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Delaying bud break in 'Edelweiss' grapevines to avoid spring frost injury by NAA and vegetable oil applications

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Abstract: Delaying bud break is an approach to avoid spring frost damage. Field experiments were conducted during the winters of 2009 and 2010 at James Arthur Vineyards in Raymond, Nebraska to study the effect of spraying naphthaleneacetic acid (NAA) and Amigo Oil to delay bud break in 'Edelweiss' grapevines to avoid such damage. In 2009, the experiment consisted of five treatments: NAA (500, 750, and 1000 mg/l), oil applied at 10%, and a non-sprayed control. There were four application dates: 6 January, 3 February, 3 March, and 1 April. In 2010, treatments were NAA at 500, 1000, and 1500 mg/l, 10% oil, and the control; application dates were 28 January, 25 February, and 25 March. Bud break was evaluated throughout spring. During harvest, number and weight of clusters were recorded. Berry samples were analyzed for pH, °Brix, and titratable acidity (TA). Pruning weights and number of clusters of the 2009 treated vines were recorded in March and August 2010, respectively. In the 2009 field experiment, oil and NAA at 1000 mg/l significantly delayed bud break by two to six days, compared to the control. Pruning weights were not significantly affected by the treatments. In 2010, oil applications significantly delayed bud break by eight to 12 days compared to the control and no significant differences were found between NAA at 1500 and 1000 mg/l. In both years, treatments had no significant effects on yields, cluster weights, berry weights, °Brix, pH or TA.

1. Introduction

Grapes are considered one of the world's major fruit crops. Grapes in the Midwest states of the USA are greatly influenced by frost injury. Particularly in Nebraska, spring frost is one of the major limitations to grape production. In 2007, grapevines were severely damaged because extraordinarily warm temperatures at the end of March were followed by extremely cold temperatures during the first week of April. Losses in affected areas in the Midwest states due to that particular freeze event were estimated to exceed one billion dollars (Guinan, 2007).

Heaters, wind machines, and sprinkler irrigation have been employed to minimize frost impact. These methods help reduce frost injury but are very costly. Due to their expense many grape growers do not utilize them, hoping that frost injury will affect only the primary bud and that secondary buds will recover growth after primary bud damage. Protecting the primary bud is essential because, for example in 'Concord', they produce 300 to 400% more fruit with 135 to 190% larger clusters compared to secondary buds (Wiggans, 1926). Some grape cultivars are not productive on secondary buds, as is the case for 'Edelweiss' (Smiley *et al*., 2008).

A different approach is to delay bud break until the period of frost risk passes, thus reducing frost injury damage. Growers have used many methods to delay bud break. Late or delayed pruning was shown to delay bud break and bloom date (Loomis, 1939). Call and Seeley (1989) reported a five day delay in bud break by using dormant oils on peach trees. Dami and Beam (2004) obtained a 20-day delay compared to the control after applying Amigo Oil on 'Chancellor' grapevines. Nigond (1960) applied NAA in the 500 to 1000 ppm range on 'Aramon' grapevines on various dates from October until March and noticed retarded bud break by 16 to 27 days. Many American hybrids such as 'Edelweiss' are known to exhibit cold hardiness. Nevertheless, these hybrids can remain under frost threat until frost period passes.

The objectives of the present study were to compare NAA and vegetable "Amigo Oil" applications on 'Edelweiss' vines to determine the best treatment for delaying bud break and to determine the effect of delaying bud

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break on fruit yield and characteristics such as juice pH, °Brix, and titratable acidity (TA).

2. Materials and Methods

Field experiment 2009

The research was conducted during the 2009 winter season at James Arthur Vineyards located in Raymond, Nebraska within Lancaster County. Treatments were applied on 12-year-old vines. The vines are trained according to the Geneva Double Curtain trellis system. Planting distances are 2.44 m between plants and 3.66 m between rows. Rows are oriented north to south. The experiment consisted of five treatments: NAA (500, 750, and 1000 mg/l) purchased from Phyto Technology Laboratories (Shawnee Mision, KS), Amigo Oil (Loveland Industries, Greely, CO) applied at 10% v/v which consisted of 9.3% oil and 0.7% emulsifier, and the control which was not sprayed. A randomized complete block design was used with three blocks of 20 vines each. There were four application dates: 6 January, 3 February, 3 March, and 1 April 2009. Most canes were pruned to leave the five proximal buds before applying treatments. The remaining distal portion of the canes was removed by the normal pruning practices employed at James Arthur Vineyards. Thus, the canes studied in the first year all had the same number of buds (i.e. five) and the total number of buds on vines were approximately the same. The whole vine was sprayed using a hand sprayer with each vine receiving approximately 0.33 l. After spraying, two canes per vine, each having five buds, were randomly selected and labeled. In the spring of 2009, vines were visually evaluated for bud break. Bud break was determined as stage five of the Eichhorn and Lorenz (1977) scale of grapevine development, where bud scales have expanded to the point at which a green shoot is visible. Bud break was evaluated day by day throughout the spring until each cane reached 60% bud break (three buds opened out of the five left after pruning). The number of Julian Days starting from 1 January 2009 until achieving 60% bud break was used as the basis of calculating number of days for bud break.

