Research Note

Japanese Beetle Defoliation Reduces Primary Bud Cold Hardiness during Vineyard Establishment

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Abstract: Insect defoliation could reduce winter hardiness of young grapevines, but such effects have not previously been quantified for field-grown vines. The impact of Japanese beetle (JB) defoliation on midwinter primary bud cold hardiness of Norton, Chambourcin, and Cabernet Sauvignon grapevines was measured during the first two years of vineyard establishment. Effect on shoot length and periderm browning by late autumn was also evaluated for first-year vines. Three treatments were used to manipulate levels of defoliation: carbaryl applied either every 7 or every 14 days during the JB flight period (mid-June to mid-August) or no insecticide spray. Cuttings from dormant canes were subjected to a controlled freezing stress each February, and the temperature causing 50% lethal injury (LT_{50}) to primary buds was compared among cultivars and treatments. All three cultivars sustained similar levels of JB defoliation, which ranged from light (3 to 8%), to moderate (13 to 26%), to severe (38 to 48%) under the 7-day, 14-day, and no-spray regimes, respectively. Norton and Chambourcin primary buds were more cold tolerant (i.e., had lower LT_{50}) than Cabernet Sauvignon in both years. Japanese beetle defoliation of nonsprayed vines significantly reduced cold hardiness of all cultivars in one or both winters. Notably, biweekly cover sprays were as effective as weekly sprays in mitigating the adverse impact of JB defoliation on vine cold hardiness. Defoliation also reduced shoot length of first-year vines, and may be associated with earlier termination of late-season vine growth. The previously undocumented potential for JB injury to reduce winter hardiness of young grapevines exacerbates its impact as a serious vineyard pest.

Key words: defoliation, grapevine, *Popillia japonica*, LT_{50}

Grapevine cold hardiness is often a primary factor influencing cultivar selection in a particular growing region. Cold hardiness is genetically predetermined and varies among the many cultivars in production (Clore et al. 1974, Wolf and Cook 1994, Zabadal et al. 2007). Because of market preferences, however, growers sometimes plant cultivars that are less than optimally adapted to their regional climate. Given the high cost of vineyard establishment and 3 to 4 year delay in economic return, costs for reestablishing a vineyard severely damaged by winter injury could be devastating for growers.

Cold hardiness is not static, and hardiness levels differ among cane and bud tissues within the vine (Howell and Shaulis 1980, Wolpert and Howell 1984, Zabadal et al. 2007). Damage to the less hardy primary dormant bud is most frequently responsible for crop reductions (Clore et al. 1974, Wolf and Cook 1991). Environmental stress and cultural management practices can alter vine cold hardiness. Such factors as site selection (Stergios and Howell 1977), cropping level (Howell et al. 1978), sunlight exposure of shoots during the previous growing season (Stergios and Howell 1977, Howell and Shaulis 1980), dormant pruning date (Wolpert and Howell 1984), and rootstock selection (Miller et al. 1988, Striegler and Howell 1991) may affect cold hardiness of vines within a given cultivar.

Defoliation by insects is another factor that can stress vines and could potentially affect winter hardiness. Simulated or real herbivory can reduce whole-vine photosynthesis, lower carbohydrate reserves, and negatively impact vegetative growth, berry development, and total cluster weights the following season compared to uninjured vines (Mercader and Isaacs 2003a, 2003b, 2004, Bennett et al. 2005). Although complete or 50% defoliation by hand during veraison reduced primary bud hardiness of Concord grapevines (Stergios and Howell 1977, Mansfield and Howell 1981), no previous studies have quantified relationships between differing levels of insect defoliation and winter hardiness of field-grown vines.

In the southeastern and southcentral United States where viticulture is a growing industry, proximity of pasture and other grassy larval habitats often leads to high numbers of Japanese beetles (JB), *Popillia japonica* Newman (Coleoptera: Scarabaeidae), in vineyards (Hammons

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et al. 2008). The beetles, which are strongly attracted by blends of feeding-induced volatiles (Loughrin et al. 1996), skeletonize the foliage from mid-June to mid-August and also feed on ripe berries (Hammons et al. 2008, 2009). Cultivars vary in susceptibility, and unprotected vines of more susceptible cultivars may sustain ≥50% defoliation (Hammons et al. 2010). JB defoliation negatively impacts whole-vine carbon assimilation more severely than does comparable levels of mechanical defoliation (Mercader and Isaacs 2003a). Many growers in Kentucky and elsewhere apply weekly insecticide sprays (6 to 9 applications), typically with carbaryl, for JB management.

