Improving Flower Production and Reducing Pre-Harvest Fruit Drop of Gold Kiwifruit (*Actinidia chinensis*)

by

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Abstract

The *Actinidia chinensis* cultivars, ‘AU Golden Sunshine’ and ‘AU Gulf Coast Gold’ produce heavy crop loads of large, commercially acceptable kiwifruit in central Alabama. However, due to fluctuations in annual winter temperatures, these vines may not always receive sufficient amounts of chilling hours or units or portions they require to break bud and flower effectively. When these vines do not receive 700–900 chilling hours, they can exhibit nominal and intermittent bud break and flowering. This leads to insufficient cropping and negative economic impacts for growers. Experiments were conducted over 2 years on ‘AU Golden Sunshine’ and ‘AU Gulf Coast Gold’ to determine the effectiveness of the dormancy breaking chemical, hydrogen cyanamide (HC). Hydrogen cyanamide is known to help overcome lack of chilling accumulation, increase bud break and bloom intensity, and to reduce unwanted lateral flower buds when applied to kiwifruit. Applications of HC were made ~28 (1 Feb.) and ~14 (15 Feb.) days before natural bud break to assess the effect on timing of application. When HC was used, an advancement in bud break and flowering was observed compared to unsprayed vines. Applications made ~28 days before natural bud break were most advanced and broke bud before the other treatment and control vines. In year one, vines receiving applications ~14 days before natural bud break showed increased bud break and flowering intensity as well as a reduction of lateral flower buds over the vines treated at ~28-day before natural bud break. The unsprayed controls had the fewest lateral buds throughout the study. During year two, all vines were injured by a late frost event that occurred 7 Mar. 2019. Vines receiving the earliest HC applications were damaged the most, followed by the vines receiving the second HC application, which led to reduced flower production compared to unsprayed vines. Control vines had the greatest number of floral shoots after the frost event because they were the last to experience bud break.

Hydrogen cyanamide may be a beneficial tool to growers experiencing lower than normal chilling, however caution is advised due to the possibility of late spring frost.

'AU Golden Sunshine' exhibits a pre-harvest fruit drop that has never been reported in kiwifruit literature before. This drop occurs just before harvest and could cause economic hardship to growers if it is not curtailed. A two-year study was conducted to determine the effectiveness of 88mg/L^{-1} NAA (Fruitone®), 264mg/L^{-1} AVG (ReTain®), and 158mg/L^{-1} 1-MCP (Harvista®) at preventing pre-harvest fruit drop of this kiwifruit cultivar. Treatments consisted of each chemical alone, AVG + NAA, and 1-MCP + NAA applied at approximately 7, 14, and 21 days before anticipated harvest (though this timing varied between the 2 years of this study). During year one of the study, pre-harvest fruit drop ranged from 5.6–13.6% on average with no conclusive efficacy of the treatments to deter fruit drop. During year two, the incidence of pre-harvest fruit drop was substantially higher than the previous year with averages ranging from 32–66%. No differences were observed between treatments. Fruit maturity and quality were largely unaffected by treatment applications throughout the entire study. Further research is required to better understand the causes of the pre-harvest fruit drop behavior that 'AU Golden Sunshine' displays as recommendations cannot be made at this time.

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List of Abbreviations

PGR	Plant growth regulator
HC	Hydrogen cyanamide
GDH	Growing degree hours
SSC	Soluble solids content
DMC	Dry matter content
h˚	Hue angle
RH	Relative humidity
BB	Bud break
˚C	Degrees Celsius
Kgf	Kilograms of force
h	Hour
g	Gram
KFl	King flower
DB	Dormant Bud
LB	Lateral flower bud
DBH	Days before harvest
DAH	Days after harvest

Chapter One

Introduction

Alabama has had a vested interest in kiwifruit since the crop was first introduced to the region during the middle of the 1980's. Trials of green kiwifruit were established in central Alabama as well as in the Gulf Coast region of the state to investigate the potential for a commercial industry. These initial plantings of *Actinidia deliciosa* (A.Chev.) C.F.Liang and A.R. Ferguson 'Hayward' began to flower during the first few springs of the 1990's and showed promise in the central counties of the state where chilling accumulations reached 800-1200 hours below 7.2 ˚C. However, in counties along the Gulf Coast region, vines were not floral because of low chilling accumulations (600-900 h) in the region (Powell et al., 2000).

During the 1990's, Auburn University received several golden kiwifruit (*Actinidia chinensis* Planch.) selections to trial at research stations around Alabama. These selections were trialed and later, two of the selections were co-patented with the Institute of Fruit and Tea, Hubei Academy of Agricultural Sciences of PR China. These new cultivars were thought to be a better fit for the region because of lower chilling requirements and other factors (Spiers et al., 2018). In 2014, a commercial orchard was established in the east-central portion of the state near Reeltown, AL. Two cultivars were planted for commercial production, 'AU Golden Sunshine' (*A. chinensis)* and its bud sport 'AU Gulf Coast Gold'. These two cultivars are heavy bearers of large fruit and can consistently produce a crop year after year when chilling hour accumulations are met. Both cultivars require 700–900 h of chilling accumulation to break bud and flower adequately (Wall et al., 2008). The region receives roughly, 700–1100 h of chilling annually in most years. However, due to fluctuations of annual winter temperatures that are typical of the

region, chilling does not always accumulate to accommodate some of the perennial fruit bearing plants grown in the region.

When kiwifruit do not receive adequate chilling, the vines show a sporadic and prolonged bud break with minimal flower production (Brundell, 1975). Insufficient flowering cuts into the production strength of an orchard, creating economic decline. Therefore, it is vitally important to ensure optimal flower production is acquired to facilitate sufficient fruit production.

Hydrogen cyanamide is a tool that growers can use to help them overcome insufficient chilling during years when it may be suboptimal. It has been heavily researched by many authors for over 3 decades and the effects of its use on green kiwifruit are well known (Hernández and Craig, 2015; McPherson et al., 2001; Powell et al., 2000; Shuck and Petri, 1995; Walton and Fowke, 1993). On green kiwifruit, HC application can result in advanced bud break and flowering, increased bud break and flowering, a compact bud break and flowering period, and a reduction in lateral flower buds. There is limited knowledge on how this chemical interacts with golden kiwifruit. Some research has been conducted on the cultivar 'Gold3' with success however, there are some minor differences in reaction between green and golden kiwifruit (Hernández and Craig, 2016; Hernández and Craig, 2015; Hernández et al., 2015). When this dormancy breaking chemical is used on 'Gold3', the same advancement in bud break and flowering is observed as with green kiwifruit. An adverse impact of hydrogen cyanamide use on 'Gold3' was the increased production of lateral flower buds (Hernández and Craig, 2015; Hernández et al., 2015). Hydrogen cyanamide has not been tested on 'AU Golden Sunshine' nor 'AU Gulf Coast Gold' and the impacts of its use are unknown on these cultivars. The goals of this research were to determine the potential advantages of hydrogen cyanamide applications on these cultivars and the effects of application timing.

‘AU Golden Sunshine’ exhibits an annual pre-harvest fruit drop just a few weeks before harvest is expected to occur. This has never been reported in kiwifruit and is a new phenomenon in the commodity. The cause of the drop is unknown and could make the cultivar commercially unacceptable. For a better understanding of pre-harvest fruit drop in kiwifruit and potential mitigation methods, we researched the apple industry. There are many apple cultivars that exhibit a severe pre-harvest fruit drop issues but are still used commercially because of the use of stop-drop chemicals (Byers, 1997). The “stop-drop” chemicals 1-methylcyclopropene (1-MCP), aminoethoxyvinylglycine (AVG), and naphthalene acetic acid (NAA) have been widely used commercially to reduce the instances of pre-harvest fruit drop of many apple cultivars (Yuan and Carbaugh, 2007; Yuan and Li, 2008). Ethylene plays a role in fruit drop and its inhibition can extend the life of the fruit pre-harvest as well as postharvest. Aminoethoxyvinylglycine and 1-MCP are ethylene biosynthesis inhibitors and work by filling the receptor for ethylene and not allowing its production. The plant hormone, auxin, also plays a role in fruit drop prevention. Normally, auxin helps regulate the production of ethylene until the end of the maturation phase of fruit development (Osborne and Morgan, 1989). The chemical NAA is a synthetic auxin that can be applied to regulate ethylene production. When these chemicals are used, alone or in combination, they show effectiveness at preventing pre-harvest fruit drop in apples. Postharvest benefits can also be observed when using these chemicals because the fruit can be left to further develop on the mother plant. Increases in soluble solids content, dry matter, and prolonged flesh firmness are common side effects of ethylene biosynthesis inhibitors (Yuan and Carbaugh, 2007; Yuan and Li, 2008). When synthetic auxin is applied, fruit do not show these enhanced postharvest affects (Marini et al., 1993). Pre-harvest fruit drop issue is new to kiwifruit cultivar ‘AU Golden Sunshine’ and must be addressed before recommendations can be made on its

viability for commercial production. This research was designed to address the issue of pre-harvest fruit drop of this cultivar using tactics observed in the apple industry.

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Chapter Two

Literature Review

Actinidia

The genus *Actinidia* Lindl. originated in the Yangtze Valley of China. The genus is represented by over 60 perennial vining or climbing species (Ferguson,1990; Schroeder and Fletcher, 1967). Vines of *Actinidia* are mostly deciduous and produce a non-dehiscent berry. The species within *Actinidia* are dioecious plants and successful fruit production requires both male and female plants. Leaves are often alternating and simple with elongated petioles. Flowers can be white, yellow, red or shades of like colors and originate in the leaf axils (Brundell, 1975b). The fruit, commonly known as kiwifruit, are often hairy, oblong, spotted with lenticels and show numerous seeds within the flesh (Ferguson, 1990; Li, 1952). Fruit flesh is commonly green to golden, but in some species can be observed as brown, white, or red. Non-woody stems are covered in yellowish, brownish, red, or sometimes pink pubescence that is typically more prevalent on newer growth (Li, 1952). New growth arises on the previous growing season's wood from axillary buds. The most commonly cultivated species of the genus *Actinidia* are *A. deliciosa*, *A. chinensis*, and *A. arguta* (Garcia et al., 2012).

Actinidia chinensis Planch.

Rising in popularity is *A. chinensis* or more commonly known as golden kiwifruit. These vines are very similar to *A. deliciosa*, except for the golden fruit flesh and little or no fruit pubescence. In 1986, Liang and Ferguson separated *A. deliciosa* and *A. chinensis* into two separate species (Ferguson and Allison, 2004). Other differences between the species are the

number of chromosomes, geographical distribution, flower size, and shoot pubescence color. However, the main distinguishing factors are the pubescence and flesh color of the fruit. The fruit of *A. chinensis* is known to be sweeter and more aromatic than green-fleshed kiwifruit (*A. deliciosa*). *Actinidia chinensis* first arrived in New Zealand around 1977 where it was thought to show great promise as a commercial species. The first commercial cultivar of the species was identified in 1991 as 'Hort16A' and marketed under the name ZESPRI™ GOLD Kiwifruit (Ferguson, 1999).

Actinidia chinensis 'Hort16A'

'Hort16A' was one of the first commercial cultivars of the golden type kiwifruit. While green fleshed fruit are hexaploid, 'Hort16A' is diploid and flowers a month before the standard green variety (Lowe et al., 1999). The fruit has an ovoid shape with a protruding distal end. The fruit has a yellow flesh and a yellow-brown skin when ripe. The pubescent are very fine, almost peach-like, and easily scuffed off to reveal the nearly shiny leathery skin. The flavor is sweet and almost creamy. In comparison, *A. deliciosa* 'Hayward' has a rounded distal end with fruit that has green flesh and brown skin when ripe. The fruit skin has thicker pubescence and a sweet, but acidic flavor (Huang, 2016). Flowering for 'Hort16A' is around April 1. Today, 'Hort16A' has been largely replaced by 'Gold3' marketed as ZESPRI™ Sungold Kiwifruit. This is due to its superior storage life and some tolerance to bacterial canker (*Pseudomonas syringae* pv. *Actinidiae* [Psa]), a major disease that severely affects many kiwifruit growing regions of the world. 'Gold3' flowers about 9 days before 'Hayward' and 3 weeks after 'Hort16A' (Lowe, 2011).

Actinidia chinensis '*AU Golden Sunshine'*

The cultivar *A. chinensis* 'AU Golden Sunshine' has been successfully cultivated and cropped since the mid-1990's in central Alabama (Spiers et al., 2018). Chilling requirements for this cultivar were determined to be 700 hours of chill accumulation for maximum bud break and flowering to occur. Roughly 15,000 growing degree hours (GDH) are required for bud break while 10,600 GDH were required for first flower emergence in this cultivar (Wall et al., 2008). This cultivar reaches full bloom between 20 April and 30 April reducing risk for damage to blooms by a late spring frost event (Spiers et al., 2018). Fruit borne on 'AU Golden Sunshine' is cylindrical and has a rounded stylar end with rounded and flattened shoulders on the stalk end similarly to 'Gold3'. Fruit are often large and will easily exceed 100g in weight when appropriate growing conditions are met (Spiers et al., 2018; Wall, personal communication). Having a marketable fruit shape and size along with later flowering and early harvest allows 'AU Golden Sunshine' to be more suitable for commercial orchard conditions in Alabama (Dozier et al., 2011a; Spiers et al., 2018). Harvest for 'AU Golden Sunshine' is typically during the last week of August through the first week of September. A pre-harvest fruit drop has been observed for 'AU Golden Sunshine' in some years in Alabama and in the first year of fruit production in California (Spiers, personal communication). The amount of fruit drop has not been closely recorded, but has been observed in several seasons on research plots. This pre-harvest fruit drop is a detriment to commercial production and in fact, could prevent this cultivar from becoming a viable commercial cultivar.

Actinidia chinensis 'AU Gulf Coast Gold'

‘AU Gulf Coast Gold’, a bud sport of the golden kiwifruit cultivar ‘AU Golden Sunshine’, has shown promise to be a cultivar fit for commercial production. ‘AU Gulf Coast Gold’ has been cultivated and cropped in central Alabama since 1999 (Spiers et al., 2018). This cultivar was discovered in Fairhope, Alabama, USA which is situated in the warmer Gulf Coast region of southern Alabama (Spiers et al., 2018). Chilling requirement is thought to be lower on ‘AU Gulf Coast Gold’ than on ‘AU Golden Sunshine’ however, chilling requirements have not yet been determined for this cultivar. Future study is needed to make further chilling determinations. Fruit from ‘AU Gulf Coast Gold’ are large, cylindrical, and uniform having a rounded but somewhat pointed stylar end. The shoulders on the stalk end of the fruit are rounded and narrow (Dozier et al., 2018). In postharvest analysis, it was found that ‘AU Gulf Coast Gold’ has a high SSC content and higher dry matter content than ‘AU Golden Sunshine’ (Spiers et al., 2018). Bud break and bloom occur ~ 2 days before ‘AU Golden Sunshine’ however, fruit ripen a full 2 to 3 weeks later than ‘AU Golden Sunshine’ (Dozier et al., 2018; Spiers et al., 2018). A prominent attribute of ‘AU Gulf Coast Gold’ is that it does not experience a pre-harvest fruit drop. If methods are not developed to alleviate pre-harvest fruit drop of ‘AU Golden Sunshine’, then the cultivar will not be recommended for commercial use and ‘AU Gulf Coast Gold’ will likely be the main cultivar recommended (Spiers, personal communication).

Actinidia chinensis ‘AU Golden Tiger’

The cultivar ‘AU Golden Tiger’ was discovered in a collection of seed amassed from fruit of the golden kiwifruit cultivar ‘AU Golden Dragon’ (Dozier et al., 2011b). The paternal donor of the cultivar is unknown as the maternal ‘AU Golden Dragon’ vine was open pollinated.

This new male cultivar initiates bud break in the last 10 days of March and later, and flowers between April 25 to May 7 in central Alabama depending on the climatic conditions of that season. This male cultivar is one of the only males available that is known to have an overlapping bloom period with ‘AU Golden Sunshine’ and ‘AU Gulf Coast Gold’ (Dozier et al., 2011b, Spiers et al., 2018).

Actinidia deliciosa ‘Hayward’

The most commonly cultivated species of kiwifruit is *Actinidia deliciosa* or more commonly known as green kiwifruit. As denoted in the common name, the flesh of these fruit is green in color with a sweet but slightly acidic flavor. The fruit is slightly flattened and oblong in shape with the presence of pubescence (Salinero et al., 2009). Though there are several cultivars of *A. deliciosa*, the most economically important is *A. deliciosa* ‘Hayward’. It is prized for its large fruit, good flavor, and long shelf life after harvest (Huang, 2016). ‘Hayward’ was selected by Hayward Wright, a nurseryman in New Zealand, from fruit collected in China. In the 1960s, ‘Hayward’ rose to prominence due to preference for the fruit in the North American market (Huang, 2016). By 1975, ‘Hayward’ was the only fruit accepted for export in New Zealand. It was during this time that the kiwifruit market expanded production to other parts of the world. For many years, it has been the standard cultivar in California and Europe (Ferguson, 1999). ‘Hayward’ vines grow in temperate climates where winters provide 900 to 1200 chilling hours for adequate flower and fruit production and flowering begins in late April to early May (Caldwell, 1989; Wall et al., 2008).

Though it is the standard cultivar grown around the world, it is not without defects. ‘Hayward’ is known for fruits that are flattened, which are not allowable by the grading

standards. These flattened fruit are often the result of ovaries fusing together (Ferguson, 1999). Adherence of the stamen to the developing fruitlet is known as the "Hayward Mark" and the fruit are rejected from the market. Even with these imperfections 'Hayward' will continue to be of economic importance for many years to come and is the standard by which other cultivars are compared (Ferguson, 1999).

Chilling

Chilling accumulation in annual bearing woody perennials is necessary for proper vegetative bud break along with flower and fruit production. Kiwifruit are no exception, and require a certain number of chilling hours to acquire maximum flower production (Brundell, 1976). Bud break in kiwifruit can be poor in climates that receive warmer winter temperatures and less than optimal chilling. In the Richardson model, chilling hour requirements are defined as temperatures above 0°C, but below 7°C (32°F – 45°F) (Wall et al., 2008). Coupled with accumulation of heat units, accumulation of chilling hours is needed to help the plants resist low winter temperatures and break dormancy when temperatures are conducive for growth (Erez, 1995). Less than optimal chilling accumulation leads to non-uniform bud break and bloom. Results from optimal accumulation are a higher percent bud break and optimum flowering (Brundell, 1976). Vegetative buds require less chilling accumulation than floral buds, and similarly terminal buds require less accumulation than do lateral buds (Erez, 1995). For this reason, vines will begin to flower at the end of canes and proceed to bloom from the terminal end to the origin. This behavior can decrease the level of uniformity at bud break and bloom. Alabama's climatic conditions do not always afford growers the luxury of accumulating the proper amount of chilling hours required for their orchard. When optimal conditions are not met,

there are a few methods growers can use to overcome the lack of chilling and supply uniform bud break and bloom (McPherson et al., 2001; Powell et al. 2000; Walton et al. 2009; Erez, 1995). Dormancy breaking chemicals have been researched on green kiwifruit for 4 decades and provide options growers can take if chilling requirements are not met (Costa et al., 1997; Erez, 1995; McPherson et al., 2001; Powell et al. 2000; Shuck and Petri, 1995; Walton et al., 1991; Walton and Fowke, 1993; Walton et al., 2009). These chemicals typically work by inducing stress on a dormant bud which in turn breaks dormancy, releasing the bud to begin to grow (Harnández and Craig, 2011). Currently, the chemical most suitable for dormancy breaking in a commercial setting is hydrogen cyanamide though newer chemistries such as alkoxylated fatty alkylamine polymer (Armobreak™) and a patented nitrogen and calcium solution formulated by Valagro (Erger®) do show some promise (Hernández and Craig, 2016).

Budbreak and Flowering

During the growing season, kiwifruit grow shoots originating from a bud that developed on the previous year's growth (New Zealand Kiwifruit Growers Inc., 2016; Brundell, 1975a; Walton et al., 1997). In winter, older unproductive wood is removed to make way for these new shoots. These new shoots, now known as canes, will be the origin for next spring's shoots and flowers (NZKGI, 2016; Walton et al., 1997). Roughly 2 weeks before bud break, buds along these new canes begin to differentiate floral primordia. Buds will begin to swell ~5 d before bud break and begin the opening period that lasts ~2 weeks (Brundell, 1975a). Upon opening, buds will exhibit a dome shaped meristem and it is at this point that these buds are now vulnerable to late spring frost events (Brundell, 1975a). From the dome stage, a small shoot emerges and unpacks a whorl of leaves. These first few leaves will provide the photoassimilates needed to

accelerate further shoot growth and elongation (Brundell, 1975a; Walton et al., 1997). Shoots can be determinate or indeterminate in growth. Determinate shoots will arise, and the meristem may abort which ceases elongation. However, determinate shoots can produce flowers and set fruit (Walton et al., 1997). Indeterminate shoots will arise and begin to rapidly elongate up to a centimeter a day. During the first 10 d of bud break, shoots that arise are typically floral. These shoots will suppress floral productivity of the shoots that follow, making them more likely to become vegetative (Walton et al., 1997). Flower development begins within the leaf axils and a small flower bud arises and elongates (Brundell, 1975a; Walton et al., 1997). While encapsulated by sepals, the ovary begins to develop several loculi that house many ovules. Theses ovules will, upon fertilization, develop into seeds. Simultaneously, anthers and filaments are developed and within a few days will begin to produce pollen (Brundell, 1975b). Bracts may develop along the pedicel and later form lateral flower buds. Inflorescences of two or three flowers are not uncommon in kiwifruit (Brundell, 1975b). Later, these lateral flower buds will be thinned to a single king flower to maximize fruit size and decrease pollination costs (NZKGI, 2016). A week before bloom occurs, petals can hardly be seen as the sepals begin to expose them. This stage of development is known as the popcorn stage and last for ~7-10 days. King flowers typically open first and are quickly followed by laterals if not previously thinned. Open flowers are considered so when they can be easily worked by pollinators (Brundell, 1975b).