Harvest date was determined by taking random samples of berries and measuring their °Brix with a refractometer. On 14 August 2009 the number and weight of clusters from the two selected canes were recorded. From the total clusters, 50 berries were randomly counted, placed in a plastic storage bag, and placed at 0°C until berry sample analysis could be conducted. On 14 September 2009 berry samples were analyzed for pH, °Brix, and titratable acidity (TA). The 50 berries/vine were weighed, allowed to thaw to reach room temperature, wrapped in cheese cloth, and crushed manually using a mortar and pestle. The extracted juice was poured into test tubes to conduct the analyses. Juice pH was measured with a Pope pH/ion meter model 1501. Soluble solids (°Brix) content was measured using an Atago PR-101 digital refractometer purchased from Nova-Tech International, Inc. (Houston, TX). TA was determined by titration with NaOH, using the procedure of Dharmadhikari and Wilker (2001). On 18, 22, and 25 March 2010 pruning weights were recorded using an upright balance scale. In order to obtain data regarding cumulative effects of the treatments on fruiting the following year, the total number of clusters/vine were counted on 10 August 2010. Temperatures for Raymond, Nebraska throughout 2009 were obtained from the High Plains Regional Climate Center, University of Nebraska, Lincoln. All statistical analyses were performed using SAS/STAT Version 9.2 and Analysis of Variance was conducted by the PROC GLIMMIX procedure.

Field experiment 2010

Treatments were applied on 13-year-old vines. These vines were not the ones sprayed in 2009. The experiment consisted of five treatments: NAA (500, 1000, and 1500 mg/l), Amigo Oil applied at 10% v/v, and the control which was not sprayed. A randomized complete block design was used with three blocks of 15 vines each. There were three application dates: 28 January, 25 February, and 25 March 2010. The entire unpruned vines were sprayed and each vine received approximately 1l of treatment solution. The treated vines were then pruned on 30 March 2010. The total number of buds/vine was recorded to determine when 50% of the total buds showed bud break and not 60% as in 2009 because of the differences in treatment procedures. In the spring of 2010, vines were visually evaluated for bud break, which was determined as previously mentioned. The total number of buds/vine was counted and bud break was evaluated day by day throughout the spring until each vine reached 50% bud break of the total number of buds that were recorded in March. The number of Julian Days starting from 1 January 2010 until 50% bud break was achieved was used as the basis to calculate the number of days for bud break. On 11 August 2010 the number of clusters and weight of clusters/vine were recorded. From the clusters, 30 berries were randomly counted, placed in a plastic storage bag, and placed in the freezer until berry sample analysis could be conducted. On 18 August 2010 berry samples were analyzed for pH, °Brix, and titratable acidity (TA) using the same procedures as described for the 2009 field experiment. Similarly to 2009, temperatures for Raymond, Nebraska throughout 2010 were obtained from the High Plains Regional Climate Center, University of Nebraska, Lincoln. All statistical analyses were performed using SAS/STAT Version 9.2 and Analysis of Variance was conducted by the PROC GLIMMIX procedure.

3. Results and Discussion

Field experiment 2009

As revealed from the analysis of variance, there was a significant treatment by month interaction for bud break and pH at ($P \leq 0.05$) (Table 1). Similar interactions were found by Dami and Beam (2004) in 'Chancellor' and 'Chambourcin' but not in 'Chardonel'. Due to interaction effects, month effects within treatments and treatment effects within the month are presented in Table 2.

