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Delaying Bud Break in 'Edelweiss' Grapevines to Avoid Spring Frost Injury by NAA and Vegetable Oil Applications

Issam M. Qrunfleh
University of Nebraska - Lincoln

DELAYING BUD BREAK IN 'EDELWEISS' GRAPEVINES TO AVOID SPRING FROST INJURY BY NAA AND VEGETABLE OIL APPLICATIONS

by

Issam M. Qrunfleh

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DELAYING BUD BREAK IN 'EDELWEISS' GRAPEVINES TO AVOID SPRING FROST INJURY BY NAA AND VEGETABLE OIL APPLICATIONS

Issam M. Qrunfleh, Ph.D.

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Advisor: Paul E. Read

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 NAA and Amigo Oil on delaying 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 the non-sprayed control. There were four application dates: January 6, February 3, March 3, and April 1. Bud break was evaluated throughout spring. During harvest, the number of clusters and weights 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 2010, NAA concentrations were 500, 1000, and 1500 mg/l, 10% oil, and the control. Application dates were: January 28, February 25, and March 25. Similarly to 2009, bud break was evaluated throughout spring, number of clusters and weights per vine were recorded, and berry samples were analyzed for the same parameters mentioned as in 2009.

A forcing solution experiment was conducted on 'Edelweiss' canes collected on the same dates as the field experiments. For each date, 20 canes were headed back to the first five buds, then cut into five single-bud cuttings and the bases immersed in forcing solution. The same treatments as used in the field experiments were applied by adding one drop on each bud. Days to bud break and shoot length one week after bud break were recorded.

In the 2009 field experiment, oil and NAA at 1000 mg/l significantly delayed bud break 2-6 days compared to the control. In 2010, oil applications significantly delayed bud break 8-12 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, and TA. The forcing solution experiment showed a month, position, and treatment interaction regarding bud break delay in both years. No treatment effects were found regarding shoot length.

This Dissertation is Dedicated to My Beloved Father and Mother for Loving me, Supporting me, and Teaching me the Value of Education

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Introduction:

Grapes are considered one of the world's major fruit crops. Fennell (2004) reported that the total area planted with grapes was 7.5 million hectares producing 60.7 million tons of fruit. The Food and Agriculture Organization (FAO) of the United Nations estimated that world production of grapes was 67.7 million tons in 2008. According to the statistics of United States Department of Agriculture (USDA), total grape production in the United States during 2009 was 7.04 million tons. Furthermore, the National Association of American Wineries publications indicate that according to USDA and National Agricultural Statistics Service (NASS), grapes are the highest value fruit crop in the nation and the sixth largest crop overall. In addition to the healthy products, grape production is a vital contributor to the United States economy and stimulates the economy by exporting produce, generating jobs, and many vineyards are listed as attractive places to be seen in many tourist visitor guides. Grape growing started in Nebraska in the late 19th Century (Read et al., 2004) and now boasts over 500 acres of grapes and over 23 wineries (Nebraska Winery and Grape Growers Association, 2008). In 1994, the first winery to open in Nebraska was Cuthills Vineyards in Pierce, Nebraska.

Grapes in the Midwest states are greatly influenced by frost injury. Particularly in Nebraska, spring frost is one of the major limitations to grape production. In 2007, severe damage of grapevines occurred because of extraordinarily warm temperatures at the end of March followed by extremely cold temperatures during the first week of April. The loss of affected areas in Midwest states due to that particular freeze event was estimated to exceed one billion dollars (Guinan, 2007). Frost injury causes significant losses by damaging vines and reducing yields. Furthermore, replacing a dead vine is another

indirect loss cost that some grape growers have to face. Moreover, the susceptibility of injured vines to crown gall disease increases (Zabadal et al., 2007).

Establishing a vineyard starts with proper site selection. This is the critical first step in reducing frost injury incidence. Unfortunately, ideal sites are hard to locate and many vineyards are established on sites that are considered not preferable. Attempts to protect grape vines from cold temperature injury began at least 2000 years ago when Roman growers scattered burning piles of canes that had been pruned during winter, dead vines and other waste to heat their vineyards when spring frost events occurred (Evans, 2000). Additionally in modern times, heaters, wind machines, and sprinkler irrigation have also been employed for minimizing frost impact. These methods help reduce frost injury but are very costly. Since these methods are expensive, many grape growers do not utilize them, hoping that frost injury will affect only the primary bud, and secondary buds will recover growth after primary bud damage. Protecting the primary bud is essential as they produce 300 to 400% more fruit with clusters 135 to 190% larger than are produced by secondary buds (Wiggans, 1926). Some grape cultivars are not productive on secondary buds such as 'Edelweiss' (Smiley et al., 2008).

A different approach is to delay bud break until the frost risk period passes and reduce frost injury damage. Growers have used many methods to delay bud break. Early in the 20th Century, late or delayed pruning was shown to delay bud break and bloom date (Loomis, 1939). Call and Seely (1989) reported a five day delay in bud break by using dormant oils on peach trees. Low-cost methods of delaying bud break consist of applying chemicals, such as growth regulators and dormant oils (Dami and Beam, 2004). They obtained a 20 day delay compared to the control after applying Amigo Oil on

'Chancellor' grapevines. They recommended testing oil applications on various grape cultivars since responses could be different due to phytotoxity problems when spraying the buds with oil. Nigond (1960) applied NAA in the range of 500 to 1000 ppm on 'Aramon' grapevines on various dates from October up to March and noticed retarded bud break by 16 to 27 days. He mentioned that NAA application is very promising to delay bud break and thus prevents damage from spring frosts, but exact schedules of dates and concentrations must be worked out for different vines under various different conditions.

Being able to delay bud break 2 to3 weeks is important to grape growers because spring frost losses can reach up to 90%, especially for early cultivars such as 'Edelweiss'. Thus, finding a chemical that is easy to apply, non-toxic to grapes as well as the environment, requires minimal labor or energy, and is effective in delaying bud break was the primary goal of this study. The objectives of this study were to:

1) Compare NAA and vegetable oil "Amigo Oil" (Loveland Industries, Greely, CO) applications on 'Edelweiss' vines to determine the best treatment in delaying bud break.
2) Determine the effect of delaying bud break on fruit yield and characteristics such as juice pH, °Brix, and titratable acidity (TA).
3) Study the effect of NAA and Amigo Oil on 'Edelweiss' single-bud cuttings on delaying bud break by use of forcing solution technology (Read et al., 1984).

References:

Call, R. and S. Seeley. 1989. Flower bud coatings of spray oils delay dehardening and bloom in peach trees. HortScience 24 (6): 914-915.

Dami, I. and B. Beam. 2004. Response of grapevines to soybean oil application. American Journal of Enology & Viticulture 55 (3): 269-275.

Evans, R. 2000. The art of protecting grapevines from low temperature injury. In Proceedings of the ASEV 50th Anniversary Annual Meeting. J. M. Rantz (Editor). American Society for Enology and Viticulture: 60-72.

Fennell, A. 2004. Freezing tolerance and injury in grapevines. Journal of Crop Improvement 10: 201-235.

Guinan, P. 2007. Understanding and preventing freeze damage in vineyards. Workshop Proceedings. University of Missouri Extension: 7-12.

Loomis, N. 1939. Note on grape foliation as affected by time of pruning. Proceedings of the American Society for Horticultural Science 37: 653-654.

Nigond, J. 1960. Delaying bud break in vines by the use of α-naphthaleneacetic acid and defense against frost. Compt. Rend. Acad. Agr. France 46: 452-457.

Read, P., A. Economou and C. Fellman. 1984. Manipulating stock plants for improved in vitro mass propagation. Proc. Int. Symp. Plant Tissue and Cell Culture: Application to Crop Improvement. Novak, J., L. Havel, and J. Dolezel (Editors). Czech. Acad. Sci. Prague: 467-473.

Read, P., S. Gu, S. Gamet and J. Schild. 2004. Testing of varieties and selections under challenging climatic conditions. Ó. A. de Sequeira and J. C. Sequeira (Editors). Acta Hort. Proc. 1st International Symposium on Grapevine: 65-71.

Smiley, L., P. Domoto, G. Nonnecke, and W. Miller. 2008. A review of cold climate grape cultivars. Iowa State University.

Wiggans, C. 1926. A study of the relative value of fruiting shoots arising from primary and secondary buds of the 'Concord' grape. Proceedings of the American Society for Horticultural Science 23: 293-296.

Zabadal, T., I. Dami, M. Goffinet, T. Martinson, and M. Chien. 2007. Winter injury to grapevines and methods of protection. Michigan State University Extension Bulletin E2930: 23-34.

Review of Literature:

Grapes:

Grapevines culture began in the Trans-Caucasus region where the classical wine grape *Vitis vinifera* originated (Read and Gu, 2003). Commercial grapes belong to the family *Vitaceae* and the genus *Vitis*. The genus *Vitis* includes more than 70 species (Alleweldt and Possingham, 1988). Some species of the genus *Vitis* that are found in Nebraska include: *V. aestivalis* Michx., *V. cinerea* (Engelm)., *V. riparia* Michx., and *V. vulpina* L. (Kaul et al., 2006).

'Edelweiss':

'Edelweiss' originated in Osceola, Wisconsin and was developed from crosses that started in 1949 (Swenson et al., 1980). The pedigree of 'Edelweiss' is 'MN 78' X 'Ontario' (Smiley et al., 2008). 'Edelweiss' was introduced by the University of Minnesota in 1980. It was introduced as a table grape with the goal of improving table grape quality in cold winter regions but then became an important cultivar for white wine especially when grown in Nebraska. Swenson et al. (1980) mentioned that it does show considerable cold hardiness in south-central Minnesota. On the other hand, Brooks and Olmo (1997) reported that it is considered very cold hardy.

The vine is considered very vigorous and productive (Swenson et al., 1980). In Nebraska, this cultivar is usually trained using the Geneva Double Curtain (GDC) trellis system. Clusters have a conical shape (Brooks and Olmo, 1997), are medium in size, very loose to moderately compact and often double-shouldered (Swenson et al., 1980). Berries are round, medium sized and green skinned with a white bloom (Swenson et al., 1980). Berries are also of a slip skin, tender flesh and have the *labrusca* fruit flavor

(Brooks and Olmo, 1997). Bud break is early, making it vulnerable to spring frosts and it is not productive on secondary buds (Smiley et al., 2008). The juice is relatively low in acidity (0.6-0.8%) and has moderate soluble solids (14-16%) (Swenson et al., 1980). It is also known to be an early maturing cultivar and Nebraska grape growers usually harvest 'Edelweiss' in August at 14-15 °Brix.

Figure 1: 'Edelweiss' cluster at James Arthur Vineyards in Raymond, Nebraska in 2009 one week before harvesting.

The Grape Bud:

Understanding the structure and physiology of the bud is vital for vineyard management. This section briefly describes the grape bud, the main vine organ focus of this study. Hellman (2003) defined the bud as "a growing point that develops in the leaf axil" and mentioned that two buds are associated with a grape leaf: the lateral bud and the dormant (latent bud). The true axillary bud of the leaf foliage is the lateral bud (Hellman

2003). The axillary's basal bud is referred to the dormant bud, winter bud, or latent bud (Lavee and May, 1997). These are formed in the bract axil of the lateral bud and they develop in the summer season. These are the major concern and emphasis during the pruning season. The dormant bud is sometimes called an eye. It is a compound bud that usually consists of three growing points: primary, secondary, and tertiary. Normally, the primary buds grow and the secondary and tertiary buds serve as a "backup system" in case the primary bud has been damaged because of frost or freeze (Hellman, 2003). Winkler et al. (1974) mentioned that in some cases such as severe pruning, destruction of part of the vine, or boron deficiency, two or all three of the buds could burst into growth and develop shoots. Dormant buds are protected by bud scales that are impregnated with suberin and contain hairs (Winkler et al., 1974). Drawings of the developmental stages of the dormant bud were illustrated by Eichhorn and Lorenz (1977) and more detailed stages by Meier (2001) (see Appendices 1 and 2). It is well documented that primary buds produce more and larger clusters than are produced by secondary buds (Wiggans, 1926).

Frost vs. Freeze:

An understanding of these two events and how they occur is essential in order to provide protection for the grapevines. Both the terms "frost" and "freeze" have been used interchangeably. In the Glossary of Meteorology, Rieger (1989) it is reported that frost is a synonym for hoarfrost, the formation of ice crystals on surfaces. If ice crystals are not formed the event is termed "Black" frost. On the other hand, Perry (1998) described them as two distinct phenomena radiation frost and advective or windborne freeze. A radiation frost occurs when the skies are clear and the wind is calm (‹5 mph). It includes two types: hoar (white) and black frosts. Ice formation depends on the dew point (frost point).

Meanwhile, advective freeze occurs when a cold air mass moves into an area carrying freezing temperatures. Clouds could be present and wind speed is >5mph. Evans (2000) used the same terms and mentioned that there are basically two dominant types of frost situations: radiant frosts and advective freezes.

According to Evans (2000), radiation frost is the easiest type of frost to protect against and it emphasizes the main reason why site selection is so important. Meanwhile, not much can be done during advective conditions. This could be explained by inversion, temperature increase with height during night. The inversion layer is the warm air over the cold air and this layer is very stable with little vertical motion or mixing (Trought et al., 1999). Radiation frost allows inversion to develop and no inversion exists during advective freeze events.

In the last 20 years, the majority of frost events occurred during the months of April and May (see Appendix 3). This period is very critical for early bud break cultivars such as 'Edelweiss' that often shows bud break in that critical period.

Protecting Grapevines from Frosts:

It has been said that "wine is made in the vineyard"; similarly frost protection starts with site selection. Trought et al. (1999) emphasized that: "when developing a vineyard, three factors should be taken into consideration: Location, Location, Location". Sites of poor air drainage have lower air temperatures compared with well air drained sites (Stergios and Howell, 1977). Site selection is the first critical decision in establishing a vineyard. There are many factors that are related in selecting a site: climate, topography, slope, soil physical and chemical characteristics, and other biological factors such as weeds, insects, and diseases. A grape grower can easily obtain

such information from textbooks and many extension publications. Ideal sites are difficult to locate and the process will involve some compromising. Some features such as growing season should never be compromised and others such as soil characteristics can be accepted as less than ideal (Wolf and Boyer, 2003).

Researchers have tested frost protection by aqueous foam (Choi et al., 1999) and hydrophobic particle film and an acrylic polymer that is capable of forming an elastic coating on the leaves (Fuller et al., 2003). The researchers considered them promising in protecting grapevines from frost, yet large-scale field applications have yet to be developed. Grape growers use wind machines, various kinds of heaters, and irrigation to protect grapevines from frost injury. Similarly, information can be obtained from various sources regarding their uses and mechanisms of protection. Vineyards of large areas even use helicopters which are characterized as the most effective method (Paul Read, personal communication). Helicopters take advantage of the inversion layer that develops over the vineyard by mixing layers and thus reduce frost injury (Creasy and Creasy, 2009). It is recommended for areas where frost is not a regular concern because of the cost of operation. Evans (2000) referred to all previous methods as active frost protection strategies and estimated the cost per hectare of wind machines, covers, and some types of heaters and irrigation methods that are used commercially. Estimated costs range from $1000- $10,000 depending on vineyard acreage and method to be used. For grape growers, less expensive methods are more preferable. Gu (2003) reported that mulching could also be an effective method for protecting grapevines from cold winters. He concluded that mounding protected ‘Gewürztraminer’ vines from the cold winter and significantly increased pruning weights.

Cold Hardiness:

Dami (2007) defined cold hardiness as "the ability of dormant grapevine tissues to survive freezing temperature stress during autumn and winter". The ability to survive is accomplished by two mechanisms described by Levitt (1980) as freeze avoidance and freeze tolerance. Cane and trunk tissues during the dormant season tolerate ice outside the living cells. Meanwhile, buds avoid freezing by supercooling; which is defined as "the ability of the contents of a cell to remain liquid at subfreezing temperatures" (Dami, 2007).

Cold hardiness is measured by the term "lethal temperature 50" which is referred to as the LT_{50}, the single temperature value that kills 50% of the primary bud population in midwinter (Dami, 2007 and Gu, 1999). Furthermore, two important methods are used to measure cold hardiness: oxidative browning and thermal analysis (Dami, 2007). Other methods are described in Zabadal et al., (2007). Since cold hardiness plays an important role in grape production regions, measuring cold hardiness has become an important technique to evaluate performance of grape cultivars especially because acclimation and deaaclimation of cold hardiness are unknown for most of the non-*vinifera* cultivars (Gu et al., 2001). 'Norton' was found to be the hardiest when compared with 'Vignoles' and 'St. Vincent' (Gu et al., 2001). 'Riesling' was the hardiest among 'Chardonnay', 'Pinot Gris', and 'Viognier' (Mills et al., 2006). Meanwhile for red cultivars, 'Cabernet Sauvignon' was the hardiest among 'Merlot', 'Malbec', and 'Syrah' (Mills et al., 2006).

The three stages of cold hardiness are: acclimation, mid-winter hardiness, and deacclimation. Acclimation is the transfer from non hardy to a cold hardy state. Responses to short days and low temperatures cause the transition. Fennell and Hoover

(1991) determined that native American species begin to acclimate in response to short days. Meanwhile, V. *vinifera* grapevines acclimate in response to both short days and low temperatures (Fennell, 2004). Mid-winter hardiness occurs in midwinter and losing hardiness in the spring is referred to as deacclimation.

Cold hardiness is associated with changes in proteins, enzymes and carbohydrate changes. Among the previous three, carbohydrate changes received the most attention (Howell, 2000). An association between cold hardiness and endogenous sugar content was found by Hamman et al. (1996). Glucose, fructose, raffinose, and stachyose increased from the onset of cold acclimation and decreased during deacclimation in 'Chardonnay' and 'Riesling' grapevines (Hamman et al., 1996).

Cold hardiness is described to be dynamic and complex. It depends on three factors: genotype, environment, and vine culture and management (Howell, 2000). In spite of being cold hardy, a grapevine could still be injured by frost especially when the deacclimation occurs quickly in response to warm temperatures that could occur in early spring.