This study examined the impact of JB defoliation on midwinter primary bud hardiness of three grape cultivars during the first two years of vineyard establishment. Defoliation levels were manipulated by cover spray frequency, so the data also address to what extent JB management can be reduced without compromising grapevine cold hardiness.

Materials and Methods

Plant material and site. Experiments were conducted using dormant cuttings of three cultivars of grapes selected from a research vineyard established in mid-May 2006 at the University of Kentucky (UK) Horticultural Research Farm in Lexington. The cultivars evaluated were Norton/own rooted (*Vitis aestivalis*), Cabernet Sauvignon #7/C3309 (*V. vinifera*), and French-American hybrid Chambourcin/own rooted (*V. vinifera* × *V. riparia)*. The research site rests at an absolute elevation of 314 m, and the soil type is Maury silt loam (a fine, mixed, semiactive, mesic, typic Paleudalfs). Vines were trained to a 1.8 m high, single high-wire bilateral cordon system with 2.4 × 3.0 m (vine × row) spacing and managed according to UK recommendations (Brown et al. 1997). During the first (2006) and second (2007) growing seasons, vines were defruited and not cropped.

Insecticide treatments. Three treatments were used for two years to provide varying levels of protection from JB defoliation: either carbaryl (Sevin XLR Plus, Bayer, Research Triangle Park, NC) applied every 7 or 14 days during the JB flight period or no insecticide treatment. The insecticide (5 mL product per liter of water) was applied until drip with a backpack sprayer (Solo, Newport News, VA) in early morning, to minimize spray drift. The experimental design was a randomized complete block with eight replications and two vines per experimental unit. Vines treated every 7 or 14 days received seven or three applications, respectively, between 23 June and 4 Aug 2006 and between 21 June and 1 Aug 2007. Standard JB traps and lures (Trécé, Adair, OK) were placed in two locations near vineyards and orchards at the UK farm to monitor JB flight (Hammons et al. 2008).

Defoliation estimates. Extent of JB defoliation (overall leaf area loss from skeletonization) of each vine was visually estimated to the nearest 5% by two independent observers in late July 2006 and 2007, after JB flight had peaked and started to decline. Defoliation estimates (two ratings per vine, two vines per replicate) were averaged to provide a single value per experimental unit.

First-year vine growth and periderm browning. The newly planted vines were trained to two primary shoots in 2006 to establish trunks. Impact of JB defoliation on current season shoot extension and periderm browning of the first-year vines was evaluated 19 Oct 2006, 6 days after subfreezing temperatures occurred. The dominant primary shoot (one per vine, two vines per replication) was evaluated for total length and number of mature nodes per shoot (a measure of termination of late-season growth). A node was considered mature if the entire node, bud, and basipetal buds had turned from green to brown (Edson et al. 1995). Percentages of defoliation and periderm browning were arcsine square root-transformed. Data were compared between spray regimes within cultivar by randomized complete block analysis of variance (ANOVA), with Fisher's LSD for mean separation if the main treatment effect was significant. Statistix 9 (Analytical Software, Tallahassee, FL) was used for all analyses.

Lethal injury (LT_{50}) analysis. Midwinter hardiness of primary buds was determined by subjecting cuttings from dormant canes to a controlled freezing stress (Stergios and Howell 1972) in mid-February of 2007 and 2008. Dormant canes (6–10 mm diam) were randomly selected from pooled samples from all vines of a given cultivar/spray treatment. Selected canes were pruned to three buds and bundled in groups of four cuttings for four replications and nine temperature evaluation points for each spray regime (16 cuttings [48 buds]/spray treatment/temperature). Progressive freeze treatments began at 0°C and ended at -40°C. Temperatures in the chamber were lowered by -5°C progressively for 2 hr ramp time and then held constant at that temperature for 1 hr soak time. Samples from each temperature treatment were removed from the freeze chamber every 3 hr and allowed to thaw for 48 hr before evaluation. Buds were dissected by making lateral cross-section cuts across the dormant bud with a razor blade, exposing the primary bud which was examined for oxidative browning, an indicator of lethal injury (Stergios and Howell 1972). Numbers of live or dead primary buds were recorded. The temperature at which 50% lethal injury (LT_{50}) occurred was estimated by fitting a sigmoidal nonlinear regression model (Logistic 4-P; $Y = a + b/(1 + Exp(c-d*X))$, where Y = % dead buds and X = temperature) to the data from each treatment combination using Statistix 9 software, then computing the predicted value from the regression equation. Estimated LT_{50} values were compared among treatments (spray regimes) within cultivars by one-way ANOVA and Fisher's LSD as described above.

