0.440| -0.250| 0.030 | 0.137 | -0.238| -0.031| -0.341| 0.099 | -0.388| 1.000 | -0.644| -0.047| 0.465 | 0.576 | 0.609 | -0.071| -0.760 |
| ST  | 0.246 | 0.028 | 0.192 | -0.178| -0.034| -0.101| 0.038 | -0.049| 0.000 | 0.282 | -0.644| 1.000 | -0.030| -0.603| -0.506| -0.816| -0.515| 0.526 |
| pH  | 0.496 | 0.133 | -0.286| 0.320 | 0.052 | -0.065| -0.156| 0.168 | 0.059 | 0.222 | -0.047| -0.030| 1.000 | 0.005 | 0.311 | 0.005 | 0.029 | 0.026 |
| OM  | -0.282| -0.163| -0.126| 0.125 | -0.158| 0.127 | -0.187| -0.077| 0.106 | -0.348| 0.465 | -0.603| 0.005 | 1.000 | 0.460 | 0.325 | 0.439 | -0.245 |
| CEC | -0.100| -0.222| -0.255| 0.042 | -0.098| -0.302| -0.061| -0.238| -0.104| -0.157| 0.576 | -0.506| 0.311 | 0.460 | 1.000 | 0.299 | -0.006| -0.424 |
| CSA | -0.208| -0.036| -0.160| 0.138 | 0.238 | 0.195 | 0.035 | 0.042 | 0.106 | -0.280| 0.609 | -0.816| 0.005 | 0.325 | 0.299 | 1.000 | 0.407 | -0.538 |
| CST | -0.122| 0.508 | 0.122 | 0.126 | 0.008 | 0.334 | -0.099| 0.443 | 0.098 | -0.022| -0.071| -0.515| 0.029 | 0.439 | -0.006| 0.407 | 1.000 | 0.294 |
| SS  | 0.232 | 0.583 | 0.280 | -0.048| -0.068| 0.138 | 0.016 | 0.466 | -0.076| 0.291 | -0.760| 0.526 | 0.026 | -0.245| -0.424| -0.538| 0.294 | 1.000 |

*Missing values were imputed with the mode of the quantified variable. C = Cultivar, RS = Rootstock, A = Vine age, PA = Planting area, I = Irrigation, GC = Ground cover, WC = Weed control, NI = Number of insecticide spray, D = Distance to forest, BD = Soil bulk density, SM = Soil mass moisture, ST = Soil texture, pH = Soil pH, OM = Soil organic matter, CEC = Cation exchange capacity, CSA = Clay/sand ratio, CST = Clay/silt ratio, SS = Sand/silt ratio*
Table 2.3 Binary logistic regression parameters and associated statistics derived from an analysis using six selected predictor variables measured from 48 vineyard blocks in Virginia

<table>
<thead>
<tr>
<th>Variables</th>
<th>$\beta$</th>
<th>SE</th>
<th>Wald $\chi^2$</th>
<th>df</th>
<th>$P$-value</th>
<th>Odds ratio ($e^\beta$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil mass moisture</td>
<td>-0.542</td>
<td>0.259</td>
<td>4.380</td>
<td>1</td>
<td>0.036</td>
<td>0.582</td>
</tr>
<tr>
<td>Ground cover</td>
<td>-0.671</td>
<td>1.024</td>
<td>0.430</td>
<td>1</td>
<td>0.512</td>
<td>0.511</td>
</tr>
<tr>
<td>Soil pH</td>
<td>1.036</td>
<td>1.113</td>
<td>0.867</td>
<td>1</td>
<td>0.352</td>
<td>2.819</td>
</tr>
<tr>
<td>Clay/sand ratio</td>
<td>9.746</td>
<td>4.813</td>
<td>4.100</td>
<td>1</td>
<td>0.043</td>
<td>17091.49</td>
</tr>
<tr>
<td>Clay/silt ratio</td>
<td>-9.322</td>
<td>5.102</td>
<td>3.339</td>
<td>1</td>
<td>0.068</td>
<td>0.00</td>
</tr>
<tr>
<td>Sand/silt ratio</td>
<td>5.009</td>
<td>4.266</td>
<td>1.378</td>
<td>1</td>
<td>0.240</td>
<td>149.68</td>
</tr>
<tr>
<td>Constant</td>
<td>2.158</td>
<td>11.328</td>
<td>0.036</td>
<td>1</td>
<td>0.849</td>
<td>8.65</td>
</tr>
</tbody>
</table>

Table 2.4. Binary logistic regression parameters and associated statistics derived from the analysis using the two final predictor variables measured from 48 vineyard blocks in Virginia

<table>
<thead>
<tr>
<th>Variables</th>
<th>$\beta$</th>
<th>SE</th>
<th>Wald $\chi^2$</th>
<th>df</th>
<th>$P$-value</th>
<th>Odds ratio ($e^\beta$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil mass moisture</td>
<td>-0.392</td>
<td>0.135</td>
<td>8.46</td>
<td>1</td>
<td>0.004</td>
<td>0.68</td>
</tr>
<tr>
<td>Clay/sand ratio</td>
<td>3.244</td>
<td>1.268</td>
<td>6.54</td>
<td>1</td>
<td>0.011</td>
<td>25.63</td>
</tr>
<tr>
<td>Constant</td>
<td>7.423</td>
<td>2.666</td>
<td>7.75</td>
<td>1</td>
<td>0.005</td>
<td>1674.03</td>
</tr>
</tbody>
</table>
Fig. 2.1 Seasonal mean ±SE number of grape root borer pupal exuviae per vine collected from 48 vineyard blocks in Virginia.
Fig. 2.2 Mean ± SE number of pupal exuviae per vine from four vineyard blocks in Virginia surveyed in 3 or 4 consecutive years
**Fig. 2.3** Scree plot that includes 18 vineyard variables measured in 48 vineyard blocks in Categorical Principal Component Analysis (CATPCA).
Fig. 2.4 Predicted probability of grape root borer infestation in vineyard blocks calculated using hypothetical values of predictors (i.e. percent soil mass moisture and clay/sand ratio) in a risk prediction model.
CHAPTER 3: SPATIAL DISTRIBUTION OF GRAPE ROOT BORER INFESTATIONS IN VIRGINIA VINEYARDS AND IMPLICATIONS FOR SAMPLING

This chapter was published as:

Abstract
Grape root borer, *Vitacea polistiformis* (Harris) (Lepidoptera: Sesiidae) is a potentially destructive pest of grapevines in the eastern United States. After feeding on grape roots for ≈2 yr in Virginia, larvae pupate beneath the soil surface around the vine base. Adults emerge during July and August, leaving empty pupal exuviae on or protruding from the soil. Weekly collections of pupal exuviae from a ≈1-m diameter weed-free zone around the base of a grid of sample vines in Virginia vineyards were conducted in July and August, 2008–2012, and their distribution was characterized using both non-spatial (dispersion) and spatial techniques. Taylor’s Power Law showed a significant aggregation of pupal exuviae, based on data from 19 vineyard blocks. Combined use of geostatistical and Spatial Analysis by Distance Indices (SADIE) methods indicated evidence of an aggregated pupal exuviae distribution pattern in seven of the nine blocks used for those analyses. Grape root borer pupal exuviae exhibited spatial dependency within a mean distance of 8.8 m, based on the range values of best-fitted variograms. Interpolated and clustering index-based infestation distribution maps were developed to show the spatial pattern of the insect with the vineyard blocks. The temporal distribution of pupal exuviae showed that the majority of moths emerged during the 3-wk period spanning the third week of July and the first week of August. The spatial distribution of grape root borer pupal exuviae was used in combination with temporal moth emergence patterns to develop a quantitative and efficient sampling scheme to assess infestations.

Key words: *Vitacea polistiformis*, *Vitis vinifera*, geostatistics, SADIE, sampling
Introduction
Grape root borer, *Vitacea polistiformis* (Harris), has been considered an economically important pest of grapevines in parts of the eastern United States for more than 150 years (Harris 1854, Brooks 1907, Clark and Enns 1964, Pollet 1975, All and Dutcher 1978). The oligophagous larvae feed on the roots of commercially important *Vitis* species and rootstocks, causing extensive root damage that can lead to reduced vine vigor and productivity and eventual vine death in extreme cases (Clark and Enns 1964, Dutcher and All 1976, All et al. 1987). Adult females deposit ≈350–400 eggs (Brooks 1907, Dutcher and All 1978) indiscriminately on the above-ground parts of vines and vegetation in vine rows over a period of 7–8 d after mating (Brooks 1907, Clark and Enns 1964, Dutcher and All 1979). After hatching, neonates enter the soil to find and establish on vine roots, during which time their mortality is highest (Dutcher and All 1978b). In Virginia, larvae feed for ≈22 mo (reviewed in Bergh 2012) before leaving the root system to pupate beneath the soil surface around the vine base. Emerging moths leave an empty pupal exuviae at the soil surface, the presence and number of which provide an indication of the infestation status of individual vines.

Compared with many other insect pests of economically important crops, the development of a truly integrated approach for managing grape root borer has lagged significantly (reviewed in Bergh 2012). Importantly, there are no symptoms expressed by the above-ground parts of vines that can be unequivocally ascribed to the cumulative effects of grape root borer feeding on roots. Symptoms known collectively as ‘slow vine decline’ (All et al. 1987), which include discolored and smaller leaves, reduced shoot growth, fewer and smaller berries, and vine wilting (Sorensen 1975, All et al. 1987), can be caused by grape root borer or a number of other horticultural or pathological conditions.

There are only two registered management options for grape root borer, soil drench applications of chlorpyrifos and mating disruption. In regions where grape root borer larvae have a 2-yr developmental period, managing an infestation requires implementing control measures in at least two consecutive seasons. In Virginia, chlorpyrifos is typically used as only a rescue treatment against severe infestations, creating a toxic barrier to neonates seeking roots in soil. However, many growers are reluctant to use chlorpyrifos in this manner due to perceptions of its negative impacts on soil biodiversity and associated effects on vine health, berry quality, and ultimately, wine quality (Bergh 2012). Mating disruption, therefore, is currently the
preferred option for managing grape root borer. Entomopathogenic nematodes have shown efficacy against this pest (Williams et al. 2010), but have not been widely adopted, due largely to the lack of coordinated efforts to educate growers about their use (Bergh 2012).