A preferable characteristic of a cultivar would be to acclimate quickly in fall and slowly deacclimate in spring. Gu et al., (2002) found that greater cold hardiness of non-*vinifera* cultivars is due to the ability to acclimate faster and deeper at low temperatures. In order to achieve this feature, and since the third factor (vine culture and management) is what grape growers can have a direct impact on, grafting onto cold-tolerant rootstocks is a feasible approach. Miller et al., (1988 a) found that canes and buds on rootstock 'C-3309' were the most cold hardy. Cane and bud acclimation were faster in fall and deacclimation in spring was slower compared to '5BB' and 'SO4' rootstocks. Moreover,

grafted 'White Riesling' was significantly hardier than own-rooted vines (Miller et al., 1988b). The different rootstocks studied had a differential influence on cold hardiness observed by measuring LT_{50} values. They concluded that '3309 C' was the most cold hardy and therefore the most desirable for winter survival. Gu (2003) reported that 'Gewürztraminer' scions on '3309 Couderc' and 'MG 420A' rootstocks were the most cold hardy and that the rootstocks had no significant effects on scion vegetative growth. On the other hand, he found that scions on mounded '110 Richter', 'St. George', and '*Riparia* Gloire' rootstocks showed earlier bud break than the non-mounded rootstocks.

Methods to Delay Bud Break:

Some of the methods that have been used to delay bud break include: delayed pruning, using various types of cryoprotective treatments (Dami et al., 1997), alginate and dormant oils (Dami et al., 2000; Dami and Beam 2004), and the use of plant growth regulators (Weaver et al., 1961).

1. Delayed Pruning:

Late or delayed pruning has been shown to delay bud break and bloom date (Loomis, 1939). Another advantage is a more uniform bud break and this was achieved in 'Perlette' and 'Thompson Seedless' from January pruning dates compared with November and December dates (Hatch and Ruiz, 1987). It is well known among grape growers that early pruning can accelerate bud break. Grape growers usually start the pruning season by pruning cultivars that show late bud break and end the season by pruning early bud break cultivars such as 'Edelweiss'. Evans (2000) mentioned that a general recommendation for grapes grown in a spring frost prone area is to delay pruning as late as possible and to prune lightly.

Friend et al., (2001) reported greater frost tolerance could result by delaying winter pruning until after bud break of apical buds which delays the onset of basal bud development. Friend and Trought (2007) delayed pruning from July (usual winter pruning time in New Zealand) up to October (when apical shoots on canes were ~ 5 cm long) which resulted in yield increases over three consecutive seasons. Late pruning increased the proportion of large seeded berries while the number of smaller seeded berries on clusters was reduced. Delayed pruning also resulted in lower levels of sugar accumulation and higher titratable acidity.

2. Dormant Oil Applications:

Attempts to delay bloom were first reported in the late sixties and early seventies. Call and Seeley (1989) delayed bud break five days using dormant oil on 'Johnson Elberta' peaches. Phytoxicity damage occurred at concentrations of 20%. Deyton et al. (1992) applied dormant oil on 'Biscoe' peaches and measured the internal CO_2 bud concentration. They concluded that the internal CO_2 concentration was higher compared to the control. In addition, repeated applications of lower concentrations of dormant oils had less phytotoxic effects on the buds compared to single applications of higher concentrations. Myers et al., (1996) applied soybean oil on 'Georgia Belle' peach trees. They reported that applications of the oil increased internal CO_2 concentrations and delayed bud break by six days when using 10% oil.

Dami and Beam (2004) treated 'Chancellor' (an early cultivar), 'Chambourcin' (late cultivar), and 'Chardonel' (mid-season cultivar) grapevines with two soybean oil-based adjuvants (Prime and Amigo Oil). They found that Prime Oil was phytotoxic to dormant buds. Regarding bud break, both treatments led to a significant bud break delay

in all three cultivars ranging from 1 to 20 days as compared to the control. Prime Oil reduced yield, whereas Amigo Oil did not affect the yield or the berry composition. Dami and Beam (2004) suggested that cultivars that are late in bud break require a later application compared to cultivars with an early bud break. They concluded that grape growers may consider oil applications as a method of frost protection by delaying bud break.

Dami (2007) reported that a study was conducted in Virginia and continued in Illinois and Ohio regarding the use of several oil types (mineral-based oils such as JMS stylet oil and soybean-based oils, including crude soybean oil, and oils with adjuvants, such as Amigo, Prime Oil, and Soydex) on several grape cultivars: 'Cabernet Franc', 'Cabernet Sauvignon', 'Chambourcin', 'Chancellor', 'Chardonel', 'Chardonnay', 'Concord', 'Lemberger', 'Pinot Gris', 'Norton', 'Seyval', and 'Vignoles'. Oil rates above 10% v/v of all oils were phytotoxic to most cultivars. Stylet Oil was even phytotoxic for 'Cabernet Franc' using 2.5% v/v and in general was considered more phytotoxic than soybean oils. The oils did not affect mid-winter bud cold hardiness, but the treated buds deacclimated at a slower rate compared to untreated. Bud break was delayed between 2 and 19 days. Higher rates of oil caused a reduction in yield. Dormant oils did not affect fruit maturation or uneven ripening. In addition, berry composition was not affected except when bud break was delayed more than 19 days.

3. Plant Growth Regulators:

A distinction should be made between plant growth hormones and plant growth regulators. A plant hormone is an organic compound, produced in a plant organ site in low concentrations, and transported from the site of synthesis to another location where it

will affect growth and development. Plant growth regulators "include plant hormones-natural and synthetic-but also, other nonnutrient chemicals not found naturally in plants but that, when applied to plants, influence their growth and development" (McMahon et al., 2007). Thus, it does not include sugars or vitamins (Preece and Read, 2005).

With the idea of breaking dormancy of dormant buds in fall, Weaver (1959) applied gibberellin at 0, 10, 50, and 250 ppm on 'Zinfandel' vines in September while foliage was still green. He reported that the number of shoots decreased with the increase of gibberellin concentration and this indicated that dormancy was prolonged by gibberellin. In another experiment, basal cuttings of 'Tokay' were treated with gibberellin at 0, 0.01, 0.1, 1, 10, and 100 ppm. He reported that the higher the concentration of gibberellin the longer it took for buds to develop.

Lavee and May (1997) mentioned that applications of exogenous gibberellic acid during the previous growing season will delay and inhibit bud opening in the following growing season. It seems that, prolonging the rest period by gibberellin application is achieved by fall applications (Weaver et al., 1961). One of the limitations of using gibberellin is its cost compared with other plant regulators (Erez, 1987).

Nigond (1960) sprayed 'Aramon' vines with NAA at 500 to 1000 ppm in October, January, February, and March. He reported that no effect in delaying bud break was achieved with the October application. On the other hand, spraying the vines early in January, in the third week of February, and the second week of March delayed bud break by 16-27 days. Applications caused some reduction of the percentage of buds that broke, but there was no effect on the growth or health of the plant. Auxins are known for inhibiting lateral bud growth (Saure, 1985) because of apical dominance.

Apical dominance has been extensively studied for a long period of time and auxin was thought to control lateral bud growth by a classical hypothesis (inhibiting cytokinin action). Auxin transport hypothesis; regulation is exerted by auxin movement and bud transition hypothesis; buds enter different developmental stages that vary in sensitivity to auxin and other signals were also used to explain auxin's involvement (Dun et al., 2006). With the advances in molecular biology techniques, shoot-multiplication signal (SMS) was reported by Beveridge (2006). Now a new model of apical dominance suggests that auxin's control is by root SMS regulation; if the source of auxin is removed acropetal SMS transport declines and lateral bud growth occurs (Malladi and Burns, 2007). In addition to its role in apical dominance, NAA was used to inhibit sprouting in muscadine grapes when used in conjunction with white latex paint (Takeda et al., 1982).

Effects of GA_3, ethephon, B-9 (Alar), CCC (Cycocel) at various concentrations on bud burst of 'Chaush' grape cuttings in February were investigated by Eris and Celik (1981). They reported that GA_3 (50 ppm), ethephon (200, 400, or 800 ppm), and B-9 (500 and 1000) markedly delayed bud burst. Ethephon at 800 ppm was the most effective concentration and delayed bud break 19 days. On the other hand, cycocel hastened bud growth significantly. All treatments had no effect on bud break percentage, but cuttings treated with GA_3 did not show normal bud growth and dried after bud break.

Paclobutrazol's effect on grapevine vegetative growth, cold hardiness, yield, and quality were studied by Ahmedullah et al. (1986). They reported that paclobutrazol strongly inhibited vegetative growth by reducing the shoot length. Paclobutrazol had no phytotoxic effect on buds and bud break was delayed 3 to 5 days. The chemical showed no effect on cold hardiness, yield, quality, and pruning weights.

Patterson and Howell (1995) studied the effect of September and October applications of GA and NAA on 'Concord' (*Vitis labrusca* L.) and only GA September applications on 'Riesling' (*Vitis vinifera* L.) grapevines. They reported 250 ppm rate of GA in October and 400 ppm rate of NAA in September delayed 'Concord' bud break. Compared to the control, GA rates were effective in delaying 'Riesling' bud break.

In a recent study, a formulation of abscisic acid known as VBC-30025 (Valent BioSciences Corporation, Libertyville, IL) was tested by Hellman et al. (2006) on dormant 'Sangiovese' cuttings, container-grown 'Sangiovese' and 'Cabernet Sauvignon' vines, and field grown 'Sangiovese' vines. They reported that soil application of the formulation was more effective than bud spraying of the cuttings. Bud break of cuttings was delayed about four days whereas soil application delayed bud break seven days.

Dormancy:

In general, deciduous fruit trees cease their growth in late fall, drop their leaves, enter a dormant phase in winter, and resume growth in spring. Westwood (1993) considered the synchronization between plant and the environment very important for the survival of the plant. Dormancy has been extensively studied. This plant phenomenon has been a major interest for many physiologists. Dormancy of grapevine buds has been reviewed by Lavee and May (1997). Arora et al., (2003) reviewed the induction and release of bud dormancy in woody perennials in general and regulation of tillering by apical dominance in grasses has been reviewed by Murphy and Briske (1992). Though much is known, there is still much to learn. This review mostly emphasizes grape bud dormancy.

Terminology and Definitions:

Dormancy has been described by many terms and definitions. Some of the early terms used include: rest, true dormancy, deep dormancy, winter dormancy, primary dormancy, and endogenous dormancy. These terms were used to describe when growth is inhibited by internal factors (Samish, 1954) even if favorable environmental conditions are present. The terms: quiescence, imposed dormancy, relative dormancy, exogenous dormancy, and environmental dormancy are used when growth is delayed because of unfavorable environmental conditions (external conditions). All of the previous terms and many other terms and definitions were collected by Lang et al., (1987).

Examples of early definitions include: "rest period of plants" (Howard, 1910), "temporary suspension of visible growth" (Samish, 1954), "no visible growth" (Romberger, 1963), "partial or growth dormancy" and "temporary cessation of growth" (Vegis, 1964) to distinguish between bud and organ dormancy, respectively. Based on the previous definitions and others, Lang et al., (1987) formulated the definition: "dormancy is a temporary suspension of visible growth of any plant structure containing a meristem" and proposed the three terms: endo-, para-, and ecodormancy. Endodormancy is "regulated by physiological factors inside the affected structure". Chilling response is an example of this type of dormancy. Paradormancy is "regulated by physiological factors outside the affected structure" such as apical dominance. Ecodormancy is "regulated by environmental factors" such as temperature extremes, nutrient deficiency and water stress. It seems that dormancy in woody perennials involves an interconnected series of phenomena regulated by internal and external factors (Lang, 1994).

Following the proposed nomenclature, Junttila (1988) made some comments on the definition that widens the meaning of dormancy from the past definitions. In addition, Junttila (1988) did not agree with the proposed terms because in many cases dormancy could be a combination of all three types. Lavee and May (1997) considered that dormancy of grapevine buds passes from one phase to the next phase in a diffuse way it would be unwise to use the previous terminology and instead they used pre-dormancy, dormancy, and post-dormancy terms to refer to Lang's para-, endo-, and ecodormancy terms, respectively. Okubo (2000) argued that the term dormancy should only be applied to endodormancy using his "one bud-one growth cycle theory" because the forced cessation for eco- and paradormancy are caused by the environment and physiological factors outside the affected structure, respectively. Although the nomenclature has been criticized and questioned by many researchers, many have accepted the use of the proposed terms and definitions. Meanwhile, others prefer to use their own terms and definitions.

Bud Dormancy Induction:

Arora et al., (2003) considered not separating bud dormancy induction from other processes such as cold hardiness is a fundamental reason for the gaps present in bud dormancy induction research. Arora et al., (2003) considered that shorter photoperiods and colder temperatures are the two environmental factors that induce shifting from paradormancy to endodormancy and simultaneously initiate cold acclimation. Seeley (1994) stated that endodormancy induction in mature trees begins in middle to late summer. Both *Vitis labruscana* and *Vitis riparia* showed onset of bud dormancy in response to shorter photoperiods, but little cold acclimation was found in *V. labruscana*

in response to short photoperiods (Fennell and Hoover, 1991). By developing controlled environmental treatments, Salzman et al., (1996) concluded that expression of the 47 kD glycoprotein is related to endodormancy but not cold acclimation, while the 27 kD protein appears to be more related to cold acclimation. It seems that grapevine buds can enter endodormancy without cold acclimation when responding to short photoperiods (Arora et al., 2003).

Tanino (2004) stated there is no doubt that plant hormones are involved in bud dormancy induction. In the linear hormonal hypothesis, dormancy induction and termination occur because of changes in the balance between inhibiting and stimulating endogenous substances. This has been a major hypothesis in the topic of dormancy and has received the most attention of physiologists (Arora et al., 2003). For example, the dormancy of 'Merlot' buds was associated with an increase of cis-ABA content and the break period was associated with a decrease (Koussa et al., 1994). Gu (2003) detected ABA, GA_4 and GA_1 but no GA_3 in dormant grapevines. He reported correlations between ABA content and bud dormancy but no correlations between gibberellins and ABA.

Changes During Dormancy:

After dormancy induction, metabolic changes occur at the biochemical and molecular levels. Lavee and May (1997) stated that moisture content drops from 80% to 50% when buds enter dormancy. Bounding and elimination of free water must occur so that water can freeze harmlessly in tissue intercellular spaces (Zabadal et al., 2007). Bound versus free water was used as a theory to explain endodormancy (Faust et al., 1991). Fennell et al., (1996) preferred to use bound versus free water as an indicator of

endodormancy and mentioned free water movement is restricted with the onset of dormancy.

Winkler and Willams (1945) reviewed the changes in starch and soluble sugars in *Vitis vinifera*. According to their findings, sugar content in above ground sections start to increase and grapevine starch starts to decrease when grapevines enter endodormancy. Jones et al., (1999) mentioned that the increase of soluble sugar content is correlated to the increased level of cold hardiness.

Changes in membrane lipids occurring during dormancy were found in apple buds (Wang and Faust, 1990) and in blackberry buds (Izadyar and Wang, 1999). These findings and others led Erez (2000) to suggest a new bud dormancy control mechanism that involves activation of two membrane-bound enzymes: oleate desaturase which is activated by low temperatures and linoleate desaturase which is activated by high temperatures. In spite of the suggested mechanism, Dennis (1994) mentioned that the role of temperature in dormancy induction is not well defined.

Some research shows that thiols are involved in dormancy. Fuchigami and Nee (1987) speculated the involvement of glutathione (GSH) in dormancy and breaking dormancy. They proposed a degree growth stage and rest breaking models based upon the GSH:GSSG (oxidized form of glutathione) ratio. This ratio is low during dormancy and increases after bud break.

Regarding amino acids and proteins, two proteins of 14 and 18 kD were produced in small traces until mid August and reached a peak at the beginning of October (Lavee and May 1997). In addition, a protein of 30 kD appeared in January and a new 52 kD was found before active growth in spring (Lavee and May 1997).

Changes of DNA and RNA content and gene expression occur during dormancy induction and release. Catalase activity in grape buds decreases naturally from a maximal level in October to a minimal level in January (Or et al., 2002).

The length of bud dormancy is genetically controlled. For example, *Vitis vinifera* has a shorter bud dormancy length compared with *Vitis labruscana* and *Vitis riparia* (Fennell and Hoover, 1991). *Vitis amurensis* has a short endodormancy period and its chilling requirements are lower and are met more rapidly than the French American hybrid 'Vignoles' (Kovács et al., 2003).

Dormancy Termination:

Chilling is required to break endodormancy and chilling requirement varies among fruit trees including grapevines (Westwood, 1993). The chilling requirement for almonds is very little while it could reach up to 2000 hours in some grape cultivars. Lavee and May (1997) mentioned that chilling is essential for dormancy termination and allowing normal bud break. Dokoozlian (1999) found a temperature range 0-10 °C is effective as chilling temperatures to break dormancy of 'Perlette' cuttings. On the other hand, Erez (2000) mentioned that the optimum curve for chilling effect is at 6-8 °C.

Chemicals can also end the rest period. Erez (1987) reported that oil + dinitro-o-cresol (DNOC), KNO_3, thiourea and cyanamides, as well as growth regulators have been used as rest-breaking chemicals. Or (2009) mentioned that hydrogen cyanamide is the most effective chemical for breaking dormancy of grapevine buds by inactivating catalase. This chemical is used in the table grape industry because it compensates for the lack of chilling in warm winter regions (Or, 2009). Catalase is an enzyme containing an

iron heme prosthetic group in each of its subunits and seems to be involved in grapevine bud break since its activity was inhibited by hydrogen cyanamide (Pérez and Lira, 2005).

Bud scales also seem to be involved in dormancy. Iwasaki and Weaver (1977) found that removal of bud scales of 'Zinfandel' cuttings accelerated bud break as well as rooting due to the ABA presence in bud scales. In addition, Iwasaki (1980) showed that bud scale removal reduced the rest period of 'Muscat of Alexandria' single bud cuttings.

