5-2014

Delay of Bud Break on 'Edelweiss' Grapevines with Multiple Applications of Amigo Oil and NAA

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DELAY OF BUD BREAK ON 'EDELWEISS' GRAPEVINES WITH MULTIPLE APPLICATIONS OF AMIGO OIL AND NAA

By

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A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Horticulture

Under the Supervision of Professor Paul E. Read

Lincoln, Nebraska

May, 2014

DELAY OF BUD BREAK ON 'EDELWEISS' GRAPEVINES WITH MULTIPLE APPLICATIONS OF AMIGO OIL AND NAA

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University of Nebraska, 2014

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'Edelweiss' is an important grape cultivar grown in the Midwestern part of the USA. This grapevine is tolerant to extreme winter temperatures which can be experienced in the areas where it is most widely grown. 'Edelweiss' is one of the earliest cultivars in the vineyard to break bud, making it very susceptible to late spring freezes. The primary buds of 'Edelweiss' produce a significant amount of fruit, while unlike many other hybrids, the secondary and tertiary buds will have little to no yields, thus making it important to protect the primary buds from a late freeze. The objective of this research was to determine if multiple applications of Naphthaleneacetic acid (NAA) or Amigo Oil has a greater effect on bud delay when compared to single applications. 'Edelweiss' vines were treated with one, two, or three applications of NAA or Amigo Oil at monthly intervals starting in early January. The purpose of the Amigo Oil and NAA application was to delay bud break without affecting desired characteristics such as yield or fruit composition. Amigo Oil was applied at 10% concentration (v/v) and the NAA at 1000 ppm with a custom built all-terrain vehicle (ATV) sprayer. All treatments of Amigo Oil led to a significant bud break delay ranging from 3 to 11 days as compared to

the control. None of the treatments resulted in negative effects on yield or fruit characteristics. A controlled laboratory experiment was also conducted, where single bud cuttings were forced in forcing solution containing 200 ppm 8-hydroxyquinoline citrate and 2% sucrose at 25°C under 12 hour days. Treatments of one, two, or three applications of 1000 ppm NAA and 10% (v/v) Amigo Oil were applied to single buds at weekly intervals. Julian days until bud break were recorded and treatment-related bud break delays were observed. Two and three applications of oil significantly delayed bud break ranging from 14 to 24 days. All NAA treatments led to significant bud delay ranging from 6 to 9 days. Grape growers in climates with the potential of late spring freezes may consider the use of Amigo Oil as a potential means to protect their vines from freeze injury.

This Thesis is dedicated to my beautiful wife and beloved parents for inspiring me and supporting me through this process.

Acknowledgments

Foremost, I would like express my sincere gratitude to my advisor Dr. Paul Read for his continuous support, patience, motivation, and enthusiasm. You have become a important role model in my academics and life; I find enjoyment in working with you every day and appreciate all of the opportunities you have provided.

Besides my advisor, I would like to thank the rest of the viticulture group: Steve Gamet, Christina Bavougian and Dongxue Shi for your assistance throughout my project and for your friendship. I would not have been able to complete this project without your dedicated assistance.

I would also like to extend my sincere appreciation to my graduate committee members: Dr. Ellen Paparozzi and Dr. Gary Yuen for the commitment of their time to my project and studies. This project could not have been completed without your ideas, suggestions and knowledge. You have taught me the importance of community within an academic environment. I am also extremely grateful for the help of Dr. Erin Blankenship on the statistical design and analysis portion of this thesis. I appreciate you always answering my constant questions with a smile. The dissection of buds and pictures would not have been possible without the assistance of Liz Conley.

This project was conducted at James Arthur Vineyards near Raymond, Nebraska. Their assistance and generosity does not go unnoticed and the help of Josh Rockemann is greatly appreciated.

My beautiful wife Jess, I love you very much. You have always pushed me to do better. I am so thankful that you are in my life; none of these accomplishments would mean anything without you. I owe my success to you!

This would not have been accomplished without the love, support and inspiration from my father (Tim), mother (Lisa), and brothers (Nate and Nick). I love you all very much and appreciate everything you have done for me.

Lastly, and most importantly, I am grateful to God for blessing me with wonderful family and friends and providing opportunities to get me where I am today. He has given me strength and patience throughout these many years of school.

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CHAPTER 1

INTRODUCTION

Grapes have recently become an exciting new alternative crop planted in the Midwest United States. With the breeding of cold hardy cultivars capable of withstanding Midwestern winters, grape growing has begun to expand exponentially in many states. According to the Usda-Nass (2012) total acreage of grapes planted in the United States was 962,100 acres. The first ever comprehensive study measuring the full economic impact of the grape, wine, grape juice, table grape and raisin industries, reported grape and grape products contributes $162 billion annually to the American economy (Mkf-Research, 2007). With an increasing percentage of that economic contribution coming from Midwestern states it is important to begin focusing on potential problems grape growers encounter in these areas.

Grapes grown in the Midwest states are commonly subjected to inconsistent temperature fluctuations. Particularly in Nebraska, spring freeze is a major limiting factor of grape production (Qrunfleh and Read, 2010). Grape production in areas that are susceptible to spring freezes is risky and can occasionally cause large economic losses to the vineyard. In March of 2007, the second warmest March on record for the lower 48 states temperatures were recorded to be an average of 6°F above normal (Guinan, 2007). The arrival of an early spring, like this, resulted in fruit crops and other crops being developmentally far ahead of schedule making them extremely susceptible to an oncoming freeze event. The loss in the affected areas in Midwest states due to that particular freeze event was estimated to exceed one billion dollars (Guinan, 2007).

Appropriate site selection is the best practice for avoiding winter and spring freeze injury. However, in many cases site selection is not a priority or not possible and vineyards are established in less than the most suitable locations. To offset poor site selection, many methods to provide frost protection have been attempted and include wind machines, overhead irrigation, and cryoprotective chemicals. With these methods being very costly, it is not economical for small growers to employ them. In the event of a late spring freeze, growers hope the bud injury will affect only the primary buds, and secondary buds will grow after primary bud damage (Qrunfleh and Read, 2010). However, protecting the primary bud is essential as they produce 300 to 400% more fruit with clusters 135 to 190% larger than those produced by secondary buds (Wiggans, 1926).

One of the best strategies for protecting against spring freezes in the vineyard is delaying the onset of bud break in the spring. Some methods that have been used to delay bud break include: delayed pruning, using various types of cryoprotective treatments (Dami et al., 1997), plant growth regulators and the use of alginate and dormant oils (Dami et al., 2000). The first attempts of using oil were reported in the late 1960s and early 1970s (Qrunfleh and Read, 2010). Dormant oil was used on 'Johnson Elberta' peaches to control insects, and delayed bloom was also observed. Applications of 10% soybean oil on 'Georgia Belle' peach trees increased internal CO_2 concentrations and delayed bud break by six days (Myers et al., 1996).

Use of dormant oils on grapevines was first reported using petroleum and vegetable-based oils (Dami et al., 2000). 'Chancellor' (an early cultivar to break bud), 'Chambourcin' (late bud break cultivar), and 'Chardonel' (mid-season bud break

cultivar) grapevines were treated with two soybean oil-based adjuvants (Prime Oil and Amigo Oil). Prime Oil however, was found to be highly phytotoxic to the dormant buds. Both treatments led to a significant delay in bud break in all cultivars where total delay ranged from one to twenty days (Dami and Beam, 2004).

Plant growth regulators have also been used in the attempt to delay bud break in grapevines. Applications of exogenous gibberellic acid (GA_3) during the previous growth season delayed and inhibited bud opening in the following growing season (Lavee and May, 1997). Spraying 'Aramon' vines with NAA at 500 to 1000 ppm in October had no effect, but spraying the vines in January, February, and March delayed bud break by 16-27 days (Nigond, 1960). Qrunfleh and Read (2010) did a similar study in southeast Nebraska on 'Edelweiss' vines and found Amigo Oil significantly delayed bud break up to 12 days when compared to the non-sprayed control. NAA at 1000 ppm also delayed bud break by three days when compared to the non-sprayed control vines.

'Edelweiss' is one of the most common wine grapes planted in Nebraska. It is one of the earliest cultivars to break bud in the spring, making it highly susceptible to spring freeze events. With most of the vineyards in Nebraska being less than 20 acres, growers cannot afford to employ freeze protection methods. Thus it is necessary to find a chemical that can delay bud break by several days. It must be cheap, easy to apply, non-toxic to grapes and humans and require minimal labor, equipment and energy to apply. The objectives of this study were to:

1. Compare the effects on bud break with multiple applications of NAA or Amigo Oil to 15-year-old 'Edelweiss' grapevines.

2. Determine if two or three applications of NAA or Amigo Oil have a greater effect on bud break than single applications of either compound.
3. Observe any phytotoxic effects of the spray treatment to the buds and determine a percentage of bud mortality due to the treatments.
4. Determine the effect of the NAA and oil on harvest and fruiting characteristics including: cluster number per cane, average cluster weight, °Brix, pH and titratable acidity (TA).
5. Develop an efficient and effective method for applying the NAA and Amigo Oil to the grapevines in the winter months.
6. Confirm the effects of NAA and Amigo Oil on 'Edelweiss' single-bud cuttings forced in a controlled laboratory environment.

References

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

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* 48:376 (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

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

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

MKF-Research. 2007. The impact of wine, grapes and grape products on the American economy. The Wine Business Center, St. Helena, California.

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. *Journal of the American Society for Horticultural Science* 121:96-100.

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.

Qrunfleh, I.M. and P.E. Read, 2010. Delaying bud break in 'Edelweiss' grapevines to avoid spring frost injury by NAA and vegetable oil applications. University of Nebraska, Lincoln, Nebraska, PhD http://digitalcommons.unl.edu/agronhortdiss/14/.

USDA-NASS, 2012. National Statistics for Grapes. In: U.S.D.o. Agriculture (ed.).

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

CHAPTER 2

LITERATURE REVIEW

Grape

The grapevine (*Vitis vinifera*) belongs to the family Vitaceae which comprises about 60 inter-fertile wild species distributed in Asia, North America and Europe under subtropical, Mediterranean and continental – temperate climatic conditions (Terral et al., 2010). The genus *Vitis* includes more than 70 species (Alleweldt and Possingham, 1988) and some of the species currently found in Nebraska include *V. aestivalis* Michx., *V. cinerea* (Engelm)., *V. riparia* Michx., and *V. vulpine* L. (Kaul et al., 2006). The North American *V. rupestris, V.riparia* or *V. berlandieri*, are used in breeding rootstock due to their resistance against grapevine pests, such as *Phylloxera*, *Oidium* and mildews (Terral et al., 2010).

'Edelweiss'

'Edelweiss' originated in Osceola, Wisconsin and was developed from crosses that date back to 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 (Qrunfleh and Read, 2010).

The 'Edelweiss' vine is considered highly vigorous, producing conical shaped clusters that are medium in size, very loose to moderately compact and often double-shouldered (Swenson et al., 1980). The vine is usually trained to a Geneva Double

Curtain (GDC) trellis system. Berries are round, medium sized and green skinned with a white bloom (Swenson et al., 1980). Berries are also of a slip skin, have tender flesh and have the *lubrusca* fruit flavor (Brooks and Olmo, 1997). 'Edelweiss' breaks bud early, making it highly susceptible to spring freeze. In addition, 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 (Qrunfleh and Read, 2010).

The Grape Bud

It is of utmost importance for grape growers to understand the anatomy and physiology of the grapevine in order to be successful vineyard managers. The grape bud is the origin of all fruit the plant will produce so it is important to understand the anatomy of this structure. The first bud which arises in the axil of the leaf subtended by a current season's shoot is known as the "prompt bud". The bud which develops in the axil of the bract is the "latent bud". The latent bud grows slowly within the bract (Srinivasan and Mullins, 1981).

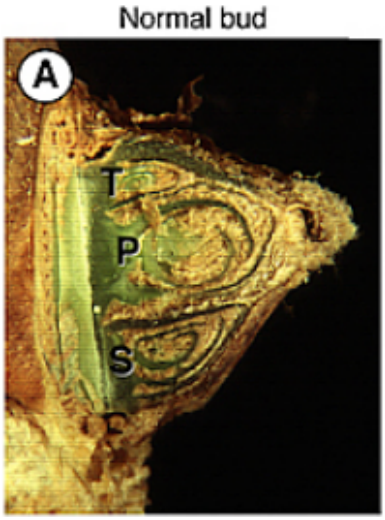


Figure 1. Normal healthy bud showing primary bud (P) bordered by the secondary (S) and Tertiary buds (T) (Zabadal et al., 2007).

The primary, secondary, and tertiary buds, enclosed in the prophyll of the summer lateral and the two basal bracts of the primary bud, constitute the compound winter bud (viticulturally termed 'eye') of the dormant cane (Pratt, 1974). The primary shoot normally grows from the previous year's shoot (viticulturally termed 'cane'); either because the terminal bud of the latter is killed by freeze or the cane has been pruned. 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). The secondary and tertiary shoots are axillary to the two basal prophylls on the primary shoot and also develop a few nodes and undergo dormancy. The secondary shoot usually bears inflorescences, although the tertiary usually bears none (Pratt, 1974). It has been shown that physical damage such as severe pruning, destruction of part of the vine, or a boron nutrient deficiency can result in two or all three of the buds bursting into growth and developing shoots (Winkler et al., 1974). Drawings of the developmental

stages of the dormant bud were illustrated by Eichhorn and Lorenz (1977) and were modified and updated by Coombe (1995) (Appendix 1).

Grape Cold Hardiness

During active growth grapevines are susceptible to freeze damage, but during the dormant season they have the ability to supercool, which allows the bud, cane, and trunk tissues to become acclimated to temperatures well below -10°C (Andrews et al., 1984). 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". Cold hardiness has been defined as "the ability of dormant grapevine tissues to survive freezing temperature stress during autumn and winter" (Dami, 2007).

Measuring freeze injury has become an important component in evaluating current grape cultivars for acclimation and deacclimation of cold hardiness. Cold hardiness is measured by the term "lethal temperature 50" which is referred to as the LT50, the single temperature value that kills 50% of the primary bud population in midwinter (Dami, 2007; Gu, 1999).

The two most commonly used methods for measuring freeze injury are oxidative browning and thermal analysis. Oxidative browning is the most common and relatively inexpensive method of measuring tissue viability and is based on the color change of bud or cane tissues that occurs after freezing and thawing (Dami, 2007). In thermal analysis, supercooled water can be detected using thermal analysis (TA). By using thermocouples

(or thermoelectric modules) to detect the latent heat release (called an exotherm) by water in the bud tissue as it freezes (Dami, 2007).

The three stages of cold hardiness are: acclimation, mid-winter hardiness, and deacclimation. Acclimation is the transfer from a non-hardy to a cold hardy state. Response to short days and low temperatures are the natural factors which cause the transition (Qrunfleh and Read, 2010). Cold hardiness is also increased when the temperature drops below freezing and remains below freezing through midwinter. Periderm formation; mobilization of carbohydrate reserves to canes, trunks and roots; and isolation of dormant buds from the vascular tissues in canes and trunks are complete shortly after leaf fall. However, cold hardiness continues to increase as a result of redistribution of water within bud tissues and desiccation.

Cold hardiness is associated with changes in proteins, enzymes and carbohydrates (Qrunfleh and Read, 2010). Among the previous three, research on carbohydrate changes have received the most attention (Howell, 2000). Along with carbohydrate changes, three other factors play an important role on cold hardiness: genotype, environment, and vine culture and management (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).

A combination of the three above factors is important for growing cold hardy grapevines and growers must address each to protect their crop from spring freeze. Within genotype, a preferable characteristic of a cultivar would be to acclimate quickly in

the fall and slowly deacclimate in the spring (Qrunfleh and Read, 2010). 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. This is where the third factor, vineyard culture and management comes into play, and grape growers can directly control this factor. One of the most often utilized strategies is grafting non-cold tolerant cultivars onto cold tolerant rootstocks. Miller et al. (1988) found that canes and buds on rootstock 'C-3309' had the most cold hardiness. Cane and bud acclimation were faster in fall and deacclimation in spring was slower compared to '5BB' and 'SO4' rootstocks. Moreover, grafted 'White Riesling' plants were significantly hardier than own-rooted vines. The different rootstocks studied had a differential influence on cold hardiness by measuring LT50 values. They concluded that vines of '3309 C' had the most cold hardiness and therefore the most desirable for winter survival. Gu (2003) reported 'Gewurztraminer' scions on '3309 Couderc' and 'MG 420A' rootstocks were the most cold hardy and 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.

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 (Qrunfleh and Read, 2010). Compared to many other deciduous fruit crops, grapevines require relatively little exposure to chilling to terminate rest (Chandler et al., 1937). Erratic and/or delayed bud break, decreased shoot and cluster numbers per vine, and poor uniformity of fruit development are commonly reported in regions where grapevines suffer from inadequate

winter chilling (Mccoll, 1986; Shulman et al., 1983). Chilling is required to break endodormancy and the chilling requirement varies among fruit trees including grapevines (Westwood, 1993). The percentage of grapevine bud break generally improves with increased exposure to chilling temperatures (Dokoozlian, 1999). Though much is known, there is still much to learn.

Bud scales are also an important component 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.

The factors responsible for terminating dormancy are equally important for normal bud break. Reaching the chilling requirement for dormancy termination allows for normal bud break (Lavee and May, 1997). In warm-winter regions where the chilling requirement is not met, chemicals can be used to end the rest period. However, currently available chemicals are expensive and risk phytotoxicity to the buds (Erez, 1987; Erez, 1994; Or and Viloznyi, 1999). Hydrogen cyanamide has been used by Or (2009) as an effective chemical for breaking dormancy of grape buds by inactivating catalase. 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 is inhibited by hydrogen cyanamide (Pérez and Lira, 2005).

Surprisingly, inducing bud break has also been achieved using electricity. Treating scions on two year old 'Kyoho' grapevines with 48 or 60 V hastened bud break

(Kurooka et al., 1990). A greater effect was found when direct current was applied at the time of deepest endodormancy. Direct current has also been used for adventitious root formation of the grapevine rootstock *Vitis champini* 'Ramsey' (Köse, 2007).

Methods of Reducing Frost Damage

It has been said "The most effective weapon against frost damage is preventative action" (Trought et al., 1999). Humphreys (1914) stated "The best time to protect an orchard (or vineyard) against frost is when it's being established". The location is one aspect of determining the proper site of a vineyard that should not be overlooked. There are many characteristics to consider when deciding which location would make for a successful vineyard. The climate, topography, slope, and soil characteristics are just a few that should be assessed before making a final decision. In areas where spring freeze events are likely, choosing a site with proper air-drainage can have a major impact on the severity of bud injury in a freeze event. Cold air drains downhill until it is impeded by an obstruction (i.e. fences, windbreaks, tree lines) large enough to pool the cold air until the topography flattens (Jones and Hellman, 2003).

In many cases the most important aspect of starting a vineyard, the location, is not necessarily a factor that can be controlled. In the Midwest where many small vineyards exist, the location is usually dependent upon the availability of land and price. Considering land for its characteristics listed above is not always a priority. With this in mind, cultural practices must be employed to protect the grapevines from freezing. Trought et al. (1999) mention many practices that can protect grapevines from freezing and they include: late spur pruning to delay bud break, ensure inter-row herbage is

closely mown and provide significant protection to radiation frost by establishing vines on a high cordon and using a hanging curtain trellis system. Caspari and Montano (2013) emphasize the use of "spare parts" when pruning the vines. "Spare parts" indicate that more than one trunk, cordon and/or canes are left on the plant after pruning. The extra "spare parts" make it possible to use those pieces when canes/cordon tied to the wire fail to grow after frost/freeze damage (Caspari and Montano, 2013).