Delaying pruning until March had a significant effect in delaying bud break by two days compared to vines pruned in February (Table 2). While a difference between the pruning carried out in February and March was detected, that difference does not exist when considering pruning in January and April. Therefore, the treated vines in 2010 study were pruned on the same date (30 March 2010) to avoid pruning effects. The idea of pruning, then applying treatments was to maintain apical dominance since pruning can signal vine bud growth, increase the effectiveness of spraying since 'Edelweiss' exhibits a vigorous growth, and reduce chemical applications since the canes will eventually be headed back to a certain number of buds in the pruning season. From the results of NAA applications, it seems that auxin applications failed to maintain apical dominance and inhibit lateral bud growth because grapevines exhibit such a strong dominance (Friend *et al.*, 2001). No significant differences were found among NAA 500, 750, and 1000 ppm between the months (Table 2). Regarding oil, no significant differences were found within months except in March (Table 2). This was due to the improper mixing of the oil with water because the oil was mistakenly frozen on the day of spraying.

With regard to the effects of treatments within months, it appeared that desirable results were achieved by oil and NAA at 1000 ppm treatments in all months (Table 2). Except in March, oil applications significantly delayed bud break by five days compared to the control (Table 2). Oil and NAA at 1000 ppm were only significantly different in January and April (Table 2). Overall, there were no significant differences between the control, NAA at 500, and 750 ppm.

Table 1 reveals neither a treatment by month interaction nor a treatment effect, but it does show a month effect at ($P \leq 0.05$) regarding the number of clusters in 2009. The largest average number of clusters per cane was found in April-treated vines but they were not significantly different from averages for vines treated in March and January (Table 3). The average number of clusters was lowest in February-treated vines and significantly different from the other three treatment dates (Table 3). Most importantly, no treatment effect was detected. The number of clusters per shoot ranged from 5.5 to 8.2 (data not shown).

Five buds were retained after pruning. Usually, each bud can produce one to two clusters (personal observation), hence ten clusters would have been an optimum production in this case. Nevertheless, the above averages were acceptable to James Arthur Vineyards and the average difference between April- and February- treated vines, although statistically different, is only 1.5 clusters (Table 3). This difference could be explained by cluster characteristics of 'Edelweiss' which are known to be very loose and many clusters can simply fall down on the ground by any

Table 3 - Least significant difference test (LSD) for average number of clusters per cane of 12-year-old 'Edelweiss' grapevines

Pruning time	Average number of clusters per cane
April	7.3 a
March	7.2 a
January	7.0 a
February	5.8 b

Different letters in a column indicate significant differences at $P \leq 0.05$ according to Fisher's protected LSD.

Table 1 - Analysis of variance table for the experiment conducted in 2009

Source of variance	DF	Bud break	No. of clusters produced in summer 2009	CW	BW	°Brix	pH	TA	PW	No. of clusters produced in summer 2010
Treatment	4	< 0.0001	0.49	0.18	0.68	0.60	0.29	0.16	0.88	0.98
Month	3	0.60	0.02	0.32	0.44	0.51	0.38	0.63	0.53	0.43
Interaction	12	0.02	0.63	0.68	0.47	0.63	0.0039	0.38	0.34	0.47

CW=Cluster weight, BW=Berry weight, TA=Titratable acidity, PW=Pruning weight.

Table 2 - Pruning time and spray treatment effects on average days to show 60% bud break in 12-year-old 'Edelweiss' grapevines

Pruning time	Spray treatments				
	Control	NAA 500 ppm	NAA 750 ppm	NAA 1000 ppm	Oil
January	125.0 ab (c)	126.0 a (c)	126.5 a (bc)	128.0 a (b)	130.0 a (a)
February	124.0 b (c)	124.5 a (bc)	126.0 a (b)	129.0 a (a)	129.0 a (a)
March	126.0 a (b)	126.0 a (b)	127.0 a (ab)	128.3 a (a)	126.5 b (ab)
April	125.5 ab (c)	125.0 a (c)	126.0 a (bc)	127.7 a (b)	129.7 a (a)

Different letters in a column indicate significant differences at $P \leq 0.05$ according to Fisher's Protected LSD. The lower case letters in parenthesis are related to treatment effects within months.

means of physical contact (Swenson *et al*., 1980; Brooks and Olmo, 1997; Smiley *et al*., 2008).

No significant interaction, month, or treatment effect was found in average weights of ‘Edelweiss’ clusters at ($P\leq$ 0.05) (Table 1). Cluster weights of the two selected canes ranged from 1.33 to 2.22 kg (data not shown). This supports the lack of differences found regarding the average number of clusters per cane since cluster weights were recorded as averages of the two canes selected and the differences found in average number of clusters per cane is attributed to the looseness characteristic of ‘Edelweiss’ clusters.

