FY 2013 Final Report to the Virginia Wine Board

Determination of Abundance, Distribution and Risk Factors Associated with the Infestation of Virginia Vineyards by Grape Root Borer

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Objective 1: Develop a database of the occurrence and abundance of grape root borer pupal cases in vineyard blocks and identify key horticultural, cultural and/or environmental variables associated with grape root borer infestations

Collection of grape root borer pupal case data: Between 2008 and 2012, we used weekly collections of grape root borer pupal cases during July and August to assess the infestation status of 50 vineyard blocks from 19 vineyards in 8 counties in northern and central Virginia (Fig. 1).

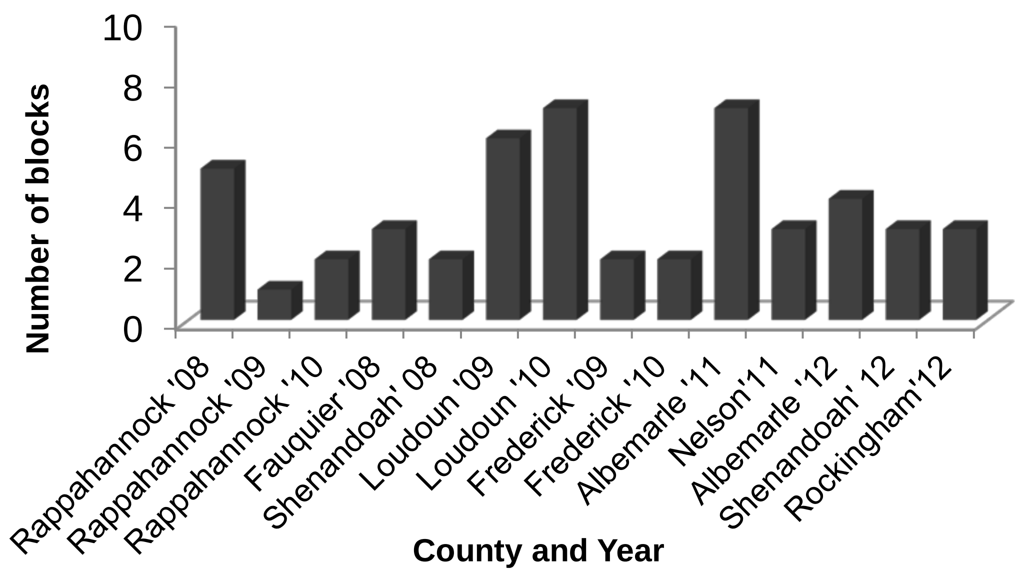

Fig. 1 Number of vineyard blocks in Virginia surveyed for pupal cases between 2008 and 2012

A few of the blocks sampled were discrete blocks that were spatially separate from others on the same farm, but most were derived from a portion of a larger, contiguous planting. Within each block sampled, the grape variety, rootstock, vine age, and other environmental and cultural practices were essentially uniform. The survey included 11 grape varieties (Fig. 2A) and >7 commercially important rootstocks (Fig. 2B).