Evans (2000) stated "Any crop can be protected against any freeze if economically warranted. The selection of a freeze protection system is primarily a question of economics" In large scale vineyards, where the potential economic loss due to a freeze event is much higher, more complex mechanisms are used. These can include wind machines, various kinds of heaters, and overhead irrigation (similar to what is used in the citrus industry) (Bearden and Elkins, 1997). Wind machines are one of the most commonly used form of freeze protection, however with a high cost (i.e. $1,500 - $1,800 /ac) they are not logical options for small scale growers (Evans, 2000). Wind machines (and even costly helicopters) take advantage of the inversion layer that develops over the vineyard by mixing layers and thus reducing freeze injury (Creasy and Creasy, 2009; Trought et al., 1999). Methods such as aqueous foam (Choi and Giacomelli, 1999), hydrophobic particle film and a leaf coating acrylic polymer (Fuller et al., 2003) have also been used for freeze protection. Unfortunately these methods have not been thoroughly tested and have not yet generated desired results. A more labor intensive practice, but much cheaper is the use of mulching. Gu (2003) concluded that mounding protected 'Gewürztraminer' vines from the cold winter and significantly increased pruning weights.

Inter-row management can also have a great influence on the minimum temperature reached in a vineyard (Trought et al., 1999). Slater and Ruxton (1954) showed that temperatures 7.5 cm above a firm surface could be ~1.0°C higher than over loose soil. Ground cover can also have major influence and grass cover or mulches may reduce temperatures by 4-6°F (Cornford, 1938; Rogers, 1957).

Cut Flowers and Woody Plants in Forcing Solution

Since the availability of plant material for in vitro purposes is limited to a short period of time during early spring (Yang and Read, 1990), it has been suggested that the same forcing solution used to extend vase life in cut flowers could be used to promote growth in woody cuttings (Read et al., 1984). A chemical for forcing cuttings studied by many researchers is 8-hydroxyquinoline citrate (8-HQC) (Qrunfleh and Read, 2010). 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 to tap water in snapdragons (Larsen and Scholes, 1966). The mechanism of prolonging life by 8-HQC was due to decreasing vascular blockage in stems and increasing water absorption and stomatal closure (Qrunfleh and Read, 2010). The same 8-HQC was investigated for effects on stem cuttings of privet and arrow-wood viburnum (Read and Yang, 1989). They concluded that indolebutric acid delivered via forcing solution increased root numbers per cutting and promoted root elongation while gibberellic acid inhibited rooting of the forced dormant stems. (Hamooh, 2001) found that adding silver thiosulfate to forcing solution hastens bud break and shoot elongation. Less time to bud break and longer shoots were also achieved when GA_3 was combined with silver thiosulfate in the forcing solution.

The use of forcing solution is important for obtaining fresh plant material for micropropagation and could also be a useful tool to enhance bud break as well as a method for studying bud dormancy in woody plants (Qrunfleh and Read, 2010). Forcing solution has most recently been used by Qrunfleh and Read (2010), to force single bud cuttings of 'Edelweiss' grapevines and observe effects of NAA and Amigo Oil on bud break.

Methods to Delay Bud Break

Buds on woody plants constitute a very small part of the mass of the plant, however during the growing season they are organs of high physiological activity (Pallardy, 2008). Typical woody plant buds maintain low, stable respiration rates during the dormant season. The same is true for grapevines where respiratory activities steadily increase from the ecodormant to bud break stage (Gardea et al., 1994). The ability of chemicals to slow respiratory activity within the bud could in turn delay grapevine response to spring environmental factors such as increased temperature and day length. Myers et al. (1996) reported that soybean oil on peach flower buds interferes with the escape of respiratory CO_2, which results in an increase of internal CO_2 concentrations. This would result in decreased respiratory activity as a result of a feedback inhibition (Isenberg, 1979).

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

Growth Regulators

Plant hormones are a group of naturally occurring, organic substances that influence physiological processes at low concentrations (Davies, 2010). The synthesis of plant hormones may be localized (as occurs for animal hormones), but may also occur in a wide range of tissues, or cells within tissues (Davies, 2010). 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).

Originally, plant growth regulators were tested for use in breaking the dormancy of dormant buds in the fall. Weaver (1959) applied gibberellin (GA_3) at 1, 10, 50, and 250 ppm on 'Zinfandel' vines in September while foliage was still green. He reported the number of shoots decreased with the increase of GA_3. In another experiment, basal cuttings of 'Tokay' were treated with GA_3 a 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.

Levels of abscisic acid (ABA), initially considered to be the 'dormancy hormone', were found to increase as buds entered dormancy and to decrease during dormancy release (Düring and Bachmann, 1975). Hellman et al. (2006) used an experimental formulation of abscisic acid (ABA; Valent biosciences VBC-30025) for the potential to delay bud burst of *Vitis vinifera* L. wine grapes. Two application methods were tested in the greenhouse – spray application to buds or soil application. They found that spray applications of ABA solutions to unopened buds increased the number of days to bud

burst by 3.5 days. Soil applications of ABA to container-grown vines provided the greatest delay in bud burst (up to 7 days) and gave the most consistent response.

For the general relationship between ABA and bud dormancy in grapevines, the general statement by Walton (1980) seems to be still valid "…a role for ABA in the induction and maintenance of bud and seed dormancy has been neither unequivocally demonstrated nor disproven…we do not know the precise biochemical events leading to or from dormancy and are thus unable to determine whether ABA can affect these events" (Lavee and May, 1997).

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. However, the vines that were sprayed in early January, 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. Qrunfleh and Read (2010) did a similar study in southeast Nebraska and found that 1000 ppm delayed bud break by three days when compared to the control in 12-year-old 'Edelweiss' vines.

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 where the apical meristem contained within the shoot apex provides a source of basipetally moving auxin that inhibits lateral bud out growth. However, a new model of apical dominance states that auxin synthesized in intact shoot apices controls axillary bud outgrowth through the up regulation of root Shoot multiplication signal (SMS) (Malladi and Burns, 2007).

NAA has also been 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 Celic (1981). They reported that GA_3 (50 ppm), ethephon (200, 400, or 800 ppm), and B-9 (500 and 1000 ppm) markedly delayed bud burst. Ethephon at 800 ppm was the most effective concentration and delayed bud break by 19 days. Cycocel hastened bud burst significantly. All treatments had no effect on bud break percentage, but cuttings treated with GA3 did not show normal bud growth and died after bud break.

1. Delayed Pruning and/or Double Pruning

One of the simplest and most practical measures to avoid freeze damage in a vineyard is to delay prune and/or double prune the grapevines. These pruning techniques take advantage of apical dominance. Delayed pruning has been shown to delay bud break and bloom date (Loomis, 1939) and can also result in more uniform bud break. This was achieved in 'Perlette' and 'Thompson Seedless' which were pruned in January compared with November and December pruning dates (Hatch and Ruiz, 1987). Pruning dates and bud break may also be influenced by translocation and storage of carbohydrates or other endogenous compounds. Early pruning could stimulate metabolic activity which delays the onset of rest (Hatch and Ruiz, 1987). In the Midwest, 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'. For example, in 2013, most 'Edelweiss' in Nebraska grapevines were pruned in the later weeks of March.

In many large scale vineyards, late or delayed pruning is not possible. Vineyard managers with limited labor force often need to start pruning early in the winter to ensure completion of work before bud break (Weber et al., 2007). Double pruning is a practice that can be used in spur-pruned vineyards to allow for more final pruning to occur later in the winter. It involves two pruning passes through the vineyard. The first pruning is usually made in November or December after leaf fall. Then a second pruning is made in late February or March, at which time, canes are cut to their final length (Weber et al., 2007). This pruning technique is not usually employed in Midwestern vineyards, as premature bud break is a possibility which could result in freeze damage.

2. Alginate and Dormant Oils

Attempts to delay bloom with alginate and dormant oils were first studied and reported on in the late sixties and early seventies. Experiments were first done on peach trees and Call and Seeley (1989) delayed bud break five days using dormant oil on 'Johnson Elberta' peaches. However, phytotoxicity was noticed at concentrations greater than 20%. Deyton et al. (1992) also applied dormant oil to 'Biscoe' peaches and measured the internal CO_2 bud concentration. They concluded that the internal CO_2 concentration was higher compared to the control. They also found repeated applications of lower concentrations of oil had less phytotoxic effects on the buds when compared to single applications of higher concentrations of dormant oil.

Some of the most recent attempts to delay bud break in grapes with dormant oils has been done on 'Chancellor', 'Chambourcin' and 'Chardonel' grapes by Dami and Beam (2004). They treated these grapes with two soybean oil-based adjuvants (Prime

and Amigo Oil); with the goal of delaying bud break without affecting fruit ripening, yield, or fruit composition. Prime and Amigo Oil were applied at 10% (v/v) on three different dates. They found that Prime Oil but not Amigo Oil was phytotoxic to dormant buds in all three cultivars. Both treatments led to significant bud delay, ranging from 1 to 20 days as compared to the control. Prime Oil reduced yield, whereas Amigo Oil did not affect the yield or berry composition. They found that Amigo Oil treated nodal sections had 41% less CO_2 emitted than that of the controls. They concluded from this work that oil coating of dormant buds may have hindered CO_2 escape from treated samples, which resulted in a decrease rather than an increase in respiration. Bud scales on Norway maple have been shown to hinder the entrance of oxygen and the respiration rate (oxygen uptake) of buds was only half as high as that of buds from which the scales had been removed (Pallardy, 2008). The second grapevine growth stage in the Modified E-L system (Coombe, 1995) shows bud scales beginning to open. The "cracking" of the bud scales allows for increased oxygen uptake and increased respiration. Dami and Beam (2004) also suggested that cultivars that are late in bud break may require a later application compared to cultivars with early 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 and Soydex) on several grape cultivars. They found that oil rates above 10% (v/v) of all oils that were applied were phytotoxic to most cultivars and Stylet was even phytotoxic at concentrations of 2.5% (v/v). Dormant oils applied at non-toxic rates delayed bud break of several cultivars from 2 to 19 days. However, they also noted

that bud break delay beyond 10 days has deleterious effects on shoot and fruit growth. They demonstrated that bud break delay of 'Chardonel' was also associated with a 30% reduction in respiratory activities of oil-treated buds as compared to untreated buds.

Mcfarland and Mcfarland (2008) treated 'Marachel Foch', 'St. Croix', and 'Brianna' at Mac's Creek vineyard in central Nebraska with Amigo Oil and Alginate Gel. Amigo Oil was applied with a backpack sprayer until runoff and single buds were manually coated with the alginate gel using a paint brush. They concluded that Alginate Gel did not result in a significant delay in bud development for all three cultivars. Amigo Oil resulted in a delay in bud break from 7 to 21 days depending upon the cultivar.

References

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

Andrews, P.K., C. Sandidge, and T. Toyama, 1984. Deep supercooling of dormant and deacclimating Vitis buds. *American Journal of Enology and Viticulture* 35:175-177.

Bearden, B. and R. Elkins, 1997. Vineyard Frost Protection. *UC Cooperative Extension, Mendocino and Lake County*.

Brooks, R. and H. Olmo, 1997. The Brooks and Olmo Register of Fruit and Nut Varieties. Third Edition ed. ASHS Press.

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

Caspari, H. and A. Montano, 2013. Re-training and "spare parts" pruning of grape vines, Colorado State University, Western Colorado Research Center.

Chandler, W.H., M.H. Kimball, G.L. Philip, W.P. Tufts, and G.P. Weldon. 1937. Chilling requirements for opening of buds on deciduous orchard trees and some other plants in California. University of California, College of Agriculture, Agricultural Experiment Station, Berkley.

Choi, C.Y. and G. Giacomelli, 1999. Freeze and frost protection with aqueous foam—Field experiments. *HortTechnology* 9:670-676.

Coombe, B., 1995. Growth Stages of the Grapevine: Adoption of a system for identifying grapevine growth stages. *Australian Journal of Grape and Wine Research* 1:104-110.

Cornford, C., 1938. Katabatic winds and the prevention of frost damage. *Quart. J. R. Met. Soc.* 64:553-587.

Creasy, G. and L. Creasy, 2009. Grapes. CABI, Cambridge, MA.

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

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

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* 48:376 (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

Davies, P.J., 2010. The plant hormones: their nature, occurrence, and functions, p. 1-15, Plant Hormones. Springer.

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

Dokoozlian, N., 1999. Chilling temperature and duration interact on the budbreak of „Perlette grapevine cuttings. *HortScience* 27:1304-1305.

Düring, H. and O. Bachmann, 1975. Abscisic acid analysis in Vitis vinifera in the period of endogenous bud dormancy by high pressure liquid chromatography. *Physiologia Plantarum* 34:201-203.

Eichhorn, K. and D. Lorenz, 1977. Phenological development stages of the grapevine. *Nachrichtenbl. Deut. Pflanzenschutz* 29:119-120.

Erez, A., 1987. Chemical control of budbreak. *HortScience* 22:1240-1243.

Erez, A., 1994. Means to compensate for insufficient chilling to improve bloom and leafing. *Dormancy and the related Problems of Deciduous Fruit Trees 395*:81-96.

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* 32:122-124.

Evans, R.G. 2000. Frost protection in orchards and vineyards, Proceedings of the ASEV 50th Anniversary Annual Meeting. 60-72

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* 143:93-98.

Gardea, A., Y. Moreno, A. Azarenko, P. Lombard, L. Daley, and R. Criddle, 1994. Changes in metabolic properties of grape buds during development. *Journal of the American Society for Horticultural Science* 119:756-760.

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

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., 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* 77:635-639.

Hamman, R., I.-E. Dami, T. Walsh, and C. Stushnoff, 1996. Seasonal carbohydrate changes and cold hardiness of Chardonnay and Riesling grapevines. *American journal of enology and viticulture* 47:31-36.

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

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

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

Hellman, E., S. Shelby, and C. Lowery, 2006. Exogenously applied abscisic acid did not consistently delay budburst of deacclimating grapevines. *American Pomological Society* 60:178.

Howell, G.S. 2000. Grapevine cold hardiness: Mechanisms of cold acclimation, mid-winter hardiness maintenance, and spring deacclimation., Proceedings of the ASEV 50th Anniversary Annual Meeting Davis, CA. 35-48

Humphreys, W., 1914. Frost protection. *Monthly Weather Review* 42:562.

Isenberg, F.M.R. 1979. Controlled atmosphere storage of vegetables, Horticultural Reviews Westport, CT. 337-394

Iwasaki, K., 1980. Effects of bud scale removal, calcium cyanamide, GA3, and ethephon on bud break of'Muscat of Alexandria'grape (Vitis vinifera L.). *Journal of the Japanese Society for Horticultural Science* 48:395-398.

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.). *Journal of the American Society for Horticultural Science* 102:584-587.

Jones, G.V. and E. Hellman, 2003. Site assessment. *Oregon Viticulture*:44-50.

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.

.

Köse, C., 2007. Effects of direct electric current on adventitious root formation of a grapevine rootstock. *American journal of enology and viticulture* 58:120-123.

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.

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-dimethylamino 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. Volume II. Water, radiation, salt, and other stresses. 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* 42:1113-1117.

McColl, C., 1986. Cyanamide advances the maturity of table grapes in central Australia. *Animal Production Science* 26:505-509.

McFarland, S. and M. McFarland. 2008. Mac's Creek - Yr 2 Cold Hardiness - #18-13-060.

McMahon, M.J., A.M. Kofranek, and V.E. Rubatzky, 2007. Hartmann's Plant Science. Pearson Education Inc. New Jersey, USA.

Miller, D., G. Howell, and R. Striegler, 1988. Cane and bud hardiness of own-rooted White Riesling and scions of White Riesling and Chardonnay grafted to selected rootstocks. *American journal of enology and viticulture* 39:60-66.

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. *Journal of the American Society for Horticultural Science* 121:96-100.

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

Or, E., 2009. Grapevine Molecular Physiology & Biotechnology. In: K.A. Roubelakis- and Angelakis (eds.), Grape bud dormancy release-the molecular aspect.

Or, E. and I. Viloznyi, 1999. Timing ofhydrogen cyanamide application to grapevine buds. *Vitis* 38:1-6.

Pallardy, S.G., 2008. Physiology of Woody Plants. Third ed. San Diego: Academic Press.

Pérez, F.J. and W. Lira, 2005. Possible role of catalase in post-dormancy bud break in grapevines. *Journal of Plant Physiology* 162:301-308.

Pratt, C., 1974. Vegetative anatomy of cultivated grapes--a review. *American Journal of Enology and Viticulture* 25:131-150.

Qrunfleh, I.M. and P.E. Read, 2010. Delaying bud break in 'Edelweiss' grapevines to avoid spring frost injury by NAA and vegetable oil applications. University of Nebraska, Lincoln, Nebraska, PhD http://digitalcommons.unl.edu/agronhortdiss/14/.

Read, P.E., 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 Czech. Acad. Sci. Prague. 467-473

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

Rogers, W.S., 1957. Protection from spring frosts. *Grower* 47:984-985.

Shulman, Y., G. Nir, L. Fanberstein, and S. Lavee, 1983. The effect of cyanamide on the release from dormancy of grapevine buds. *Scientia Horticulturae* 19:97-104.

Slater, C. and J. Ruxton, 1954. The effect of soil compaction on incidence of frost. *Rep. E. Malling Res. Stn for*:88-91.

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

Srinivasan, C. and M.G. Mullins, 1981. Physiology of flowering in the grapevine—a review. *American Journal of Enology and Viticulture* 32:47-63.

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

Takeda, F., V. Drane, and M.S. Saunders. 1982. Inhibiting sprouting in Muscadine grapes, Proc. Florida State Hortic. Soc 95). 127-128

Terral, J.-F., E. Tabard, L. Bouby, S. Ivorra, T. Pastor, I. Figueiral, S. Picq, J.-B. Chevance, C. Jung, and L. Fabre, 2010. Evolution and history of grapevine (Vitis

vinifera) under domestication: new morphometric perspectives to understand seed domestication syndrome and reveal origins of ancient European cultivars. *Annals of Botany* 105:443-455.

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

Walton, D.C., 1980. Does ABA play a role in seed germination? *Israel Journal of Botany* 29:68-80.

Weaver, R.J., 1959. Prolonging dormancy in Vitis vinifera with gibberellin.

Weaver, R.J., S.B. Mccune, and B.G. Coombe, 1961. Effects of various chemicals and treatments on rest period of grape buds. *American Journal of Enology and Viticulture* 12:131-142.

Weber, E.A., F.P. Trouillas, and W.D. Gubler, 2007. Double pruning of grapevines: A cultural practice to reduce infections by Eutypa lata. *American Journal of Enology and Viticulture* 58:61-66.

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

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

Yang, G. and P.E. Read, 1990. Influence of plant growth regulators and season on growth responses of forced woody stems. *In Vitro Culture, 23rd International Horticulture Congress. 300*:173-176.

Zabadal, T.J., I.E. Dami, M.C. Goffinet, T.E. Martinson, and M.L. Chien, 2007. Winter injury to grapevines and methods of protection. Michigan State University Extension.

CHAPTER 3

Year 1: Pilot Study, 2012

Purpose: The first year's experiment was set up as a pilot study to obtain a variance of bud break that would be used to design the following year's experiment.

Materials and Methods:

Site Selection

Applications were made during the winter of 2012 at James Arthur Vineyards located near Raymond, Nebraska (40° 57' 19.8396" N, 96° 45' 4.8312" W). Soil types across the vineyard are Aksarben silty clay loam, Mayberry silty clay loam, and Nodaway silt loam.

Figure 2: Aerial view of 14-year-old 'Edelweiss' plot used for experimentation in 2012 (Google-Maps, 2013).

Grapevines

'Edelweiss' grapevines were chosen for this experiment for their early bud break potential and wide popularity amongst grape growers and wine makers in Nebraska. The vines were 14 years old and were trained to a Geneva Double Curtain (GDC) trellis system. Plant spacing was 8 feet (2.44 m) and row spacing was 12 feet (3.66 m). Row orientation is north to south.