Kubota and Miyamuki (1992) used a garlic paste to break dormancy in one- year old grapevine dormant canes. Garlic paste was more effective when applied at a deeper dormancy stage in December rather than January. Kubota and other Japanese researchers studied garlic paste and published most of their findings in the Journal of Japanese Society for Horticultural Science during the nineties. Kubota et al., (2002) studied other volatile *Allium* species compounds as a paste on bud break of 'Muscat of Alexandria' and 'Kyoho' single bud cuttings. They suggested it could be a useful replacement of calcium and hydrogen cyanamides although the mechanism is unknown.

Bud break has been achieved by using electricity. Treating scions of two year old 'Kyoho' grapevines with 48 or 60 V hastened bud break (Kurooka et al., 1990). Again, a more pronounced effect was when direct current was applied at the time of deepest endodormancy. Köse (2007) used direct current for adventitious root formation of the grapevine rootstock *Vitis champini* 'Ramsey' and mentioned it has the potential to improve propagation of grapevine rootstocks that are difficult to root.

Forcing Solution and Cut Flowers:

Extending the life of many cut flowers is accomplished by adding chemicals to vase water (Hamooh, 2001). One of the chemicals studied by many researchers is 8-hydroxyquinoline citrate (8-HQC). Larsen and Scholes (1965) noticed more than a doubling of vase-life in cut carnation flowers compared with tap water and a 2.7 fold vase life increase compared with tap water in snapdragons (Larsen and Scholes, 1966). In addition, cut flower characteristics such as flower diameter in carnation and spike length in snapdragon were shown to be improved in the two previously mentioned studies. Furthermore, Marousky (1969 b) reported that 8-HQC and sucrose doubled vase-life in cut roses compared to ones held in water. The mechanism of prolonging life by 8-HQC was due to decreasing vascular blockage in stems and increasing water absorption and stomatal closure. Regarding sucrose, it was found to reduce stomatal opening. The same mechanisms were found to be the reasons of extending vase-life and improving quality of gladiolus (Marousky, 1969 a). Paulin (1986) added that sugar addition delays the loss of membrane integrity and degradation of phospholipids. Other studies that showed an increase in cut flower life and improvement in quality include: Abdel-Kader and Rogers (1986) in gerbera, Kofranek (1986) in *Triteleia laxa*, and Nooh et al. (1986) in *Ruscus hypoglossum* and *Nephrolepis exaltata*. These previous studies were published in The Third International Symposium on Post-Harvest Physiology of Ornamentals. After the applications of forcing solutions to extend vase-life of many cut flowers, investigators studied the possibility of forcing solution applications on bud break and rooting of woody plants, especially for micropropagation purposes.

Forcing Solution and Woody Plants:

The same forcing solution employed for vase life extension was suggested by Read et al., (1984) to be used to produce explants by soaking cut stem ends in the solution to force new growth. Since the availability of plant materials for in vitro purposes is limited to a short period of time during early spring (Yang and Read, 1991), forcing solution and plant growth regulators have been investigated to accelerate bud break and enhance rooting of cuttings. Read and Yang (1989) concluded that infusion of plant growth regulators via forcing solutions enhanced in vitro performance. Read and Yang (1992) concluded that indolebutyric acid delivered via the forcing solution increased root numbers per cutting and promoted root elongation while gibberellic acid inhibited rooting of forced dormant stems of privet and arrow-wood. Similarly, (Hamooh, 2001) found that GA_3 decreased rooting ability of some softwood cuttings. *In vitro* shoot proliferation in Vanhoutte's spirea was stimulated by adding BA in the forcing solution, while GA_3 resulted in less *in vitro* shoot proliferation (Yang and Read, 1993). The use of forcing solution and some wetting agents hastened bud break in lilac, privet, and Vanhoutte spirea (Yang and Read, 1992). In another study, Yang and Read (1997 b) found that GA_3 hastened bud break while BA and IBA delayed break. Hamooh (2001) found that adding silver thiosulfate to the forcing solution hastens bud break and shoot elongation. Less time to bud break and longer shoots were achieved when GA_3 was combined with silver thiosulfate in the forcing solution. An advantage of the use of forcing solution technique was demonstrated by the reduction of time from culture to potted plants of 5-leaf aralia (Yang and Read, 1997 a).

It can be concluded that the forcing solution technique could be a useful method for micropropagation purposes to enhance bud break as well as a method for studying bud break dormancy in woody plants.

Forcing Solution and Grape Cutting Studies:

According to Gu and Read, (2004), there are many studies on bud dormancy of grapevines, but none of the studies used forcing solutions. Gu (2003) studied the effects of forcing solution and some plant growth regulators namely: GA_3, BA, IAA, and ABA on bud break of 'Lacrosse', own rooted 'Chambourcin', and grafted 'Chambourcin' on the '3309 Couderc' rootstock. Forcing solution did not accelerate bud break compared to water treatments which is contrary to the previous studies on other woody plants. All of the added plant growth regulators delayed bud break with GA_3 delaying the most. Furthermore, interactions between cultivar, month, and the plant growth regulators were present.

References:

Abdel-Kader, B. and M. Rogers. 1986. Postharvest treatment of *Gerbera jamesonii*. Acta Horticulturae 181: 169-176.

Ahmedullah, M., A. Kawakami, C. Sandidge and R. Wample. 1986. Effect of paclobutrazol on the vegetative growth, yield, quality and winter hardiness of buds of 'Concord' grape. HortScience 21 (2): 273-274.

Alleweldt, G. and J. Possingham. 1988. Progress in grapevine breeding. Theoretical and Applied Genetics 75: 669-673.

Arora, R., L. Rowland, and K. Tanino. 2003. Induction and release of bud dormancy in woody perennials: a science comes of age. HortScience 38 (5): 911-921.

Beveridge, C. 2006. Axillary bud outgrowth: sending a message. Current Opinion in Plant Biology 9:35-40.

Brooks, R. and H. Olmo. 1997. The Brooks and Olmo Register of Fruit and Nut Varieties. Third Edition. Published by ASHS Press (American Society for Horticultural Science).

Call, R., and S. Seeley. 1989. Flower bud coatings of spray oils delay dehardening and bloom in peach trees. HortScience 24 (6): 914-915.

Choi, C., W. Zimmt and G. Giacomelli. 1999. Freeze and frost protection with aqueous foam- foam development. HortTechnology 9 (4): 654-661.

Creasy, G. and L. Creasy. 2009. Grapes. Crop Production Science in Horticulture. CABI, Cambridge, MA.

Dami, I. 2007. Understanding and preventing freeze damage in vineyards. Workshop Proceedings. University of Missouri Extension.

Dami, I. and B. Beam. 2004. Response of grapevines to soybean oil application. American Journal of Enology & Viticulture 55 (3): 269-275.

Dami, I., R. Hamman, and C. Stushnoff. 1997. Delay of bud break and deacclimation in grapevines to overcome spring frost. American Journal of Enology & Viticulture 48 (3): 376 (Abstract).

Dami, I., R. Hamman, C. Stushnoff, and T. Wolf. 2000. Use of oils and alginate to delay bud break of grapevines. Proceedings of the ASEV 50th Anniversary Annual Meeting.73-76.

Dennis, F. Jr. 1994. Dormancy- what we know (and what we don't know?). HortScience 29 (11): 1249-1255.

Deyton, D., C. Sams and J. Cummins. 1992. Application of dormant oil to peach trees modifies bud-twig internal atmosphere. HortScience 27 (12): 1304-1305.

Dokoozlian, N. 1999. Chilling temperature and duration interact on the budbreak of 'Perlette' grapevine cuttings. HortScience 34 (6): 1054-1056.

Dun, E., B. Ferguson, and C. Beveridge. 2006. Apical dominance and shoot branching. Divergent opinions or divergent mechanisms. Plant Physiology. 142: 812-819.

Eichhorn, K., Lorenz, D. 1977. Phänologische Entwicklungsstadien der Rebe. Nachrichtenbl. Deut. Pflanzenschutz 29:119-120.

Erez, A. 1987. Chemical control of budbreak. HortScience 22 (6): 1240-1243.

Erez, A. 2000. Bud dormancy: a suggestion for the control mechanism and its evolution. In J. D. Viémont and J. Crabbé (Editors) "Dormancy in Plants: From Whole Plant Behaviour to Cellular Control".

Eris, A. and H. Celic. 1981. Effects of some plant growth regulators on bud burst and rooting of *Vitis vinifera* L. cv. Chaush cuttings. American Journal of Enology & Viticulture. 32 (2): 122-124.

Evans, R. 2000. The art of protecting grapevines from low temperature injury. In Proceedings of the ASEV 50th Anniversary Annual Meeting. 60-72.

Faust, M., D. Liu, M. Millard and G. Stutte. 1991. Bound versus free water in dormant apple buds- a theory for endodormancy. HortScience 26 (7): 887-890.

Fennell, A. 2004. Freezing tolerance and injury in grapevines. Journal of Crop Improvement 10: 201-235.

Fennell, A., C. Wake, and P. Molitor. 1996. Use of ^{1}H-NMR to determine grape bud water state during the photoperiodic induction of dormancy. J. Amer. Soc. Hort. Sci. 121 (6): 1112-1116.

Fennell, A. and E. Hoover. 1991. Photoperiod influences growth, bud dormancy, and cold acclimation in *Vitis labruscana* and *V. riparia*. J. Amer. Soc. Hort. Sci. 116: 270-273.

Friend, A., C. Stushnoff, G. Creasy and M. Trought. 2001. Manipulating bud break date in grapevines. In Proceedings of the ASEV 52nd Anniversary Annual Meeting. Page 16 (Abstract).

Friend, A. and M. Trought. 2007. Delayed winter spur-pruning in New Zealand can alter yield components of 'Merlot' grapevines. Australian Journal of Grape and Wine Research 13 (3): 157-164.

Fuchigami, L. and C. Nee. 1987. Degree growth stage model and rest-breaking mechanisms in temperate woody perennials. HortScience 22 (5): 836-845.

Fuller, M., F. Hamed, M. Wisniewski and D. Glenn. 2003. Protection of plants from frost using hydrophobic particle film and acrylic polymer. Annals of Applied Biology 143: 93-97.

Gu, S. 1999. Lethal temperature coefficient – a new parameter for interpretation of cold hardiness. Journal of Horticultural Science & Biotechnology 74 (1): 53-59.

Gu, S., P. Ding and S. Howard. 2002. Effect of temperature and exposure time on cold hardiness of primary buds during the dormant season in 'Concord', 'Norton', 'Vignoles' and 'St. Vincent' grapevines. Journal of Horticultural Science & Biotechnology 77 (5): 635-639.

Gu, S., S. Dong, J. Li, and S. Howard. 2001. Acclimation and deacclimation of primary bud cold hardiness in 'Norton', 'Vignoles' and 'St. Vincent' grapevines. Journal of Horticultural Science & Biotechnology 76 (6): 655-660.

Gu, S. 2003. Rootstock and mounding effect on growth and cold hardiness of 'Gewürztraminer' (*Vitis vinifera*) and bud dormancy of 'Lacrosse' and 'Chambourcin' (*Vitis* Spp.). Ph D. Dissertation, University of Nebraska, Lincoln.

Gu, S. and P. Read. 2004. The influence of plant growth regulators included in the forcing solution on bud break of dormant grapevines (*Vitis* spp.). Acta Horticulturea 640: 225-234.

Hamman, R., I. Dami, T. Walsh, and C. Stushnoff. 1996. Seasonal carbohydrate changes and cold hardiness of 'Chardonnay' and 'Riesling' grapevines. American Journal of Enology & Viticulture 47 (1): 31-36.

Hamooh, B. 2001. Application of Forcing Solution Technology to Micro and Macropropagation Woody Plant Species. Ph D. Dissertation, University of Nebraska, Lincoln.

Hatch, R. and M. Ruiz. 1987. Influence of pruning date on budbreak of desert table grapes. American Journal of Enology & Viticulture 38 (4): 326-328.

Hellman, E. 2003. Oregon Viticulture. Oregon State University Press.

Hellman, E., S. Shelby, and C. Lowey. 2006. Exogenously applied abscisic acid did not consistently delay budburst of deacclimating grapevines. Journal of the American Pomological Society 60 (4): 178-186.

Howard, W. 1910. The rest period in plants. Proceedings of the Society for Horticultural Science 7: 33-46.

Howell, G. 2000. Grapevine cold hardiness: mechanisms of cold acclimation, mid-winter hardiness maintenance, and spring deacclimation. In Proceedings of the ASEV 50th Anniversary Annual Meeting.

Iwasaki, K. 1980. Effects of bud scale removal, calcium cyanamide, GA_3, and ethephone on bud break of 'Muscat of Alexandria' grape (*Vitis vinifera* L.). J. Japan. Soc. Hort. Sci. 48 (4): 395-398.

Iwasaki, K. and R. Weaver. 1977. Effects of chilling, calcium cyanamide, and bud scale removal on bud break, rooting, and inhibitor content of buds of 'Zinfandel' grape (*Vitis vinifera* L.). J. Amer. Soc. Hort. Sci. 102 (5): 584-587.

Izadyar, A. and S. Wang. 1999. Changes of lipid components during dormancy in 'Hull Thornless' and 'Triple Crown Thornless' blackberry cultivars. Scientia Horticulturae 82: 243-254.

Jones, K. S., J. Paroschy, B. McKerise, and S. Bowley. 1999. Carbohydrate composition and freezing tolerance of canes and buds in *Vitis Vinifera*. Journal of Plant Physiology 155: 101-106.

Junttila, O. 1988. To be or not to be dormant: some comments on the new dormancy nomenclature. HortScience 23 (5): 805-806.

Kaul, R. B., D. Sutherland, and S. Rolfsmeier. 2006. The Flora of Nebraska: keys, description, and distribution maps of all native and introduced species that grow outside cultivation. School of Natural Resources. University of Nebraska, Lincoln, NE.

Kofranek, A. 1986. Studies on the post harvest handling of *Triteleia laxa*. Acta Horticulturae 181: 231-236.

Köse, C. 2007. Effects of direct electric current on adventitious root formation of a grapevine rootstock. American Journal of Enology & Viticulture 58 (1): 120-123.

Koussa, T., M. Broquedis and J. Bouard. 1994. Changes of abscisic acid level during the development of grapevine latent buds, particularly in the phase of dormancy break. Vitis 33: 63-67.

Kovács, L., P. Byers, M. Kaps, and J. Saenz. 2003. Dormancy, cold hardiness, and spring frost hazard in *Vitis amurensis* hybrids under continental climatic conditions. American Journal of Enology & Viticulture 54 (1): 8–14.

Kubota, N. and M. Miyamuki. 1992. Breaking dormancy in grapevines with garlic paste. J. Amer. Soc. Hort. Sci. 117 (6): 898-901.

Kubota, N., Y. Yamane, and K. Toriu. 2002. Breaking bud dormancy in grape cuttings with non-volatile and volatile compounds of several *Allium* species. J. Japan. Soc. Hort. Sci. 71 (4): 467-472.

Kurooka, H., S. Horiuchi, S. Fukunga, and E. Yuda. 1990. Effects of electric current on breaking bud dormancy in grapes. Bulletin of the University of Osaka Prefecture 42: 111-119.

Lang, G. 1994. Dormancy-the missing links: molecular Studies and integration of regulatory plant and environmental interactions. HortScience 29 (11): 1255-1263.

Lang, G., J. Early, G. Martin, and R. Darnell. 1987. Endo-, para-, and ecodormancy: physiological terminology and classification for dormancy research. HortScience 22 (3): 371 – 377.

Larsen, F. and J. Scholes. 1965. Effects of sucrose, 8-hydroxyquinoline citrate, and N-dimethyl amino succinamic acid on vase-life and quality of cut carnations. Proc. Amer. Soc. Hort. Sci. 87: 458-463.

Larsen, F. and J. Scholes. 1966. Effects of 8-hydroxyquinoline citrate, N-dimethyl amino succinamic acid, and sucrose on vase-life and spike characteristics of cut snapdragons. Proc. Amer. Soc. Hort. Sci. 89: 694-701.

Lavee, S. and P. May. 1997. Dormancy of grapevine buds – facts and speculation. Australian Journal of Grape and Wine Research 3: 31-46.

Levitt, J. 1980. Responses of Plants to Environmental Stresses. Second Edition. Volume 1. Academic Press.

Loomis, N. 1939. Note on grape foliation as affected by time of pruning. Proceedings of the American Society for Horticultural Science 37: 653-654.

Malladi, A. and J. Burns. 2007. Communication by plant growth regulators in roots and shoots of horticultural crops. HortScience.42 (5): 1113-1117.

Marousky, F. 1969 a. Physiological role of 8-hydroxyquinoline citrate and sucrose in extending vase-life and improving quality of cut gladiolus. Florida State Horticultural Society 409-414.

Marousky, F. 1969 b. Vascular blockage, water absorption, stomatal opening, and respiration of cut 'Better Times' roses treated with 8-hydroxyquinoline citrate and sucrose. J. Amer. Soc. Hort. Sci. 94: 223-226.

McMahon, M., A. Kofranek and V. Rubatzky. 2007. Hartmann's Plant Science: Growth, Development and Utilization of Cultivated Plants. Fourth Edition. Prentice Hall.

Meier, U. 2001. Grapevine. Growth stages of mono-and dicotyledonous plants. BBCH Monograph. Federal Biological Research Center for Agriculture and Forestry, Berlin, Germany.

Miller, D., G. Howel, R. Striegler. 1988 a. Cane and bud hardiness of selected grapevine rootstocks. American Journal of Enology & Viticulture 39 (1): 55-59.

Miller, D., G. Howel, R. Striegler. 1988 b. Cane and bud hardiness of own-rooted white Riesling and scion of 'White Riesling' and 'Chardonnay' grafted to selected rootstocks. American Journal of Enology & Viticulture 39 (1): 60-66.