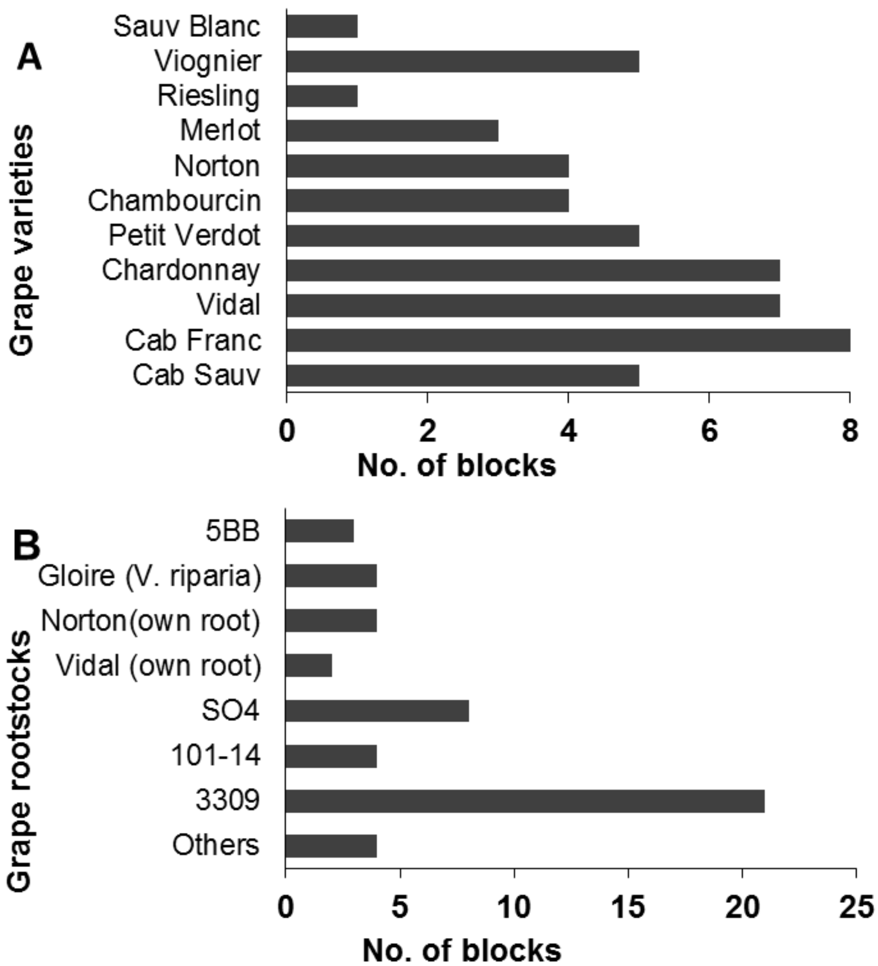

Fig. 2 Grape varieties (A) and roostocks (B) from 50 vineyard blocks in Virginia surveyed between 2008 and 2012

Pupal cases were collected from around the base of sample vines in a grid of 39 – 118 vines per block; the sampling grid in most blocks (n = 28) consisted of 80 vines. In all blocks, the grid of sample vines consisted of the first vine in each panel in every second row. Numbers of sample vines per row and number of rows varied according to block size and shape. For example, in blocks with 80 vines, the grid of sample vines consisted of the first vine in the first 10 panels per row in every second row across 16 rows. The average size of the area sampled was ~0.5 acre. Prior to the onset of adult emergence each year (ca. early July), the area around the base of each sample vine was cleared of vegetation using a "weed-eater" followed by raking, creating a ~1-m diameter area of clean soil. Most blocks were sampled in one season, although 4 blocks were sampled in 3 - 4 consecutive years. During the final field season in 2012, 10 new blocks from 5 vineyards were sampled, thereby completing our targeted dataset of 50 blocks.

Collection of potential risk factor data: To identify key risk factors underlying the differences in grape root borer infestation levels among the vineyard blocks sampled, we collected data on horticultural, cultural and environmental variables from each of 48 blocks.

Horticultural variables. Information about grape cultivar, rootstock, vine age, and block size for each vineyard block was collected from participating growers. The distance of each block sampled to the nearest forested area was measured using Google Earth, although we did not collect data on the presence or abundance of wild grape in those area. Five distances (including maximum and minimum) from the border of each vineyard block that was closest to the forest were measured and averaged.

Cultural variables. Information on the vineyard cultural practices (insecticide use, ground cover, weed control and irrigation) used from late June through early September two years before the sampling year (i.e. in the year during which eggs were laid, given a 2-year larval development period) were collected from growers using a questionnaire in 2012. Insecticide use information included type, rate and frequency of insecticides applied. Qualitative ratings of individual blocks for ground cover, including weeds under the vine trellis (1= 1-20%, 2 = 21-40%, 3 = 41-60%, 4 = 61-80%, 5 = 81-100%), weed management (1 = none, 2 = light, 3 = medium, 4 = heavy), and irrigation (yes/no) were made based on grower feedback.