Experiment

The treatments consisted of 1000 ppm NAA (*Phyto*Technology Laboratories, Shawnee Mission, KS), 10% (v/v) Amigo Oil (Loveland Industries, Greely, CO) which consisted of 9.3% soybean oil, 0.7% emulsifier and 90% water and a control which had no spray application. Treatments were applied to two rows consisting of 18 vines each. Within each row were three treatments where each treatment was applied to six vines, representing one experimental unit. NAA concentrations were prepared by weighing out 1000 mg of NAA and dissolving in roughly 10 ml 1M sodium hydroxide (NaOH) and the volume was completed to 1000 ml with deionized distilled water. The pH of the NAA solution was measured and adjusted to 7 by adding a few drops of 1M hydrochloric acid (HCl). Two control rows received no spray applications and were compared against the treated rows.

The first spray date occurred on January 26, 2012 where the 18 unpruned vines in each row were sprayed with either NAA or oil using a small one gallon hand sprayer. On the second spray date (February 25, 2012) only 12 of the original 18 vines were sprayed and on the final spray date (March 27th, 2012) only 6 of the original 18 vines were

sprayed. Within each of the two rows, six-vine experimental units received one, two or three applications of NAA or Amigo Oil. The spray solution was applied to the entire cordon and canes until runoff, which resulted in approximately 0.33 L per vine. Past studies have recommended spraying until runoff; however, the volume used per vine was 0.7 L (Dami and Beam, 2004; Mcfarland and Mcfarland, 2008; Qrunfleh and Read, 2010). All vines were cane pruned to five buds following the last spray application according to James Arthur's Vineyards normal vineyard management practices.

Data Collection

Bud break was visually evaluated in the spring of 2012. Grapevine bud break was determined at stage four of the modified Eichhorn-Lorenz system (E-L) scale of grapevine development (Coombe, 1995). Stage four indicates the bud scales have expanded to where the first leaf tissue is visible. Total bud counts for each vine were taken. Bud break was evaluated daily during the spring starting on April 1st. Bud break counts were taken until bud break had reached 60% of the total number of buds allocated per vine during pruning. The number of Julian days starting from January 1, 2012 was recorded once 60% of the buds had reached stage four.

Harvest

Harvest occurred on August 2, 2012. Each plant within the six plant experimental unit was completely harvested. The total cluster number and weight were recorded for each plant. The data for the six vines were combined and averaged together to attain a single value. Once the clusters had been weighed, 100 berries were randomly chosen and

placed in a plastic freezer bag, and stored in the freezer (0°F) until berry sample analysis could be conducted.

Berry Analysis

Berry analysis was conducted on August 10, 2012, where berry size, pH, °Brix, and titratable acidity (TA) were measured. Berries were removed from the freezer the day before testing and placed in a cooler (40°F) to thaw. On the day of testing, berries were removed from the cooler and allowed to warm to room temperature. The 100 berry samples were weighed and average berry size was found. Berry samples were then crushed within their plastic bag and the juice was then extracted by cutting a small hole in the bag and allowing the clear juice to run out into a 100 ml beaker. 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).

Results and Discussion:

Variance and Bud Break

Data were gathered in the spring of 2012 in order to obtain a reliable variance (σ^2) estimate of bud break to use in the following year's experiment. The variance within this field of 14-year-old 'Edelweiss' grapevines had a value of nine.

Small differences were found amongst the treatments. The control treatments had a mean bud break on April 9, 2012. The three oil treatments were consistent and also had

a mean bud break date of April 9, 2012. Lastly, the three NAA treatments delayed bud break up to five days. One application of NAA had a bud break range from April 10, 2012 to April 14, 2012. While not statistically analyzed, it was clear that little or no difference was found with any of the treatments when compared to the control. With this being the case, it was even more vital to run a power analysis to obtain the proper number of replications to be used in the following year's experiment. A 95% power analysis was run where 12 replications were found to be the optimal number. It was also noted that the hand sprayer used for applying NAA and oil was not supplying sufficient consistent coverage to the vines.

Table 1: Mean Julian date of 'Edelweiss' grapevines treated with one, two and three applications of 1000 ppm NAA or 10% (v/v) Amigo Oil.

Treatment	Julian Days until Bud Break
NAA 1	104.3
NAA 2	100.0
NAA 3	103.0
Oil 1	99.2
Oil 2	99.7
Oil 3	98.5
Control	99.1
Control	98.2

*1, 2, and 3 corresponds to the number of treatments of NAA or Amigo Oil applied in January, January and February, or January, February and March, respectively.

Harvest Results:

Although harvest and yield components (cluster weight, berry size, pH, °Brix, and titratable acidity) can vary greatly from year to year depending upon environmental

conditions, harvest data were collected in the August, 2012 as a baseline for the following year's experiment and presented in Appendix 3 & 4.

References

Coombe, B., 1995. Growth Stages of the Grapevine: Adoption of a system for identifying grapevine growth stages. *Australian Journal of Grape and Wine Research* 1:104-110.

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

Dharmadhikari, M.R. and K.L. Wilker, 2001. Micro vinification: a practical guide to small scale wine production. Midwest Viticulture and Enology Center, Dept. of Fruit Science, Southwest Missouri State University.

Google-Maps. 2013. James Arthur Vineyard. https://maps.google.com/maps?q=james+arthur+vineyards&ie=UTF-8&ei=lKD7UvThOOy62gXq-IGABQ&ved=0CAwQ_AUoBA

McFarland, S. and M. McFarland. 2008. Mac's Creek - Yr 2 Cold Hardiness - #18-13-060.

Qrunfleh, I.M. and P.E. Read, 2010. Delaying bud break in 'Edelweiss' grapevines to avoid spring frost injury by NAA and vegetable oil applications. University of Nebraska, Lincoln, Nebraska, PhD http://digitalcommons.unl.edu/agronhortdiss/14/.

CHAPTER 4

Year 2: Delaying Bud Break with Multiple Applications of NAA and Amigo Oil

Purpose: The second year's experiment was done to observe the effects of multiple applications of Amigo Oil and NAA on bud break, harvest parameters and fruit characteristics.

Materials and Methods:

Site Selection

The second year's experiment performed in 2013 was done in the same vineyard as the previous year's experiment at James Arthur Vineyards located just outside Raymond, Nebraska (40° 57' 19.8396" N, 96° 45' 4.8312" W). Soil types across the vineyard are Aksarben silty clay loam, Mayberry silty clay loam, and Nodaway silt loam.

Figure 3: Aerial view of 15-year-old 'Edelweiss' plot used for experimentation in 2013 (Google-Maps, 2013).

Grapevines

Treatments were again applied to the same vineyard consisting of 15-year-old 'Edelweiss' grapevines. Vine and row spacing were the same as the previous year with vine spacing being 8 feet (2.44 m) and row spacing being 12 feet (3.66 m). The vines are trained and trellised on a Geneva Double Curtain (GDC) design. The vines were under standard vineyard management practices throughout the year.

Experiment

A Youden Square incomplete randomized block design was used and replicated three times. Each Youden Square consisted of a 4 x 7 blocking scheme (row x column) and contained a total of 28 experimental units. There were four experimental units per treatment; each unit consisted of four vines, with data being taken from the second and third vines (center two). Blocking was done both on the row and column, accounting for the elevation change from the top of the row to the bottom and for the elevation and soil differences across the vineyards. Each row consisted of no less than 24 vines and within each row four treatments were randomly assigned. The first plant of each row acted as a buffer and did not receive a treatment. Vines two through five on each row received the first treatment, vines six and seven acted as buffer plants and the next treatment was applied to vines eight through eleven (Figure 4). Between each four vine experimental unit a two vine buffer was assigned. In two instances, in rows 32 and 34, a series of vines had been replanted and required the reassignment of two treatments laterally across the vineyard to rows 20 and 22. In two more cases, control treatments were mistakenly

sprayed, making it necessary to reassign two new control experimental units in row 20 and 22 (Figure 4).

The experiment consisted of seven treatments, including the control and included one, two or three applications of either 1000 ppm NAA (*Phyto*Technology Laboratories, Shawnee Mission, KS) or 10% (v/v) Amigo Oil (Loveland Industries, Greely, CO) applied at monthly intervals. NAA concentrations were prepared by weighing out 49.4g NAA powder and dissolving in roughly 100 ml of .5 M sodium hydroxide (NaOH) and the volume was then completed to 1000 ml by adding distilled water, which was then mixed on site with 13 gallons of water.

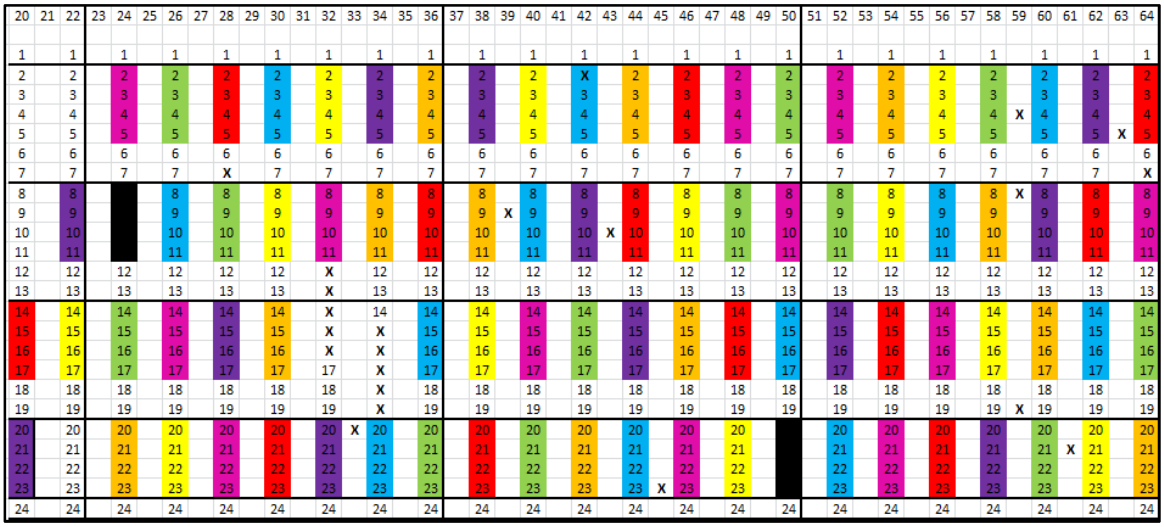


Figure 4: Layout of treatments within the vineyard at James Arthur Vineyards near Raymond, Nebraska.

Custom Sprayer

It was determined during the prior year's pilot experiment that the sprayer previously used provided inadequate coverage for the increased scale of this experiment.

Backpack and hand sprayers have been most commonly used in similar experiments (Dami and Beam, 2004; Mcfarland and Mcfarland, 2008; Myers et al., 1996; Qrunfleh and Read, 2010). However, with the large size of this experiment hand sprayers would be insufficient. With around 40 gallons of spray solution of both NAA and Amigo Oil being applied, a backpack sprayer would have to be refilled a total of 16 times. With this in mind, it was decided that a new sprayer would be developed to meet the needs of this large scale experiment. It was decided the best option would be to modify a conventional all-terrain vehicle (ATV) sprayer and build a spray apparatus mountable to the front rack of the ATV. The articulating spray nozzles would increase the coverage and the steady pressure of the electric pump would increase consistency. In addition, time necessary to make applications would be dramatically decreased as would human fatigue when compared to using a backpack sprayer. A basic 25 gallon ATV sprayer was used and outfitted with a tank agitator. In the absence of a tank agitator the oil separates from the water in mere minutes (Figure 5). As the oil separates, vines receive inconsistent concentrations of oil across the vineyard. The first vines sprayed receive less than the recommended 10% oil while the last vines sprayed receive far more than 10%. The presence of a tank agitator is a factor that should equalize the concentration of oil that each plant receives.

Figure 5. Example showing the time it takes for Amigo Oil at a 10% concentration (9.3% oil and 0.7% emulsifier) to separate from water in the absence of agitation.

The sprayer apparatus was mounted to steel brackets on the front of an ATV and was adjustable horizontally and vertically to accommodate for changes in trellis height. The sprayer used three CountyLine® Multi-Range Flat Spray Tips (LU 80-04S), each of which was on its own adjustable arm, allowing fine-tuning adjustment for the most optimum spray angle. At the conclusion of multiple applications, it was quickly realized that a mechanical, electric pump driven sprayer is much more effective at attaining the optimum coverage for applying NAA and Amigo Oil (Figure 6 & 7). It was also observed that when vines were sprayed until runoff, the solution tended to run down the cane until it hit a node/bud where it then accumulated (Figure 8).

Figure 6. ATV Sprayer modified to spray Amigo Oil and NAA on unpruned vines.

Figure 7. Flat fan spray nozzle attached to the adjustable arms of the sprayer. The small droplets easily penetrate into the cordon area and cover all buds.

Figure 8. Visual evidence of "spraying until runoff", where droplets are accumulating at the buds and nodal sections of the cane.

The first NAA and Amigo Oil applications were made on January 4th, 2013 starting at 10:00 am. The weather was clear with temperatures in the morning around 15°F and reaching a high of about 32°F in the afternoon with wind speeds around 10 mph. About 8 inches of snow was on the ground. The first applications were made with NAA and an initial volume of 13 gallons was mixed and sprayed. The sprayer was set up to spray the cordon and the canes 12" above or below the cordon. Canes outside of this region would eventually be pruned off. Also, with the vines being trained to a GDC, it was necessary to spray both sides of each row. After NAA applications, the tank and lines were flushed and cleaned to remove any excess NAA. Amigo Oil was the second treatment and was mixed on site by adding 2.5 gallons of oil to 22.5 gallons of water. The extremely low temperatures caused the build-up of Amigo Ice in the tank, lines and

spray tips when the sprayer was shut down for more than a few minutes. As long as the sprayer was running the system would not freeze, however, when the solution ran out, the lines would quickly freeze and the system would have to be thawed out after each refill. Conditions such as these are what growers would expect to encounter when making applications in winter months.

A total of 35 gallons of both NAA and Amigo Oil were sprayed on the first spray date. Plants were sprayed until runoff; however, the snow slowed down the ATV and plants received 0.9 L, which was slightly more than the expected 0.7 L.

The second treatment date occurred on February 7th, 2013, starting at 8:00 am. Conditions were slightly more optimal than the previous date with temperatures ranging from the upper 30s to low 40s °F and winds from the north at 15 mph. The same procedures were used to mix the spray solutions. The vines identified to receive two and three applications were sprayed on this date. Spray was again applied until runoff with a total of 17 gallons sprayed of both the NAA and Amigo Oil, equating to a total of 0.7 L per vine.

The third and final application was made on March 7th, 2013 starting at 8:30 am. Conditions were similar to the second application date in February, with temperatures ranging from the 30s to 40s °F and the wind from the south at 10-15 mph. The experimental units that received an application on this date were only the treatments which were meant to receive an application of NAA or oil in all three months, meaning only 1/3 of the original treatments was sprayed. Fourteen gallons total of NAA and ten gallons total of Amigo Oil were sprayed. It was noticed that at the conclusion of this

spray date, a small breeze may be conducive for "swirling" the spray around the cordon increasing coverage.

Once all applications had been completed, the vineyard was pruned by the crew to normal standards in the third week of March. Four canes from the center two vines of each replication were randomly selected and marked with ribbon. All vines were cane pruned to five buds following the last spray application according to James Arthur's normal vineyard management practices.

Data Collection

Bud counts were taken every three days and began on May 6, 2013 and concluded June 6, 2013 after 80% of buds had opened. Bud break was determined as stage four of the modified Eichhorn-Lorenz (E-L) scale of grapevine development (Coombe, 1995). Stage four indicates that the bud scales have expanded to the point where the first leaf tissue is visible. Buds on each of the four preselected canes per experimental unit were counted and recorded. Bud break counts were taken on each preselected cane until bud break had reached 80% (four out of five buds open). Grapevine bud break considered complete when 80% of the buds had reached stage four. The Julian date (beginning January 1, 2013) when the cane had reached 80% bud break was determined. The Julian dates of bud break on each of the four canes were averaged together to obtain a mean Julian date of bud break for that experimental unit.

Harvest

Harvest occurred on August 21, 2013. Each experimental unit was harvested by removing only the grape clusters only from the four preselected canes. To keep harvest

data consistent with bud break data it was necessary to harvest grape clusters growing only from the preselected canes and therefor, only a small percentage of the total fruit per plant was harvested. The total number of clusters and weight was recorded for each two plant experimental unit. The total cluster weight was divided by the total cluster number for each experimental unit to obtain the average cluster weight. Once the clusters had been weighed, 100 berries were randomly chosen and placed in a plastic freezer bag, and stored in the freezer (-17.8°C) until berry sample analysis could be conducted.

Berry Analysis

Berry analysis was conducted on September 13, 2013, to measure pH, °Brix, and titratable acidity (TA). Berries were removed from the freezer the day before testing and placed in a cooler (40°F) to thaw. On the day of testing, the berries were removed from the cooler and warmed to room temperature. Berry samples were then crushed within the plastic freezer bag and the juice was extracted by cutting a small hole in the bag and allowing the clear juice to run out into a 100 ml beaker. The extracted juice was then poured into test tubes to conduct the analyses. Juice pH was measured with a Hanna pH/ORP meter model HI 2211. Soluble solids (°Brix) content was measured using an Atago PR-101 digital refractometer. TA was determined with the use of a Hanna HI 900 automated titration system.

NAA and Oil Phytotoxicity to Dormant Buds

Bud phytotoxicity resulting from the treatments was also evaluated. The eight, (five bud) preselected canes from each experimental unit were evaluated every two days starting May 6, 2013 to June 5, 2013 for bud death (as well as bud break). The total of

unopened buds per experimental unit were recorded and divided by 80% of the potential total number of buds (16) to obtain percent of bud mortality. The three NAA and oil treatments were statistically compared to the control to test for effects on bud phytotoxicity. In two experimental units, a single cane was split due to winter injury. Buds on these canes were not factored into the total bud death.

Pruning Weights

Pruning weights were collected on March 11, 2014. Only shoots originating from the original four preselected canes were collected and measured. Pruned shoots from the four canes were then combined and weighed yielding a total pruning weight for that specific experimental unit.

Statistical Analysis

Data were analyzed using the PROC GLIMMIX procedure to test the effects of multiple applications of NAA and oil on bud break, phytotoxicity and yield parameters. Three Youden Squares were assigned to the field where blocking occurred in both the rows and columns and were random. P-values were adjusted according to Tukey's method. PROC GLIMMIX procedure was used to test for effects at different measurement dates and the AICC covariance model was used for this procedure. (SAS/STAT Version 9.3, SAS Institute, Cary, NC).

Results and Discussion:

Bud Break

Treatments LS-Means were adjusted for multiple tests and compared at α=0.05. Bud break had occurred in all control experimental units by May 6, 2013 (136 Julian days). A significant difference in bud delay was found between oil treatments and both control and NAA treatments at ($p \leq 0.05$) (Appendix 7).

Bud break was significantly delayed by all three treatments of Amigo Oil when compared to the control. One, two and three applications of oil had a significant effect on delaying bud break with a total delay of four days (p=0.0027), six days (p<0.0001), and seven days (p<0.0001), respectively. With one application of oil, bud break was observed between May 16th and May 21st yielding a total bud delay between two and seven days. Bud delay ranged four to nine days on May 19th to May 23nd with two applications of oil. Three applications of oil had a bud break range from May 19th to May 25th giving a total bud delay between five and eleven days.

According to differences in LS-Means, there was no significant difference between any of the three Amigo Oil treatments. However, one application of oil was just slightly not significantly different than three applications of oil (p=0.0608) (Appendix 7).

Table 2: Julian days until 80% bud break of 'Edelweiss' grapevines treated with 1000 ppm NAA or 10% (v/v) Amigo Oil in each of the three Youden Squares and the mean of the three squares.