Hydrogen Cyanamide (H_2CN_2)

Overcoming lack of chilling can be obtained with the use of hydrogen cyanamide (HC) in years with marginal chilling. Hydrogen cyanamide is a restricted use plant growth regulator that, when used properly, can help plants overcome inadequate chilling, thus inducing bud break.

Other impacts of HC are increases in flower numbers, decreases in lateral flower buds (LB), and increased bloom uniformity (Costa et al., 1997; Erez, 1995; McPherson et al., 2001; Powell et al., 2000; Shuck and Petri, 1995; Walton, 1991; Walton and Fowke, 1993; Walton et al., 2009). The mode of action of HC is not well known but is thought to be associated with sub-lethal stressors that lead to bud break (Walton et al., 2009). When HC is applied to a dormant bud, a down regulation of catalase occurs, and this induces stress on any bud that comes into contact with the chemical. The increases in stress caused by HC change amino acid accumulations at the metabolic level and this is thought to facilitate early bud break (Walton et al., 2009).

In kiwifruit, HC is typically applied at a rate of 15 liters (4 gallons) of product in 378 liters (100 gallons) of water (2% a.i.), 4 weeks before natural bud break to promote more uniform bud break in areas where marginal chilling was acquired (Dormex®, AlzChem Trostberg GmbH, 2019). Much research has been done using HC to overcome lack of proper chilling in green kiwifruit (Costa et al., 1997; Erez, 1995; McPherson et al., 2001; Powell et al. 2000; Shuck and Petri, 1995; Walton et al. 2009). Overcoming lack of chill and increasing flower production relate directly to final yields and returns for growers. However, few studies have been done using HC on golden kiwifruit. Hernandez and Craig (2015) reported that when HC was used to concentrate bud break, it was discovered that the later HC was sprayed on the gold cultivar ‘Gold3’, the more compact and intense the flowering. This study indicated that applications of 3% made 31 d before natural bud break improved bud break intensity over applications made 57–36 d before natural bud break. A more intense bud break leads to more buds breaking simultaneously which facilitates a compact bud break and more intense flowering period (Hernández and Craig, 2015). In the same study, timing of application did not significantly improve bud break on the green kiwifruit ‘Hayward’ (Hernández and Craig, 2015).

In another study conducted by Hernández et al. (2015), applications of HC at 3% made 25 d before natural bud break on ‘Gold3’ had the greatest intensity of bud break when compared to applications made 33–45 d before natural bud break. As applications were made closer to natural bud break less LB were developed on ‘Gold3’ (Hernández and Craig, 2015; Hernández et al., 2015). Lateral bud development was low no matter the timing of application on ‘Hayward’ (Hernández and Craig, 2015).

Green kiwifruit dormancy has been intensively researched over the last 5 decades and more is understood about the impacts of low chilling accumulations for these vines (Costa et al., 1997; Erez, 1995; Hernández and Craig, 2015; McPherson et al., 2001; Powell et al., 2000; Shuck and Petri, 1995; Walton et al. 2009). It is now known that HC is an effective tool to manipulate bud break and flowering in areas of temperate zone climates with marginal chilling. Hydrogen cyanamide shows less effectiveness when used in areas that receive optimal chilling requirements (Costa et al., 1997; Powell et al., 2000; Walton and Fowke, 1993). When trialed, Walton (1991) observed that bud break typically occurred 37 d after HC application when used on green kiwifruit, which is similar to the findings of Erez (1995), Shuck and Petri (1995), Powell et al., (2000), McPherson et al., (2001), and others. This coincides with optimal application windows for green kiwifruit being 45–30 d before natural bud break when applied to green kiwifruit (Costa et al., 1997; Erez, 1995; McPherson et al., 2001; Powell et al. 2000; Shuck and Petri, 1995; Walton et al. 2009). If applications are made earlier than this period, chilling may have not reached a point that will allow HC to affect dormant buds. Hydrogen cyanamide is only effective at overcoming 30% of chilling for a given winter period (Erez, 1995).

Phytotoxicity is a concern when using HC and care must be considered when deciding how much product should be applied and when applications will be made (Erez, 1995; Shuck

and Petri, 1995; Costa, 1997; Powell et al., 2000). Applications made no later than 30 d before natural bud break can cause tip burn of shoots, leading to loss of apical dominance which in turn allows for more vegetative growth when concentrations greater than 3% are applied (Erez, 1995). Phytotoxicity can also negatively impact flower production of vines (Erez, 1995; Shuck and Petri, 1995). It was discovered that applications can be made closer to bud break without ill effects at lower rates (≤ 2%) if the buds were not visibly active (Powell et al., 2000; Shuck and Petri, 1995). More damage can be seen with increased amounts of active ingredient (>3% a.i.) as well as ill effects, such as more lateral flower buds (Costa, 1997; Erez, 1995; Shuck and Petri, 1995). Powell et al. (2000) observed that HC applications made 3 to 4 weeks before natural bud break at a rate of 1–2% increased bud break and flowering intensity of 'Hayward' without any phytoxicity. Optimal efficacy of HC is seen when applied at 1–2% which decreases the risk of phytotoxicity and increases bud break and flowering activity (Powell et al., 2000; Shuck and Petri, 1995). These findings are in agreement with many other authors who have also studied the phytotoxic implications of HC use (Costa, 1997; Erez, 1995; McPherson et al., 2001; Shuck and Petri, 1995). In any instance, if HC is applied to green kiwifruit there is an advancement of bud break that occurs (Walton, 1991; Costa et al., 1997; Walton et al., 2009; Engin et al., 2010; Inglese et al., 1998; Hernández et al., 2015). Typically, the earlier HC is applied in respect to natural bud break, the earlier bud break will occur (Walton, 1991; Costa et al., 1997; Walton, 2009; Engin et al., 2010; Inglese et al., 1998; Hernández et al., 2015). A study by Inglese et al. (1998) revealed that the effect of HC is directly dependent on timing of applications. Hydrogen cyanamide applications of 2% advanced bud break by 34 and 24 d when applied 8 and 6 weeks ahead of natural bud burst compared to vines that were left unsprayed. However, in the same study, Inglese et al., (1998) observed that the closer to bud break that HC is applied, the more

positive the effect on flower production, recommending applications as close as 2 weeks before natural bud break. This increase in flower production is observed because of a compact bud break and bloom exhibited on vines receiving HC. Shoots arising 10 or more days after bud break show floral suppression from shoots that arose before (Shuck and Petri, 1995). More flowers will arise from vines treated with HC because bud break is condensed, allowing for more buds to break before floral suppression occurs (Engin et al., 2010; Inglese et al., 1998; Hernández et al., 2015; McPherson et al., 2001; Shuck and Petri, 1995). Condensing bloom period directly impacts the pollination period of the vines. A more condensed bloom is auspicious for the reason that growers spend less effort ensuring adequate pollination (Engin et al., 2010; Ingleseet al., 1998; Hernández et al., 2015; McPherson et al., 2001; Shuck and Petri, 1995). Hydrogen cyanamide showed a negative correlation on lateral flower bud (LB) instances in green kiwifruit (Hernández and Craig, 2015; McPherson et al., 2001). McPherson et al. (2001) made applications of HC with a rate of 3.12%, 33 and 92 d before natural bud break. When applied at this rate and timing, LB were reduced over unsprayed vines on green kiwifruit (McPherson et al., 2001). Upon HC application, stress is induced causing an abscission or abortion of LB which can reduce the cost of fruit thinning for growers cultivating green kiwifruit. This reduction in LB also increases likelihood of larger fruit being produced (Erez, 1995; Hernández and Craig, 2011; McPherson et al., 2001). Contrary to this, Powell et al., (2000) did see some increases in LB when HC was applied at a rate of 2% to green kiwifruit in the Gulf Coast region of the U.S., however these instances were minimal. When HC was applied to the golden kiwifruit ‘Gold3’, an increase in LB percentages as KFl/DB increased was observed (Hernández and Craig, 2015; Hernández et al., 2015). Hernández and Craig (2015) used a 3% solution of HC on ‘Gold3’ applied 57, 51, 45, 36, and 31 d before natural bud break.

Applications made 31 d before natural bud break had not only the highest intensity of bud break (90%), but also the lowest instances (12%) of LB development. This study agrees with the findings of Hernández et al. (2015) using the same application rates of HC on ‘Gold3’, but different timings. Applications made 25 d before natural bud break showed the highest amount of bud break (79%) for the study and had only 20% LB development. The treatment with the lowest amount of LB (18%) was applied just 17 d ahead of natural bud break (Hernández et al., 2015).

Pre-harvest fruit drop

Ethylene is produced during many processes of plant development. Ethylene is present during germination, root development, damage, abscission, senescence and fruit ripening (Abeles et al., 1992). Kiwifruit are climacteric fruit and will ripen on or off the vine. Typically, ethylene production is regulated during fruit development until after the maturation stage to induce ripening (Wright and Heatherbell, 1967). Abscission of fruit just before harvest has been observed on ‘AU Golden Sunshine’ and could be a detriment to those who cultivate this commodity. Pre-harvest fruit drop has not been previously recorded in kiwifruit and very little is known of the causes.

When a plant sheds leaves, branches, flowers, fruits, and/or seeds, the process is known as abscission. Abscission typically occurs in response to damage, the spreading of propagules, or when a plant part has lost its function and is no longer needed (Osborne and Morgan, 1989). When fruit fall from the mother plant, they typically abscise to drop to the ground to spread seeds inside. This process is regulated by the plant with cells that are programmed to die along a predetermined location within the plant. Usually, this is not a random event and cells within the

abscission zone are created early in the development of fruit and other plant parts (Osborne and Morgan, 1989). Ethylene production plays a key role in the abscission process and it typically induces the programmed cell death that leads to the ablation of fruit and other plant parts. Ethylene is held in check mainly by auxin, a plant hormone that regulates growth within a plant. When auxin is not present, ethylene is freely produced and abscission can occur (Osborne and Morgan, 1989). Ethylene is a catalyst for abscisic acid, which is a key component of separation of fruit from the maternal body. Abscisic acid accelerates separation in the abscission zone leading to fruit drop. Once abscisic acid has begun the process of cell separation it cannot be reversed (Osborne and Morgan, 1989). Therefore, in a pre-harvest fruit drop situation, ethylene must be inhibited before abscisic acid initiates cell separation. This can be achieved by applications of naphthalene acetic acid (NAA), a synthetic auxin that may help regulate ethylene production. Ethylene can also be inhibited by ethylene biosynthesis inhibitors that block ethylene receptors, limiting its production (Yuan and Carbaugh, 2007; Yuan and Li, 2008; Marini et al., 1993).

1-Methylcyclopropene (1-MCP)

The chemical 1-MCP is used to aid in extending postharvest life of fresh commodities that are ethylene sensitive. It works similarly to aminoethoxyvinylglycine (AVG), and inhibits ethylene response by binding to ethylene receptors in cell membranes (Yuan and Carbaugh, 2007). Typically, this product is used in small volumes as a pretreatment for fruits that are in long-term postharvest storage (Byers et al., 2005; Elfving et al., 2007; Yuan and Carbaugh, 2007). Recently, a new formulation was developed by AgroFresh, Inc. known as Harvista™. Harvista™ is formulated to be able to be dispersed in a water-spray solution in the field to help

delay harvest and prevent pre-harvest fruit drop (AgroFresh Inc., 2018). This product has recently been labeled as a PGR for kiwifruit. Harvista™ is applied 3 to 21 d before anticipated harvest at a rate of 15–60 g/ha to decrease pre-harvest fruit drop, enhance fruit size, maintain firmness, and improve harvest management of kiwifruit (AgroFresh Inc., 2018). Research has tested 1-MCP on kiwifruit for delaying softening and ripening (Boquete et al., 2004; Ilina et al., 2010; Menniti et al., 2005). A study by Ilina et al. (2010) made applications of 1-MCP in postharvest settings to the *A. deliciosa* 'Hayward'. Applications of 1-MCP made 40, 80, and 120 d after being placed in cold storage delayed fruit softening and extended the ripening period of fruit rewarmed at 20˚C (Ilina et al., 2010). These findings agree with those of Boquete et al. (2004) and Menniti et al. (2005). Little is known about the effectiveness of 1-MCP to alleviate pre-harvest fruit drop in kiwifruit.

Pre-harvest fruit drop prevention has been extensively studied in apples to determine the efficacy of many PGRs (Amarante et al., 2002; Basak and Buczek, 2010; Byers, 1997; Byers et al., 2005; Dal Cin et al., 2008; McArtney et al., 2008; Rath et al., 2006; Schupp and Greene, 2004; Yildiz et al., 2012; Varanasi et al., 2013; Yuan and Carbaugh, 2007; Yuan and Li, 2008). In a study conducted by Yuan and Carbaugh (2007), it was determined that high rates of 1-MCP (396 mg/L) were more effective 7 days before anticipated harvest than AVG, NAA, or control vines at preventing pre-harvest fruit drop for up to 21 d after application on *Malus* × *domestica* 'Golden Delicious'. In 2008, Yuan and Li tested other rates and timings of these products and found that 1-MCP concentrations ($160mg/L^{-1}$ and $80mg/L^{-1}$) performed significantly better than controls at 15 d before anticipated harvest for prevention of pre-harvest fruit drop, however, the higher rate ($160mg/L^{-1}$) was more effective 7 d before anticipated harvest. Rates of 125 and $250mg/L^{-1}$ were tested on 'Scarletsour Delicious' and 'Cameo' apples 1 to 3 weeks before

anticipated harvest (McArtney et al., 2008). Higher rates closer to anticipated harvest were shown to be most effective at controlling fruit softening and ethylene production for up to 225 d after harvest. Little impact was seen on other postharvest parameters like SSC and titratable acidity (McArtney et al., 2008). This agrees with findings from similar studies conducted by Elfving et al, (2007) and Yuan and Li (2008).

There have also been many studies conducted on the postharvest impacts of 1-MCP when used in postharvest storage environments on kiwifruit (Boquete et al., 2004; Jianguo et al., 2003; Koukounaras and Sfakiotakis, 2007; Menniti et al., 2005; Sharma et al., 2012; Xiucai and Jishu, 2001; Zhao et al., 2005). In a study conducted by Menniti et al, (2005) *A. deliciosa* 'Hayward' were harvested and exposed to 1-MCP at room temperature for 12 h or at <5 ˚C for 24 h. Rates varied from 100nl/L^{-1} (single or double applications) or 250 nl/L^{-1} (single application). They discovered that both rates slowed softening of postharvest kiwifruit in both 20 ˚C as well as <5 ˚C. Applications of 1-MCP made before harvest resulted in fruit staying firmer and remaining viable longer than control fruit in postharvest storage (Menniti et al., 2005). Another study, Koukounaras and Sfakiotakis (2007) treated *A. deliciosa* 'Hayward' with 1-MCP for different durations in cold storage. After cold storage, fruit were left at 20 ˚C for shelf-life testing. In this study, ethylene production was suppressed when fruit were treated with 1-MCP over fruit that were not treated. Fruit treated with 1-MCP showed a delay in fruit softening over control fruit when shelf-life was evaluated after 8 to 20 weeks in cold storage (Koukounaras and Sfakiotakis, 2007). When Sharma et al, (2012) used 1-MCP on *A. deliciosa* 'Allison', they showed that the chemical could reduce weight loss of fruit in storage because of its action on respiration of kiwifruit. Production of ethylene was also reduced when 1-MCP was applied, agreeing with

Menniti et al, (2005) and Koukounaras and Sfakiotakis (2007). Firmness was delayed in fruit that received 2ml/L^{-1} of 1-MCP over fruit that were left untreated (Sharma et al., 2012).

Aminoethoxyvinylglycine (AVG)

Aminoethoxyvinlyglycine (AVG) under the trade name ReTain®, is a chemical that, when absorbed into plant tissue, irreversibly binds to a key enzyme that is a precursor to ethylene. This binding prevents the enzyme from attaching to the receptor and limits ethylene production (Byers, 1997). This product slows all processes associated with the production of ethylene including flesh softening, the disappearance of starches, stem loosening and, in apples, red color formation. In apples, it is applied at a rate of 333–666 g/ha^{-1} 1 to 4 weeks before harvest and is allowed 6 hours of drying time after application (Greene, 2005; ReTain®, Valent Biosciences LLC, 2018). It is recommended that 900 L of water be used per hectare for proper distribution of the product. The AVG has been used on kiwifruit to help reduce fruit softening due to ethylene production, but little is known about its ability to reduce abscission in this commodity, though it is not specifically labeled for this (Manriquez et al., 1999; ReTain®, Valent Biosciences LLC, 2018). A study was conducted by Manriquez et al. (1999) that used AVG to reduce softening and extend storage life of the green kiwifruit cultivar 'Hayward'. AVG was applied at rates of 20, 100, and 500mg/L^{-1} in either a pre-harvest spray application 56 to 14 d before harvest or a submersion, postharvest. The highest firmness was reported on fruit that had been sprayed with AVG 28 d before harvest at all rates. Firmness was no longer statistically significant when applications were made more than 42 d from harvest. No differences were observed between treatments and controls in relations to SSC (Manriquez et al., 1999).
Kim et al, (1999) showed that when 500ppm AVG is applied to *A. deliciosa* pre-harvest, a reduction of ethylene production and softening can be observed in fruit placed in cold storage for

two months over fruit left untreated. Firmness of the fruit was extended on fruit receiving AVG and left at 20˚C to ripen however, this effect was not seen in fruit placed in cold storage (Kim et al., 1999).

In apples, AVG has been studied to prevent pre-harvest fruit drop at many rates and timings (Byers, 1997; Dal Cin et al., 2008; Greene and Schupp 2004; Schupp and Greene, 2004; Stover et al., 2003; Varanasi et al., 2013; Webster et al., 2006; Yildiz et al., 2012; Yuan and Carbaugh, 2007; Yuan and Li, 2008). Byers (1997) concluded that AVG at a rate of 132mg/L^{-1} was the most effective at controlling pre-harvest fruit drop in various apple cultivars. These fruit also had better firmness retention over lower applications of AVG and all applications of NAA in the study. Soluble solids content was unaffected by both AVG and NAA applications. Lower rates of AVG were less effective as were applications of NAA against pre-harvest fruit drop (Byers, 1997). Schupp and Greene (2004) studied the effects of AVG on pre-harvest fruit drop and maturation of 'McIntosh' apples. They found that the higher rates of AVG were more effective at preventing fruit drop, with the rate of 783 mg/L^{-1} being the most effective on this cultivar. The minimum amount of AVG that was effective at keeping the drop ≤10% was 106 mg/L^{-1} (Schupp and Greene, 2004). Applications made 28 to 14 days before anticipated harvest at higher rates (150-225mg/L^{-1}) were most effective at extending harvest and preventing ethylene production (Greene and Schupp, 2004). The most effective timing and concentration of AVG to prevent pre-harvest fruit drop were applications made 14 d before anticipated harvest and these applications also delayed ripening more than daminozide (Greene and Schupp, 2004). Fruit firmness was impacted positively with increasing AVG concentrations. The soluble solids content was not impacted by AVG applications (Schupp and Greene, 2004). Fruit size can be increased when AVG is used to extend harvest because fruit can be left on the tree for longer,

allowing for a larger size to be obtained (Schupp and Greene, 2004). A study by Yuan and Li (2008) showed that rates of 125mg/L^{-1} of AVG applied 7 d before anticipated harvest were effective at mitigating pre-harvest fruit drop of 'Bisbee Delicious' apples. They also learned that AVG (125mg/L^{-1}) + NAA (20mg/L^{-1}) were effective at preventing pre-harvest fruit drop over NAA or AVG alone (Yuan and Li, 2008). Optimum efficacy what observed with applications of AVG (125mg/L^{-1}) + NAA (20mg/L^{-1}) at 3 weeks before anticipated harvest followed by an additional NAA (20mg/L^{-1}) application 1 week before harvest. Similar results were seen when (125mg/L^{-1} AVG was applied 3 weeks before harvest followed by 125mg/L^{-1} AVG + 20mg/L^{-1} NAA 1 week before anticipated harvest (Yuan and Li, 2008).