Environmental variables. The physical and chemical properties of soil and the longitude and latitude of individual vineyard blocks were measured. Soil sample collection and preparation were conducted following standard protocols. In fall 2012, five soil samples (corners and center) of each block surveyed were collected using a soil sampling auger. For each block, one composite sample was prepared from the five initial samples and analyzed for soil organic matter content, pH, particle size, water holding capacity. Soil analysis from each of 48 blocks was conducted at Virginia Tech's Soil Testing Laboratory in Blacksburg. In addition, one core ring sampler (5.08 cm diam, 5.08 cm length) was used to collect one soil sample from each block for calculation of soil bulk density. Soil from each core ring was oven-dried at 105^0C for 24 hours and weighed. Bulk density for each of 48 blocks was calculated using the following formula.

$$bulk\ density\ = \frac{mass\ of\ dry\ soil}{volume\ of\ core\ ring}$$

Latitude and longitude of each vineyard block were determined using Google Earth.

Statistical analysis: Nonlinear principal component analysis (NLPCA) and multiple regression were used for data analysis. Principal component analysis was first used to reduce the number of variables (19 variables measured) into a few uncorrelated variables (i.e. principal components) so that the relationship between the most important variables and grape root borer infestation status could be determined. This non-linear principal component method enabled inclusion of data from nominal (e.g. cultivar, rootstock), ordinal (e.g. ground cover, weed control), and continuous (e.g. vine age, block size, proximity to forest, soil pH, soil bulk density) variables in the model. Multiple regression analyses then used the selected principal components as independent variables and average number of pupal cases per vine from each block as the dependent variable. Variables that were represented by significant Principal Components and that had higher factor loadings, indicated by the NLPCA analyses, were considered important risk factors.

Results: Our data revealed that the level of infestation by grape root borer larvae varied widely among vineyards and vineyard blocks in Virginia, ranging from severe to negligible (Fig. 3). Most blocks surveyed (47 of 50) had detectable infestations. To categorize grape root borer infestation status among blocks, we ranked them according to three relative infestation levels that were based on the mean total number of pupal cases per vine (heavily infested: ≥1 pupal case/vine; moderately infested: ≥0.4 - <1 pupal case/vine; lightly infested: <0.4 pupal case/vine). Based on this, a total of 5, 10, and 32 vineyard blocks were fell into heavy, moderate, and light infestation rankings, respectively. The maximum number of pupal cases collected from a single vine in one season was 28. This vineyard was in serious decline from grape root borer and was the most severe example of infestation that we observed during the study. Although 38 of 50 sample blocks had pupal case counts higher than the grape root borer economic threshold (0.074 cases/vine) developed by Dutcher and All (1979) for ‘Concord’ grape, *Vitis labrusca*, most of those blocks, which were predominantly *V. vinifera,* were not considered to be in decline by the growers. Based on our experience working in Virginia vineyards, many or most of the vineyard mangers were unaware of the extent to which their vineyards were infested by grape root borer. This may be partly due to the lack of specific symptoms associated with grape root borer feeding on roots until vines begin to show severe effects of prolonged and heavy infestations. That the vine health and productivity of lightly and moderately infested blocks were not apparently affected highlights the importance of developing an economic threshold (discussed below) for grape root borer in *V. vinifera* vines in Virginia (i.e. when is intervention against this pest warranted?).