Treatment	Youden Square 1	Youden Square 2	Youden Square 3	Squares Combined
Control	135.06 a	136.50 ace	133.63 a	135.06 a
NAA 1	135.50 a	135.25 ae	135.13 a	135.29 ac
NAA 2	135.06 a	137.25 ade	134.56 a	135.63 ac
NAA 3	136.25 a	134.13 a	137.81 adc	136.06 ad
Oil 1	135.81 a	141.56 e	138.38 aef	138.58 bcd
Oil 2	139.06 a	142.81 cdfb	140.13 bdeg	140.67 b
Oil 3	139.06 a	144.56 b	142.31 cfg	141.98 b

*1, 2, and 3 corresponds to the number of treatments of NAA or Amigo Oil applied in January, January and February, or January, February and March, respectively.

* Values in the same column with same letters are not significantly different at $p \leq 0.05$.

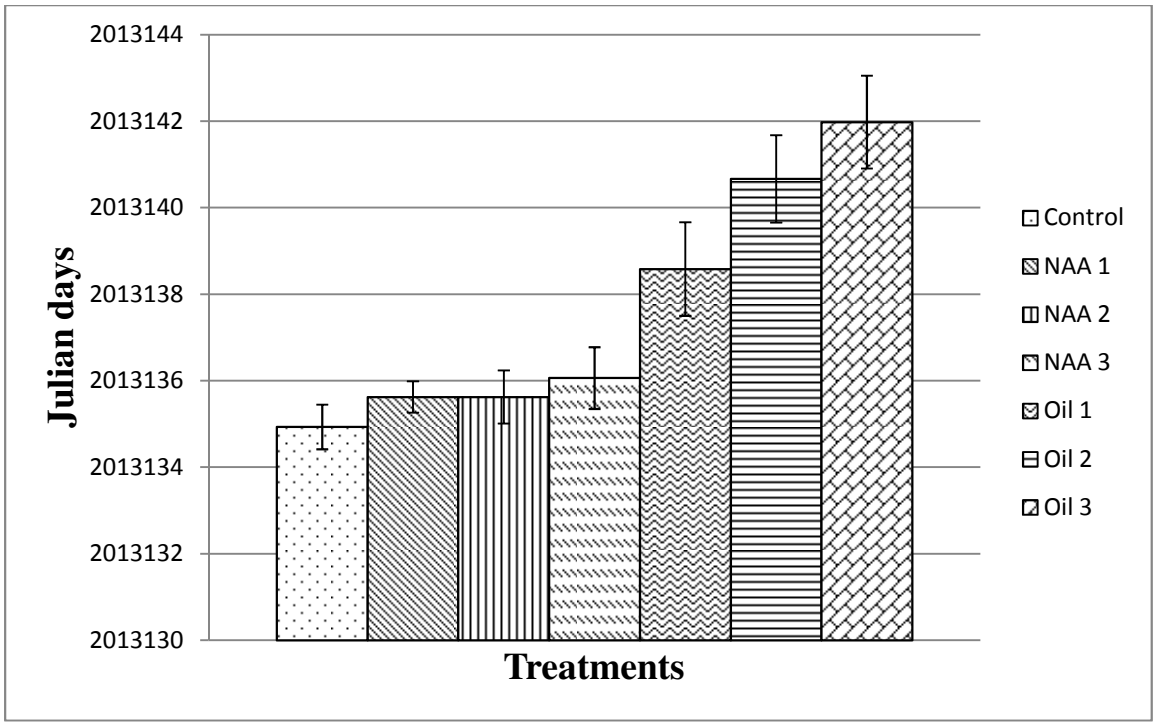


Figure 9. Mean Julian date until 80% bud break of 'Edelweiss' grapevines when treated with one, two, and three applications of 1000 ppm NAA or 10% (v/v) Amigo Oil. Vertical bars represent standard error of means.

In some cases, one or more of the five buds on a cane failed to open and were included in the bud mortality percentage. Instances such as this were semi-frequent in all treatments, resulting in 80% bud break not being achieved in some of the experimental units. To account for this, the point at which the percent bud break ceased to increase for that cane was determined to be the date of bud break.

A repeated measures ANOVA model was fit to determine the differences of each treatment at each of the dates on which bud counts were taken. Bud counts were taken on twelve separate dates beginning on May 4th and ending on June 5th, 2013. Once the bud counts ceased to increase the vine was determined to be budded out.

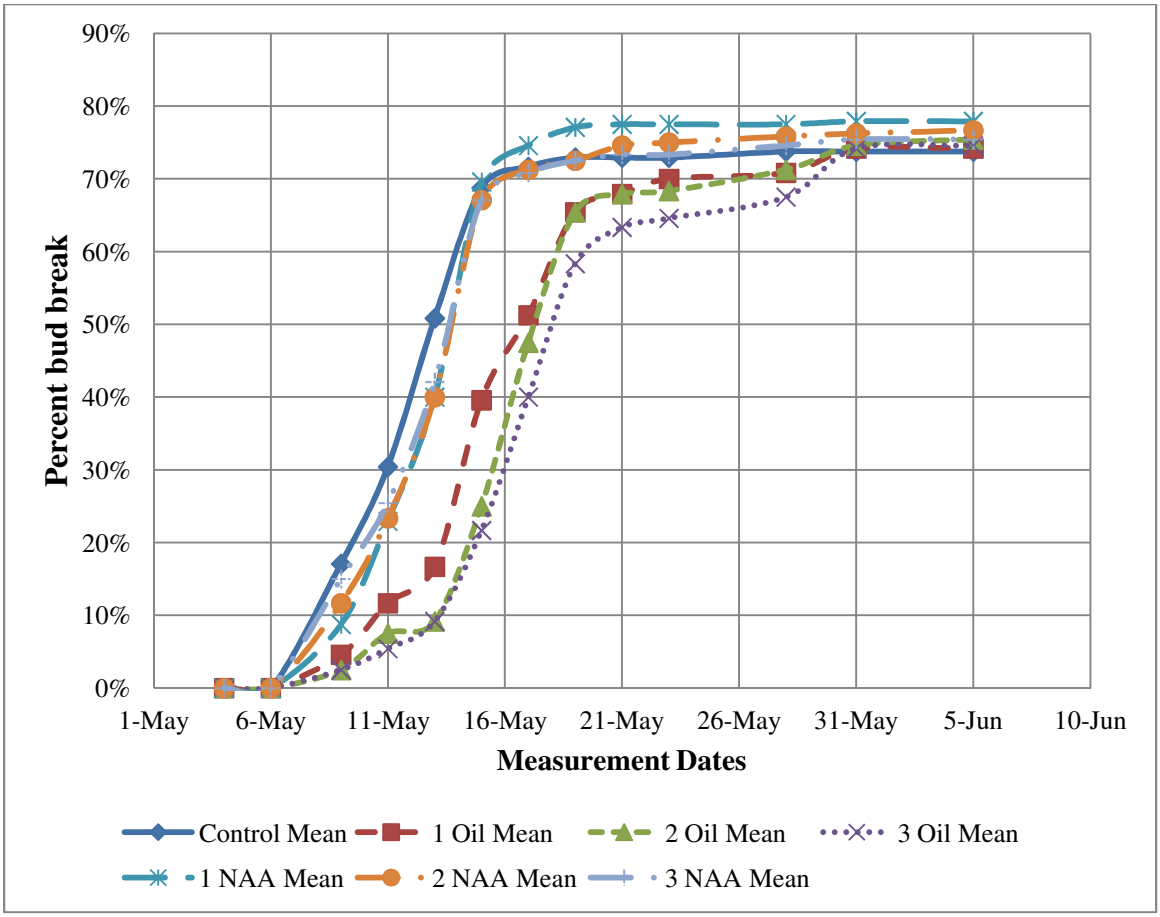


Figure 10. Plot showing the rate of bud break of one, two or three applications of 1000 ppm NAA or 10% (v/v) Amigo Oil at each measurement date.

It is important when analyzing bud break to consider the speed of bud development and opening in addition to mean Julian days until bud break. For instance, it would be important to know what percent of the buds are open on a certain date as compared to the control. Figure 10 shows the control having 50% buds open on May 15 while vines sprayed with three applications of oil have only 9% buds open. If a freeze event occurred on this date only 9% of the vineyard would have primary bud damage had it been sprayed three times with oil. The buds of the control and NAA treatments opened sooner than (i.e. 10%) each of the three oil treatments (Figure 10). Bud break in the

control had almost completely occurred (i.e. >70%) by May 17 while three applications of oil eventually ceased increasing on May 31. When bud break was complete (~75%), a 14 day delay was found between the control and three applications of oil.

NAA treatments showed a similar response to the control, with no significant differences between the control and the three NAA treatments. Thus, one, two and three applications of 1000 ppm NAA had no effect on bud break.

The three oil treatments exhibited a greater delay in bud opening than control and the NAA treatments. Buds that received the control and NAA treatments developed quickly after the first buds began to open reaching 75% bud break within eleven days. The buds receiving oil treatments initially developed much slower. On May 13, when the control was at 50% bud break, treatments of one and three applications of oil were only at 9% bud break. However, after 10% of buds had opened the process had begun and all treatments then opened at the same rate. The oil affected the buds by keeping the open bud percentage below 10% for a longer period of time. All three oil treatments were significantly different from that of the control ($p<0.0001$ – $p=0.0466$) through May 17 (Figure 10). The curve began to level off around May 21, indicating the effect of three applications of oil was longer lasting on roughly 10% of the buds. Oil treatments were no longer significantly different from the control or the NAA treatments after May 21 and remaining buds gradually opened to a final date of May 31.

The delayed bud opening response occurred successively in conjunction with the number of times oil was applied. Three applications of Amigo Oil showed the greatest suppression of bud development and open bud counts stopped increasing on May 31st (14

days later than the control). The control leveled off and reached its highest bud break on May 17th with 73% of its buds open. On the same date, bud break of buds receiving three applications of oil was only 40%. One and two applications of oil were only significantly different from one another on May 15 (p=0.0363) where their percentages of bud break were 25% for two oil applications and 40% for one oil application. On remaining dates there were no significant differences between one and two applications of oil (Figure 10).

Delaying bud break up to eleven days and slowing initial bud opening can encourage grape growers to use Amigo Oil as a preventative tool for protecting their crop from spring freeze injury. The ability of the oil to delay bud break could be attributed to the reduced respiratory activity in the bud. Dami and Beam (2004) concluded nodal sections treated with Amigo Oil had 41% less emitted CO_2 than that of the controls. They concluded from this work that oil coating of dormant buds may have hindered CO_2 escape from buds, which resulted in decreased respiration. This also agrees with past research where dormant oil was applied to 'Biscoe' peaches and internal CO_2 concentrations were measured. It was concluded that the internal CO_2 concentration was higher compared to the control (Deyton et al., 1992). Myers et al. (1996) applied soybean oil to 'Georgia Belle' peach trees and also reported applications of 10% oil increased internal CO_2 concentrations and delayed bud break. Although there were no internal CO_2 concentration measurements done in our work, conclusions from previous studies provide clear reasons as to why soybean oil delays bud break. Filling air spaces between bud scales and reducing internal bud CO_2 concentrations is an important characteristic contributing to oils ability to delay bud break.

NAA and Oil Phytotoxicity to Dormant Buds

Bud mortality ranged from 3.13% (one application NAA) to 8.72% (control) (Table 3). The control had the highest percentage of bud death of all the treatments. There were no significant differences found between any of the treatments at $p \leq 0.05$. It can be concluded that treatments were not phytotoxic and did not cause increase bud mortality. Dead buds observed may have been the result of winter or mechanical damage to the buds. Dami and Beam (2004) reported vines treated with Amigo Oil sustained 4% to 5% injury. However, in this research injury in Amigo-treated vines was not different from that of the control vines, indicating that at a 10% rate Amigo Oil is not phytotoxic. Dami and Beam (2004) also mentioned that increased bud death (>12%) in his experiment may have been caused by the oils not being thoroughly mixed before application. This corresponds to previous observations where oil can separate from water within ten minutes, making it necessary to include a tank agitator in the spray tank.

Table 3: Bud mortality in 15-year-old 'Edelweiss' grapevines in response to multiple application of 1000 ppm NAA or 10% (v/v) Amigo Oil.

Treatment	Injury (%)
Control	8.7 a
NAA 1	3.1 a
NAA 2	4.8 a
NAA 3	5.7 a
Oil 1	7.3 a
Oil 2	5.7 a
Oil 3	6.3 a

*1, 2, and 3 corresponds to the number of treatments of NAA or Amigo Oil applied in January, January and February, or January, February and March, respectively.

*Values with same letters are not significantly different at $p \leq 0.05$.

Harvest Results:

Number of Clusters per Cane

Equally important to effectiveness of the NAA or Amigo Oil on bud break is treatments not negatively affecting harvest parameters. Table 4 shows there was no treatment effect on the number of clusters per cane when 'Edelweiss' grapevines were treated with multiple applications of NAA and Amigo Oil when compared to the control ($p \leq 0.05$). Total cluster counts were within the acceptable range when considering the implemented grapevine management strategy. In addition, James Arthur Vineyards had one of the largest harvests on record out of the treated 'Edelweiss' block.

Table 4: Total cluster count per two vine experimental unit treated with one, two and three applications of 1000 ppm NAA or 10% (v/v) Amigo Oil to 'Edelweiss' grapevines.

Treatment	Total Cluster Count
Control	19.6 a
NAA 1	22.1 a
NAA 2	23.7 a
NAA 3	18.8 a
Oil 1	20.4 a
Oil 2	18.6 a
Oil 3	16.2 a

*1, 2, and 3 corresponds to the number of treatments of NAA or Amigo Oil applied in January, January and February, or January, February and March, respectively.

* Values with same letters are not significantly different at $p \leq 0.05$.

Table 5: Mean cluster number per cane treated with one, two and three applications of 1000 ppm NAA or 10% (v/v) Amigo Oil to 'Edelweiss' grapevines.

Treatment	Mean Cluster Number per Cane
Control	2.2 a
NAA 1	2.8 a
NAA 2	3.0 a
NAA 3	2.3 a
Oil 1	2.5 a
Oil 2	2.3 a
Oil 3	2.0 a

*1, 2, and 3 corresponds to the number of treatments of NAA or Amigo Oil applied in January, January and February, or January, February and March, respectively.

*Values with same letters are not significantly different at $p \leq 0.05$.

Cluster Weight

Table 7 shows there was no treatment effect on the average cluster weight when compared to the control ($p \leq 0.05$). This supports previous research which has shown little or no interaction between NAA or oil treatments and cluster weights (Dami, 2007; Dami and Beam, 2004; Qrunfleh and Read, 2010). There was however a slight difference in total cluster weight between two applications of NAA and three applications of oil. In addition, two and three applications of oil had significantly different mean cluster weights (p=0.04) (Appendix 11). These small differences can be attributed to inconsistency of berry ripeness at harvest. The experiment was conducted at a commercial winery and the entire 'Edelweiss' block was harvested in one day. It was not possible to only pick fruit at the proper ripeness.

Table 6: Total cluster count per two vine experimental unit treated with one, two and three applications of 1000 ppm NAA or 10% (v/v) Amigo Oil.

Treatment	Total Cluster Weight (lbs)
Control	7.2 ab
NAA 1	8.1 ab
NAA 2	8.8 a
NAA 3	7.8 ab
Oil 1	7.0 ab
Oil 2	7.0 ab
Oil 3	4.7 b

*1, 2, and 3 corresponds to the number of treatments of NAA or Amigo Oil applied in January, January and February, or January, February and March, respectively.

*Values with same letters are not significantly different at $p \leq 0.05$.

Table 7: Mean cluster weight after harvest of 'Edelweiss' grapevines treated with one, two and three applications of 1000 ppm NAA or 10% (v/v) Amigo Oil.

Treatment	Mean Cluster Weight (lbs)
Control	0.4 ab
NAA 1	0.4 ab
NAA 2	0.4 ab
NAA 3	0.4 ab
Oil 1	0.4 ab
Oil 2	0.4 a
Oil 3	0.3 b

*1, 2, and 3 corresponds to the number of treatments of NAA or Amigo Oil applied in January, January and February, or January, February and March, respectively.

*Values with same letters are not significantly different at $p \leq 0.05$.

Berry Analysis

No significant difference between the control and any of the treatments was found in °Brix of 100 berry samples of 'Edelweiss' berries at $p \leq 0.05$ °Brix ranged from 12.87 (control) to 13.51 (NAA 2 & Oil 2) A significant difference was found in pH of the 100 berry samples between the control and three oil applications (p=0.0438) (Table 8). Again, this is the result of inconsistent fruit ripeness across the vineyard. There were no other significant differences found between the control and any of the treatments. The pH of berries ranged from 3.12 to 3.28. Similarly to °Brix, there were no significant differences observed between the control and any of the treatments when measuring TA (titratable acidity) of the 100 berry samples (Table 8). TA ranged from 12.02 g/l to 13.76 g/l.

Table 8: Measured values of pH, °Brix, and titratable acidity (TA) from 100 berry samples of 'Edelweiss' grapevines treated with one, two and three applications of 1000 ppm NAA or 10% (v/v) Amigo Oil.

	Treatments						
	Control	**1 Oil**	**2 Oil**	**3 Oil**	**1 NAA**	**2 NAA**	**3 NAA**
pH	3.28 a	3.14 ab	3.19 ab	3.12 b	3.18 ab	3.19 ab	3.19 ab
°Brix	12.87 a	13.33 a	13.51 a	13.42 a	12.97 a	13.51 a	13.14 a
TA (g/L)	12.02 ab	12.76 ab	13.21 ab	13.76 a	12.36 ab	11.58 ab	12.30 b

*1, 2, and 3 corresponds to the number of treatments of NAA or Amigo Oil applied in January, January and February, or January, February and March, respectively.

*Values in the same row with same letters are not significantly different at $p \leq 0.05$.

Regarding harvest parameters, Dharmadhikari and Wilker (2001) mentioned that optimum ranges for white wine would be 21-22 °Brix, 3.2-3.4, and 0.7-0.9% for total soluble solids, pH, and the TA, respectively. 'Edelweiss' is harvested at an earlier stage regarding °Brix when compared to a cultivar such as 'Vignoles'. Swenson et al. (1980), mentioned 'Edelweiss' juice is relatively low in acidity (0.6-0.8%) and has moderate soluble solids (14-16%) and should be picked at an early mature stage (14 °Brix). There has been much controversy amongst winemakers about which harvest parameter is most important, pH or soluble solids. However, harvesting the grapes at optimum pH may be the most beneficial because adjusting sugar levels in the juice is far easier than adjusting pH.

The harvest results of this study were generally in the 21-22% recommended range for total soluble solids (Dharmadhikari and Wilker, 2001). 'Edelweiss' grapes are generally harvested with total soluble solids of 12.5-14 °Brix as compared to recommended rates of similar cultivars.

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 studies in eastern states found high oil applications could delay ripening and reduce yields. This corresponds to what was found when applying different concentrations of Prime Oil and Amigo Oil to grapevines, which showed high concentrations of either oil caused phytotoxicity and bud death resulting in reduced yields (Dami and Beam, 2004). The article reported two studies were conducted in California and 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. Clearly, bud break delay and

yield components are dependent upon the concentration and type of oil used, making it necessary to find the proper concentration for the most optimum results. Multiple applications do not appear to have a compounding effect on phytoxicity. For example, three applications of 10% oil do not act similar to a single application of 30% oil

Pruning Weights

Pruning weights were collected on March 11, 2014. Only the pruning from the four preselected canes from each experimental unit were collected and weighed. The pruning weights depicted in Table 9 are just a fraction of the total weight of prunings that were removed from the entire experimental unit. However, there were no significant differences in pruning weights between NAA, oil or control treatments.

Table 9: Mean pruning weights of each of the treatments, taken and weighed on March 11, 2014.

Treatment	Total Pruning Weight (lbs)
Control	1.00 a
NAA 1	0.92 a
NAA 2	0.91 a
NAA 3	1.16 a
Oil 1	0.87 a
Oil 2	0.82 a
Oil 3	0.87 a

*1, 2, and 3 corresponds to the number of treatments of NAA or Amigo Oil applied in January, January and February, or January, February and March, respectively.

