Manipulating Crop Load Using Plant Growth Regulators

By

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DECLARATION

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SUMMARY

Flower or fruit thinning plays an important role in deciduous fruit production in ensuring optimal yield, fruit size and quality. The stone- and pome fruit industries still rely heavily on hand thinning. Due to the increase in labor costs and the time constraints of hand thinning, alternative methods of thinning are required. Chemical thinning is the most promising tool that growers have to reduce the hand thinning requirement.

1-Aminocyclopropane-1-carboxylic acid (ACC) was evaluated on stone fruit and showed promising results on the Japanese plums 'Laetitia' and 'Fortune'. ACC at 400 $\mu l \cdot L^{-1}$ applied at 8 – 10 mm fruitlet diameter would be the recommended rate and application timing for both cultivars. ACC was not an effective thinning agent on 'August Red' nectarines, but ACC at 400 $\mu l \cdot L^{-1}$ consistently gave promising results and would be the recommended rate on 'Keisie' cling peaches.

The efficacy of ACC as chemical thinner on apples was cultivar dependent. In the apple trials, an industry standard was included in order to compare the efficacy of ACC against chemical thinning agents that are currently used in industry. The industry standard varied from grower to grower. Not ACC, nor the industry standard 6-benzyladenine (6-BA) and 6-BA tank-mixed with 1-naphthaleneacetic acid (NAA) sufficiently thinned 'Fuji' in either of the two seasons. The lack of thinner efficacy was accredited to environmental and intrinsic plant factors on the "difficult-to-thin" 'Fuji'. ACC gave promising results on mature 'Cripps' Red' trees when applied at 15 - 20 mm fruitlet diameter in the 2018/2019 season. The recommended rate of ACC on 'Cripps' Red' would be between 250 and 500 $\mu l \cdot L^{-1}$. ACC over thinned smaller, immature 'Cripps' Red' trees in the 2017/2018 season. ACC was evaluated in 2018/2019 on 'Royal Gala' where 250 $\mu l \cdot L^{-1}$ ACC applied at 8 - 10 mm fruitlet diameter showed promising results and performed better than the grower application of NAA.

In the chemical fruit thinning trials on 'Forelle', S-abscisic acid (S-ABA) was a successful thinner at one of the trial sites in 2017/2018 season. The Glen Fruin trial site experienced poor fruit set and therefore chemical thinning would not have been advisable in the 2017/2018 season. S-ABA subsequently over thinned. At the site where adequate fruit set occurred, S-ABA proved to be a promising thinner at a rate of 300 to 400 $mg \cdot L^{-1}$. In the fruit set trials on young 'Packham's Triumph' trees, gibberellins and cytokinin (GA_{4+7} plus 6-BA) and aminoethoxyvinylglycine (AVG) tank-mixed with prohexadione-calcium (ProCa) was not

effective in increasing fruit set, while AVG on its own significantly increased yield. In the 2018/2019 season, NAA was applied seven to 14 days before harvest to reduce fruit drop in 'Forelle' at two trial sites. However, a large number of fruit dropped due to strong wind before these trials could commence, and we can therefore not confidently report on the efficacy of NAA on 'Forelle'.

OPSOMMING

Manipulasie van oeslading met behulp van plantgroeireguleerders

Blom- en/of vruguitdunning is ʼn belangrike praktyk in die sagtevrugtebedryf om optimale opbrengs, vruggrootte en – kwaliteit te verseker. Die kern- en steenvrugindustrieë maak nog steeds staat op handuitdunning om vruglading te verlaag. Handuitdunning is egter tydrowend en weens die toenemende arbeidskoste het ʼn vraag na alternatiewe uitdunningstegnieke, veral vir steenvrugte, ontstaan. Chemiese uitdun is die mees belowende opsie om handuitdunning te verminder.

1-Aminosiklopropaan-1-karboksielsuur (ACC) is op steenvrugte geëvalueer en het belowende resultate op die Japannese pruime, ‘Laetitia’ en ‘Fortune’, opgelewer. ACC toegedien teen ʼn dosis van 400 $\mu l \cdot L^{-1}$ by ʼn vrugdeursnee van 8 – 10 mm blyk die optimale tydsberekening, sowel as dosis van ACC-toediening vir albei kultivars te wees. ACC was nie ʼn doeltreffende uitdunningsmiddel in die geval van ‘August Red’ nektariens nie. ACC toegedien teen ʼn dosis van 400 $\mu l \cdot L^{-1}$ wanneer ‘Keisie’ geelperske vruggies se deursnee 8 – 10 mm was, het konstant belowende resultate opgelewer en word dus teen hierdie dosis en tydsberekening aanbeveel.

Die effektiwiteit van ACC as chemiese uitdunmiddel op appels was kultivar-afhanklik. ʼn Standaard-industrie chemiese uitdunprogram is by die appelproef ingesluit om die effektiwiteit van ACC met chemiese uitdunningsmiddels wat tans in die industrie gebruik word, te vergelyk. Die industrie-standaard het van produsent tot produsent verskil. Nie ACC, nóg die industrie-standaarde, 6-bensieladenien (6-BA), en 6-BA gemeng met 1-naftaleenasynsuur (NAA), het ‘Fuji’ in enige van die twee seisoene genoegsaam uitgedun. Die onvermoë van hierdie produkte om doeltreffend uit te dun word toegeskryf aan omgewingsfaktore, asook intrinsieke boomkaraktereienskappe van die ”moeilik-om-uit-te-dun” ‘Fuji’. ACC, toegedien by ʼn vrugdeursnee van gemiddeld 8 – 10 mm, het belowende resultate op volwasse ‘Cripps’ Red’ bome in die 2018/2019 seisoen getoon. ʼn Dosis tussen 250 en 500 $\mu l \cdot L^{-1}$ ACC word aanbeveel vir ‘Cripps’ Red’, maar opvolgproewe word benodig. ACC het te sterk uitgedun in die geval van kleiner, onvolwasse ‘Cripps’ Red’ bome in die 2017/2018 seisoen. ACC is in die 2018/2019 seisoen op ‘Royal Gala’ geëvalueer. ʼn Dosis van 250 $\mu l \cdot L^{-1}$

ACC, toegedien by ŉ vrugdeursnee van 8 – 10 mm, het die mees belowende resultate getoon. Dit het ook beter resultate as die industrie-standaard NAA, gelewer.

Tydens chemiese vruguitdunningsproewe op 'Forelle'-pere het S-absisiensuur (S-ABA) in die 2017/2018 seisoen suksesvol uitgedun by een van die proefpersele. Die Glen Fruin proefperseel het natuurlike swak set in die 2017/2018 seisoen getoon, en chemiese uitdunning was nie kommersieel toegepas nie. S-ABA het dus tot oorbodige uitdunning gelei in ons proef. S-ABA het belowende uitdunningsresultate getoon teen ŉ dosis van 300 tot 400 $mg \cdot L^{-1}$ by die perseel waar voldoende vrugset plaasgevind het. In die vrugsetproewe op jong 'Packham's Triumph' bome het gibberelliene plus sitokiniene (GA_{4+7} plus 6-BA) en amino-etoksifinielglisien (AVG) gemeng met proheksadioon-kalsium (ProCa) nie vrugset verbeter nie, terwyl AVG op sy eie die opbrengs verhoog het. In die 2018/2019 seisoen, is NAA voor oes toegedien om die vooroes vrugval van 'Forelle'-pere by twee proefpersele te verlaag. Groot hoeveelhede vrugte het egter reeds geval as gevolg van sterk wind voor die proewe kon begin en daarom kan ons nie oortuigend verslag lewer oor die effektiwiteit van NAA om vooroes vrugval by 'Forelle' pere te verminder nie.

NOTE

This thesis is a compilation of chapters, starting with a literature review, followed by three research papers. Each paper was prepared as a scientific paper for submission to *HortScience*. Repetition or duplication between papers might therefore be necessary. The language used was therefore English (United States).

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GENERAL INTRODUCTION

The South African deciduous fruit industries, consisting of pome- and stone fruit, are export driven with high volumes of fruit being exported annually. Due to export markets having minimum standards to which fruit must adhere in order to be exported, it is of utmost importance that growers produce fruit with adequate size and quality. One way in which to ensure this, is through flower or fruitlet thinning. By adjusting the number of fruit on the tree, the remaining fruit will develop to a size that is commercially viable (Njoroge and Reighard, 2008). In South Africa, there is a high dependence on hand thinning by laborers. Hand thinning is time consuming and expensive, and with labor costs constantly increasing, the costs of hand thinning will further increase. Chemical thinning is the main alternative to hand thinning (Rosa et al., 2008).

In the literature review of this thesis, the current literature on chemical thinning was evaluated. Evidently, there are still unanswered questions with regards to chemical thinning. Furthermore, there is a need for alternative chemical thinning agents, which could provide growers with more flexibility and options.

Various chemical thinning agents have been evaluated on stone fruit, but few have delivered consistent results. One strategy is to reduce flower induction in the preceding season with gibberellins. However, growers would rather prefer to first evaluate flower density and tree health before deciding on a chemical thinning strategy, as poor fruit set could lead to over thinning. Another option is to thin flowers in the current season by using caustic blossom thinners such as ammonium thiosulphate (ATS), Tergitol-TMN-6 and hydrogen cyanamide (Fallahi et al., 2006). These have all been effective to an extent, but have not been consistent enough to become general practice. Many different chemicals have been evaluated as fruitlet thinners on stone fruit. Although some have proven promising, rather inconsistent results are generally obtained (Costa et al., 2004). No satisfactory chemical thinning in peach and nectarine have been achieved despite the numerous agents being evaluated (Costa and Vizzotto, 2000; Steenkamp, 2015). 1-Aminocyclopropane-1-carboxylic acid (ACC) has shown promise as a fruitlet thinner in stone fruit (Theron et al., 2017a; Steenkamp, 2015). In Paper 1, we report on the efficacy of ACC as a thinner on Japanese plum, nectarine and cling peach.

In the apple industry, two of the most frequently used post bloom thinners are 6-benzyladenine (6-BA) and 1-naphthaleneacetic acid (NAA) (Schupp et al., 2012). These chemicals successfully thin a wide range of apple cultivars, usually when applied from petal fall until 10 to 12 mm fruitlet diameter (Greene, 1992). However, it is not always possible to apply thinning agents during these phenological stages due to unfavorable environmental conditions, uncertainty about fruit set and/or failure of these compounds to adequately thin when previously used (Schupp et al., 2012). Thus there is a need for a chemical thinner that can be applied during a later application window. Currently there are two chemical thinners registered for use in the late thinning window (17 to 25 mm fruitlet diameter), viz., ethephon and carbaryl (Schupp et al., 2012). Unfortunately, ethephon thins erratically and its efficacy highly temperature dependent. (Jones and Koen, 1985). Carbaryl is considered to be a mild thinner and is mostly used in combination with other chemical thinners to increase the thinning effect (Schupp et al., 2012). However, carbaryl harms beneficial insects and water organisms and is already banned in certain countries (Wertheim, 1997). There is thus a need to find a predictable chemical thinner that fits into sustainable fruit production, and can be used as a rescue thinning agent (17 - 25 mm fruitlet diameter) in years when primary thinning agents could not adequately reduce fruit set. ACC is a chemical thinning agent that shows potential when applied in a late thinning window on apples (Schupp et al., 2012; McArtney and Obermiller, 2012. In Paper 2, we report on the efficacy of ACC on a number of apple cultivars.

In the pear industry, new chemical thinners are required that are predictable, efficient and fit into sustainable fruit production. Abscisic acid (ABA) is a naturally occurring plant hormone and has shown potential as a chemical thinner at 8 – 10 mm fruitlet diameter on ‘Forelle’ and ‘Bartlett’ pears (Green, 2012; Theron et al., 2017b). In Paper 3, we report on trials that further evaluated the efficacy of S-ABA on ‘Forelle’. Two other challenges that the pear industry face, is pre-harvest fruit drop and poor fruit set of young trees. Aminoethoxyvinylglycine (AVG) is an ethylene biosynthesis inhibitor, and can be used to increase fruit set by decreasing the ethylene concentration across the abscission zone (AZ) (Webster, 2000). We therefore also report in Paper 3 on the efficacy of AVG in increasing fruit set in young ‘Packham’s Triumph’ trees. In addition, we evaluated the efficacy of 1-naphthaleneacetic acid (NAA) in reducing pre-harvest fruit drop in ‘Forelle’ pears.

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LITERATURE REVIEW: 1-Aminocyclopropane Carboxylic Acid and Abscisic Acid as Chemical Thinners on Stone and Pome Fruit.

Contents

1. Natural fruit abscission

The fruit load of a fruit tree is firstly determined by the number of flowers that are borne by the tree, which is dependent on several factors occurring in the season preceding anthesis. These factors include; phase transition from vegetative to reproductive, whether or not adequate winter chilling was received and conditions during spring (bloom) (Costa et al., 2018). This review will not cover these factors. During fruit set there is a high requirement for resources from the tree, often before adequate leaf surface area has developed to support both the vegetative and reproductive growth and development. In spite of the lack of resources, many fruit species still bear a surplus of fruitlets which they are not able to sustain and support during fruit development (Keller and Loescher, 1989). Thus some fruit species have developed self-regulatory mechanisms in order to obtain an optimal balance between reproductive and vegetative parts (Costa and Vizzotto, 2000). The physiological fruit drop, which ultimately determines natural fruit load in various fruit tree species, is caused by the activation of the abscission zone (AZ). The AZ is an anatomical region of the fruit pedicle which is located at different positions in different fruit species. A series of events causes the activation of the AZ, and these events culminate in the development of an abscission signal (Costa et al., 2018). In higher plants, organ shedding is achieved by the dissolution of the middle lamella of specific layers of undifferentiated cells called abscission cells (Osborn, 1989). These cells make up the AZ, a predetermined zone that is able to respond to both internal and external signals, with hormones being the most important signals (Costa et al., 2018). Current abscission models have divided the abscission process into four steps. The first step is the differentiation of the abscission zone, the second step is gaining in ability to respond to abscission signals, during the third step cell separation is triggered and during the fourth step organ shedding occurs (Patterson, 2001).

This review will mainly focus on the effect of hormones on abscission and abscission signalling. It is, however, important to note that there are many other factors that play a role in abscission and abscission signalling. In terms of hormones, auxin, ethylene and abscisic acid (ABA) are important in abscission (Taiz and Zeiger, 2010). Ethylene biosynthesis is not essential for abscission to take place, although it has been found that inhibitors of ethylene interfere with the abscission process (Patterson and Bleecker, 2004). Several enzymes, which are involved either at the transcriptional/translational level are regulated by ethylene (Ruperti et al., 1998). Ethylene along with ABA can inhibit auxin biosynthesis which will counteract the auxin induced suppression of abscission (Sexton et al,. 1985). As long as there are sufficient levels of auxins moving from a plant organ across the abscission zone, no fruit drop will occur. If the auxin flux drops below a certain level, the continuously produced ethylene stimulates abscission (Wertheim, 1997). Therefore, abscission is a process stimulated by ethylene and suppressed by auxins (Wertheim, 1997). Fruitlet and flower abscission is stimulated when pollination related processes are inhibited, due to fluctuation in hormonal concentrations in the abscission zone. The biggest increase in ethylene concentration occurs when the endosperm inside the seed is consumed by the growing embryo (Wertheim, 1997). During this time the concentration of other hormones are reduced and an increase in abscission occurs (Wertheim, 1997).

Competition between fruitlets also causes abscission. Older and more developed fruit initiate fruit drop in younger fruitlets, as older fruit have stronger indole-3-acetic-acid (IAA) transport connections with the main plant (Taiz and Zeiger, 2010). Consequently the unidirectional transport of auxin is responsible for the abscission in younger fruitlets, as auxin from older fruits inhibit the auxin transport from more immature fruit causing abscission (Bangerth, 2000). In pome fruit trees, auxin transport from competing bourse shoots subtending clusters of pome fruit can also inhibit auxin transport from fruitlets (Bangerth, 2000).