Mills, L., J. Ferguson, and M. Keller. 2006. Cold-hardiness evaluation of grapevine buds and cane tissues. American Journal of Enology & Viticulture 57 (2): 194-200.

Murphy, J. and D. Briske. 1992. Regulation of tillering by apical dominance: chronology, interpretive value, and current perspectives. Journal of Range Management 45 (5): 419-429.

Myers, R., D. Deyton, and C. Sams. 1996. Applying soybean oil to dormant peach trees alters internal atmosphere, reduces respiration, delays bloom, and thins flower buds. J. Amer. Soc. Hort. Sci. 121 (1): 96-100.

Nigond, J. 1960. Delaying bud break in vines by the use of α-naphthaleneacetic acid and defense against frost. Compt. Rend. Acad. Agr. France 46: 452-457.

Nooh, A. T. El-Kiey, and M. Khattab. 1986. Studies on the keeping quality of cut green *Ruscus hypoglossum* L. and *Nephrolepis exaltata* Schott. Acta Horticulturae 181: 223-230.

Okubo, H. 2000. Growth cycle and dormancy in plants. In J. D. Viémont and J. Crabbé (Editors) "Dormancy in Plants: From Whole Plant Behaviour to Cellular Control".

Or, E. 2009. Grape bud dormancy release-the molecular aspect. In K. A. Roubelakis-Angelakis (Editor.) "Grapevine Molecular Physiology & Biotechnology". Second Edition.

Or, E., I. Vilozny, A. Fennell, Y. Eyal, and A. Ogrodovitch. 2002. Dormancy in grape buds: isolation and characterization of catalse cDNA and analysis of its expression following chemical induction of bud dormancy release. Plant Science 162: 121-130.

Patterson, W. and D. Howell. 1995. Effect of fall-applied plant growth regulating compounds on bud break of 'Concord' and 'Riesling'. American Journal of Enology & Viticulture 46 (3): 414.

Paulin, A. 1986. Influence of exogenous sugars on the evolution of some senescence parameters of petals. Acta Horticulturae 181: 183-194.

Pérez, F. and W. Lira. 2005. Possible role of catalase in post-dormancy bud break in grapevines. Journal of Plant physiology. 162 (3): 301-308.

Perry, K. 1998. Basics of frost and freeze protection for horticultural crops. HortTechnology 10 – 15.

Preece, J. and P. Read. 2005. The Biology of Horticulture: an Introductory Textbook. Second Edition. John Wiley & Sons.

Read, P., A. Economou and C. Fellman. 1984. Manipulating stock plants for improved in vitro mass propagation. Proc. Int. Symp. Plant Tissue and Cell Culture: Application to Crop Improvement. Novak, J., L. Havel, and J. Dolezel (Editors). Czech. Acad. Sci. Prague: 467-473.

Read, P. and G. Yang. 1989. Influencing propagation by stock plant PGR treatments. Acta Horticulturae (ISHS) 251: 121-128.

Read, P. and G. Yang. 1992. Plant growth regulators effects on rooting of forced softwood cuttings. Acta Horticulturae (ISHS) 300: 197-200.

Read, P. and S. Gu. 2003. A century of American Viticulture. HortScience 38 (5): 943 – 951.

Rieger, M. 1989. Freeze protection for horticultural crops. Horticultural Reviews 11: 45-109. Edited by J. Janick. Published by Timber Press. Portland, Oregon

Romberger, J. 1963. Meristems, growth, and development in woody plants. USDA Technical Bulletin No. 1293.

Salzman, R., R. Bressan, P. Hasegawa, E. Ashworth, and B. Bordelon. 1996. Programmed accumulation of LEA-like proteins during desiccation and cold acclimation of overwintering grape buds. Plant, Cell and Environment. 19: 713-720.

Samish, R. 1954. Dormancy in woody plants. Annual Review of Plant Physiology 5:183-204.

Saure, M. 1985. Dormancy release in deciduous fruit trees. Horticultural Reviews 7:239-300. Edited by J. Janick.

Seeley, S. 1994. Dormancy – The Black Box. HortScience. 29 (11): 1248.

Smiley, L., P. Domoto, G. Nonnecke, and W. Miller. 2008. A review of cold climate grape cultivars. Iowa State University.

Stergios, B. and G. Howell. 1977. Effect of site on cold acclimation and deacclimation of 'Concord' grapevines. American Journal of Enology & Viticulture 28 (1): 43-48.

Swenson, E., P. Pierquet, and C. Stushnoff. 1980. 'Edelweiss' and 'Swenson Red' grapes. HortScience 15 (1): 100.

Takeda, F., V. Drane, and M. Saunders. 1982. Inhibiting sprouting in muscadine grapes. Proc. Fla. State Hort. Soc. 95: 127-128.

Tanino, K. 2004. Hormones and endodormancy induction in woody plants. Journal of Crop Improvement 10: 157-199.

Trought, M., G. Howell and N. Cherry. 1999. Practical considerations for reducing frost damage in vineyards. Frost Damage and Management in New Zealand Vineyards.

Vegis, A. 1964. Dormancy in higher plants. Annual Review of Plant Physiology 15:185-224.

Wang, S. and M. Faust. 1990. Changes of membrane lipids in apple buds during dormancy and budbreak. J. Amer. Soc. Hort. Sci. 115 (5): 803-808.

Weaver, R. 1959. Prolonging dormancy in *Vitis vinifera* with Gibberellin. Nature 183: 1198-1199.

Weaver, R., S. McCune, and B. Coombe. 1961. Effects of various chemicals and treatments on rest period of grape buds. American Journal of Enology & Viticulture 12 (3): 131-142.

Westwood, M. 1993. Temperate-Zone Pomology, Physiology and Culture. Third Edition. Timber Press, Portland, Oregon.

Wiggans, C. B. 1926. A study of the relative value of the fruiting shoots arising from primary and secondary buds of 'Concord' grape. Proceedings of the American Society for Horticultural Science 23: 293-296.

Winkler, A., J. Cook, W. Kliewer, and L. Lider. 1974. General Viticulture. University of California Press.

Winkler, A. and W. Williams. 1945. Starch and sugars of *Vitis vinifera*. Plant Physiology 20: 412-432.

Wolf, T., and J. Boyer. 2003. Vineyard Site Selection. Virginia Cooperative Extension Publication Number 463-020. Virginia Polytechnic Institute and State University, Blacksburg, VA. http://www.ext.vt.edu/pubs/viticulture/463-020/463-020.html.

Yang, G. and P. Read. 1991. Influence of plant growth regulators and season on growth responses of forced woody stems. Acta Horticulturae 300: 173-176.

Yang, G. and P. Read. 1992. Pre-forcing treatments influence bud break and shoot elongation in forced woody species. J. Environ. Hort. 10 (2): 101-103.

Yang, G. and P. Read. 1993. *In vitro* culture of *Vanhoutte's spirea* explants from 'secondary cultures' and dormant stems forced in solutions containing plant growth regulators. Plant Cell, Tissue, and Organ Culture 33: 25-30.

Yang, G. and P. Read. 1997 a. *In vitro* shoot proliferation of 5-leaf aralia explants from field grown plants and forced dormant stems. Plant Cell, Tissue, and Organ Culture 47: 289-291.

Yang, G. and P. Read. 1997 b. Plant growth regulators in the forcing solution influenced bud break and shoot elongation of dormant woody plant species. PGRSA Quarterly 25 (3): 145-152.

Zabadal, T., I. Dami, M. Goffinet, T. Martinson, and M. Chien 2007. Winter injury to grapevines and methods of protection. Michigan State University Extension Bulletin E2930.

CHAPTER 1

Delaying Bud Break: Field Experiment, 2009

Materials and Methods:

Site Selection:

The research was conducted during the 2009 winter season at James Arthur Vineyards located in Raymond, Nebraska within Lancaster County. James Arthur Vineyards was the second winery opened in Nebraska, officially opened in September of 1997, and is the largest commercial winery in the state.

Edelweiss Vines:

Treatments were applied on 12 year old vines. The vines are trained using the Geneva Double Curtain trellis system. Planting distances were 8 feet (2.44 m) between plants and 12 feet (3.66 m) between rows. Rows are oriented north to south.

Experiment:

The experiment consisted of five treatments: NAA (500, 750, and 1000 mg/l) purchased from Phyto Technology Laboratories, 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. NAA concentrations were prepared by weighing out 0.5, 0.75, and 1 g, respectively. NAA was dissolved by adding a few drops of 1M NaOH and the volume was completed to 1000 ml by adding distilled water. The pH of each of the NAA solutions was measured and readjusted to about 7 by adding a few drops of 1M HCL. A randomized complete block design was used with three blocks of 20 vines each. There were four application dates: Jan. 6, Feb. 3, Mar. 3, and Apr. 1, 2009. Most vines were pruned to five buds before applying treatments. Treatments were applied on one year old

canes. 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 by tying with a yellow ribbon.

Bud Break:

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. Stage five indicates that the bud scales have expanded to the point at which a green shoot is visible as shown in Figure 2. 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 January 1, 2009 until achieving 60% bud break was used as the basis of calculating number of days for bud break.

Figure 2: Bud break of 'Edelweiss' grapevines at stage five.

Weather Data:

Temperatures for Raymond, Nebraska throughout the year of 2009 were obtained from the High Plains Regional Climate Center, University of Nebraska, Lincoln.

Harvesting and Berry Samples:

On August 14, 2009, the number and weight of clusters from the two selected canes were recorded. From the clusters, 50 berries were randomly counted, placed in a plastic storage bag, and placed in the freezer (32.0 °F) (0 °C) until berry sample analysis could be conducted.

Berry Analysis:

On September 14, 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. TA was determined by titration with NaOH, using the procedure of Dharmadhikari and Wilker (2001).

Pruning Weights in Winter 2010:

On March 18, 22, and 25, 2010 pruning weights of the whole treated vines were cut from the base and weighed using an upright balance scale (Figure 3).

Figure 3: Issam Qrunfleh weighing ‘Edelweiss’ prunings in winter 2010.

Harvest of Summer 2010:

In order to obtain data regarding cumulative effects of the treatments on fruiting the following year, total number of clusters/vine were counted on August 10, 2010.

Statistical Analysis:

All statistical analyses were performed using SAS/STAT Version 9.2 and Analysis of Variance was conducted by the PROC GLIMMIX procedure.

Results and Discussion:

Bud Break:

According to the analysis of variance, there was a significant treatment by month interaction at ($P \leq 0.05$) (Table 1).

Table 1: Analysis of variance table for number of days to show 60% bud break in 12-year-old 'Edelweiss' grapevines:

Effect	**DF**	**F Value**	**Pr > F**
TRT	4	22.97	< 0.0001
MONTH	3	0.63	0.60
TRT*MONTH	12	2.10	0.02

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 are presented in Table 2 and treatment effects within the month are presented in Table 3.

Table 2: Monthly effects within treatments on average days to show 60% bud break in12-year-old 'Edelweiss' grapevines:

Months	Treatments				
	Control	**NAA 500 ppm**	**NAA 750 ppm**	**NAA 1000 ppm**	**Oil**
January	125±0.98 ab	126 a	126.5 a	128 a	130 a
February	124 b	124.5 a	126 a	129 a	129 a
March	126 a	126 a	127 a	128.3 a	126.5 b
April	125.5 ab	125 a	126 a	127.7 a	129.7 a

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

Delaying pruning until March had a significant effect in delaying bud break two days compared to vines pruned in February (Table 2). The idea of pruning then applying treatments was to maintain apical dominance (Preece and Read, 2005), increase the effectiveness of spraying since the canes are too crowded, and canes will be eventually 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 because grapevines exhibit strong apical dominance (Friend et al., 2001). No significant differences were found between NAA 500, 750, and 1000 ppm within 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. The same improper mixing occurred in the study conducted by Dami and Beam (2004) using the same oil.

Regarding effects of treatments within months, it appeared that desirable results were achieved by oil and NAA at 1000 ppm treatments in all months (Table 3). Except in March, oil applications significantly delayed bud break five days compared to the control (Table 3). Oil and NAA at 1000 ppm were only significantly different in January and April (Table 3). Overall, there were no significant differences between the control, NAA at 500, and 750 ppm. See Appendix 4 regarding the number of days delaying bud break in 2009.

Table 3: Treatment effects within months on average days to show 60% bud break in 12-year-old 'Edelweiss' grapevines:

Months	**Treatments**				
	Control	**NAA 500 ppm**	**NAA 750 ppm**	**NAA 1000 ppm**	**Oil**
January	125±0.98 c	126 c	126.5 bc	128 b	130 a
February	124 c	124.5 bc	126 b	129 a	129 a
March	126 b	126 b	127 ab	128.3 a	126.5 ab
April	125.5 c	125 c	126 bc	127.7 b	129.7 a

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

Number of Clusters per Cane:

Table 4 shows neither treatment by month interaction nor a treatment effect but does show a month effect at ($P \leq 0.05$).

Table 4: Analysis of variance table for number of clusters in 12-year-old 'Edelweiss' grapevines:

Effect	DF	F Value	Pr > F
TRT	4	0.86	0.49
MONTH	3	3.56	0.02
TRT*MONTH	12	0.82	0.63

The largest average number of clusters per cane was found in April treated vines but they were not significantly different from the average of vines treated in March and January (Table 5). Average number of clusters was lowest in February treated vines and significantly different from the other three treatment dates (Table 5). Most importantly, no treatment effect was detected. Number of clusters per shoot ranged from 5.5 to 8.17 (see Appendix 5).

Table 5: Least Significant Difference Test (L.S.D.) for average number of clusters per cane of 12-year-old 'Edelweiss' grapevines:

Month	Average Number of Clusters per Cane
April	7.3±0.72 a
March	7.2±0.72 a
January	7.0±0.72 a
February	5.8±0.72 b

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

"The number of flower clusters per shoot varies with cultivar, management, and environmental conditions, but can range from none to five or even more" (Creasy and Creasy, 2009). Five buds were retained after pruning. Usually, each bud can produce one to two clusters (Paul Read, personal communication), hence ten clusters would have been an optimum production in this case. Nevertheless, the above averages are accepted by growers and average difference between April treated vines and February although statistically different is only a 1.5 difference (Table 5). This difference could be explained by cluster characteristics of 'Edelweiss' which is known to be very loose (Brooks and Olmo, 1997; Smiley et al., 2008; Swenson et al., 1980).

Weight of Clusters:

No significant interaction, month, or treatment effect was found in average weights of 'Edelweiss' clusters at ($P \leq 0.05$) (Table 6). Cluster weights of the two selected canes ranged from 1.33 to 2.22 kg (see Appendix 6).

Table 6: Analysis of variance table for cluster weights of 12-year-old 'Edelweiss' grapevines:

Effect	**DF**	**F Value**	**Pr > F**
TRT	4	1.61	0.18
MONTH	3	1.17	0.32
TRT*MONTH	12	0.77	0.68

This supports neglecting differences found regarding average number of clusters per cane since cluster weights were recorded as the average cluster weights of the two canes that were selected and that the differences found in average number of clusters per cane is attributed to the looseness characteristic of 'Edelweiss' clusters.

Berry Analysis:

Also, no significant interaction, month, or treatment effect was found in weights of 50 berry samples of 'Edelweiss' berries at ($P \leq 0.05$) (Table 7). Once again, this will support neglecting the differences detected in the average number of clusters per cane. The 50 berry sample weights ranged from 116.01 to 125.19g (see Appendix 7).

Table 7: Analysis of variance table for weight of 50 berries sampled from 12-year-old 'Edelweiss' grapevines:

Effect	**DF**	**F Value**	**Pr > F**
TRT	4	0.58	0.68
MONTH	3	0.92	0.44
TRT*MONTH	12	0.99	0.47

Tables 8, 9, and 10 show analysis of variance regarding berry characteristics °Brix, pH and TA, respectively. A treatment by month interaction was only found in pH analysis at ($P \leq 0.05$) (Table 9).

Table 8: Analysis of variance table for °Brix of berries sampled from 12-year-old 'Edelweiss' grapevines:

Effect	**DF**	**F Value**	**Pr > F**
TRT	4	0.69	0.60
MONTH	3	0.79	0.51
TRT*MONTH	12	0.82	0.63

Table 9: Analysis of variance table for pH of berries sampled from 12-year-old 'Edelweiss' grapevines:

Effect	**DF**	**F Value**	**Pr > F**
TRT	4	1.29	0.29
MONTH	3	1.05	0.38
TRT*MONTH	12	3.06	0.0039

Table 10: Analysis of variance table for TA of berries sampled from 12-year-old 'Edelweiss' grapevines:

Effect	DF	F Value	Pr > F
TRT	4	1.73	0.16
MONTH	3	0.59	0.63
TRT*MONTH	12	1.11	0.38

Due to interaction effects in pH analysis, month effects within treatments are presented in Table 11 and treatment effects within the month are presented in Table 12.

Table 11: Monthly effects within treatments on pH of 12-year-old 'Edelweiss' grapevines:

Months	Treatments				
	Control	**NAA 500 ppm**	**NAA 750 ppm**	**NAA 1000 ppm**	**Oil**
January	3.14±0.04 b	3.26 ab	3.26 a	3.22 a	3.26 a
February	3.21 ab	3.27 a	3.23 a	3.17 a	3.25 a
March	3.23 a	3.18 c	3.20 a	3.21 a	3.22 a
April	3.25 a	3.20 bc	3.24 a	3.20 a	3.14 b

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

NAA at 750 and 1000 ppm showed no significant differences in pH values for all four months (Table 11). Meanwhile, oil treatment showed a significant different pH in April and the control in January although not significantly different from February (Table 11). More obvious differences are observed in NAA at 500 ppm (Table 11).