*Values with same letters are not significantly different at $p \leq 0.05$.

Climatic Data

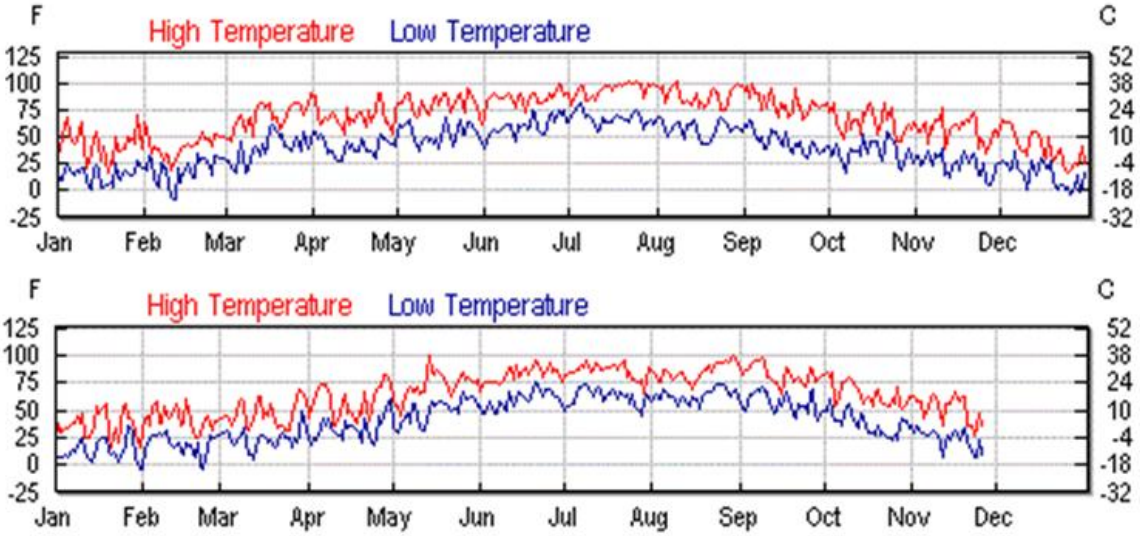


Figure 11: 2012 (upper) and 2013 (lower) temperature data from the Oak Creek Vineyard, located roughly two miles from research site. Source: Weather Underground.

The average temperature for the growing months in 2013 was 68.3°F (20.17°C) with a high temperature reaching 100.0°F (37.78°C) as shown in Figure 11. This compared to 2012 which showed an average temperature of 73.7°F (23.17°C) and a high temperature of 102.9°F (39.39°C). More interestingly, comparing March and April of 2012 and 2013, there were major differences in average and high temperatures between these months. In March 2012, the average temperature was 53.9°F (12.17°C) with a high of 91.0°F (32.78°C) and in April 2012 the average temperature was 56.1°F (13.38°C) with a high of 91.0°F (32.78°C). In comparison, March 2013 had an average temperature of only 34.4°F (1.33°C) and a high of 70.0°F (21.11°C). The average temperature in April 2013 was 45.3°F (7.39°C) with a high of 82.9°F (28.18°C). The spring of 2012 was abnormally warm for this area, while the next year (2013) was the complete opposite

and was uncharacteristically cool. With such a late and cool spring in 2013, we were concerned the NAA or oil treatments would not show bud delay effects as the plants were already behind schedule. However, significant differences were found amongst the oil treatments and the control, regardless of the abnormally cool spring. We would expect that in a typical temperature based year, bud delay could be extrapolated out even further than what was observed. Vines stayed dormant far into the spring in 2013. However, when temperatures suddenly increased, bud break of treatments responded in parallel with temperatures (Figure 10 & 11). Oil appeared to slow the physiological response to warmer temperatures and increased day length for a period of time, until it could no longer hold back this response. At this point, we then see a dramatic rate increase in bud break of oil treatments several days later.

It was also observed before any bud break counts were taken that oil was clearly still on the vines in the spring and summer. Wood of trunks and canes appeared to have been rubbed with furniture oil, giving it a dark chocolate brown color. Vines sprayed with oil were easily distinguished from the control and NAA treated vines. It is crucial that the oil remain on the vines and Dami and Beam (2004) stated that oil effectiveness may vary from one season to another according to the degree of oil "weathering". Multiple applications could possibly overcome the "weathering" of the oil throughout the winter.

References

Coombe, B., 1995. Growth Stages of the Grapevine: Adoption of a system for identifying grapevine growth stages. *Australian Journal of Grape and Wine Research* 1:104-110.

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

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

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

Dharmadhikari, M.R. and K.L. Wilker, 2001. Micro vinification: a practical guide to small scale wine production. Midwest Viticulture and Enology Center, Dept. of Fruit Science, Southwest Missouri State University.

Google-Maps. 2013. James Arthur Vineyard. https://maps.google.com/maps?q=james+arthur+vineyards&ie=UTF-8&ei=lKD7UvThOOy62gXq-IGABQ&ved=0CAwQ_AUoBA

McFarland, S. and M. McFarland. 2008. Mac's Creek - Yr 2 Cold Hardiness - #18-13-060.

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. *Journal of the American Society for Horticultural Science* 121:96-100.

Qrunfleh, I.M. and P.E. Read, 2010. Delaying bud break in 'Edelweiss' grapevines to avoid spring frost injury by NAA and vegetable oil applications. University of Nebraska, Lincoln, Nebraska, PhD http://digitalcommons.unl.edu/agronhortdiss/14/.

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

CHAPTER 5

Delaying Bud Break in the Laboratory 2013

Experiment 1, 2013

Materials and Methods:

Plant Material

Dormant canes of 'Edelweiss' grapevines were collected from James Arthur Vineyards near Raymond, Nebraska on January 16, 2013. A total of 150 canes were taken from the same 'Edelweiss' block in which the field experiment was done, however, the canes were taken from rows that had not been sprayed. The age, vigor, and growing conditions of the plants were identical to those of the main experiment. Canes were headed back to the fifth bud and stems with buds six through nine were collected. It was not possible to take the first five buds of the cane as James Arthur is a commercial vineyard and we did not want to cause potential fruit losses.

After collection, canes were brought back to the lab, wrapped in moist newspaper, placed in a plastic bag and put into a 1.7°C cooler until experimentation began. The day of experimentation, canes with the seventh position buds were removed from the cooler and sorted based upon stem length and diameter. Only canes with similar sized stems were used for experimentation. The single bud cuttings were soaked in a solution containing 10% bleach (Clorox™, 6% Sodium Hypochlorite) for 15 seconds and then rinsed with distilled water. A 4x4 Latin Square design was used and each GA7 vessel contained each treatment of either the Amigo Oil or the NAA. The single bud cuttings

were then randomly assigned to a specific treatment by marking with a certain color of marking tape.

Preparing Forcing Solutions

A stock of the forcing solution 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 deionized distilled water to reach 1000 ml (Read et al., 1984). The forcing solution was stored in a dark cooler at 35°F (1.67°C) when not being used.

Treatments

The treatments in this experiment were the same as the field experiment. They consisted of seven treatments: one, two, and three applications of 10% (v/v) Amigo Oil (Loveland Industries, Greely, CO) which consisted of 9.3% oil 0.7% emulsifier and 90% water; one, two, and three applications of 1000 ppm NAA (*Phyto*Technology Laboratories, Shawnee Mission, KS), and the control. One tray was set up for the three treatments of the Amigo Oil and the control, and another tray was set up for the three NAA treatments. NAA concentrations were prepared as described earlier. Treatments were applied according to Qrunfleh and Read (2010) on buds by adding one drop per bud using a sterile transfer pipette. Oil and NAA treatments were applied at weekly intervals, up to three weeks. After treatment, the single-bud canes were placed vertically (proximal ends down) in GA7 vessels containing approximately 100 ml of freshly prepared forcing solution. The solutions in the GA7 vessels were replaced with freshly prepared forcing solution every two or three days as the volume of the solution decreased. The GA7 vessels (*Phyto*Technology Laboratories, Shawnee Mission, KS) were placed under

artificial light at 12 hour days and at 25°C. Days to bud break starting from the date of treatment 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 phytotoxic effects of the treatments.

Experiment 2, 2013

Materials and Methods:

'Edelweiss' canes collected from James Arthur Vineyards from the previous experiment were used; however, bud positions six and eight were used for experimentation. Canes were cut into single bud cuttings and separated based upon either bud position six or eight. The single bud cuttings were sorted by stem length and diameter to ensure uniformity. The single bud cuttings were soaked in a solution containing 10% bleach for 15 seconds and rinsed with distilled water. A randomized complete block design was used where each GA7 vessel contained each treatment of either the Amigo Oil or NAA. The single bud cuttings were then randomly assigned to a specific treatment by wrapping with a certain color of marking tape.

Preparing Forcing Solutions

A stock of forcing solution 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 deionized distilled water to reach 1000 ml (Read et al., 1984). The forcing solution was stored in a dark cooler at 1.67°C when not being used.

Treatments

The experiment consisted of the same treatments as the previous forcing solution experiment and the field experiment. The seven treatments were: one, two, and three applications of 10% (v/v) Amigo Oil (Loveland Industries, Greely, CO) which consisted of 9.3% oil, 0.7% emulsifier and 90% water; one, two, and three applications of 1000 ppm NAA (*Phyto*Technology Laboratories, Shawnee Mission, KS), and the control. One tray was set up for three treatments of the Amigo Oil and the control, and another tray was set up for NAA treatments. NAA concentrations were prepared as described earlier. Treatments were applied differently from the previous experiment. It was found that placing a single drop of the oil or NAA on the bud did not completely cover the entire bud and in some cases the drop fell off the bud. Insufficient and inconsistent coverage was occurring by using the single drop method. A new simple method of applying the treatments involved mixing up the 10% (v/v) oil or 1000 ppm NAA, placing the solution into a 100 ml beaker and dipping the single bud cutting into the solutions for five seconds. Oil and NAA treatments were applied at weekly intervals, up to three weeks. After treatment, single-bud canes were placed vertically (proximal ends down) in GA7 vessels containing approximately 100 ml of freshly prepared forcing solution. Because of the previously failed experiment, the forcing solution was 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 under 12 hour days of artificial light at room temperature 25°C. Days to bud break starting from the date of treatment application were recorded throughout the study. Buds that did not break were examined according to Qrunfleh and Read (2010) by cutting

into longitudinal sections and examining under a stereomicroscope to examine the viability of the bud and any phytotoxic effects caused by treatments.

Analysis of Variance was conducted using the PROC GLIMMIX procedure to test the effects of multiple applications of NAA and oil on bud break. All analyses were conducted using SAS/STAT Version 9.3, SAS Institute, Cary, NC.

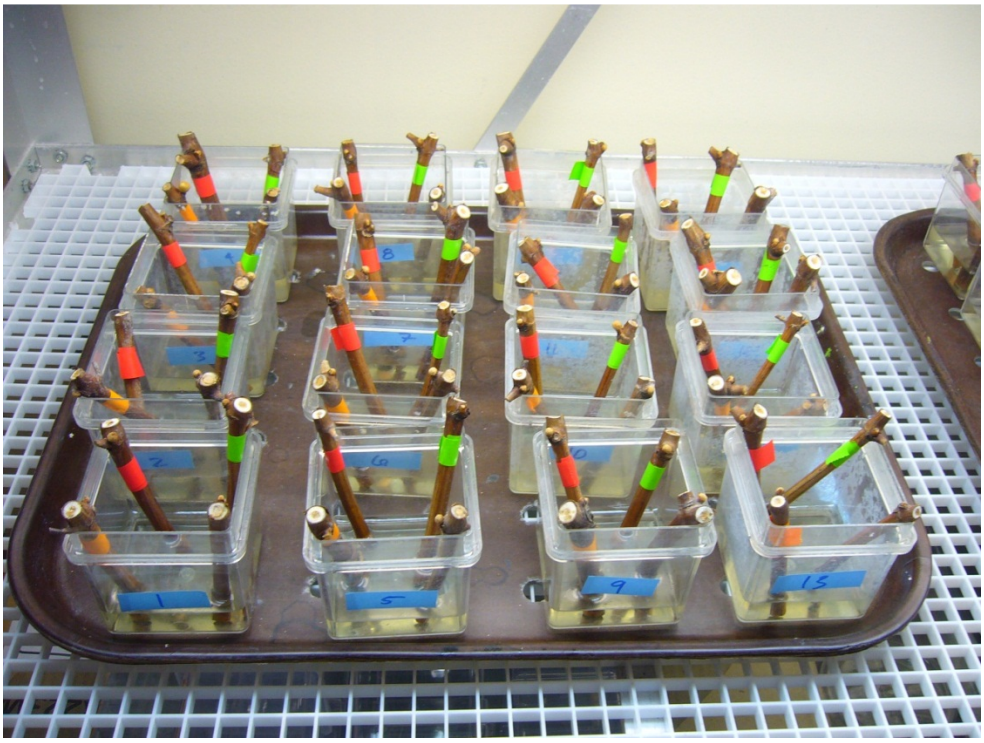


Figure 12: 'Edelweiss' single-bud cuttings being forced in forcing solution consisting of 200 mg 8-hydroxyquinoline citrate and 2% sucrose.

Results and Discussion:

A total of 192 single-bud cuttings were used in this experiment. With the exception of one bud in the NAA experiment, the only buds which showed phytotoxic effects came from the oil experiment. A total of 5 out of 96 buds showed phytotoxic

effects and failed to grow. Dami and Beam (2004) reported 6-10% bud injury with Prime Oil and 4-5% with Amigo Oil. In this experiment Amigo Oil showed phytotoxic effects just over 10%. These phytotoxic effects may be the result of the oil not being physically degraded from the buds by environmental conditions. It is also possible that buds on the preselected canes may have already been injured or dead, as there is no way to check for bud health in the field without killing the bud. During the selection process, only canes with plump healthy appearing buds were chosen.

Lavee and May (1997) discussed reasons for the lack of growth could be explained by: physical or chemical conditions external to the bud or bud scale restriction by enclosing bract tissue. Qrunfleh and Read (2010) experienced similar difficulties and had 10% bud mortality in laboratory trials forcing single-bud cuttings. This was explained by single-bud cuttings taken too early where the grapevines were still in the endodormant stage. In addition, cuttings may have not received adequate chilling hours because the month of January had abnormally high temperatures.

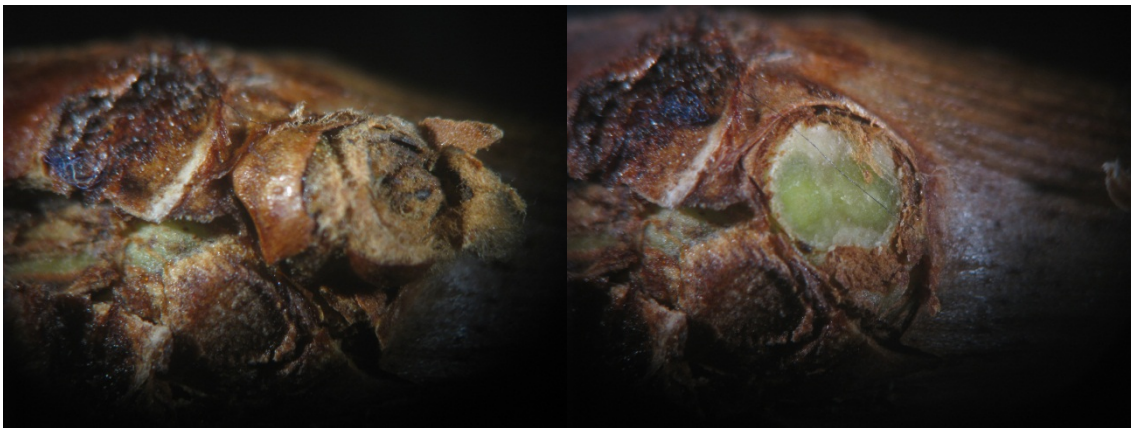


Figure 13: ‘Edelweiss’ single bud cutting treated with oil showing phytotoxicity and complete bud death (left). ‘Edelweiss’ single-bud cutting treated with oil showing no phytotoxicity effects to the primary and secondary buds (right).

No significant differences were found between bud position (#6 and #8) in either the oil or NAA experiments as seen in Table 10. As a result the #6 and #8 bud position experiments were combined and analyzed together to increase statistical power.

Table 10: Type III test of fixed effects, testing for differences amongst bud position #6 and #8 treated with one, two and three applications of 1000 ppm NAA or 10% (v/v) Amigo Oil.

Effect	F-value	Pr > F
Bud Position * NAA Treatments	0.6	0.6196
Bud Position * Oil Treatments	0.63	0.6008

Table 11: Comparison of one, two, and three applications of 1000 ppm NAA or 10% (v/v) Amigo Oil to 'Edelweiss' single bud cuttings to the control. Buds were forced under laboratory conditions using 200 mg 8-hydroxyquinoline citrate and 2% Sucrose.

Treatments	Estimate	Standard Error	Adjusted P-value
Control vs NAA 1	-5.292	1.552	0.0060
Control vs NAA 2	-7.034	1.570	0.0002
Control vs NAA 3	-8.500	1.552	<.0001
Control vs Oil 1	-5.136	3.519	0.4689
Control vs Oil 2	-14.307	3.570	0.0011
Control vs Oil 3	-24.266	3.611	<.0001

*1, 2, and 3 corresponds to the number of treatments of NAA or Amigo Oil applied at weekly intervals.

*Values are significantly different at $p< 0.05$

Significantly different delays were observed between all three NAA treatments and the control at $P\leq 0.05$. There was not a difference in the amount of bud delay between one application of oil and the control; however, there was a significant delay between the two and three oil applications and the control (Table 11).

Figure 14: ‘Edelweiss’ single-bud cutting treated with 1000 ppm NAA showing bud expansion.

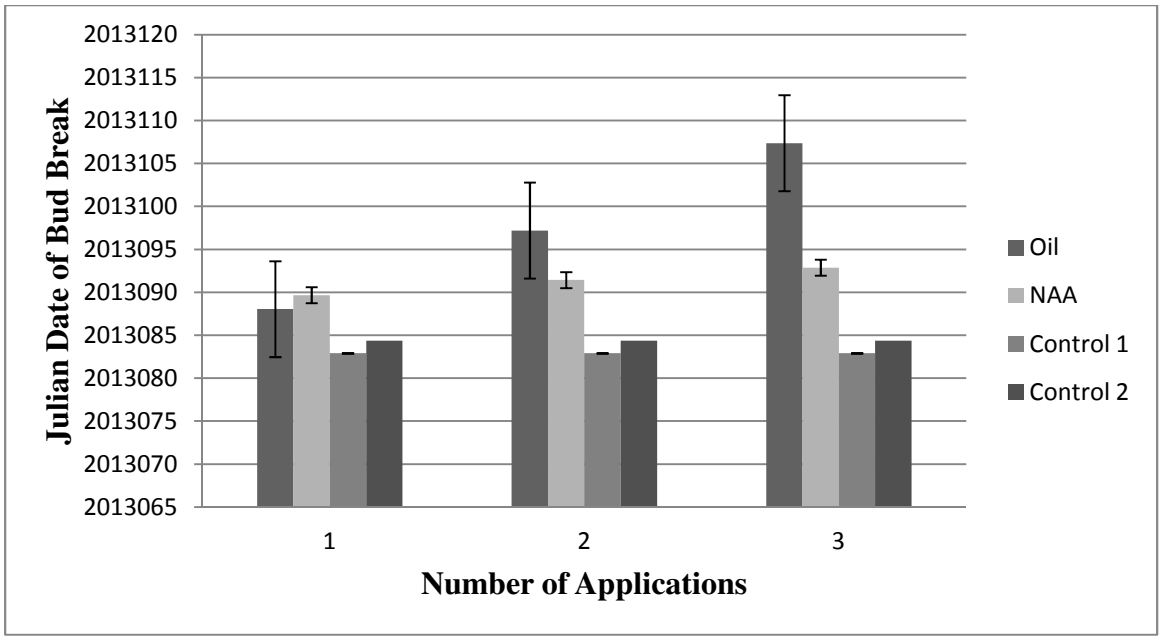


Figure 15: Number of Julian days until 'Edelweiss' single-bud cuttings showed bud break at bud position six and eight treated with one, two or three applications of 1000 ppm NAA or 10% (v/v) Amigo Oil. Control 1 is associated with the oil treatments and control 2 is associated with the NAA treatments. Vertical bars represent standard error of means.