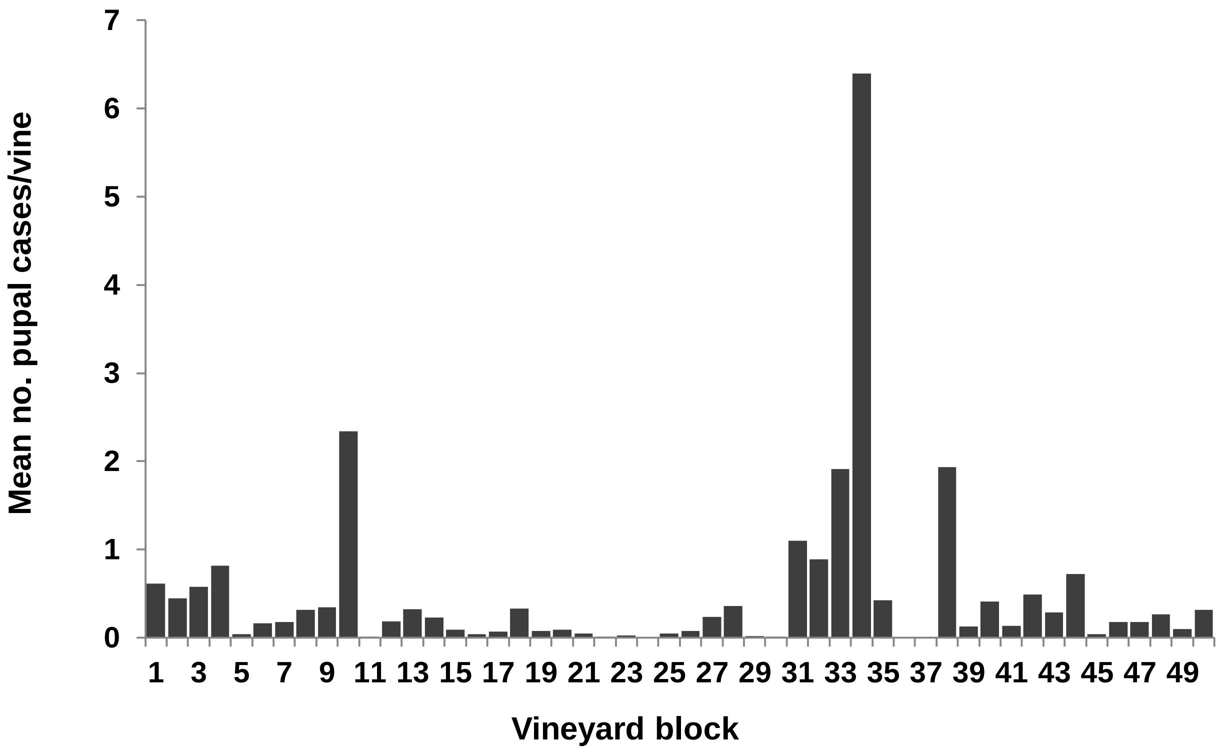

Fig. 3 Mean ± SE number of grape root borer pupal cases per vine from 50 vineyard blocks in Virginia surveyed from 2008 to 2012

Although our exploration of these data are ongoing and will be reported in a scientific publication in preparation, our initial results showed that soil factors (soil mass moisture, soil texture, percentage of sand in soil and cation exchange capacity of soil) were most important in explaining the differences in infestation level among the blocks sampled. A higher proportion of clay in soil and higher soil mass moisture were most strongly associated with higher borer infestations while higher sand content was negatively associated with infestations. Since grape root borer infestations are, in the first instance, dependent upon the success of newly hatched larvae at finding and establishing on grape roots, it is plausible that vineyard soil properties might have effects in this process by influencing larval mobility or mortality in soil during their search for food. Low soil moisture content is known to have especially profound effects on the mortality of young larvae seeking roots. Another possibility is that the physical properties of vineyard soil may differentially affect the diffusion of behaviorally active volatiles from grape roots in soil. Rijal et al. (2013) showed that newly hatched larvae are strongly attracted to these volatiles, suggesting that they have a significant role in facilitating larval food-finding. Another, albeit speculative, factor that might have contributed to differences in levels of grape root borer infestation among the blocks sampled is the populations of entomopathogenic nematodes (EPN) in the soil. Recent studies have shown that at least two species of native, soil-dwelling EPNs attacked larval grape root borer (reviewed in Bergh 2012) and that their effectiveness varied with species, location and soil texture and soil moisture content. Interestingly, clay soil (which seems to be positively associated with grape root borer infestations) had a negative effect while sandy or silty soil (which seems to be negatively associated with infestations) had a positive effect on EPN occurrence and virulence (reviewed in Campos-Herrera et al. 2008). The relationship between EPN populations and grape root borer larval abundance in commercial vineyards is an important question for future research and may explain further the effects that we have measured.

We also conducted a related study examining pupal case abundance in four blocks in which the same vines were surveyed in 3 or 4 consecutive years (Fig. 4). At sites 1 and 2, which were blocks from the same vineyard, the mean number of pupal cases per vine declined between 2008 and 2011. At site 3, pupal cases showed instances of increased and decreased abundance during the same interval, while pupal case abundance at site 4 remained essentially constant between 2010 and 2012. During March/April of 2011, site 3 received a soil application of imidacloprid targeting Japanese beetle. Imidacloprid is a systemic insecticide and might have contributed to reduced grape root borer pupal case counts at site 3 in 2011 and 2012, although this is purely speculative, given that we do not understand the effects of this compound on grape root borer larvae. Among these blocks, the maximum number of pupal cases recovered from an individual live vine over a 4-year period was 19.