Another important and long-standing issue with grape root borer has been that most growers do not monitor or scout for this pest routinely and thus remain unaware of infestations until vines begin to show symptoms of decline (Brooks 1907, Dutcher and All 1979b). It is often the case that infestations are detected only upon removal of affected vines and inspection of roots for larvae and their feeding channels. Researchers have monitored or assessed grape root borer populations using pheromone traps (Snow et al. 1987, Bergh et al. 2005), pupal exuviae counts (Townsend 1991, Johnson et al. 1991, Pearson 1992), and root inspection of extracted vines (Sarai 1972), although the latter is not often a pragmatic option. Captures of male grape root borer in pheromone traps accurately reflect the presence and seasonal phenology of adults, and have been used extensively for those purposes (Snow et al. 1991, Bergh et al. 2005, Weihman and Liburd 2007). However, no useful relationship has been found between the number of male moths captured in pheromone traps and pupal exuviae counts from individual vineyard blocks (J.P.R., unpublished data), likely because of the capture of moths emerging from other blocks and from wild vines (Snow et al. 1991, Webb et al. 1992, Bergh 2006). Consequently, the presence of pupal exuviae near the base of vines is the only unequivocal, non-destructive indicator of vine infestation by grape root borer larvae (Johnson et al. 1991, Bergh 2012).

Although an understanding of the spatial distribution of a pest species is crucial to the development of reliable sampling plans for estimating and predicting their densities and for implementing rational management decisions (Taylor 1984, Legendre and Fortin 1989, Liebhold et al. 1993), to our knowledge, the spatial distribution of grape root borer infestations has not been investigated, and its management has suffered from a lack of this basic information. Non-spatial techniques for studying insect distributions, using the relationship between mean and variance, have been employed extensively to calculate dispersion indices (Southwood 1978, Taylor 1984, Kuno 1991, Young and Young 1998). However, the spatial patterns of insect densities can be characterized directly using geostatistical methods (Isaaks and Srivastava 1989, Rossi et al 1992, Liebhold et al. 1993) that analyze and model the spatial relationships among individuals in a population (Schotzko and O’Keeffe 1990, Williams et al. 1992). Spatial dependence (autocorrelation) derived from geostatistical analyses can be used to predict
populations at unsampled locations (e.g. by kriging), define sampling scales for independent samples, and to quantify the spatial pattern of insect species (Williams et al. 1992). The spatial distribution pattern of insect species can also be quantified using the method, Spatial Analysis by Distance Indicies (SADIE), which is particularly useful for count data (Perry 1995, Perry et al. 1999).

The objectives of this study were to characterize the distribution of grape root borer infestations in commercial vineyard blocks in Virginia, based on pupal exuviae sampling and the use of non-spatial and spatial techniques, and to develop a quantitative sampling plan for reliable and efficient assessment of the infestation status of individual vineyard blocks.

**Materials and Methods**

**Study Sites and Pupal Exuviae Sampling.** Grape root borer pupal exuviae were sampled from 48 vineyard blocks across 18 vineyards (geographic range: 38° 0' 36" N to 39° 19' 12" N and 77° 37' 48" W to 78° 50' 60" W) in Virginia (see Chapter 2). Each block was sampled once between 2008 and 2012. For the study reported here, pupal exuviae data from 19 of the 48 blocks that met the following criteria were used for the analyses described below: 1) the block was large enough so that a grid of 80 sample points (vines) could be overlaid on the area and, 2) the block ultimately yielded a seasonal mean of ≥ 0.1 pupal exuviae per vine. The 19 blocks selected were located in the following counties: Loudoun (2), Rappahannock (1), Shenandoah (4), Rockingham (3), Albemarle (6), and Nelson (3). The area sampled in each block (≈ 0.4 ha) was a portion of a larger contiguous planting (1.86 ± 0.43 SE ha) of vines that were 11.8 ± 2.7 SE years old. Within each block, the cultivar, rootstock, vine age, and other environmental and cultural practices were essentially uniform. Seven grape cultivars (Chardonnay, Vidal, Petit Verdot, Viognier, Cabernet Franc, Sauvignon Blanc, and Chambourcin) and four rootstocks (3309, V. riparia Gloire, SO4, 5BB) were represented.

In vineyards, a panel is defined as the space between consecutive wood or metal posts to which the trellis wire is attached, and usually contains 3–5 vines. In each block, the grid of 80 sample vines consisted of the first vine in the first 10 panels per row in every second row across 16 rows. Before or at the beginning of adult emergence, the area around the base of each sample vine was cleared of vegetation using a “string trimmer” and by raking, leaving a ≈1-m diameter area of clean soil (Johnson et al. 1991). At weekly intervals for 6–8 wk from early July through
late August, spanning most of the adult emergence period in Virginia (Pfeiffer et al. 1990, Bergh et al. 2005), pupal exuviae on or protruding from the soil around the base of the vine at each sampling point were collected and recorded.

Non-Spatial Measure of Aggregation. Taylor’s Power Law (TPL), a mean-variance based measure of aggregation, was used to characterize the dispersion of grape root borer pupal exuviae in the blocks. With TPL, the variance ($s^2$) is related to mean ($\bar{x}$) by a simple power law $s^2 = a\bar{x}^b$ where, $a$ and $b$ are parameters representing population characteristics (Taylor 1961). Logarithmic transformations of pupal exuviae variance and mean were used to convert the exponential relationship into a linear relationship, expressed by the regression equation of log variance with log mean, $\log s^2 = b \log \bar{x} + \log a$. The regression coefficient, $b$ is the index of aggregation; an aggregated distribution of counts is indicated when $b > 1$ (Taylor 1961, Taylor et al. 1988). Hypothesis testing of the regression slope ($b$) equal to 1 was conducted using $t$-tests [$t = (\text{slope} - 1)/\text{SE of slope}$] with $(n - 2)$ df at $P < 0.05$ (Davis 1994, Reay-Jones 2010a, 2010b). Regression analysis and the creation of regression plots were carried out in JMP Pro (SAS Institute 2010).

Spatial Measures of Aggregation. Of the 19 vineyard blocks used in the analysis of TPL, nine blocks were selected to characterize the spatial distribution patterns of grape root borer infestations within each using geostatistical analysis (variograms) and SADIE. The nine blocks were chosen because they each had a mean seasonal pupal exuviae count (ranging from 0.4 – 6.4 per vine) that was greater than the median value of 0.33 exuviae per vine that was estimated from the 19 blocks.

Geostatistical Analysis. Variography is a geostatistical technique used to assess spatial autocorrelation (or spatial dependence) in a sampled variable through the development of an experimental semivariogram that describes the relationship between sample values with distance and/or direction within the sampling space. Mathematically, the semivariogram ($\gamma$) can be represented by (Cressie 1993, Davis 1994),

$$\hat{\gamma}(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(x_i) - z(x_i + h)]^2,$$

where $\hat{\gamma}(h)$ is the estimated semivariance for the entity of interest (z) at all points ($x_i$) separated by lag distance ($h$), and $N(h)$ is the number of pairs of samples separated by lag distance $h$. 47
There are generally two types of variation in spatial data, large scale (trend) and small scale (stochastic) (Fortin and Dale 2005). Removing existing trend in the data is a critical step in variography (Isaaks and Srivastava 1989, Cressie 1993, Young and Young 1998). To test for trends in the data, multiple linear regression analysis was used, with pupal exuviae counts as the dependent variable and the spatial references (i.e., X and Y values) of 80 sample points as the independent variables (Park and Tollefson 2006, Karimzadeh et al. 2011) using SAS (SAS Institute 2010). A significant regression ($P < 0.05$) indicated the presence of a trend in the data, which were then subjected to median polishing. The median polishing technique extracts large scale variation from the data so that the remnant or median polished residuals can be used in variogram analysis to model small-scale variation (Bakhsh et al. 2000, Costa 2009, Frank et al. 2011). The median polished residuals for the pupal exuviae data were derived using code written in Matlab (MathWorks Inc, Natick, MA) and were then used to develop the semivariograms. Pupal exuviae count data for the vineyard blocks without significant trends were transformed using log$(x + 1)$ to meet normality assumptions (Isaaks and Srivastava 1989). All of the semivariograms were developed using the geostatistical software, GS$^+$ (Gamma Design Software 2008).

The semivariogram has three parameters, range, sill, and nugget that determine its shape. The range is the physical distance within which data among sample points are spatially correlated or dependent (Liebhold et al. 1993, Fortin and Dale 2005) and is the lag distance beyond which the variogram plot stops increasing (Legendre and Fortin 1989). The semivariance value at which the plotted points level off is the sill, and the nugget is the semivariance value at zero lag distance (Liebhold et al. 1993).

Semivariograms without a sill are fitted either into Nugget or linear models, indicating no detectable spatial dependence and a random distribution pattern of the data (Liebhold et al. 1991, Rossi et al. 1992). Semivariograms with a definite sill are fitted either to exponential, spherical, or Gaussian models (Journel and Huijbregts 1978, Isaaks and Srivastava 1989), indicating an aggregated distribution pattern (Schotzko and O'Keeffe 1989, 1990). Both anisotropic (directional) and omnidirectional variograms (Isaaks and Srivastava 1989, Liebhold et al. 1993) were developed for the pupal exuviae data. Anisotropic variograms were calculated at four directions (0, 45, 90, and 135 degrees) with a 22.5 degree offset tolerance. An anisotropy factor ($AF$), the quotient between minor and major range of anisotropic variograms, was also calculated.
and used to determine the presence of directional effects in the pupal exuviae count data (Karimzadeh et al. 2011). The best fitted omnidirectional variogram model for the pupal exuviae data in each of the 9 blocks was selected based on the smallest value of residual sum of squares (RSS) and the greatest $r^2$ value (Park and Tollefson 2005, Robinson and Metternicht 2006, Frank et al. 2011). Evaluation of the degree of aggregation in pupal exuviae counts was based on the nugget-to-sill ratio ($C_0/ C_0 + C$) (Trangmar et al. 1986), where $< 0.25$, $0.25 – 0.75$, and $> 0.75$ indicate strong, moderate, and weak aggregation, respectively (Farias et al. 2002, Frank et al. 2011).