Results

Flight activity of JB in the vineyard peaked in late July in both years (Hammons et al. 2008, 2010). The carbaryl spray regimes provided three distinct levels of JB defoliation within each cultivar in both years (Table 1). Vines that were sprayed weekly sustained <10% defoliation, biweekly treatment resulted in intermediate (13–26%) damage, whereas unprotected vines were 38 to 48% defoliated by late July. All three cultivars were similarly susceptible to JB ($F_{2,14}$ = 0.2, p = 0.8 in 2006; $F_{2,14}$ = 0.6, p = 0.6 in 2007; between-cultivar comparisons for nonsprayed vines). Japanese beetle feeding aggregations were abundant in the vineyard, especially on nonsprayed foliage, and no other insect defoliators were observed damaging the vines.

Nonlinear regression models fitted to the 2007 bud freeze data for nontreated and 7-day sprayed Norton vines (Figure 1) illustrate the sigmoidal response seen for all three cultivars and the decreased cold hardiness associated with JB defoliation of Norton. Between-cultivar comparison of JB-protected (7-day sprayed) vines indicates that primary buds of Norton and Chambourcin were inherently more cold tolerant than those of Cabernet Sauvignon ($F_{2,9}$ =57.4 and 46.1 in 2007 and 2008, respectively; p < 0.001). Buds from nonsprayed, JB-defoliated Norton vines, however, showed significantly reduced cold tolerance (higher LT_{50}) in both the first and second winters after vine planting compared to buds from Norton vines that had been sprayed weekly or biweekly (Table 1). Chambourcin buds from nontreated vines also showed significantly reduced cold hardiness during the first winter after establishment and the same trend, although nonsignificant (p < 0.14), after the second growing season. Buds from nontreated Cabernet Sauvignon showed a trend (p = 0.09) for reduced cold hardiness after their first growing season, and significant reduction in LT_{50} after two summers of JB defoliation (Table 1). Cabernet Sauvignon showed more variable response to the temperature treatments than did the other cultivars, likely contributing to lack of statistical significance in 2007. Notably, the 14-day spray interval provided the same benefit as the weekly sprays as far as mitigating the impact of JB defoliation on midwinter hardiness of primary buds (Table 1).

The first subfreezing temperatures (-0.6°C) occurred on 13 and 15 Oct in 2006. Japanese beetle defoliation during that year significantly reduced primary shoot growth of Norton and Cabernet Sauvignon, and there was a similar trend of impact on Chambourcin (Table 2). However, biweekly cover sprays were as effective as weekly sprays in mitigating adverse impacts of JB defoliation on vine growth. Japanese beetle defoliation was associated with a greater percentage of mature nodes with periderm browning by 19 Oct 2006, on nonsprayed, first-year Norton and Chambourcin (Table 2). Following the second (2007) growing season,

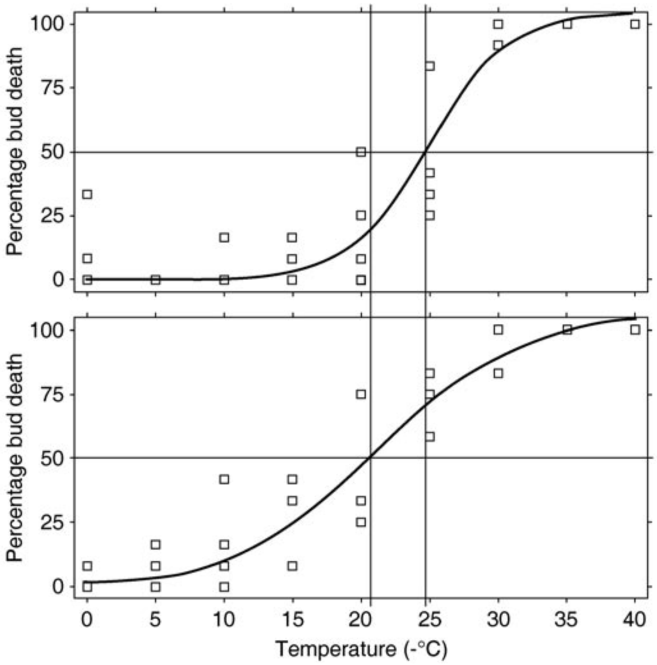

Figure 1 Nonlinear regression models fitted to the 2007 bud freeze data for 7-day sprayed (top) and nontreated (bottom) Norton vines. Note that LT_{50} is ~4° higher for nontreated, Japanese beetle-defoliated vines.