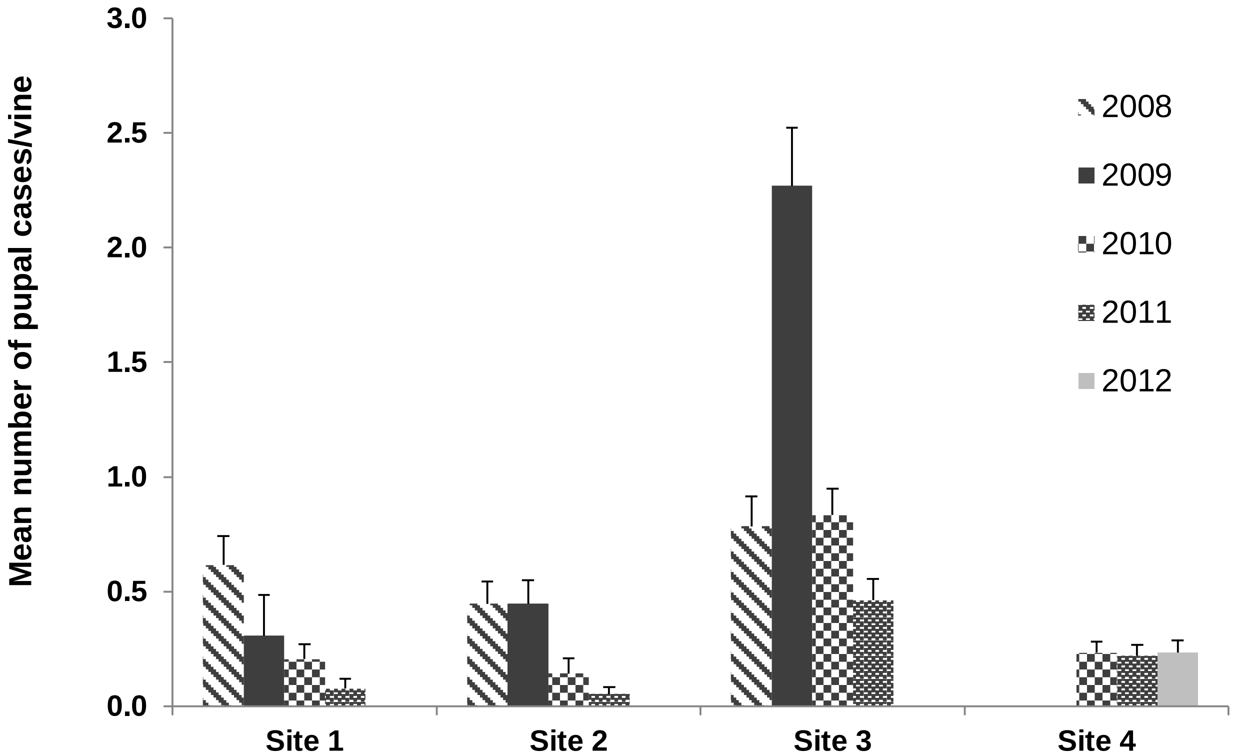

Fig. 4 Mean ±SE total number of pupal cases per vine from 4 vineyard blocks in Virginia surveyed in 3 or 4 consecutive years

Objective 2: Characterize the spatial distribution of grape root borer in vineyards and develop a model to predict the occurrence and distribution of the insect within vineyard blocks

Characterizing the spatial distribution of grape root borer pupal cases: Pupal case data from Objective 1 were used to characterize the distribution of grape root borer infestations in vineyard blocks and to develop a quantitative sampling plan. Both non-spatial (Taylor's Power Law, i.e. TPL) and spatial (Variograms and SADIE) techniques were used. Nineteen blocks that met the following criteria were used in TPL analyses; 1) enabled a grid of 80 sample vines and, 2) yielded a seasonal mean of ≥ 0.1 pupal cases per vine. These 19 vineyard blocks sampled were located in Loudoun (2 blocks), Rappahannock (1 block), Shenandoah (4 blocks), Rockingham (3 blocks), Albemarle (6 blocks), and Nelson (3 blocks) counties. Seven grape cultivars (Chardonnay, Vidal, Petit Verdot, Viognier, Cabernet Franc, Sauvignon Blanc, Chambourcin) and four rootstocks (3309, *V. riparia* Gloire, SO4, 5BB) were included, and mean vine age was 11.8 ± 2.7SE years. Data from nine blocks with mean total pupal cases counts per vine that were greater than the median value of 0.33 cases per vine (range = 0.4 - 6.4 pupal cases per vine) were used to characterize the spatial distribution pattern of grape root borer infestations using geospatial analysis (variograms and SADIE). As well, interpolated maps of infestation patterns were developed for five blocks using the kriging method.

