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Black rot control and bud cold hardiness of 'Noiret' winegrape

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Abstract

Black rot, caused by *Guignardia bidwellii* (Ellis) Viala and Ravaz, and bud cold hardiness are both management issues in eastern U.S. viticulture. Black rot infections lead to vine stress, resulting in premature defoliation and rotten fruit, potentially compromising cold acclimation of the vine. No studies have targeted bud cold hardiness in relation to severity of prior season black rot infection. Thus, in 2011, 'Noiret', a hybrid winegrape, was subjected to four black rot control treatments: conventional (C), organic 1 (O1), organic 2 (O2), and no spray (N). Leaves and fruit were scored for black rot severity. The O1 and N treatments had the highest level of leaf and fruit disease severity and were not significantly different. The C treatment had the least amount of leaf and fruit disease severity and the O2 treatment was intermediate and significantly different from the O1, N, and C treatments. Bud samples were taken in January, February, and March 2012 and exposed to subzero temperatures (-21 °C, -23 °C, -26 °C, -29 °C) in an ethylene glycolbath to assess if prior season black rot infection impacted primary bud hardiness. In January and March nearly all buds were still alive at -21°C and -23°C, but -29°C caused more damage. Black rot control treatments were not a statistically significant factor in the bud hardiness experiment. This could be due to black rot severity being below a critical threshold for impact or the vines had enough time to recover in late summer and fall to reach full mid-winter hardiness.

Key words: Disease control, disease severity, *Guignardia bidwellii*, interspecific hybrid, organic, vine stress, *Vitis* spp.

Introduction

Black rot, caused by *Guignardia bidwellii* (Ellis) Viala and Ravaz, requires constant disease management during the summer and it can lead to vine stress (Louime *et al.*, 2010), leaf drop, and rotten fruit, potentially compromising overall vine ability to prepare for cold temperatures. 'Noiret', an interspecific hybrid wine grape (*Vitis* spp. L.) cultivar released in 2006 (Reisch *et al.*, 2006) has the potential to be important in the Oklahoma wine industry. It has been tested extensively in New York, Indiana, and other states (Reisch *et al.*, 2006), where it was reported to be susceptible to black rot in Indiana and slightly susceptible in New York.

Previous studies have not examined bud cold hardiness in relation to severity of prior season black rot infection. Monitoring bud cold hardiness in the winter is a concern for grape growers in the eastern U.S. Bud cold hardiness experiments using differential thermal analysis showed that the LTE_{50} mid-winter primary bud cold hardiness for 'Noiret' in New York was -25.7 °C (Pool *et al.*, 1990). Mid-winter cold hardiness is stable in most locations if temperatures remain consistently low (Ferguson *et al.*, 2011) however, Oklahoma often has extreme temperature fluctuations during the vine dormancy period, thus the variation in bud cold hardiness could change in response to the fluctuations in ambient temperatures (Hubackova, 1996; Ferguson *et al.*, 2011).

Grape cultivars differ in their response to fluctuating mid-winter temperatures (Mills *et al.*, 2006; Ferguson *et al.*, 2011). Cultivars may also respond differently when grown in different locations (Howell, 2000). A majority of the vine response is genetically controlled and can be due to the grape species involved in the cultivar's parentage (Howell, 2000; Londo and Johnson, 2014). *Vitis* species respond differently during dormancy to ambient temperatures (Jiang and Howell, 2002; Fennell, 2004; Londo and Johnson, 2014) and possibly other factors that impact cold hardiness levels. Studies to assess mid-winter primary bud hardiness have largely been conducted in more northern areas, where cold temperatures are more consistent (Pool *et al.*, 1990; Hubackova, 1996; Jiang and Howell, 2002; Rekika *et al.*, 2005; Ferguson *et al.*, 2011). Poor vine management that leads to vine stress can also reduce vine cold hardiness (Howell, 2000), including injury from diseases which can interact with other vine stressors to reduce cold hardiness (Zabadal *et al.*, 2007).

This study was designed to test the efficacy of organic and conventional black rot control in relation to bud cold hardiness. The following hypotheses were tested: higher levels of black rot would result in elevated primary bud mortality due to enhanced vine stress and fungicide treatments would reduce vine stress and provide increased protection against primary bud mortality when subjected to freezing events.

Materials and methods

The trial was conducted at the Cimarron Valley Research Station located in Perkins, OK, USA. The vineyard was planted in 2008 on Konawa loamy fine sand with Teller fine sandy loam intrusions. 'Noiret', an interspecific hybrid cultivar that has some susceptibility to black rot was chosen for this study (Reisch *et al.*, 2006). The vines were not grafted to a rootstock. The experimental design was a randomized complete block with four replicates. Single vine plots were separated by at least one non-treated vine. Plants were spaced 2.4 m apart in-row with a between-row spacing of 3.7 m. Recommended maintenance

Table 1. Black rot control treatments applied to 'Noiret' grape within an 8-spray program in 2011[a].