During apple anthesis, the ovary exhibits modest growth and auxin production. Even though ethylene production is high, the exact role of this hormone during anthesis is disputed. Therefore, the chance of fruit drop during flowering is relatively high due to a high ethylene to auxin ratio (Wertheim, 1997). For example, an application of Ethephon, 1-aminocyclopropane carboxylic acid (ACC) or other ethylene producing compounds to apple flowers or spur leaves was found to induce flower abscission. Once flowers have been fertilised, the subsequent processes greatly increases the hormonal activity of the embryo and endosperm, and therefore the chance of drop decreases (Wertheim, 1997). Therefore, flowers are likely to drop when no fertilization occurs.

1. Importance of thinning

Stone and pome fruit industries benefit from regular, annual crops with high external and internal fruit quality. One of the prerequisites to reach these goals of high fruit quality is to have the right number of flowers on a tree, thus sufficient flower-bud formation in the preceding season. This can only be achieved if crop load is not excessive (Wertheim, 2000). Therefore a thinning action is needed to manipulate the yield in order to increase both fruit size, return bloom and other aspects of fruit quality.

Adjusting the number of fruit on the tree by thinning results in a higher availability of assimilates for the remaining fruit. Therefore the remaining fruit are more likely to reach a commercially viable size (Njoroge and Reighard, 2008). Reducing the crop load also reduces the occurrence of biennial bearing, whilst increasing tree vigour and reducing susceptibility to pathogens (Reighard and Byers, 2009).

Thinning strategies. Timing is of vital importance in fruit thinning. There are three periods during which fruit thinning could be implemented; pre-bloom, full-bloom and post-bloom (Njoroge and Reighard 2008). The earlier thinning takes place, the more intense the effect of thinning will be (Bergh, 1990; 1992). Many growers prefer to thin post-bloom as they can ensure that adequate fruit set has occurred. The cheapest and easiest method of fruit thinning is pruning. However, even with adequate pruning, trees still tend to set too many fruit and additional thinning actions are required at a later stage (DeJong and Grossman, 1994).

The differences between stone and pome fruit must also be considered when choosing a thinning strategy. Fruit growth of stone fruit can be divided into three stages (Costa and Vizzotto, 2000). During stage 1, rapid fruit growth takes place due to cell division and elongation at the beginning of the season (Day and DeJong, 1998). During growth stage 2 pit hardening takes place, using a high amount of assimilates in the process. The final growth stage consists of cell expansion as well as mesocarp maturation. This stage is once again a rapid growth stage. Therefore thinning fruit during stage 1 is considered optimal as the cell number will be established during this stage. Fruit growth occurs logarithmically and therefore it is beneficial to thin during this stage as a potential loss of fruit size can occur if fruit are thinned at a later stage (Day and DeJong, 1998). Thinning during stage 1 reduces the competition for assimilates during the early growth of fruitlets, increasing the potential for increased fruit size (Stover, 2001). During stage 2, fruit pit hardening requires a lot of assimilates for endocarp lignification. Therefore, delaying fruit thinning until stage 2 of fruit growth will result in a substantial amount of assimilates not being utilized for fruit growth. An advantage, however, of delaying fruit thinning until stage 2 is that it is easier to select fruit of the right size to be thinned (Costa and Vizzotto, 2000). Delaying fruit thinning up until 30 days after full bloom (DAFB) will allow the grower to thin fruit on a shoot very selectively, as at this stage there will be a distinct difference in fruit size. However, waiting this long until thinning compromises the gain in reduced fruit- fruit competition for assimilates that would have been obtained from thinning earlier (Southwick and Glozer, 2000).

Pome fruit have two growth stages. During growth stage 1 cell division takes places (Bain and Robertson, 1951). Thereafter, during stage 2, fruit growth occurs due to cell enlargement (Bain and Robertson, 1951). Therefore the final size of a mature fruit is due to the amount of cell division and the degree of cell enlargement (Bain and Robertson, 1951).

Major differences also exist between stone and pome fruit in terms of carbohydrate availability during the thinning period. Peach trees, for example, are much more efficient at photosynthesis and therefore they will have more assimilates available for developing sinks throughout the season compared to apple trees (Costa et al., 2018). There is also an increased amount of reserves which are built up and stored throughout the season in peach trees. Therefore, peach trees can be "more difficult to thin" than apples trees due to the increased availability of assimilates (Costa et al., 2018).

Other factors to consider before applying thinning strategies. The influence of spur quality, position of the spur, flower bud size and fruit size must be considered, as these factors could influence the chemical thinner efficacy. Another important aspect to take into account is the different responses of trees to available thinners. Climatic conditions also have an effect on thinner efficacy. Light and temperature, especially three to four days after the thinner application, has a marked effect on thinner efficacy, and can be incorporated into various models and should be practically implemented when choosing when and which thinner to use (Lakso et al., 2005).

2. Chemical thinning

The problem with hand-thinning is that it is very labour intensive and time consuming. With continuously increasing labour costs, there is a need to look at alternative ways in which to thin fruit (Stern and Ben-Arie, 2009). Thus thinning flowers and/or fruitlets using chemicals have become customary practice in pome fruit (Forshey, 1976, 1987; Williams, 1994). There are currently many chemical thinning agents available on the market, but some may not remain available or may not be used under certain circumstances (Wertheim, 2000). For example, the insecticide and fruit thinner, Carbaryl, does not fit the current standards of sustainable fruit production in certain production areas and has been banned from many markets. Some manufactures may also decide the cost required for the re-registration of certain chemical agents was too high as was the case with 1-naphthalene acetic acid (NAA) and its amide (NAAm) in some countries (Wertheim, 2000). Another compound that has been removed from many markets is the flower thinner dinitro-ortho-cresol (DNOC) (Fallahi et al,. 1997).

Producers are still continuing to implement hand thinning in orchards because of the inconsistent results achieved by chemical fruit thinning agents. Therefore, there is a need to look at new fruit and flower thinning agents that are predictable and reliable to satisfy the needs of the growers, manufactures and society (Wertheim, 2000).

3. Chemical thinning agents currently available

Different chemical thinning agents are applied at different periods during fruit growth.

"Thinning" flowers for the subsequent season. Gibberellic acid (GA_3) is an option for growers to "thin" (reduce numbers) flowers for the subsequent season, by preventing flower initiation and induction. In peach trees, GA_3 has been found to reduce crop load in the subsequent season when applied during flower differentiation in the current season (Costa and Vizzotto, 2000). De Villiers (2014) also found that a GA_3 application could have a beneficial effect on fruit quality in the subsequent season in Japanese plum. Gibberellic acid is transported from the fruit to the nodes in the nearby vicinity, inhibiting the initiation of new floral primordia (Webster and Spencer, 2000). The vegetative phase of bud development which precedes flower initiation is of critical importance in determining the amount of flowers produced (Luckwill, 1977). Therefore, applying GA_3 in the current season partially reduces return bloom and indirectly reduces crop load, subsequently resulting in decreased hand thinning costs (Gonzalez-Rossia et al., 2006). The use of gibberellins have not become a standard commercial practice due to the possibility of frost or poor flower set in the next season, which results in poor fruit set. Growers prefer to first evaluate the intensity of fruit set before applying a thinning action (Byers et al., 1990b). There have been various studies conducted to evaluate GA as a potential thinning agent and these are summarised in Table 1a and 1b.

Table 1a. A summary of research conducted on stone fruit using gibberellins GA_3 and GA_4 to reduce flower induction and initiation, and therefore return bloom.

Fruit type and Cultivar	**Concentration of active ingredient**	**Time of application and comments**	**References and comments**
'Patterson' Apricot	75-100 $mg \cdot L^{-1}$	Late May (approximately 2 weeks after harvest)	Southwick and Glozer, (2000). 100 $mg \cdot L^{-1}$ effectively reduced flowering
'Patterson' Apricot	50-100 $mg \cdot L^{-1}$ GA_3	First week of July (approximately 2 weeks after harvest)	Southwick et al., (1995). 50 and 100 $mg \cdot L^{-1}$ yielded like hand thinned trees and had larger fruit at harvest. These effects were most noticeable in the seasons where cropping was greater.
Sweet cherry	100 $mg \cdot L^{-1}$ GA_3	43 DAFB*	Proebsting and Mills, (1974). Reduced flowering in the following season
'Royal/Blenhaim' apricot.	60 $mg \cdot L^{-1}$ GA_4	Three different dates from 8 May to 8 June (Northern Hemisphere)	Southwick and Glozer (2000). GA_4 successfully reduced flowering
Sweet cherry	20 $mg \cdot L^{-1}$ GA_3	19 days before harvest (DBH)	Facteau et al., (1989). Reduced flowering the following season
'Sunlight' nectarine	GA_3 at 90, 120, 150 and 180 $mg \cdot L^{-1}$	Treatments where applied 4 weeks before harvest as well as in between the 1st and 2nd harvest. Double application of 90 $mg.L^{-1}$ 4 weeks before harvest as well as during harvest	Coetzee and Theron (1999). No interaction between concentration and time of application, all early applications thinned excessively
'Scarlet-Snow' peach 'Queen-Giant', and 'Arctic Mist' nectarine	25 $mg.L^{-1}$ GA_3 to the basal part of shoots	60 DAFB*	Stern and Ben-Arie (2009). Successfully reduced flowering the following season

* Days after full bloom

Table 1b. A summary of research conducted on pome fruit using gibberellins GA_3 and GA_4 to reduce flower induction and initiation, and therefore return bloom.

Fruit type and Cultivar	**Concentration of active ingredient**	**Time of application and comments**	**References and comments**
'Braeburn' apples	GA_3 from 10 to 330 µg·L^{-1}	Full bloom	McArtney (1994). Increasing concentrations of GA_3 applied in the light-flowering year caused a linear decrease in proportion of flowering spurs in the following year and linearly increased the proportion of flowering spurs 2 years after application. All GA treatments elongated the fruit in the year of treatment.
'Cameo', 'Honeycrisp', and 'Fuji' apples	'Honeycrisp' and 'Fuji' 300 mg·L^{-1} GA_{4+7} was applied. 'Cameo' 400 mg·L^{-1} GA_{4+7} was applied.	At petal fall, trees were manually adjusted shortly before anthesis to one of three levels of crop load (100%, 50%, and 0%)	Schmidt et al., (2009). Initial crop load was the primary determinant of return bloom. GA_{4+7} consistently reduced floral initiation
'Delicious' applies	250 to 500 mg·L^{-1} GA_{4+7}	Four sprays spaced one month apart, approximately 4.5, 9, 13 and 18 weeks, respectively, after petal fall.	Unrath and Whitworth (1991). 500 mg·L^{-1} sprays were not significantly more effective than 250 mg·L^{-1}. GA_{4+7} reduced return bloom percentage on trees by up to 95 percent.
Cox's Orange Pippin trees	GA_3, GA_4, GA_7 or GA_{4+7} all at 500 µg·L^{-1}	At full bloom and two or four weeks thereafter on two-year-old trees.	Tromp (1982). None of the treatments greatly affected shoot growth. At full bloom applications, GA_3 and especially GA_7 and GA_{4+7} markedly reduced flowering on spur buds. At the two later timings, GA_3 and GA_4 had no effect, whereas GA_7, alone or combined with GA_4, still showed distinct activity.

Flower thinning in the current season. Most growers would prefer to thin flowers after observing flower intensity as well as overall tree health (Byers et al., 1990b). Pelargonic acid, monocarbamidedihydrogen sulphate (MCDS, WilthinTM), endothallic acid, the rest-breaking agent hydrogen cyanamide (Dormex) have emerged as new flower thinners (Fallahi et al., 1997; Byers, 1997). However, MCDS, pelargonic and endothallic acid, are all rather phytotoxic and not always effective and can cause fruit-skin damage. Dormex gave encouraging results without adverse effects (Fallahi et al., 1997; Byers, 1997), but the registration of this chemical will be difficult in many countries as it is also phytotoxic to bees (Wertheim, 2000). Lime sulphur is an option as a chemical flower thinner for organic farmers (Stopar, 2004). However, lime sulphur has been known to over thin and also cause leaf phytotoxicity (Stopar, 2004). One promising option is ammonium thiosulfate (ATS). The mode of action of ATS is presumably through the desiccation of flowers and damage to the base of the flower peduncle (Byers and Lysons, 1985; Byers et al., 1986). The efficacy of ATS is therefore largely dependent on the number of flowers present at the vulnerable stages of floral development at the time of spraying. Flowers at balloon stage and flowers that have been open for two days are the most sensitive to ATS (Webster and Hollands, 1993). Climatic conditions during the season preceding flowering influence the time of flower initiation and the speed of floral development within the bud. There have been various studies conducted to evaluate ATS as a potential thinning agent and summarised in Table 2a and 2b.

Table 2a. Summary of studies using ammonium thiosulfate (ATS) as a chemical thinning agent for stone fruit.

Fruit type and Cultivar	**Concentration of active ingredient**	**Time of application and comments**	**References and comments**
'Garnet Beauty' and 'Red Haven' peach	37.4 $L.ha^{-1}$ and 74.8 $L.ha^{-1}$ ATS	Full bloom	Greene et al., (2001). ATS reduced fruit set significantly and increased final fruit size at harvest of both cultivars.
'Victoria' plum	High volume sprays 1.5% ATS	3 sprays at or post anthesis	Webster and Hollands, (1993). ATS thinned significantly and improved fruit size
'Opal' and 'Victoria' plum	1-1,5 % ATS	Single application at full bloom	Meland, (2007). High volume sprays until runoff was more effective than low volume sprays. ATS reduced fruit set and increased fruit size. 'Opal' was more sensitive to ATS than 'Victoria' and a low dosage is recommended
'Bing' sweet cherry	2% ATS	Single application at full bloom	Whiting et al., (2004). ATS consistently reduced fruit set and increased fruit quality compared to the control trees.
'Redhaven' peach	1 and 2% ATS	50-60% full bloom	Turk et al., (2014). The 2% treatment caused the largest increase in fruit size however the thinning action was too strong. 1% ATS over-thinned in 2 of the 3 years of the experiments with the thinning action not differing significantly from the control in one year.

Table 2b. Summary of studies using ammonium thiosulfate (ATS) as a chemical thinning agent for pome fruit.

Fruit type and Cultivar	Concentration of active ingredient	Time of application and comments	References and comments
'Manchurian' crab apple	ATS at concentrations of 1-5%	Full bloom	Janoudi and Flore (2005). ATS at 5% concentration followed by washing with water within 1 h of application. Delayed washing and higher concentrations of ATS caused excessive thinning and moderate to severe damage to trees.
'Gala' apple	0.5-1.5% ATS	Full bloom	Basak, (2000). ATS markedly reduced the initial fruit set, but fruit size was only slightly improved
'Jonagold' apple	1.0%, 2.0% and 3.0% ATS	Single application at full bloom	Kacal and Koyuncu (2012). ATS was found to be an ineffective fruit thinner and was not effective in reducing biennial bearing severity in 'Jonagold' apples
'Delicious' apple	1% ATS	First application at 20% full bloom and second application at 80% full bloom	Bound and Wilson (2007). Evaluated the efficacy of single application vs multiple applications of ATS and found that a multiple application was the most effective,
'Braeburn' apple	1% ATS	20% full bloom	Milić et al., (2011). ATS increased fruit size significantly. 1% ATS can successfully reduce fruit set of younger trees.