Temporal distribution of pupal cases: To examine the temporal distribution of pupal cases collected throughout the majority of the emergence period of adult grape root borer, data from 35 of the 50 blocks sampled between 2008 and 2012 were used (n = 9, 7, 3, 8, and 8 blocks in consecutive years from 2008 to 2012, respectively). These blocks were selected for this purpose based on the following criteria; 1) pupal cases were collected weekly for at least 6 weeks and, 2) the mean total number of cases per vine was ≥ 0.10. Based on the seasonal total number of pupal cases collected per block, weekly proportions were calculated. Temperature and rainfall data from June through August for all sampling years were collected from a weather station located at Virginia Tech's AREC, Winchester, VA.

Results: Taylor's Power Law analyses showed a strong aggregation of grape root borer pupal cases in all 19 blocks evaluated. Use of geospatial techniques resulted in six of nine vineyard blocks showing significant aggregation, and revealed that the likelihood of aggregation is greater in blocks with relatively higher infestation levels. There were no indications that the density of pupal cases was greater at the edges of the blocks sampled. All five blocks mapped using the kriging technique had an actual border on least one side of the sampling grid. The aggregated distribution of pupal cases in many blocks, especially those with higher borer populations, is an important consideration for the development of a sampling plan to assess the infestation status of individual blocks. In order to obtain an accurate estimate of relative population density, it is necessary that data from sequential sampling points (i.e. vines) are not affected by infestation "hot spots" within the block. Our analysis indicated that independent samples of grape root borer pupal cases can be obtained by sampling from vines separated by >8.8 m (~29 feet).

Our calculations of the temporal patterns of emergence of grape root borer also factors importantly into sampling considerations. Figure 5 A-E shows the seasonal distributions of pupal cases collected between 2008 and 2012. In 2008, 81% of pupal cases (n = 9 blocks) were collected between the third week of July and the first week of August (Fig. 5A). In 2009 (n = 7 blocks) and 2012 (n = 8 blocks), respectively, 82% and 79% of cases were collected between the second week of July and the first week of August (Fig. 5B and D). In 2010, 67% of cases (n = 3 blocks) were collected between the second and fourth week of July (Fig. 5C), while in 2011, 77% of cases (n = 8 blocks) were collected between the fourth week of July and the second week of August (Fig. 5E). Figure 5F shows combined data from all five years and revealed that ~65% of pupal cases (n = 35 blocks) were collected during the 3-wk period between the third week of July and the first week of August.

The average daily temperature from June through August in the consecutive years during which pupal cases were sampled (2008-2012) was respectively, 22.1, 21.8, 24.3, 23.7, and 22.7^{0}C, whereas average daily rainfall was 3.4, 2.5, 1.6, 1.7, 3.4 mm, respectively. Relatively higher average daily temperature and lower rainfall during this period in 2010 may have resulted in earlier peak emergence than in other years. Although peak emergence of adult grape root borer may occur about 1 week earlier in years with unusually warm and dry weather during spring and summer, sampling during the 3-wk period mentioned above should provide a reasonable estimation of its abundance in most seasons, with least effort. Importantly, this recommended sampling period coincides with a period during which labor required for other tasks in mid-Atlantic vineyards is much reduced (T. K. Wolf, personal communication).