Naphthalene acetic acid (NAA)

Naphthalene acetic acid (NAA) is a synthetic auxin that directly interacts with the enzymes associated with the abscission zone (Yuan and Carbaugh, 2007). It interacts with the genes associated with cell separation. Apples treated with NAA do not show a delay in maturation. For NAA to be an effective drop preventing chemical, it must be applied at a rate of 10–88mg/L^{-1} and 7 to 28 days before harvest, and fruit maturity must be monitored intensively (Valent U.S.A. LLC., 2017). The product is only effective for 7 to 14 days and some producers make two applications. Application of NAA in conditions that are not favorable can lead to early fruit abscission (Valent U.S.A. LLC., 2017).

The use of NAA to reduce pre-harvest fruit drop has been documented in several studies (Anthony and Coggins, 2001; Dal Cin et al., 2008; Hoying and Robinson, 2010; Marini et al., 1993; Stover et al., 2003; Yuan and Carbaugh, 2007; Yuan and Li, 2008). Marini et al. (1993) found that repeated applications of NAA were more effective at preventing fruit drop than a

single application. It was discovered that treatments of 25mg/L^{-1} NAA were effective at stopping pre-harvest fruit drop in citrus however, rates of over 100mg/L^{-1} were more effective (Anthony and Coggins, 2001). Stover et al. (2003) showed that application of NAA did not slow pre-harvest fruit drop over unsprayed 'McIntosh' apples in year 1 of study, however, in year 2 of their study NAA did slow pre-harvest fruit drop when applied 7 d after applications were made. Fruit drop can be prevented in 'Delicious' apples when applied at a rate of 20mg/L^{-1} 21 to 7 days before anticipated harvest, however, two applications (21 and 7 d before harvest) of NAA at the same rate showed better efficacy for ~7 more days (Yuan and Li, 2008). Yuan and Carbaugh (2007) found that 'Golden Supreme' and 'Golden Delicious' apples that had been treated with NAA tended to lose firmness faster than fruit treated with AVG and this agrees with many other studies to date (Byers, 1997; Greene, 2005; Greene and Schupp, 2004; Yuan and Li, 2008). When NAA is applied in tandem with AVG, pre-harvest fruit drop of some apple cultivars are prevented more effectively than NAA or AVG alone (Hoying and Robinson, 2010; Yildiz et al., 2012; Yuan and Carbaugh, 2007; Yuan and Li, 2008). Similar results are seen when NAA is applied in combination with 1-MCP with the chemicals working better in tandem than alone (Yuan and Carbaugh, 2007).

Fruit Maturity and Harvest

Kiwifruit are borne from the fertilized ovary of pistillate flowers. Two rows of seeds will develop in each locule, holding several ovules in each. Ovules are fertilized by male germ cells delivered by a pollen tube to ovule (Brundell, 1975b). A single pollen tube can only fertilize one ovule and the number of fertilized ovules will influence final fruit size and quality. Fertilized seeds produce hormones that drive the growth of cells around them (Hasey, 1994). Kiwifruit are

no different than most other climacteric fruit in that they go through three main stages of growth during development. First, the fruit is fertilized and enters a stage of rapid growth due to cells dividing rapidly (Hasey, 1994). This first stage can last for 4 to 9 weeks after fertilization. The second stage last for another 4 to 6 weeks and is designated by a slowing of fruit growth. During this stage, cell numbers are not increasing as rapidly as they had in the first stage (Hasey, 1994; Pratt and Reid, 1974). However, cells are elongating and becoming larger. It is during this stage that carbohydrates are being deposited in the fruit (Hasey, 1994; Pratt and Reid, 1974). The final stage of fruit growth is known as maturation and shows the least amount of growth. During maturation, carbohydrates continue to be stored until the fruit are mature and begin to convert to sucrose and glucose spurring on ripening (Hasey, 1994). During the ripening process, the flesh color of *A. chinensis* cultivars will convert from green (h˚, >110) to yellow (h˚, < 98) as chlorophyll is overcome by esterified carotenoids (Montefiori et al., 2009). This ripening is encouraged by the production of ethylene. Ethylene impacts the conversion of sugars from starches and the structural integrity of the fruit (Montefiori et al., 2009; Pratt and Reid, 1974).

Harvest takes place during the maturation and ripening process. Fruit are sampled and measured for weight, SSC, h˚, flesh firmness, and DW to determine readiness (Hasey, 1994). Later harvested fruit tend to retain their firmness longer and show increased dry matter than fruit harvested earlier (Boukouvalas and Chouliaras, 2005). Harvest is conducted by hand picking into picking bags and from there into storage bins for curing (Hasey, 1994). Curing the fruit is a process in which the fruit are left in bins in a barn or storage facility at temperatures ranging from 10 ˚C to 20 ˚C and a RH of 92% for up to 3 days before moving the fruit to cold storage (Bautista-Baños et al., 1997). Curing is critical for harvested fruit to develop defenses against postharvest such as *Botrytis* rot. Fruit that is left to cure for too long can exhibit shriveling

making the fruit unmarketable (Bautista-Baños et al., 1997). Once cured, the bins will be placed into a cold storage room at 0 ˚C with a RH of 95%. If the fruit are stored at temperatures greater than 5 ˚C, rapid softening can occur making the fruit less marketable. One-third to one-half of fruit firmness can be lost per month in storage (Hasey, 1994).

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Chapter Three

Effect of Hydrogen Cyanamide on Flower Production of 'AU Golden Sunshine' and 'AU Gulf Coast Gold' Kiwifruit

Introduction

In temperate regions around the world, perennial plants require a minimum amount of chilling to break dormancy and initiate normal growth in the spring. Woody fruit bearing perennials require a certain amount of chilling below 7.2 ˚C and above 0 ˚C to initiate bud break and flower bud production. Kiwifruit have exhibited these characteristics in temperate zone growing regions around the world. Unsatisfactory chill hour accumulations can result in meager and desultory bud break and flower production for these vines that will impact profitability to producers cultivating the commodity (Brundell, 1975a; Brundell, 1975b; Brundell, 1976). It is standard practice in commercial growing regions to combat the negative effects of low chilling with dormancy breaking chemicals like hydrogen cyanamide (HC). This chemical is applied before natural bud break to assist in the release of dormant buds from rest (McPherson et al., 2001; Nee, 1991; Powell et al., 2000).

The Southeastern United States experiences inadequate chilling some years due to milder winter temperatures in the region. This can limit production of perennial fruiting plants grown there and reduce profitability of producers. Green kiwifruit (*Actinidia deliciosa* C.F. Liang et A.R. Ferguson) was introduced to the region in the mid-1980's by a commercial nursery in South Carolina (Powell et al., 2000). Later, plantings were established in Central and Southern Alabama to be trialed for a possible commercial industry. These plantings were mostly

unsuccessful due to the high amount of chilling required by the green kiwifruit cultivar ‘Hayward’ (Powell et al., 2000). Investigations into golden kiwifruit (*A. chinensis* Planch) began in the 1990’s. Two cultivars that were observed to have commercial potential in the region are ‘AU Golden Sunshine’ and ‘AU Gulf Coast Gold’ (Spiers et al., 2018). The chilling requirements for ‘AU Golden Sunshine’ is thought to be between 700-900 h chilling for optimal bud break and flowering, while chilling is not yet known for ‘AU Gulf Coast Gold’ (Dozier et al., 2011; Wall et al., 2008).

Southeast Kiwi Farming Cooperative, a 180-acre commercial orchard, was established in 2014 near Reeltown, Alabama, USA and began growing ‘AU Golden Sunshine’ and ‘AU Gulf Coast Gold’. The potential to utilize HC to encourage fruit production of these new cultivars warranted research. During years with low chill hour accumulation, vines can exhibit sporadic bud break and insufficient flower production, leading to very few saleable fruit (Brundell, 1976). Hydrogen cyanamide has been extensively researched as a tool to overcome insufficient chill accumulation for kiwifruit in temperate zone regions. Not only has HC been researched to overcome lack of chill, but also as a means to advance and consolidate bud break and bloom. Flower numbers are also increased when the product is used appropriately (Costa et al., 1997; Erez, 1995; Hernández et al., 2015; Inglese et al., 1998; McPherson et al., 2001; Nee, 2012; Powell et al., 2000; Shuck and Petri, 1995; Walton, 1991; Walton et al., 1997). Research on the effects of HC on *A. chinensis* cultivars is lacking and a better understanding of the effects of HC on these cultivars of golden kiwifruit is needed before it can be recommended to growers of the commodity.

It is known that timing and concentration of applications play a critical role in efficacy of products containing HC. Hernández and Craig (2011) determined that applications made 45 to 25

d before natural bud break advanced bud break on *A. deliciosa* ‘Hayward’. These findings agree with research addressing effects of HC application timing on green kiwifruit varieties conducted by others (Erez, 1995; Shuck and Petri, 1995; McPherson et al., 2001; Powell et al., 2000). However, Hernández et al. (2015) showed that applications of HC at a rate of 3% on *A. chinensis* ‘Gold3’, applied 17 d before natural bud break, were comparable to those made 33 d before natural bud break in regards to bud break intensity. Importantly, lateral flower bud development of ‘Gold3’ was reduced when applications were made closer to anticipated bud break (Hernández et al., 2015). Incidence of lateral flower buds have also been decreased when applications of 3% HC were made on green kiwifruit, which could lead to lower pollination and thinning expenses for growers (Erez, 1995; McPherson et al., 2001). This reduction in lateral flower development is thought to be related to chilling and timing of HC application. Sites receiving less chilling will have fewer inflorescences than sites with higher chilling when HC is used (McPherson et al., 2001). Applications of HC made closer to natural bud break show a reduction in lateral flowers due to controlled damage of lateral flowers when adequate chilling is accumulated (Hernández and Craig, 2011; Hernández and Craig, 2015). The instance of lateral buds increased when HC was applied to ‘Gold3’ 25 or more days before anticipated bud break (Hernández et al., 2015). Concentrations at or below 2% HC decrease the risk of phytotoxicity that are associated with higher concentrations and later application times (Erez, 1995; Shuck and Petri, 1995; Costa, 1997; Powell et al., 2000). When HC is used properly, flowering is increased because more buds arise before floral suppression begins (Shuck and Petri, 1995). This is because effective applications of HC condense bud break and flowering (Engin et al., 2010; Inglese et al., 1998; Hernández et al., 2015; McPherson et al., 2001; Shuck and Petri, 1995).

Applications made to vines ~14 d before natural bud break can increase flower production over earlier applications on *A. deliciosa* (Inglese et al., 1998).

The objectives of this research were to determine the effectiveness of HC on bud break and flower development of 'AU Golden Sunshine' and 'AU Gulf Coast Gold'. Timing of application of HC and the influence of chilling were also studied.

Materials and Methods

Experimental Design

All experiments were conducted over 2 years (2018-2019) at Southeastern Kiwi Farming Cooperative in Reeltown, Alabama. Vines chosen were 4 to 5 years of age at initiation and were managed according to the best management practices recommended for commercial production of the commodity (Hasey, 1994). Three studies were conducted to determine the efficacy of 2% HC applied approximately 28 or 14 d before natural bud break, as compared to unsprayed (control) vines. Each of the studies were arranged in a randomized complete block design with one vine per treatment replication.

Study one was conducted on 'AU Golden Sunshine' grafted onto *A. deliciosa* 'Hayward' seedlings. During year one (2018), there were 15 blocks with three vines per block (one vine/treatment) while year two (2019) had 10 blocks. Year two of this study also included the male *A. chinensis* cultivar 'AU Golden Tiger' that was represented by five additional blocks with three vines per block. Study two contained 11 blocks of 'AU Golden Sunshine' on its own roots during year one and 10 blocks during year two. 'AU Gulf Coast Gold' was the subject of study three, having 15 blocks during year one and 10 during year two. To reduce variability, four canes, two on either side of the cordon were chosen per vine for flowering and bud break data

collection in all fields. These canes were in various locations along the cordon and were marked using flagging tape.

Treatment Application

Treatments were randomly applied in each block. Three treatments were applied to better understand application timing impact on uniformity of bud burst and bloom period. Hydrogen cyanamide was applied at a concentration of 2% to the experimental vines during both years of the study. In 2018, the first treatment of HC was applied approximately 28 days before anticipated natural bud break on 1 Feb. The second treatment of HC was applied approximately 14 days before anticipated natural bud break on 15 Feb. The third treatment remained unsprayed and was used as a control. A total of 1198h below 7.2 ˚C accumulated at the orchard during 2018. At the time of the first HC application (1 Feb.) 1013h had accumulated and a total of 1079h were accumulated by the time of the second application (15 Feb.) in 2018. In 2019, the first treatment of HC was applied on 31 Jan. and the second treatment was applied on 14 Feb. Chilling hour accumulations in 2019 totaled at 891 h below 7.2 ˚C. Chill hours of 749 h and 806 h had accumulated by the time of the first (31 Jan.) and second (14 Feb.) applications respectively. Hydrogen cyanamide was applied via backpack sprayer (Solo 475-B Backpack Sprayer, Solo Inc. Newport News, VA, USA) to entire vines until run-off.

Data Collection

Cane diameter and cane length were measured on each of the four canes used per vine. Dormant buds were counted prior to bud break as a basis to determine king flower to dormant bud (KFl/DB) ratios. This ratio can be used by growers to make crop estimations during the

growing season. Bud break was assessed twice weekly and each emerging bud was counted when it reached the dome shape described by Brundell (1975a). Bud break counts ceased when buds were no longer breaking. After bud break, floral and vegetative shoots were counted to calculate a floral shoot percentage. Shoots were considered to be floral if flower buds were present on the shoot and considered vegetative if no flower buds were observed on the shoot. These counts continued until flowering occurred. Flower counts were made every 3 d and flowers were considered open when the petals permitted easy access to bees (Hernández and Craig, 2011). Flower counts began upon opening of the first bloom until all blooms had successfully opened. Vines had reached full bloom when 95% of flowers had opened.

Statistical Analysis

An analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). The experimental design was a randomized complete block with canes as subsamples, and cultivars were analyzed separately. For number of king flowers (#KFl), number of lateral flower buds (# LB), king flower to dormant bud ratio (KFl/DB), lateral bud to king flower ratio (LB/KFl), total number of shoot, number of veg shoot, KFl/shoot, percent floral shoots, and number of floral shoots, ANCOVA was used when a linear relationship was found between cane length, cane caliper, and/or dormant bud number and the response; otherwise, ANOVA was used. Models were fit with each of the covariates and combinations, and the model with the lowest corrected Akaike information criterion (AICC) value was used. The treatment design was 1-way. The negative binomial distribution was used with the Laplace method for # KFl, # LB, total shoots, vegetative shoots, and # floral shoots. The beta distribution was used for % floral shoots; otherwise, the Gaussian distribution was used.

Linear and quadratic trends over treatment dates were examined using simple model regressions. ANCOVA was used for number of bud breaks with the number of dormant buds as the covariate in a 2-way treatment design of treatment and date. ANOVA was used for percent bud breaks. Number of bud breaks was analyzed using the negative binomial distribution while percent bud breaks was analyzed using the beta distribution. The experimental design was completely randomized with repeated measures over date. The experimental design for open flower number and full bloom percent was completely randomized with canes as subsamples. Open flower number was analyzed using the negative binomial distribution while full bloom percent was analyzed using the beta distribution. A 2-way treatment design of treatment rate and date was used. Linear and quadratic trends over treatment rates and dates were examined in all models with 2-way designs using qualitative/quantitative model regressions. Reported are least squares means. All significances were at $\alpha=0.05$.

Results

Study 1: Effect of 2% hydrogen cyanamide application timing on 'AU Golden Sunshine' on A. deliciosa seedling rootstocks.

Bud break

The date of natural bud break varied by year and cultivar. During the 2018 season, natural bud break occurred on 2 March for 'AU Golden Sunshine' on 'Hayward' seedling rootstocks. The 1 Feb. treatment advanced bud break by ~7 d in comparison to natural bud break (Fig. 3.1). Bud break on vines receiving this early application began 23 d after application. The 15 Feb. treatment advanced bud break by ~5 d when compared to natural bud break, or bud break occurred 12 d after HC application. Though vines treated on 1 Feb. broke bud prior to the 15

Feb. treated plants, the 15 Feb. application resulted in a greater bud break percentage (Table 3.1). The 15 Feb. treatment had a higher percentage of total bud break over other treatments. The 1 Feb. application did not result in a higher bud break percentage than the control.

During the 2019 season, the region experienced a spring frost event after a prolonged duration of warm temperature that occurred on 7 March with temperatures getting as low as -3.3 ˚C. This had a negative impact on any bud that broke before 7 March 2019, causing termination. Secondary buds did arise that were later discovered to be vegetative. Flower production was also limited due to this frost event. Nevertheless, natural bud break occurred on 25 Feb. 2019. This was slightly earlier than in 2018 though less chilling had accumulated. This earlier natural bud break could be due to an abnormal warming period that occurred the month before bud break commenced. The first HC application (31 Jan.) showed the most advancement with buds breaking 8 days before the unsprayed vines. There was no advancement of bud break observed when the later application (14 Feb.) was compared to control vines (Fig. 3.2). Applications made on 31 Jan. resulted in 25 d advancement of bud break when compared to the 'AU Golden Sunshine' control vines grafted on *A. deliciosa* rootstocks, 16 d advanced bud break in comparison to own rooted 'AU Golden Sunshine' plants, and occurred 18 d before natural bud break of 'AU Gulf Coast Gold'. Applications made on 15 Feb. resulted in bud break of 11, 2, and 4 d before natural bud break, respectively. Bud break percentage was highest among the vines that received the first application of HC and though the second application did not break bud before unsprayed vines, it did surpass them on bud break percentage until the frost event. Post frost event, all buds that had broken were terminated, however, bud break began again with no difference in advancement or bud break percentage between treatments.

Flowering

‘AU Golden Sunshine’ on *A. deliciosa* rootstocks showed promise during the 2018 season when 2% HC applications were made, with heavy flower loads and ample vigor. The first flowers for this cultivar emerged during the first week of April (~60 d after application) on vines receiving the early application (1 Feb.) of HC (Fig. 3.3). This was advanced over unsprayed vines by two full weeks which bore their first flowers on 16 April. The later application (15 Feb.) of HC also showed advancement in flowering on ‘AU Golden Sunshine’ on *A. deliciosa* rootstocks over unsprayed vines with the first flowers opening on 10 April, 8 d after vines receiving the early application began to bloom (Fig. 3.3). Both treatments reached full bloom (≥ 95% bloom) by 23 April, only 2 d ahead of unsprayed vines during 2018. However, ~50% bloom was achieved on treated vines on 18 April, just as unsprayed vines were beginning to bloom. By 20 April, 2% HC treated vines reached full bloom while control vines were only half way through bloom (Fig. 3.3). The greatest number of KFl/shoot were observed on vines receiving the later application with ~2× the number of KFl/shoot than on unsprayed vines. King flower to dormant bud ratios for this cultivar were highest on vines receiving the later application having a ~2.5× greater KFl/DB than control vines (Table 3.1). Vines sprayed on 1 Feb. had higher KFl/DB than control vines, but lower than the vines treated on 15 Feb. Control vines and vines receiving the 2% HC application on 15 Feb. had relatively few LB compared to vines treated with 2% HC on 1 Feb. Shoots that arose were mostly floral on all treatments. The highest percentage of floral shoots were found on vines sprayed later, on Feb. 15. Control vines had the lowest percentage of floral shoots (Table 3.1).

During the 2019 season, ‘AU Golden Sunshine’ flowered at approximately the same time for all treatments. There were no effects of treatments on %BB, KFl/DB, KFl/shoot, or %LB however, floral shoots were greater on control vines than on vines receiving applications of 2%

HC. Blooms began to appear on 15 April for all treatments. This was due to the late spring frost which caused shoots to die back and reemerge as vegetative shoots. The vines most affected by the late freeze were vines receiving the early applications of 2% HC because they were far more advanced (Fig. 3.4). More buds had broken and were terminated than on control vines leading to less flowers. Control vines, though also injured by the frost event, had a greater percentage of floral shoots, followed by vines receiving the 14 Feb. application (Table 3.1).

Study 2: Effect of 2% hydrogen cyanamide application timing on own rooted 'AU Golden Sunshine'

Bud break

When studied on its own roots, 'AU Golden Sunshine' naturally broke bud on 23 Feb. 2018. The early application advanced bud break by ~7 days while the second application aligned with natural bud break (Fig. 3.5). Bud break in the early treatment occurred 16 d after application while in the later treatment vines broke bud 9 d after application. There were no differences in bud break percentage across treatments (Table 3.2).

'AU Golden Sunshine' on its own roots performed similarly in both years with natural bud break occurring earlier than grafted vines. Natural bud break occurred on 16 Feb. 2019, just 2 d after the later application of HC was applied. Despite this, no phytotocity was observed on any vines. The early application resulted in the most advanced bud break of treated vines for which bud break occurred approximately ~7 d before unsprayed vines initiated the process. There was no advancement of bud break on vines that received 2% HC on 14 Feb. (Fig. 3.6). Again, this is likely related to tardiness of the later application.