Fruitlet thinning in the current season. Growers generally prefer to thin fruitlets after flowering in order to evaluate fruit set and lower the risk of over thinning (Meland, 2007). There are many options available to thin fruitlets in the current season, 6-benzyladenine (6-BA), 1-naphthaleneacetic acid (NAA), ethephon (C_2H_4), carbaryl and metamitron are the most commonly used chemical thinning agents. The cytokinin 6-BA and has received a lot of attention. It does not thin apples directly by affecting the movement of carbohydrates from the leaves to fruit, but instead, 6-BA influences the carbohydrate supply by increasing mitochondrial respiration and decreasing net photosynthesis (Pn). The decreased Pn leads to a limited supply of carbohydrates to the fruit and thus increased fruit abscission. This theory is supported by evidence that 6-BA only thinned apples on a girdled small fruiting spur when one leaf was present per fruit. However, when the spurs had two or more leaves, thinning did not take place (Yuan and Greene, 2000). Another theory is that 6-BA stimulates abscission through "correlative abscission" (Bangerth, 2000) due to 6-BA stimulating bourse shoot growth which increases auxin auto-inhibition. This causes a decrease in auxin flow from younger fruit resulting in the auxin concentration to decrease across the AZ ultimately causing abscission (Bangerth, 2000). It is well documented that cytokinins increase cell division (Letham, 1969). At the time when 6-BA is normally applied as a thinner (14-18 DAFB), cell division is still taking place (Patricia Denne, 1963). An increase in cell numbers in the fruit should contribute to an increase in fruit size. There have been various studies conducted to evaluate 6-BA as a potential thinning agent and these are summarised in Table 3a and b.

Table 3a: Summary of studies using 6-benzyladenine (6-BA) as a fruit thinning agent for pome fruit.

Fruit type and Cultivar	**Concentration of active ingredient**	**Time of application and comments**	**References and comments**
Early 'Bon Crétien' pear	100-150 mg.L^{-1}	8 DAFB* Smaller fruit (6 - 8 mm) more susceptible to 6-BA than larger fruit (8 to 12mm).	Theron et al., (2010a). 150 mg.L^{-1} had the largest decrease in crop load and the largest increase in fruit size
'Forelle' pear	100, 125, 150, 200 mg.L^{-1} as well as a split application of 3 x 50 mg.L^{-1}	8, 11 and 17 DAFB*	Theron et al., (2010b). None of the treatments had a significant effect on fruit size and return bloom
'Fuji' apple	50, 100, 200 or 400 mg I^{-1}	20 DAFB*	Bound et al., (1991). Increased thinning with increase in concentration of 6-BA. Return bloom was significantly improved. 400 mg.L^{-1} increased russet.
'McIntosh' apple	50 or 100 mg·L^{-1}	10 mm stage of fruit development	Green (2002). 6-BA thinned fruit and increased fruit size sufficiently.
'Empire' apple	75 or 150 mg·L^{-1}	3.6 mm (6 DAFB*) to 17 mm (29 DAFB*)	Elfving and R.A. Cline (1993). 6-BA increased fruit weight more effectively than either NAA or carbaryl. 6-BA increased return bloom as much or more than NAA or carbaryl.

* Days after full bloom

Table 3b: Summary of studies using 6-benzyladenine (6-BA) as a fruit thinning agent for pome fruit.

Fruit type and Cultivar	**Concentration of active ingredient**	**Time of application and comments**	**References and comments**
‘McIntosh’, ‘Delicious’, ‘Golden Delicious’, ‘Empire’, and ‘Idared’ apple	75-100 mg·L^{-1}	6-BA applied at Full bloom plus 14-23 DAFB*	Greene and Wesley (1990). In all incidences 6-BA increased flesh firmness and increased the fruit size. 6-BA at 75 to 100 mg·L^{-1} appears to compare very favourably with other commercially used thinners of apples (NAA, Carbaryll and Ethephon)
McIntosh’, ‘Delicious’, ‘Golden Delicious’, ‘Mutsu, ‘Empire’, and ‘Abas’ apple	Between 50-100 mg·L^{-1}	Most effective at 10mm fruit diameter stage	Green (1993). Rates of higher than 150 mg·L^{-1} may result in spur elongation, asymmetric fruit and over-thinning
‘Red Delicious’ apple	100 mg·L^{-1}	10 mm fruit diameter stage	Elfving (1994). Difficult to thin cultivars may require rates up to 100 mg·L^{-1}
‘Spadona’ pear	100 mg·L^{-1}	10 mm fruit diameter stage (2 weeks after full bloom)	Stern and Flaishman (2003). Fruit size was increased without a reduction in yield, thus the fruit size increase can be directly attributed to the increase in the rate cell division
‘Coscia’ pear	100 mg·L^{-1}	10 mm fruit diameter stage (2 weeks after full bloom)	Stern and Flaishman (2003). Fruit size was increased but yield was decrease, thus the increase in fruit size was attributed to the thinning effect
‘Summerred’ apple	100 mg·L^{-1}	10 mm fruit diameter stage	Stopar and Lokar (2003). 6-BA sprays resulted in a significant thinning effect, together with a substantial increase in fruit size.

* Days after full bloom

The auxin, 1-naphthaleneacetic acid (NAA) is another chemical available to thin fruitlets. It has been suggested that the mode of action of NAA is by reducing the energy that is available to the young developing fruit. This is done either by interference with photosynthesis (Stopar et al., 1997) or by a reduction in the translocation of metabolites, including photosynthates from the leaves to the fruit (Schneider, 1975, 1978). NAA also causes a reduction in the export of diffusible auxins, especially from weaker fruitlets (Crowe, 1965). NAA is known to thin fruit and reduce the fruits IAA export at the same time (Crowe, 1965; Ebert and Bangerth, 1982). Auxin transport inhibitors are also effective thinners (Stahley and Williams, 1972; Bangerth, 1997). NAA is one of the most reliable thinners and is often used on "difficult to thin" cultivars. High concentrations of NAA may cause the formation of pygmy fruit (Marini, 1996). The amide of NAA, naphthalacetamide (NAD), is considered to be more reliable than NAA in variable climates, as climate has been known to affect the thinning efficacy of NAA more than NAD (Wertheim, 2000). With both, NAA and NAD, the thinning action is directly proportional to the concentration (Forshey, 1976). NAD is less effective than NAA and is preferred on cultivars which are not difficult to thin (Wertheim, 2000). One disadvantage of NAD is that it does not always result in an increase in fruit size, as NAD has been known to slow down fruit growth (Wertheim, 2000). Some of the studies that have evaluated NAA as a fruit thinner is summarized in Table 4a and 4b.

Table 4a: Summary of studies using 1-naphthaleneacetic acid (NAA) as a chemical thinning agent for pome fruit.

Fruit type and Cultivar	**Concentration of active ingredient**	**Time of application and comments**	**References and comments**
‘Fuji' apple	5 - 15 mg·L^{-1} 5 mg·L^{-1} under thinned and 10 and mg·L^{-1} over thinned	At full bloom or 14 DAFB*	Jones et al., (1989). Jones could make no firm recommendations on NAA application on ‘Fuji' apples
‘Elstar’ apple	50 mg·L^{-1} NAA-ethyl	Full bloom	Wertheim (2000). The treatment did not break severe bianualism, did thin sufficiently
‘Elstar’ apple	10 mg·L^{-1} NAA-methyl	10 mm fruit diameter stage	Wertheim (2000). The treatment did not break severe bianualism, no effect on fruit size
‘Delicious’ apple	2.5 - 10 mg·L^{-1}	Near petal fall ≥9mm fruit diameter	Marini (1996). NAA was a strong thinner when applied close to petal fall. When applied after the 10mm fruit diameter stage it increased the number of pigmy fruit significantly.
‘Empire’ apple	NAA (7.5 mg·L^{-1}) + carbaryl (600 mg·L^{-1})	Petal fall to 5mm fruit diameter stage	Stover (2001). Combination of NAA and Carbonyl improved fruit size, thinned effectively and improved return bloom. Elfving and Cline (1993) found that BA was more effective than a combination of NAA and Carbonyl, Stover’s results contradicted their findings.
‘Summerred’ apple	10 mg·L^{-1}	10 mm fruit diameter stage	Stopar and Lokar (2003). NAA applied alone had a significant thinning effect. However this thinning effect did not significantly increase crop load, and half of the crop was lost due to the thinning effect.

* Days after full bloom

Table 4b: Summary of studies using 1-naphthaleneacetic acid (NAA) as a chemical thinning agent for pome fruit.

Fruit type and cultivar	Concentration of active ingredient	Time of application and comments	References and comments
Spur type 'Delicious' apple	10 - 15 mg·L^{-1}	5 to 15 days after petal fall or 10 to 12 mm king fruit diameter.	Black et al., (1995). Thinning response was significant however fruit size was not increased significantly after the thinning effect
'Priana' and 'Beliana' apricot	20 mg·L^{-1}	14-18 DAFB*	Son (2004). Son evaluated the effect of NAA on fruit quality of 'Priana' and 'Beliana' apricots and found that an NAA application significantly improved fruit size.
'Gala' apple	10 mg·L^{-1}	10 fruit diameter stage	Basak (2006). When NAA was applied shortly after blossoming fruit size was significantly increased compared to the un-thinned control. However fruit sized was not greater than the hand thinned treatment.
'Nijisseiki' pear	7.5 mg·L^{-1}	15 DAFB	McArtney and Wells (1995). NAA had no effect on yield efficacy, crop density as well as mean fruit weight at harvest. NAA did however reduce flesh firmness at harvest and after cold storage.
'Fuji' and 'Delicious' apple	5 - 10 mg·L^{-1}	Petal fall	Williams (1993). Fruit set was reduced however undesirable side effects such as low seed number, small fruit size and foliage curling was observed.

*Days after full bloom

Ethephon (C_2H_4) is a fruit thinner that gives variable results. This variability is due to the changing sensitivity of the developing apple flowers/fruitlets and is highest at pink-bud stage and declines to almost zero at petal fall (Costa and Vizzotto, 2000; Williams, 1994). Temperature is also involved in the efficacy of ethephon as thinning increases linearly with an increase in temperature between 12 to 24 ◦C (Jones and Koen, 1986). Ethylene reduces the concentration of auxin in various tissues, either by inhibition of auxin biosynthesis (Valdovinos et al., 1967) or by a direct inhibition of auxin transport (Schröder and Bangerth, 2005). There have been various studies conducted to evaluate ethephon as a potential thinning agent as summarized in Table 5a and 5b.

Table 5a: Summary of studies using ethephon (C_2H_4) as a chemical thinning agent for pome fruit.

Fruit type and Cultivar	**Concentration of active ingredient**	**Time of application and comments**	**References and comments**
'Red Fuji' apple	800 mg·L^{-1}	Full bloom	Jones et al., (2015). Jones found that ethephon was a more effective thinner at full bloom instead of 14 DAFB as was previously founded. A mean fruit weight of 200g per apples was reached.
'Golden Delicious' apple	350 mg·L^{-1}	Balloon blossom-stage and at 42 DAFB*	Jones et al., (1983). Satisfactory thinning was achieved using this lower concentration of ethephon as mean fruit diameter was increased by an average of 70mm or more and fruit set was decreased.
'Nijisseiki' and 'Hosui' pear	400, 600 and 800 mg·L^{-1}	15 DAFB*	McArtney and Well (1995). Increase in concentrations resulted in increase in reduced fruit set as well as fruit size and increased the incidence and severity of flesh spot decay. Ethephon increased the return bloom of 'Nijisseiki' pears.
'Delicious' apple	1000 to 1500 mg·L^{-1}	12 to 26 DAFB*	Byers (1993). Ethephon caused tree growth to decrease as well as causing fruit abscission. Return bloom was greatly increased by the treatments, although there was no increase in fruit size.
'Spartan' apple	500 mg·L^{-1}	Balloon stage of flowering	Knight et al., (1987). Treatments reduced the number of fruit per tree and increased fruit size. May be an effective method to thin 'Spartan' apples.

'Kosui', 'Chojuro', 'Niitaka', and 'Imamuraaki' pear	400 mg·L^{-1}	14 DAFB*	Kim et al., (1988). Treatment resulted in a thinning and shifted the distribution of fruit weights. There was less small fruit (below 280 grams) and less very large fruit (above 401 grams).
'Hosui' and 'Okusankichi' pear	400 mg·L^{-1}	14 DAFB*	Kim et al., (1988). Treatment over-thinned and shifted the distribution of fruit weights. There was less small fruit (below 280 grams) and less very large fruit (above 401 grams).
'Golden Delicious' apple	400 mg·L^{-1}	20 mm stage of fruit growth	Yuan (2007). Treatment thinned efficiently

*Days after full bloom

Table 5b: Summary of studies using ethephon as a chemical thinning agent for stone fruit.

Fruit type and cultivar	Concentration of active ingredient	Time of application and comments	References and comments
'Jubileum' plum	375 mg·L^{-1}	Full bloom	Meland and Birken, 2010 Thinned to 10-15% fruit set. Treatments increased fruit size but significantly reduced yield.
'Jubileum' plum	250 mg·L^{-1}	10 mm fruit diameter	Meland and Birken, 2010 Thinned to 10-15% fruit set. Treatments increased fruit size but significantly reduced yield.
'Victoria' plum	mixture 10 mg·L^{-1} NAA and 75 mg·L^{-1} ethephon	27 DAFB*	Meland, 2007 Both treatments increased fruit quality and return bloom
'Victoria' plum	250, 375 and 500 mg·L^{-1}	Full bloom	Meland, 2007 Effective thinning
'Victoria' plum	125, 250 and 375 mg·L^{-1}	10-12 mm fruitlet diameter	Meland, 2007 Effective thinning

*days after full bloom

Carbaryl (marketed as Sevin®) is the most versatile thinner available. It is a mild thinner that can be applied from petal fall to 18 mm fruitlet diameter (Knight and Spencer, 1987). Carbaryl hardly ever over-thins (Forshley, 1987) and is considered a mild thinner and is often used in combination with other chemical thinners like 6-BA. Carbaryl disrupts seed development, which leads to a decrease in auxin export by the fruit stalk (Wertheim, 1997). Carbaryl is toxic to bees and water organisms and must be used at petal fall when the bees are already out of the orchard. It is currently banned in Europe (Hehnen et al., 2012). There have been various studies conducted to evaluate carbaryl as a potential thinning agent as summarized in Table 6a and 6b.

Table 6a: Summary of studies using carbaryl as a chemical thinning agent for pome fruit

Fruit type and Cultivar	**Concentration of active ingredient**	**Time of application and comments**	**References and comments**
'Laxton's Superb and Worcester Pearmain' apple	0.0083% to 0.075%	From petal fall to 4 - 5 weeks after petal fall carbaryl is effective	Way (1967). Thinning resulted in an increase in fruit size. Increased blossoming and cropping was most marked with biennial trees of 'Laxton's Superb', 'Worcester Pearmain' showed a moderate increase in subsequent flowering
'Delicious' apple	900 mg·L^{-1}	Petal fall to 18.5 mm fruit diameter stage	Marini (1996). At petal fall carbaryl was mild thinner. At an average fruit diameter of 8-9 mm fruit diameter carbaryl was an effective thinner.
'Cox's Orange Pippin' apple	1500 mg·L^{-1}	12 mm fruit diameter	Knight and Spenser (1987). Significantly reduced crop load and improved fruit size. In years of high fruit set additional thinning action might be needed.
'Delicious' and 'Fuji' apple	0.125 - 0.188%	Petal fall	Williams (1993). Servin® applied later than petal fall may cause seed abortion unless the temperature is high enough to cause fruit with aborted seeds to abscise
'Redchief Delicious' apple	900 mg·L^{-1} and 900 mg·L^{-1} together with 92% polypropylene shade over the entire tree for 4 days	17 DAFB* (fruit size: 9.95 ± 0.41 mm)	Byers et al., (1990a). Spraying trees with carbaryl reduced fruit set by 25%. The combination of shade + carbaryl spraying reduced fruit set by 89%
'Golden Delicious' apple	0.075%	21 to 28 day DAFB*	Wertheim (1970). Carbaryl was found to be a reliable thinner of 'Golden Delicious' apples

*days after full bloom

Metamitron is the most recently added chemical thinning agent used mainly on pome fruit. Metamitron acts by inhibiting photosystem II of photosynthesis (Abbaspoor et al., 2006). The decrease in the photosynthetic efficacy causes a decrease in assimilate production, thereby increasing the competition for the remaining assimilates. The concentration and time of application depend largely on the climatic conditions from one week before to one week after application (Costa et al., 2018). When conditions are favourable for carbon assimilation (low night temperatures and high irradiation), concentrations must be increased in order to retain effectiveness, and vice versa if climatic conditions are not favourable for carbon assimilations (high temperatures and low irradiation) as over thinning could occur (Costa et al., 2018). Phytotoxicity has been seen is some cultivars although it is transient and does not adversely affect final fruit quality, yield or return bloom (Costa et al., 2018). Some of the studies that have evaluated metamitron as a fruit thinner are summarized in Table 7a.