Table 12: Treatment effects within months on pH of 12-year-old 'Edelweiss' grapevines:

Months	**Treatments**				
	Control	**NAA 500 ppm**	**NAA 750 ppm**	**NAA 1000 ppm**	**Oil**
January	3.14±0.04 b	3.26 a	3.26 a	3.22 a	3.26 a
February	3.21 ab	3.27 a	3.23 ab	3.17 b	3.25 a
March	3.23 a	3.18 a	3.20 a	3.21 a	3.22 a
April	3.25 a	3.20 ab	3.24 a	3.20 ab	3.14 b

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

Regarding treatment effects within months, no significant differences were found among treatments within March (Table 12). The control was significantly different from all other treatments in January (Table 12) and the oil treatment was significantly different from the control and NAA at 750 ppm in April. From the results in Tables 11 and 12, it seems that differences in pH values are not due to the delay in bud break but to environmental conditions. Creasy and Creasy (2009) mentioned that berry characteristics are totally dependent on environmental conditions especially the microclimate (climate within canopy).

°Brix ranged from 12.27 to 13.23, pH values were 3.14 to 3.27, and TA values were 0.83 to 1.13 g/100ml (see Appendices 8, 9, and 10). 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% for the total soluble solids, pH, and the TA, respectively. 'Edelweiss' is harvested at an earlier stage regarding °Brix. Swenson et al., (1980) mentioned that 'Edelweiss' juice is relatively low in acidity (0.6-0.8%) and has moderate soluble solids (14-16%). They recommended for wine making that it should be picked at an early mature stage (14 °Brix). I discussed 'Edelweiss' harvest parameters with 'Edelweiss' wine makers at the 13th Annual Nebraska Winery and Grape Growers Forum and Tradeshow that was held at the Holiday Inn, Kearney, Nebraska March 4-6, 2010. According to six wine makers, °Brix is the most important harvest parameter. On the other hand, five wine makers mentioned that pH is the most important because a wine maker can harvest earlier than the suggested range and add sugar. According to them, adjusting juice pH is more difficult than adjusting sugar levels. All of them were in agreement that TA is the least important among the three parameters. Five wine makers mentioned that they prefer a little higher range of °Brix up to 16. Regarding pH and TA, the wine maker's ranges were in the proposed ideal ranges by Dharmadhikari and Wilker (2001).

Harvest parameter results of this study in 2009 were in the recommended ranges except for soluble solids. Lower °Brix values of the samples harvested in 2009, were due to the cooler July temperatures. The average maximum temperature was 80.9 °F (27.16 °C) where as the minimum average temperature was 60.8 °F (15.99 °C) as shown in Figure 4. Higher temperatures during that month would have been more preferable for the

vines to produce more photosynthates and accumulate more sugar. Weaver (1976) and (Winkler et al., 1974) mentioned that optimum temperatures for photosynthesis ranges from 77-86 °F (25-30 °C). In addition, lower night temperatures would have been preferable for reducing respiration rates and breakdown of the sugars that have accumulated. Winkler et al., (1974) mentioned that 40 °F (4.44 °C) halves the respiration rate and is an advantage to suppress fungal disease.

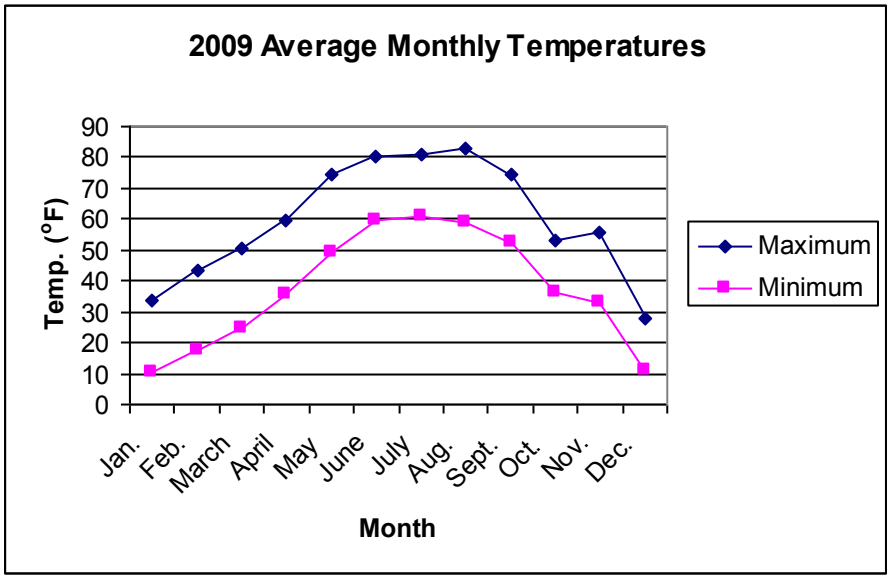


Figure 4: The maximum and minimum monthly average temperatures during 2009. Source: High Plains Regional Climate Center.

Seth McFarland of Mac's Creek Winery and Vineyards in Lexington, Nebraska has been using the same oil as that used in this study for three years. He reported his results at the 13th Annual Nebraska Winery and Grape Growers Forum and Tradeshow that was held at the Holiday Inn, Kearney, Nebraska March 4-6, 2010. Oil applications were sprayed at the same ratio (10%) on 'Marechal Foch', 'St. Croix', and 'Brianna' on March 17, April 4, April 21, and April 28. 'Marechal Foch' and 'St. Croix' showed a

delay of 12 days and 7-10 days delay for 'Brianna'. The applications did not affect the yields nor the quality of grapes harvested. He reported higher average weights of 'Marechal Foch' compared to the controls because the oil did protect the primary buds without showing any phytotoxity effects. Although he did repeat spraying, the results reported in this study are not because single applications, but because of the effect of early pruning.

An article entitled "Do Oil Sprays Delay Ripening for Winegrapes?" was published in the Wines and Vines Magazine in May 2010. It was reported that some studies in eastern states found that high oil applications could delay ripening and reduce yields. On the other hand, the article reported two studies that were conducted in California that showed no effects of JMS Stylet -Oil on ripening, number of clusters per vine, cluster weight, berry weight, juice pH, juice TA, total sugar per berry or total sugar per vine. From the results of this study, it seems that such a delay will not affect berry characteristics and that berry characteristics depend mainly on environmental conditions.

Pruning Weights in 2010:

The treatments had no effect on pruning weights taken in winter 2010 as shown in Table 13 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.

Table 13: Analysis of variance table for pruning weights of 13-year-old 'Edelweiss' grapevines:

Effect	DF	F Value	Pr > F
TRT	4	0.30	0.88
MONTH	3	0.74	0.53
TRT*MONTH	12	1.17	0.34

Pruning weights ranged from 1.05 to 1.43 kg (see Appendix 11). 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, this could be an indication that treatments in this study had no effect on 'Edelweiss' cold hardiness, but it is proof that such applications have no negative effects on grapevines vegetative growth and that such delays in bud break should be of no concern.

Number of Clusters in 2010:

There is always a concern regarding cumulative effects especially with plant growth regulator applications. Number of clusters per vine ranged from 12 to 16 (see Appendix 12). Analysis of variance in Table 14 show that there were no such effects regarding number of clusters that were produced in the following harvest year 2010 ($P \leq 0.05$). Low number of clusters per vine is attributed to above average precipitation that

occurred in May and particularly in June, 2010. Further details regarding number of clusters are discussed in Chapter 2 with the 2010 field experiment results.

Table 14: Analysis of variance table for number of clusters produced in 2010 for 13-year-old 'Edelweiss' grapevines following 2009 treatments:

Effect	**DF**	**F Value**	**Pr > F**
TRT	4	0.09	0.98
MONTH	3	0.94	0.43
TRT*MONTH	12	1.00	0.47

NAA can induce fruit set and it can be applied early in the growing season to prevent abscission of flower buds (Preece and Read, 2005). NAA applications in 2009 did not improve fruit set the following year. In many fruit trees, NAA is also used as a fruit thinning plant growth regulator (Westwood, 1993) and it was expected not to show any negative effects on yields the following year. The results of this study confirm this expectation.

References:

Brooks, R. and H. Olmo. 1997. The Brooks and Olmo Register of Fruit and Nut Varieties. Third Edition. Published by ASHS Press (American Society for Horticultural Science).

Creasy, G. and L. Creasy. 2009. Grapes. Crop Production Science in Horticulture. CABI, Cambridge, MA.

Dami, I. and B. Beam. 2004. Response of grapevines to soybean oil application. American Journal of Enology & Viticulture 55 (3): 269-275.

Dharmadhikari, M. and K. Wilker. 2001. Micro Vinification: A practical guide to small-scale wine production. Missouri State Fruit Experiment Station, Mountain Grove, Missouri.

Eichhorn, K., Lorenz, D. 1977. Phänologische Entwicklungsstadien der Rebe. Nachrichtenbl. Deut. Pflanzenschutz 29:119-120.

Friend, A., C. Stushnoff, G. Creasy and M. Trought. 2001. Manipulating bud break date in grapevines. In Proceedings of the ASEV 52nd Anniversary Annual Meeting. Page 16 (Abstract).

Gu, S. 2003. Rootstock and mounding effect on growth and cold hardiness of 'Gewürztraminer' (*Vitis vinifera*) and bud dormancy of 'Lacrosse' and 'Chambourcin' (*Vitis* Spp.). Ph D. Dissertation, University of Nebraska, Lincoln.

Preece, J. and P. Read. 2005. The Biology of Horticulture: an Introductory Textbook. Second Edition. John Wiley & Sons.

Smiley, L., P. Domoto, G. Nonnecke, and W. Miller. 2008. A review of cold climate grape cultivars. Iowa State University.

Swenson, E., P. Pierquet, and C. Stushnoff. 1980. 'Edelweiss' and 'Swenson Red' grapes. HortScience 15 (1): 100.

Weaver, R. 1976. Grape Growing. John Wiley & Sons, Inc.

Westwood, M. 1993. Temperate-Zone Pomology, Physiology and Culture. Third Edition. Timber Press, Portland, Oregon.

Winkler, A., J. Cook, W. Kliewer, and L. Lider. 1974. General Viticulture. University of California Press.

CHAPTER 2

Delaying Bud Break: Field Experiment, 2010

Materials and Methods:

Site Selection:

The research was conducted during the 2010 winter season at James Arthur Vineyards located at Raymond, Nebraska in Lancaster County.

Edelweiss Vines:

Treatments were applied on 13 year old vines. The vines are trained using the Geneva Double Curtain trellis system. Planting distances were 8 feet (2.44 m) between plants and 12 feet (3.66 m) between rows.

Experiment:

The experiment consisted of five treatments: NAA (500, 1000, and 1500 mg/l) purchased from Phyto Technology Laboratories, 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. NAA concentrations were prepared by weighing out 0.5, 1, and 1.5 g, respectively. NAA was dissolved by adding a few drops of 1M NaOH and the volume was completed to 1000 ml by adding distilled water. The pH of each of the NAA solutions was measured and readjusted to about 7 by adding a few drops of 1M HCL**.** A randomized complete block design was used with three blocks of 15 vines each. There were three application dates: Jan. 28, Feb. 25, and Mar. 25, 2010. Treatments were applied on one year old canes. The whole vine was sprayed using a hand sprayer with

each vine receiving approximately 1 L. For observation purposes only, five random vines were sprayed on February 25 and March 25 to observe the effects of double applications.

Pruning:

The treated vines were pruned on March 30, 2010. The total number of buds/vine was recorded to determine when 50% of the total buds showed bud break.

Bud Break:

In the spring of 2010, vines were visually evaluated for bud break. Bud break was determined as stage five of the Eichhorn and Lorenz (1997) scale of grapevine development. Stage five indicates that the bud scales have expanded to the point at which a green shoot is visible. 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 January 1, 2010 until 50% bud break was achieved was used as the basis of calculating number of days for bud break.

Weather Data:

Temperatures for Raymond, Nebraska throughout the year of 2010 were obtained from the High Plains Regional Climate Center, University of Nebraska, Lincoln.

Harvesting and Berry Samples:

On August 11, 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.

Berry Analysis:

On August 18, 2010 berry samples were analyzed for pH, °Brix, and titratable acidity (TA). The 30 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. TA was determined by titration with NaOH, using the procedure of Dharmadhikari and Wilker (2001).

Statistical Analysis:

All statistical analyses were performed using SAS/STAT Version 9.2 and Analysis of Variance was conducted by the PROC GLIMMIX procedure.

Results and Discussion:

Bud Break:

Unlike the study in 2009, there was no significant treatment by month interaction but a significant treatment effect was present at ($P \leq 0.05$) (Table 15).

Table 15: Analysis of variance table for number of days to show 50% bud break of 13-year-old 'Edelweiss' grapevines:

Effect	**DF**	**F Value**	**Pr > F**
TRT	4	20.78	<.0001
MONTH	2	1.40	0.26
TRT*MONTH	8	1.51	0.20

The oil treatment significantly delayed bud break up to 12 days compared to the control. Furthermore, it significantly delayed bud break four and five days compared to NAA 1500 ppm and 1000 ppm, respectively (Table 16). No significant differences were found between NAA 500 ppm treatment and the control but there were significant differences between NAA 1500 ppm, NAA 1000 ppm and NAA 500 ppm (Table 16). See Appendix 13 regarding the number of days delaying bud break in 2010.

Table 16: Least Significant Difference Test (L.S.D.) for average number days of bud break for 13-year-old 'Edelweiss' grapevines:

Treatment	Average Number Days to Bud Break
Oil	122.22±0.70 a
NAA 1500 ppm	118.44±0.70 b
NAA 1000 ppm	117.33±0.70 b
NAA 500 ppm	115.22±0.70 c
Control	114.00±0.70 c

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

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. Although there was no month effect on bud break, March overall applications delayed one to two days more than February and January. Regarding double spraying observations, the five selected vines showed bud break after 122 Julian Days, so they were in the same range as for single applications. It is interesting to mention that Seth McFarland of Mac's Creek Winery and Vineyards in Lexington, Nebraska obtained his results from several applications meanwhile, Dami and Beam (2004) obtained results from single applications. Nevertheless, this aspect requires further investigation.

Number of Clusters per Vine:

Table 17 shows no effects on the total number of clusters per vine at ($P \leq 0.05$). Similar results were obtained when total number of clusters per vine of the 2009 study was counted in 2010 to study cumulative effects of treatments. Total number of clusters per vine ranged from 12 to 19 clusters per vine (see Appendix 14). This range is almost similar to the range (12 to 17 clusters) detected when number of clusters was counted in 2010 of the 2009 study to observe any cumulative treatment effects on the vine. In addition, similar analysis of variance was 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 (Figure 5). It is important to notice the low number of clusters per vine in 2010 because such production is not acceptable to grape growers.

Table 17: Analysis of variance table for number of clusters in 13-year-old 'Edelweiss' grapevines:

Effect	**DF**	**F Value**	**Pr > F**
TRT	4	0.26	0.90
MONTH	2	1.41	0.26
TRT*MONTH	8	0.35	0.94

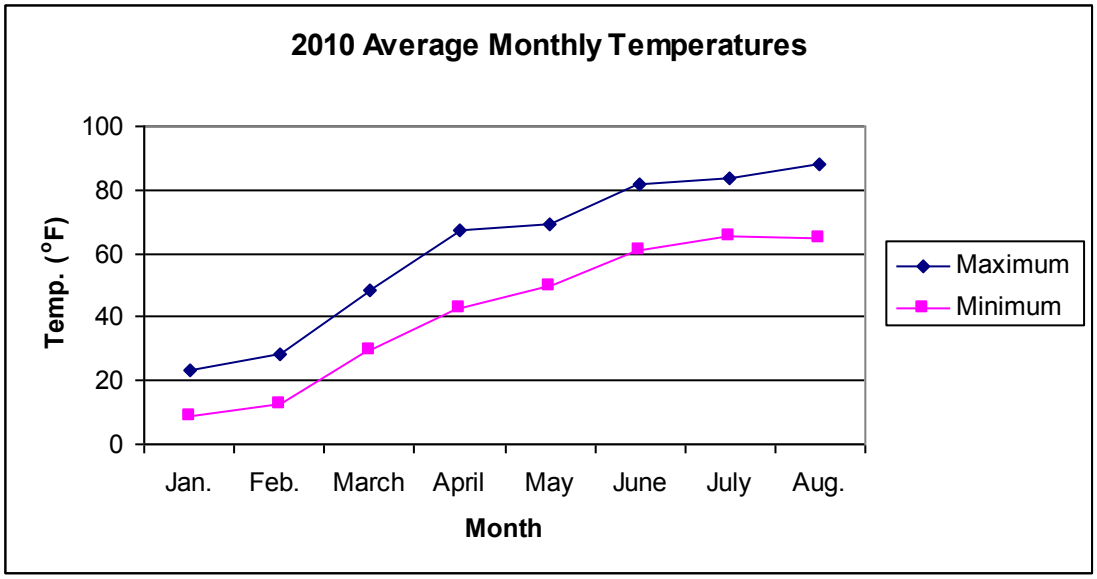


Figure 5: The maximum and minimum monthly average temperatures during 2010. Source: High Plains Regional Climate Center.

According to the High Plains Regional Climate Center, normal precipitation for Raymond, Nebraska is 4.76 and 3.74 inches (121 mm and 95 mm) for May and June, respectively. In 2010, monthly precipitation in June was 9.81 inches (249 mm) which is almost three times the monthly average. This had a negative impact on 'Edelweiss' fruit set and explains the low number of clusters produced per vine. In fact, 'Edelweiss' yields harvested at James Arthur Vineyards were 8 tons/acre in 2009 and only 3 tons/acre in 2010. Creasy and Creasy (2009) mentioned that the most important factors that affect fruit set percentage are: availability of light, moderate temperatures, and dry weather. The vines showed vigorous vegetative growth in 2010 with low number of clusters as shown in Figure 6. Weaver (1976) noted that calyptras may not fall in cold rainy weather and this will reduce the amount of fruit set.

Figure 6: 'Edelweiss' vines at James Arthur Vineyards, Raymond, Nebraska showing vigorous vegetative growth in summer 2010.

In addition to high precipitation, vigorous growth possibly affected the availability of light even with the GDC training system which provides higher light transmittance than other trellising systems (Huck, 2009). Thus, low yields were due to the wet weather and vigorous vegetative growth which reduces availability of light.