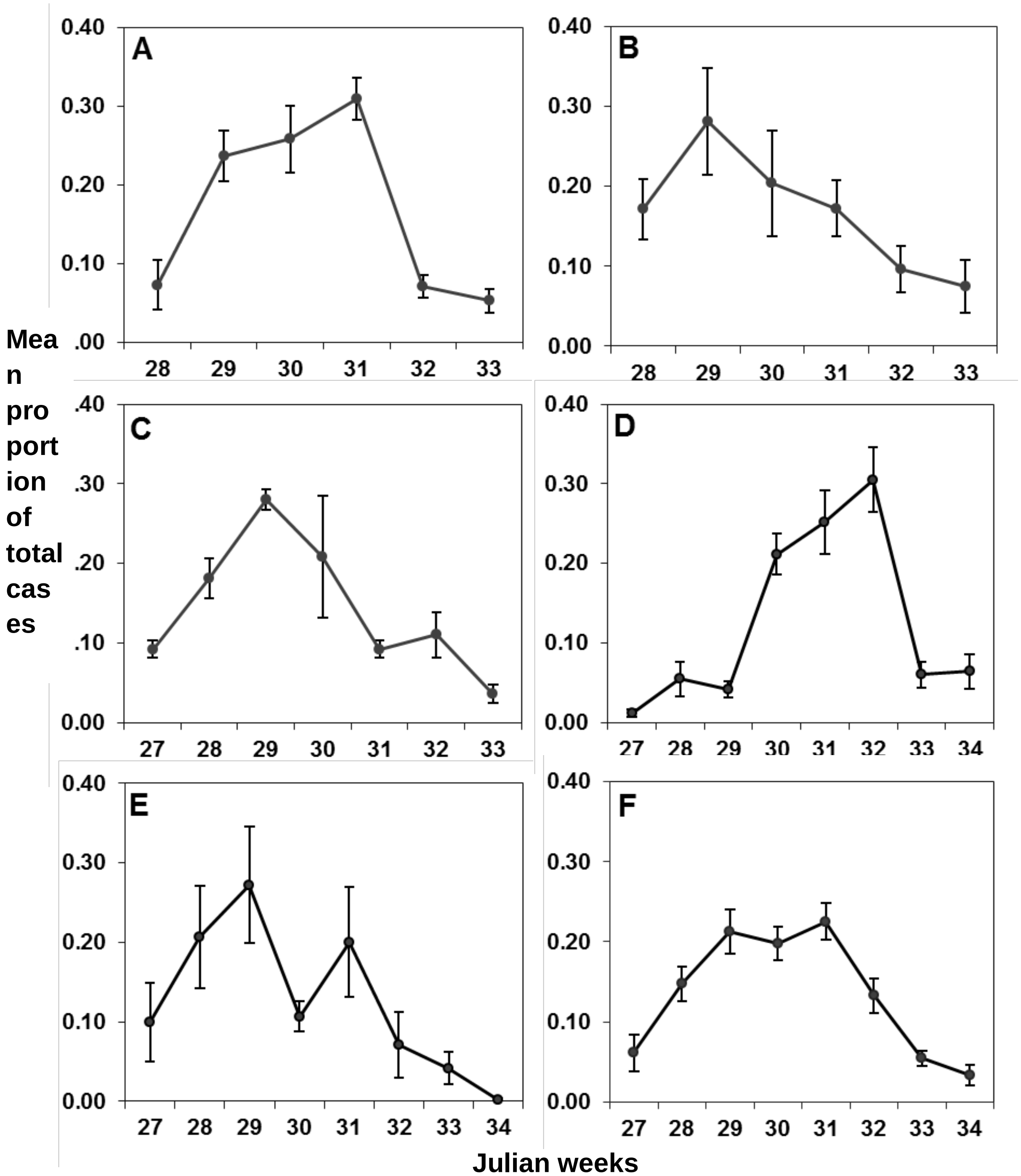

Fig. 5. Mean ± SE weekly proportion of total grape root borer pupal cases collected from vineyard blocks in Virginia in, (A) 2008, (B) 2009, (C) 2010, (D) 2011, (E) 2012 and (F) 2008-2012 combined. In consecutive years from 2008, 9, 7, 3, 8, and 8 blocks were sampled, respectively. Julian week 28 and 32, represent 9 -15 July and 6 – 12 August, respectively.