Flowering

‘AU Golden Sunshine’ on its own roots began to flower much earlier than vines on *A. deliciosa* rootstocks and behaved erratically. Vines receiving early applications of 2% HC began to bloom in late March 2018 (~54 d after application) and had completed flowering by 20 April (Fig. 3.7). Control vines flowered second on 29 March 2018 with vines receiving the later application not flowering until 3 April. Vines sprayed with 2% HC had a greater KFl/DB than controls during 2018 (Table 3.2). A higher KFl/DB occurred on vines sprayed on 15 Feb. however, all KFl/DB were extremely low for this cultivar on its own roots. There were no effects of treatments on % LB, as LB production was similarly low. The percentage of floral shoots were greater on vines that received the later application (15 Feb.) compared to control vines and vines that received the early application (1 Feb.).

There were no differences in KFl/DB, KFl/shoot, or %LB during the 2019 season (Table 3.1). Flowers first appeared 11 April in 2019 for vines sprayed on 31 Jan. Both of the other treatments bloomed 4 d later on 15 April (Fig. 3.8). All vines showed similar results for flower numbers and floral shoots during 2019 (Table 3.2). This was likely due to the tardiness of the later application in relation to the frost event that spring. The late spring frost did more damage to these vines because of the preeminence of break bud in the spring. This is another indication that there were some scion/rootstock interactions that delay bud break.

Study 3: Effect of 2% hydrogen cyanamide application timing on ‘AU Gulf Coast Gold’ grafted on A. deliciosa seedling rootstocks.

Bud break

Natural bud break for grafted ‘AU Gulf Coast Gold’ vines also occurred on 2 March during 2018. The early application showed an advancement of ~7 d ahead of natural bud break while the later application was ~5 d advanced. Bud break occurred 23 d after application for the

early treatment and 12 d after application for the later treatment. Again, both treatments were more advanced than control vines (Fig. 3.9). The later application had a higher bud break percentage than the early application and the control (Table 3.3).

Natural bud break for grafted 'AU Gulf Coast Gold' plants occurred on 18 Feb. during 2019. Sensitivity to growing degree hours (GDH) may have played a role in the advancement of bud break of this cultivar when compared to 'AU Golden Sunshine' also on *A. deliciosa* rootstocks. Advances in bud break were observed for the early application of 2% HC. Bud break was advanced by ~5 days by the early application which was 14 d after application. No advancement in bud break was observed between the later application and the control (Fig. 3.10). The early application had a higher bud break percentage than the later application and control before the frost event, however, all buds were terminated and secondary growth resumed, equalizing bud break percentage across treatments (Table 3.3).

Flowering

Flowering for grafted 'AU Gulf Coast Gold' began on 30 March (~58 d after application) for vines receiving the early application of 2% HC in 2018. This was followed by vines that received the later application, which began to bloom on 2 April. Unsprayed vines did not flower until 14 April and bloom duration was longer than the other two treatments (Fig. 3.11). Vines receiving 2% HC completed bloom on 19 April, 4 d before unsprayed vines. Vines treated with 2% HC had more advanced flowering compared to the control vines. The least number of KFl were counted on control vines while vines sprayed on 15 Feb. had the most. The highest KFl/DB was observed on vines receiving the later application (Table 3.3). Both 2% HC treatments had a higher KFl/DB than the control. The percentage of LB were similar to that of 'AU Golden Sunshine' on *A. deliciosa* rootstocks. The early application showed the highest LB percentage at

10% and control vines showed the least LB (3%). Floral shoots emerged at a higher rate on vines receiving the later application of HC (90.8%). During 2019, the frost event limited flower production of these vines. There were no differences among treatments for 'AU Gulf Coast Gold' in 2019 (Table 3.3). Flowers began opening on 11 April for all treatments (Fig. 3.12).

Study 4: Effect of 2% hydrogen cyanamide application timing on 'AU Golden Tiger'.

Bud break

The male cultivar, 'AU Golden Tiger' was also studied during 2019 to determine effect of HC on male flower production and synchronization with flowering period of female cultivars. Natural bud break began on 25 Feb. for the male cultivar (Fig. 3.13). Before the frost event, the early application showed a higher bud break percentage, however, there was not an advancement of bud break over unsprayed vines (Table 3.4). This could indicate that use of HC could increase floralness of this male cultivar though more experimentation is needed. There were no differences in bud break percentage between unsprayed vines and vines receiving the later application.

Flowering

The male 'AU Golden Tiger' showed no advancement in flowering which was delayed compared to the female cultivars. This put the male flowering slightly behind the female vines which is typical of this cultivar. The first flowers opened on 19 April with no differences among treatments (Fig. 3.14). This cultivar was the most floral overall having nearly double the percentage of floral shoots as the other cultivars in the study during 2019 (Table 3.4).

Discussion

Recommendations of HC use to advance bud break in selected gold kiwifruit cultivars can be risky and vary from season to season depending on a number of factors. Chilling

accumulation plays a vital role in the decision-making process for growers applying HC, however, date of natural bud break and accumulation of GDH should be considered before moving forward with applications. Our results for 2018 show that applying HC ~14-16 d before natural bud break on 'AU Golden Sunshine' and 'AU Gulf Coast Gold' may provide the highest amount of bud break percentages and the least percentage of lateral flower buds which agrees with the findings of Inglese et al. (1998) and Hernández et al. (2015). This could be due to the amount of chilling that was received after 1 Feb. and before 15 Feb. More chilling could have helped accelerate bud break for the 15 Feb. (2018) application (Erez, 1995). Applications of 2% HC could advance bud break and could increase susceptibility to a late spring frost event like the one experienced in 2019. Buds do recover and secondary shoots will arise, however, most will not be floral.

Bud break was advanced with all treatments of 2% HC over unsprayed vines during 2018 and this is concurring with results found by many other authors (Costa et al., 1997; Erez, 1995; McPherson et al., 2001; Powell et al. 2000; Shuck and Petri, 1995). Vines receiving the early application (1 Feb. 2018) were most advanced but had lower KFl/DB and greater %LB than vines receiving the late application (15 Feb. 2018), which could increase the amount of effort required for fruit thinning. Highest bud break percentages were seen on vines receiving the later application (15 Feb. 2018), just 2 weeks out from natural bud break. Also, during 2018, flowering was advanced on all vines receiving HC, though the late application (15 Feb. 2018) had the highest KFl/DB and fewer LB than the early application. These findings agree with those of Hernández and Craig (2015) and Hernández et al. (2015) who found that applications made closer to natural bud break decreased the amount of LB observed on *A. chinensis* 'Gold3'. When grown on its own roots, 'AU Golden Sunshine' showed erratic bud break with or without the use

of HC. More research is needed to better understand rootstock interactions with relation to bud break and flowering of ‘AU Golden Sunshine’.

In 2019, the most advancement in bud break resulted from the early application (31 Jan. 2019), however, this treatment also received the most damage from the late spring frost event. There were less flowers and shoots were mostly vegetative on vines receiving the early application (31 Jan. 2019). This is directly associated with the frost event. Though chilling was lower in 2019, there was an advancement in bud break from previous years due to a warm spell the months before natural bud break. There were no signs of phytotoxicity caused by HC throughout the two-year study.

In this experiment, flowering was advanced and bloom was consolidated when HC was used in 2018. Vines were considered to have reached full bloom when 95% of flowers had opened. As with bud break, the most advancement of bloom occurred on vines that received the early application of HC on 1 February 2018. The early application also showed increased lateral flower buds compared to the late application (15 Feb.) and the control during 2018. There were no significant differences in bloom during the 2019 season due to the late frost event experienced.

Flowering was significantly advanced when HC was applied during 2018 across both grafted cultivars. Applications made ~14 d (15 Feb.) before natural bud break had higher KFl/DB and lower %LB that the other treatment. ‘AU Golden Sunshine’ on its own roots performed unsatisfactory when HC was applied producing a lower KFl/DB compared to grafted vines. When grafted on *A. deliciosa* rootstocks, ‘AU Golden Sunshine’ showed the highest KFl/DB when HC was applied 2 weeks before natural bud break. ‘AU Gulf Coast Gold’ had a lower KFl/DB than the ‘AU Golden Sunshine’ when both were grafted on *A. deliciosa*

rootstocks, however, ‘AU Gulf Coast Gold’ had the highest percentage of floral shoots. This indicates that ‘AU Golden Sunshine’ produced more vegetative shoots than did ‘AU Gulf Coast Gold’.

Conclusions

Based on results from 2018 in the absence of frost injury, 2% HC may be used to advance bud break and flowering of these female cultivars with optimum applications being applied ~15 d before anticipated bud break. Vines receiving HC applications ~2 weeks before natural BB showed the highest KFl/DB, KFl/Shoot, and greatest percentage of floral shoots when frost injury did not occur (2018). Applications made too early can lead to fewer flowers and increase the amount of unwanted LB. This could lead to higher thinning costs to growers. No advancements occurred for the male ‘AU Golden Tiger’ vines and more research is required to make recommendations on the cultivar. The risk of frost injury increases with an advancement in BB. Though even untreated vines experienced significant frost injury in 2019, flower production was reduced to a greater extent on vines treated with HC compared to untreated vines due to BB advancement.

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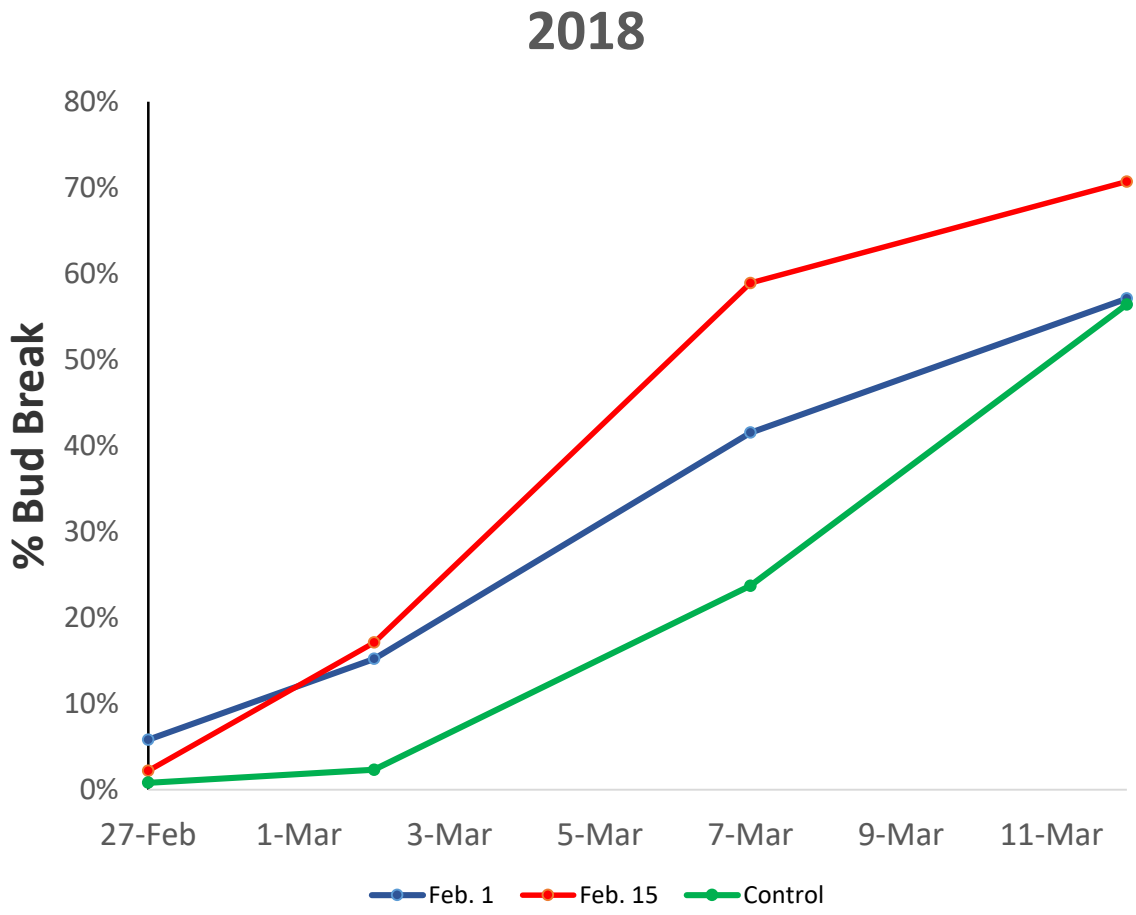
Figure 3.1: Percent bud break of 'AU Golden Sunshine' grafted on *A. deliciosa* rootstocks over time during the spring of 2018 (Feb.-March).



Date	2/27	3/2	3/7	3/12	Sign.
Feb. 1	5.8	15.2	41.5	57.1	Q***
Feb. 15	2.2	17.1	58.9	70.7	Q***
Control	0.8	2.3	23.7	56.4	Q**
Sign.	L***	Q***	Q***	Q***	

* Linear (L) or quadratic (Q) trends using model regressions at α=0.01(**) or 0.001(***). Non –significant (NS) or Sign. = Significance.

Table 3.1: Effects of hydrogen cyanamide (2% a.i.) applications on bud break, flowering, and lateral bud development of 'AU Golden Sunshine' *Actinidia chinensis* grafted on seedling *A. deliciosa* rootstock during 2018 and 2019.

Year	Treatment timing	Chilling (<7.2˚C)	Chilling (0˚C-7.2˚C	BB[z] (%)	KFl/DB[y]	KFl/Shoot[x]	LB[w] (%)	Floral Shoots[v] (%)
2018	Feb. 1	1013	575	57.1	1.10	2.0	17.0	77.5
	Feb. 15	1079	639	70.7	1.72	3.0	3.0	84.4
	Control	1198	718	56.4	0.67	1.0	1.0	62.0
Sign.[u]				Q***	Q***	Q***	L***	Q***
2019	Jan. 31	749	588	41.0	0.10	0.24	0.0	9.7
	Feb. 14	806	634	41.9	0.16	0.38	0.0	16.1
	Control	891	691	42.8	0.39	0.90	0.1	27.6
Sign.				NS	NS	NS	NS	L***

[z] %BB = Percent bud break = Percentage of total dormant buds that broke dormancy

[y] KFl/DB = Number of king flowers per dormant bud

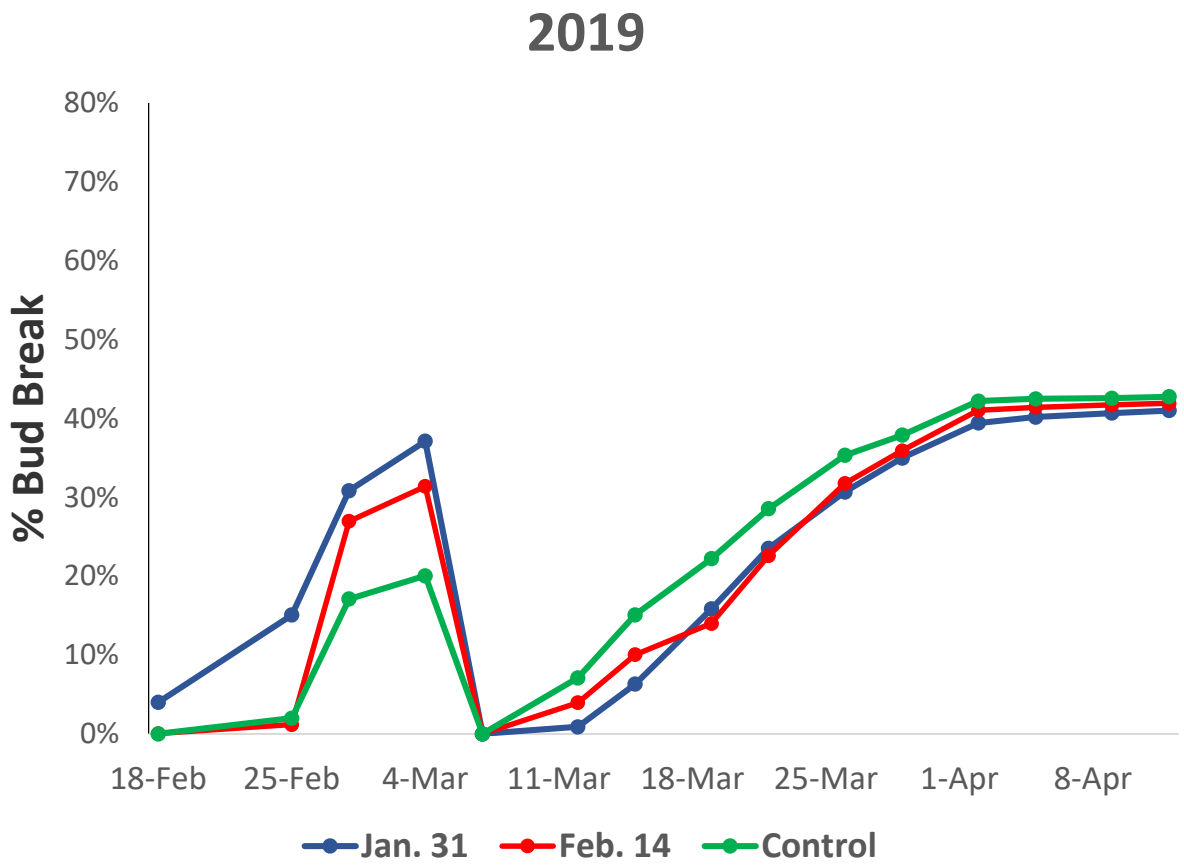
[x] KFl/Shoot = Number of king flowers per shoot

[w] LB (%) = Lateral flower bud percentage = total number of lateral flowers divided by total number of flowers present

[v] % Floral Shoot = Percent floral shoots based on number of shoots with flowers over total number of shoots

[u]Linear (L) or quadratic (Q) trends using model regressions at $\alpha = 0.001$(***). Non-significant (NS) or Sign. = Significance.

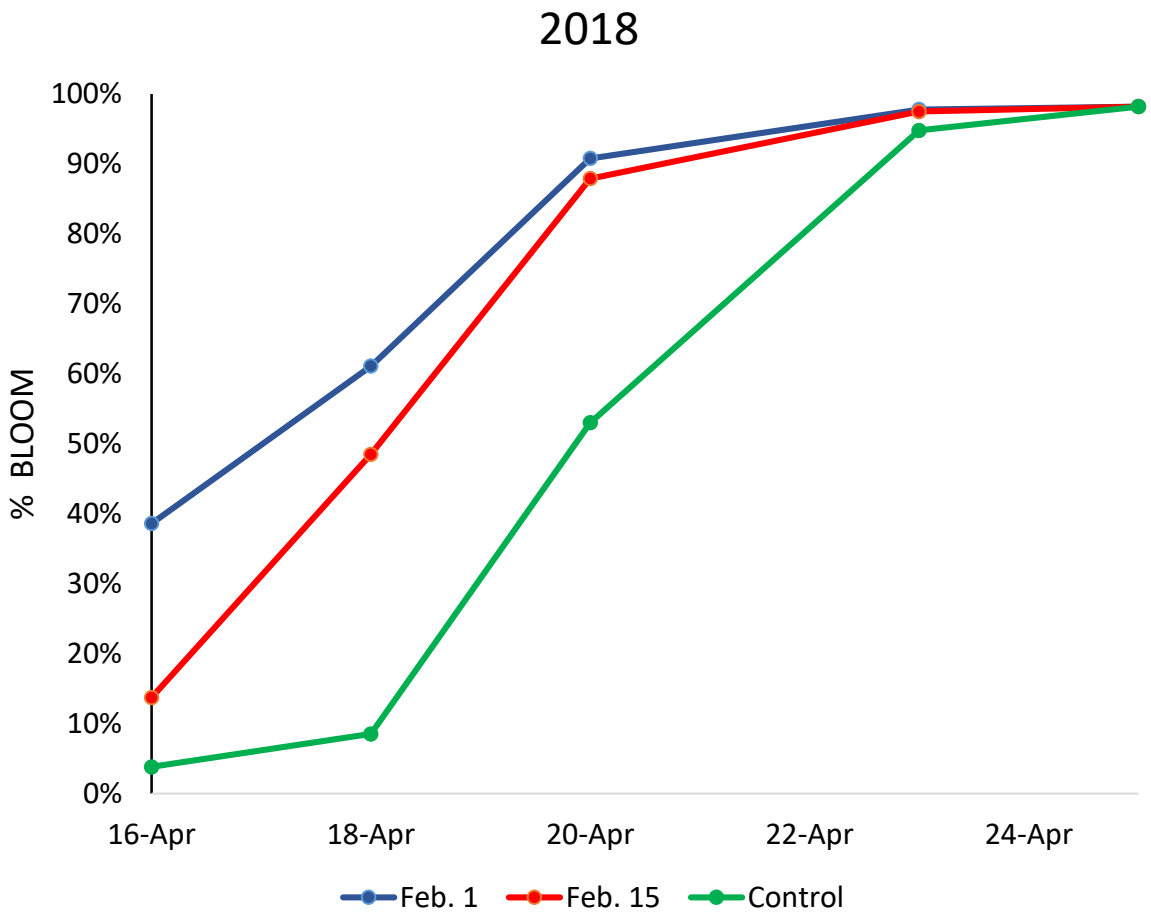
Figure 3.2: Percent bud break of 'AU Golden Sunshine' on *A. deliciosa* rootstocks over time during the spring of 2019 (Feb.-April).