Interpolated maps of grape root borer infestations were developed using the geostatistical technique, kriging, in GS+ (Gamma Design Software 2008). Kriging provides estimates of the variable at unsampled locations based on the weights derived from the variogram model (Isaaks and Srivastava 1989, Roberts et al. 1993, Liebhold et al. 1991, 1993). In particular, ordinary kriging has been used to estimate insect numbers throughout a sampling space (Isaaks and Srivastava 1989, Roberts et al. 1993). Mathematically, ordinary kriging is identical to multiple linear regression with the exception that the dependent and independent variables are the same, but are measured at different locations (Liebhold et al. 1993) such that,

$$
\hat{Z}(x_0) = \sum_{i=1}^{n} \lambda_i z(x_i)
$$

, where $\hat{Z}(x_0)$ is the value to be estimated at location $x_0$, $z(x_i)$ are the sampled values at their respective locations, $\lambda_i$ are the weights to be given to each sampled value, and $n$ is the number of sample points within the estimated area.

*Spatial Analysis by Distance Indices–SADIE.* The method known as SADIE has been used to quantify spatial patterns of insect counts from spatially-referenced sample points with $x$ and $y$ coordinates (Perry 1995, Perry et al. 1999). SADIE measures the overall aggregation based on the distance to regularity ($D$), which represents the minimum total distance that individuals would need to move in order to achieve the same number (i.e. mean) for each sample point within a sampling area. Higher $D$ values indicate stronger aggregation. The magnitude of $D$ is assessed by a randomization test in which permutations of all observed counts among sample points are performed (Perry and Dixon 2002). The assessment provides an index of aggregation,
I_a with an associated probability, P_a. Aggregated, uniform, and random distribution patterns are indicated by I_a > 1, I_a = 1, and I_a < 1, respectively (Perry 1995). The associated probability (i.e. P_a < 0.025 or P_a > 0.975) determines whether or not the resultant distribution pattern is significantly different from randomness (Perry 1998).

Estimated and contoured pupal exuviae distribution maps providing a quantitative description of the location, size, and dimension of each cluster were developed by calculating clustering indices for each spatially-referenced sample point in SADIE (Perry et al. 1999, Perry and Dixon 2002, Kamdem et al. 2012). Cluster refers to the area of neighboring sampling units with all counts that are either larger or smaller than the sample mean. Clustering indices for each sample point were used to display the clustering area in the form of a patch (i.e. density above average counts) and/or in a gap (i.e. density below average counts) in a sampling space. Patch cluster refers to areas of strong clustering (cluster indices >1.5) containing counts greater than the overall mean count. Gap cluster is an area with clustering indices < −1.5 caused by relatively fewer counts in neighboring sample points (Perry et al. 1999). Clustering indices representing a patch are denoted by \( v_i \) with associated P-value, \( P_{v_i} \), while cluster indices representing a gap are denoted by \( v_j \) with associated P-value, \( P_{v_j} \). The presence of significant patches and gaps are indicated by \( P_{v_i} < 0.025 \) and \( P_{v_j} < 0.025 \), respectively. The calculation of the index of aggregation and index of clustering in SADIE was carried out using SADIEShell (Rothamsted Experimental Station 2008). In total, 1950 randomizations with a non-parametric option were used for the analysis. Estimated infestation distribution maps containing patches and gaps of the vineyard blocks were developed using the clustering index values (i.e. \( v_i \) and \( v_j \)) of each sample point in JMP (SAS Institute 2010).

**Temporal Distribution of Pupal Exuviae.** To examine the temporal distribution of pupal exuviae collected during the period of adult emergence, data from 35 of the 48 blocks sampled between 2008 and 2012 were used (n = 9, 7, 3, 8, and 8 blocks in consecutive years from 2008 to 2012, respectively). The blocks were selected based on the following criteria: 1) pupal exuviae were collected weekly for at least 6 wk and, 2) the seasonal mean number of exuviae per vine was \( \geq 0.10 \). Based on the seasonal total number of pupal cases collected per block, weekly mean percentage of total pupal exuviae was calculated.

**Predicting Cumulative Pupal Exuviae Counts.** Pupal exuviae data from the 35 blocks used for the temporal distribution evaluation were used to calculate mean cumulative counts
(weekly) as a percentage of total seasonal counts (Cockfield and Mahr 1994, Kamminga et al. 2009, Milanos and Savopoulou-Soultani 2006). Percent cumulative pupal exuviae counts across Julian weeks during the moth emergence period were fitted to the Weibull function using the formula (Wagner et al. 1984, Dodson 2006),

\[ f(x) = 100 \left(1 - e^{-(x/a)^\beta}\right), \]

where \( f(x) \) is the percent cumulative mean count of pupal exuviae at each Julian week \( x \), \( \alpha \) is a rate parameter, and \( \beta \) describes the shape of the curve. The fit of the data to the Weibull function was carried out using nonlinear least squares regression in TableCurve 5.01 (SYSTAT Software 2002).

**Results**

**Non-Spatial Measure of Aggregation.** The seasonal mean number of grape root borer pupal exuviae collected per vine varied considerably among the 19 vineyard blocks used for the analysis of Taylor’s Power Law (Fig. 3.1). The analysis showed a significant \( t = 28.22; \text{df} = 17; P < 0.001 \) relationship between logarithmic variance and logarithmic mean of pupal exuviae per vine (Fig 2). The \( r^2 = 0.98 \) and \( s_e b/b = 0.03 \) indicated that the data satisfied the criteria (i.e. \( r^2 > 0.8 \) and \( s_e b/b < 0.2 \)) for statistical validity (Downing 1986). The slope \( (b) \) of the fitted line was also significantly greater than 1 \( (t = 7.6; \text{df} = 17; P < 0.001) \), indicating an aggregated dispersion pattern for pupal exuviae within the vineyards (Fig. 3.2).

**Spatial Measures of Aggregation.** Grape root borer pupal exuviae were found to be spatially aggregated in vineyard blocks in which mean pupal exuviae densities were \( \geq 0.5 \) per vine.

**Geostatistical Analysis.** The active lag distance, which is the distance over which the semivariance was calculated, ranged from 34.21–44.74 m. The uniform lag intervals used ranged from 5.5–6.6 m. None of the nine blocks selected for geostatistical analysis had an anisotropic \( AF \) value < 0.5, suggesting no directional component to pupal exuviae counts. Therefore, only omnidirectional semivariograms were considered further.

Development of omnidirectional semivariograms revealed that five blocks (A, B, C, D, and H) showed an aggregated spatial distribution pattern for pupal exuviae based on the
variogram model, model $r^2$ and RSS, and nugget-to-sill ratio ($C_0/C_0 + C$) (Table 3.1). The $r^2$ and RSS showed that the best fitted variogram models among the blocks were exponential (block A; $r^2 = 0.56$), spherical (blocks B, C, D, H; $r^2 = 0.96, 0.73, 0.74, 0.35$, respectively), linear (block E), and nugget (blocks F, G, I). Since blocks E, F, G, and I produced either a linear or nugget variogram model (Table 3.1), no evidence of an aggregated distribution was indicated. The nugget-to-sill ratio ($C_0/C_0 + C$), which measures the degree of aggregation, was $< 0.25$ in all blocks in which aggregation was observed, indicating strong aggregation of pupal exuviae (Table 3.1). Range values for the semivariograms were between 7.24 and 13.58 m, with a mean of $8.8 \pm 2.7$ SE m (Table 3.1). Interpolated maps developed by kriging for the blocks in which aggregation was observed are shown in Fig. 3.3.

Spatial Analysis by Distance Indices–SADIE. The analysis using SADIE also showed spatial aggregation of pupal exuviae in five of the nine blocks analyzed (i.e., A, B, D, E, and I), based on indices of aggregation ($I_a > 1$ (Table 2). However, spatial aggregation of pupal exuviae was indicated by both SADIE and the geostatistical analysis only in blocks B and D (Table 2). Clustering maps showing patches and/or gaps were developed using the $v_1$ and $v_2$ values for each sample point from blocks A, B, and E (Fig. 4). Visual comparison indicated a high degree of similarity between infestation distribution maps produced using geostatistics (Fig. 3.3a & b) and SADIE (Fig. 3.4a & b).

Temporal Distribution of Pupal Exuviae. In 2008, 81% of grape root borer pupal exuviae (n = 9 blocks) were collected between the third week of July, Julian week (JW) 29 and the first week of August, JW 31 (Fig. 3.5a). In 2009 (n = 7 blocks) and 2012 (n = 8 blocks) 82% and 79% of exuviae, respectively, were collected between the second week of July (JW 28) and the first week of August (JW 31) (Fig. 3.5b and d). In 2010, 67% of exuviae (n = 3) were collected between the second and fourth week of July, (JW 28 and 30) (Fig. 3.5c), while in 2011, 77% of exuviae (n = 8) were collected between the fourth week of July (JW 30) and the second week of August, (JW 32) (Fig. 3.5e).

Predicting Cumulative Pupal Exuviae Counts. The fit of percent cumulative mean count of pupal exuviae to the Weibull function was significant ($F = 1710.08; df = 1, 6; P = < 0.0001; r^2 = 0.995$) (Fig. 3.6), with $\alpha = 30.28 \pm 0.06$ SE and $\beta = 20.25 \pm 1.07$ SE. Based on the Weibull model, 34% of total seasonal exuviae were collected by the third week of July (JW 29), 56% by the fourth week of July (JW 30), 80% by the first week of August (JW 31), and 95% by
the second week of August (JW 32). In predicting the peak moth emergence period using the Weibull function, 80% of total seasonal exuviae were collected between Julian weeks 27 and 31, 70.7% were collected during Julian weeks 28–31 and 61.5% were collected during Julian weeks 29–31.