Spray regimen and rate[b]	Times applied	Application sequence
Conventional		
Mancozeb (DithaneRainshield® 75DF 0.29 L· ha^{-1}) + Quinoxyfen (Quintec® 2.08SC 0.29 L· ha^{-1})	1	1
Tebuconazole (Elite® 45 WP 0.29 L· ha^{-1})	3	2, 5, 8
Trifloxystrobin (Flint® 50 WG 0.14 L· ha^{-1})	2	3, 6
Mycobutanil (Nova® 40 WP 0.36 L· ha^{-1})	2	4, 7
Organic 1		
Basic Copper Sulfate (Cuprofix Ultra® 40 DF 0.22 L· ha^{-1})	1	1
Basic Copper Sulfate (Cuprofix Ultra® 40 DF 0.22 L· ha^{-1}) + *Bacillus subtilis* (Serenade Max® 14.6 WP 3.36 kg· ha^{-1})	4	2, 4, 6, 8
Basic Copper Sulfate (Cuprofix Ultra® 40 DF 0.22 L· ha^{-1}) + *Bacillus pumilus* (Sonata® ASO 9.35 L· ha^{-1})	3	3, 5, 7
Organic 2		
Basic Copper Sulfate (Cuprofix Ultra® 40 DF 0.22 L· ha^{-1})	1	1
Basic Copper Sulfate (Cuprofix Ultra® 40 DF 0.22 L· ha^{-1}) + *Bacillus subtilis* (Serenade® ASO 1.34F 14.03 L· ha^{-1})	4	2, 4, 6, 8
Basic Copper Sulfate (Cuprofix Ultra® 40 DF 0.22 L· ha^{-1}) + *Bacillus pumilus* (Sonata® ASO 9.35 L· ha^{-1})	3	3, 5, 7
No Spray	0	0

[a]Spray regimen previously described in Smith *et al*. (2012). [b]Trade names are used only for illustrative purposes and it is not implied as an endorsement of these products to the exclusion of other suitable products by Mississippi State University, Oklahoma State University, or the University of Wisconsin.

practices were followed throughout the growing season (Stafne, 2010). Four black rot control treatments (conventional (C), organic 1 (O1), organic 2 (O2), and no spray (N)) were imposed on the vines (Table 1). Fungicides were applied with a CO_2-pressurized wheel barrow sprayer with a vertical boom equipped with three TX8015 flat fan nozzles. The system was calibrated to deliver 935 L ha^{-1}. Fungicide applications were first made on 13 Apr. 2011 and continued on 14-day intervals until veraison for a total of eight sprays (Table 1). Ratings of leaf severity (average percent of leaf area with symptoms of black rot) and fruit severity (average percent of cluster area with symptoms of black rot) were taken on 22 June 2011 based on the methods described by Nutter *et al.* (2006).

Five individual bud samples, replicated four times for each month and treatment temperature, were taken at approximately 3-week intervals beginning 19 January and ending 1 March 2012 and exposed to sub-zero temperatures (-21°C, -23 °C, -26 °C, and -29 °C) in a temperature-controlled ethylene glycol ($C_6H_6O_2$) bath (Thermo-Fisher Scientific, ULT-80, Newington, NH, USA) to assess if previous season black rot infection had an impact on primary bud hardiness (Rekika *et al.*, 2005). Due to sampling error, the data for February was not included in the analysis. Buds were collected and separated by month and vine from which they were collected. A single bud from each cutting was placed in a labeled plastic bag with four bud bags per numbered vine per month. The bags were labeled 1 through 4 for each vine corresponding to the temperatures at which they were removed from the bath. After the bags were filled, they were separated by the number indicated on the bag. All treatments were placed in a jar covered with parafilm to help seal the lids. A temperature sensor (Watchdog model A110, Spectrum Technologies, Aurora, IL, USA) was double bagged and placed in a plastic container with four weights. The container was placed in the bottom center of the bath to record the bath temperature.The initial temperature was held at 1°C for 48 h, and then the temperature decreased 5 °C each hour until -21°C was reached. The temperature was then held for 24 h, after which it dropped 1°C each 30 min to the next programmed test temperature. Each time a set temperature (-21 °C, -23 °C, -26 °C, and -29 °C) and incubation period (24 h) was reached, the appropriate jar of buds was removed and replaced with an empty jar containing five weights to maintain fluid levels in the bath. After a jar was removed, it sat at room temperature for 3-6 h before buds were examined under a microscope.

All disease and bud data were arcsine-transformed for statistical analysis. Results presented are based on back-transformed means. Data were analyzed by analysis of variance ($P \leq 0.05$) using JMP 9.0 (SAS Institute, Cary, NC, USA). Transformed treatment means were compared by Tukey's HSD ($P \leq 0.05$).

Results and discussion

Black rot control treatments: Significant differences in leaf and fruit disease severity were identified with the highest level of disease recorded in the N plots (Table 2). The O1 and N treatments had the highest level of leaf and fruit lesion severity and were not significantly different (Table 2). The C treatment had the least amount of leaf and fruit lesion severity and the O2 treatment was intermediate and significantly different from the O1, N, and C treatments. Thus, the conventional spray regimen was better than the organic spray in the control of black rot. There were differences between organic spray treatments, showing that a change in product formulation (wettable powder (WP) vs. aqueous solution (AS)) can reduce spray efficacy. The O1 treatment was no different than the N treatment, suggesting that it did not provide a reduction in black rot disease severity.