Table 7a: Summary of studies using metamitron as a chemical thinning agent for pome fruit.

Fruit type and Cultivar	Concentration of active ingredient	Time of application and comments	References and comments
'Fuji' apple	50% metamitron, 350 $mg \cdot L^{-1}$	Single treatment at 6 mm fruitlet diameter stage and double application at 6 and 12 mm fruitlet diameter stage.	Dorigoni and Lezzer (2007). Single application was close to the target of 100 fruits per tree with fruit size at least 250 grams. Double application strongly over-thinned.
'Gala' apple	350 $mg \cdot L^{-1}$	Single application at 18 mm fruitlet diameter stage	McCartney and Obermiller (2012). Reduced fruit set, with metamitron having a greater thinning activity than standard recue agent and ACC.
'Conference' pear	175 - 350 $mg \cdot L^{-1}$	8 - 12 mm fruitlet diameter	Maas and Van der Steeg (2011). Fruit load decreased linearly with an increase in concentration of metamitron.
'Elstar' apple	350 $mg \cdot L^{-1}$	single and double application at 6-8 mm and at 12 - 14 mm fruitlet diameter	Lafer (2009). Repeated treatments of Metamitron produced a significant reduction in fruit set. The fruit size was improved according to the crop load reduction.
'Gala Must' apple	Single and double application of 305 $mg \cdot L^{-1}$	Single 6 - 8 mm fruitlet diameter and double application at 10 - 14 mm fruit diameter	Basak (2011). In 2006, only the double metamitron treatment caused significant reduction of fruit set as well as an increase in fruit size with no negative effects. In 2008, the good effect of thinning was noticed after one spray with metamitron, while a double treatment caused over-thinning.

4. Hormonal influence of Ethylene

The role of ethylene in flower abscission is well documented. The exposure of flowers to exogenous ethylene result in an increase in the rate and number of inflorescences that abscise in plants such as olive trees (*Olea europaea)* (Weis et al., 1991) and cut flowers such as *Euphorbia fulgens, Clerodendrum, Hibiscus, Fuchsia* as well as several other species (Cameron and Reid, 1981; van Leeuwen, 1985; Rewinkel-Jansen, 1985).The rate of ethylene production often increases before flower abscission as seen in tomato flowers (Roberts et al., 1984). Dostal et al. (1991) found that amino-oxyacetic acid (AOA), an inhibitor of ethylene, reduced the onset of flower abscission in many species. Serval mutants in *Arabidopsis* like EIN3, EIL1 and EIL2, which are defected in ethylene perception, show a delay in floral organ abscission but not a block. This indicates that floral abscission can occur through ethylene dependent and ethylene independent pathways (Chao et al., 1997). The effect of ethylene inhibitors and mutants deficient in ethylene perception strongly suggests that natural flower abscission is controlled at least in some part by the endogenous ethylene concentration within the plant. Other external factors that promote an increase in ethylene production by the plant also results in an increase in abscission e.g. increased temperatures (Konsens et al., 1991), mineral deficiencies (Addicott, 1970) as well as water stress or a low soil water potential (Apelbaum and Yang, 1936). The primary role of ethylene in abscission is to stimulate the synthesis of terminal hydrolytic enzymes that degrade the cell walls in the abscission zone (Addicott, 1970). Other factors promote abscission by causing an increase in ethylene such as temperature and drought stress; ethylene can then be considered to function as a secondary messenger in the control sequences of abscission. Ethylene has several effects on auxin metabolism all resulting in a decrease in auxin concentration in the abscission zone (Addicott, 1970).

5. Hormonal influence of abscisic acid (ABA)

Abscisic acid (ABA) is a plant hormone associated with abscission and plant dormancy (Taiz and Zeiger, 2010). During periods of drought, ABA is also the hormone responsible for stomatal closure (Taiz and Zeiger, 2010). An external application of ABA can induce stomatal closure even when the plant is not under water stress (Correia et al., 1999). Stomatal closure results in a decrease in carbon fixation in plants as net photosynthesis is decreased and a decrease in carbon fixation has been associated with an increase in thinner efficacy (Lakso et al., 2006). Therefore, ABA may induce fruit thinning by causing a carbohydrate shortage within the plant. An increase in ABA also caused an increase in ethylene production and abscission in peaches treated with ABA at pit hardening (Giovanaz et al., 2015).

6. New products

1-Aminocyclopropane Carboxylic Acid (ACC). Ethylene is formed naturally in plants by the conversion of S-adenosylmethionine (SAM) to ACC and is catalysed by the enzyme ACC synthase (ACS). ACC is then converted to ethylene by ACC oxidase (ACO) (Kende, 1993). ACS and ACO are encoded by small multi-gene families that are differentially regulated by biotic and abiotic factors. A total of 5 ACO genes and 3 ASO genes have been isolated from apple trees (Li and Yuan, 2008; Binnie and McManus, 2009). Ethylene can only be formed from exogenously applied ACC in tissues that have the ACO enzyme; the tissue also has to be sensitive to ethylene before there will be any response to exogenously applied ACC (McArtney, 2011). In general, when exogenous ACC is applied to plant tissues, ethylene synthesis increases substantially. This indicates that the synthesis of ACC is usually the limiting biosynthetic step in the production of ethylene in plant tissues.

McArtney (2011) conducted a study to evaluate the potential of using exogenously applied ACC, as well as other commercially available fruit thinning agents on 32 uniform ‘Gold-Rush/Mark’ apple trees in a 12-year-old orchard in North Carolina. He reported a positive linear relationship between the dose rate of ACC applied and the amount of ethylene a detached fruiting spur released 1 day after the treatment application. The ethylene production had decreased by 90% 4 days after ACC was applied. The application of 5 $mg \cdot L^{-1}$ NAA reduced fruit set in ‘Gold-Rush’ in 2009 ($P = 0.05$) but there was no significant difference in 2010 ($P = 0.09$). An application of various dose rates of ACC significantly reduced fruit set in ‘Gold-Rush’ in both years ($P < 0.0001$). In 2009 there was a significant effect on fruit set ($P = 0.0004$) with combination of ACC and NAA. It must be noted, however, that 2009 experienced a low natural fruit set for this cultivar in the particular area (McArtney, 2011). A negative linear relationship was found between the rate of ACC applied to spurs and the number of fruit that those spur set in both 2009 and 2010. According to the data collected in 2010, McArtney concluded that ACC could be used to effectively reduce fruit set in apples, and furthermore ACC could be used in combination with NAA as their effects were additive. A concentration of 200 $mg \cdot L^{-1}$ ACC resulted in an excessive decrease in fruit set in the ‘Gold-Rush’ apples.

There was a rapid increase in ethylene expression of detached fruiting spurs when ACC was applied at full bloom and at 16 DAFB when the fruitlet diameter was 10 mm; however, the mean ethylene expression significantly reduced at 31 DAFB when the mean fruitlet diameter was 20 mm (McArtney, 2011). McArtney, (2011) found that concentrations of ACC between 50 – 200 $mg{\cdot}L^{-1}$ had no effect on the fruit set of 'Cripps' Pink' apples.

McArtney and Obermiller (2012) conducted an experiment to evaluate whether or not ACC could be applied as a fruit thinner during times when the fruit is insensitive to other commercially available fruit thinners (16 – 25 mm fruitlet diameter). An application of 400 $mg{\cdot}L^{-1}$ ACC on 'Royal Gala' apples when the mean fruit diameter was 18 mm significantly reduced fruit set in the 2010 season. However, metamitron had a greater thinning effect than ACC. A combination of metamitron and ACC had a more severe thinning effect on 'Royal Gala' than either treatment on their own, which suggests an additive effect of carbohydrate stress or ethylene synthesis on fruit abscission. None of the treatments had a significant effect on fruit size. A "rescue thinning programme" is used when chemical thinning agents could not be applied during the normal application timings, for whatever reason. An application of a standard "rescue thinning programme" of (carbaryl + ethephon + NAA) to 'Royal Gala' fruit which had a mean fruit diameter of 20 mm had a significant effect on fruit set; however, the treatment had no significant effect when applied to fruit that had a mean diameter of more than 25 mm (McArtney and Obermiller, 2012). On the other hand, a combination of metamitron (175 $mg{\cdot}L^{-1}$) and ACC (200 $mg{\cdot}L^{-1}$) caused a significant reduction in fruit set even when applied to 'Royal Gala' fruit which had a mean diameter of 20 mm or 25 mm (2010). However, there was no significant effect at the 31 mm diameter stage. In 2012, an application of a standard "rescue thinning programme" of (carbaryl + ethephon + NAA) to 'Royal Gala' fruit reduced fruit set when applied at the 20 mm and 27 mm diameter stage, but had no significant effect when applied at the 31 mm diameter stage. When ACC (400 $mg{\cdot}L^{-1}$) was applied in combination with metamitron (350 $mg{\cdot}L^{-1}$) in 2012 at the 20 mm diameter stage it resulted in aggressive thinning as well as some leaf yellowing and leaf abscission. McArtney and Obermiller (2012) found that a combination of ACC and metamitron also had a significant effect at the 27 mm and 33 mm diameter fruit stages. Thus, following these results, it was concluded that ACC and metamitron applied separately could have a significant effect on fruit set when applied before and up to the 20 mm diameter stage. A Combination of the ACC and metamitron, however, could have a significant effect on fruit set even when the application is delayed until the 33 mm diameter fruit stage depending on the climatic conditions of that particular year. This is due to the thinning activity of metamitron being dependent on the ambient temperature and the irradiation directly after application. Thus the authors concluded that ACC could be an effective fruit thinner in apples and that ACC is also effective in thinning fruit during times when fruit are not sensitive to other commercially available fruit thinners.

Schupp et al. (2012) evaluated ACC at concentrations of 0, 100, 300 and 500 $mg{\cdot}L^{-1}$ on 'Golden Delicious' apples. An application of ACC at the 20 mm fruit diameter stage was effective in reducing crop load and increasing return bloom, whereas an application of ACC at the 10 mm fruit diameter stage was ineffective. Schupp et al. (2012) found a linear increase in thinning efficacy with an increase in ACC concentration. A concentration of 300 or 500 $mg{\cdot}L^{-1}$ was found to be the most effective concentrations with both 300 and 500 $mg{\cdot}L^{-1}$ having similar thinning effects when applied at the 20 mm fruit diameter stage. Schupp et al. (2012) did not find any signs of phytotoxicity in the two seasons, and concluded that ACC is a potential late chemical thinning agent for 'Golden Delicious' apples. The observed rate responses to ACC concentration occurred both seasons under hot temperatures, a condition under which over-thinning with ethephon has been observed (Jones and Koen, 1985).

Theron et al. (2017a) evaluated ACC (75 - 300 $\mu L{\cdot}L^{-1}$) on 'Forelle' pears in two production areas in the 2016/2017 season in South Africa. All the treatments were applied at the 8 - 10 mm fruitlet diameter stage. In the Elgin production area ACC at rates of 225 and 300 $\mu L{\cdot}L^{-1}$ significantly reduced fruit set per cluster compared to the untreated control and increased fruit size. The amount off hand thinning required was significantly reduced by 150, 225 and 300 $\mu L{\cdot}L^{-1}$ ACC treatments in the Elgin production area. These results were not reflected in the Warm Bokkeveld production area.

Steenkamp (2015) conducted trials to evaluate ACC on 'Keisie' and 'Sandvliet' peaches. All the foliar applications were made when the fruitlets were at 8 - 10 mm diameter. ACC had a significant fruit thinning effect on 'Keisie' peaches in two seasons. ACC reduced fruit set linearly as the concentration of ACC increased. No reduction in yield was observed and fruit size was not effected in both seasons. ACC application significantly reduced fruit set in 'Sandvliet' peaches in one season; however; the amount of hand thinning required was not significantly reduced. The yield of 'Sandvliet' peaches and the yield efficiency was significantly reduced which is an indication that hand thinning was too severe. Due to the yield reduction, the fruit size was significantly increased with ACC at 400 and 600 $\mu l{\cdot}L^{-1}$. Steenkamp (2015) concluded that the preferred application rate of ACC for 'Keisie' peaches is 600 $\mu l{\cdot}L^{-1}$

applied at the 8 - 10 mm fruitlet diameter stage. Based on one season results, Steenkamp (2015) does not recommend the use of ACC on 'Sandvliet' peaches. ACC did not cause split pit in either cultivar, while slight and severe leaf drop was observed in 'Keisie' and 'Sandvliet', respectively (Steenkamp, 2015).

Theron et al. (2017b) evaluated ACC over two seasons (2014/2015) on Japanese plums 'Laetitia', 'African Rose™' and 'Fortune'. All the foliar applications were made when fruitlets had a diameter of 7 - 10 mm. In the first season only the highest rate of ACC (500 $\mu l \cdot L^{-1}$) had a significant thinning effect on 'African Rose™' compared to the control. The rate of 500 $\mu l \cdot L^{-1}$ ACC had no significant effects on yield, harvest distribution or fruit weights indicating that the treatment did not over thin. In the subsequent season higher rates of ACC (600 and 800 $\mu l \cdot L^{-1}$) were applied. Even with the higher rates of ACC a large number of fruitlets still had to be thinned by hand at commercial hand thinning. The higher rates of ACC had a quadratic effect on the yield, with the highest rate of ACC (800 $\mu l \cdot L^{-1}$) over thinning and causing a significant reduction in yield. The yield of the treatment of 600 $\mu l \cdot L^{-1}$ ACC did not significantly differ from the control and had the best effect on fruit size out of all the ACC treatments. Therefore 600 $\mu l \cdot L^{-1}$ ACC was established as the recommended rate on 'African Rose™' (Theron et al., 2017b). ACC at rates of 200, 400 and 600 $\mu l \cdot L^{-1}$ was applied to 'Fortune', with the two highest rates significantly reducing the hand thinning required (Theron et al., 2017b). The yields were significantly lower compared to the control indicating that over thinning occurred. The lowest rate of ACC (200 $\mu l \cdot L^{-1}$) did not cause a significant thinning effect. The rate of 400 $\mu l \cdot L^{-1}$ resulted in over thinning but resulted in an increase in average fruit size. It was speculated that the ideal thinning rate for 'Fortune' would be somewhere between 200 and 400 $\mu l \cdot L^{-1}$ (Theron et al., 2017b). On 'Laetitia' plums the two highest rates of ACC (400 and 600 $\mu l \cdot L^{-1}$) significantly thinned fruitlets. The overall yield was not significantly affected by ACC (400 $\mu l \cdot L^{-1}$) compared to the control and significantly increased average fruit size. Therefore, 400 $\mu l \cdot L^{-1}$ was the recommended ACC rate on 'Laetitia' (Theron et al., 2017b). Severe leaf drop was observed on 'Laetitia' when ACC was applied at midday at temperatures exceeding 30 °C (Theron et al., 2017b).