Figure 15 corresponds directly to data gathered in the field where each additional application of oil significantly extends the date of bud break. Each of the three oil applications was significantly different from one another at $P \leq 0.05$ (Table 11). NAA treatments showed similar response oil where each additional application extended the date of bud break. However, there was not a statistically significant difference between one, two or three applications of NAA (Table 11).

Table 12: Comparison of single bud cuttings treated with one, two and three applications of 10% oil and 1000 ppm NAA.

Treatments	Estimate	Standard Error	Adjusted P-value
NAA 1 vs NAA 2	-1.742	1.570	0.6848
NAA 1 vs NAA 3	-3.208	1.552	0.1749
NAA 2 vs NAA 3	-1.466	1.570	0.7868
Oil 1 vs Oil 2	-9.170	3.570	0.0612
Oil 1 vs Oil 3	-19.129	3.611	<.0001
Oil 2 vs Oil 3	-9.959	3.661	0.0426

*1, 2, and 3 corresponds to the number of treatments of NAA or Amigo Oil applied at weekly intervals.

*Values are significantly different at $p< 0.05$

Results from NAA treatments in the laboratory experiment showed the opposite effect of what was found in the field. This can be explained by the "weathering" of NAA after application to the vines in the field. Within the field, buds undergo harsher conditions than buds in a controlled laboratory environment. This "weathering" effect in the field may be the primary reason NAA treatments showed no bud break delay in the field but showed significant delays in the laboratory. The lack of "weathering" in the lab allows the NAA to stay on the canes and buds where it slows the escape of respiratory CO_2 and suppresses buds response to increased light and temperature.

Solely through visual observations, oil treated buds showed secondary growth bud before primary bud growth. This may be explained by oil and NAA slowing respiration in the primary bud to an extent that plants push the secondary bud. However, once the oil is weathered away or the signal is overcome the primary bud begins to grow. This

phenomenon was also observed in the field experiment where both primary and secondary buds pushed simultaneously (Figure 16 & 17).

Figure 16: The primary and secondary buds opening simultaneously on 15-year-old ‘Edelweiss’ grapevines.

Figure 17: Primary bud opening after the secondary bud on 15-year-old ‘Edelweiss’ grapevines.

Laboratory Experiment Comparing January Application and the Control

It was unclear if the application in the first week of January would delay bud break. During the time of application weather was not optimal where temperatures were between 28°F and 32°F and 8 inches of snow was on the ground. Below freezing temperatures caused the buildup of “Amigo Ice” within the sprayer, requiring thawing each time the tank was refilled. Low temperatures caused spray solution to freeze instantly as it coated the vines. A combination of these problems made it unclear as to whether or not the treatments would be effective. To test for treatment effects, dormant single-bud cuttings were collected from the one oil, one NAA and the control treatments sprayed in January. Single-bud cutting were brought back to the lab and forced under a

controlled laboratory environment. Effects of the treatments were analyzed in an identical way to the field experiment and related to the field results.

Materials and Methods:

Preparing Forcing Solutions

A stock of the forcing solution containing 200 mg 8-hydroxyquinoline citrate (8-HQC)/l and 2% sucrose was prepared by weighing out 0.2 g 8-HQC, 20 g of sucrose and adding deionized distilled water to reach 1000 ml (Read et al., 1984). The forcing solution was stored in a dark cooler at 1.7°C when not being used.

Plant Material

Single bud cuttings were collected on April 1, 2013 from vines receiving one application of NAA, Amigo Oil and the control. Canes were selected from the two outside plants of the four plant experimental unit since the center two plants were used for data collection in the field experiment. A single bud cutting (5^{th} position bud) was taken on either side of the row from each plant, with a total of four cuttings taken per experimental unit. A total of twelve replications of the control, NAA, and Amigo Oil treatments were collected, yielding a total of 144 cuttings.

The single bud cuttings were brought back to the lab, immediately had the basal 0.2 cm ends cut off and were placed basal-end-first into baby food jars containing 100 ml of 8-HQC. Cuttings were placed on a light rack under 12 hour days at 25°C. The baby food jars were arranged identically to their orientation and blocking in the vineyard. Each jar contained four single bud cuttings of the same experimental unit. The solutions

were replaced with 100 ml of freshly prepared 8-HQC every four days and the basal 0.2 cm ends of the cuttings were cut off each time the solutions were changed. Julian days to bud break beginning from the date of when the cuttings were placed in forcing solution were recorded throughout the study. After all cuttings had broken bud, the four single-bud cuttings for each experimental unit were averaged together to obtain a final bud break date for that treatment experimental unit. 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 phytotoxic effects of the treatments according to Qrunfleh and Read (2010).

Analysis of Variance was conducted using the PROC GLIMMIX procedure to test the effects of multiple applications of NAA and oil on bud break. All analyses were conducted using SAS/STAT Version 9.3, SAS Institute, Cary, NC.

Results and Discussion:

A total of 144 single-bud cuttings were used in this experiment. Bud mortality was minimal, where in both the oil and NAA treatments a total of just two buds failed to open (≈4% bud mortality). The single-bud cuttings used for this experiment were collected later in the winter than the cuttings used for the forcing experiment, resulting in a faster rate of bud break. This response can be attributed to the buds being in the early stages of ecodormancy.

The three Youden Squares were analyzed separately and combined after no block effect was found. The control broke bud April 22, 2013 and buds treated with one

application of NAA occurred two days later on April 24, 2013. Finally, buds treated with one application of Amigo Oil broke bud ten days later on May 2, 2013.

Table 13: Number of Julian days until bud break of single bud cuttings treated with one application (in January) of 1000 ppm and 10% (v/v) Amigo Oil in each of the three blocks and the mean of the blocks.

Treatment	Youden Square 1	Youden Square 2	Youden Square 3	Squares Combined
Control	113.13	111.88	112.19	112.40 a
NAA 1	116.50	116.00	109.69	114.06 a
Oil 1	113.88	127.96	124.13	121.99 bc

*1 corresponds to the number of treatments of NAA or Amigo Oil.

*Values in the same row with same letters are not significantly different at $p \leq 0.05$.

Similar to the field experiment, there was a significant difference between one application of oil and the control *(p*=0.0225) (Table 13). However, it was interesting that the statistical significance was greater in the laboratory than the field experiment *(p*=0.0403) (Table 13). This reinforces the fact that "weathering" degrades treatments in the field, making it necessary to apply multiple applications. The cuttings that were removed from the corrosive environment of the vineyard and brought into a controlled laboratory setting showed a more significant delay than the buds that were left out in field.

One application of NAA also showed similar results to the field experiment and was not significantly different from the control *(p*=0.0833) (Table 13). However, in the laboratory experiment the single application of NAA was much more significant than the

single application in the field experiment *(p*=0.9999). Again, “weathering” of the NAA is a major factor in deteriorating the effectiveness of the NAA.

References

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

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

Qrunfleh, I.M. and P.E. Read, 2010. Delaying bud break in 'Edelweiss' grapevines to avoid spring frost injury by NAA and vegetable oil applications. University of Nebraska, Lincoln, Nebraska, PhD http://digitalcommons.unl.edu/agronhortdiss/14/.

Read, P.E., 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 Czech. Acad. Sci. Prague. 467-473

CHAPTER 6

Conclusions:

1. Bud break is greatly dependent upon spring temperatures (growing degree days) and the effect of applying Amigo Oil will either be magnified or minimized depending upon the rate of the accumulation of growing degree days in the spring.
2. The initial delay of bud opening after treatments is or as more important than the actual final date of bud break. It was observed that none of the treatments suppressed 100% of the buds, but the initial rate at which buds opened was significantly different between one and two applications of oil when compared to the control.
3. Amigo Oil did not exhibit the 20-day delay reported by Dami and Beam (2004), or the 12 days reported by Qrunfleh and Read (2010). However, with three applications of oil a bud delay between 5-11 days was observed. In years where an earlier spring occurs it may be expected that these delays could be extended.
4. Amigo Oil applications showed better performance compared to the control, with one application of oil delaying bud break four days compared to the control, two applications delaying bud break six days, and three applications delaying bud break by seven days.
5. 1000 ppm NAA did not exhibit the 7-day delay reported by Qrunfleh and Read (2010) using 'Edelweiss' grapevines. There was absolutely no effect of one, two, or three applications of NAA on bud break in the field study.
6. Delaying bud break with oil and trying to delay bud break with 1000 ppm NAA showed no negative impact on berry characteristics. Differences that occurred in

pH of the berries appeared to be the result of differences in vine sampling location.

7. In forcing solution studies, one, two and three applications of NAA significantly delayed bud break when compared to the control. Two and three applications of oil significantly delayed bud break when compared to the control. Forcing buds in controlled laboratory environment eliminated the "weathering" of the oil and maximized the effects of the treatments. The favorable conditions (i.e. light quality, day length, temperature) of the environment also contributed to the increased effectiveness of the auxin NAA.
8. To achieve optimum vine coverage a hand sprayer will not suffice. For large scale vineyard a mechanical sprayer must be built for consistent spraying. A tank agitator must also be installed within the tank when using Amigo Oil, as separation begins within 10 minutes.

As a result of this research, it can be recommended to apply 10% (v/v) Amigo Oil a minimum of two times at monthly intervals to vineyards prone to spring freeze events and on cultivars that exhibit early bud break, such as 'Edelweiss'. It would be recommended to begin oil applications in the first week of February followed by another application in March. If a later than normal spring occurs, there may be enough time to make a third application. Rather than looking at the final date of bud break, growers should examine slowed rate of bud break on grapevines treated with oil. Slowing the rate of bud break in the spring will provide a lower percentage of primary buds being injured should a spring freeze occur.

NAA at 1000 ppm should not be used in vineyards to delay bud break. These treatments showed no delay in bud break on ‘Edelweiss grapevines. In addition, it is not feasible for growers to mix up hundreds of gallons of NAA by dissolving the NAA powder in 1M sodium hydroxide. If a bud delay is shown in the future using NAA, a liquid NAA product must be developed with the inclusion of a spreader sticker.

Cost would be an important deciding factor for the grower to contemplate. According to Qrunfleh and Read (2010) the total price per acre to apply Amigo Oil is $106. Are the costs of applying $106 worth of Amigo Oil (not including labor, machinery, fuel) per acre lower than what the grower would see in extra profits at the end of the season? Unfortunately, it is not possible for growers to predict when a spring freeze will occur, but the use of Amigo Oil as a precautionary measure can help protect the vineyard. It is important to note that it would not be recommended for growers to apply oil to the entire vineyard but rather to freeze prone areas or early bud breaking cultivars. In order to protect vineyards sites in spring freeze prone areas, growers must be proactive and implement either cultural practices, chemical practices, or both to avoid spring frost damage.

Future research is necessary to explore the mechanisms of how Amigo Oil delays bud break in grapevines. The timing of application should also be further explored, such as applying oil immediately prior to a freeze event. Making multiple applications at weekly intervals, rather than monthly, later in the winter may provide similar or better results. It would also be interesting if a spreader sticker was incorporated into the NAA solution. This change would allow the NAA to stick to the vines and buds more effectively and would possibly delay bud break as does the Amigo Oil.

References

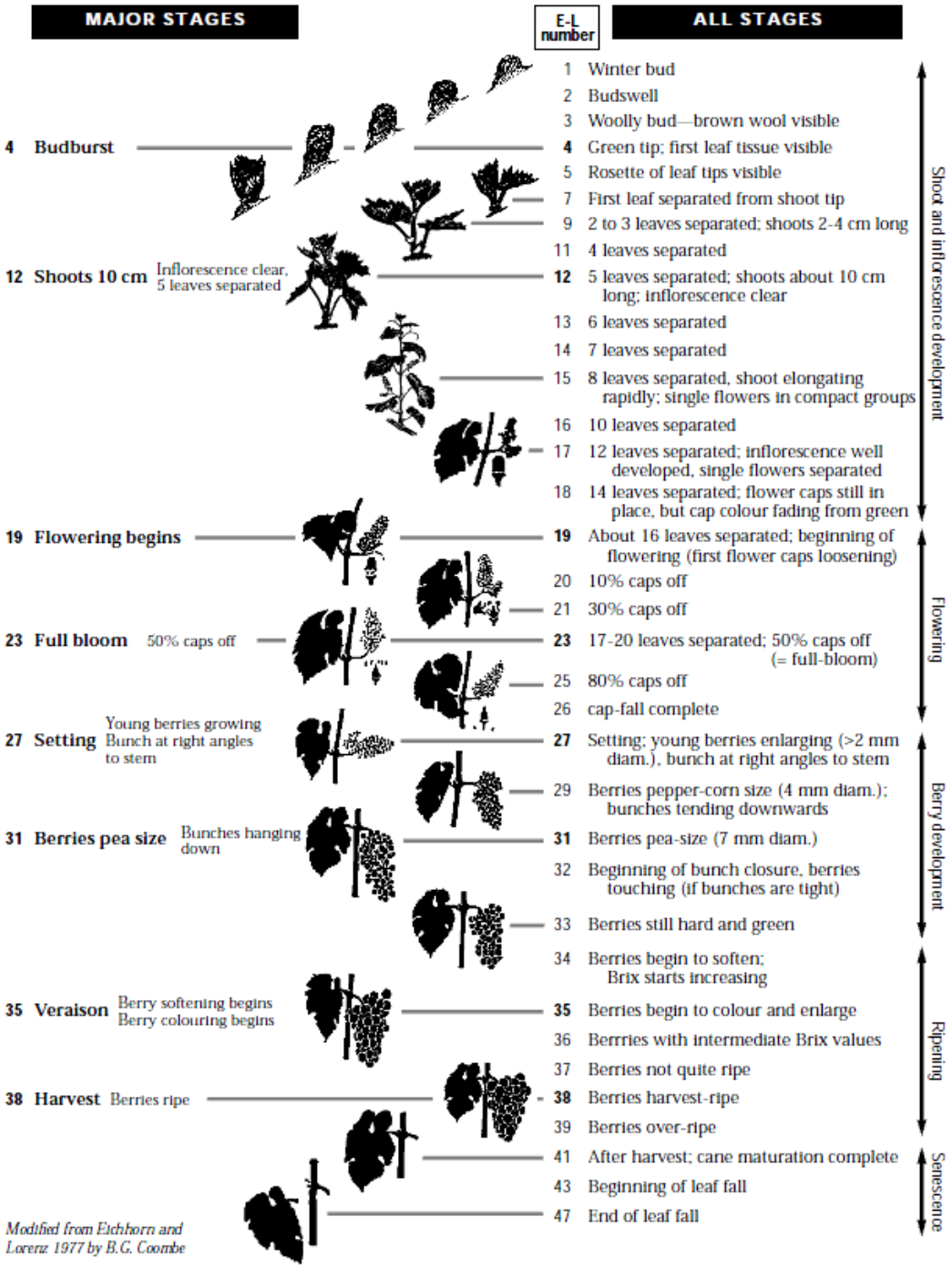
Dami, I. and B.A. Beam, 2004. Response of grapevines to soybean oil application. *American Journal of Enology and Viticulture* 55:269-275.

Qrunfleh, I.M. and P.E. Read, 2010. Delaying bud break in 'Edelweiss' grapevines to avoid spring frost injury by NAA and vegetable oil applications. University of Nebraska, Lincoln, Nebraska, PhD http://digitalcommons.unl.edu/agronhortdiss/14/.

Appendices:

Appendix 1: Modified Eichhorn and Lorenz Bud Growth Stages

Source: Coombe (1995)



Appendix 2: Julian Date Calender

JULIAN DATE CALENDAR
PERPETUAL

Day	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Day
1	001	032	060	091	121	152	182	213	244	274	305	335	1
2	002	033	061	092	122	153	183	214	245	275	306	336	2
3	003	034	062	093	123	154	184	215	246	276	307	337	3
4	004	035	063	094	124	155	185	216	247	277	308	338	4
5	005	036	064	095	125	156	186	217	248	278	309	339	5
6	006	037	065	096	126	157	187	218	249	279	310	340	6
7	007	038	066	097	127	158	188	219	250	280	311	341	7
8	008	039	067	098	128	159	189	220	251	281	312	342	8
9	009	040	068	099	129	160	190	221	252	282	313	343	9
10	010	041	069	100	130	161	191	222	253	283	314	344	10
11	011	042	070	101	131	162	192	223	254	284	315	345	11
12	012	043	071	102	132	163	193	224	255	285	316	346	12
13	013	044	072	103	133	164	194	225	256	286	317	347	13
14	014	045	073	104	134	165	195	226	257	287	318	348	14
15	015	046	074	105	135	166	196	227	258	288	319	349	15
16	016	047	075	106	136	167	197	228	259	289	320	350	16
17	017	048	076	107	137	168	198	229	260	290	321	351	17
18	018	049	077	108	138	169	199	230	261	291	322	352	18
19	019	050	078	109	139	170	200	231	262	292	323	353	19
20	020	051	079	110	140	171	201	232	263	293	324	354	20
21	021	052	080	111	141	172	202	233	264	294	325	355	21
22	022	053	081	112	142	173	203	234	265	295	326	356	22
23	023	054	082	113	143	174	204	235	266	296	327	357	23
24	024	055	083	114	144	175	205	236	267	297	328	358	24
25	025	056	084	115	145	176	206	237	268	298	329	359	25
26	026	057	085	116	146	177	207	238	269	299	330	360	26
27	027	058	086	117	147	178	208	239	270	300	331	361	27
28	028	059	087	118	148	179	209	240	271	301	332	362	28
29	029		088	119	149	180	210	241	272	302	333	363	29
30	030		089	120	150	181	211	242	273	303	334	364	30
31	031		090		151		212	243		304		365	31

Appendix 3: Year 1, Multiple Applications of 1000 ppm NAA

Number of Applications	Plant Number	Julian Days Until Bud Break	Total Cluster Number per Vine	Total Fruit Harvested per Vine (lbs)	Mean Cluster Weight (g)	pH	°Brix	TA (g/100ml)
3	4	104	48	9.40	0.196	3.92	15.7	0.92
3	5	101	39	5.90	0.151	3.99	16.2	0.83
3	6	101	26	5.10	0.196	3.90	16.5	0.80
3	7	101	43	7.15	0.166	4.01	16.7	0.84
3	8	101	55	8.10	0.147	3.87	16.6	0.81
3	9	108	48	6.65	0.139	3.64	15.1	1.07
2	10	95	49	6.90	0.141	3.59	13.8	1.08
2	11	101	64	13.10	0.205	3.72	16.1	1.04
2	12	95	51	7.60	0.149	3.59	14.8	1.16
2	13	101	27	7.00	0.259	3.91	15.7	0.78
2	14	104	32	7.95	0.248	3.65	14.8	0.95
2	15	104	44	8.75	0.199	3.70	14.6	1.11
1	16	104	44	6.05	0.138	3.89	16.6	0.84
1	17	104	40	7.65	0.191	3.76	16.4	0.87
1	18	101	74	11.95	0.161	3.84	16.2	0.93
1	19	108	42	7.25	0.173	3.76	15.9	0.77
1	20	101	48	7.75	0.161	3.63	15.2	1.01
1	21	108	47	8.30	0.177	3.77	15.4	1.08
Control	4	97	47	9.50	0.202	3.53	16.5	0.69
Control	5	97	39	5.90	0.151	3.89	17.8	0.59
Control	7	108	39	8.15	0.209	3.70	16.1	0.80
Control	10	97	22	4.40	0.2	3.58	16	0.95
Control	15	97	77	9.60	0.125	3.95	16.6	0.69
Control	18	97	46	10.45	0.227	3.83	16.6	0.74