**Discussion**

Characterization of the spatial distribution of grape root borer pupal exuviae within commercial vineyard blocks has improved the ability to monitor infestations of this important and insidious below-ground pest, and by extension, should assist future research efforts and enable more informed management decisions by growers. Although a non-spatial technique indicated that grape root borer pupal exuviae showed an aggregated dispersion pattern within the vineyard blocks, the use of geostatistical analysis and SADIE showed true spatial aggregation only in blocks with ≥0.5 exuviae per vine. Based on the extent of spatial dependency indicated by the range value of the variogram model, counts of pupal exuviae appeared to be aggregated within a mean distance of 8.8 m.

Both exogenous and endogenous factors can influence insect distributions and contribute to aggregated distribution patterns. Exogenous factors such as climate, soil type, topography, stochastic disturbances, and solar activities may contribute to large-scale variation by creating microenvironments favoring aggregation (Elsbury et al. 1998, Toepfer et al. 2007). Endogenous influences include biological (e.g. host-finding and oviposition behavior) and ecological factors (e.g. predation or parasitization risks) characteristic of individual species (Dormann 2007). Spatial distribution patterns of immature insects can also be influenced by adult distributions during their period of peak emergence (Toepfer et al. 2007). For example, both adult and larval cutworms, *Agriotes sordidus* Illiger and *Agriotes litigiosus* Rossi, were found to be spatially clustered based on separate experiments conducted in two different cropping systems (i.e. a mixed cropping system of organic vegetable and fruit crops and a cereal cropping system) in northern Italy (Furlan and Burgio 1999, Burgio et al. 2005). Since grape root borer larvae are thought to have a 2-yr developmental period in Virginia, the abundance and distribution of pupal exuviae measured in an individual vineyard block in any given year would have been influenced primarily by adult emergence and oviposition, and larval establishment on roots, two years previously. Upon emergence, adult female grape root borer typically rest on the
lower trunk of a vine near the emergence site, and eventually walk up into the canopy (Pearson 1992) where calling and mating occur (Dutcher and All 1978a). Females carrying a full egg complement exhibit rather sluggish flight, and as is often the case with other moths (Bernays and Chapman 1994), and may at least initially oviposit in the vicinity of the emergence and mating site, where larval resources in this perennial plant are often spatially and temporally stable (Greenfield 1981). Although the distance over which ovipositing female grape root borers move has not been quantified, Brooks (1907) observed a female depositing single eggs at intervals of a few inches over a distance of about 3 m along a vine cordon. Limited flight distance of ovipositing females could also reduce the risk from predation by natural enemies (Greenfield 1981), including insectivorous birds such as the barn swallow (Hirundo rustica erythrogaster Boddaert), mocking bird (Mimus polglottos polglottos L.) and great crested fly catcher (Myiarchus crinitus L.), that are known to attack it in vineyards (Brooks 1907, Clark and Enns 1964).

Unlike the contribution of nearby wild Vitis to higher infestations of grape berry moth, Paralobesia viteana (Clemens), in vineyard border rows (Hoffman and Dennehy 1989), interpolated pupal exuviae distribution maps from these vineyard blocks showed no evidence of higher densities at or near the edges (Fig. 3.3). Of the nine blocks used in the spatial analysis, four blocks had at least one edge in close proximity (≤ 65 m) to a wooded area from which grape root borer infestations in commercial plantings are thought to originate (Snow et al. 1991, Bergh 2006), although the presence and abundance of wild Vitis in those areas was not assessed.

Spatial distribution is a complicated and multidimensional phenomenon and the use of more than one analytical approach, including traditional and spatial techniques, has been recommended for better clarity and interpretation of results (Madden and Hughes 1995, Perry et al. 2002). The suitability of different methods for spatial data analysis and their interpolation techniques were reviewed by Legendre and Fortin (1989). Although differing results from such uses of traditional and spatial techniques to determine aggregations of several insect species have been reported (Young and Young 1990, Midgarden et al. 1993, Fortin and Dale 2005), results from one method should not negate those from others, since the different approaches elucidate different aspects of insect distribution patterns, and together provide better accuracy (Perry et al. 2002, Queiroz et al. 2010). While traditional measures of aggregation such as Taylor’s Power Law have been widely used to determine insect dispersion and distribution, they do not represent
true spatial distribution patterns. In this study, Taylor’s Power Law showed a strong aggregation of grape root borer pupal exuviae in vineyards, while variogram analyses provided additional information on the spatial autocorrelation or spatial dependence of pupal exuviae with distance and direction within the vineyard blocks (Fortin and Dale 2005). The variograms and their parameters indicated a strongly aggregated distribution of grape root borer pupal exuviae in 5 of 9 vineyard blocks. Wright et al. (2002) reported that feeding injury from larval European corn borer, *Ostrinia nubilalis* (Hübner), showed an aggregated distribution pattern at 4 of 7 sites. Farias et al. (2003) showed that the distribution of three sharpshooter species trapped in yellow sticky cards over several seasons in a citrus orchard resulted in fitted variograms for 9 of 36 datasets. Similarly, the distribution of plant pathogenic nematodes in cotton showed an aggregated distribution in some, but not all fields (Farias et al. 2002). Of the 9 blocks included in the geospatial analysis, 7 blocks (A, B, C, D, E, H, and I) showed evidence of aggregation of pupal exuviae based on the combined results from the variogram and SADIE analyses. The different results from the two types of spatial analyses could possibly be due to the different ways by which spatial weights for individual sample points are calculated (Kamdem et al. 2012). Our use of SADIE indicated an aggregated pupal exuviae distribution pattern in 5 of 9 vineyard blocks, although only 2 showed statistically significant aggregation. Since SADIE measures true clustering among neighboring sample points, isolated higher values from individual sample points do not contribute to aggregation. Variogram analyses, however, incorporate these higher values in the analysis and are more appropriate for characterizing and estimating local population abundance and density (Perry et al. 2002, Perry and Dixon 2002). Other studies in which both geostatistical analyses and SADIE were used to quantify spatial distributions of insects yielded variations similar to those reported here (Perry et al. 2002, Karimzadeh et al. 2011, Kamdem et al. 2012).

Our results showed that grape root borer pupal exuviae tended to be spatially aggregated in vineyard blocks in which pupal exuviae densities were $\geq 0.5$ per vine. Similar density-dependent aggregation patterns have been reported for western corn rootworm, *Diabrotica virgifera virgifera* LeConte, and grass thrips, *Anaphothrips obscurus* (Müller) (Midgarden et al. 1993, Reisig et al. 2011). Aggregation is usually not detected from sampled areas with a low density of the variable of interest (Taylor et al. 1978, Taylor 1984, Lepš 1993, Blom and Fleischer 2001), since variance and mean are often equal, rendering spatial distributions
indistinguishable from random (Chiang and Hodson 1959, Taylor et al. 1978). In blocks F and G, which showed no evidence of aggregation from either variogram or SADIE analyses, at least 70% of the sampled vines yielded no pupal exuviae, likely contributing to our inability to detect an aggregated distribution in those blocks.

Understanding the spatial distribution of an insect species is a key component for developing a quantitative and efficient sampling scheme (Legendre and Fortin 1989, Fortin and Dale 2005, Park and Tollefson 2006). Sampling schemes developed for other pest insects that were based on spatial distributions employed an appropriate sampling method (systematic, random, or stratified) in a gridded (square, triangular, or hexagonal) sampling space (Schotzko and O'Keeffe 1990, Williams et al. 1992, Liebhold et al. 1993, Wright et al. 2002, Frank et al. 2011). Sampling distance can differ according to the intended purpose. If the intention is to develop infestation density maps for site-specific management (Fleischer et al. 1999), as has been used for some agricultural pests (Weisz et al. 1996, Blom et al. 2002), sampling should be within the distance of the range value of the variogram (8.8 m for grape root borer exuviae) (Weisz et al. 1995, Park and Tollefson 2005, Frank et al. 2011). However, there are several reasons why a map-based precision approach for grape root borer management may not be a pragmatic option for growers. First, collecting the data to develop prediction maps for this pest over large vineyard acreage would be very labor intensive. Second, map development requires specialized software and expertise that is relatively uncommon and not readily accessible to many growers. Finally, there are only two registered products considered effective for grape root borer management: 1) chlorpyrifos applied as a soil drench around the vine base as a barrier to neonate movement to roots, and 2) mating disruption using Isomate-GRB. Most growers are averse to chlorpyrifos applications unless infestations have reached critical levels (Bergh 2012) and mating disruption requires treatment of entire vineyards or vineyard blocks. Consequently, sampling grape root borer pupal exuviae to quantify its relative abundance or to evaluate the effectiveness of control measures appear to be the best uses of this monitoring approach. For these purposes, use of a sampling distance that is higher than the range value of the variogram is necessary to obtain independent samples.

Regardless of the intended use of data from grape root borer pupal exuviae sampling, accurate assessment of its presence and abundance requires removal of all living and dead vegetation around the base of sample vines in early July. We recommend a cleaned area around
each sample vine of about 1-m diameter, since Dutcher and All (1978c) showed that ≈90% of grape root borer pupal exuviae were recovered from a circular area of ≈35 cm radius around the vine base. This can be accomplished easily with herbicide or a “string trimmer” and raking, typically involving only a few minutes per vine. We also recommend weekly visits to all sample vines during the assessment period, since exuviae that are left on the soil surface are prone to being displaced by storms, mowers, or airblast sprayers. Exuviae left protruding from the soil surface are not as prone to being dislodged (J.C.B., unpublished data).