Steenkamp (2015) conducted trials to evaluate the efficiency of ACC as a chemical fruit thinner on the nectarines 'Turquois', 'Alpine' and 'August Red'. All the foliar applications were made when the average fruit size was 8 - 10 mm in diameter. The only trial where a significant thinning effect was observed was on 'Turquois' where the highest rate of ACC (500 μl·L^{-1}) resulted in a significant thinning effect, although at this rate a significant reduction in yield was recorded, without a significant increase in fruit size. The two highest rates of ACC caused a significant increase in fruit firmness. In 'Alpine', none of the ACC treatments significantly reduced the fruit set compared to the untreated control, and slight ACC-induced leaf drop was observed. 'Alpine' showed a decrease in fruit firmness as the concentration of ACC increased. Steenkamp (2015) found no significant effect on fruit set or thinning requirement, yield or fruit size with ACC in 'August Red'. The only significant effect was that the harvest date shifted slightly earlier. 'August Red' displayed slight ACC-induced leaf drop, thus at this current stage ACC would not be recommended for thinning on nectarines (Steenkamp, 2015) although different timings of ACC application could be worth investigating.

S-Abscisic Acid (S-ABA). Giovanaz et al. (2015) evaluated S-ABA as a possible fruit thinner of 'Chiripá' peach. S-ABA was applied at different rates and at different times during fruit development. The time of S-ABA application was chosen to coincide with a fruit development stage which was based on the amount of endocarp lignification that had taken place. Giovanaz et al. (2015) therefore applied S-ABA at fruit growth stage 1, 2 and 3. A concentration of 500 mg•L^{-1} S-ABA was applied at stage 1 (24 DAFB), stage 2 (40 DAFB) and stage 3 (52 DAFB). The S-ABA application at stage 2 (40 DAFB) was the only treatment that was effective in reducing set and the number of fruit per tree. Giovanaz et al. (2015) then evaluated three different rates of S-ABA at 40 DAFB (350, 500, and 750 mg• L^{-1}) and all three applications increased fruit ethylene production while decreasing fruit set. Giovanaz et al. (2015) found leaf chlorosis following all three rates of S-ABA.

Einhorn and Arrington (2018) evaluated the combined effect of S-ABA and shading on gas exchange and fruit set of 10-year-old 'Bartlett' pear trees on OH×F 97 rootstock. S-ABA concentrations of 0 and 125 mg·L^{-1} were evaluated at 0, 44 and 77% shading. Einhorn and Arrington (2018) found that single leaf stomatal conductance was controlled by S-ABA alone, but both shade and S-ABA had an effect on Pn. At increasing levels of shade, Pn inhibition was not promoted by S-ABA, but by the shade alone. Fruit set was significantly reduced by both S-ABA and shading. The data suggests that S-ABA and shading have different mechanisms by which they decrease in fruit set (Einhorn and Arrington, 2018). Einhorn and Arrington (2018) concluded that shading caused a decrease in fruit set by limiting light supply and S-ABA caused a decrease in fruit set by limiting the CO_2 supply. When the 125 mg·L^{-1} S-

ABA application was combined with the highest amount of shading (77 %) the shading had the highest effect on reducing Pn. If S-ABA limited Pn by a hormonal effect an additive effect would have been observed, thus Einhorn and Arrington (2018) concluded that the effect of S-ABA on reducing Pn was likely due to a limiting effect on CO_2.

Greene (2012) evaluated the effect of S-ABA alone or in combination with 6-benzyl adenine on the fruit set and fruit quality of 'Bartlett' pears. Greene (2012) applied S-ABA at 50, 125, 250 and 500 mg·L^{-1} at full bloom, petal fall and at the 10 mm fruitlet diameter stage. Thinning was significant when applied at all three times. The later the application of S-ABA, the greater was the thinning efficacy. At the 10 mm fruitlet diameter stage the trees where nearly defruited. This stage of fruit development has frequently been cited as the stage where fruitlets are the most sensitive to thinners (Greene, 2002; Schwallier, 1996; Wertheim, 2000; Williams and Edgerton, 1981). Lakso et al. (2006) found that the 10 mm fruitlet diameter stage may also be a period where there is limited carbohydrate availability due to strong competition among competing sinks, which include growing fruit and rapidly growing shoots. As S-ABA is linked to stomatal function and closure, it makes sense that S-ABA will be very effective in causing fruit abscission during this stage as it would reduce the carbohydrate supply even further by limiting Pn through stomatal closure (Greene, 2012). S-ABA caused severe thinning, with rates of as low as 125 mg·L^{-1} judged to be excessive. The thinning treatments did significantly improve return bloom, increased fruit weight, diameter, flesh firmness and soluble solids at harvest. Excessive and commercially unacceptable leaf yellowing and abscission was noted after the S-ABA application, especially at 250 and 500 mg·L^{-1}. 6-BA treatments where unable to rectify this yellowing in contrast to what was found in other plant species (Greene, 2012).

Theron at al. (2017a) evaluated S-ABA on 'Forelle' pears in two trial sites over two seasons. S-ABA caused a significant thinning effect and increased fruit size when applied at rates of 200-300 mg·L^{-1} at both trial sites in the 2015/2016 season. In the 2016/2017 season there was a linear decrease in fruit set with an increase in S-ABA rate at one of the trial sites, which led to a decrease in yield when the highest concentration of S-ABA (600 mg·L^{-1}) was applied. All the S-ABA treatments, except for the lowest concentration (100 mg·L^{-1}), increased fruit weight at harvest. At the other trial site no significant effect on thinning or fruit set was obtained for any of the treatments. However, the two highest rates of S-ABA (400 and 600 mg·L^{-1}) increased average fruit weight at harvest. Theron et al. (2017a) concluded that S-ABA at 300 mg·L^{-1} applied at the 8-10 mm fruit diameter stage has potential as a chemical thinner for ‘Forelle’ pears.

Green et al. (2011) also evaluated the effects on S-ABA and 6-BA alone or in combination on ‘Fuji’ and ‘McIntosh’ apples. In this trial S-ABA thinned ‘McIntosh’ in 2 of the 3 and ‘Fuji’ in 1 of the 2 seasons. Temperatures were very similar during the time of application in all the seasons thus not explaining the differences in efficacy. In the years in which S-ABA thinned effectively, it was applied at petal fall, full bloom and 10 mm fruitlet diameter stage. Green et al. (2011) noted that S-ABA had no effect on return bloom even when a thinning effect occurred. Leaf yellowing was not quantified but did occur in ‘McIntosh’ and could be a deterrent in using it as a thinner. However, when S-ABA was applied together with 6-BA, leaf yellowing was reduced or eliminated. In 2003, S-ABA did not have a thinning effect but 6- BA did, but when 6-BA and S-ABA where used in combination the thinning effect of 6-BA was reduced. However, Greene et al. (2011) found that in 2004 when 6-BA and S-ABA were applied separately both had a thinning effect and when used in combination their thinning effect was additive, regardless of the time of application. In 2008, both 6-BA and S-ABA thinned ‘Fuji’ when applied separately; however, when combined there was no significant interaction between 6-BA and S-ABA. Thus Green et al. (2011) concluded that more work will be required to clearly define the relationship between S-ABA and 6-BA when used as a tank-mixed thinning programme.

7. Conclusion

Often a thinning action is needed in pome and stone fruit production in order to reach the maximum potential of a particular orchard. Currently hand thinning is still the most widely used method of thinning. However, with the cost and time constraints associated with hand thinning there is a need to look at alternative methods to thin. Chemical thinning shows huge potential to reduce the dependence on hand thinning. S-ABA and ACC shows potential as chemical thinning agents. However, there are many unanswered questions with regards to how climate or production area, timing, cultivar and dosage effect the efficacy of both S-ABA and ACC. Further studies are needed in order to optimise these two thinning agents in industry.

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PAPER 1: The Efficacy of 1-Aminocyclopropanecarboxylic Acid (ACC) as a Chemical Thinning Agent on Stone Fruit

***Abstract.* By optimizing thinning practices, annual cropping can be achieved. Currently most of the thinning is done by hand in the South African stone fruit industry. There is a great need to find chemical thinning strategies that are reliable and efficient and which could decrease the growers' dependence on hand thinning. The purpose of this study was to further evaluate the efficacy of 1-aminocyclopropane carboxylic acid (ACC) at a range of concentrations and phenological application stages, with the hope of finding an ideal concentration and application stage, for possible registration of ACC. In the 2017/2018 season, ACC was evaluated on the Japanese plums 'Laetitia' and 'Fortune' as well as on 'August Red' nectarines. ACC applied at a rate of 400 µl·L^{-1} at 8 - 10 mm fruit diameter gave promising results on 'Laetitia' and 'Fortune'. ACC significantly increased fruit size in 'Laetitia'. ACC significantly reduced the hand thinning requirement and increased fruit size for 'Fortune'. ACC was not an effective thinning agent for 'August Red' nectarines. ACC did not cause any negative effects on color development, fruit firmness and split pit in the 2017/2018 season. Promising results were found with ACC on 'Keisie' cling peaches. A rate of 400 µl·L^{-1} thinned significantly and increased fruit size. No negative effects on split pit were observed. Leaf drop did occur in the 'Keisie' trials, but was most severe at the 800 µl·L^{-1} application, which was only included for registration purposes. Based on these trials, the application stage should provisionally be at 4 - 6 mm fruitlet diameter.**

Key words: 1-aminocyclopropane-1-carboxylic acid (ACC), thinning, yield, leaf drop, fruit quality.

In the stone fruit industry, annual cropping of enough, good quality fruit is very important. One way to achieve this is by flower and fruit thinning (Stover, 2000). Thinning manipulates the crop load in order to increase fruit size and quality in the current season and to improve return bloom the following season (Wertheim, 2000). Peaches and nectarines are self-fertile, therefore do not need any cross pollination and most cultivars tend to set heavy crop loads, which necessitates a thinning action (Day and DeJong, 1998). Even though most

Japanese plum cultivars are not self-fertile, fruit set is often still too high and thinning is needed for the remaining fruit to reach an adequate size (Day and DeJong, 1998).

Hand thinning is a time consuming and expensive process. Therefore, most growers prefer to wait as long as possible for larger and smaller fruit to become easily distinguishable and thereby making it easier to thin small fruit selectively (Njoroge and Reighard, 2008). The resultant increase in fruit size due to thinning does not always compensate for the decrease in yield; therefore the balance between yield and fruit size should be such that economic returns are optimized (Njoroge and Reighard, 2008). However, continuous increases in labor costs together with the time constraints of hand thinning have led growers to search for other ways of thinning orchards (Stern and Ben-Arie, 2009). One of the most promising ways to thin fruit and flowers, which has become a standard practice in many pome fruit cultivars, is chemical thinning (Williams, 1994).

Various chemical thinning agents have been evaluated on stone fruit, but few have delivered consistent results. One strategy is to reduce flower induction in the preceding season with gibberellic acid (GA_3). Endogenous GA_3 is transported from the fruit to the nodes in its vicinity, inhibiting the initiation of new floral primordia (Webster and Spencer, 2000). Since GA_3 must be applied when flower bud induction can be inhibited, this developmental stage of every cultivar must be known in order for GA_3 application to be successful (Southwick and Glozer, 2000). It is not only the specific application period that has resulted in GA_3 not becoming a standard thinner, but also the fact that growers prefer to first evaluate the intensity of bloom or fruit set before thinning (Byers et al., 1990). The possibility of frost during bloom, which results in low fruit numbers, is another deterring factor.

Many growers would prefer to first observe the flower intensity as well as tree health before choosing a chemical thinning agent (Byers et al., 1990). Therefore, another strategy is to thin flowers in the current season by using caustic blossom thinners such as ammonium thiosulphate (ATS). ATS thins flowers by causing damage to the flower peduncle or other flower parts thus causing the desiccation of flowers. One advantage of blossom thinners is that damage to the flowers causes the early reallocation of assimilates to other sinks (Fallahi, 1997). Turk et al. (2014) conducted experiments using ATS on ‘Red Haven’ peaches and found 2% ATS thinned excessively. In one season, 1% ATS did not thin enough as it did not differ from the control, but in two other seasons it thinned too severely, and therefore Turk et al. (2014) concluded that ATS is an inconsistent thinner on ‘Red Haven’ peaches. Other caustic thinners like Tergitol-TMN-6 and hydrogen cyanamide (Fallahi et al., 2006) have been effective to an extent, but not to the point where they have become general practice.

The abscission of young fruit is correlatively driven (Bangerth, 2000). The abscission zone (AZ)-model states that the AZ must be activated in order for fruit drop to occur (Wertheim, 1997). Polar auxin transport, which prevents the activation of the AZ, is reduced as soon as the distal organ starts to senesce and produces more ethylene. Ethylene decreases the auxin concentration across the AZ by directly inhibiting auxin biosynthesis or by inhibiting auxin transport (Valdovinos et al., 1967). Thus ethylene is an abscission promoting hormone (Wertheim, 1997). However, for young fruit to senescence they must be pre-determined to drop by a correlative event (Bangerth, 2000). A decrease in auxin transport to the abscission zone, characteristic of senescent leaves, is not observed with young fruit (Bangerth, 2000). In apple fruit for example, auxin export from young fruit and transport to the AZ generally increases shortly after fruit set (Gruber and Bangerth 1990), whereas ethylene production drops during the same time (Blanpied 1972; Ebert and Bangerth 1985; Miller et al. 1988). Therefore, these fruit should not abscise. However, some fruit do abscise and this can be explained by correlatively-regulated abscission of young fruit by a “correlative dominance effect” of adjacent fruit or nearby shoot tips that correlatively inhibits IAA export from neighboring fruit (Bangerth, 2000).

No satisfactory chemical thinning in peach and nectarine have been achieved despite the numerous agents (3-CPA, CGA, Orthonil, Morphactins, NAA, NAAm, Ethrel) employed and the extensive body of research devoted to the subject (Costa and Vizzotto, 2000). Ethephon is an ethylene releasing product which has shown some interesting results, but has not been reliable enough to become a standard cultural practice (Costa and Vizzotto, 2000). Ambient temperatures greatly affects the thinning efficiency of ethephon (Jones et al., 1983). Meland (2007) evaluated ethephon as a chemical fruit thinner on ‘Victoria’ plum *(Prunus domestica* L.) applying 250 mg·L^{-1} at full bloom. Fruit set was significantly reduced, crop load decreased by ca. 50%, and fruit weight increased, soluble solids concentration and ground colour improved compared to untreated trees (Meland, 2007). Steenkamp (2015) evaluated ACC and 6-BA on ‘Keisie’ and ‘Sandvliet’ peaches, and concluded that 6-BA was not an effective thinner and would not be recommended on these cultivars. 1-Aminocyclopropane carboxylic acid (ACC) is a precursor of ethylene and was evaluated on the Japanese plums ‘Laetitia’, ‘African Rose™’ and ‘Fortune’ by Theron et al. (2017). They concluded that the recommended rates of ACC should be determined separately for each cultivar as they differed in sensitivity, to ACC, for example the recommended rates for ‘African Rose™’ are 600 $\mu l \cdot L^{-1}$ and for ‘Laetitia’ 400 $\mu l \cdot L^{-1}$ (Theron et al., 2017). Theron et al. (2017) could not establish a recommended rate of ACC for ‘Fortune’ and stated that further trials were needed to determine this, but suggested that the recommended rate would be between 200 and 400 $\mu l \cdot L^{-1}$ ACC. Steenkamp (2015) conducted trials on ‘Keisie’ and ‘Sandvliet’ peaches at the 8 - 10 mm fruitlet diameter stage at different rates. Steenkamp (2015) concluded that the preferred application rate of ACC for ‘Keisie’ peaches is 600 $\mu l \cdot L^{-1}$ at the 8 - 10 mm fruitlet diameter stage. Based on one season results, ACC is not recommended as a chemical thinner on ‘Sandvliet’ peaches due to phytotoxicity that could have been related to very high temperatures after application

In the following trials, ACC was evaluated on ‘Keisie’ cling peach, ‘August Red’ nectarine and ‘Laetitia’ and ‘Fortune’ Japanese plums. These cultivars were chosen as ‘Keisie’ peach is the most widely planted peach cultivar in South Africa contributing 24% of the total peach production, while ‘August Red’ nectarine is the second most widely planted nectarine cultivar in South Africa contributing 8% of the total nectarine production area and sets quite heavily, thus needing substantial thinning (HORTGRO, 2018). ‘Laetitia’ plum is the most widely panted Japanese plum cultivar in South Africa contributing 10% of the total production area while ‘Fortune’ is the fourth most widely planted cultivar and sets quite heavily, and makes up 7% of the total plum production area (HORTGRO, 2018). The aim of this study was to evaluate the thinning efficacy of ACC on these cultivars in order to see whether a recommended rate could be determined for ACC to be registered as a chemical thinner on stone fruit in South Africa.