Date	2/18	2/25	2/28	3/4	3/7	3/12	3/15	3/19	3/22	3/26	3/29	4/2	4/5	4/9	4/12	Sign.
Jan. 31	4.0	15.1	30.8	37.1	0.0	0.9	6.3	15.8	23.5	30.7	35.0	39.4	40.2	40.7	41.0	NS
Feb. 14	0.0	1.2	27.0	31.4	0.0	3.9	10.0	14.0	22.6	31.8	35.9	41.0	41.4	41.7	41.9	NS
Control	0.0	2.0	17.1	20.0	0.0	7.1	15.1	22.2	28.5	35.3	37.9	42.2	42.5	42.6	42.8	NS
Sign.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

* Linear (L) or quadratic (Q) trends using model regressions at α=0.05(*), 0.01(**), or 0.001(***). Non –significant (NS) or Sign. = Significance.

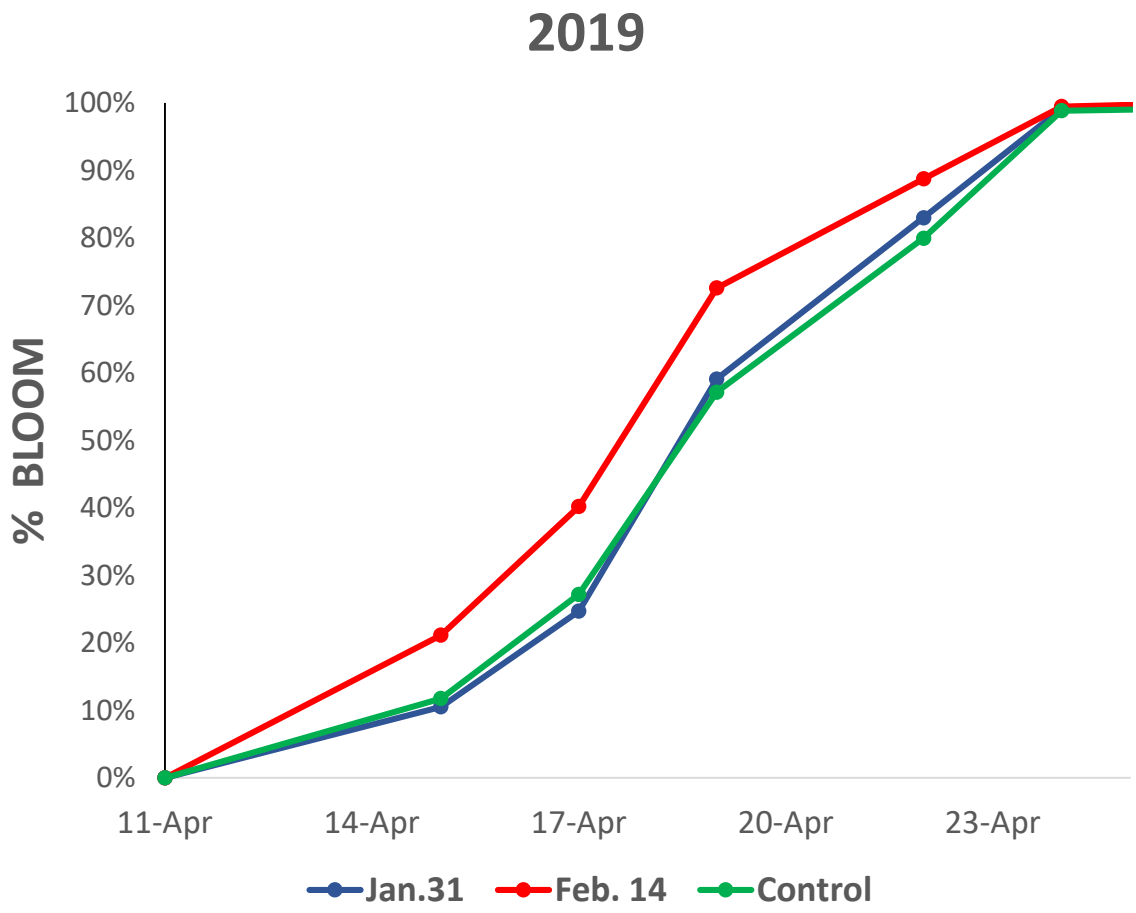
Figure 3.3: Percent bloom of 'AU Golden Sunshine' on *A. deliciosa* rootstocks over time during the spring of 2018 (April).



Date	4/16	4/18	4/20	4/23	4/25	Sign.
Feb. 1	3.8	8.5	53.0	94.8	98.2	L***
Feb. 15	13.7	48.5	87.9	97.5	98.2	Q***
Control	38.6	61.1	90.8	97.8	98.2	Q*
Sign.	L***	Q***	Q**	L*	NS	

* Linear (L) or quadratic (Q) trends using model regressions at α=0.05(*), 0.01(**), or 0.001(***). Non –significant (NS) or Sign. = Significance.

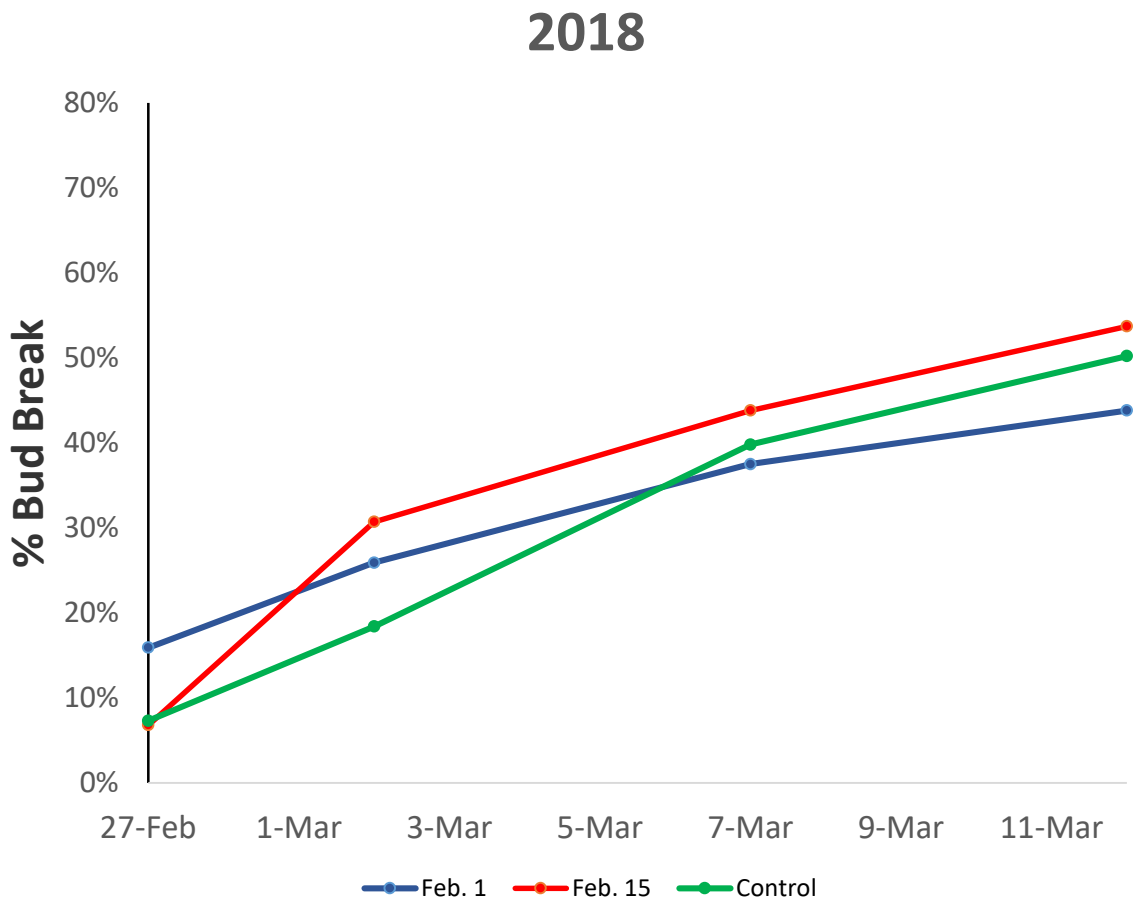
Figure 3.4: Percent bloom of 'AU Golden Sunshine' on *A. deliciosa* rootstocks over time during the spring of 2019 (April).



Date	4/11	4/15	4/17	4/19	4/22	4/24	4/26	4/29	Sign.
Jan. 31	0.0	10.5	24.7	59.1	83.0	99.2	100.0	100.0	NS
Feb. 14	0.0	21.1	40.2	72.6	88.8	99.5	100.0	100.0	NS
Control	0.0	11.8	27.2	57.2	80.0	98.9	99.2	99.7	NS
Sign.	NS	NS	NS	NS	NS	NS	NS	NS	

* Linear (L) or quadratic (Q) trends using model regressions at α=0.05(*), 0.01(**), or 0.001(***). Non – significant (NS) or Sign. = Significance.

Figure 3.5: Percent bud break of 'AU Golden Sunshine' on its own roots over time during the spring of 2018 (Feb.-March).



Date	27-Feb	2-Mar	7-Mar	12-Mar	Sign.
Feb. 1	15.9	25.9	37.5	43.8	Q**
Feb. 15	6.8	30.7	43.8	53.7	Q***
Control	7.3	18.4	39.8	50.2	L***
Sign.	L**	Q*	NS	NS	

* Linear (L) or quadratic (Q) trends using model regressions at α=0.05(*), 0.01(**), or 0.001(***). Non – significant (NS) or Sign. = Significance.

Table 3.2: Effects of hydrogen cyanamide (2% a.i.) applications on bud break, flowering, and lateral bud development of 'AU Golden Sunshine' on its own roots during 2018 and 2019.

Year	Treatment timing	Chilling (<7.2˚C)	Chilling (0˚C-7.2˚C	BB[z] (%)	KFl/DB[y]	KFl/Shoot[x]	LB[w] (%)	Floral Shoots[v] (%)
2018	Feb. 1	1013	575	43.8	0.55	1.3	3.9	77.5
	Feb. 15	1079	639	53.7	0.63	1.1	0.0	81.3
	Control	1198	718	50.2	0.29	0.7	0.03	69.7
Sign.[u]				NS	Q**	L**	NS	Q**
2019	Jan. 31	749	588	44.5	0.04	0.09	0.0	3.7
	Feb. 14	806	634	51.1	0.07	0.15	0.6	6.1
	Control	891	691	48.9	0.11	0.26	0.0	7.2
Sign.				NS	NS	NS	NS	NS

[z] %BB = Percent bud break = Percentage of total dormant buds that broke dormancy

[y] KFl/DB = Number of king flowers per dormant bud

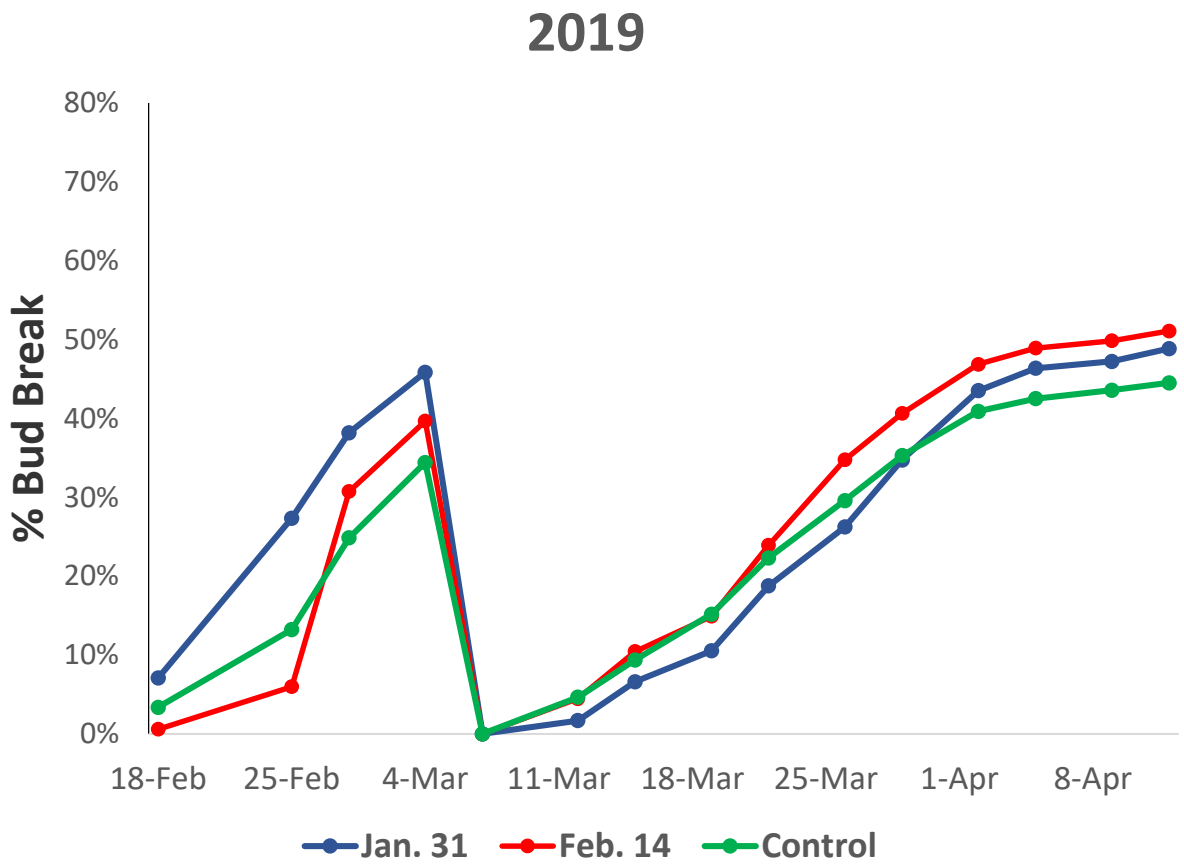
[x] KFl/Shoot = Number of king flowers per shoot

[w] LB (%) = Lateral flower bud percentage = total number of lateral flowers divided by total number of flowers present

[v] % Floral Shoot = Percent floral shoots based on number of shoots with flowers over total number of shoots

[u]Linear (L) or quadratic (Q) trends using model regressions at α=0.01(**). Non-significant (NS) or Sign. = Significance.

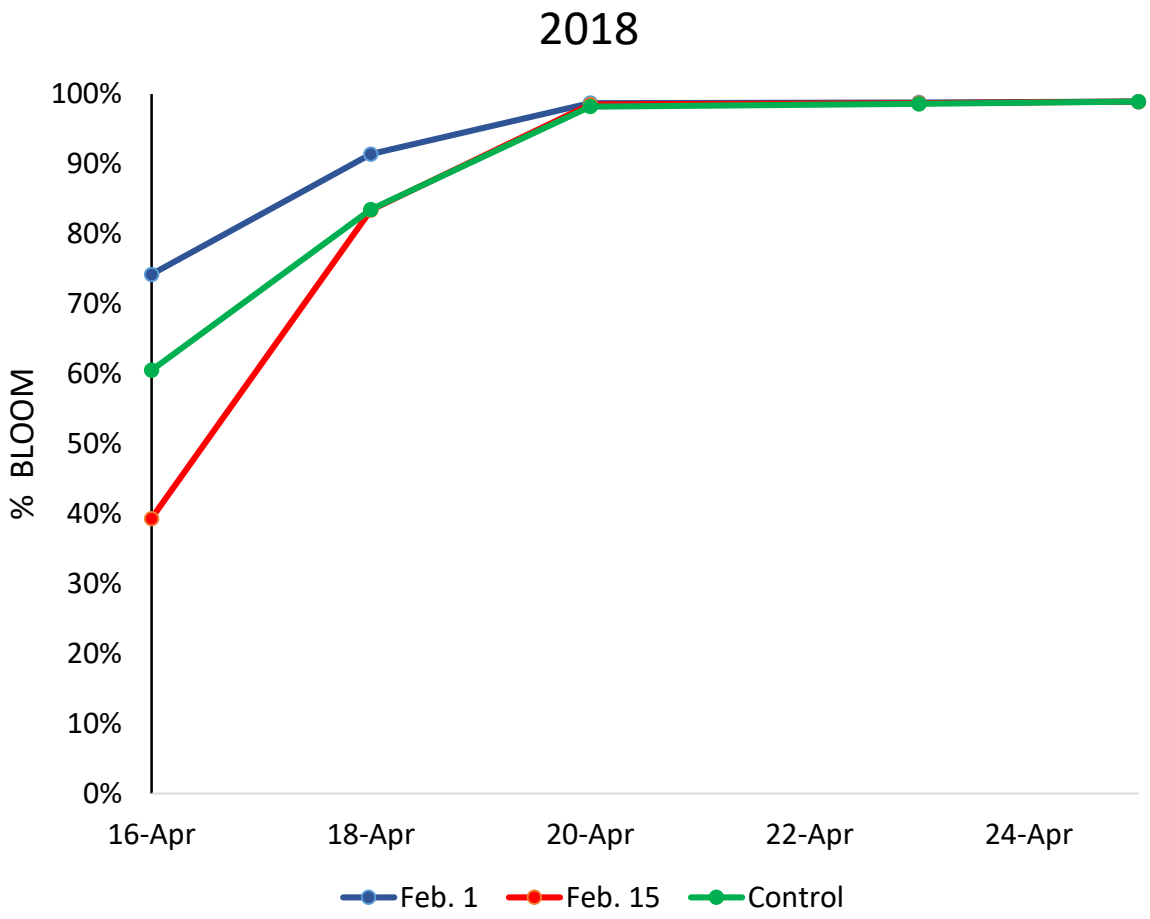
Figure 3.6: Percent bud break of 'AU Golden Sunshine' on its own roots over time during the spring of 2019 (Feb.-April).



Date	2/18	2/25	2/28	3/4	3/7	3/12	3/15	3/19	3/22	3/26	3/29	4/2	4/5	4/9	4/12	Sign.
Jan. 31	7.1	27.4	38.2	45.9	0.0	1.7	6.6	10.5	18.8	26.2	34.7	43.5	46.4	47.2	48.9	NS
Feb. 14	0.6	6.0	30.7	39.7	0.0	4.5	10.4	14.9	23.9	34.8	40.6	46.9	48.9	49.9	51.1	NS
Control	3.4	13.2	24.8	34.4	0.0	4.6	9.4	15.2	22.3	29.6	35.3	40.9	42.5	43.6	44.5	NS
Sign.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

* Linear (L) or quadratic (Q) trends using model regressions at α=0.05(*), 0.01(**), or 0.001(***). Non – significant (NS) or Sign. = Significance.

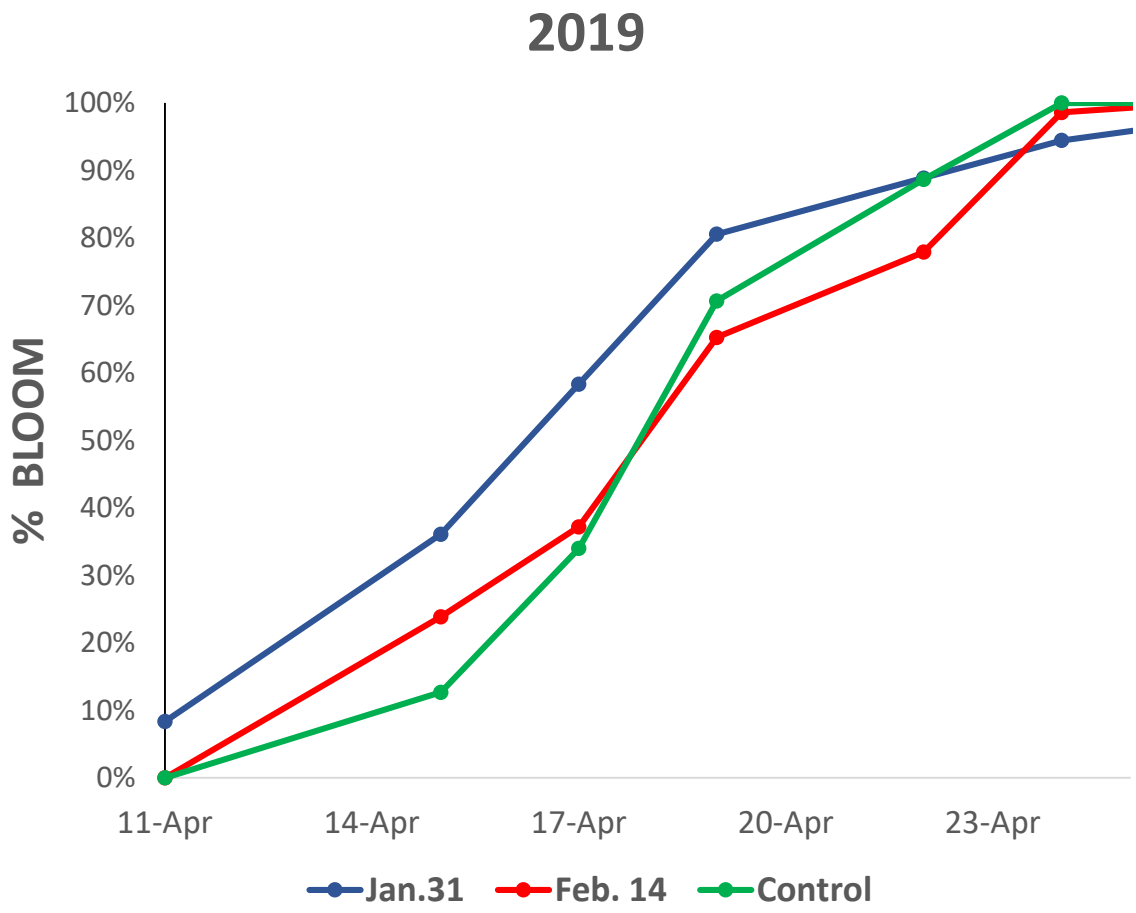
Figure 3.7: Percent bloom of 'AU Golden Sunshine' on its own roots over time during the spring of 2018 (April).