Our sampling from 2008–2012 revealed that, on average, 63.2% of all pupal exuviae were found between the third week of July and the first week of August, and 73.6% were found between the second week of July and the first week of August. Based on pupal exuviae counts from three vineyards in two consecutive years in North Carolina, Pearson (1992) found that peak emergence of adult grape root borer ranged from the first to the last week of August, and that the duration of adult emergence was somewhat longer than reported here. Similar latitude-dependent differences in the seasonal patterns of adult grape root borer emergence were reported by Brooks (1918) and Snow et al. (1991). Air temperature and rainfall are two factors that likely influence the onset, peak, and duration of grape root borer emergence (Clark and Enn 1964, Sarai 1972). The average daily temperature from June through August in the consecutive years from 2008 through 2012 recorded at Virginia Tech’s Alson H. Smith, Jr. Agricultural Research and Extension Center, Winchester, VA was 22.1, 21.8, 24.3, 23.7, and 22.7 °C, and average daily rainfall was 3.4, 2.5, 1.6, 1.7, 3.4 mm, respectively. The unusually hot and dry conditions in Virginia that prevailed during late spring and summer in 2010 may explain the earlier onset and peak of grape root borer emergence than was recorded in other years. Although peak emergence of adult grape root borer may occur about one week earlier in such years, weekly sampling throughout the 3 or 4 wk periods mentioned above should provide a reasonable estimation of its abundance in most seasons, with least effort. Nowatzki et al. (2002) noted that scouting during the period of peak adult emergence of northern and western corn rootworm, *Diabrotica barberi* Smith & Lawrence and *D. virgifera virgifera*, was efficient for assessing infestations. Importantly, our recommended sampling period for grape root borer pupal exuviae coincides with a period during which labor required for other tasks in mid-Atlantic vineyards is much reduced (T. K. Wolf, personal communication). In other eastern states where grape root borer is
an economic pest, the appropriate sampling period may differ somewhat from that reported here, due to different seasonal patterns of moth emergence (Snow et al. 1991, Pearson 1992).

By coupling the results of our analyses of the spatial distribution of grape root borer infestations with its seasonal patterns of emergence in Virginia vineyards, we recommend a systematic sampling scheme for this pest that involves weekly pupal exuviae collections between mid-July and early August from a grid of sample vines in blocks to be assessed. The strong fit of the cumulative percentage of emergence from pooled data across all years to the Weibull function should enable growers or researchers to accurately capture specific ranges of percentage emergence, according to individual needs. Variogram analyses indicated an appropriate inter-vine sampling distance of ≈9 m to achieve independent samples for population density assessment. Based on vine spacing that is typical for vineyards in Virginia, a sample vine spacing of 9 m is equivalent to one vine per 1.5 panels along the row. We recommend a grid of sample vines constituted of vines in every second row and of a minimum of 50 vines per vineyard block of average size (≈1–2 ha). With relatively little training and experience, one person could survey 50 vines in about 30 min. All pupal exuviae found must be removed to avoid recounting during subsequent surveys.

Dutcher and All (1979b) calculated an economic threshold of 0.074 larvae per vine for grape root borer feeding on roots of ‘Concord’ vines (Vitis labrusca L.) in Georgia and recommended intervention upon detection of the pest. Since 38 of the 48 blocks surveyed in Virginia between 2008 and 2012 exceeded this recommended threshold (J.P.R., unpublished data) based on total numbers of pupal exuviae collected per vine, but most showed no apparent effects from the pest, Bergh (2012) suggested that the threshold recommendation by Dutcher and All (1979b) may be conservative for grape root borer on V. vinifera in the mid-Atlantic region. While the development of economic thresholds for grape root borer on V. vinifera in Virginia and surrounding states would add importantly to the utility and interpretation of sampling data, in the interim the sampling scheme we propose should greatly improve the ability to assess and manage grape root borer infestations in vineyard blocks.
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Table 3.1 Variogram models and parameters for grape root borer pupal exuviae distribution in vineyard blocks in Virginia

<table>
<thead>
<tr>
<th>Block</th>
<th>Mean exuviae per vine† (± SE)</th>
<th>Range (m)</th>
<th>Model</th>
<th>$r^2$</th>
<th>RSS</th>
<th>Active lag (m)</th>
<th>$C_0$</th>
<th>$C_0+C$</th>
<th>$C_0/C_0+C$</th>
<th>AF</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.34 ± 0.26</td>
<td>7.50</td>
<td>Ex</td>
<td>0.56</td>
<td>0.1490</td>
<td>37.65</td>
<td>0.370</td>
<td>3.38</td>
<td>0.109</td>
<td>0.99</td>
</tr>
<tr>
<td>B</td>
<td>1.94 ± 0.36</td>
<td>13.58</td>
<td>Sp</td>
<td>0.96</td>
<td>0.0017</td>
<td>34.21</td>
<td>0.045</td>
<td>0.632</td>
<td>0.071</td>
<td>0.99</td>
</tr>
<tr>
<td>C</td>
<td>6.40 ± 0.55</td>
<td>7.44</td>
<td>Sp</td>
<td>0.73</td>
<td>0.0003</td>
<td>35.81</td>
<td>0.038</td>
<td>0.43</td>
<td>0.088</td>
<td>0.94</td>
</tr>
<tr>
<td>D</td>
<td>1.10 ± 0.16</td>
<td>8.21</td>
<td>Sp</td>
<td>0.74</td>
<td>0.0013</td>
<td>43.42</td>
<td>0.050</td>
<td>0.382</td>
<td>0.131</td>
<td>0.99</td>
</tr>
<tr>
<td>E</td>
<td>0.88 ± 0.14</td>
<td>-</td>
<td>Li</td>
<td>0.02</td>
<td>0.0270</td>
<td>43.42</td>
<td>0.965</td>
<td>0.997</td>
<td>0.968</td>
<td>-</td>
</tr>
<tr>
<td>F</td>
<td>0.41 ± 0.09</td>
<td>-</td>
<td>Nu</td>
<td>0.80</td>
<td>0.0020</td>
<td>35.81</td>
<td>0.198</td>
<td>0.198</td>
<td>1.000</td>
<td>-</td>
</tr>
<tr>
<td>G</td>
<td>0.43 ± 0.09</td>
<td>-</td>
<td>Nu</td>
<td>0.64</td>
<td>0.0028</td>
<td>43.42</td>
<td>0.172</td>
<td>0.172</td>
<td>1.000</td>
<td>-</td>
</tr>
<tr>
<td>H</td>
<td>0.49 ± 0.09</td>
<td>7.24</td>
<td>Sp</td>
<td>0.35</td>
<td>0.0024</td>
<td>44.74</td>
<td>0.038</td>
<td>0.203</td>
<td>0.188</td>
<td>1.0</td>
</tr>
<tr>
<td>I</td>
<td>0.73 ± 0.11</td>
<td>-</td>
<td>Nu</td>
<td>0.47</td>
<td>0.0006</td>
<td>35.81</td>
<td>0.272</td>
<td>0.272</td>
<td>1.000</td>
<td>-</td>
</tr>
</tbody>
</table>

† Means were calculated based on the total pupal exuviae counts from each block in one season.

RSS: Residual sum of squares; $C_0$ = Nugget; $C_0+C$ = Sill; $C_0/C_0+C$ = Nugget-to-Sill ratio; AF: Anisotropy Factor;

Nu: Nugget model ($C_0 = C_0+C$); Ex: Exponential model; Sp: Spherical model; Li: Linear model
Table 3.2 SADIE parameters for grape root borer pupal exuviae distribution in vineyard blocks in Virginia

<table>
<thead>
<tr>
<th>Block</th>
<th>Mean exuviae per vine† (± SE)</th>
<th>$I_a$</th>
<th>$P_a$</th>
<th>$\bar{v}_j$</th>
<th>$\bar{v}_i$</th>
<th>$P\bar{v}_j$</th>
<th>$P\bar{v}_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.34 ± 0.26</td>
<td>1.383</td>
<td>0.036</td>
<td>-1.346</td>
<td>1.279</td>
<td>0.048</td>
<td>0.069</td>
</tr>
<tr>
<td>B</td>
<td>1.94 ± 0.36</td>
<td>1.393</td>
<td>0.022*</td>
<td>-1.399</td>
<td>1.312</td>
<td>0.025*</td>
<td>0.047</td>
</tr>
<tr>
<td>C</td>
<td>6.40 ± 0.55</td>
<td>0.919</td>
<td>0.640</td>
<td>-0.901</td>
<td>0.902</td>
<td>0.730</td>
<td>0.715</td>
</tr>
<tr>
<td>D</td>
<td>1.10 ± 0.16</td>
<td>1.014</td>
<td>0.359</td>
<td>-1.024</td>
<td>0.985</td>
<td>0.351</td>
<td>0.441</td>
</tr>
<tr>
<td>E</td>
<td>0.88 ± 0.14</td>
<td>2.031</td>
<td>&lt;0.001**</td>
<td>-1.977</td>
<td>1.977</td>
<td>0.001**</td>
<td>0.002**</td>
</tr>
<tr>
<td>F</td>
<td>0.41 ± 0.09</td>
<td>0.854</td>
<td>0.862</td>
<td>-0.853</td>
<td>0.848</td>
<td>0.837</td>
<td>0.856</td>
</tr>
<tr>
<td>G</td>
<td>0.43 ± 0.09</td>
<td>0.869</td>
<td>0.769</td>
<td>-0.861</td>
<td>0.903</td>
<td>0.794</td>
<td>0.661</td>
</tr>
<tr>
<td>H</td>
<td>0.49 ± 0.09</td>
<td>0.912</td>
<td>0.629</td>
<td>-0.917</td>
<td>0.904</td>
<td>0.624</td>
<td>0.660</td>
</tr>
<tr>
<td>I</td>
<td>0.73 ± 0.11</td>
<td>1.209</td>
<td>0.099</td>
<td>-1.197</td>
<td>1.158</td>
<td>0.111</td>
<td>0.137</td>
</tr>
</tbody>
</table>

† Means were calculated based on the total pupal exuviae counts from each block in one season.