Materials and Methods

Plant material, site description and treatments of the 2017/2018 season. In the 2017/2018 season, two trials were conducted on ‘Laetitia’ and ‘Fortune’, respectively. Both the trials were on the farm Goedemoed (33°50’51”S 19°57’57”E) in the Ashton-Robertson region in the Western Cape, South Africa. ‘Fortune’, on the rootstock ‘Maridon’, was planted in 2013 at a 5 x 0.75 m spacing. The cross pollinator in the orchard is ‘Angelino’ and the training system is a V-hedge. ‘Laetitia’, on the rootstock ‘Marianna’, was also planted at the same spacing in 2013 as a V-hedge with ‘Larry Ann’ as cross-pollinator. ‘August Red’, on the rootstock ‘SAPO 778’, was planted in the Koue Bokkeveld in the Western Cape, South Africa on the farm Bo-Bokfontein (32°49'14.4"S 19°16'01.2"E). Trees were planted at a spacing of 4 m x 1.5 m and trained as slender spindles.

For the plum trials, ACC (Valent BioSciences Corporation, Libertyville, Illinois 60048, USA) was evaluated as summarized in Table 1. One treatment was included using an older formulation of ACC in order to compare the efficacy to previous trials done with this older formulation. Dates of treatment application, hand thinning and harvest can be found in Table 2. For the 'August Red' nectarine trial ACC (Valent BioSciences Corporation, Libertyville, Illinois 60048, USA) was evaluated as summarized in Table 3 and dates of application, hand thinning and harvest can be found in Table 4.

Plant material, site description and treatments of the 2018/2019 season. In the 2018/2019 season, two trials were conducted on 'Keisie' peaches. One 'Keisie' trial was conducted on the farm La Plaisante (33°27'08"S 19°12'01"E) near Wolseley in the Western Cape, South Africa. This 'Keisie' orchard on the rootstock 'Flordaguard' was planted in 2015 at a tree spacing of 2.5 m × 4.5 m. The other 'Keisie' trial was conducted on the farm Lucerne (33°51'10"S 19°58'07"E) in the Robertson region in the Western Cape, South Africa. This 'Keisie' orchard on the rootstock 'GF 677' was planted in 1997 at tree spacing of 2.5 m × 5 m.

In both the 'Keisie' trials, ACC (Valent BioSciences Corporation, Libertyville, Illinois 60048, USA) was evaluated as summarized in Table 5. Dates of application, hand thinning and harvest can be found in Table 6.

Trial lay-out and treatment application: Randomized complete block designs were used with 10 single tree replications. Applications were made using a motorized knap sack sprayer (STIHL, Pietermaritzburg, South Africa) and product was applied at an equivalent of approximately 1000 L per ha. Treatments where applied early morning before the temperature exceeded 20 C° and when the wind speed did not exceed 3 $m \cdot s^{-1}$. Weather data during and after application is presented in Fig. 1 - 5. At least one tree was left untreated between the treated trees and at least one buffer row was left in between treated rows in order to prevent any drift effects.

Data collection. For all the trials the following procedure was followed. After the application of treatments, a period of at least two weeks was given in order for the fruitlets to drop. Leaf drop was recorded on a scale of 1 - 3, with 1 being no leaf drop and 3 being severe leaf drop. Fruit set was only determined for 'August Red' by tagging five one-year-old shoots (± 45 cm) on the lower half of the tree canopy in 2017/2018. At full bloom, the number of flowers on each shoot was counted and fruit number determined before hand thinning and expressed as percentage fruit set per shoot. Hand thinning was done according to standard commercial practices. All the fruitlets that were thinned by hand were collected and brought back to the laboratory where the number of fruitlets thinned per tree were calculated. At each commercial harvest date the yield of fruit per tree was recorded. After harvest the tree circumference was measured in order to calculate the tree cross sectional area. This was done in order to calculate the yield efficacy expressed as kg fruit per trunk cross sectional area ($kg \cdot cm^{-2}$). For the plum and nectarine trials a sample of 30 fruit per replicate per harvest was collected and brought back to the laboratory for destructive and non-destructive fruit quality analysis. For the 'Keisie' trials the same was done, but on a sample of 20 fruit. In the case of the 'August Red' and the 'Keisie' trial on La Plaisante, the first harvest was so small that fruit samples were not taken to the laboratory. For all fruit samples taken to the laboratory length, diameter and weight were recorded. Fruit color was determined for 'Laetitia' using the color chart PL. 25 (Deciduous Fruit Board) with values ranging from 1 to 12 where 12 = dark red. No color chart was available for 'Fortune' plums, so color was not recorded. On a sample of 15 fruit per harvest, firmness and split pit/broken stones were recorded per fruit. Split pit/broken stones was recorded as either present or not and expressed as a percentage of fruit from the sample displaying the disorder. Fruit firmness was measured for the 'August Red' trial using the GÜSS texture analyzer with an 11.1 mm probe (Güss electronic model GS 20, Strand, South Africa).

Statistical analysis. The data were analyzed using SAS Enterprise guide 7.1 (SAS Institute Inc., Cary, North Carolina, USA) using the linear model procedure and the pairwise t-test to determine the Least Significant Difference (LSD) when the F-statistic indicated significance at $P<0.05$. Single degree of freedom, orthogonal, polynomial contrast were fitted where applicable.

Results

Results from the 2017/2018 season: 'Laetitia'. None of the ACC treatments significantly influenced the number of individual fruitlets that had to be hand thinned during commercial hand thinning compared to the untreated control (Table 7). ACC at 200 and 800 $\mu l \cdot L^{-1}$ applied at 4 – 6 mm fruitlet diameter, as well as ACC at 800 $\mu l \cdot L^{-1}$ applied at the 8 – 10 mm fruitlet diameter were the only treatments which significantly influenced yield and yield efficiency compared to the untreated control (Table 7). All three of these treatments reduced yield and yield efficiency (Table 7). On average, the early application (4 - 6 mm fruitlet diameter) of ACC reduced yield per tree more than the second applications at the 8 - 10 mm fruitlet stage (Table 7). During the early applications the effect of rate of ACC was quadratic with the 400 $\mu l \cdot L^{-1}$ not reducing yield per tree. An increase in rate of ACC decreased yield per tree linearly with the later applications. The same effects were found in yield efficiency except that the effectiveness of early applications vs. later applications did not differ. The old and new formulation of ACC at 400 $\mu l \cdot L^{-1}$ did not significantly differ from each other in yield or yield efficiency (Table 7). Only the 4 - 6 mm fruitlet diameter application of 800 $\mu l \cdot L^{-1}$ ACC advanced the harvest, compared to the untreated control (Table 8). For both application times, the percentage fruit picked at the first harvest increased quadratically with an increase in ACC rate. The quadratic response in % fruit picked during the first harvest was due to the 200 and 400 $\mu l \cdot L^{-1}$ at the 4 - 6 mm stage being similar while at the 8 - 10 mm stage the two higher rates were similar in response (Table 8).

The only treatment which significantly affected average weight over both harvests compared to the untreated control, was the old formulation of ACC at 400 $\mu l \cdot L^{-1}$ at 8 – 10 mm fruit diameter which increased fruit weight over both harvests (Table 8). When the average fruit weight of both harvest where combined, the later applications on average increased fruit weight compared to earlier applications (Table 8). For the first harvest the same trend was observed, but for the second harvest there were no significant effects on fruit weight (Table 9 and 10). The new and old formulations of ACC at 400 $\mu l \cdot L^{-1}$, as well as 800 $\mu l \cdot L^{-1}$ all applied the 8 – 10 mm fruitlet diameter significantly increased fruit weight compared to the untreated control. At the first harvest date, fruit weight increased linearly with rate of ACC with the earlier applications, while this increase was quadratic at the later application stage with 400 and 800 $\mu l \cdot L^{-1}$ not differing from each other. ACC 200 $\mu l \cdot L^{-1}$ applied at the earlier stage significantly reduced fruit weight compared to the untreated control (Table 9). No such trends where observed at the second harvest date (Table 10). None of the treatments significantly affected average fruit length or -diameter at either harvest (Table 9 and 10). Treatments did not have a significant effect on fruit firmness, although a quadratic trend was observed after the first applications with 400 $\mu l \cdot L^{-1}$, fruit had a lower firmness than those receiving 200 or 800 $\mu l \cdot L^{-1}$ (Table 11). None of the treatments had a significant effect on fruit firmness at the second harvest or on fruit color at either harvest (Table 11 and 12). No leaf drop was observed in this trial.

Results from the 2017/2018 season: 'Fortune'. ACC on average reduced fruit set as indicated by the reduced hand thinning requirement (p=0.0123) (Table 13). The highest rates of ACC (800 $\mu l{\cdot}L^{-1}$) applied at both application timings, as well as the old formulation of ACC at 400 $\mu l{\cdot}L^{-1}$ applied at the 8 – 10 mm fruit diameter, significantly decreased the hand thinning requirement compared to the untreated control (Table 13). The earlier vs later application timing did not differ from each other, but with both timings the hand thinning requirement decreased linearly with increasing rate of ACC (Table 13). No significant differences were found in total yield or yield efficiency (Table 13). None of the treatments had a significant effect on average fruit weight, -length and -diameter except that a linear decrease in average fruit length was observed with an increase in ACC rate during the early application timing (Table 14). No significant differences were found in fruit firmness (Table 15). Leaf drop was observed when ACC was applied at 800 $\mu l{\cdot}L^{-1}$ during both application timings (Fig. 6).

Results from the 2017/2018 season: 'August Red'. The highest concentration of ACC applied at the earlier application time significantly decreased percentage fruit set on five tagged one-year-old shoots per tree compared to the untreated control (Table 16). There was a quadratic trend in percentage fruit set in the early application window with an increase in set up to an ACC rate of 600 $\mu l{\cdot}L^{-1}$ where after it decreased (Table 16). None of the treatments significantly influenced the number of fruitlets that were hand thinned during commercial hand thinning (Table 16). On average, significantly more fruit were harvested from ACC treated trees compared to the untreated control at the first harvest date (p= 0.0122) (Table 17). There was a linear increase in the percentage of fruit picked at the first harvest date with an increase in ACC rate following the early ACC applications, but no such trend was found following the later applications (Table 17). At the second harvest date a quadratic trend was observed in the percentage fruit picked following the later ACC applications, with significantly fewer fruit harvest from 600 $\mu l{\cdot}L^{-1}$ treated trees (Table 17). The reverse was observed at the third harvest date. A nearly significant linear (p=0.0526) decrease in percentage fruit picked during the third harvest was found with higher ACC rate during the early application window (Table 17).

ACC at 400 $\mu l{\cdot}L^{-1}$ applied at 8 - 10 mm fruitlet diameter significantly increased the total yield compared to the untreated control, but there were no significant differences between the different ACC treatments (Table 18). The yield efficiency per tree was higher when 600 $\mu l{\cdot}L^{-1}$ was applied at 8 - 10 mm fruitlet diameter compared to the same rate applied at 4 - 6 mm fruitlet diameter (Table 18). There were no significant differences in the average fruit weight of fruit from the two harvests combined compared to the untreated control (Table 18). The first harvest was too small for fruit to be sampled for fruit size measurements. No significant differences were found between treatments in the average fruit weight of the second and third harvest (Table 19 and 20). At the second harvest, average fruit diameter was smaller following the later 400 $\mu l \cdot L^{-1}$ ACC compared to the same treatment applied earlier (Table 19). The average fruit length decreased linearly with increase in early applied ACC rate at the second harvest (Table 19). No differences in fruit length or diameter were found at the third harvest (Table 20). No significant differences in fruit firmness at either harvest date (Table 21). ACC application on average increased the percentage fruit with split pit at both harvests (p= 0.0123 and 0.0052, respectively), but values were very low ranging from 0.3 to 1.0 and therefore not horticulturally significant (Table 22).

Results from the 2018/2019 season: 'Keisie' at Lucerne farm. The highest concentration applied at the early application and all the treatments applied at the later period significantly decreased the number of fruitlets thinned by hand compared to the untreated control (Table 23). There was a quadratic trend with the number of fruitlets that were thinned with an early application with ACC at 400 $\mu l \cdot L^{-1}$ needing more thinning than the 200 or 800 $\mu l \cdot L^{-1}$ applications. There was a linear decrease in the number of fruitlets thinned with an increase in ACC rate when treatments were applied at the later stage. On average, ACC applied at the later stage had a more significant thinning effect than treatments applied at the earlier stage (Table 23). Later applications caused a more significant increase in leaf drop than early applications (Table 23). The highest rate of ACC caused the most significant leaf drop during both application timings. There was a linear increase in leaf drop with an increase in concentration when treatments were applied at 4 - 6 mm fruitlet diameter while there was a quadratic trend with later applications where both the higher rates resulted in highest leaf drop (Table 23).

The highest rate of ACC applied at the later stage significantly decreased the average yield and yield efficiency per tree compared to all other treatments and the untreated control (Table 24). There was a quadratic increase in yield efficacy up to 400 $\mu l \cdot L^{-1}$ ACC when treatments were applied at the early stage while there was a linear decrease in total yield and yield efficiency with a later application. The later applications reduced yield and yield efficiency on average compared to the earlier applications (Table 24). The highest rate of ACC applied at the later stage significantly shifted the harvest distribution later compared to the untreated control and all the early applications (Table 25). A significant linear decrease in percentage fruit harvested during the first pick followed an increase in ACC rate applied at 8 - 10 mm fruitlet diameter. On average, average fruit weight of all harvests combined was decreased by the later ACC treatments compared to the early applications, while fruit weight decreased linearly with increasing ACC rate during the later application window. The highest rate of ACC applied at the later stage significantly decreased average fruit weight compared to the control (Table 25).

There were no significant differences in average fruit weight at the first harvest compared to the untreated control, but a linear decrease in fruit weight -length and -diameter was found with an increase in ACC rate for treatments applied during the later stage and the average fruit weight and length was lower on average for the later treatments compared to the earlier treatments (p=0.0492) (Table 26). At the first harvest, fruit diameter was larger on average following ACC treatment compared to the untreated control (p=0.0106) (Table 26). There were no significant differences in fruit weight at the second harvest (Table 27). On average, the fruit diameter and length was larger following the early application compared to the later application. Average fruit length decreased linearly with increasing rate of ACC for both the earlier and the later applications (Table 27). ACC at 200 and 800 $\mu l \cdot L^{-1}$ significantly increased percentage split pit compared to the untreated control (Table 28). Percentage split pit ranged from 0 to 2.5 % in the fruit from the first harvest, with a small quadratic increase in percentage split pit during the early applications (Table 28). During the first harvest, the earlier treatments application caused a small increase in percentage split pit on average compared to the later applications timing. No significant differences were found in the percentage split pit from the second harvest. These differences are not horticulturally significant (Table 28).