Table 2. Leaf and fruit severity ratings for black rot infection on 'Noiret' grape during the 2011 growing season in Perkins, Oklahoma

Treatment	Leaf severity rating[a] (%)	Fruit severity rating[a](%)
Organic 1	17.5 a[b]	26.3 a
No spray	16.3 a	23.8 a
Organic 2	7.5 b	11.3 b
Conventional	1.7 c	0.0 c

[a]Ratings of leaf and fruit severity are the average percent of leaf area and cluster area with symptoms of black rot, respectively as described by Nutter *et al*. (2006). Data presented are from back-transformed means. [b]Means separated by the same letter within a column are not significantly different based on Tukey's HSD ($P \leq 0.05$).

Primary bud cold hardiness: Primary bud response to cold temperatures did differ among temperatures within months. At -21°C and -23°C for buds taken in January and March nearly all were still alive (Table 3). Buds exposed to colder temperatures of -26°C and -29°C had greater mortality, especially for January (Table 3). As in many eastern U.S. states, the continental climate prevalent in Oklahoma has a strong impact on vine growth and development, especially fluctuating winter and spring temperatures (Stafne, 2007). For January and March, weather was dry and unseasonably warm with an average temperature of 5°C

14 days prior to sampling and no rainfall. Average temperature 14 days before the March sampling was 9 °C with only 2.8 mm of rain.There were no significant interactions of black rot control treatment and temperature for the bud hardiness experiment. Black rot control treatment main effect was also not significant for January (P = 0.0518) and March (P = 0.2994). Temperature was found to be the dominant factor that led to bud mortality in this study. Hubackova (1996) stated that hardiness of primary buds in grapevine were dependent upon the maximum and mean temperatures to which they were exposed prior to imposed controlled freeze testing; however, even though unseasonably warm temperatures prevailed during this experiment, the primary bud hardiness of 'Noiret' was found to be slightly different than reported by others (Pool *et al.*, 1990; Reisch *et al.*, 2006). This may be attributable to the difference in methods used to determine bud hardiness or to the different environmental conditions under which the vines were grown. Drought conditions during the winter may have led to a decrease in tissue water content (Jiang and Howell, 2002) and a subsequent increase in cold hardiness, but other studies have not found consistent results in establishing a relationship between water deficit and primary bud hardiness in grapevines (Basinger and Hellman, 2006).

Table 3. 'Noiret' grape primary bud cold hardiness at four subzero temperatures taken in January and March 2012

Treatment temperature (°C)	Alive primary buds, January (%)	Alive primary buds, March (%)
-21	100.0 a[a]	100.0 a
-23	100.0 a	100.0 a
-26	70.7 b	85.3 a
-29	61.3 b	70.7 b

[a]Means separated by the same letter within a column are not significantly different based on Tukey's HSD ($P \leq 0.05$).

Interaction effects of black rot and temperature on cold hardiness: There was no statistically significant interaction of black rot control treatment and temperature in relation to bud hardiness (P=0.37 and P=0.98 for January and March, respectively). This could be due to black rot severity being below a critical threshold for impact or the vines had enough time to recover in late summer and fall to reach full mid-winter hardiness. This may be due to 'Noiret' having a moderate susceptibility to black rot (Reisch *et al.*, 2006). In our trials in 2011, low to moderate levels of black rot leaf severity were observed (26% severity in the Nvines). Zabadal *et al.* (2007) stated that black rot may not be as serious a factor in potential cold hardiness reduction as late season fungi like powdery mildew (*Uncinula necator* (Schw.) Burr.) and downy mildew (*Plasmopara viticola*), so the vines may have had time to recover sufficiently in this study to avoid detrimental impacts. Under conditions that favor greater black rot disease pressure that lead to more leaf and fruit disease severity, the results may have been different. So, even though in this study black rot had no effect on primary bud cold hardiness, further studies that incorporate disease as a factor should be conducted.

Black rot control was not a statistically significant factor in the bud hardiness experiment. This could be due to the low to moderate levels of observed black rot severity being below a critical threshold for impact on 'Noiret' or the infections were no longer impacting vines later in the summer and they had enough time to recover to reach full mid-winter hardiness. Based on these data, primary bud mortality never reached 50% for 'Noiret' when subjected to subzero temperatures as low as -29 °C which was slightly hardier than was reported when the cultivar was first released (Reisch *et al.*, 2006). Buds began to show a significant loss in cold hardiness between -23 and -26 °C. More work can be done in this area to assess potential negative impacts on bud cold hardiness as it relates to disease infection in the prior season. Differences were observed between the two organic spray treatment suggesting that product formulation can impact efficacy. We determined that low to moderate levels of black rot infection did not affect 'Noiret' primary bud cold hardiness in the following winter, rather temperature was the driving factor in bud mortality.

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