Also, no significant interaction, month, or treatment effect was found in weights of 50 samples of ‘Edelweiss’ berries ($P\leq$ 0.05) (Table 1). Once again, this will supports neglecting the differences detected in the average number of clusters per cane. The 50 berry sample weights ranged from 116.0 to 125.2 g (data not shown).

A treatment by month interaction was only found in pH analysis at ($P\leq$0.05) (Table 1). Due to interaction effects in pH analysis, month effects within treatments and treatment effects within the month are presented in Table 4.

NAA at 750 and 1000 ppm showed no differences in pH values for all four pruning times (Table 4). Meanwhile, oil treatment gave a significantly lower pH for April pruning, as did the control in January, although the latter was not significantly different from February’s result (Table 4). More obvious differences were observed with NAA at 500 ppm for the four pruning times (Table 4).

Regarding treatment effects within months, no significant differences were found among treatments within March (Table 4). The control was significantly different from all other treatments in January (Table 4) and the oil treatment was significantly different from the control and NAA at 750 ppm in April. From the results in Table 4 and Table 1, it seems that differences in pH values are not due to the delay in bud break but to environmental conditions. In this regard, Creasy and Creasy (2009) mentioned that berry characteristics are totally dependent on environmental conditions, especially the microclimate (climate within canopy). This was also confirmed by Huck (2009). In her study, training systems influenced sunlight penetration, canopy structure and thus fruit composition of ‘Frontenac’ was totally dependent on climate within the canopy. The vines showed vigorous vegetative growth in 2010 with a low number of clusters. Weaver (1976) noted that calyptras may not fall in cold rainy weather and this will reduce the amount of fruit set.

The °Brix ranged from 12.3 to 13.2, pH values were 3.14 to 3.27, and TA values were 0.83 to 1.13 g/100 ml (data not shown). Regarding harvest parameters, Dharmadhikari and Wilker (2001) mentioned that optimum ranges for white wine would be 21-22%, 3.2-3.4, and 0.7-0.9 g/100 ml for the total soluble solids, pH, and the TA, respectively. ‘Edelweiss’ is purposely harvested at an earlier stage regarding °Brix and then chaptalized. Swenson *et al*. (1980) mentioned that ‘Edelweiss’ juice is relatively low in acidity (0.6-0.8%) and has moderate soluble solids (14-16%), and recommended that for wine making it should be picked at an early mature stage (14°Brix).

Harvest parameter results of the 2009 study were in the recommended ranges, except for soluble solids. Lower °Brix values than the preferable ranges (14-16%) of the samples harvested in 2009 were due to the cooler July temperatures (Fig. 1). Higher temperatures during that month would have been preferable for the vines to produce more photosynthates and accumulate more sugar. Weaver (1976) and Winkler *et al*. (1974) mentioned that an optimum temperature for photosynthesis ranges from 25 to 30°C. In addition, lower nighttime temperatures would have been preferable to reduce respiration rates and breakdown the accumulated sugars. Winkler *et al*. (1974) mentioned that 4.4°C halves the respiration rate and is an advantage to suppress fungal disease.

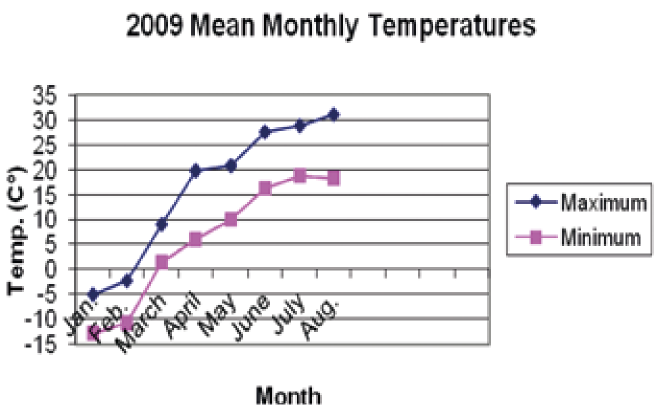

Fig. 1 - The maximum and minimum monthly average temperatures for Raymond, Nebraska in 2009. Source: High Plains Regional Climate Center.