Weight of Clusters:

No significant interaction, month, or treatment effect at ($P \leq 0.05$) was found in 'Edelweiss' cluster weights (Table 18). Cluster weights ranged from 2.26 to 3.65 kg (see Appendix 15).

Table 18: Analysis of variance table for cluster weights of 13-year-old 'Edelweiss' grapevines:

Effect	DF	F Value	Pr > F
TRT	4	0.24	0.91
MONTH	2	1.32	0.28
TRT*MONTH	8	0.38	0.92

Berry Analysis:

Analysis of variance for berry weights showed a similar trend in 2010 as in 2009. No treatment by month interaction, month, or treatment effects were present at ($P \leq 0.05$) (Table 19). Berry sample weights ranged from 97.94 to 104.45 g (see Appendix 16).

Table 19: Analysis of variance table for weight of 30 berries sampled from 13-year-old 'Edelweiss' grapevines:

Effect	DF	F Value	Pr > F
TRT	4	0.47	0.76
MONTH	2	1.13	0.34
TRT*MONTH	8	1.89	0.10

Tables 20, 21, and 22 show analysis of variance regarding berry characteristics °Brix, pH and TA, respectively. Similar trends were present in 2010 °Brix and TA results with no treatment by month interaction, month, or treatment effects at ($P \leq 0.05$).

Regarding pH, unlike 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 12.73 to 13.47, pH values were 3.26 to 3.41, and TA values were 1.1 to 1.4 g/100ml (see Appendices 17, 18, and 19). °Brix ranges were higher in 2010 which was expected since average July temperatures were 80.9 °F (27.16 °C) and 83.9 °F (28.83 °C) in 2009 and 2010, respectively. Regarding pH and TA, pH 2010 results are in the same as range recommended (3.2-3.4) by Dharmadhikari and Wilker (2001), but were a little higher than the recommended TA ranges (0.7-0.9%). TA is probably the least important parameter to wine makers as discussed earlier in Chapter 1.

Table 20: Analysis of variance table for °Brix of berries sampled from 13-year-old 'Edelweiss' grapevines:

Effect	**DF**	**F Value**	**Pr > F**
TRT	4	0.27	0.89
MONTH	2	0.18	0.84
TRT*MONTH	8	0.55	0.81

Table 21: Analysis of variance table for pH of berries sampled from 13-year-old 'Edelweiss' grapevines:

Effect	**DF**	**F Value**	**Pr > F**
TRT	4	0.40	0.81
MONTH	2	4.16	0.03
TRT*MONTH	8	1.41	0.23

Table 22: Analysis of variance table for TA of berries sampled from 13-year-old 'Edelweiss' grapevines:

Effect	**DF**	**F Value**	**Pr > F**
TRT	4	0.64	0.64
MONTH	2	2.92	0.07
TRT*MONTH	8	0.54	0.82

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 is important for recommendation purposes. In spite of that, no significant differences were found between months Table 23.

Table 23: Least Significant Difference Test (L.S.D.) for pH of berry samples in 2010:

Month	**Average pH**
February	3.37±0.02 a
January	3.34±0.02 ab
March	3.29±0.02 b

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

References:

Creasy, G. and L. Creasy. 2009. Grapes. Crop Production Science in Horticulture. CABI, Cambridge, MA.

Dami, I. and B. Beam. 2004. Response of grapevines to soybean oil application. American Journal of Enology & Viticulture 55 (3): 269-275.

Dharmadhikari, M. and K. Wilker. 2001. Micro Vinification: A practical guide to small-scale wine production. Missouri State Fruit Experiment Station, Mountain Grove, Missouri.

Eichhorn, K., Lorenz, D. 1977. Phänologische Entwicklungsstadien der Rebe. Nachrichtenbl. Deut. Pflanzenschutz 29:119-120.

Friend, A., C. Stushnoff, G. Creasy and M. Trought. 2001. Manipulating bud break date in grapevines. In Proceedings of the ASEV 52nd Anniversary Annual Meeting. Page 16 (Abstract).

Huck, C. 2009. Training system effects on sunlight penetration, canopy structure and fruit composition of 'Frontenac' grape (*Vitis* spp.). M. Sc. Dissertation, University of Nebraska, Lincoln.

Weaver, R. 1976. Grape Growing. John Wiley & Sons, Inc.

CHAPTER 3

Forcing Solution Experiment 2009

Materials and Methods:

Plant Material:

Dormant canes of 'Edelweiss' grapevines were collected from James Arthur Vineyards in Raymond, Nebraska on four dates: Jan. 6, Feb. 3, Mar. 3, and Apr. 1, 2009. For each date, 20 canes were randomly selected, similar in diameter and length, and headed back to the first five buds. Each cane was then cut into five single-bud cuttings. A 5 x 5 Latin Square design was used for this experiment. The single-bud cuttings were soaked in a solution containing 10 % Clorox for 15 seconds and then rinsed with tap water. The identity of the bud position was retained using four different colored tapes and the control without any tape. The single-bud cuttings with tags were placed in plastic storage bags and placed in the cooler at 4-5 °C prior to treatment the following day.

Preparing Forcing Solutions:

A stock of the forcing solution developed by Read et al., (1984) containing 200 mg 8-hydroxyquinoline citrate (8-HQC)/l and 2% sucrose was prepared by weighing out 0.2 g 8-HQC and 20 g of sucrose and adding distilled water to reach 1000 ml.

GA7 Containers:

The GA7 containers into which the forcing solution treatments were to be placed were autoclaved at 121 °C for 20 minutes.

Treatments:

The experiment consisted of five treatments: NAA (500, 750, and 1000 mg/l) purchased from Phyto Technology Laboratories, Amigo Oil (Loveland Industries, Greely, CO) applied at 10% v/v which consisted of 9.3 % oil and 0.7% emulsifier, and the non-treated control. NAA concentrations were prepared as described earlier. Treatments were applied on buds by adding one drop per bud using a sterile transfer pipette. After treatment, the single-bud canes were placed vertically (proximal ends down) in GA7 containers containing 100 ml of forcing solution as shown in Figure 7. The solutions were replaced with 100 ml of freshly prepared forcing solution every four days and the basal 0.2 cm ends of the cuttings were cut off each time the solutions were changed. The GA7 containers were placed in a room where the temperature was 25 °C. Days to bud break starting from the date of treatment and shoot length after one week of bud break were recorded throughout the study as shown in Figure 8. Buds that did not show bud break were cut into longitudinal sections and examined under a stereomicroscope to examine the viability of the bud and any phytotoxity effect of any of the treatments.

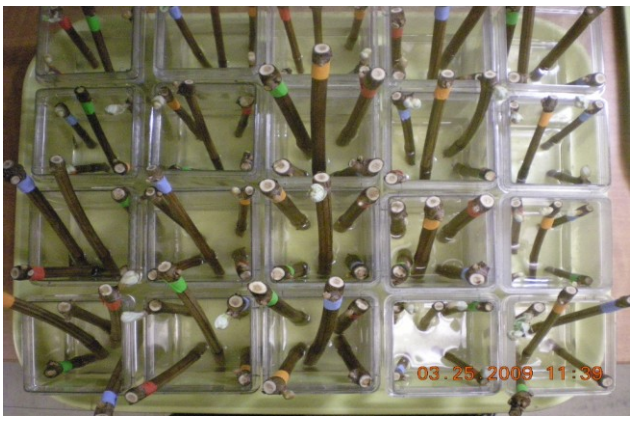


Figure 7: 'Edelweiss' single-bud cuttings placed in GA7 containers containing forcing solution.

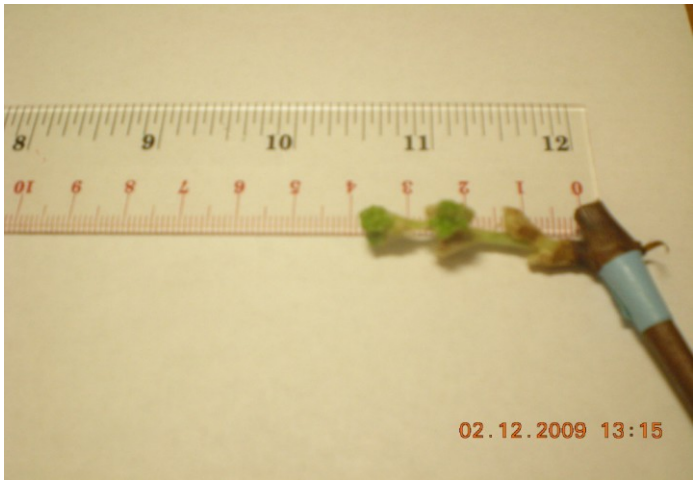


Figure 8: Measuring shoot length of forced ‘Edelweiss’ single-bud cuttings after one week of bud break.

Forcing Solution Experiment 2010

Materials and Methods:

Plant Material:

Dormant canes of 'Edelweiss' grapevines were collected from James Arthur Vineyards in Raymond, Nebraska on three dates: Jan. 28, Feb. 25, and Mar. 25, 2010. For each date, 20 canes were randomly selected, similar in diameter and length, and headed back to the first five buds. Each cane was then cut into five single-bud cuttings. A 5 x 5 Latin Square design was used for this experiment. The single-bud cuttings were soaked in a solution containing 10 % Clorox for 15 seconds and then rinsed with tap water. The identity of the bud position was retained using four different colored tapes and the control without any tape. The single-bud cuttings with tags were placed in plastic storage bags and placed in the cooler at 4-5 °C prior to treatment the following day.

Preparing Forcing Solutions:

A stock of the forcing solution developed by Read et al., (1984) containing 200 mg 8-hydroxyquinoline citrate (8-HQC)/l and 2% sucrose was prepared by weighing out 0.2 g 8-HQC and 20 g of sucrose and adding distilled water to reach 1000 ml.

GA7 Containers:

The GA7 containers into which the forcing solution treatments were to be placed were autoclaved at 121 °C for 20 minutes.

Treatments:

The experiment consisted of five treatments: NAA (500, 1000, and 1500 mg/l) purchased from Phyto Technology Laboratories, Amigo Oil (Loveland Industries, Greely, CO) applied at 10% v/v which consisted of 9.3 % oil and 0.7% emulsifier, and the non-treated control. NAA concentrations were prepared as described earlier. Treatments were applied on buds by adding one drop per bud using a sterile transfer pipette. After treatment, the single-bud canes were placed vertically (proximal ends down) in GA7 containers containing 100 ml of forcing solution. The solutions were replaced with 100 ml of freshly prepared forcing solution every four days and the basal 0.2 cm ends of the cuttings were cut off each time the solutions were changed as shown in Figure 9. The GA7 containers were placed in a room where the temperature was 25 °C. Days to bud break starting from the date of treatment and shoot length after one week of bud break were recorded throughout the study. Buds that did not show bud break were cut into longitudinal sections and examined under a stereomicroscope to examine the viability of the bud and any phytotoxity effect of any of the treatments.

Figure 9: Issam Qrunfleh cutting the basal ends of 'Edelweiss' single-bud cuttings and changing the forcing solution.

Statistical Analysis:

All statistical analyses were performed using SAS/STAT Version 9.2 and ANOVA was conducted by the PROC GLIMMIX procedure.

Results and Discussion:

Bud Break in Forcing Solution Experiment 2009:

A total of 400 single-bud cuttings were used in this experiment. Only the single-bud cuttings treated with oil showed missing data values. Among the 20 single-bud cuttings in January, February, March, and April (a total of 80), only 12 showed bud break stage five in April. Some of the single-bud cuttings showed only bud swelling and others showed no growth symptoms at all (Figure 10).

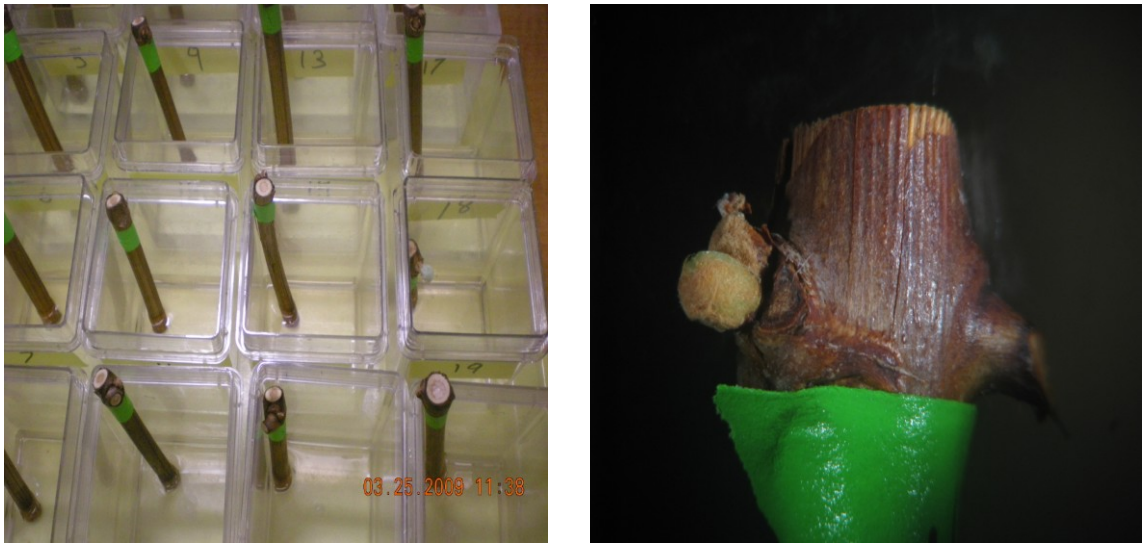


Figure 10: 'Edelweiss' single-bud cuttings treated with oil showing no bud break (left) and some bud swelling (right).

Dami and Beam (2004) reported 6-10% bud injury with Prime Oil and 4-5% with Amigo Oil. They found no differences between Amigo Oil treated vines and the controls which led them to conclude that only Prime Oil was phytotoxic to the grapevines studied. Furthermore, they noticed that November treated vines showed the most injury. In addition, a bud position effect in 'Chambourcin' was detected showing that basal buds had more injury than apical buds (Dami and Beam, 2004). They considered buds one to

five as basal buds, the same bud positions used in this study. No explanation was given for their results.

Buds that did not show bud break were longitudinally sectioned and examined under a stereomicroscope to determine bud viability and any treatment phytotoxity. Total number of buds, position, and the cane number from which they were taken are presented in Table 24:

Month	**Cane**	**Bud Position**	**Total Number of Buds**
January	14, 15	1, 4	2
February	7, 15	2, 4	2
March	8, 14	4, 1	2
April	1, 10	4, 3	2
Total			8

Table 24: Month, cane number, bud position on cane, and total number of 'Edelweiss' single-bud that showed oil phytotoxicity damage in 2009.

Total number of buds that were treated with oil was 80. Phytotoxity damage as shown in Figure 11 is 10% which may be because these cuttings were placed inside with no environmental factors to help break down oil effects.

Figure 11: ‘Edelweiss’ single-bud cuttings treated with oil showing phytotoxity and primary bud damage.

The remaining buds showed no phytotoxity symptoms and a live primary bud as shown in Figure 12.

Figure 12: ‘Edelweiss’ single-bud cuttings treated with oil showing no phytotoxity and a live primary bud.

Lavee and May (1997) mentioned that reasons for no growth could be explained by: physical or chemical conditions external to the bud, restriction by enclosing bract tissues, or correlative inhibition. Our expectation is that the single-bud cuttings were

taken too early because grapevines are in the endodormant stage in January (Gu, 2003). Another contributing factor is that these cuttings probably did not receive the adequate chilling hours especially considering that average monthly temperatures in January and February were 33.4 °F (0.7 °C) and 43.5 °F (6.4 °C), respectively. “It is well known that grapevine buds can remain dormant for long periods without losing their vitality” (Lavee and May, 1997).

Analysis of variance for number of days to show bud break are shown in Table 25.

Effect	**DF**	**F Value**	**Pr > F**
MONTH	3	112.49	<.0001
POSITION	4	2.17	0.075
MONTH*POSITION	12	2.39	0.01
TRT	4	19.75	<.0001
MONTH*TRT	9	2.18	0.03
POSITION*TRT	16	3.96	<.0001
MONTH*POSITION*TRT	36	2.07	0.0011

Table 25: Analysis of variance table for number of days to show stage five bud break in the 2009 forcing solution experiment for ‘Edelweiss’ single-bud cuttings.

A significant month by position by treatment interaction was detected at ($P \leq 0.05$) as shown in Table 25. Single-bud cuttings at bud position five treated with NAA at 1000 ppm clearly showed a delay in bud break (Figure 13).

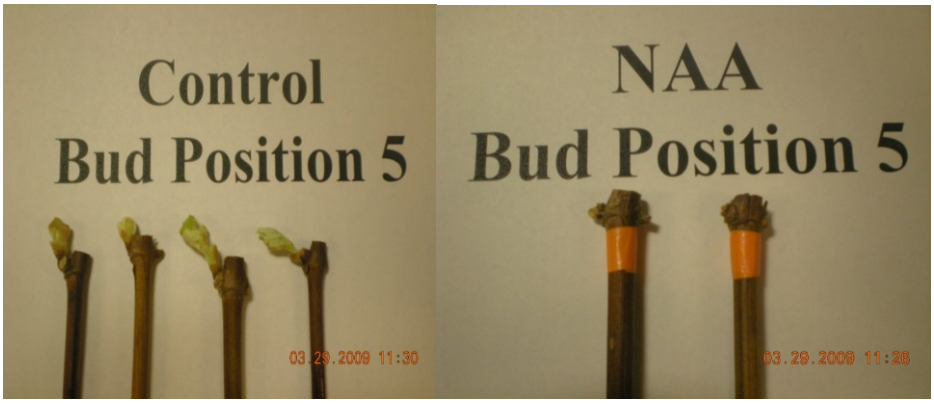


Figure 13: 'Edelweiss' single-bud cuttings at bud position five showing a delay in bud break by using NAA at 1000 ppm.