Results from the 2018/2019 season: 'Keisie' at La Plaisante farm. The concentrations of 400 and 800 $\mu l \cdot L^{-1}$ ACC applied at both application times significantly decreased the number of fruitlets which had to be hand thinned (Table 29). The highest concentration applied at the earlier stage caused the most significant thinning effect, although it did not differ from the same application applied at the later stage (Table 29). There was a linear decrease in the number of fruitlets thinned with an increase in ACC rate with early applications while there was a quadratic decrease with later applications where the reduction between 200 and 400 $\mu l \cdot L^{-1}$ ACC was steep. There was a quadratic increase in leaf drop with an increase in ACC rate for treatments applied at the early stage due to the lowest concentration not causing any drop while the increase was linear for treatments applied at the later stage. The earlier applications caused less severe leaf drop compared to the later applications. The highest concentration applied at the later stage caused the most significant leaf drop (Table 29).

The highest rate of ACC applied at both application times caused the most significant reduction in the yield and yield efficiency per tree (Table 30). Both the highest concentrations of ACC (800 μl·L^{-1}) applied at both applications timings, significantly decreased yield and yield efficiency compared to the untreated control (Table 30). There was a linear decrease in total yield and yield efficiency with an increase in concentration for both application periods (Table 30). A linear increase in percentage fruit picked during harvest one was found with an increase in ACC rate applied early (Table 31). For the second harvest a linear increase in percentage fruit picked was found with increasing ACC rate for both application times while this was reversed in the third harvest (Table 31). The average fruit weight (over the second and third harvest) was reduced by the high ACC rate in the second application window compared to the untreated control, the highest rate applied early and to the medium rate applied late (Table 31). The first harvest was so small that a sample of 20 fruit per tree could not be collected, thus fruit size and percentage split pit were not determined. There were no significant differences in fruit size (weight, diameter or length) at the second and third harvest (Table 32 and 33), although there was a linear increase in average fruit weight during the second harvest with an increase in ACC rate following the early applications (Table 32). There was no significant differences in percentage split pit at the second harvest (Table 34). During the third harvest there was a very small linear increase in percentage fruit with split pit from 0 to 2.9% with an increase in ACC rate when treatments were applied at the earlier stage (Table 34).

Discussion

'Laetitia'. None of the ACC treatments, not even 800 μl·L^{-1}, significantly reduced the hand thinning requirement. This is in contrast to previous trials with ACC on 'Laetitia' where rates of 400 and 600 μl·L^{-1} caused significant thinning (Theron et al., 2017). The 800 μl·L^{-1} ACC was included in the trial as a double rate for possible registration of ACC at 400 μl·L^{-1}. The reason why ACC did not seem to induce fruitlet abscission could be that the hand thinning was done too early before fruit drop was completed. This could also explain why yield was reduced by some ACC applications indicating that some fruit might have dropped subsequent to hand thinning. ACC thinned 'African Rose™' more severely when applied at an earlier application timing (4 - 6 mm fruitlet diameter) (Theron et al., 2017). In this trial, earlier applications (4 - 6 mm fruit diameter stage) caused a slight decrease in yield compared to the

later applications. This supports the theory that hand thinning was implemented too soon in the orchard, as it appears that there was indeed some thinning effect. However the newer and older formulations of ACC at 400 $\mu l \cdot L^{-1}$ did not significantly affect yield and yield efficiency, thus ACC at 400 $\mu l \cdot L^{-1}$ did not reduce the crop load below the optimum level. There was a slight advancement in harvest maturity, coinciding with the largest reduction in yield (ACC 800 $\mu l \cdot L^{-1}$ at the 4 – 6 mm fruit diameter stage). Steenkamp (2015) noticed a similar effect of ACC on 'Keisie' peaches and Wünsche et al. (2000) on apples. Prior to the ACC applications at 4 - 6 mm fruit diameter, there was a heat wave with temperatures rising above 35°C (Fig. 1). Although temperatures during and three days after the 4 - 6 mm fruit diameter application, were more mild ranging from about 24 to 27 °C, it is possible that the fruitlets had not yet recovered from the carbohydrate deficit, caused by the heat wave, which could explain the yield reduction that occurred at this application timing (Fig. 1).

A well-known thinning response is an increase in fruit size of thinned trees compared to un-thinned trees (Costa and Vizzotto, 2000). We also found that the later applications on average caused a bigger increase in average fruit weight than the earlier applications probably due to a decrease in yield (Costa and Vizzotto, 2000). Theron et al. (2017) found a positive effect on fruit size when ACC was applied at 400 and 600 $\mu l \cdot L^{-1}$ on 'Laetitia'. In this trial, 400 $\mu l \cdot L^{-1}$ applied at 8 - 10 mm fruitlet diameter was the most effective treatment, even though there was no significant thinning effect at the time hand thinning was performed, fruit weight was significantly increased without decreasing yield or yield efficiency. No negative effects were observed on fruit firmness, color development or leaf drop. This concurs with the recommended rate for 'Laetitia' of 400 $\mu l \cdot L^{-1}$ (Theron et al., 2017).

'Fortune'. On average, ACC reduced fruit set compared to the untreated control and the hand thinning requirement decreased linearly with an increasing rate of ACC during both application windows, which is in agreement with Theron et al. (2017). In addition, Schupp et al. (2012) reported that thinning increased linearly with an increase in ACC rate when applied to 'Golden Delicious' apple trees. Schupp et al. (2012) measured daily light and temperature data for calculating the MaluSim carbon balance model (Lakso et al., 2000). Schupp et al. (2012) found that periods of severe carbohydrate stress coincided with both the 10 mm and 20 mm fruit diameter applications in one season, and ACC subsequently reduced crop load at both application timings below optimal. During this trial, the maximum temperature was 18 °C on the day of application at the 4 - 6 mm fruit diameter stage and rose to a maximum of 29 °C three days after the treatments (Fig. 2). During the 8 - 10 mm fruit diameter applications,

maximum temperatures where considerably higher, with 35 ℃ on the day of application and 24 ℃ three days after the applications (Fig. 2). The reason why yield and yield efficiency was not significantly reduced when ACC was applied during these high temperatures could be due to the minimum temperatures not being very high. Therefore, respiration at night was not too high thus not causing a severe carbohydrate deficit within the trees.

In contrast to what Theron et al. (2017) found, ACC did not over thin in this trial, as yield and yield efficiency was not significantly affected. Trees with lower crop loads tend to have larger fruit compared to trees with higher crop loads (Pavel and DeJong, 1993), but in this trial none of the treatments affected yield and yield efficiency, which explains why no significant effects on average fruit weight, -length and -diameter are reported. The efficacy of the earlier applications were not better than the late applications. One could expect the earlier thinning to benefit fruit size as competing fruitlets were removed earlier, but this was not the case. Theron et al. (2017), however, found significant increases in fruit size with ACC at 400 and 600 $\mu l \cdot L^{-1}$, but as mentioned earlier, these treatments had over-thinned. ACC at 800 $\mu l \cdot L^{-1}$ was included in this trial as a double rate for the possible registration of ACC at 400 $\mu l \cdot L^{-1}$ on 'Fortune'. The new formulation of ACC at 400 $\mu l \cdot L^{-1}$ lowered the number of fruitlets that had to be hand thinned at both application windows, although not significantly compared to the untreated control. While the old formulation of ACC at 400 $\mu l \cdot L^{-1}$ applied at the 8 – 10 mm fruit diameter stage did cause a significant thinning effect compared to the untreated control, but did not differ from the same new formulation applied at the same rate and timing. Thus, considering previous results (Theron et al., 2017), it would appear that 400 $\mu l \cdot L^{-1}$ ACC is indeed the appropriate concentration for 'Fortune'. The thinning effect of the 400 $\mu l \cdot L^{-1}$ treatment applied at the 8 – 10 mm fruit diameter stage did not differ significantly from the double rate (800 $\mu l \cdot L^{-1}$) applied at the early application time. Thus the 400 $\mu l \cdot L^{-1}$ application at 8 – 10 mm had a slightly stronger thinning effect, albeit not significantly so, than when applied at the 4 - 6 mm fruit diameter stage. The 8 – 10 mm fruit diameter stage therefore appears to be the appropriate application time for 400 $\mu l \cdot L^{-1}$ ACC on 'Fortune'. ACC did cause leaf drop when applied at 800 $\mu l \cdot L^{-1}$. However, as mentioned before, this rate was included as a double dose rate for registration purposes and commercially ACC would not be applied at such high rates. In this trial, ACC did not cause any negative effects on fruit firmness, or fruit color development, which could prohibit registration on this cultivar.

'August Red'. ACC at 800 µl·L^{-1} applied at 4 - 6 mm fruitlet diameter was the only treatment that significantly decreased the percentage fruit set on tagged one-year-old shoots compared to the untreated control. It must be noted that this treatment did not differ significantly from the other ACC treatments except 600 µl·L^{-1} ACC applied at the same time, and overall ACC treatments did not differ from the untreated control (p=0.1701). The percentage fruit set for the different ACC treatments ranged from 88.30 to 94.73 percent. Thus the decrease in fruit set percentage was relatively small and could possibly be due to natural variation within the orchard rather than being ACC-induced, also considering that the hand thinning requirement was not affected by any treatments. The fruit set was determined on one-year-old shoots in the lower part of the tree canopy while the hand thinning requirement gives an indication of fruit set throughout the whole tree canopy and is a better indication of the efficacy of chemical thinning applications. Temperatures during both application times were conducive to ACC action (Fig. 3), as maximum temperatures ranged from 27 to 17 °C during and three days prior to treatment application at 4 - 6 mm fruit diameter, while during and three days after the 8 - 10 mm fruit diameter application, maximum temperatures ranged from 22 to 25 °C (Fig. 3). Steenkamp (2015) also found no effect of ACC on percentage fruit set or hand thinning requirement on 'August Red' and 'Alpine' nectarines, although the highest concentration of ACC used in his trials was 600 µl·L^{-1} ACC applied at 8 - 10 mm fruitlet diameter. Ceccarelli et al. (2014) evaluated ACC on 'Stark Red Gold' nectarines at a high concentration of 750 µl·L^{-1} applied at petal fall and 20 mm fruit diameter. They reported that even at such high rate ACC had no significant thinning effect on 'Stark Red Gold' nectarines. In our trial, significantly more 'August Red' fruit were harvested from ACC treated trees compared to the untreated control at the first harvest date, although it should be noted that this harvest was small. There was an increase in the percentage of fruit picked at the first harvest date with an increase in ACC rate following the early ACC application, but no such trend was found following the later applications. At the second harvest date a quadratic trend was observed in the percentage fruit picked following the later ACC application, with significantly fewer fruit harvested from 400 µl·L^{-1} treated trees . The reverse was observed at the third harvest date. This advancement in fruit maturity was also noticed in 'Laetitia' as discussed earlier. ACC treatments did not differ in yield per tree and only 400 µl·L^{-1} ACC applied at the 8 - 10 mm diameter increased yield compared to the untreated control, while yield efficiency was higher in 600 µl·L^{-1} ACC applied late compared to the same application early. It is difficult to explain these small differences in yield. Due to ACC not effecting the crop load to a large

degree, no significant differences in fruit size were expected (Pavel and DeJong, 1993). As per expectation, ACC did not have a significant effect on fruit size at either harvest date, except for a slight decrease in fruit size (length and diameter, but not weight) following the late 400 $\mu l \cdot L^{-1}$ ACC application possibly due to the slightly higher yield of this treatment. Steenkamp (2015) also did not find an effect on fruit size. Results from this trial, as well as previous trials of ACC on 'August Red' and 'Alpine' nectarines by Steenkamp (2015) and 'Stark Red Gold' nectarines by Ceccarelli et al. (2014), indicate that ACC might not be an effective chemical thinning agent on 'August Red' when applied at 4 - 6 or 8 - 10 mm fruitlet diameter. As some leaf drop was observed, it may be interesting to evaluate ACC on 'August Red' at the pink bud stage or during full bloom before the start of vegetative development.

'Keisie'. In both trials, the 800 $\mu l \cdot L^{-1}$ treatment caused the most significant thinning effect. At the Lucerne site later applications thinned more severely than earlier applications. It should be noted that maximum temperatures where considerably higher in the three days following the later applications compared to the earlier applications (Fig. 4 and 5), with temperatures reaching as high as 38 °C at the Lucerne trial site following the later application (Fig. 4). Yoon et al. (2008) evaluated the effect of temperature as well as irradiance on 6-BA plus carbaryl, and NAA plus carbaryl efficacy on 'Empire' apple trees and found that temperature following a chemical thinner application is a dominant factor with higher temperatures intensifying the response to the chemical thinner. Warm temperatures also intensify the competition between sinks during a time where there is a great demand for reserves (Greene, 2002). Yoon et al. (2008) concluded that the effects of temperature, shade and chemical application is triggered at, or just prior to three days after the treatment application. Thus it could be expected that this increase in temperature could contribute to the thinning response following the later application. In both trials, a linear (sometimes quadratic) increase in thinning action occurred with an increase in rate of ACC during both application windows. This is in agreement with Steenkamp (2015) who also evaluated ACC on 'Keisie' peaches and found a linear thinning response with an increase in ACC rate at 8 - 10 mm fruitlet diameter. Ceccarelli et al. (2014) also found an increased thinning response with an increase in ACC application when applied at petal fall on 'Flaminia' peaches. Schupp et al. (2012) found a linear response in thinning efficacy in 'Golden Delicious' apples with increasing rates of ACC from 100, 300, and 500 $mg.L^{-1}$.

Later applications caused a more severe incidence of leaf drop than early applications at both trial sites. This is expected as the leaf surface area is more developed during the later application window. The highest rate of ACC (the double rate for registration purposes) caused the most severe leaf drop during both application timings. The trends in leaf drop response to ACC was the same as the trends in fruit thinning response at both trial sites. Steenkamp (2015) found that adding 6-BA to ACC did not reduce the occurrence of leaf drop. However, the addition of 6-BA caused an additional thinning response in one trial. Thus the addition of 6-BA could possibly result in over thinning when ACC is applied at higher rates and it did not combat reducing leaf drop.

The highest rate of ACC applied at the later stage significantly decreased the total yield. The fruit thinning effect of increasing rate of ACC was generally reflected in yield and yield efficiency per tree at both trial sites. The effect was more severe at La Plaisante where a young, 4-year-old orchard was used compared to the 22-year-old orchard at Lucerne. The decrease in yield, especially with the double rate of 800 $\mu l \cdot L^{-1}$ ACC, with an increase in thinning action indicates that the thinning action was too severe and that over-thinning occurred. Steenkamp (2015) evaluated ACC on 'Sandvliet' peaches and found that ACC caused a decrease in yield and fruit set, without significantly effecting the number of fruitlets thinned. Steenkamp (2015) found that ACC-induced leaf drop was more severe in 'Sandvliet' peaches and speculated that the yield could have been decreased due to over-thinning by the team of labors as more fruitlets could be seen due to the leaf drop. This could have also been a possible reason or contribution to the decrease in yield in our 'Keisie' trials.

At the Lucerne trial site the highest rate of ACC applied at the later timing significantly shifted the harvest distribution later compared to the untreated control. A significant linear decrease in percentage fruit harvested during the first pick occurred following a higher ACC rate applied at 8 - 10 mm fruitlet diameter. This is in contrast to what was observed at the La Plaisante trial site where a linear increase in the percentage fruit picked during the first harvest occurred with an increase in ACC during the early application. Thus harvest maturity was advanced in ACC treated trees at one site but delayed at the second. The advancement in maturity is expected when yield is decreased as discussed earlier (Steenkamp, 2015; Wünsche et al., 2000). Why it did not occur at Lucerne is unclear.