* significant at $P \leq 0.025$, ** significant at $P \leq 0.005$

$I_a$: Index of aggregation; $P_a$: $P$ value of $I_a$

$\bar{v}_j$: Mean value of clustering index over the gap units; $P\bar{v}_j$: $P$ value of $\bar{v}_j$

$\bar{v}_i$: Mean value of clustering index over the patch units; $P\bar{v}_i$: $P$ value of $\bar{v}_i$
**Fig. 3.1** Seasonal mean (±SE) number of grape root borer pupal exuviae per vine collected from 19 vineyard blocks in Virginia. Blocks A – S used for Taylor’s Power Law analysis and Blocks A – I used for geostatistical and SADIE analyses.
Fig. 3.2 Fit of grape root borer pupal exuviae counts from 19 vineyard blocks in Virginia to Taylor’s Power Law (solid line), indicating aggregation, and a hypothetical fitted equation (dashed line) for variance equal to the mean, indicating random distribution.
Fig. 3.3 Interpolated maps, developed using kriging based on the asymptotic variogram models, of grape root borer pupal exuviae distributions in five vineyard blocks in Virginia. Figs, a, b, c, d, and e represent vineyard blocks A, B, C, D, and H from Fig. 3.1, respectively.
**Fig. 3.4** Estimated maps of grape root borer pupal exuviae distribution using clustering index values (i.e. $v_1$ and $v_j$) of each of 80 sample points. Figs. a, b, and c represent vineyard blocks, A, B, and E from Fig. 1, respectively. Sampled areas having all sample points with cluster indices $> 1.5$ and $< -1.5$ are referred to as ‘patches’ and ‘gaps’, respectively.
Fig. 3.5 Mean (± SE) weekly percentage of total grape root borer pupal exuviae collected from vineyard blocks in Virginia in; (a) 2008, (b) 2009, (c) 2010, (d) 2011, and (e) 2012. In consecutive years between 2008 and 2012, 9, 7, 3, 8, and 8 blocks were sampled, respectively. Julian weeks 28 and 32, represent 9 – 15 July and 6 – 12 August, respectively.
Fig. 3.6 Percent cumulative mean grape root borer pupal exuviae from pooled data (2008 – 2012) collected from 35 vineyard blocks in Virginia (solid circle) fitted to the Weibull function (solid line with solid triangle), $f(x) = 100 \left(1 - e^{-\left(x/\alpha\right)^{\beta}}\right)$. In the Weibull function, $\alpha = 30.28 \pm 0.06$ SE and $\beta = 20.25 \pm 1.07$ SE.
CHAPTER 4: BEHAVIORAL RESPONSE OF GRAPE ROOT BORER NEONATES TO GRAPE ROOT VOLATILES

This chapter was published as:

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 belowground interactions with host plants. The behavioral response of groups of grape root borer neonates to stimuli from host and non-host 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 (non-host) 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, belowground herbivory
Introduction

Grape root borer, *Vitacea polistiformis* (Harris) (Lepidoptera: Sesiidae), is an important, oligophagous pest of grapevines 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 (Rijal, 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 aboveground 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 belowground 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* Fabr. (Herron 1953), and black vine weevil, *Otiorhynchus sulcatus* (Fabr.) (Clark et al. 2011).

Given that vineyard infestation by grape root borer ultimately depends upon neonates finding and establishing on vine roots and that, for reasons as yet unknown, infestation severity can vary widely among and within vineyards (Rijal, unpublished data), understanding the plant-insect interactions between this pest and its wild and cultivated Vitaceae hosts will provide important insights that may guide research toward its sustainable management. In nature, the
roots of wild *Vitis* species likely occur in close 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 belowground 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 belowground herbivory directly or indirectly by affecting insect behavior or development (Hiltiola and Turlings 2012). Polyphagous root-feeding insects often utilize primary metabolites such as CO$_2$, 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, Soni and Finch 1979, Jones and Coaker 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, Soni and Finch 1979, Jones and Coaker 1979, Koštál 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), Johnson and Nielsen (2012), and Hiltiola and Turlings (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 non-host (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 AM, 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 long x 35 cm wide x 35 cm deep) 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 deep x 14 cm diam) with a screened lid and water-soaked cotton ball under shaded conditions outdoors. Eggs were collected daily and kept in 50 x 9 mm tight-lock Petri dishes (Falcon 35-1006, Becton Dickinson and Co., Franklin Lakes, NJ) in a covered plastic box (31 cm long x 21.5 cm wide x 7.5 cm deep) in a controlled environment chamber (Percival I-36LL, Percival Scientific, Perry, IA) set at a 14 h photoperiod and constant 15°C to slow egg development. Bergh (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 *V. berlandieri* x *V. riparia* hybrid and was widely planted in Virginia between 1988 and 2000, while the 420-A rootstock (*V. riparia* x *V. rupestris*) has been less commonly used but has commercial importance (T. Wolf, personal communication). Grape roots (≈1.5-3.0 mm diam) 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, 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 long, ≈1.5-3.0 mm diam) were placed individually in 125 ml wide-mouth glass jars with Teflon-lined caps containing 50 ml histological grade ethanol (95% purity, Fisher Scientific, Pittsburg, 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 ≈2 ml in a Buchi RE 111 Rotavapor evaporator (Buchi Labortechnik AG, Flawil, Switzerland) with vacuum in a water bath at ≈35°C. Concentrated extracts were stored at 0°C.

Headspace volatiles were collected from the roots (Fig. 4.1B) of own-rooted 3309 and 420-A grapevines (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 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 mm x 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 long; 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 (≈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 ≈200 µl under a nitrogen stream and stored at -30°C until use.

**Bioassays.** Bioassays were conducted using clear, 50 x 9 mm tight-lock Petri dishes (Falcon 35-1006, Becton Dickinson and Co., Franklin Lakes, NJ) (Fig. 4.1B & D). Dishes were ventilated via a 12 mm diam 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 diam hole drilled in the center of two opposing quadrants in the bottom of each dish was covered with a piece of metal screen (1 x 1 mm mesh) glued to the outer surface. A 12 mm diam hole was drilled in the plastic cap of threaded glass vials (2 ml, 2.7 cm long; 12 mm ID) (Fisher Scientific, Pittsburg, 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 (Fig. 4.1B), in which single or paired treatments were presented.

In bioassays using freshly collected roots, two root pieces (2-3 cm long, ≈1.5-3.0 mm diam) 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 diam filter paper discs (Whatman No 1) were treated with 50 µl 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- versus 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 head-space volatiles from different plant sources used filter paper discs that were treated either with 10 µl 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) (Fig 4.1C & D) and the lid was placed on each. In a test in which one larva was transferred to each of 24 bioassay 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, 5 neonates per dish were used in all other bioassays. Since most larvae eclosed in the morning, all bioassays were initiated before noon. The dishes were placed in test tube racks (Nalge Nanc 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 (Fig. 1.4E). The racks were placed in a clear, covered plastic box (28 cm long x 16.5 cm wide x 10 cm deep) with damp paper towel lining the bottom and the box was placed in a dark drawer of a laboratory bench for 1 h (Fig. 1.4E-F). During the bioassays, room lights were turned off and air temperature was 24.8°C ± 0.08 SE.

Between bioassays, the glass vials were washed in hot water with detergent, rinsed with clean water then with 99.9% HPLC grade acetone (Fisher Scientific, Pittsburg, PA) and oven-dried at 100°C for ~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.** Prior to 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 µl 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 6-7 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 diam) 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 about 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 diam) 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 (α = 0.05) using SAS-Ver. 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 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. 4.2A-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. 4.3A-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. 4.4A-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. 4.4C).

**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. 4.5A-B). Paired stimuli comparisons between apple roots and 420-A roots from 1-year-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. 4.5C). 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. 4.6A), 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. 4.6B).

**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. 4.7A-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 non-host 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 very 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 due to the positive geotactic behavior exhibited by grape root borer neonates that must burrow downward through soil to find food; i.e. 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. Interestingly, 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 non-host fruit volatiles in no-choice laboratory assays in which the outcomes were due to an all-or-nothing response by individuals, like those reported here. Second, larvae may have responded to the presence of CO\textsubscript{2} presumably released by actively respiring tissue from fresh apple root pieces and used this as a non-specific plant orientation cue (Johnson and Nielson 2012). Perception of general, nonspecific plant cues such as CO\textsubscript{2} 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 upon 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 CO\textsubscript{2} in combination with the induced plant volatiles, (E)-\(\beta\)-caryophyllene or dimethyl disulfide, compared with either CO\textsubscript{2} or the volatiles alone. Evidence that oligophagous insect herbivores are attracted to CO\textsubscript{2} (Jones and Coaker 1979, Bernklau and Bjostad 1998, Johnson and Nielson
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 and Prokopy (2000) reported a significantly stronger response of adult plum curculio to host than non-host 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 different 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 due to different compounds or to 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 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 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). Recently, Bernklau et al. (2013) demonstrated that D. virgifera virgifera larvae demonstrated a strong positive response to a blend of two or more compounds combining both polar and non-polar compounds isolated from the corn root. A polyphagous white grub, Costelytra zealandica (White), utilized 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 present 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 (Zhang, 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 belowground 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. While 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, *V. shuttleworthii* House, showed significantly less borer injury than other rootstocks without *V. shuttleworthii* in their parentage. An extensive and intensive survey by Rijal (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 semi-field experiments, food-finding by western corn rootworm larvae was disrupted by incorporating a source of CO$_2$ (Bernklau et al. 2004, Schumann et al. 2014), attractants from corn roots or CO$_2$ combined with insecticides (Hibbard et al. 1995, Bernklau and Bjostad 2005, Schumann et al. 2014) into soil. Larval and adult cabbage root fly, *Delia radicum* L., respond to sulfur-based volatiles from brassica crops (Koštál 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 (Dosdall 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.
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Fig. 4.1 A) Grape root head-space volatile collection at USDA-ARS facility, Beltsville, MD, B) Petri dish bioassay design with ventilated top, C) transferring grape root borer neonates, D) bioassay arena with transferred larvae at the center, E) Petri dishes with neonates arranged in test tube racks, F) Petri dishes within a plastic boxes placed inside drawer of the laboratory bench.
Fig. 4.2 Mean (±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. Bars with the same letter are not significantly different at the 5% level of significance (Student’s *t*-test).
Fig. 4.3 Mean (±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. Bars with the same letter are not significantly different at the 5% level of significance (Student’s *t*-test).
Fig. 4.4 Mean (±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. Bars with the same letter are not significantly different at the 5% level of significance (Student’s *t*-test).
Fig. 4.5 Mean (±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. Bars with the same letter are not significantly different at the 5% level of significance (Student’s *t*-test).
Fig. 4.6 Mean (±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. Bars with the same letter are not significantly different at the 5% level of significance (Student’s *t*-test).
Fig. 4.7 Mean (±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. Bars with the same letter are not significantly different at the 5% level of significance (Student’s *t*-test).
CHAPTER 5: CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

The results of the studies reported in this dissertation provide valuable new insights into grape root borer pest status, monitoring and sampling, and plant-insect interactions between larvae and their *Vitis* hosts, and have numerous practical implications to the sustainable management of this pest in commercial vineyards in the eastern United States.