Interest of this experiment was to examine the capability of treatments to delay bud break mainly at bud position five in the presence of a forcing solution. Grape buds normally show bud break starting from distal toward proximal ends. A comparison between NAA at 1000 ppm and the control at bud position five is shown in Figure 14.

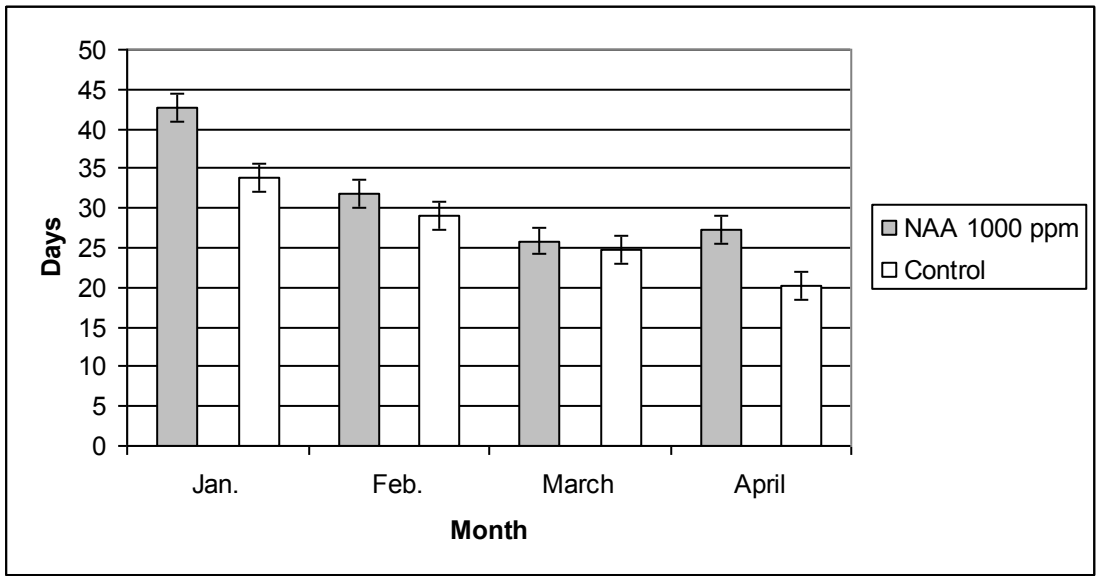


Figure 14: Number of days required by 'Edelweiss' single-bud cuttings to show bud break at bud position five treated with NAA at 1000 ppm and the control in 2009.

Figure 14 shows that there was a significant difference in bud break delay times nine days in January and seven days in April at ($P \leq 0.05$). Obtaining such results in January could be because the treatment was most effective in the deepest endodormant stage as shown in other studies (Kubota and Miyamuki, 1992; Kurooka et al., 1990) or the delay was because single-bud cuttings were taken too early and that they did not receive enough chilling hours.

Shoot Length in Forcing Solution Experiment 2009:

Analysis of variance for shoot length after one week of bud break only shows a significant monthly effect (Table 26):

Effect	**DF**	**F Value**	**Pr > F**
MONTH	3	61.11	<.0001
POSITION	4	0.88	0.48
MONTH*POSITION	12	1.79	0.05
TRT	4	1.64	0.17
MONTH*TRT	9	1.14	0.34
POSITION*TRT	16	0.95	0.51
MONTH*POSITION*TRT	36	1.10	0.34

Table 26: Analysis of variance table for shoot length of 'Edelweiss' single-bud cuttings after one week of bud break.

Average shoot lengths from January, February, March, and April treatments were 2.64, 6.07, 4.86, and 3.74 cm, respectively. Shorter shoot lengths were obtained from

single-bud cuttings taken early in January. This is also an indication that the vines were at the endodormant stage and had not received adequate chilling hours as mentioned previously. Overall winter temperatures in 2009 were warmer than normal as explained and displayed earlier in Figure 4. Most importantly, there was no treatment effect which also gives support that such applications seem to have no effect on the vegetative growth.

Bud Break in Forcing Solution Experiment 2010:

Similarly to 2009, analysis of variance shows a significant month by position by treatment interaction at ($P \leq 0.05$) as shown in Table 27.

Effect	DF	F Value	Pr > F
MONTH	2	80.4	<.0001
POSITION	4	3.5	0.01
MONTH*POSITION	8	1.37	0.21
TRT	4	7.18	<.0001
MONTH*TRT	7	0.74	0.64
POSITION*TRT	16	1.1	0.36
MONTH*POSITION*TRT	27	1.92	0.01

Table 27: Analysis of variance table for number of days to show stage five bud break in the 2010 forcing solution experiment for 'Edelweiss' single bud cuttings.

For this study, a total of 300 single-bud cuttings were used. Only the single-bud cuttings treated with oil showed missing values. Among the 20 single-bud cuttings collected and treated in January, February, and March, only 11 showed bud break stage

five from February treatments and 10 in March. No bud break was observed in January. Similarly, buds that did not show bud break were longitudinally sectioned and examined under a stereomicroscope. Total number of buds, position, and the cane number from which they were taken are presented in Table 28:

Month	Cane	Bud Position	Total Number of Buds
January	7, 8, 12	2, 4, 5	3
February	3, 9	2, 1	2
March	19	1	1
Total			6

Table 28: Month, cane number, bud position on cane, and total number of 'Edelweiss' single-bud that showed oil phytotoxicity damage in 2010.

Total number of buds that were treated with oil was 60. The phytotoxity damage was also 10%. In order to explain failure of single-bud treated with oil, xylem and phloem tissues were examined under a stereomicroscope. Possible failure could be attributed to problems in the conducting tissues. However, examination showed normal xylem and phloem tissues as shown in Figure 15.

Figure 15: A cross section of one of the 'Edelweiss' single-bud cuttings treated with oil showing normal xylem and phloem tissues.

Single-bud cuttings treated with oil that did not show bud break in 2009 and 2010 could be explained by bud scale and oil effects on bud respiration and cell membranes. Bud scales were shown to be involved in dormancy (Iwasaki, 1980 and Iwasaki and Weaver, 1977). Respiration was shown to be decreased with oil applications (Dami and Beam, 2004). Furthermore, there is also a possibility that oil applications are affecting cell membranes, since membrane lipids were also found to be involved in dormancy (Erez, 2000; Izadyar and Wang, 1999; and Wang and Faust, 1990). Further studies are required to prove this interpretation.

Single-bud cuttings treated with oil only showed a 10% phytotoxity damage in both years which is probably explained by the indoor placement of the cuttings without any environmental factors that could help in breaking down oil effects. On the other hand, the remaining buds showed a primary living bud which is a critical issue when applying chemicals on vines to delay bud break. Figure 16 shows a delay in bud break at bud position five by using oil applications.

Figure 16: 'Edelweiss' single-bud cuttings showing no bud break at bud position five by using oil applications (far right) and the non-treated control at bud position one (far left) showing more advanced bud break stage than NAA at the three concentrations applied 500 (blue tag), 1000 (red tag), and 1500 ppm (orange tag).

NAA at 1500 ppm was also effective in delaying bud break at bud position five (Figure 17). Similarly to NAA at 1000 ppm in the 2009 study.

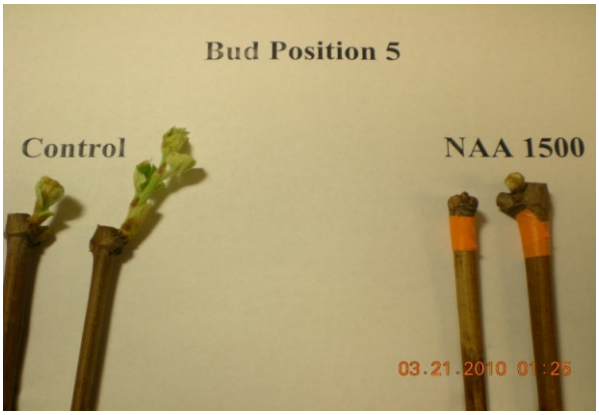


Figure 17: 'Edelweiss' single-bud cuttings at bud position five showing a delay in bud break by using NAA at 1500 ppm compared to the non-treated control.

Similarly, a comparison between NAA at 1000 ppm and the control at bud position five is shown in Figure 18. A similar comparison between NAA at 1500 ppm is shown in Figure 19.

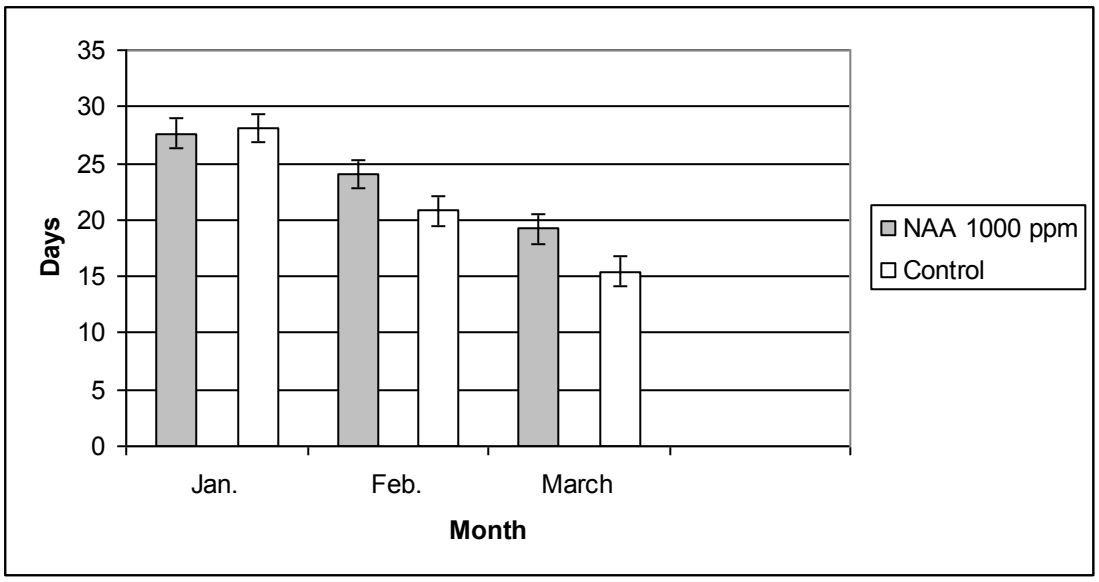


Figure 18: Number of days required by single-bud cuttings to show bud break at bud position five treated with NAA at 1000 ppm and the control in 2010.

In January, NAA at 1000 ppm failed significantly to delay bud break at bud position five as shown in Figure 18. This supports the interpretation of January 2009 results where it was assumed that results were related to time of taking cuttings and inadequate chilling hours. However, NAA at 1000 ppm significantly delayed bud break three days where cuttings were treated in February and four days when treated in March. A similar trend was shown by NAA at 1500 ppm, where it failed to significantly delay bud break in January but significantly delayed bud break four days with February treatments and five days with March treatments.

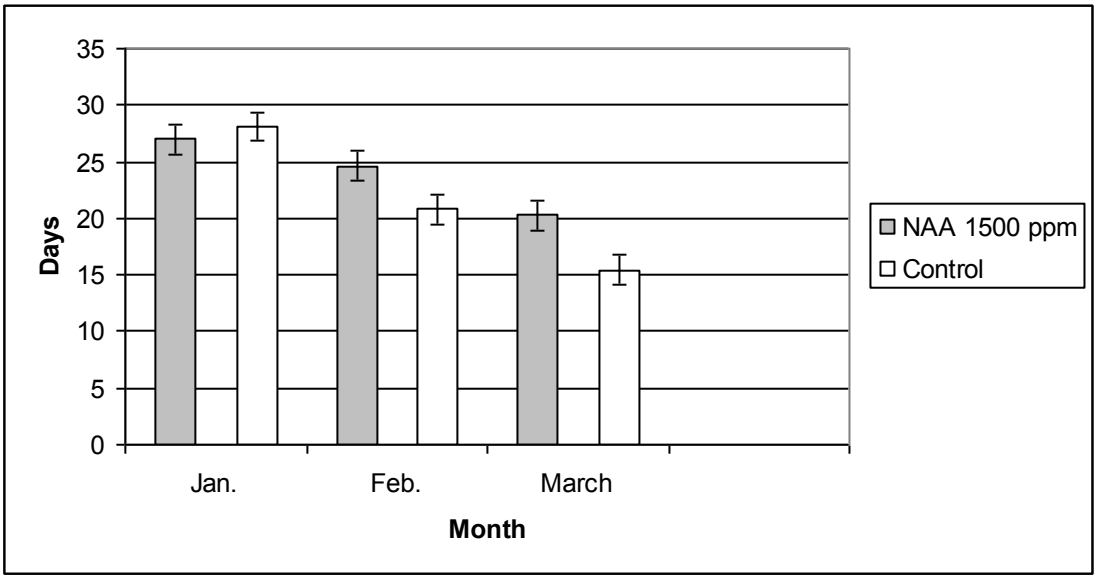


Figure 19: Number of days required by single-bud cuttings to show bud break at bud position five treated with NAA at 1500 ppm and the control in 2010.

Shoot Length in Forcing Solution Experiment 2010:

Analysis of variance shows only a month by treatment interaction at ($P \leq 0.05$) (Table 29).

Effect	DF	F Value	Pr > F
MONTH	2	0.29	0.75
POSITION	4	0.99	0.41
MONTH*POSITION	8	0.52	0.84
TRT	4	2.28	0.06
MONTH*TRT	7	2.23	0.04
POSITION*TRT	16	0.73	0.76
MONTH*POSITION*TRT	27	1.02	0.45

Table 29: Analysis of variance table for shoot length of 'Edelweiss' single-bud cuttings after one week of bud break.

Average shoot lengths in January, February, and March were 5.01, 4.71, and 4.78 cm, respectively. Differences in shoot length results for both years are probably because of different winter weathers and the time of taking cuttings.

References:

Dami, I. and B. Beam. 2004. Response of grapevines to soybean oil application. American Journal of Enology & Viticulture 55 (3): 269-275.

Erez, A. 2000. Bud dormancy: a suggestion for the control mechanism and its evolution. In J. D. Viémont and J. Crabbé (Editors) "Dormancy in Plants: From Whole Plant Behaviour to Cellular Control".

Gu, S. 2003. Rootstock and mounding effect on growth and cold hardiness of 'Gewürztraminer' (*Vitis vinifera*) and bud dormancy of 'Lacrosse' and 'Chambourcin' (*Vitis* Spp.). Ph D. Dissertation, University of Nebraska, Lincoln.

Iwasaki, K. 1980. Effects of bud scale removal, calcium cyanamide, GA_3, and ethephone of 'Muscat of Alexandria' grape (*Vitis vinifera* L.). J. Japan. Soc. Hort. Sci. 48 (4): 395-398.

Iwasaki, K. and R. Weaver. 1977. Effects of chilling, calcium cyanamide, and bud scale removal on bud break, rooting, and inhibitor content of buds of 'Zinfandel' grape (*Vitis vinifera* L.). J. Amer. Soc. Hort. Sci. 102 (5): 584-587.

Izadyar, A. and S. Wang. 1999. Changes of lipid components during dormancy in 'Hull Thornless' and 'Triple Crown Thornless' blackberry cultivars. Scientia Horticulturae 82: 243-254.

Kubota, N. and M. Miyamuki. 1992. Breaking dormancy in grapevines with garlic paste. J. Amer. Soc. Hort. Sci. 117 (6): 898-901.

Kurooka, H., S. Horiuchi, S. Fukunga, and E. Yuda. 1990. Effects of electric current on breaking bud dormancy in grapes. Bulletin of the University of Osaka Prefecture 42: 111-119.

Lavee, S. and P. May. 1997. Dormancy of grapevine buds – facts and speculation. Australian Journal of Grape and Wine Research 3: 31-46.

Read, P., A. Economou and C. Fellman. 1984. Manipulating stock plants for improved in vitro mass propagation. Proc. Int. Symp. Plant Tissue and Cell Culture: Application to Crop Improvement. Novak, J., L. Havel, and J. Dolezel (editors). Czech. Acad. Sci. Prague: 467-473.

Wang, S. and M. Faust. 1990. Changes of membrane lipids in apple buds during dormancy and budbreak. J. Amer. Soc. Hort. Sci. 115 (5): 803-808.

Conclusions:

1. Delaying pruning until March, especially for cultivars that show early bud break such as 'Edelweiss' will delay bud break.
2. Amigo Oil did not exhibit the 20-day delay reported by Dami and Beam (2004) using 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).
3. Amigo Oil showed better performance compared to NAA, even at higher NAA concentrations. It delayed bud break slightly longer (four to five days) and did not affect either quantity or quality of fruit produced. NAA at 1000 and 1500 ppm showed similar potentials of Amigo Oil in delaying bud break.
4. Oil applications and NAA at 1000 to 1500 ppm in March and up to early April, could give grape growers acceptable delay of bud break. This is based on the performance of both oil and NAA applications in the field experiments and the forcing solution studies.
5. In forcing solution studies, oil and NAA treatments did delay bud break of 'Edelweiss' single-bud cuttings placed in forcing solution.
6. NAA applications at 1000 ppm in April significantly delayed bud break seven days. In the 2010 forcing solution experiment, bud break was significantly delayed four days by NAA at 1000 ppm and five days by NAA at 1500 ppm with March applications. One of the possible drawbacks found when using Amigo Oil observed in the forcing solution studies was phytotoxity to the treated buds.

7. Delaying bud break shows no negative impact on berry characteristics. Although differences within climate canopy were not measured, berry characteristics appeared to vary more depending on vine sampling location than whether bud break was delayed or not. Research has shown that climate within the canopy is significantly important regarding berry maturity and characteristics.