Appendix 4: Year 1, Multiple Applications of 10% (v/v) Amigo Oil

Number of Applications	Plant	Julian Days Until Bud Break	Total Cluster Number per Vine	Total Fruit Harvested per Vine (lbs)	Mean Cluster Weight (g)	pH	°Brix	TA (g/100ml)
3	4	97	79	12.10	0.153	3.97	17.3	0.705
3	5	104	21	2.90	0.138	3.97	17.1	0.600
3	6	97	49	16.30	0.333	4.05	17.8	0.600
3	7	99	89	15.30	0.172	4.01	18.1	0.660
3	8	99	66	11.15	0.169	3.89	16.5	0.750
3	9	99	32	5.00	0.147	3.78	16.6	0.750
2	10	104	41	7.00	0.171	3.88	16.5	0.765
2	11	108	43	11.75	0.273	3.93	15.5	0.720
2	12	97	44	7.35	0.167	3.91	16.9	0.765
2	13	99	39	8.05	0.206	4.03	17.6	0.720
2	14	95	27	5.55	0.207	3.98	16.7	0.690
2	15	95	68	7.55	0.111	3.73	16.5	0.870
1	16	95	59	10.45	0.177	3.99	16.2	0.750
1	17	104	25	2.60	0.104	3.99	17.4	0.645
1	18	101	57	8.75	0.148	3.75	16.0	0.855
1	19	95	68	12.65	0.186	3.85	16.2	0.825
1	20	99	23	3.65	0.159	3.60	14.7	1.125
1	21	97	55	8.80	0.160	3.89	16.7	0.780
Control	4	97	191	32.10	0.168	3.92	15.4	0.705
Control	8	99	34	5.00	0.147	3.81	16.9	0.765
Control	11	97	32	5.20	0.164	3.96	17.3	0.735
Control	13	101	57	7.50	0.132	3.90	16.7	0.690
Control	16	104	28	5.00	0.179	3.79	16.5	0.840
Control	19	101	31	2.00	0.065	3.64	15.2	1.065

Appendix 5: Date of 80% Bud Break of all Measured Canes

Treatment & Row Number	Cane #	Date of 80% Bud Break	Julian Date	Mean Julian Date		Treatment & Row #	Cane #	Date of 80% Bud Break	Julian Date	Mean Julian Date
1 NAA 20	1	13-May	2013133			**1 NAA 28**	1	13-May	2013133	
	2	15-May	2013135				2	15-May	2013135	
	3	15-May	2013135				3	19-May	2013139	
	4	13-May	2013133	2013134			4	15-May	2013135	2013136
Control 22	1	15-May	2013135			**1 Oil 28**	1	13-May	2013133	
	2	28-May	2013148				2	19-May	2013139	
	3	15-May	2013135				3	15-May	2013135	
	4	13-May	2013133	2013138			4	11-May	2013131	2013135
3 NAA 22	1	15-May	2013135			**Control 28**	1	15-May	2013135	
	2	15-May	2013135				2	13-May	2013133	
	3	15-May	2013135				3	15-May	2013135	
	4	13-May	2013133	2013135			4	13-May	2013133	2013134
3 Oil 24	1	28-May	2013148			**3 Oil 28**	1	15-May	2013135	
	2	15-May	2013135				2	13-May	2013133	
	3	15-May	2013135				3	19-May	2013139	
	4	17-May	2013137	2013139			4	11-May	2013131	2013135
1 Oil 24	1	15-May	2013135			**2 Oil 30**	1	15-May	2013135	
	2	15-May	2013135				2	15-May	2013135	
	3	15-May	2013135				3	15-May	2013135	
	4	15-May	2013135	2013135			4	19-May	2013139	2013136
2 NAA 24	1	15-May	2013135			**3 NAA 30**	1	15-May	2013135	
	2	15-May	2013135				2	17-May	2013137	
	3	15-May	2013135				3	15-May	2013135	
	4	15-May	2013135	2013135			4	21-May	2013141	2013137
1 Oil 26	1	11-May	2013131			**2 NAA 30**	1	13-May	2013133	
	2	11-May	2013131				2	11-May	2013131	
	3	15-May	2013135				3	15-May	2013135	
	4	13-May	2013133	2013133			4	28-May	2013148	2013137
2 Oil 26	1	13-May	2013133			**1 NAA 30**	1	15-May	2013135	
	2	19-May	2013139				2	15-May	2013135	
	3	17-May	2013137				3	17-May	2013137	
	4	19-May	2013139	2013137			4	17-May	2013137	2013136
3 Oil 26	1	17-May	2013137			**3 NAA 32**	1	11-May	2013131	
	2	17-May	2013137				2	15-May	2013135	
	3	17-May	2013137				3	15-May	2013135	
	4	28-May	2013148	2013140			4	13-May	2013133	2013134
3 NAA 26	1	17-May	2013137			**3 Oil 32**	1	17-May	2013137	
	2	17-May	2013137				2	5-Jun	2013156	
	3	31-May	2013151				3	17-May	2013137	
	4	15-May	2013135	2013140			4	23-May	2013143	2013143

Appendix 5 (cont.): Date of 80% Bud Break of all Measured Canes

Treatment & Row Number	Cane #	Date of 80% Bud Break	Julian Date	Mean Julian Date		Treatment & Row #	Cane #	Date of 80% Bud Break	Julian Date	Mean Julian Date
Control 32	1	15-May	2013135			**3 NAA 38**	1	15-May	2013135	
	2	15-May	2013135				2	15-May	2013135	
	3	15-May	2013135				3	15-May	2013135	
	4	15-May	2013135	2013135			4	13-May	2013133	2013135
Control 34	1	13-May	2013133			**1 NAA 38**	1	13-May	2013133	
	2	11-May	2013131				2	15-May	2013135	
	3	13-May	2013133				3	15-May	2013135	
	4	17-May	2013137	2013134			4	19-May	2013139	2013136
2 NAA 34	1	15-May	2013135			**3 NAA 40**	1	15-May	2013135	
	2	15-May	2013135				2	15-May	2013135	
	3	15-May	2013135				3	17-May	2013137	
	4	15-May	2013135	2013135			4	15-May	2013135	2013136
2 Oil 34	1	28-May	2013148			**2 Oil 40**	1	21-May	2013141	
	2	17-May	2013137				2	19-May	2013139	
	3	19-May	2013139				3	19-May	2013139	
	4	28-May	2013148	2013143			4	5-Jun	2013156	2013144
2 NAA 36	1	15-May	2013135			**3 Oil 40**	1	21-May	2013141	
	2	11-May	2013131				2	28-May	2013148	
	3	15-May	2013135				3	21-May	2013141	
	4	13-May	2013133	2013134			4	19-May	2013139	2013142
1 NAA 36	1	17-May	2013137			**1 Oil 40**	1	15-May	2013135	
	2	15-May	2013135				2	31-May	2013151	
	3	17-May	2013137				3	19-May	2013139	
	4	17-May	2013137	2013137			4	31-May	2013151	2013144
2 Oil 36	1	28-May	2013148			**2 Oil 42**	1	15-May	2013135	
	2	17-May	2013137				2	31-May	2013151	
	3	17-May	2013137				3	28-May	2013148	
	4	19-May	2013139	2013140			4	21-May	2013141	2013144
1 Oil 36	1	15-May	2013135			**Control 42**	1	15-May	2013135	
	2	28-May	2013148				2	15-May	2013135	
	3	23-May	2013143				3	15-May	2013135	
	4	19-May	2013139	2013141			4	17-May	2013137	2013136
End Youden Square 1						**1 Oil 42**	1	19-May	2013139	
Control 38	1						2	19-May	2013139	
	2	17-May	2013137				3	17-May	2013137	
	3	15-May	2013135				4	19-May	2013139	2013139
	4	19-May	2013139	2013137		**2 NAA 42**	1	15-May	2013135	
2 NAA 38	1	21-May	2013141				2	15-May	2013135	
	2	17-May	2013137				3	21-May	2013141	
	3	15-May	2013135				4	17-May	2013137	2013137
	4	15-May	2013135	2013137						

Appendix 5 (cont.): Date of 80% Bud Break of all Measured Canes

Treatment & Row Number	Cane #	Date of 80% Bud Break	Julian Date	Mean Julian Date		Treatment & Row #	Cane #	Date of 80% Bud Break	Julian Date	Mean Julian Date
2 NAA 44	1	15-May	2013135			**1 NAA 48**	1	17-May	2013137	
	2						2	15-May	2013135	
	3	15-May	2013135				3	15-May	2013135	
	4	19-May	2013139	2013135			4	21-May	2013141	2013137
1 NAA 44	1	15-May	2013135			**3 NAA 48**	1	11-May	2013131	
	2	17-May	2013137				2	15-May	2013135	
	3	15-May	2013135				3	13-May	2013133	
	4	15-May	2013135	2013136			4	13-May	2013133	2013133
Control 44	1	19-May	2013139			**1 Oil 50**	1	15-May	2013135	
	2	17-May	2013137				2	19-May	2013139	
	3	17-May	2013137				3	19-May	2013139	
	4	15-May	2013135	2013137			4	21-May	2013141	2013139
2 Oil 44	1	19-May	2013139			**3 Oil 50**	1	19-May	2013139	
	2	17-May	2013137				2	19-May	2013139	
	3	17-May	2013137				3	28-May	2013148	
	4	19-May	2013139	2013138			4	21-May	2013141	2013142
1 NAA 46	1	15-May	2013135			**2 Oil 50**	1	31-May	2013151	
	2	9-May	2013129				2	15-May	2013135	
	3	13-May	2013133				3	5-Jun	2013156	
	4	15-May	2013135	2013133			4	21-May	2013141	2013146
3 NAA 46	1	13-May	2013133			**End Youden Square 2**				
	2	13-May	2013133			**3 Oil 52**	1	23-May	2013143	
	3	15-May	2013135				2	17-May	2013137	
	4	13-May	2013133	2013134			3	17-May	2013137	
2 NAA 46	1	31-May	2013151				4	19-May	2013139	2013139
	2	15-May	2013135			**1 Oil 52**	1	13-May	2013133	
	3	17-May	2013137				2	17-May	2013137	
	4	17-May	2013137	2013140			3	19-May	2013139	
3 Oil 46	1	19-May	2013139				4	19-May	2013139	2013137
	2	28-May	2013148			**Control 52**	1	15-May	2013135	
	3	28-May	2013148				2	15-May	2013135	
	4	31-May	2013151	2013147			3	15-May	2013135	
3 Oil 48	1	31-May	2013151				4	15-May	2013135	2013135
	2	21-May	2013141			**2 Oil 52**	1	19-May	2013139	
	3	31-May	2013151				2	31-May	2013151	
	4	28-May	2013148	2013148			3	23-May	2013143	
1 Oil 48	1	28-May	2013148				4	28-May	2013148	2013145
	2	21-May	2013141			**2 NAA 54**	1	11-May	2013131	
	3	21-May	2013141				2	15-May	2013135	
	4	31-May	2013151	2013145			3	15-May	2013135	
							4	13-May	2013133	2013134

Appendix 5 (cont.): Date of 80% Bud Break of all Measured Canes

Treatment & Row Nnumber	Cane #	Date of 80% Bud Break	Julian Date	Mean Julian Date		Treatment & Row #	Cane #	Date of 80% Bud Break	Julian Date	Mean Julian Date
3 NAA 54	1	15-May	2013135			**Control 58**	1	15-May	2013135	
	2	28-May	2013148				2	13-May	2013133	
	3	15-May	2013135				3	15-May	2013135	
	4	17-May	2013137	2013139			4	9-May	2013129	2013133
1 NAA 54	1	15-May	2013135			**2 Oil 60**	1	19-May	2013139	
	2	19-May	2013139				2	17-May	2013137	
	3	15-May	2013135				3	19-May	2013139	
	4	17-May	2013137	2013137			4	17-May	2013137	2013138
3 Oil 54	1	28-May	2013148			**Control 60**	1	13-May	2013133	
	2	28-May	2013148				2	15-May	2013135	
	3	17-May	2013137				3	11-May	2013131	
	4	31-May	2013151	2013146			4	15-May	2013135	2013134
3 NAA 56	1	19-May	2013139			**2 NAA 60**	1	28-May	2013148	
	2	9-May	2013129				2	15-May	2013135	
	3	19-May	2013139				3	15-May	2013135	
	4	13-May	2013133	2013135			4	13-May	2013133	2013138
2 Oil 56	1	17-May	2013137			**1 Oil 60**	1	19-May	2013139	
	2	15-May	2013135				2	17-May	2013137	
	3	19-May	2013139				3	15-May	2013135	
	4	17-May	2013137	2013137			4	23-May	2013143	2013139
3 Oil 56	1	19-May	2013139			**Control 62**	1	15-May	2013135	
	2	21-May	2013141				2	15-May	2013135	
	3	28-May	2013148				3	11-May	2013131	
	4	19-May	2013139	2013142			4	11-May	2013131	2013133
1 NAA 56	1	17-May	2013137			**1 NAA 62**	1	13-May	2013133	
	2	17-May	2013137				2	13-May	2013133	
	3	17-May	2013137				3	15-May	2013135	
	4	13-May	2013133	2013136			4	15-May	2013135	2013134
1 Oil 58	1	17-May	2013137			**2 Oil 62**	1	17-May	2013137	
	2	15-May	2013135				2	28-May	2013148	
	3	19-May	2013139				3	19-May	2013139	
	4	31-May	2013151	2013141			4	17-May	2013137	2013140
2 NAA 58	1	13-May	2013133			**3 NAA 62**	1	28-May	2013148	
	2	11-May	2013131				2	15-May	2013135	
	3	13-May	2013133				3	15-May	2013135	
	4	13-May	2013133	2013133			4	15-May	2013135	2013138
3 NAA 58	1	19-May	2013139			**1 NAA 64**	1	15-May	2013135	
	2	15-May	2013135				2	15-May	2013135	
	3	28-May	2013148				3	13-May	2013133	
	4	15-May	2013135	2013139			4	13-May	2013133	2013134

Appendix 5 (cont.): Date of 80% Bud Break of all Measured Canes

Treatment & Row Number	Cane #	Date of 80% Bud Break	Julian Date	Mean Julian Date
3 Oil 64	1	31-May	2013151	
	2	19-May	2013139	
	3	19-May	2013139	
	4	21-May	2013141	2013143
1 Oil 64	1	19-May	2013139	
	2	15-May	2013135	
	3	19-May	2013139	
	4	17-May	2013137	2013138
2 NAA 64	1	21-May	2013141	
	2	11-May	2013131	
	3	15-May	2013135	
	4	11-May	2013131	2013135
End Youden Square 3				

Appendix 6: Harvest Data of each Experimental Unit

Treatment & Row Number	Total Cluster Count	Mean Cluster Number per Cane	Total Cluster Weight	Mean Cluster Weight (lbs)	pH	°Brix	TA (g/l)
Control 20	35	4.38	8.10	0.231	3.07	9.6	16.10
1 NAA 20	27	3.38	10.30	0.381	3.01	9.2	15.35
Control 22	17	2.13	5.65	0.332	3.19	13.1	11.40
3 NAA 22	24	3.00	10.10	0.421	3.28	13.8	9.71
3 Oil 24	24	3.00	6.80	0.283	3.20	14.1	9.72
1 Oil 24	19	2.38	5.20	0.274	3.17	12.0	10.94
2 NAA 24	27	3.38	6.20	0.230	3.30	14.6	10.77
1 Oil 26	30	3.75	9.75	0.325	3.16	14.7	10.64
2 Oil 26	21	2.63	10.20	0.486	3.16	9.6	11.23
3 Oil 26	23	2.88	7.45	0.324	3.02	11.7	12.68
3 NAA 26	12	1.50	3.20	0.267	3.30	14.2	10.97
1 NAA 28	12	1.50	4.85	0.404	3.16	14.0	12.31
1 Oil 28	18	2.25	8.50	0.472	3.16	12.9	12.45
Control 28	15	1.88	5.85	0.390	3.93	14.1	11.01
3 Oil 28	19	2.38	5.00	0.263	3.22	13.3	11.91
2 Oil 30	27	3.38	10.90	0.404	3.24	15.0	11.70
3 NAA 30	19	2.38	8.95	0.471	3.12	12.0	12.70
2 NAA 30	21	2.63	9.85	0.469	3.14	12.5	12.85
1 NAA 30	19	2.38	2.95	0.155	3.35	14.4	10.13
3 NAA 32	10	1.25	4.20	0.420	3.21	14.6	11.07
3 Oil 32	24	3.00	5.60	0.233	3.25	14.1	11.58
Control 32	23	2.88	8.15	0.354	3.20	11.8	11.93
Control 34	20	2.50	7.20	0.360	3.12	12.1	12.31
2 NAA 34	32	4.00	9.95	0.311	3.20	13.1	11.97
2 Oil 34	14	1.75	4.20	0.300	3.22	13.7	12.89
2 NAA 36	14	1.75	2.85	0.204	3.32	14.3	10.75
1 NAA 36	25	3.13	9.60	0.384	3.15	11.8	11.77
2 Oil 36	18	2.25	5.95	0.331	3.29	14.3	11.56
1 Oil 36	25	3.13	6.55	0.262	3.18	13.5	12.83
End Youden Square 1							
Control 38	9	1.13	2.65	0.294	3.24	13.9	11.70
2 NAA 38	9	1.13	2.15	0.239	3.21	14.4	11.56
3 NAA 38	10	1.25	3.95	0.395	3.33	13.8	10.56
1 NAA 38	24	3.00	6.75	0.281	3.24	12.3	12.09
3 NAA 40	23	2.88	8.10	0.352	3.29	12.6	11.98
2 Oil 40	13	1.63	3.90	0.300	3.23	13.9	13.47
3 Oil 40	11	1.38	1.85	0.168	3.17	13.0	16.75
1 Oil 40	23	2.88	9.70	0.422	3.19	13.5	13.47
2 Oil 42	16	2.00	4.20	0.263	3.14	14.0	13.37