An unprecedented database of the infestation status of Virginia vineyards, using pupal exuviae sampling, revealed that ~94% of the 48 vineyard blocks sampled support some levels of infestation with substantial difference in their degrees of infestation ranging from light to heavy infestation. Examination of 18 biotic and abiotic factors for their potential contribution to differences in the extent of infestation among these vineyards showed that two environmental variables, water holding capacity and clay/sand ratio of the soil, were most strongly associated with the infestation status. None of the horticultural and cultural factors was significant in explaining the differences among the blocks sampled. The two significant factors identified were used to develop a risk prediction model, the validation of which may enable grape growers to predict the relative risk that newly established blocks might become heavily infested and to assess the probability that established plantings are more or less heavily infested. This information should help guide grower decisions about appropriate levels of monitoring grape root borer in their respective vineyards. Toward that end, the pupal exuviae sampling data were also used to determine the spatial distribution of grape root borer infestations in commercial vineyards. Since aggregated distributions were detected in vineyard blocks with higher pupal exuviae densities, a quantitative sampling protocol was developed to enable independent samples for population assessments. Weekly captures of male moths in sex pheromone baited traps were not a reliable predictor of weekly numbers of pupal exuviae, reinforcing the utility of pupal exuviae sampling for population assessments.

From a grower perspective, there is an important need either to prevent grape root borer infestations from reaching economically damaging levels or to respond appropriately to an existing infestation. There are three scenarios that could dictate grower response to this pest, 1) newly established vineyard that is not near an older planting, 2) newly established vineyard block that is near established blocks, and 3) older, established block(s).
In scenario 1, after evaluating the relative vulnerability of the site using the risk prediction model, it would be important to monitor the background population of grape root borer developing on wild grapes in the vicinity, as they represent the potential source of infestation. This could be achieved effectively using sex pheromone traps and should be initiated during the year of planting, since there were instances of young vines (2- to 5-yr-old) infested by grape root borer. Once an infestation has established, the utility of pheromone-baited traps for assessing the in-vineyard population of grape root borer is likely to diminish, although the sensitivity of traps to quantified changes in vineyard populations across multiple years has not been investigated. Growers would be advised to use the sampling protocol developed herein to follow the infestation status of new vineyards across years. Indeed, an important, unexplored research question is the rate at which infestations in newly established blocks increase over time.

In scenario 2, pheromone trapping could also be used in the year of planting to assess the background grape root borer population from vines in adjacent blocks and from wild grape in the vicinity. Given that most vineyard blocks sampled in this study showed some level of infestation, it may be that infestations in newly planted blocks become established more quickly and/or increase more rapidly, suggesting an increased need for vigilant monitoring.

In scenario 3, let us assume that a grower has no prior information about the level of infestation in older, established blocks. Data from pheromone traps that use a sticky liner, like those used in the present studies, will likely not provide an accurate representation of the infestation status of established blocks. However, further studies comparing the strength of the relationship between weekly pupal exuviae counts and captures in sticky traps versus non-saturating, bucket style traps may improve the utility of pheromone-based trapping for population assessment. Use of the risk prediction model may yield insights into the likelihood that a block is more or less heavily infested, although as mentioned previously, the model requires field validation. Regardless of the model output, growers should be encouraged to assess the status of their blocks periodically, if not annually, using the sampling scheme recommended. The present studies have clearly shown that a reasonable estimate of pupal exuviae density can be obtained by sampling during a relatively brief period spanning peak emergence, making this effort more palatable to growers. However, in the absence of an economic injury level or economic threshold for grape root borer on *V. vinifera* vines, data from pupal exuviae sampling will provide only qualitative guidance about whether intervention against the pest is warranted.
Indeed, although the development of these parameters will likely be very difficult, this would be an extremely significant contribution to grape root borer IPM.

Another highly relevant question is whether blocks found to be lightly infested from sampling will remain lightly infested or become more heavily infested over time. The association between two, soil-related factors and grape root borer infestations shown by these studies raises intriguing questions about the effects of different soils on the presence of entomopathogenic nematodes in vineyards. Previous studies have demonstrated that several species of entomopathogenic nematodes can infect larval grape root borer. However, since Virginia vineyards have never been surveyed for these potentially important biological control agents, such efforts may provide important new information about why vineyard blocks differed in the magnitude of grape root borer infestations. Furthermore, since naturally occurring, locally adapted species and strains of nematodes are known to persist in soil for extended periods, their presence or absence may help to explain the likelihood of temporal changes of grape root borer populations in vineyard blocks.

Ultimately, infestation of grape vines by grape root borer is dependent upon the success of larval establishment on vine roots, which may be largely dependent on their ability to perceive and locate roots. These studies have revealed new information about the behavioral response of grape root borer larvae to host roots and their movement capacity in soil. Newly eclosed larvae responded positively to grape root volatiles in laboratory bioassays. Preliminary comparisons of root headspace volatile profiles by Dr. Aijun Zhang have revealed volatile compounds from grape roots that were not detected from non-host, apple roots. Future studies of food-finding behavior by larval grape root borer should include chemical identification of these compounds and evaluations of their behavioral activity. Subsequently, a comparison of root headspace volatile profiles across a range of Vitis species and rootstocks may yield information on their relative suitability or resistance to grape root borer. Indeed, the Florida leatherleaf grape, V. shuttleworthii, showed partial resistance to grape root borer larvae and would be an appropriate candidate for such a comparison.

There are precedents in the literature for the use of behaviorally-active, host-plant compounds to disrupt food-finding by root-feeding insects. Given our demonstration of the behavioral response of grape root borer larvae to grape root volatiles, this may be a fertile area for future research with this species. The soil column bioassays developed during the studies
reported herein showed that neonate grape root borer, 1) moved horizontally and vertically in soil, 2) travelled up to 120 cm to a food source, 3), survived more than 48 h without food, and 4) responded to fresh grape root pieces over a distance of 5 cm in soil. These bioassays may prove useful to future research on the effects of adding extraneous sources of host root stimuli to soil on the rate and success of food-finding by neonate grape root borer.
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APPENDIX A

RELATIONSHIP BETWEEN CAPTURES OF MALE GRAPE ROOT BORER IN SEX PHEROMONE TRAPS AND PUPAL EXUVIAE COUNTS

Objective: To examine the relationship between the number of male grape root borer captured weekly in sex pheromone-baited traps in commercial vineyard blocks in Virginia and the number of grape root borer pupal exuviae collected weekly from sample vines in the same blocks.

Materials and Methods
This was an extension of the studies described in Chapters 2 and 3, and used some of the same vineyard blocks. In blocks that were not affected by the presence of other grape root borer pheromone traps in the same vineyard, a single “large plastic delta” (LPD) trap was baited with a pheromone lure and suspended from the upper trellis wire in the vine row in the center of the sampling grid from which pupal exuviae were collected. Trap liners were replaced as needed. The number of male grape root borer in each trap was recorded weekly throughout the period when pupal exuviae were sampled in the same block. Ultimately, data from nine blocks in which trapping and pupal exuviae sampling was conducted for ≥ 6 wk were selected for analyses. These blocks ranged in the seasonal total number of pupal exuviae collected. Accordingly, the nine blocks were assigned to each of three infestation levels (n = 3 blocks per infestation level); high (total exuviae = 71 – 140) (Fig. A.1 A-C), intermediate (total exuviae = 26 – 34) (Fig. A.1 D-F) and low (total exuviae = 4 – 6) (Fig. A.1 G-I) based on relative differences in the total numbers of pupal exuviae collected. Linear regression analysis was conducted to determine the relationship between weekly moth captures and pupal exuvium counts using SPSS 21 (IBM Corp. 2012).

Results and Discussion
Since grape root borer has a 1:1 sex ratio, this would not have affected the relationship between captures and pupal exuviae counts. There was not a significant relationship between weekly moth captures and the number of pupal exuviae collected from blocks classified as having a low infestation (block A: $r^2 = 0.181$; $P = 0.341$, block B: $r^2 = 0.075$; $P = 0.493$, block C: $r^2 = 0.010$;
\[ P = 0.847 \], an intermediate infestation (block D: \( r^2 = 0.009; P = 0.861 \), block E: \( r^2 = 0.211; P = 0.252 \), block F: \( r^2 = 0.255; P = 0.202 \)), or a high infestation (block G: \( r^2 = 0.021; P = 0.759 \), block H: \( r^2 = 0.054; P = 0.579 \), block I: \( r^2 = 0.088; P = 0.476 \)) (Fig. A.1).

Although we do not understand the “active space” of a grape root borer pheromone lure, males are strong fliers, and captures in traps likely included males that had emerged from elsewhere in the vineyard and from wild vines nearby. As well, since the trapping efficiency of the sticky delta trap liners decreases rapidly (“trap saturation”) after about 20 moths have been captured (Bergh, unpubl. data), this effect may have influenced the strength of these relationships in some blocks on some weeks. Decreased trap efficiency was likely on 5, 8, and 10 of the sampling weeks in blocks with low, intermediate, and high infestation levels, respectively (Fig. A.1). While effective for determining the presence and activity periods of adult GRB, the utility of delta traps for assessing vineyard infestation status appears limited. While “bucket” style pheromone traps for grape root borer are considered “non-saturating”, they are not as effective for capturing male grape root borer as delta traps (Bergh, unpubl. data). Although labor- and time-intensive, pupal exuviae sampling provides the most accurate representation of the infestation status of individual vineyard blocks, and was used in Chapters 2 and 3.
Fig. A.1 Weekly GRB pupal exuvium counts and male moth captures in vineyard blocks with low (A, B, C), intermediate (D, E, F), and high (G, H, I) infestation levels.
APPENDIX B

FOOD-FINDING BY GRAPE ROOT BORER NEONATES IN SOIL COLUMN BIOASSAYS

Grape root borer infestations in commercial vineyards depend primarily upon the success of neonates at finding and establishing on grape roots. This critical period may be influenced by many biotic and abiotic factors, not least of which are their capacities to detect roots and move through the soil to them. Although I showed in Chapter 4 that grape root borer neonates were attracted to volatile compounds from grape roots, no studies have been conducted to determine their movement capacity in soil in the vertical or horizontal dimensions, their rate of movement in soil in response to the presence or absence of host stimuli, or the distance over which larvae can perceive host root stimuli.