As a result of this research, it can be recommended to use Amigo Oil at 10% or NAA at 1000 to 1500 ppm in March to April for sites that prone to frost events such as 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. The question that could arise would be which is more affordable for grape growers? Assuming that planting distances are 8 x 12 feet, this means that there will be 460 grapevines in one acre. Assuming that every vine should receive one liter, this means that applications would require 460 liters and since oil is applied at 10%, a total of 46 liters would be required for one acre. Since oil price is $9.21 per gallon, and the gallon is almost four liters, the price of spraying one acre would be almost $106. Assuming that NAA would be applied at 1000 ppm and that the price of 500 grams is $25, the price of spraying one acre would be almost $23. Furthermore, this study opens doors for future studies regarding the value of repeat spraying or mixing Amigo Oil with NAA. In addition to that, investigating phytotoxicity damage to buds caused by oil applications that could possibly occur under vineyard conditions is warranted.

Appendices:

Appendix 1: Eichhorn and Lorenz Bud Growth Stages

Source: Eichhorn and Lorenz (1977)

1. Winter dormancy: Winter buds pointed to rounded, bright or dark brown according to cultivar; bud scales more or less closed according to cultivar.

2. Bud swelling: Buds expand inside the bud scales.

3. Wool stage: Brown wool clearly visible (doeskin).

5. Bud burst: Green shoot first clearly visible.

7. First leaf unfolded and spread away from shoot.

9. Two to three leaves unfolded.

12. Five to six leaves unfolded; inflorescence clearly visible.

15. Inflorescence swelling; flowers closely pressed together.

17. Inflorescence fully developed; flowers separating.

19. Beginning of flowering: First flowerhoods (calyptra) falling.

21. Early flowering: 25% of flowerhoods fallen.

23. Full flowering: 50% of flowerhoods fallen.

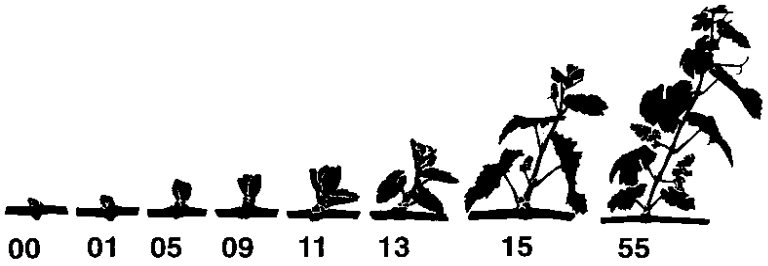
25. Late flowering: 80% of flowerhoods fallen.

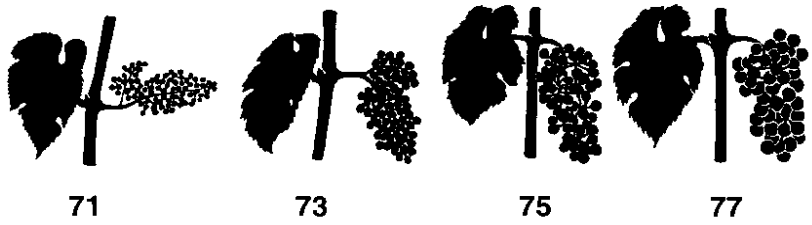
27. Fruit set: Young fruits begin to swell; remains of flowers lost (shatter).

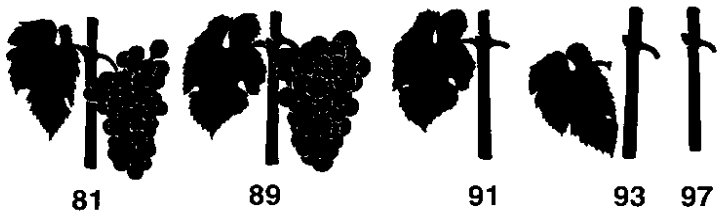
29. Berries BB-sized; bunches begin to hang.

31. Berries pea-sized; bunches hang.

Appendix 2: Meier Bud Growth Stages







Source: Meier (2001).

Appendix 3:

Dates of last spring frost and temperatures recorded at Lincoln Municipal Airport for the period 1990-2010:

Year	Spring Frost Date	Temperature (°F)	Year	Spring Frost Date	Temperature (°F)
1990	May 1	30	2001	April 18	29
1991	April 1	31	2002	April 25	29
1992	May 6	29	2003	April 22	30
1993	April 17	32	2004	May 3	26
1994	May 1	24	2005	May 3	27
1995	May 2	31	2006	April 9	32
1996	April 30	32	2007	April 15	28
1997	May 13	27	2008	April 28	30
1998	April 18	31	2009	April 28	31
1999	April 18	26	2010	April 19	32
2000	April 21	29	Source: High Plains Regional Climate Center		

Appendix 4: Treatment by month mean delay in bud break in 2009 (Julian Calendar Days):

Treatment	Month of Treatment	Bud Break Days
Control	January	125.00±0.78
Control	February	124.00±0.78
Control	March	126.00±0.78
Control	April	125.50±0.78
NAA 1000 ppm	January	128.00±0.78
NAA 1000 ppm	February	129.00±0.78
NAA 1000 ppm	March	128.33±0.78
NAA 1000 ppm	April	127.67±0.78
NAA 500 ppm	January	126.00±0.78
NAA 500 ppm	February	124.50±0.78
NAA 500 ppm	March	126.00±0.78
NAA 500 ppm	April	125.00±0.78
NAA 750 ppm	January	126.50±0.78
NAA 750 ppm	February	126.00±0.78
NAA 750 ppm	March	127.00±0.78
NAA 750 ppm	April	126.00±0.78
Oil	January	130.00±0.78
Oil	February	129.00±0.78
Oil	March	126.50±0.78
Oil	April	129.67±0.78

Appendix 5: Treatment by month mean number of clusters of the two canes selected in 2009:

Treatment	Month of Treatment	Number of Clusters
Control	January	6.67±1.03
Control	February	5.67±1.03
Control	March	7.50±1.03
Control	April	5.67±1.03
NAA 1000 ppm	January	7.67±1.03
NAA 1000 ppm	February	5.67±1.03
NAA 1000 ppm	March	6.83±1.03
NAA 1000 ppm	April	8.00±1.03
NAA 500 ppm	January	8.17±1.03
NAA 500 ppm	February	5.50±1.03
NAA 500 ppm	March	7.67±1.03
NAA 500 ppm	April	8.00±1.03
NAA 750 ppm	January	6.83±1.03
NAA 750 ppm	February	6.33±1.03
NAA 750 ppm	March	6.67±1.03
NAA 750 ppm	April	7.50±1.03
Oil	January	5.50±1.03
Oil	February	5.83±1.03
Oil	March	7.50±1.03
Oil	April	7.33±1.03

Appendix 6: Treatment by month mean cluster weights (kg) of the two canes selected in 2009:

Treatment	Month of Treatment	Cluster Weights (kg)
Control	January	1.53±0.26
Control	February	1.50±0.26
Control	March	1.92±0.26
Control	April	1.33±0.26
NAA 1000 ppm	January	1.78±0.26
NAA 1000 ppm	February	1.44±0.26
NAA 1000 ppm	March	1.58±0.26
NAA 1000 ppm	April	1.84±0.26
NAA 500 ppm	January	2.22±0.26
NAA 500 ppm	February	1.75±0.26
NAA 500 ppm	March	1.90±0.26
NAA 500 ppm	April	2.03±0.26
NAA 750 ppm	January	1.97±0.26
NAA 750 ppm	February	1.45±0.26
NAA 750 ppm	March	1.52±0.26
NAA 750 ppm	April	1.88±0.26
Oil	January	1.34±0.26
Oil	February	1.49±0.26
Oil	March	1.81±0.26
Oil	April	1.84±0.26

Appendix 7: Treatment by month mean of the 50 berry weights (g) in 2009:

Treatment	Month of Treatment	Weight (g)
Control	January	118.96±2.73
Control	February	122.13±2.73
Control	March	122.10±2.73
Control	April	123.76±2.73
NAA 1000 ppm	January	121.90±2.73
NAA 1000 ppm	February	116.38±2.73
NAA 1000 ppm	March	125.19±2.73
NAA 1000 ppm	April	123.08±2.73
NAA 500 ppm	January	119.90±2.73
NAA 500 ppm	February	122.64±2.73
NAA 500 ppm	March	116.01±2.73
NAA 500 ppm	April	121.91±2.73
NAA 750 ppm	January	120.46±2.73
NAA 750 ppm	February	118.17±2.73
NAA 750 ppm	March	117.91±2.73
NAA 750 ppm	April	122.64±2.73
Oil	January	118.96±2.73
Oil	February	123.79±2.73
Oil	March	123.27±2.73
Oil	April	122.39±2.73

Appendix 8: Treatment by month mean °Brix in 2009:

Treatment	Month of Treatment	°Brix
Control	January	12.73±0.25
Control	February	12.47±0.25
Control	March	12.40±0.25
Control	April	12.60±0.25
NAA 1000 ppm	January	12.60±0.25
NAA 1000 ppm	February	12.43±0.25
NAA 1000 ppm	March	12.63±0.25
NAA 1000 ppm	April	12.47±0.25
NAA 500 ppm	January	12.53±0.25
NAA 500 ppm	February	12.80±0.25
NAA 500 ppm	March	12.37±0.25
NAA 500 ppm	April	12.27±0.25
NAA 750 ppm	January	12.30±0.25
NAA 750 ppm	February	12.47±0.25
NAA 750 ppm	March	12.37±0.25
NAA 750 ppm	April	12.60±0.25
Oil	January	13.23±0.25
Oil	February	12.43±0.25
Oil	March	12.57±0.25
Oil	April	12.57±0.25

Appendix 9: Treatment by month mean pH in 2009:

Treatment	Month of Treatment	pH
Control	January	3.14±0.03
Control	February	3.21±0.03
Control	March	3.23±0.03
Control	April	3.25±0.03
NAA 1000 ppm	January	3.22±0.03
NAA 1000 ppm	February	3.17±0.03
NAA 1000 ppm	March	3.21±0.03
NAA 1000 ppm	April	3.20±0.03
NAA 500 ppm	January	3.26±0.03
NAA 500 ppm	February	3.27±0.03
NAA 500 ppm	March	3.18±0.03
NAA 500 ppm	April	3.20±0.03
NAA 750 ppm	January	3.26±0.03
NAA 750 ppm	February	3.23±0.03
NAA 750 ppm	March	3.20±0.03
NAA 750 ppm	April	3.24±0.03
Oil	January	3.26±0.03
Oil	February	3.25±0.03
Oil	March	3.22±0.03
Oil	April	3.14±0.03

Appendix 10: Treatment by month mean TA (g/100 ml) in 2009:

Treatment	Month of Treatment	TA (g/100 ml)
Control	January	1.07±0.08
Control	February	0.97±0.08
Control	March	0.97±0.08
Control	April	1.00±0.08
NAA 1000 ppm	January	0.97±0.08
NAA 1000 ppm	February	0.83±0.08
NAA 1000 ppm	March	0.83±0.08
NAA 1000 ppm	April	1.00±0.08
NAA 500 ppm	January	0.83±0.08
NAA 500 ppm	February	0.83±0.08
NAA 500 ppm	March	1.03±0.08
NAA 500 ppm	April	0.97±0.08
NAA 750 ppm	January	1.07±0.08
NAA 750 ppm	February	1.13±0.08
NAA 750 ppm	March	1.00±0.08
NAA 750 ppm	April	0.93±0.08
Oil	January	0.97±0.08
Oil	February	0.90±0.08
Oil	March	0.90±0.08
Oil	April	1.07±0.08

Appendix 11: Treatment by month mean pruning weights (kg) in 2010 of the 2009 treated vines:

Treatment	Month of Treatment	Pruning Weights (kg)
Control	January	1.09±0.12
Control	February	1.20±0.12
Control	March	1.30±0.12
Control	April	1.21±0.12
NAA 1000 ppm	January	1.34±0.12
NAA 1000 ppm	February	1.07±0.12
NAA 1000 ppm	March	1.10±0.12
NAA 1000 ppm	April	1.05±0.12
NAA 500 ppm	January	1.19±0.12
NAA 500 ppm	February	1.43±0.12
NAA 500 ppm	March	1.24±0.12
NAA 500 ppm	April	1.06±0.12
NAA 750 ppm	January	1.36±0.12
NAA 750 ppm	February	1.06±0.12
NAA 750 ppm	March	1.10±0.12
NAA 750 ppm	April	1.32±0.12
Oil	January	1.27±0.12
Oil	February	1.27±0.12
Oil	March	1.17±0.12
Oil	April	1.05±0.12

Appendix 12: Treatment by month mean number of clusters in 2010 of the 2009 treated vines:

Treatment	Month of Treatment	Cluster Numbers
Control	January	16.00 ±2.19
Control	February	14.00±2.19
Control	March	16.00±2.19
Control	April	13.00±2.19
NAA 1000 ppm	January	13.67±2.19
NAA 1000 ppm	February	16.67±2.19
NAA 1000 ppm	March	15.33±2.19
NAA 1000 ppm	April	13.33±2.19
NAA 500 ppm	January	15.00±2.19
NAA 500 ppm	February	13.67±2.19
NAA 500 ppm	March	15.33±2.19
NAA 500 ppm	April	14.67±2.19
NAA 750 ppm	January	13.33±2.19
NAA 750 ppm	February	14.33±2.19
NAA 750 ppm	March	15.67±2.19
NAA 750 ppm	April	15.33±2.19
Oil	January	14.67±2.19
Oil	February	11.67±2.19
Oil	March	15.00±2.19
Oil	April	15.67±2.19

Appendix 13: Treatment by month mean delay in bud break in 2010 (Julian Calendar Days):

Treatment	Month of Treatment	Bud Break Days
Control	January	113.00±1.21
Control	February	115.00±1.21
Control	March	114.00±1.21
NAA1000	January	117.33±1.21
NAA1000	February	117.33±1.21
NAA1000	March	117.33±1.21
NAA1500	January	118.00±1.21
NAA1500	February	117.33±1.21
NAA1500	March	120.00±1.21
NAA500	January	114.00±1.21
NAA500	February	117.00±1.21
NAA500	March	114.67±1.21
Oil	January	122.00±1.21
Oil	February	120.00±1.21
Oil	March	124.67±1.21

Appendix 14: Treatment by month mean number of clusters per vine in 2010:

Treatment	Month of Treatment	Number of Clusters/Vine
Control	January	12.00±2.87
Control	February	16.00±2.87
Control	March	16.67±2.87
NAA1000	January	14.67±2.87
NAA1000	February	15.33±2.87
NAA1000	March	14.67±2.87
NAA1500	January	15.00±2.87
NAA1500	February	18.33±2.87
NAA1500	March	14.00±2.87
NAA500	January	17.00±2.87
NAA500	February	18.67±2.87
NAA500	March	15.00±2.87
Oil	January	16.00±2.87
Oil	February	19.00±2.87
Oil	March	13.33±2.87

Appendix 15: Treatment by month mean cluster weights (kg) of the mean number of clusters in 2010:

Treatment	Month of Treatment	Cluster Weights (kg)
Control	January	2.26±0.55
Control	February	3.22±0.55
Control	March	3.34±0.55
NAA1000	January	2.97±0.55
NAA1000	February	2.95±0.55
NAA1000	March	2.84±0.55
NAA1500	January	2.95±0.55
NAA1500	February	3.62±0.55
NAA1500	March	2.79±0.55
NAA500	January	3.26±0.55
NAA500	February	3.64±0.55
NAA500	March	2.87±0.55
Oil	January	3.11±0.55
Oil	February	3.65±0.55
Oil	March	2.89±0.55

Appendix 16: Treatment by month mean of the 30 berry weights (g) in 2010:

Treatment	Month of Treatment	Weight (g)
Control	January	104.45±1.49
Control	February	97.94±1.49
Control	March	99.48±1.49
NAA1000	January	99.83±1.49
NAA1000	February	99.08±1.49
NAA1000	March	103.00±1.49
NAA1500	January	100.66±1.49
NAA1500	February	102.12±1.49
NAA1500	March	99.98±1.49
NAA500	January	102.11±1.49
NAA500	February	100.88±1.49
NAA500	March	102.32±1.49
Oil	January	100.91±1.49
Oil	February	100.94±1.49
Oil	March	98.68±1.49

Appendix 17: Treatment by month mean °Brix in 2010:

Treatment	Month of Treatment	°Brix
Control	January	13.13±0.35
Control	February	13.27±0.35
Control	March	13.27±0.35
NAA1000	January	13.13±0.35
NAA1000	February	12.77±0.35
NAA1000	March	13.10±0.35
NAA1500	January	13.23±0.35
NAA1500	February	12.83±0.35
NAA1500	March	13.07±0.35
NAA500	January	13.20±0.35
NAA500	February	13.47±0.35
NAA500	March	12.73±0.35
Oil	January	13.20±0.35
Oil	February	13.17±0.35
Oil	March	13.17±0.35

Appendix 18: Treatment by month mean pH in 2010:

Treatment	Month of Treatment	pH
Control	January	3.27±0.04
Control	February	3.38±0.04
Control	March	3.33±0.04
NAA1000	January	3.32±0.04
NAA1000	February	3.41±0.04
NAA1000	March	3.28±0.04
NAA1500	January	3.37±0.04
NAA1500	February	3.28±0.04
NAA1500	March	3.29±0.04
NAA500	January	3.34±0.04
NAA500	February	3.36±0.04
NAA500	March	3.30±0.04
Oil	January	3.40±0.04
Oil	February	3.41±0.04
Oil	March	3.26±0.04

Appendix 19: Treatment by month mean TA (g/100 ml) in 2010:

Treatment	Month of Treatment	TA (g/100 ml)
Control	January	1.15±0.12
Control	February	1.20±0.12
Control	March	1.35±0.12
NAA1000	January	1.40±0.12
NAA1000	February	1.20±0.12
NAA1000	March	1.55±0.12
NAA1500	January	1.35±0.12
NAA1500	February	1.10±0.12
NAA1500	March	1.40±0.12
NAA500	January	1.35±0.12
NAA500	February	1.30±0.12
NAA500	March	1.30±0.12
Oil	January	1.35±0.12
Oil	February	1.25±0.12
Oil	March	1.35±0.12