Date	4/16	4/18	4/20	4/23	4/25	Sign.
Feb. 1	74.2	91.4	98.7	98.8	98.9	NS
Feb. 15	39.3	83.4	98.5	98.7	98.9	NS
Control	60.5	83.5	98.2	98.6	98.9	NS
Sign.	NS	NS	NS	NS	NS	

* Linear (L) or quadratic (Q) trends using model regressions at α=0.05(*), 0.01(**), or 0.001(***). Non – significant (NS) or Sign. = Significance.

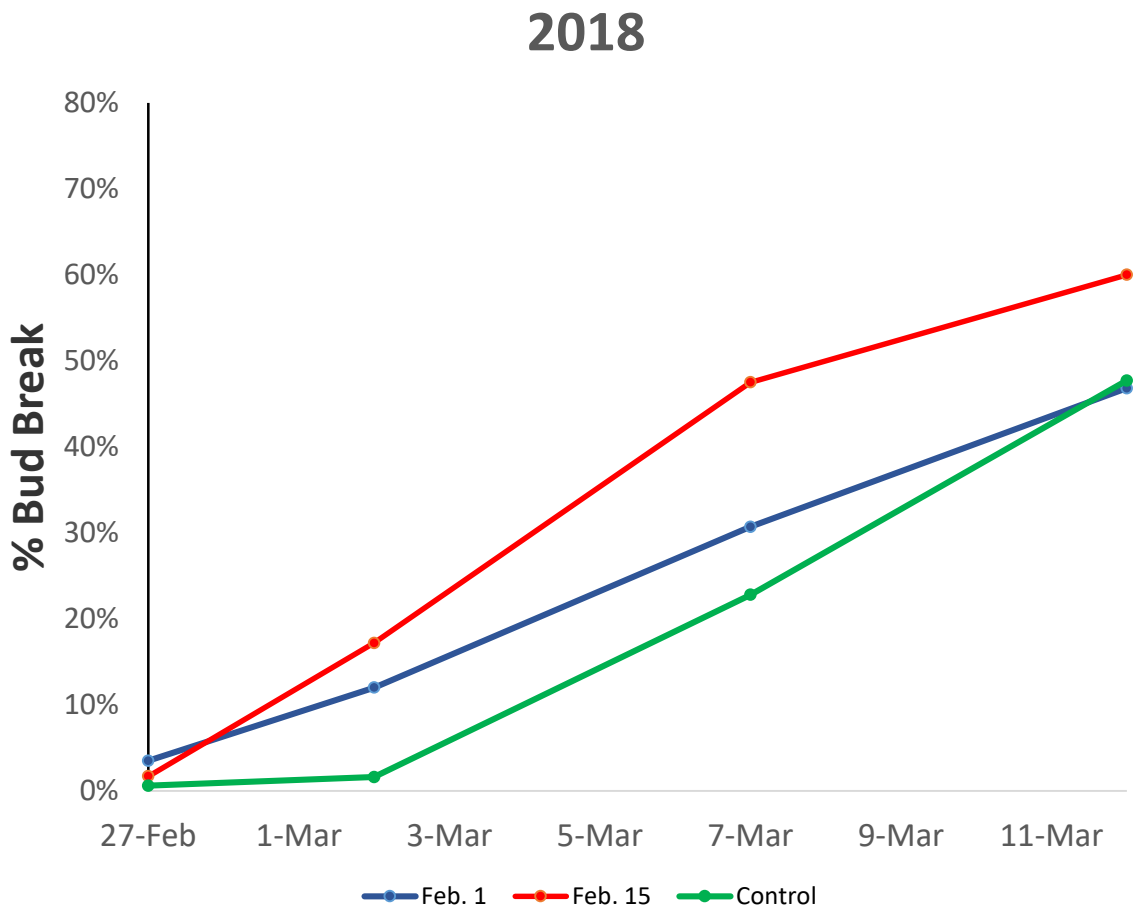
Figure 3.8: Percent bloom of 'AU Golden Sunshine' on its own roots over time during the spring of 2019 (April).



Date	4/11	4/15	4/17	4/19	4/22	4/24	4/26	4/29	Sign.
Jan. 31	8.3	36.1	58.3	80.6	88.9	94.4	97.2	100.0	NS
Feb. 14	0.0	23.9	37.2	65.3	77.9	98.6	100.0	100.0	NS
Control	0.0	12.7	34.0	70.7	88.7	100.0	100.0	100.0	NS
Sign.	NS	NS	NS	NS	NS	NS	NS	NS	

* Linear (L) or quadratic (Q) trends using model regressions at α=0.05(*), 0.01(**), or 0.001(***). Non – significant (NS) or Sign. = Significance.

Figure 3.9: Percent bud break of 'AU Gulf Coast Gold' over time during the spring of 2018 (Feb.-March).



Date	2/27	3/2	3/7	3/12	Sign.
Feb. 1	3.5	12.0	30.7	46.8	L***
Feb. 15	1.7	17.2	47.5	60.0	Q***
Control	0.6	1.6	22.8	47.7	Q**
Sign.	L**	Q***	Q***	Q***	

* Linear (L) or quadratic (Q) trends using model regressions at α=0.05(*), 0.01(**), or 0.001(***). Non – significant (NS) or Sign. = Significance.

Table 3.3 Effects of hydrogen cyanamide (2% a.i.) applications on bud break, flowering, and lateral bud development of 'AU Gulf Coast Gold' during 2018 and 2019.

Year	Treatment timing	Chilling (<7.2˚C)	Chilling (0˚C-7.2˚C	BB[z] (%)	KFl/DB[y]	KFl/Shoot[x]	LB[w] (%)	Floral Shoots[v] (%)
2018	Feb. 1	1013	575	46.8	0.88	1.7	10.0	81.9
	Feb. 15	1079	639	60.0	1.35	2.1	6.0	90.8
	Control	1198	718	47.7	0.56	1.1	3.0	69.4
Sign.[u]				Q***	Q***	Q***	L*	Q**
2019	Jan. 31	749	588	71.3	0.18	0.24	3.9	9.2
	Feb. 14	806	634	72.1	0.29	0.43	1.2	16.8
	Control	891	691	67.5	0.32	0.46	0.6	16.6
Sign.				NS	NS	NS	NS	NS

[z] %BB = Percent bud break = Percentage of total dormant buds that broke dormancy

[y] KFl/DB = Number of king flowers per dormant bud

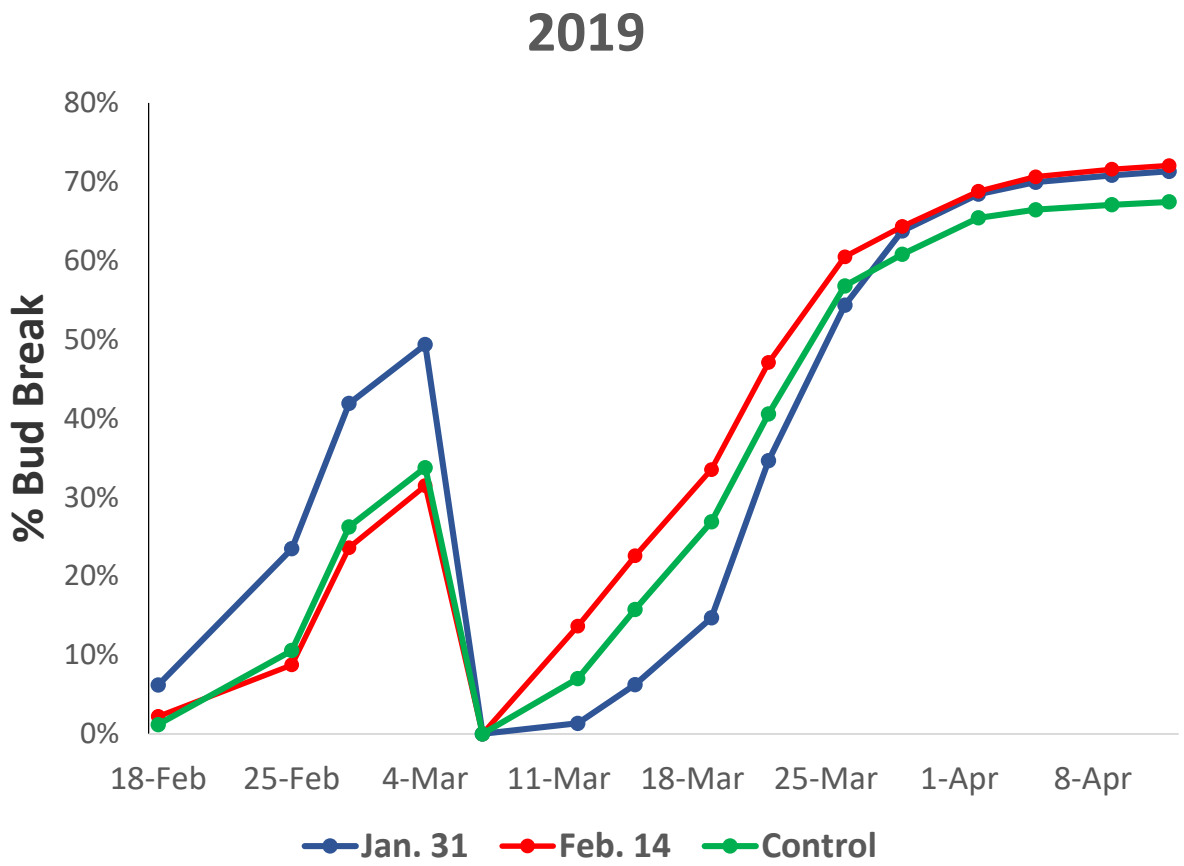
[x] KFl/Shoot = Number of king flowers per shoot

[w] LB (%) = Lateral flower bud percentage = total number of lateral flowers divided by total number of flowers present

[v] % Floral Shoot = Percent floral shoots based on number of shoots with flowers over total number of shoots

[u]Linear (L) or quadratic (Q) trends using model regressions at α=0.05(*), 0.01(**) or 0.001(***). Non – significant (NS) or Sign. = Significance.

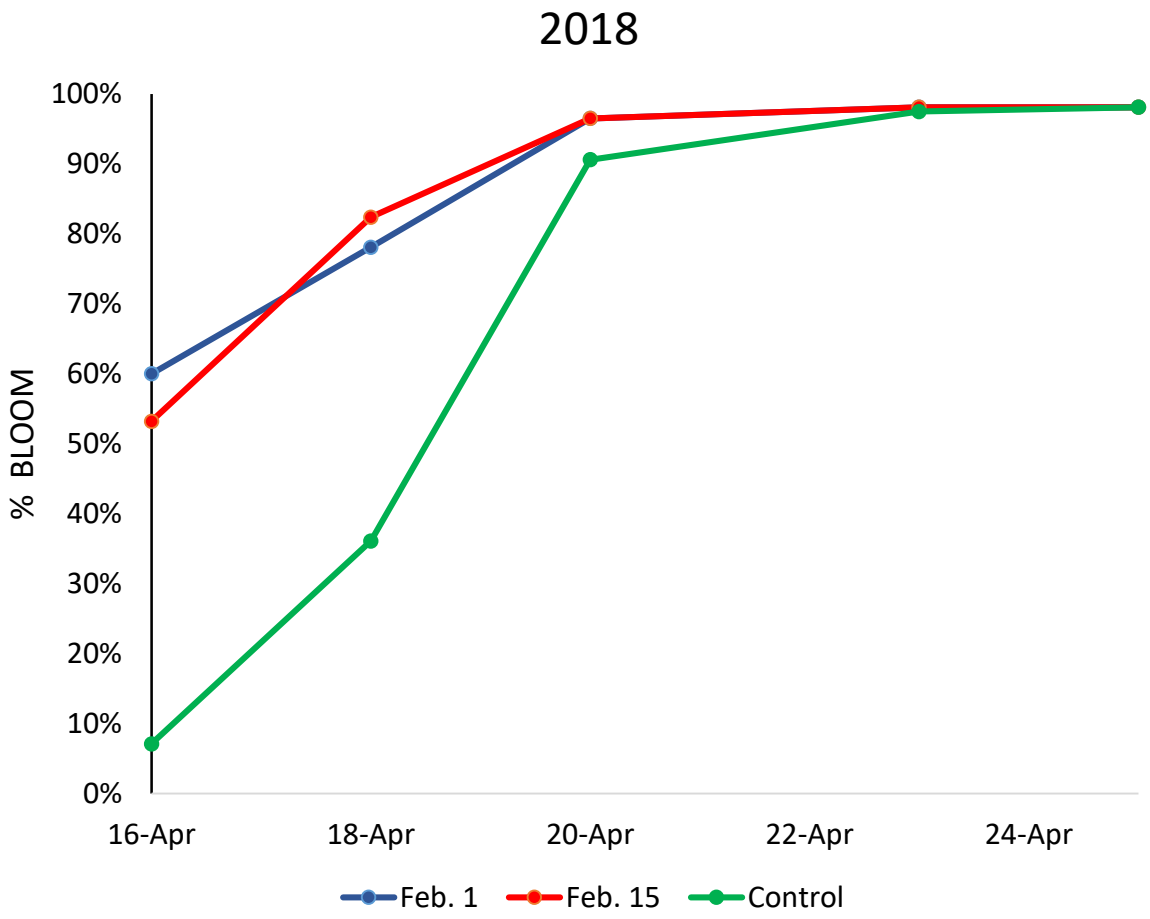
Figure 3.10: Percent bud break of 'AU Gulf Coast Gold' over time during the spring of 2019 (Feb.-April).



Date	2/18	2/25	2/28	3/4	3/7	3/12	3/15	3/19	3/22	3/26	3/29	4/2	4/5	4/9	4/12	Sign.
Jan. 31	6.2	23.5	41.9	49.4	0.0	1.3	6.2	14.7	34.7	54.4	63.8	68.4	70.0	70.8	71.3	NS
Feb. 14	2.2	8.7	23.6	31.5	0.0	13.7	22.6	33.5	47.1	60.5	64.3	68.8	70.6	71.6	72.1	NS
Control	1.2	10.6	26.2	33.8	0.0	7.0	15.8	26.9	40.6	56.8	60.8	65.4	66.5	67.1	67.5	NS
Sign.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

* Linear (L) or quadratic (Q) trends using model regressions at α=0.05(*), 0.01(**), or 0.001(***). Non – significant (NS) or Sign. = Significance.

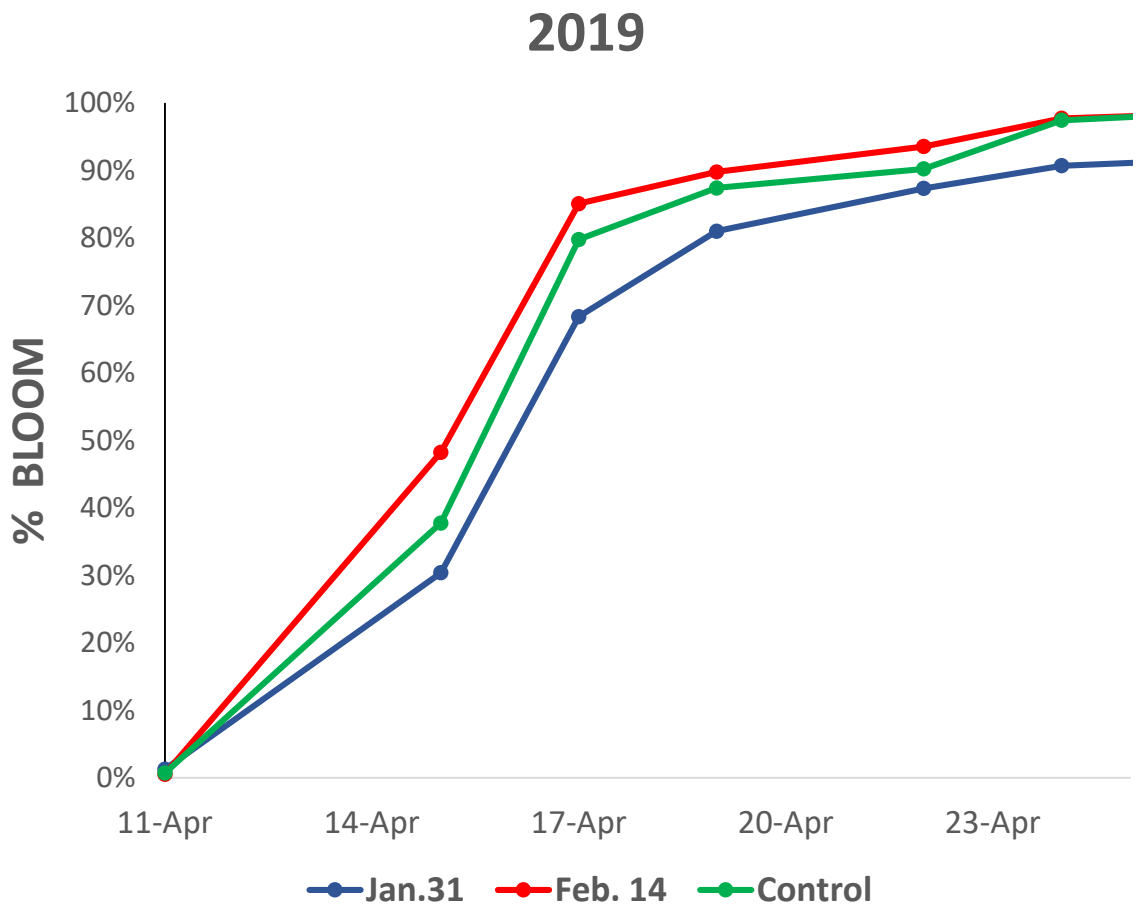
Figure 3.11: Percent bloom of 'AU Gulf Coast Gold' over time during the spring of 2018 (April).



Date	4/16	4/18	4/20	4/23	4/25	Sign.
Feb. 1	60.0	78.1	96.5	98.1	98.1	Q***
Feb. 15	53.2	82.4	96.5	98.1	98.1	Q***
Control	7.1	36.1	90.6	97.5	98.1	Q***
Sign.	Q***	Q***	L*	NS	NS	

* Linear (L) or quadratic (Q) trends using model regressions at α=0.05(*), 0.01(**), or 0.001(***). Non – significant (NS) or Sign. = Significance.

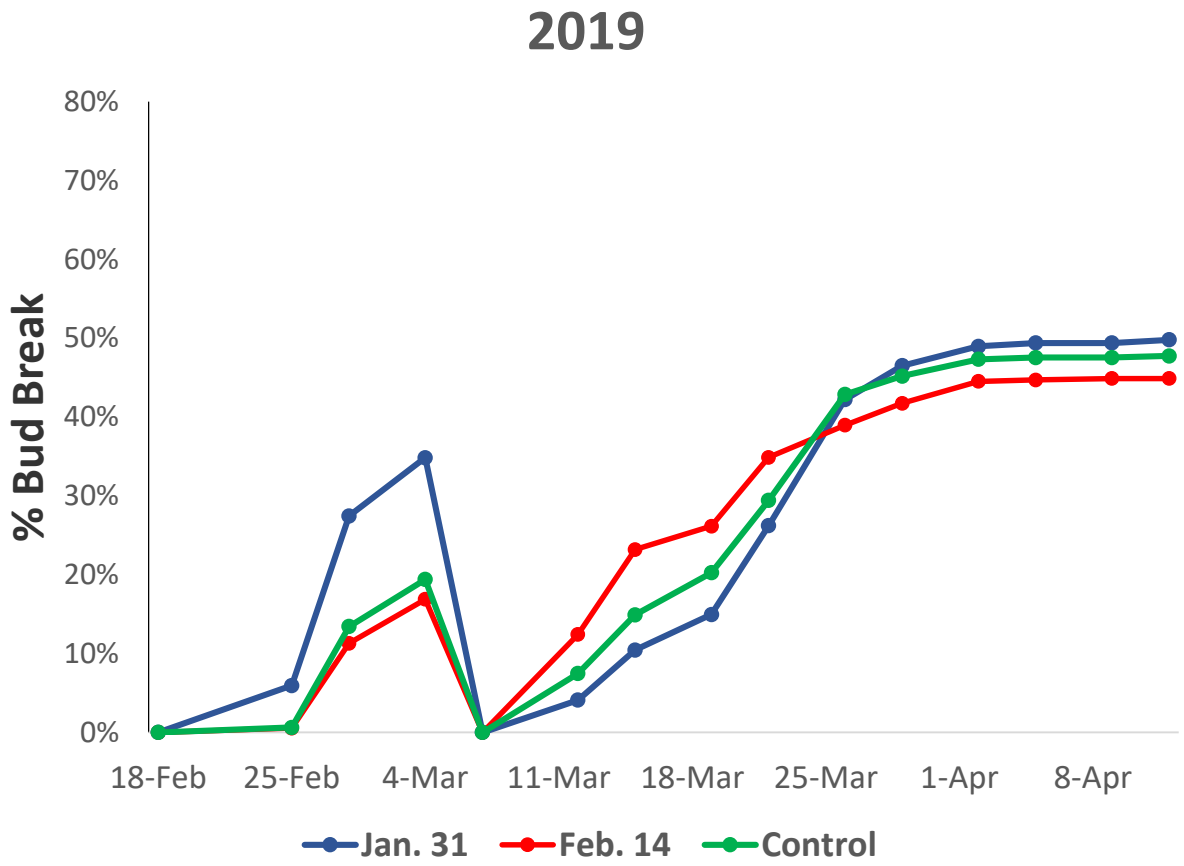
Figure 3.12: Percent bloom of 'AU Gulf Coast Gold' over time during the spring of 2019 (April).