Appendix 6 (cont.): Harvest Data of each Experimental Unit

Treatment & Row Number	Total Cluster Count	Mean Cluster Number per Cane	Total Cluster Weight	Mean Cluster Weight (lbs)	pH	°Brix	TA (g/l)
Control 42	13	1.63	3.00	0.231	3.33	12.9	12.01
1 Oil 42	12	1.50	5.05	0.421	3.31	13.6	10.97
2 NAA 42	15	1.88	7.20	0.480	3.20	13.7	13.18
2 NAA 44	18	2.25	7.00	0.389	3.20	12.0	9.17
1 NAA 44	27	3.38	9.45	0.350	3.56	13.1	11.65
Control 44	14	1.75	4.40	0.314	3.25	13.5	11.67
2 Oil 44	22	2.75	8.05	0.366	3.14	13.5	13.74
1 NAA 46	24	3.00	10.60	0.442	3.16	14.0	11.03
3 NAA 46	21	2.63	7.50	0.357	3.15	13.0	12.49
2 NAA 46	21	2.63	4.70	0.224	3.21	13.4	11.73
3 Oil 46	10	1.25	2.15	0.215	2.96	12.3	18.79
3 Oil 48	3	0.38	0.75	0.250	3.06	13.7	16.09
1 Oil 48	15	1.88	4.40	0.293	3.14	13.2	14.19
1 NAA 48	17	2.13	6.20	0.365	3.10	14.2	12.95
3 NAA 48	27	3.38	16.45	0.609	3.14	11.9	13.82
1 Oil 50	11	1.38	3.75	0.341	3.17	12.6	11.83
3 Oil 50	18	2.25	6.05	0.336	3.09	13.7	14.76
2 Oil 50	8	1.00	5.05	0.631	3.21	13.4	16.05
End Youden Square 2							
3 Oil 52	20	2.50	5.75	0.288	3.23	13.8	12.04
1 Oil 52	22	2.75	7.05	0.320	3.18	14.1	12.50
Control 52	24	3.00	9.90	0.413	3.32	13.9	11.70
2 Oil 52	4	0.50	3.55	0.888	3.39	14.4	12.50
2 NAA 54	29	3.63	17.35	0.598	3.19	13.8	11.98
3 NAA 54	7	0.88	3.30	0.471	3.20	13.3	13.01
1 NAA 54	16	2.00	6.15	0.384	3.16	14.2	11.94
3 Oil 54	14	1.75	4.10	0.293	3.14	14.0	13.76
3 NAA 56	25	3.13	13.55	0.542	3.15	12.5	11.80
2 Oil 56	27	3.38	11.35	0.420	3.18	14.8	11.43
3 Oil 56	13	1.63	4.85	0.373	3.12	13.5	13.41
1 NAA 56	28	3.50	12.95	0.463	3.11	13.2	12.41
1 Oil 58	23	2.88	8.35	0.363	2.87	11.8	15.81
2 NAA 58	46	5.75	17.35	0.377	3.12	13.0	12.42
3 NAA 58	30	3.75	7.10	0.237	3.07	12.5	14.75
Control 58	17	2.13	6.85	0.403	3.19	13.6	12.08
2 Oil 60	27	3.38	10.70	0.396	3.04	12.2	15.69
Control 60	25	3.13	11.25	0.450	3.22	14.4	11.60
2 NAA 60	26	3.25	8.75	0.337	3.11	13.1	13.11
1 Oil 60	26	3.25	7.40	0.285	3.08	14.0	13.67
Control 62	19	2.38	10.80	0.568	3.16	12.1	11.77

Appendix 6 (cont.): Harvest Data of each Experimental Unit

Treatment & Row Number	Total Cluster Count	Mean Cluster Number per Cane	Total Cluster Weight	Mean Cluster Weight (lbs)	pH	°Brix	TA (g/l)
1 NAA 62	21	2.63	7.40	0.352	3.07	12.7	14.29
2 Oil 62	24	3.00	6.05	0.252	3.02	13.3	15.12
3 NAA 62	19	2.38	8.25	0.434	3.05	13.5	14.00
1 NAA 64	24	3.00	10.75	0.448	3.15	12.5	13.15
3 Oil 64	15	1.88	6.35	0.423	3.16	14.0	11.89
1 Oil 64	19	2.38	8.55	0.450	3.13	14.0	13.17
2 NAA 64	28	3.50	11.85	0.423	3.10	14.2	10.05
End Youden Square 3							

Appendix 7: Treatment Comparison of Julian Date of Bud Break

Treatment	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjusted P-value
Control	**NAA 1**	-0.398	1.172	51	-0.34	0.736	0.9999
Control	**NAA 2**	-0.686	1.169	51	-0.59	0.560	0.9969
Control	**NAA 3**	-1.171	1.173	51	-1.00	0.323	0.9520
Control	**Oil 1**	-3.692	1.170	51	-3.15	0.003	0.0403
Control	**Oil 2**	-5.726	1.169	51	-4.90	<.0001	0.0002
Control	**Oil 3**	-7.107	1.172	51	-6.07	<.0001	<.0001
NAA 1	**NAA 2**	-0.288	1.142	51	-0.25	0.802	1.0000
NAA 1	**NAA 3**	-0.773	1.144	51	-0.68	0.502	0.9934
NAA 1	**Oil 1**	-3.294	1.146	51	-2.87	0.006	0.0802
NAA 1	**Oil 2**	-5.329	1.145	51	-4.65	<.0001	0.0004
NAA 1	**Oil 3**	-6.710	1.142	51	-5.87	<.0001	<.0001
NAA 2	**NAA 3**	-0.485	1.146	51	-0.42	0.674	0.9995
NAA 2	**Oil 1**	-3.006	1.144	51	-2.63	0.011	0.1390
NAA 2	**Oil 2**	-5.040	1.144	51	-4.41	<.0001	0.0010
NAA 2	**Oil 3**	-6.421	1.145	51	-5.61	<.0001	<.0001
NAA 3	**Oil 1**	-2.521	1.147	51	-2.20	0.033	0.3151
NAA 3	**Oil 2**	-4.555	1.146	51	-3.97	0.000	0.0039
NAA 3	**Oil 3**	-5.936	1.144	51	-5.19	<.0001	<.0001
Oil 1	**Oil 2**	-2.035	1.142	51	-1.78	0.081	0.5666
Oil 1	**Oil 3**	-3.416	1.142	51	-2.99	0.004	0.0608
Oil 2	**Oil 3**	-1.381	1.146	51	-1.21	0.234	0.8890

Appendix 8: Treatment Comparison of Total Cluster Number per Experimental Unit

Treatment	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjusted P-value
Control	**NAA 1**	-2.542	2.915	51	-0.87	0.387	0.975
Control	**NAA 2**	-4.126	2.893	51	-1.43	0.160	0.785
Control	**NAA 3**	0.777	2.903	51	0.27	0.790	1.000
Control	**Oil 1**	-0.804	2.905	51	-0.28	0.783	1.000
Control	**Oil 2**	0.962	2.894	51	0.33	0.741	1.000
Control	**Oil 3**	3.405	2.914	51	1.17	0.248	0.903
NAA 1	**NAA 2**	-1.584	2.824	51	-0.56	0.577	0.998
NAA 1	**NAA 3**	3.319	2.833	51	1.17	0.247	0.902
NAA 1	**Oil 1**	1.738	2.852	51	0.61	0.545	0.996
NAA 1	**Oil 2**	3.504	2.843	51	1.23	0.223	0.878
NAA 1	**Oil 3**	5.947	2.824	51	2.11	0.040	0.365
NAA 2	**NAA 3**	4.903	2.851	51	1.72	0.092	0.607
NAA 2	**Oil 1**	3.322	2.832	51	1.17	0.246	0.901
NAA 2	**Oil 2**	5.088	2.831	51	1.80	0.078	0.556
NAA 2	**Oil 3**	7.531	2.841	51	2.65	0.011	0.132
NAA 3	**Oil 1**	-1.581	2.842	51	-0.56	0.581	0.998
NAA 3	**Oil 2**	0.185	2.833	51	0.07	0.948	1.000
NAA 3	**Oil 3**	2.628	2.823	51	0.93	0.356	0.966
Oil 1	**Oil 2**	1.766	2.822	51	0.63	0.534	0.996
Oil 1	**Oil 3**	4.209	2.822	51	1.49	0.142	0.749
Oil 2	**Oil 3**	2.443	2.851	51	0.86	0.396	0.977

Appendix 9: Treatment Comparison of Mean Cluster Number

Treatment	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjusted P-value
Control	**NAA 1**	-0.584	0.349	51	-1.67	0.100	0.636
Control	**NAA 2**	-0.766	0.346	51	-2.22	0.031	0.305
Control	**NAA 3**	-0.134	0.347	51	-0.39	0.700	1.000
Control	**Oil 1**	-0.336	0.347	51	-0.97	0.338	0.959
Control	**Oil 2**	-0.114	0.346	51	-0.33	0.743	1.000
Control	**Oil 3**	0.162	0.349	51	0.46	0.645	0.999
NAA 1	**NAA 2**	-0.182	0.337	51	-0.54	0.592	0.998
NAA 1	**NAA 3**	0.450	0.339	51	1.33	0.190	0.836
NAA 1	**Oil 1**	0.248	0.341	51	0.73	0.471	0.990
NAA 1	**Oil 2**	0.470	0.340	51	1.38	0.173	0.808
NAA 1	**Oil 3**	0.746	0.337	51	2.21	0.032	0.308
NAA 2	**NAA 3**	0.632	0.341	51	1.85	0.070	0.521
NAA 2	**Oil 1**	0.430	0.338	51	1.27	0.210	0.862
NAA 2	**Oil 2**	0.652	0.338	51	1.93	0.060	0.472
NAA 2	**Oil 3**	0.928	0.340	51	2.73	0.009	0.111
NAA 3	**Oil 1**	-0.202	0.340	51	-0.59	0.556	0.997
NAA 3	**Oil 2**	0.020	0.339	51	0.06	0.952	1.000
NAA 3	**Oil 3**	0.296	0.337	51	0.88	0.384	0.974
Oil 1	**Oil 2**	0.222	0.337	51	0.66	0.513	0.994
Oil 1	**Oil 3**	0.498	0.337	51	1.48	0.146	0.757
Oil 2	**Oil 3**	0.276	0.341	51	0.81	0.423	0.983

Appendix 10: Treatment Comparison of Total Cluster Weight per Experimental Unit

Treatment	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjusted P-value
Control	**NAA 1**	-0.910	1.348	51	-0.67	0.503	0.993
Control	**NAA 2**	-1.590	1.341	51	-1.19	0.241	0.897
Control	**NAA 3**	-0.541	1.344	51	-0.40	0.689	1.000
Control	**Oil 1**	0.204	1.345	51	0.15	0.880	1.000
Control	**Oil 2**	0.189	1.341	51	0.14	0.889	1.000
Control	**Oil 3**	2.566	1.349	51	1.90	0.063	0.488
NAA 1	**NAA 2**	-0.680	1.310	51	-0.52	0.606	0.999
NAA 1	**NAA 3**	0.368	1.313	51	0.28	0.780	1.000
NAA 1	**Oil 1**	1.113	1.319	51	0.84	0.403	0.979
NAA 1	**Oil 2**	1.098	1.317	51	0.83	0.408	0.980
NAA 1	**Oil 3**	3.475	1.310	51	2.65	0.011	0.132
NAA 2	**NAA 3**	1.049	1.319	51	0.79	0.430	0.985
NAA 2	**Oil 1**	1.794	1.313	51	1.37	0.178	0.817
NAA 2	**Oil 2**	1.779	1.312	51	1.36	0.181	0.822
NAA 2	**Oil 3**	4.155	1.316	51	3.16	0.003	0.040
NAA 3	**Oil 1**	0.745	1.316	51	0.57	0.574	0.998
NAA 3	**Oil 2**	0.730	1.313	51	0.56	0.581	0.998
NAA 3	**Oil 3**	3.107	1.310	51	2.37	0.022	0.231
Oil 1	**Oil 2**	-0.015	1.310	51	-0.01	0.991	1.000
Oil 1	**Oil 3**	2.362	1.310	51	1.80	0.077	0.552
Oil 2	**Oil 3**	2.377	1.320	51	1.80	0.078	0.553

Appendix 11: Treatment Comparison of Average Cluster Weight

Treatment	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjusted P-value
Control	**NAA 1**	0.003	0.045	51	0.07	0.946	1.000
Control	**NAA 2**	0.004	0.044	51	0.09	0.932	1.000
Control	**NAA 3**	-0.038	0.045	51	-0.86	0.395	0.977
Control	**Oil 1**	0.018	0.045	51	0.41	0.686	1.000
Control	**Oil 2**	-0.052	0.044	51	-1.17	0.247	0.901
Control	**Oil 3**	0.088	0.045	51	1.97	0.054	0.445
NAA 1	**NAA 2**	0.001	0.043	51	0.02	0.986	1.000
NAA 1	**NAA 3**	-0.041	0.043	51	-0.95	0.347	0.962
NAA 1	**Oil 1**	0.015	0.044	51	0.34	0.732	1.000
NAA 1	**Oil 2**	-0.055	0.044	51	-1.26	0.213	0.866
NAA 1	**Oil 3**	0.085	0.043	51	1.97	0.055	0.448
NAA 2	**NAA 3**	-0.042	0.044	51	-0.96	0.341	0.960
NAA 2	**Oil 1**	0.014	0.043	51	0.33	0.743	1.000
NAA 2	**Oil 2**	-0.056	0.043	51	-1.29	0.204	0.855
NAA 2	**Oil 3**	0.084	0.044	51	1.93	0.059	0.468
NAA 3	**Oil 1**	0.056	0.044	51	1.29	0.202	0.852
NAA 3	**Oil 2**	-0.014	0.043	51	-0.32	0.753	1.000
NAA 3	**Oil 3**	0.126	0.043	51	2.92	0.005	0.072
Oil 1	**Oil 2**	-0.070	0.043	51	-1.62	0.111	0.670
Oil 1	**Oil 3**	0.070	0.043	51	1.62	0.112	0.671
Oil 2	**Oil 3**	0.140	0.044	51	3.20	0.002	0.036

Appendix 12: Treatment Comparison of pH

Treatment	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjusted P-value
Control	**NAA 1**	0.095	0.050	51	1.92	0.061	0.478
Control	**NAA 2**	0.083	0.049	51	1.70	0.094	0.617
Control	**NAA 3**	0.086	0.049	51	1.74	0.087	0.591
Control	**Oil 1**	0.139	0.049	51	2.82	0.007	0.091
Control	**Oil 2**	0.085	0.049	51	1.75	0.087	0.588
Control	**Oil 3**	0.155	0.050	51	3.12	0.003	0.044
NAA 1	**NAA 2**	-0.012	0.048	51	-0.25	0.806	1.000
NAA 1	**NAA 3**	-0.009	0.048	51	-0.20	0.846	1.000
NAA 1	**Oil 1**	0.044	0.048	51	0.91	0.369	0.970
NAA 1	**Oil 2**	-0.010	0.048	51	-0.20	0.843	1.000
NAA 1	**Oil 3**	0.060	0.048	51	1.25	0.218	0.872
NAA 2	**NAA 3**	0.002	0.048	51	0.05	0.962	1.000
NAA 2	**Oil 1**	0.056	0.048	51	1.16	0.250	0.905
NAA 2	**Oil 2**	0.002	0.048	51	0.05	0.964	1.000
NAA 2	**Oil 3**	0.071	0.048	51	1.48	0.145	0.755
NAA 3	**Oil 1**	0.053	0.048	51	1.11	0.274	0.923
NAA 3	**Oil 2**	0.000	0.048	51	0.00	0.997	1.000
NAA 3	**Oil 3**	0.069	0.048	51	1.45	0.154	0.774
Oil 1	**Oil 2**	-0.053	0.048	51	-1.12	0.266	0.918
Oil 1	**Oil 3**	0.016	0.048	51	0.33	0.745	1.000
Oil 2	**Oil 3**	0.069	0.048	51	1.43	0.160	0.785

Appendix 13: Treatment Comparison of °Brix

Treatment	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjusted P-value
Control	**NAA 1**	-0.104	0.467	51	-0.22	0.825	1.000
Control	**NAA 2**	-0.640	0.467	51	-1.37	0.176	0.814
Control	**NAA 3**	-0.271	0.467	51	-0.58	0.564	0.997
Control	**Oil 1**	-0.462	0.467	51	-0.99	0.327	0.954
Control	**Oil 2**	-0.640	0.467	51	-1.37	0.176	0.814
Control	**Oil 3**	-0.552	0.467	51	-1.18	0.243	0.898
NAA 1	**NAA 2**	-0.536	0.457	51	-1.17	0.246	0.901
NAA 1	**NAA 3**	-0.167	0.457	51	-0.37	0.716	1.000
NAA 1	**Oil 1**	-0.358	0.457	51	-0.78	0.436	0.985
NAA 1	**Oil 2**	-0.536	0.457	51	-1.17	0.246	0.901
NAA 1	**Oil 3**	-0.448	0.457	51	-0.98	0.332	0.956
NAA 2	**NAA 3**	0.369	0.457	51	0.81	0.422	0.983
NAA 2	**Oil 1**	0.178	0.457	51	0.39	0.699	1.000
NAA 2	**Oil 2**	0.000	0.457	51	0.00	1.000	1.000
NAA 2	**Oil 3**	0.089	0.457	51	0.19	0.847	1.000
NAA 3	**Oil 1**	-0.191	0.457	51	-0.42	0.677	1.000
NAA 3	**Oil 2**	-0.369	0.457	51	-0.81	0.422	0.983
NAA 3	**Oil 3**	-0.281	0.457	51	-0.61	0.541	0.996
Oil 1	**Oil 2**	-0.178	0.457	51	-0.39	0.699	1.000
Oil 1	**Oil 3**	-0.089	0.457	51	-0.20	0.846	1.000
Oil 2	**Oil 3**	0.089	0.457	51	0.19	0.847	1.000

Appendix 14: Treatment Comparison of Titratable Acidity (TA)

Treatment	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjusted P-value
Control	**NAA 1**	-0.339	0.657	51	-0.52	0.608	0.999
Control	**NAA 2**	0.437	0.649	51	0.67	0.504	0.994
Control	**NAA 3**	-0.284	0.652	51	-0.44	0.665	0.999
Control	**Oil 1**	-0.738	0.653	51	-1.13	0.264	0.916
Control	**Oil 2**	-1.192	0.649	51	-1.84	0.072	0.531
Control	**Oil 3**	-1.739	0.657	51	-2.65	0.011	0.133
NAA 1	**NAA 2**	0.776	0.633	51	1.23	0.226	0.881
NAA 1	**NAA 3**	0.055	0.636	51	0.09	0.932	1.000
NAA 1	**Oil 1**	-0.399	0.643	51	-0.62	0.538	0.996
NAA 1	**Oil 2**	-0.853	0.640	51	-1.33	0.188	0.833
NAA 1	**Oil 3**	-1.400	0.633	51	-2.21	0.032	0.307
NAA 2	**NAA 3**	-0.721	0.643	51	-1.12	0.267	0.919
NAA 2	**Oil 1**	-1.175	0.635	51	-1.85	0.070	0.522
NAA 2	**Oil 2**	-1.629	0.635	51	-2.56	0.013	0.159
NAA 2	**Oil 3**	-2.176	0.639	51	-3.41	0.001	0.021
NAA 3	**Oil 1**	-0.454	0.639	51	-0.71	0.481	0.991
NAA 3	**Oil 2**	-0.908	0.636	51	-1.43	0.160	0.785
NAA 3	**Oil 3**	-1.455	0.633	51	-2.30	0.026	0.264
Oil 1	**Oil 2**	-0.454	0.632	51	-0.72	0.476	0.991
Oil 1	**Oil 3**	-1.001	0.632	51	-1.58	0.119	0.693
Oil 2	**Oil 3**	-0.547	0.642	51	-0.85	0.398	0.978

Appendix 15: Treatment Comparison of Julian date of Bud Break in Laboratory Forcing Experiments

NAA Treatments							
Treatment	**Treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adjusted P-value**
Control	**NAA 1**	-5.292	1.552	65	-3.41	0.001	0.006
Control	**NAA 2**	-7.034	1.570	65	-4.48	<.0001	0.000
Control	**NAA 3**	-8.500	1.552	65	-5.48	<.0001	<.0001
NAA 1	**NAA 2**	-1.742	1.570	65	-1.11	0.271	0.685
NAA 1	**NAA 3**	-3.208	1.552	65	-2.07	0.043	0.175
NAA 2	**NAA 3**	-1.466	1.570	65	-0.93	0.354	0.787

Oil Treatments							
Treatment	**Treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adjusted P-value**
Control	**Oil 1**	-5.136	3.519	52	-1.46	0.150	0.469
Control	**Oil 2**	-14.307	3.570	52	-4.01	0.000	0.001
Control	**Oil 3**	-24.266	3.611	52	-6.72	<.0001	<.0001
Oil 1	**Oil 2**	-9.170	3.570	52	-2.57	0.013	0.061
Oil 1	**Oil 3**	-19.129	3.611	52	-5.30	<.0001	<.0001
Oil 2	**Oil 3**	-9.959	3.661	52	-2.72	0.009	0.043

January Single Application							
Treatment	**Treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adjusted P-value**
Control	**NAA 1**	-1.720	2.684	7	-0.64	0.542	0.803
Control	**Oil 1**	-9.451	2.667	7	-3.54	0.009	0.023
NAA 1	**Oil 1**	-7.731	2.626	7	-2.94	0.022	0.050