Table 1 Defoliation by Japanese beetle feeding manipulated with frequency of carbaryl sprays and temperature causing death of 50% of sampled buds (LT_{50}) for first-year (2006–2007) and second-year (2007–2008) vines of three grape cultivars.

	Norton		Chambourcin		Cabernet Sauvignon	
Spray[a]	**% Defoliation**[b]	**LT_{50} °C**	**% Defoliation**	**LT_{50} °C**	**% Defoliation**	**LT_{50} °C**
First-year vines						
7 d	8 ± 2 c	-24.7 ± 0.3 a	8 ± 1 c	-24.4 ± 0.3 a	5 ± 1 c	-12.2 ± 1.6
14 d	15 ± 3 b	-24.2 ± 0.3 a	26 ± 3 b	-22.4 ± 0.8 a	18 ± 4 b	-10.9 ± 0.6
NT	44 ± 6 a	-21.2 ± 1.2 b	46 ± 8 a	-18.6 ± 0.6 b	48 ± 5 a	-8.4 ± 0.8
F	24.2	6.3	19.2	22.6	39.0	3.2
p	0.001	0.05	0.001	0.001	0.001	0.09
Second-year vines						
7 d	3 ± 1 c	-25.0 ± 0.3 a	3 ± 1 c	-23.2 ± 0.7	3 ± 1 c	-16.5 ± 0.7 a
14 d	13 ± 2 b	-24.7 ± 0.5 a	18 ± 2 b	-22.7 ± 1.1	13 ± 3 b	-16.4 ± 0.8 a
NT	44 ± 3 a	-23.1 ± 0.5 b	43 ± 6 a	-20.8 ± 0.4	38 ± 7 a	-13.7 ± 0.6 b
F	166	5.3	32.8	2.5	13.4	4.7
p	0.001	0.05	0.001	0.14	0.001	0.05

[a] Vines sprayed at 7- or 14-day intervals. NT: not treated.

[b] Randomized complete block ANOVA (df = 2,14) for % defoliation; one-way ANOVA (df = 2,9) for LT_{50}; means (± SE) within columns not followed by the same letter are significantly different (Fisher's LSD, p < 0.05).

dormant pruning weight of nontreated Norton, Chambourcin, and Cabernet Sauvignon was reduced by 36, 38, and 45%, respectively, compared with treated vines (Hammons et al. 2010).

Discussion

This study is the first, to our knowledge, to quantify the effects of insect defoliation on cold hardiness of field-grown grapevines. Our midwinter LT_{50} measurements for primary dormant buds of vines protected from JB are similar to those previously reported for Norton (Gu 1999), Chambourcin (Gu 1999), and Cabernet Sauvignon (Wolf and Cook 1991). Variation in such factors as regional winter temperature, sampling date and methodology, and sampled plant material likely accounts for much of the variability in reported LT_{50} values within particular grape cultivars (e.g., Stergios and Howell 1977, Howell and Shaulis 1980, Proebsting et al. 1980, Wolpert and Howell 1984, Gu et al. 2001, 2002, Zabadal et al. 2007). Genetic differences also strongly influence winter hardiness, as is the case for Cabernet Sauvignon compared to the more cold-tolerant Norton and Chambourcin (Wolf and Cook 1991, 1994, Gu 1999).

Cold hardiness is also modified by how the vine responds to the winter conditions of a particular growing region (Gu et al. 2001). Winter trunk and root carbohydrate reserves affect the subsequent productivity of grapevines (Bennett et al. 2005). Defoliation of vines by leaf removal can significantly reduce overwintering carbohydrate reserves (Candolfi-Vasconcelos and Koblet 1990, Bennett et al. 2005) as well as the overwintering ability of primary dormant buds (Stergios and Howell 1977, Mansfield and Howell 1981). Both mechanical defoliation and JB injury were shown to reduce whole-vine carbon assimilation of young *Vitis labrusca* (L.) Niagara vines (Mercader and Isaacs 2003a). Leaf abscission associated with severe JB defoliation could also cause premature termination of vine growth and may account for the common trend of reduction in shoot length and increased periderm browning we observed for nonsprayed, first-year vines. This, too, could limit acquisition of overwintering carbohydrate reserves. Carbaryl application may also inhibit periderm formation; however, that hypothesis was not directly tested in our study.