Table 4 - Pruning time and spray treatment effects within month on pH of fruit harvested in 2009 from 12-year-old ‘Edelweiss’ grapevines treated with NAA and “Amigo Oil”

Pruning Time	Spray Treatments				
	Control	NAA 500 ppm	NAA 750 ppm	NAA 1000 ppm	Oil
January	3.14 b (b)	3.26 ab (a)	3.26 a (a)	3.22 a (a)	3.26 a (a)
February	3.21 ab (ab)	3.27 a (a)	3.23 a (ab)	3.17 a (b)	3.25 a (a)
March	3.23 a (a)	3.18 c (a)	3.20 a (a)	3.21 a (a)	3.22 a (a)
April	3.25 a (a)	3.20 bc (ab)	3.24 a (a)	3.20 a (ab)	3.14 b (b)

Different letters in a column indicate significant differences at $P\leq$ 0.05 according to Fisher’s Protected LSD. The lower case letters in parenthesis are related to treatment effects within months.

The treatments had no effect on pruning weights taken in winter 2010 as shown in Table 1 at ($P \leq 0.05$). This shows that NAA and oil applications had no negative effect on vegetative growth during spring, summer, and fall seasons after bud break.

Pruning weights ranged from 1.05 to 1.43 kg (data not shown). Cultural practices such as mounding that tend to increase cold hardiness can result in higher pruning weights (Gu, 2003). In his study, mounding protected 'Gewürztraminer' vines from the cold winter and significantly increased pruning weights. Although cold hardiness was not measured in this study, the experimental applications had no negative effects on grapevine vegetative growth and such delays in bud break should be of no concern.

Regarding the concerns of grape growers on cumulative effects, especially with applications of plant growth regulators, the number of clusters per vine ranged from 12 to 16 (data not shown). Analysis of variance in Table 1 showed that there were no such effects regarding the number of clusters produced in the following harvest year 2010 ($P \leq 0.05$).

Field experiment 2010

Unlike the study in 2009, there was no significant treatment by month interaction, but a significant pruning time and spray treatment effect was present ($P \leq 0.05$) (Table 5).

The oil treatment significantly delayed bud break by eight days compared to the control. Furthermore, it significantly delayed bud break by nearly four and five days compared to NAA 1500 ppm and 1000 ppm, respectively (Table 6).

Delaying bud break up to 12 days can encourage grape growers to use oil as an effective method to delay bud break and avoid spring frost injury. "The probability of freezing temperatures occurring decreases as spring progresses. Therefore, cultural methods that delay the onset of bud break will decrease the risk of frost damage" (Friend *et al.*, 2001). Furthermore, delaying pruning until March was very effective in improving results compared to the study in 2009 regarding delaying bud break. It is suggested that grape growers should delay pruning as much as possible and if this strategy is to be adopted to reduce frost risk, pruning should be done late in the pruning season especially for cultivars that exhibit early bud break. Although there was no month effect on bud break, overall March applications delayed by one to two days more than February and January (data not shown).

Table 5 shows no effects on the total number of clusters per vine in 2010 (it was cluster per cane in 2009) ($P \leq$ 0.05). The total number of clusters per vine ranged from 12 to 19 (data not shown), which is similar to the range found in 2009. In addition, similar analysis of variance results were obtained compared to the 2009 study regarding treatment by month interaction and treatment effects. The only difference was a month effect present in the 2009 study which was not present in 2010. Totally different weather conditions prevailed in 2009 compared to 2010 (Fig. 2).

According to the High Plains Regional Climate Center, normal precipitation for Raymond, Nebraska is 121 mm and 95 mm for May and June, respectively. In 2010, monthly precipitation in June was 249 mm, almost three times the monthly average. This had a negative impact on 'Edelweiss' fruit set. In fact, 'Edelweiss' yields harvested at James Arthur Vineyards were 8 tons/acre in 2009 and

Table 6 - Least Significant Difference Test (LSD) for average number days of bud break for 13-year-old 'Edelweiss' grapevines in 2010

Treatment	Average number days to bud break
Oil	122 a
NAA 1500 ppm	118 b
NAA 1000 ppm	117 b
NAA 500 ppm	115 c
Control	114 c

Different letters in a column indicate significant differences at $P \leq 0.05$ according to Fisher's Protected LSD.