Summary and recommendations

Our analysis of putative risk factors associated with differences in grape root borer infestation levels among the blocks sampled did not indicate that cultivar or rootstock contributed meaningfully to these differences. Interestingly, we did observe one instance in which a block of 'Norton' vines from which just a few pupal cases were collected was next to other blocks containing *vinifera* cultivars that were heavily infested. Generally, our surveys in Norton blocks (n = 4) produced very few pupal cases relative to many other blocks and further exploration of this species may yield important insights into the relative suitability of different *Vitis* species. Vine age was not an important factor, and we have observed instances of 3- to 4-year-old vineyard blocks with pupal cases. Similarly, coverage by weeds or cover crop or irrigation practices did not contribute to explaining these differences. The patterns of grape root borer infestation in individual blocks did not show edge effects or effects of proximity to forest. Our analysis revealed significant effects of soil physical properties on vineyard infestation by grape root borer that should be amenable to further validation in commercial vineyard blocks using the sampling method described below.

By combining the results from our spatial distribution study and the temporal adult emergence pattern in Virginia vineyards, we can recommend a systematic sampling scheme for grape root borer that involves weekly pupal cases collections between mid-July and early August from a grid of sample vines in blocks to be assessed. Our analyses indicated an appropriate inter-vine sampling distance of 9 m (~30 ft) to achieve independent samples for population density assessment. Based on the typical vine spacing in Virginia vineyards, a sample vine spacing of 9 m is equivalent to one vine per 1.5 panels along the vine row. We recommend a grid of sample vines comprised of vines in every second row and consisting of a minimum of 50 vines per vineyard block of average size (~3 - 5 acre). Accurate assessment of the presence and abundance of pupal cases requires removal of vegetation on soil surface around the base of sample vines prior to peak moth emergence and at least weekly visits to all sample vines during some portion of the emergence period. This is easily accomplished with a "weed wacker" and by raking, typically taking only a few minutes per vine to create a clean soil surface. We recommend a clean area around each sample vine of about 1 m diam. Pupal cases may be found with the anterior end protruding vertically from the soil surface or they may be found lying on the surface. Weekly visits to sample vines during the assessment period are necessary, since pupal cases that are lying on the soil surface are prone to being displaced by the wind from storms, mowers, or airblast sprayers. With minimal training, one experienced person could survey 50 vines in about 30 minutes. All pupal cases found should be removed so that they are not counted again during subsequent surveys.

As mentioned previously, an economic threshold for grape root borer infestation *V. vinifera* vineyards has not been developed. The economic threshold for an insect pest is the population level above which injury is likely to occur. Dutcher and All (1979) calculated an economic threshold of 0.074 larvae per vine for grape root borer feeding on roots of 'Concord' vines in Georgia and recommended intervention upon detection of the pest, although Bergh (2012) suggested that this recommendation may be conservative for grape root borer on *V. vinifera* in the mid-Atlantic region. The majority of 50 blocks surveyed in Virginia between 2008 and 2012 exceeded this recommended threshold, based on total numbers of pupal cases per vine, yet most blocks showed no apparent effects from the pest. While the development of an economic threshold for grape root borer on *V. vinifera* in Virginia and surrounding states would add importantly to the utility of sampling, in the interim the sampling scheme that we propose should greatly improve the assessment of grape root borer populations in vineyard blocks, enable researchers to address applied and basic questions more efficiently and help growers refine their management of this pest.

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Publications and Presentations

Rijal, J, C.C. Brewster, and J.C. Bergh. Spatial distribution of grape root borer (Lepidoptera: Sesiidae) infestations in Virginia vineyards and implications for sampling
In preparation for Environmental Entomology

Rijal, J, C.C. Brewster, and J.C. Bergh. 2012. Spatial distributions of grape root borer (Lepidoptera: Sesiidae) in Virginia vineyards and the development of a quantitative sampling scheme. Entomological Society of America annual meeting, November 11-14, Knoxville, TN. (2^{nd} place in President's Competition)

Rijal, J.P. 2012. Out of sight, out of mind: Grape root borer infestation in Virginia vineyards.The Grape Press. Vol. 28. No. 2.

Rijal, J.P. and J.C. Bergh. 2011. Pupal cases sampling versus pheromone trapping to assess grape root borer infestations in Virginia vineyards. Poster presented at annual meeting of Entomological Society of America, 13-16 November, 2011, Reno, NV

Rijal, J.P., J.C. Bergh and C.C. Brewster. 2011. Determination of risk factors associated with the infestation of Virginia vineyards by larvae of the grape root borer (Lepidoptera: Sesiidae). Entomological Society of America-Eastern Branch, 18-21 March, 2011, Harrisburg, PA