Date	4/11	4/15	4/17	4/19	4/22	4/24	4/26	4/29	Sign.
Jan. 31	1.3	30.4	68.4	81.0	87.3	90.7	91.6	92.4	NS
Feb. 14	0.5	48.3	85.1	89.8	93.5	97.7	98.4	99.6	NS
Control	0.7	37.8	79.8	87.4	90.2	97.5	98.4	99.2	NS
Sign.	NS	NS	NS	NS	NS	NS	NS	NS	

* Linear (L) or quadratic (Q) trends using model regressions at α=0.05(*), 0.01(**), or 0.001(***). Non – significant (NS) or Sign. = Significance.

Figure 3.13: Percent bud break of 'AU Golden Tiger' over time during the spring of 2019 (Feb.-April).



Date	2/18	2/25	2/28	3/4	3/7	3/12	3/15	3/19	3/22	3/26	3/29	4/2	4/5	4/9	4/12	Sign.
Jan. 31	0.0	5.9	27.5	34.8	0.0	4.1	10.5	15.0	26.2	42.2	46.5	49.0	49.4	49.4	49.8	NS
Feb. 14	0.0	0.6	11.3	16.9	0.0	12.4	23.2	26.2	34.9	39.0	41.7	44.5	44.7	44.9	44.9	NS
Control	0.0	0.6	13.4	19.4	0.0	7.5	14.9	20.3	29.4	42.9	45.2	47.3	47.5	47.5	47.8	NS
Sign.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

* Linear (L) or quadratic (Q) trends using model regressions at α=0.05(*), 0.01(**), or 0.001(***). Non – significant (NS) or Sign. = Significance.

Table 3.4: Effects of hydrogen cyanamide (2% a.i.) applications on bud break, flowering, and lateral bud development of 'AU Golden Tiger' during 2019.

Year	Treatment timing	Chilling (<7.2˚C)	Chilling (0˚C-7.2˚C	BB[z] (%)	KFl/DB[y]	KFl/Shoot[x]	LB[w] (%)	Floral Shoots[v] (%)
2019	Jan. 31	749	588	49.8	0.64	0.26	0.0	40.7
	Feb. 14	806	634	44.9	0.51	1.14	0.0	55.8
	Control	891	691	42.8	0.60	1.26	0.0	44.2
Sign.[u]				NS	NS	NS	NS	NS

[z] %BB = Percent bud break = Percentage of total dormant buds that broke dormancy

[y] KFl/DB = Number of king flowers per dormant bud

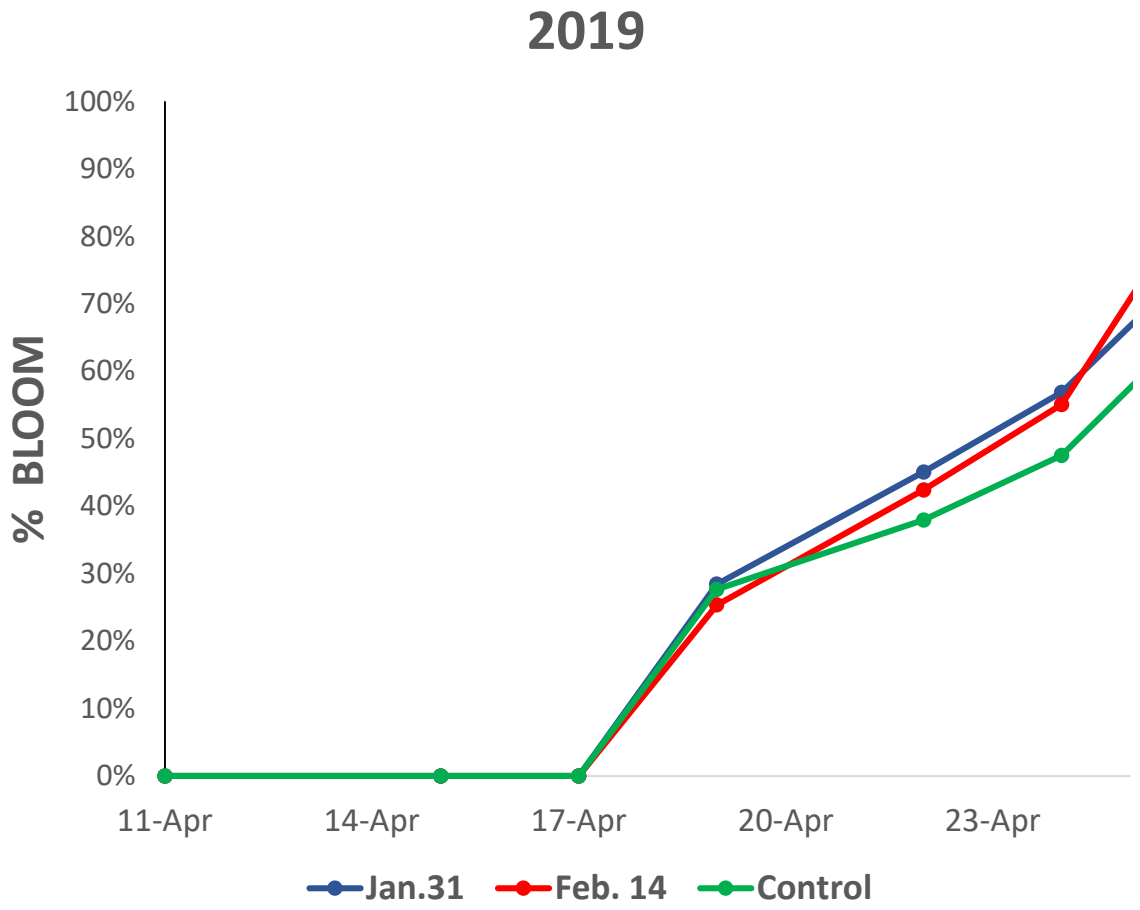
[x] KFl/Shoot = Number of king flowers per shoot

[w] LB (%) = Lateral flower bud percentage = total number of lateral flowers divided by total number of flowers present

[v] % Floral Shoot = Percent floral shoots based on number of shoots with flowers over total number of shoots

[u] Non –significant (NS) or Sign. = Significance.

Figure 3.14: Percent bloom of 'AU Golden Tiger' over time during the spring of 2019 (April).



Date	4/11	4/15	4/17	4/19	4/22	4/24	4/26	4/29	Sign.
Jan. 31	0.0	0.0	0.0	28.4	45.0	56.9	76.7	92.0	NS
Feb. 14	0.0	0.0	0.0	25.4	42.4	55.1	86.6	94.2	NS
Control	0.0	0.0	0.0	27.7	37.9	47.5	67.7	79.8	NS
Sign.	NS	NS	NS	NS	NS	NS	NS	NS	

* Linear (L) or quadratic (Q) trends using model regressions at α=0.05(*), 0.01(**), or 0.001(***). Non –significant (NS) or Sign. = Significance.

Chapter Four

Effect of NAA, AVG, and 1-MCP on Preharvest Fruit Drop, Fruit Maturity, and Quality of 'AU Golden Sunshine' Kiwifruit.

Introduction

The golden kiwifruit 'AU Golden Sunshine' *Actinidia chinensis* Planch. exhibited pre-harvest fruit drop that occured just before harvest. This can cause serious economic hardship on commercial producers that grow the commodity. Knowledge of pre-harvest fruit drop in this commodity is extremely limited as preharvest fruit drop has not been previously reported for kiwifruit. For insight into this behavior, we look to experimentation done to prevent pre-harvest fruit drop of many apple (*Malus* × *domestica* Borkh.) cultivars.

Abscission occurs naturally during the early stages of fruit development in many commodities (Osborne and Morgan, 1989). Typically, this abscission occurs due to insufficient pollination, damaged fruit, stress, or self-thinning. Later abscission that occurs just before harvest can be caused by similar factors and results in elevated ethylene production (Wright and Heatherbell, 1967). A rise in ethylene triggers abscisic acid to infiltrate the abscission zone which, in turn, accelerates abscission. The plant hormone auxin, along with other compounds, can help keep ethylene in check when at normal concentrations. When auxin is not present or is in low concentrations, ethylene production can rise and lead to fruit abscission (Osborne and Morgan, 1989). Keeping ethylene at low concentrations and auxin at normal or higher than normal concentrations can help reduce pre-harvest fruit drop in apple cultivars (Marini et al., 1993; Yuan and Carbaugh, 2007; Yuan and Li, 2008)

When faced with a pre-harvest fruit drop in apple production, researchers have looked to chemicals that can reduce ethylene biosynthesis as well as synthetic auxins to suppress ethylene production (Amarante et al., 2002; Basak and Buczek, 2010; Byers, 1997; Byers et al., 2005; Dal Cin et al., 2008; McArtney et al., 2008; Rath et al., 2006; Schupp and Greene, 2004; Varanasi et al., 2013; Yildiz et al., 2012; Yuan and Carbaugh, 2007; Yuan and Li, 2008). Chemicals like aminoethoxyvinylglycine (AVG), 1-methylcyclopropene (1-MCP), and naphthalene acetic acid (NAA) have all been shown to reduce pre-harvest abscission of fruit in many apple cultivars (Byers et al., 2005; Dal Cin et al., 2008; Yuan and Carbaugh, 2007; Yuan and Li, 2008). The chemicals, AVG and 1-MCP work by inhibiting ethylene response by binding to ethylene receptors on cell membranes (Yuan and Carbaugh, 2007). These products slow all processes associated with the production of ethylene including flesh softening, the disappearance of starches, and stem loosening (Byers, 1997). These products, in some cases, also show postharvest benefits as well by extending the amount of time the fruit can be left on the plant (Byers et al., 2005). Products containing 1-MCP and AVG were shown to extend storage life of many commodities, including kiwifruit, when applied in a pre-harvest setting (Byers et al., 2005; Ilina et al., 2010; Menniti et al., 2005; Yildiz et al., 2012). Byers (1997) reported that AVG can reduce fruit softening associated with ethylene production as well as slow pre-harvest fruit drop when applied 7 to 28 d before anticipated harvest when tested on several apple cultivars. At concentrations of 150-225 mg/L^{-1}, applied within 7 to 14 d of harvest, AVG was more effective at preventing pre-harvest fruit drop and extending postharvest life of ‘McIntosh’ and ‘Delicious’ apple cultivars (Schupp and Greene, 2004; Yuan and Li, 2008). Applications of 1-MCP are most effective at preventing pre-harvest fruit drop when applied to apples at rates between 80 to 250 mg/L^{-1} just 1 to 2 weeks before harvest (McArtney et al., 2008; Yuan and Li, 2008). High rates 1-MCP applied closer to anticipated harvest were most

effective against fruit softening in postharvest storage (Elfving et al., 2007; McArtney et al., 2008). Products containing NAA can reduce pre-harvest fruit drop in some apple cultivars (Marini et al., 1993; Stover et al., 2003; Yuan and Carbaugh 2007; Yuan and Li, 2008), but NAA does not slow down softening of fruit postharvest (Byers, 1997; Greene, 2005; Greene and Schupp, 2004; Yuan and Li, 2008). Studies have shown that NAA is most effective when applied multiple times or in combination with an ethylene biosynthesis inhibitor (Hoying and Robinson, 2010; Yildiz et al., 2012; Yuan and Carbaugh, 2007; Yuan and Li, 2008). Effective rates used to prevent pre-harvest fruit drop of apples were recommended to between 10 and 25 mg/L^{-1}, whereas rates as high as 100 mg/L^{-1} are used on citrus (Anthony and Coggins, 2001; Yuan and Li, 2008). If applications are made more that 7 to 14 d before anticipated harvest, secondary applications may need to be made as the product is only effective for up to 2 weeks (Marini et al., 1993; Valent U.S.A. LLC., 2017).

There is no published research pertaining to the efficacy of these chemicals to prevent pre-harvest fruit drop of kiwifruit. The objective of this study was to determine the effectiveness of NAA, AVG, 1-MCP, or AVG or 1-MCP combined with NAA to alleviate the pre-harvest fruit drop in 'AU Golden Sunshine'. The effect of application timing of the treatments on pre-harvest fruit drop and the impact on postharvest maturity and quality of kiwifruit was investigated.

Materials and Methods

Experimental Design

This study was conducted during the 2018 and 2019 growing seasons on 4-5-year-old fruiting 'AU Golden Sunshine' kiwifruit vines at Southeast Kiwi Farming Cooperative in Reeltown, AL. Vines were selected that showed similar characteristics and crop load. The

experiment was arranged as an augmented factorial treatment design with five plant growth regulator (PGR) treatments × three application dates, plus an unsprayed control. The experiment was arranged in a randomized complete block design with six single-plant replications. The PGR treatments were 88mg L^{-1} NAA (Fruitone™, Valent U.S.A. LLC, 2017), 264mg L^{-1} AVG (ReTain™, Valent Biosciences LLC, 2018), 158mg L^{-1} 1-MCP (Harvista™, AgroFresh Inc, 2018), 264mg L^{-1} AVG + 88mg L^{-1} NAA and 158mg L^{-1} 1-MCP + 88mg L^{-1} NAA. These rates were chosen to determine effects of these chemicals at their highest labeled rate. For year 1 (2018) of this study, two harvest dates were selected to determine if time of harvest played a role in the pre-harvest fruit drop and maturity of the fruit. Two vines per treatment/replication were selected, one for each harvest. The first harvest was on 31 Aug. 2018 and the second harvest was on 14 Sept. 2018. Vines associated with the first harvest were sprayed with the PGR treatments at 27, 20, or 15 days before harvest (DBH). Vines associated with the second harvest received applications on the same dates as the first harvest, which equated to 41, 34, or 29 DBH. For year 2 (2019) of this study, there was only one harvest date. This harvest was on 15 Sept. 2019 and PGR treatments were applied 20, 13, or 6 DBH. The PGR treatments were applied to the fruit as directed by the label, until runoff was achieved with a backpack sprayer (Solo 475-B Backpack Sprayer, Solo Inc. Newport News, VA, USA). The PGR 1-MCP is volatile when mixed with water and a great care was taken when mixing and applying the chemical. Two vines received 1-MCP applications per tank in less than 2 minutes to decrease the risk of the product losing effectiveness.

Data Collection

Fruit counts were made at study initiation and at harvest to calculate the cumulative fruit drop per vine. Fruit were also collected as they dropped to better understand when drop occurred.

At each harvest, 20 fruit were sampled from each vine for subsequent maturity and quality analysis. After harvest, fruit were "cured" for 24 h at 22 °C, then placed in cold storage at 1 °C. Fruit quality was determined on five fruit/vine at 4, 32, 61, or 89 days after harvest in year 1 (2018). During year 2 (2019) of the study, a cooler malfunction occurred after the first quality analysis (5 DAH). Temperatures exceeded 8 ˚C for several days causing an acceleration of ripening. Due to this malfunction, the experiment was terminated after the second quality analysis (33 DAH).

Each fruit was measured for soluble solids content (SSC), firmness, internal hue angle (h˚), and percent dry matter (DM). Fruit SSC were assessed twice per fruit using a Leica Mark II Plus refractometer (model 13104940; Reichert Analytical Instruments, Depew, NY, USA) with a sample taken from the proximal and distal end of each fruit and then averaged for a final SSC. Firmness was acquired using a penetrometer (McCormick Fruit Pressure Tester FT327, McCormick Fruit Tech Yakima, WA, USA) measuring each fruit twice in kilograms of force to insert an 8 mm slightly rounded probe 1 cm into the fruit's mesocarp. Skin of the fruit was removed (1 mm) in two locations, 90° adjacent to one another, along the equator of the fruit, where measurements occurred. The two measurements were then averaged for the final fruit firmness. Internal hue angle was measured using a spectrophotometer (model CM-600d; Konica Minolta Instruments, Tokyo, Japan) after 2 mm of the exocarp were removed along the equator at opposite sides of the fruit. Two readings were obtained and then averaged for a final h° per fruit. Percent dry matter was obtained by weighing two, 3 mm, transverse slices from the equator of each fruit, before and after drying. The average of the two slices were recorded to determine percent dry matter.

Statistical Analysis

Data were analyzed using a generalized linear mixed models (negative binomial distribution and log link function) using the GLIMMIX procedure of SAS (version 9.4; SAS Institute, Cary, NC, USA). The experimental design was a randomized complete block design, with block included in the models as a random effect. The augmented factorial treatment design (complete factorial plus a control) was handled by including a dichotomous variable based on the method of Piepho et al. (2006). When there was not a significant interaction between factors ($p > 0.05$), main effects were examined. When there was a significant interaction between factors, simple effects were examined. The p-values for multiple pairwise comparisons of least-squares means (proportions of dropped fruit) were adjusted using the Holm-Simulated method.

There was an irrigation failure on three of the six blocks in Aug. 2019. The vines affected by the irrigation malfunction were visibly stressed and displayed leaf loss. Despite this, there was no significant difference in pre-harvest fruit drop between irrigated and non-irrigated vines (during this period). Fruit quality analyses were similar when including all blocks or the three blocks not affected. Hence, all of the vines were included in the final analysis.

Results

Fruit Drop

There was no effect of date of application (DBH), PGR, or the interaction of PGR by DBH on incidence of pre-harvest fruit drop for either year of this study (Table 4.1 and 4.2). There was an effect of harvest date on fruit drop, with ~14% greater fruit drop observed by the 14 Sept. 2018 harvest compared to the 31 Aug. 2018 harvest (Table 4.1). All vines associated with the earlier harvest date had <10% pre-harvest fruit drop whereas vines harvested 14 Sept. had 10-29% fruit drop. The observed pre-harvest fruit drop was much greater in 2019 as the mean percent fruit drop was ~50% (Table 4.2). The total number of fruit (harvested fruit + fruit drop) was 13,188 in 2019

compared to 18,975 in 2018. Though there were less fruit produced in 2019, there were 2× (5865) more fruit that dropped than in 2018 by the second harvest (2881). Hence, the greater percent fruit drop was not just related to the lower fruit production. There was a spring frost that contributed to the lower fruit production in 2019. Late summer of 2019 was extremely dry compared to 2018 and this may have negatively impacted the vines allowing for an increased instance of pre-harvest fruit drop.

Maturity and Quality

There were no effects of date of application (DBH), PGR, or the interaction of PGR by DBH for most fruit quality attributes in year 1 (2018) (Table 4.3). There was a main effect of PGR on SSC and firmness of fruit 32 DAH, and firmness of fruit 89 DAH. The SSC of fruit 32 DAH was lower in response to 264mg L^{-1} AVG + 88mg L^{-1} NAA compared to 264mg/L^{-1} AVG and 158mg/L^{-1} 1-MCP, though all of the PGR treated fruit were similar to untreated fruit. Fruit treated with 158mg/L^{-1} 1-MCP were softer at 32 DAH than fruit that received applications of 88mg L^{-1} NAA and 264mg L^{-1} AVG combined with 88mg L^{-1} NAA, but were not different than untreated fruit. At 89 DAH, fruit that received applications of 158mg/L^{-1} 1-MCP were softer than fruit treated with 88mg L^{-1} NAA alone. All fruit were similar to untreated fruit 89 DAH. The interaction of DBH and PGR affected hue angle 4 DAH and firmness 61 DAH (Table 4.4). At 4 DAH, fruit treated with 158mg/L^{-1} 1-MCP + 88mg L^{-1} NAA applied 20 DBH had a lower hue angle, or a more yellow flesh, than applications made at 27 DBH. This is the only instance in which this occurred and was likely due more to chance than the impacts of the treatments. At 61 DAH, fruit receiving applications of 88mg L^{-1} NAA 27 DBH were firmer than fruit treated with 88mg L^{-1} NAA 15 and 20 DBH (Table 4.4).