Table 2 Influence of spray regimes resulting in differing degrees of defoliation by Japanese beetles on shoot length, number of mature nodes, and percentage of mature nodes of three cultivars during the first growing season. Parameters evaluated on 19 Oct. First subfreezing night (-0.6°C) was 13 Oct. Mature nodes based on periderm browning (see text).

Treatment[a]	Shoot length (cm)[b]	Mature nodes (n)	Mature nodes (%)[b]
Norton			
7 d	230 ± 24 a	8.3 ± 1.2 a	20 ± 2 a
14 d	201 ± 18 ab	3.8 ± 0.5 b	11 ± 1 b
NT	187 ± 19 b	10.2 ± 1.7 a	29 ± 4 a
F	2.5	6.6	8.3
Cabernet Sauvignon			
7 d	263 ± 22 a	8.1 ± 1.3	15 ± 3
14 d	258 ± 21 a	8.6 ± 2.7	14 ± 3
NT	184 ± 20 b	7.8 ± 2.1	20 ± 6
F	16.7	0.03	0.51
Chambourcin			
7 d	229 ± 24	8.6 ± 1.7	22 ± 4 ab
14 d	220 ± 19	7.2 ± 1.5	16 ± 3 b
NT	199 ± 16	11.4 ± 2.2	31 ± 7 a
F	0.8	1.5	2.6

[a] Vines sprayed at 7- or 14-day intervals. NT: not treated. See Table 1 for corresponding % defoliation.

[b] Randomized complete block ANOVA (df = 2,14). Within cultivars, means not followed by the same letter are significantly different (Fisher's LSD, $p < 0.05$).

Although the differences in LT_{50} of JB-defoliated Norton, Chambourcin, and Cabernet Sauvignon vines amount to only a few degrees, in some cases even a 1 to 2°C reduction in primary bud cold hardiness could lead to a substantial crop loss (Wolf and Cook 1991). Potentially damaging winter temperatures and late spring frost are fairly common in Kentucky (Kurtural and Wilson 2008) and other temperate growing regions (Howell and Shaulis 1980, Wolf and Cook 1991). The lowest field temperatures before sampling of canes in the first (2007) and second (2008) winters were -17°C (05 Feb) and -16°C (20 Jan), respectively. As shown herein, such low temperatures are capable of reducing primary bud survival of Cabernet Sauvignon, especially for vines defoliated by JB in the previous growing season. Susceptibility of Cabernet Sauvignon to winter injury likely accounts for the relatively high variance in LT_{50} it showed in our experiments. Moreover, although LT_{50} is used to define and compare cold hardiness of grape cultivars (Stergios and Howell 1977), greater than 50% survival of primary buds likely is needed to produce an economically sustainable crop load (Gu et al. 2002). Costs for reestablishing a vineyard severely damaged by winter injury could be devastating for growers and, therefore, it is important that they understand the benefits of JB management for increased winter hardiness and for increased vine growth and yield (Hammons et al. 2010). In the southeast, JB feeding on grape berries also facilitates aggregation and feeding by green June beetles, *Cotinis nitida* L. (Hammons et al. 2008, 2009), and probably other secondary pests of fruits.

Our data nevertheless indicate that young grapevines, at least of the cultivars evaluated, can tolerate low to moderate (10–20%) cumulative defoliation from JB feeding without significant loss of midwinter primary bud cold hardiness. Importantly, biweekly cover sprays provided sufficient protection from JB to mitigate its adverse impact on vine cold hardiness. Biweekly cover sprays similarly provided equivalent benefits as weekly sprays as far as mitigating JB impact on vegetative growth and yield of susceptible young vines (Hammons et al. 2010). This information may help growers to reduce insecticide inputs and costs.

There is little published data that quantifies the impact of insect defoliation on winter hardiness of perennial plants.

In one such example, defoliation by caterpillars for two or more consecutive years, followed by extreme cold winter temperatures, was implicated in increased oak mortality in northern Germany (Thomas et al. 2004). We showed here that levels of JB defoliation typical of those that occur in nonsprayed Kentucky vineyards have the potential to significantly reduce winter hardiness of young grapevines.

Conclusion

Japanese beetle is now established throughout most of the eastern United States and, despite regulatory control efforts, is expanding its range in the Great Plains, Great Lakes, and southcentral states. It is a continual threat to become established in California, the Pacific Northwest, and Europe. The potential for severe JB defoliation to reduce winter hardiness of young grapevines exacerbates its impact as a vineyard pest. However, reduced spray regimes that allow some (10–20%) cumulative leaf area loss from JB likely are sufficient to mitigate those adverse effects on vine cold hardiness.

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