Objectives: To measure the movement of larval grape root borer in soil column bioassays.

General Methods
Soil column bioassays were constructed from PVC pipes filled with soil that had been brought to 25% soil moisture (1.20 g/cc bulk density). The soil was collected in 2008 from a grassy field adjacent to a vineyard in Virginia that was heavily infested with grape root borer and then spread and dried on a platform in a greenhouse. Debris such as plant material and stones were removed by hand and the soil was then sifted using a metal screen (1.5×1.5 mm mesh). Between years, the soil was stored in a partially covered plastic tub in a greenhouse, in which it dried thoroughly.

The soil columns were prepared between 16 and 18 hr in advance of each set of bioassays. To prepare them, distilled water (w:w) was added to weighed batches of dry soil to standardize its moisture content and bulk density among pipes of a given length. The damp soil was added to the pipes to within ~0.5 cm of the top. The top of each pipe was sealed with Parafilm and the pipes were stored at 5°C until bioassays were initiated the following morning.
For Experiments 1-3 and Experiment 4 (see below), 2.54 cm ID and 1.27 cm ID PVC pipes were used, respectively. For Experiments 1-3, in which straight sections of vertically or horizontally oriented pipes were used, a tight-fitting PVC adaptor containing a circular, fitted piece of metal screen (1.5×1.5 mm mesh) (Fig. B.1 B & C) to prevent soil from falling out of the pipe was inserted over the bottom of each pipe. A tight-fitting, round-bottomed PVC cap was placed over the adaptor (Fig B.1 B & D). For Experiment 1, in which only pipes with grape root pieces were used, the bottom cap contained a moistened, circular sponge plug with a fitted, circular piece of filter paper covering its top, on which grape roots pieces were presented. For Experiments 2-4, which involved comparisons of larval response in pipes with and without grape roots or a filter paper disc treated with grape root extract, circular discs were cut from the sticky liner of pheromone traps and a 2-cm diam hole was cut in the middle of each. These were placed atop the filter paper on the damp sponge and grape root pieces or treated filter paper were presented in the hole (Fig B.3 C & D). For Experiment 4, the straight section of a polypropylene Y-adaptor was inserted into the bottom of a 10 cm long section of PVC pipe (Fig B.3 B). Black electrician’s tape was wrapped around the exposed parts of the Y-adaptor to prevent light penetration through the translucent material. A section of PVC pipe (5 or 7.5 cm long) was inserted over both arms of the Y-adaptor. A PVC adaptor containing a fitted, circular piece of metal screen (mesh size, 1.5×1.5 mm; Fig. 1C) was placed at the end of each PVC section and the bottom cap was placed at the end of these.

The vertical and horizontal bioassay columns and the Y-tube bioassays were secured to different frames to accommodate them (Fig. B.1 A and Fig. B.2), and the bioassays were conducted in laboratory rooms or in an environmental chamber at 25.6 °C ± 0.12 SE and 68.2% ± 0.42 RH.

The stimulus sources used in the various experiments included; 1) two pieces of freshly collected roots (~2.0 mm diam, ~2.0 cm long) from vines on 420-A rootstock growing at Virginia Tech’s Winchester research station or, 2) filter paper discs treated with an ethanol based root extract of 420-A roots. Grape root borer eggs were obtained from females collected in vineyards and the larvae used were from those eggs. See Chapter 4, for details about root collection, root extract preparation and presentation, and generation of eggs and larvae for use in these experiments.
In Experiments 1 and 2, eggs that were in the process of hatching were used; larvae could be readily seen chewing their way out of eggs. For Experiments 3 and 4, recently hatched larvae (<30 min. old) were used. Using a fine-tipped, damp paint brush, a single egg or larva was transferred from the petri dishes in which they were held to a small glass dish, which was placed on top of the soil in each column. When eggs were used, the hatch of each larva was verified by inspecting the egg at some point after the bioassay was initiated. At intervals that varied according to the experiment, the bottom cap was removed from each pipe and inspected for the presence of the larva.
Fig. B.1 Components of soil column bioassay showing: A) vertical pipes of different lengths B) adaptor/cap assembly on the bottom of the pipes, C) adaptor with metal screen, and D) bottom cap with moist filter paper and grape root pieces.
**Fig. B.2** Horizontal pipes with center hole for introducing grape root borer egg

**Fig. B.3** Y-tube bioassay showing: A) bioassays in frame within a controlled-environment room, B) Y-tube assembly, C) bottom cap without grape root pieces, and D) bottom cap with grape root pieces.
Experiment 1. What is the movement capacity of grape root borer neonates in vertical and horizontal soil columns?

Materials and Methods

*Vertical soil column*
- Soil column lengths: 15, 30, 60, 90, 120 cm with root pieces (n = 10/column length)
- Larval presence in the bottom cap evaluated at 24-h intervals for 5 d
- Data transformation conducted using Sqrt (x + 0.5) and analyzed using one-way ANOVA; mean separation performed using Tukey-Kramer HSD

*Horizontal soil column*
- Soil column (30 cm long) with a hole in the middle (Fig. B.2) (n = 15)
- Fresh root pieces in cap at bottom of perpendicular section at both ends
- Larval presence at either end evaluated at 24-h intervals for 3 d

Results
- After the first 24 h, there was a significant effect of vertical column length on the number of larvae recovered ($F = 8.84; \text{ df} = 3, 12; \; P = 0.0023$), but not a significant difference among the 15, 30, and 60 cm columns (Fig. B.4 A)
- Some larvae reached the bottom of the 120 cm vertical column after ≥48 h (Fig. B. 4 B)
- No larvae were recovered from either end of horizontal columns after 24 h (Fig. B.5). After 48 and 72 hr, respectively, the cumulative number of larvae recovered was 10 and 14, and there was no indication of differences in larval movement to either end
- The rate of larval movement was greater in vertical soil columns than in horizontal soil columns
Fig. B.4 Mean (±SE) number of grape root borer larvae recovered from the bottom of vertical soil columns, A) after 24 h and, B) at 24-h intervals for five days. Values with the same letter are not statistically significant at 5% level of significance.
Cumulative number of grape root borer larvae recovered from the ends of 30 cm horizontal soil columns at 24-h intervals.

Experiment 2. Does the presence of root pieces affect the recovery of grape root borer neonates from the bottom of a soil column?

Materials and Methods

- 15 cm vertical soil columns (n = 10/treatment with 3 independent repetitions)
- Presence or absence of fresh root pieces
- Larval presence evaluated at 24-h intervals for 3 d
- Data from three repetitions pooled and analyzed using a 2×2 contingency table

Results

- There was not a significant effect of the presence of root pieces on the number of larvae recovered ($\chi^2 = 0.36; \text{df} = 1; P = 0.546$) (Fig. B.6)
Fig. B.6 Total number of grape root borer larvae recovered after 24 h from the bottom of 15 cm vertical soil columns with and without pieces of grape root at the bottom.

Experiment 3. Does the presence of grape root pieces influence the rate of food-finding by grape root borer neonates?

Materials and Methods
- Vertical soil columns (15, 30, 60 cm long) with and without roots (n = 5/treatment and 3 independent repetitions)
- Larval presence at the bottom evaluated at 8-h intervals for 72 h
- Data from three repetitions pooled and analyzed using a 2×3 contingency table (Fisher’s Exact Probability Test)

Results
- There was not a significant difference between the cumulative number of larvae recovered at the bottom of three column lengths with and without root at all evaluation times (8 h, \( P = 1.000 \); 16 h, \( P = 1.000 \); 24 h, \( P = 0.1312 \); 32 h, \( P = 0.1218 \); 40 h, \( P = 0.8825 \); 48 h, \( P = 0.7359 \); 56 h, \( P = 0.7573 \); 64 h, \( P = 1.000 \); 72 h, \( P = 0.9321 \) ) (Fig. B.7)
Experiment 4. What is the distance over which neonates perceive root stimuli in soil?

Materials and Methods

- Y-tubes (Fig. B.3 A-D) were used (n = 10-12 tubes with 3 independent repetitions)
- The main arm of the Y-tube was of fixed length (10 cm long) with side arm lengths of 5 or 7.5 cm
- Stimulus treatments: 1) fresh root vs no root, and 2) filter paper discs treated with grape root extract vs discs treated with ethanol
- Paired stimuli presented in opposing arms of the Y-tube
• Larval presence at the bottom of both arms of the Y-tube recorded at 24 h intervals for 72 h
• Data from three repetitions were pooled and analyzed using the binomial test.

Results
• In tubes with 5.0 cm long arms, the number of larvae recovered from the arm with root was significantly higher ($N = 23; P = 0.0002$) than from the opposing arm (Table B.1).
• In tubes with 7.5 cm long arms, there was not a significant difference (need stat $N = 26; P = 0.2786$) in the number of larvae recovered from the two arms (Table B.1).
• There was not a significant difference ($N = 21; P = 0.0946$) when root extract was used in Y-tubes with 5 cm long arms (Table B.1).

Table B.1. Number of larvae recovered from Y-tube bioassays after 72 h

<table>
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<th></th>
<th>Host stimulus</th>
<th>Control</th>
<th>Not recovered</th>
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</thead>
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<td>Repetition 1 (n = 12)</td>
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<tr>
<td>Repetition 2 (n = 10)</td>
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<td>3</td>
</tr>
<tr>
<td>Repetition 3 (n = 10)</td>
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<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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<td>3b</td>
<td>9†</td>
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<tr>
<td><strong>7.5 cm arms with root</strong></td>
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<td>0</td>
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<tr>
<td>Repetition 2 (n = 10)</td>
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<tr>
<td>Repetition 3 (n = 10)</td>
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<tr>
<td><strong>Total</strong></td>
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<td>11a</td>
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<td><strong>5 cm arms with root extract</strong></td>
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<tr>
<td>Repetition 1 (n = 10)</td>
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<tr>
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<td>9a</td>
<td>7†</td>
</tr>
</tbody>
</table>

†† Not included in statistical analysis.

Values with the same letter are not statistically significant at 5% level of significance.