In year 2 (2019), there were no effects of DBH, PGR, or the interaction of DBH by PGR on hue angle or dry matter content (DMC) (Table 4.5). There were main effects of DBH and PGR on firmness 5 DAH. Fruit treated with 158mg/L^{-1} 1-MCP and 264mg L^{-1} AVG were firmer than untreated fruit 5 DAH. Interestingly, fruit receiving applications 13 and 20 DBH were firmer than fruit receiving applications at 6 DBH or no application, regardless of PGR. Fruit receiving applications of 158mg/L^{-1} 1-MCP 20 DBH had lower SSC than when it was applied 6 or 13 DBH (Table 4.6). There were no differences in fruit quality 33 DAH, but this could be attributed to the cooler malfunction (Table 4.5). Only hue angle and SSC were measured after the cooler malfunction, and these measurements were primarily done to determine the final SSC and hue of this fruit when fully ripened.

The harvest date affected all fruit quality parameters (Table 4.3). As expected, fruit were more mature in response to the later harvest date as observed by higher SSC, lower firmness, and lower flesh hue angle. These differences were maintained throughout the postharvest analysis. Fruit harvested 31 Aug. were greener than fruit harvested 14 Sept. and most fruit harvested at the earlier date never developed the appropriate internal hue angle that is acceptable for golden kiwifruit commercially. Fruit harvested on 14 Sept. had greater DMC. The SSC and the DMC were greater for the 2019 harvest, likely due to the later harvest in relation to fruit maturity and perhaps the reduced crop load for the 2019 season. The greater DMC observed for fruit harvested in 2019 contributed to higher SSC when fully ripened (~16.5 % SSC) compared to final SSC observed in 2018 (~14.6% SSC).

Discussion

The PGRs that are frequently used to alleviate pre-harvest fruit drop of apples that were used in this study did not affect the pre-harvest fruit drop of 'AU Golden Sunshine' kiwifruit. Pre-harvest fruit drop is not common on kiwifruit, and this is the first study to report this issue. 'AU Golden Sunshine' is the only cultivar that we have observed to have a pre-harvest fruit drop, though the % fruit drop had not previously been recorded. The pre-harvest fruit drop has been previously observed to occur ~7–14 d prior to anticipated harvest. However, the harvest date has been difficult to determine for this cultivar due to the variation in fruit maturity between fruit destined to drop and the fruit that will remain (Spiers, personal communication).

As observed in year 1 of this study (Table 4.1), harvesting fruit earlier can reduce the incidence of fruit drop. The earlier harvested fruit will likely be firmer and less mature overall. This strategy is confounded by the variation in fruit maturity contributed by the fruit that were destined to drop, but this appears to be the best strategy presently. In addition, fruit that has not developed a suitable internal flesh color (hue angle) prior to harvest may not develop the characteristic yellow flesh color postharvest. This was observed in this study where many of the fruit harvested earlier in 2018 remained somewhat green and did not reach the desired flesh color postharvest. The suggested hue angle for harvest of *A. chinensis* is 103 °h (Burdon et al., 2014). In addition to greater color development, an advantage of harvesting later includes greater DMC of fruit. Greater DMC has been correlated with increased consumer acceptance, and enhanced postharvest storage as long as the fruit is still firm at harvest (Burdon et al., 2004; Crisosto and Kader, 1999).

When applied to apples, AVG, 1-MCP, and NAA, alone or in combinations have been observed to alleviate pre-harvest fruit drop (Amarante et al., 2002; Basak and Buczek, 2010; Byers, 1997; Byers et al., 2005; Dal Cin et al., 2008; McArtney et al., 2008; Rath et al., 2006; Schupp and

Greene, 2004; Yildiz et al., 2012; Varanasi et al., 2013; Yuan and Carbaugh, 2007; Yuan and Li, 2008). In apples, AVG and 1-MCP showed ethylene inhibition that extended long into postharvest storage, decreasing the instance of fruit softening for a period (Elfying et al., 2007; McArtney et al., 2008; Yuan and Li, 2008). There appeared to be no substantive results of the treatments on fruit quality parameters in this study. Fruit quality was determined from 5 fruit/vine on each analysis. Perhaps, if a larger sample size were used, the effects of the PGRs to enhance postharvest longevity would have been observed. There is the possibility that the inherent variation in maturity caused by the fruit drop phenomenon in 'AU Golden Sunshine' may have made results unclear and contributed to the unexplained significant effects from the statistical analysis (Xie, 2017). In a postharvest study conducted by Manriquez et al. (1999) on 'Hayward' kiwifruit, AVG applied in concentrations of 100 and 500 mg/L, 4 weeks before harvest, reduced endogenous ethylene compared to unsprayed controls vines. Fruit receiving 20, 100, or 500 mg/L^{-1} of AVG 4–6 weeks before harvest stayed firmer longer than untreated fruit in cold storage. The sample size for each treatment was a single vine per replication while one box of fruit was used from that vine for firmness analysis (Manriquez et al., 1999). Menniti et al. (2005) conducted a similar postharvest study on 'Hayward' kiwifruit using 1-MCP. In their study, treatments with 1-MCP concentrations of 100-250 nl/L^{-1} were applied to 50 fruit per treatment at harvest. Fruit receiving applications of 100 or 250 nl/L^{-1} 1-MCP remained firmer (1.4 kgf) than untreated fruit (0.6 kgf) after 100 d in cold storage (Menniti et al., 2005).

Harvest may have been delayed for the 2019 growing season, however evidence of effects should have been observed. Fruit harvested during 2019 initially had greater SSC and DM than fruit harvested during 2018. This could be due to the lower number of fruit left on the vines during 2019 as well as the advanced maturation that had occurred by harvest.

In further research, an increase in the amount of active ingredient or the addition of a surfactant may result in greater PGR effect on 'AU Golden Sunshine'. When used on 'Red Chief' apples, rates of 600 mg/L^{-1} of AVG showed the least amount of cumulative drop percentage in a study by Yildiz et al. (2012). A study by Anthony and Coggins (2001) used rates of NAA between 25 mg/L^{-1} and 400 mg/L^{-1}. The rates of 100 and 400 mg/L^{-1} (NAA) showed the greatest reduction of mature fruit drop of navel oranges in that study. When the surfactants Latron AG-98 and Silwet L-77 were used, even greater efficacy was realized at high rates (100–400 mg/L^{-1}) of NAA (Anthony and Coggins, 2001). Our rates of NAA (88 mg/L^{-1}) and AVG (264 mg/L^{-1}) were much less than the rates of the studies mentioned above. Applications made for the 2018 season could have had better timing to get closer to the targeted DBH. Yuan and Li (2008) showed that applications of AVG at 3 weeks followed by applications of NAA at 1 week before anticipated harvest showed the greatest reduction of pre-harvest fruit drop of 'Delicious' apples. Split applications like theirs may have positively impacted fruit drop and further research will be needed to determine the effectiveness of these types of applications on 'AU Golden Sunshine' moving forward.

Conclusions

Pre-harvest fruit drop of the golden kiwifruit cultivar, 'AU Golden Sunshine' was not controlled during this study. Growers that are cultivating this cultivar could have significant economic losses due to the excessive pre-harvest fruit drop that occurs most years. Extension of postharvest quality was not be realized with applications of the PGR treatments in this study. Perhaps ethylene is not involved and there is a different mechanism causing the pre-harvest fruit drop of 'AU Golden Sunshine'. Harvest date could play a role in the amount of drop observed. Harvesting the fruit early allows for more fruit to make it to storage. However, fruit harvested early

will have decreased maturity and quality compared to fruit harvested later. Though other studies have shown these PGRs to be effective, the effectiveness of the treatments tested on fruit quality and postharvest longevity of ‘AU Golden Sunshine’ were not evident in this study and no recommendations can be made at this time. More research is required to better understand the underlying causes of the pre-harvest fruit drop of ‘AU Golden Sunshine’ and the appropriate methods to manage it.

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Table 4.1: Percent pre-harvest fruit drop: Least means squares of main effect comparisons of 'AU Golden Sunshine' during 2018.

Year	Treatment	Fruit drop (%)
Treatment least squares means grouped by days before harvest		
2018	Control	11.6 a[z]
	15[y]	8.4 a
	20	11.0 a
	27	8.8 a
Significance: p =0.1285		
Treatment least squares means grouped by PGR		
2018	Control	11.6 a
	NAA[x]	10.2 a
	1-MCP[w]	10.7 a
	1-MCP + NAA	8.6 a
	AVG[v]	10.3 a
	AVG + NAA	7.5 a
Significance: p =0.2443		
Treatment least squares means grouped by harvest		
2018	31 August	4.9 b
	14 September	19.0 a
Significance of harvest: p <0.0001		

[z]Least squares means of main effects for control vs treatment*DBH, control vs treatment*PGR, and harvest, adjusted for multiple comparisons: Holm-Simulated ($p < 0.05$). Means followed by the same letter are not significantly different from one another.
[y] Application of PGR timing in days before harvest.
[x] NAA under the tradename Fruitone-L ™.
[w] 1-MCP under the tradename Harvista™.
[v] AVG under the tradename ReTain™.
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Table 4.2: Percent pre-harvest fruit drop: Least means squares of main effect comparisons of 'AU Golden Sunshine' during 2019.

Year	Treatment	Fruit drop (%)
Treatment least squares means grouped by days before harvest		
2019	control	56.8 a[z]
	6[y]	47.5 a
	13	45.5 a
	20	43.7 a
Significance of control vs treatment*dbh: p =0.3999		
Treatment least squares means grouped by PGR		
2019	control	56.8 a
	NAA[x]	56.6 a
	1-MCP[w]	42.6 a
	1-MCP + NAA	53.2 a
	AVG[v]	46.3 a
	AVG + NAA	45.6 a
Significance of control vs treatment*chemical: p =0.3056		

[z]Least squares means of main effects for control vs treatment*DBH, control vs treatment*PGR, and harvest, adjusted for multiple comparisons: Holm-Simulated ($p < 0.05$). Means followed by the same letter are not significantly different from one another.
[y] Application of PGR timing in days before harvest.
[x] NAA under the tradename Fruitone-L ™.
[w] 1-MCP under the tradename Harvista™.
[v] AVG under the tradename ReTain™.

Table 4.3: Fruit maturity: Main effect comparisons of treatment by harvest of 'AU Golden Sunshine' maturity and quality during 2018.

		4 DAH[z]				32 DAH				61 DAH				89 DAH			
		SSC[y] (%)	Firm[x] (kgf)	Hue angle[w] (˚)	DMC[v] (%)	SSC (%)	Firm (kgf)	Hue angle (˚)	DMC (%)	SSC (%)	Firm (kgf)	Hue angle (˚)	DMC (%)	SSC (%)	Firm (kgf)	Hue angle (˚)	DMC (%)
Significance of treatment factors																	
Control by treatment		0.7177	0.9481	0.3143	0.8465	0.5055	0.1253	0.5672	0.5044	0.8103	0.0538	0.9943	0.8914	0.6725	0.5981	0.7441	0.2780
DBH		0.8823	0.9519	0.9263	0.9146	0.8529	0.0853	0.9501	0.9207	0.9119	0.0571	0.3891	0.4841	0.1391	0.0509	0.9233	0.8624
PGR		0.0899	0.1384	0.7622	0.9050	<.0001	0.0073	0.3793	0.2470	0.0944	0.0564	0.6788	0.5464	0.1067	0.0199	0.1813	0.1292
DBH by PGR*				0.0372							0.0059						
harvest		<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	0.0035	<.0001	<.0001	<.0001	0.0040	<.0001	0.0147	<.0001	<.0001
Treatment least squares means for main effects																	
PGR[u]	DBH[t]																
Control		8.7 a	4.0 a		17.0 a	12.5 abc	1.1 ab	101.13 a	17.0 a	14.3 a		101.13 a	16.3 a	14.5 a	0.3 ab	101.06 a	16.6 a
NAA[s]		8.3 a	4.1 a		17.2 a	12.1 bc	1.4 a	101.54 a	17.0 a	14.2 a		101.27 a	16.3 a	14.6 a	0.4 a	100.48 a	16.5 a
1-MCP[r]		8.9 a	4.0 a		17.1 a	12.6 ab	1.2 b	101.68 a	17.0 a	14.4 a		101.05 a	16.3 a	14.6 a	0.3 b	101.24 a	16.3 a
1-MCP + NAA		8.5 a	3.8 a		17.2 a	12.1 bc	1.3 ab	101.02 a	16.7 a	14.1 a		100.86 a	16.4 a	14.6 a	0.3 ab	100.73 a	16.2 a
AVG[q]		8.8 a	4.1 a		17.2 a	12.7 a	1.3 ab	101.52 a	16.8 a	14.3 a		101.13 a	16.3 a	14.7 a	0.3 ab	100.93 a	16.2 a
AVG + NAA		8.4 a	4.0 a		16.9 a	11.9 c	1.5 a	101.56 a	16.7 a	14.0 a		101.31 a	16.1 a	14.3 a	0.3 ab	101.06 a	16.0 a
	control	8.7 a	4.0 a		17.0 a	12.5 a	1.1 a	101.13 a	17.0 a	14.3 a		101.13 a	16.3 a	14.5 a	0.3 a	101.06 a	16.6 a
	15	8.6 a	3.9 a		17.1 a	12.3 a	1.3 a	101.44 a	16.8 a	14.2 a		101.13 a	16.2 a	14.7 a	0.3 a	100.92 a	16.2 a
	20	8.6 a	4.0 a		17.1 a	12.2 a	1.3 a	101.51 a	16.8 a	14.2 a		101.29 a	16.3 a	14.4 a	0.3 a	100.91 a	16.3 a
	27	8.8 a	4.0 a		17.2 a	12.2 a	1.4 a	101.44 a	16.8 a	14.2 a		100.94 a	16.4 a	14.6 a	0.4 a	100.83 a	16.3 a
Harvest																	
31 Aug.		7.6 b	4.3 a	106.99 a		10.9 b	1.5 a	103.52 a	16.7 b	13.6 b	0.8 a	102.68 a	16.2 b	14.1 b	0.4 a	102.17 a	16.1 b
14 Sept.		9.6 a	3.7 b	101.17 b	17.0	13.6 a	1.1 b	99.40 a	16.9 a	14.8 a	0.4 b	99.56 b	16.5 a	15.1 a	0.3 b	99.62 b	16.5 a

*When the interaction DBH*PGR is not significant ($p > 0.05$) main effect means for each treatment factor followed by the same letter are not significantly different using Holm-simulated method for multiple comparisons. When the interaction DBH*PGR is significant simple effects means are presented in Table 4.4. Experimental error occurred for DMC of fruit harvested 31 Aug. at 4 DAH so those data are not reported.

[z] DAH = Days after harvest.

[y] SSC = % Soluble solids content.
[x] Firm = fruit firmness measured in kgf.
[w] HUE angle = Internal hue angle of fruit flesh in degrees.
[v] DMC = % dry matter content.
[u] PGR = Plant growth regulator (chemical applied).
[t] DBH = Days of application before harvest.
[s] NAA under the tradename Fruitone-L™.
[r] 1-MCP under the tradename Harvista™.
[q] AVG under the tradename ReTain™.
* Least squares means by treatment and harvest, adjusted for multiple comparisons: Holm-Simulated ($p < 0.05$).

Table 4.4: Fruit maturity: simple effect comparisons of DBH*PGR after harvest of 'AU Golden Sunshine' internal hue angle and firmness during 2018.

		4 DAH[z]	61 DAH
		Hue angle[y] (˚)	Firm[x] (kgf)
Treatment least squares means grouped by dbh and chemical			
PGR[w]	DBH[v]		
Control		103.29 a	0.4 a
NAA[u]	15	103.89 a	0.6 b
	20	104.04 a	0.6 b
	27	104.98 a	1.0 a
1-MCP[t]	15	104.17 a	0.7 a
	20	104.24 a	0.6 a
	27	102.75 a	0.6 a
1-MCP + NAA	15	104.45 ab	0.7 a
	20	102.86 b	0.5 a
	27	105.30 a	0.7 a
AVG[s]	15	103.76 a	0.6 a
	20	104.98 a	0.7 a
	27	103.64 a	0.7 a
AVG + NAA	15	104.51 a	0.6 a
	20	104.12 a	0.7 a
	27	104.31 a	0.7 a

*When interactions between DBH*PGR were significant ($p < 0.05$) at the main effects level, simple effects means are presented. Simple effects means followed by the same letter are not significantly different using the Holm-simulated method. DBH*PGR significant at p = 0. 0372. Comparisons were made per PGR by DBH.

[z] DAH = Days after harvest.

[y] HUE angle = Internal hue angle of fruit flesh in degrees.

[x] Firm = fruit firmness measured in kgf.

[w] PGR = Plant growth regulator (chemical applied).

[v] DBH = Days of application before harvest.

[u] NAA under the tradename Fruitone-L™.

[t] 1-MCP under the tradename Harvista™.

[s] AVG under the tradename ReTain™.

* Least squares means by treatment and harvest, adjusted for multiple comparisons: Holm-Simulated ($p < 0.05$).

Table 4.5: Fruit maturity: Main effect comparisons of 'AU Golden Sunshine' maturity and quality during 2019 harvest maturity.

		5 DAH[z]				33 DAH			
		SSC[y]	Firmness[x] (kgf)	Hue angle[w] (˚)	DMC[v] (%)	SSC	Firmness (kgf)	Hue angle (˚)	DMC (%)
Significance of treatment factors									
Control by treatment		0.1644	0.0018	0.4550	0.5579	0.5375		0.3459	
DBH		0.1883	0.0009	0.3510	0.8958	0.7307		0.3318	
PGR		0.8666	0.0011	0.6556	0.3709	0.2429		0.1472	
DBH by PGR*		0.0122							
Treatment least squares main effects									
PGR[u]	DBH[t]								
Control			2.8 c	98.05 a	18.4 a	16.6 a		96.08 a	
NAA[s]			3.3 bc	98.21 a	18.3 a	16.5 a		96.76 a	
1-MCP[r]			3.4 ab	98.65 a	18.2 a	16.3 a		96.39 a	
1-MCP + NAA			3.3 bc	98.25 a	18.2 a	16.3 a		96.09 a	
AVG[q]			3.8 a	98.48 a	18.5 a	16.6 a		96.74 a	
AVG + NAA			3.2 bc	98.25 a	18.0 a	15.9 a		96.48 a	
	Control		2.8 b	98.05 a	18.4 a	16.6 a		96.08 a	
	6		3.1 b	98.26 a	18.2 a	16.2 a		96.33 a	
	13		3.4 a	98.25 a	18.3 a	16.2 a		96.49 a	
	20		3.6 a	98.60 a	18.3 a	16.5 a		96.66 a	

*When the interaction DBH*PGR is not significant ($p > 0.05$) main effect means for each treatment factor followed by the same letter are not significantly different using Holm-simulated method for multiple comparisons. When the interaction DBH*PGR is significant simple effects means are presented in Table 4.6.

[z] DAH = Days after harvest.

[y] SSC = % Soluble solids content.
[x] Firm = fruit firmness measured in kgf.
[w] HUE angle = Internal hue angle of fruit flesh in degrees.
[v] DMC = % dry matter content. [Z] DBH = Days of application before harvest.
[u] PGR = Plant growth regulator (chemical applied).
[t] DBH = Days of application before harvest.
[s] NAA under the tradename Fruitone-L™.
[r] 1-MCP under the tradename Harvista™.
[q] AVG under the tradename ReTain™.
* Least squares means by treatment and harvest, adjusted for multiple comparisons: Holm-Simulated ($p < 0.05$).

Table 4.6: Simple effects comparison of least squares means for soluble solids content at 5 days after harvest 2019.

		5 DAH[z]	
		SSC[y]	
Treatment least squares means grouped by dbh and chemical			
PGR[x]	DBH[w]		
Control			
NAA[v]	6	13.2	a
	13	12.1	a
	20	12.6	a
1-MCP[u]	6	13.1	a
	13	13.5	a
	20	10.8	b
1-MCP + NAA	6	12.9	a
	13	12.4	a
	20	12.2	a
AVG[t]	6	12.9	a
	13	11.5	a
	20	12.6	a
AVG + NAA	6	13.1	a
	13	12.5	a
	20	12.7	a

*When interactions between DBH*PGR were significant ($p < 0.05$) at the main effects level, simple effects means are presented. Simple effects means followed by the same letter are not significantly different using the Holm-simulated method. DBH*PGR significant at $p = 0.0122$.

[z] DAH = Days after harvest.

[y] SSC = % Soluble solids content. [X] Firm = fruit firmness measured in kgf.

[x] PGR = Plant growth regulator (chemical applied).

[w] DBH = Days of application before harvest.

[v] NAA under the tradename Fruitone-L™. abcdefghijklmnopqrstuvwxyz

[u] 1-MCP under the tradename Harvista™.

[t] AVG under the tradename ReTain™.

* Least squares means by treatment and harvest, adjusted for multiple comparisons: Holm-Simulated ($p < 0.05$).