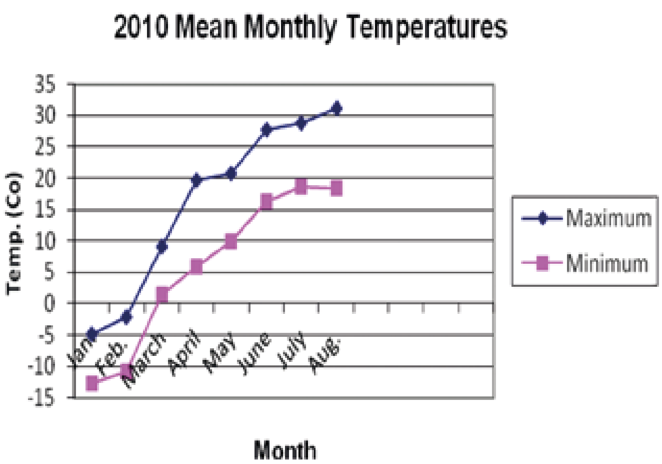

Fig. 2 - The maximum and minimum monthly average temperatures for Raymond, Nebraska in 2010. Source: High Plains Regional Climate Center.

Table 5 - Analysis of variance table for the experiment conducted in 2010

Source of variance	DF	Bud break	No. of clusters produced in summer 2010	CW	BW	°Brix	pH	TA
Treatment	4	< 0.0001	0.90	0.91	0.76	0.89	0.81	0.64
Month	2	0.26	0.26	0.28	0.34	0.84	0.03	0.07
Interaction	8	0.20	0.94	0.92	0.10	0.81	0.23	0.82

CW=Cluster weight, BW=Berry weight, TA=Titratable acidity, PW=Pruning weight.

only 3 tons/acre in 2010.

No significant effects ($P \leq 0.05$) were found in 'Edelweiss' cluster weights (Table 5), which ranged from 2.26 to 3.65 kg (data not shown).

Analysis of variance for berry weights showed a similar trend in 2010 as in 2009. No significant effects were present at $P \leq 0.05$ (Table 5). Berry sample weights ranged from 97.94 to 104.45 g (data not shown).

Similar trends were present in 2010 °Brix and TA results with no significant effects at $P \leq 0.05$. Regarding pH, unlike the results of 2009 where a treatment by month interaction was present, the 2010 study showed a month effect at ($P \leq 0.05$). °Brix ranged from 12.7 to 13.5, pH values were 3.26 to 3.41, and TA values were 1.1 to 1.4 g/100 ml (data not shown). °Brix ranges were higher in 2010, which was expected since average July temperatures were 27.2°C and 28.8°C in 2009 and 2010, respectively. Regarding pH and TA, the 2010 results for the former are in the same range as recommended (3.2-3.4) by Dharmadhikari and Wilker (2001), but TA ranges were a little higher than the recommendation of 0.7-0.9%.

The analysis of variance table for pH results showed a significant month but not a treatment effect at ($P \leq 0.05$). Absence of a treatment effect on berry characteristics is important for recommendation purposes. However, only slight differences were found between months (Table 7).

4. Conclusions

Based on the 2010 results, delaying pruning until March, especially for cultivars that show early bud break such as 'Edelweiss', will delay bud break. Amigo Oil did not exhibit the 20-day delay reported by Dami and Beam (2004) in French-American hybrids and NAA did not exhibit the 16- to 27-day delay that was obtained in the study with cut stems taken from 'Aramon' (*Vitis vinifera*) vines (Nigond, 1960). Amigo Oil gave better performance compared to NAA in both years, even at higher NAA concentrations. It delayed bud break slightly longer (four to five days) and did not affect either the quantity or quality of fruit produced. NAA at 1000 and 1500 ppm showed a potential bud break delay similar to that of Amigo Oil. Oil applications and NAA at 1000 to 1500 ppm in March and up to early April could provide grape growers with an acceptable delay of bud break. This is based on the performance of both oil and NAA applications in the field experiments. Delaying bud break shows no negative impact on berry characteristics.

Table 7 - Least Significant Difference Test (LSD) for pH of berry samples in 2010

Month	Average pH
February	3.37 a
January	3.34 ab
March	3.29 b

Different letters in a column indicate significant differences at $P \leq 0.05$ according to Fisher's Protected LSD.

As a result of this research, it can be recommended to use Amigo Oil at 10% or NAA at 1000 to 1500 ppm from March to April for sites that are prone to frost events such as in southeastern Nebraska and on cultivars that show early bud break such as 'Edelweiss'. Any resulting delay in bud break will decrease the possibility of frost injury. Furthermore, this study opens the door to future studies regarding the value of repeated spraying (Qrunfleh, 2010) or mixing Amigo Oil with NAA. Furthermore, investigation of any phytotoxicity damage to buds caused by oil applications that could possibly occur under vineyard conditions is warranted.

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