An increase in average fruit size would be expected with a decrease in yield (Pavel and DeJong, 1993; Costa and Vizzotto, 2000). However, the 800 $\mu l \cdot L^{-1}$ application applied at the later application window, which caused over thinning in both trials, significantly decreased the combined average fruit weight of all harvests compared to the untreated control. This was not expected, but could be due to the leaf drop. The high occurrence of leaf drop could have resulted in the trees not producing enough carbohydrates throughout the season to sustain adequate fruit growth. Once again it should be noted that the 800 $\mu l \cdot L^{-1}$ application was only included as a double rate for registration purposes and would not be recommended commercially. Overall, when average fruit size was calculated for each harvest date separately, most of the ACC effects on fruit size were not significant, when applied at 200 or 400 $\mu l \cdot L^{-1}$. Steenkamp (2015) also did not observe any significant increases in fruit size. Ceccarelli et al. (2014) found that ACC treated 'Flaminia' peaches, which did not receive additional hand thinning after treatment application, performed similarly with regards to fruit size than trees that only received hand thinning. Theron et al. (2017) also found that ACC had a positive effect on fruit size of 'African Rose™', 'Laetitia' and 'Fortune' plums, were fruit size increased with an increase in thinning effect.

Thus it appears that ACC at 400 $\mu l \cdot L^{-1}$ would be the recommended rate for 'Keisie' peaches. With regard to the application timing, the later application at 8 - 10 mm fruitlet diameter had a more significant thinning effect than the earlier applications at 4 - 6 mm fruitlet diameter, but possibly due to higher maximum temperatures and not necessary due to higher sensitivity to ACC *per se*. However, yields where drastically reduced at the La Plaisante trial site. Since this young orchard was severely over thinned by ACC, it appears that the ACC treatments were too harsh for these younger trees. Therefore ACC should be used with caution on young orchards and at a reduced rate. ACC might be better suited to orchards in full production. Considering that yield was significantly reduced at the later application stage compared to the earlier application stage, the 4 - 6 mm fruitlet diameter stage would be advisable. Temperature should not only be considered on the day of application but also up to at least three days subsequent to application (Yoon et al., 2008). Provisionally, from these trials, the 4 - 6 mm fruitlet diameter stage would be the advisable timing, with temperatures ideally ranging between 18 and 28 °C. ACC generally did not have any significant negative side effects e.g. on the percentage of fruit with split pit.

Conclusion

From these trials as well as previous trials conducted by Theron et al. (2017), the results of ACC on the Japanese plums 'Laetitia' and 'Fortune' showed promise as a possible thinning agent. ACC on 'Laetitia' caused an increase in fruit size, and on 'Fortune' caused a significant thinning effect without significantly effecting yield. ACC at 400 μl·L^{-1} applied at the 8 – 10 mm fruit diameter stage would be the recommended rate and application timing for both cultivars. From these trials as well as previous research (Steenkamp, 2015), ACC does not appear to be an effective thinning agent on 'August Red' nectarines. For 'Keisie' cling peaches ACC at 400 μl·L^{-1} consistently gave promising results and would be the recommended rate at the 4 – 6 mm fruitlet diameter.

McArtney and Obermiller (2012) evaluated ACC alone or in combination with metamitron on apples, and generally found that the thinning effect was additive when treatments where combined. Thus an option could be to combine ACC with other chemical thinning agents to increase the overall thinning effect whilst reducing possible negative effects on leaf drop. Another possible option for the use of ACC on peaches could be to apply ACC at pink bud stage, before leaves have developed, as this could significantly reduce the negative effects of ACC on leaf drop. ACC in combination with other chemical thinning agents and ACC applied at pink bud stage should therefore be considered for future research. When ACC is applied at the 8 - 10 mm fruit diameter stage, temperature should be considered to prevent excessive thinning and leaf drop.

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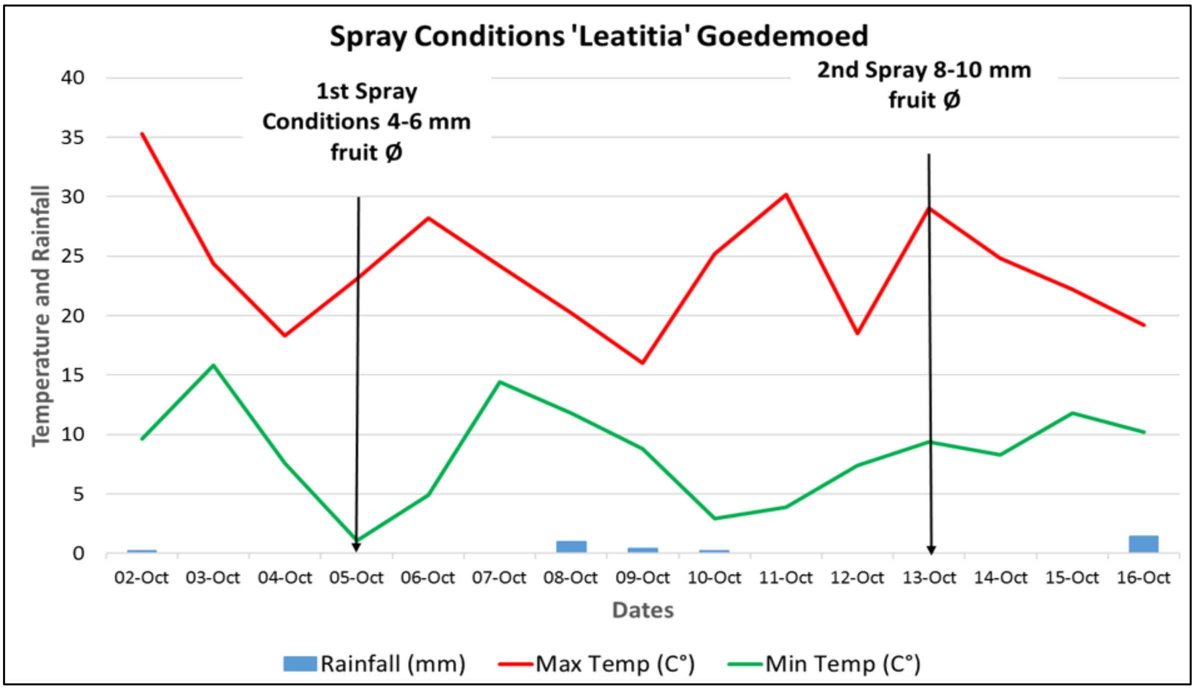

Fig. 1. Temperature and rainfall during 1-aminocyclopropane carboxylic acid (ACC) application for 'Laetitia' plums at Goedemoed, Ashton-Robertson region, South Africa (2017/2018).

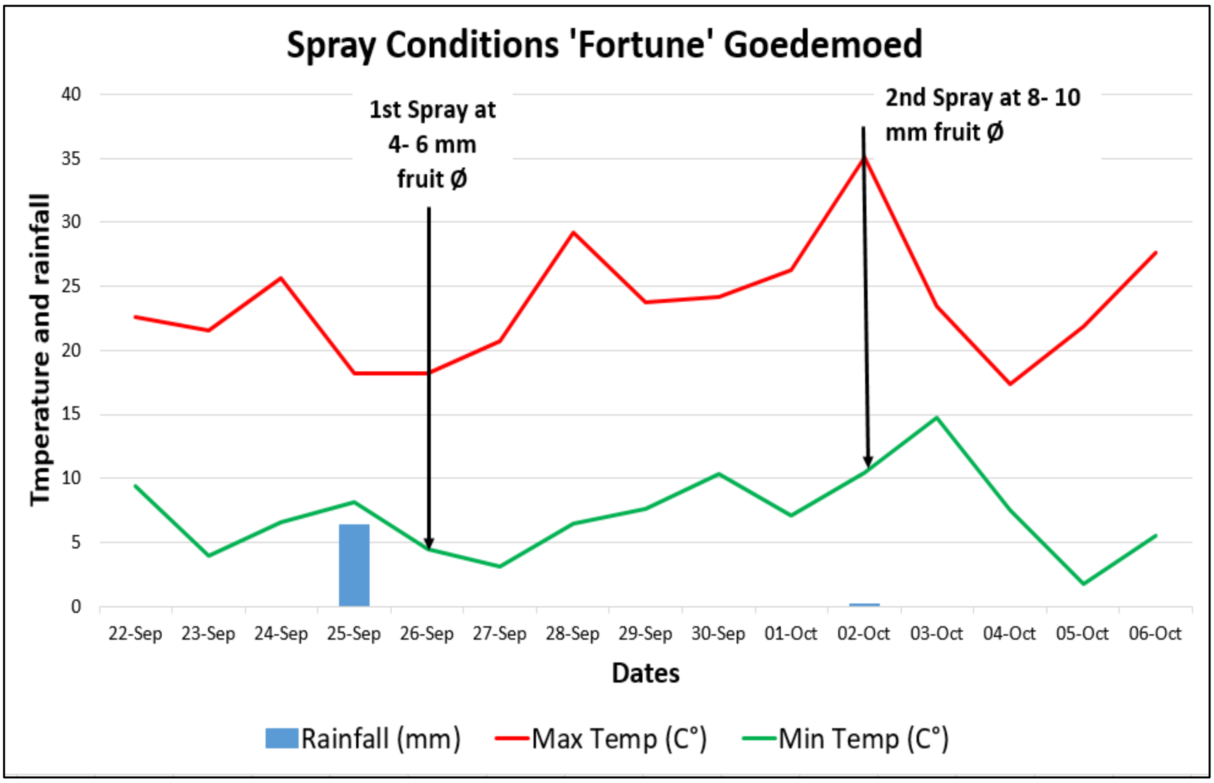

Fig. 2. Temperature and rainfall during 1-aminocyclopropane carboxylic acid (ACC) application for 'Fortune' plums at Goedemoed, Ashton-Robertson region, South Africa (2017/2018).

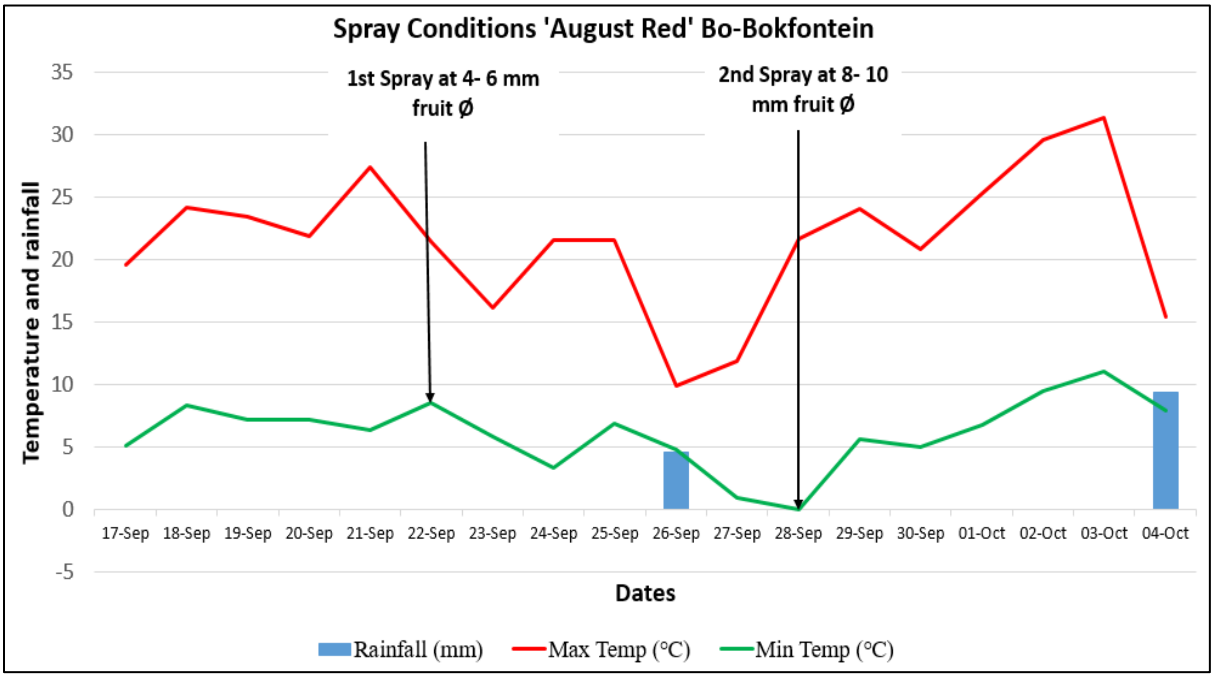

Fig. 3. Temperature and rainfall during 1-aminocyclopropane carboxylic acid (ACC) application for ‘August Red’ nectarines at Bo-Bokfontein, Koue Bokkeveld district, South Africa (2017/2018).

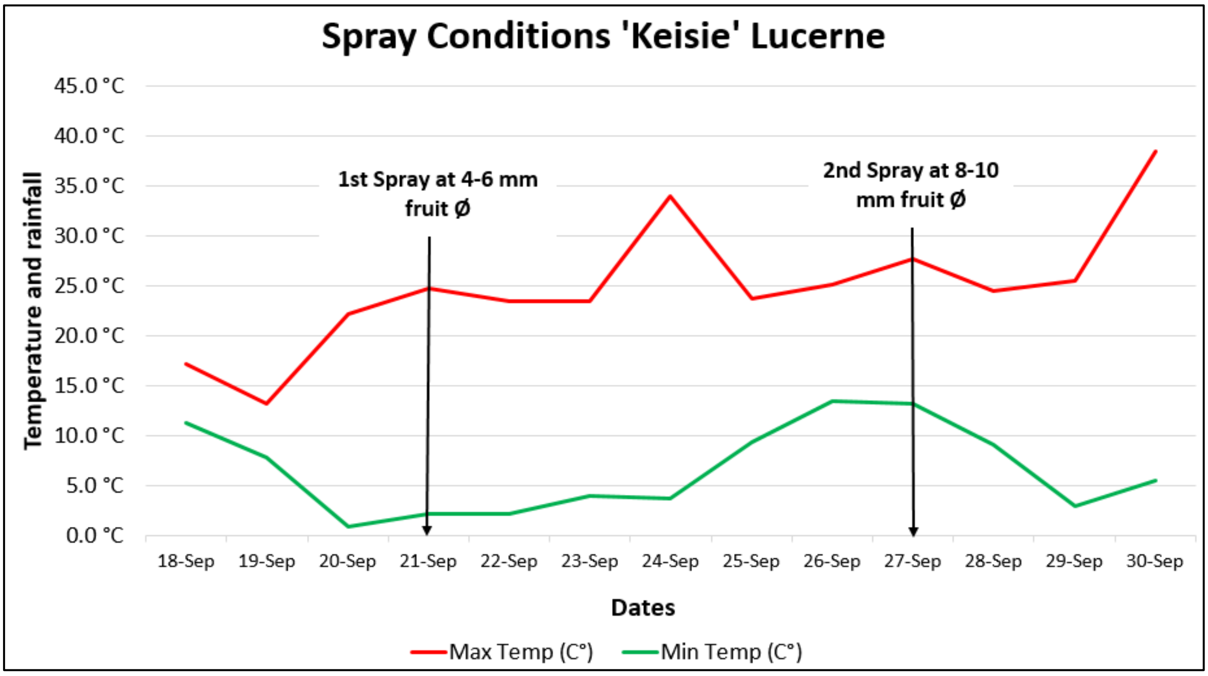

Fig. 4. Temperature and rainfall during 1-aminocyclopropane carboxylic acid (ACC) application for ‘Keisie’ peaches at Lucerne, Robertson district, South Africa (2018/2019).

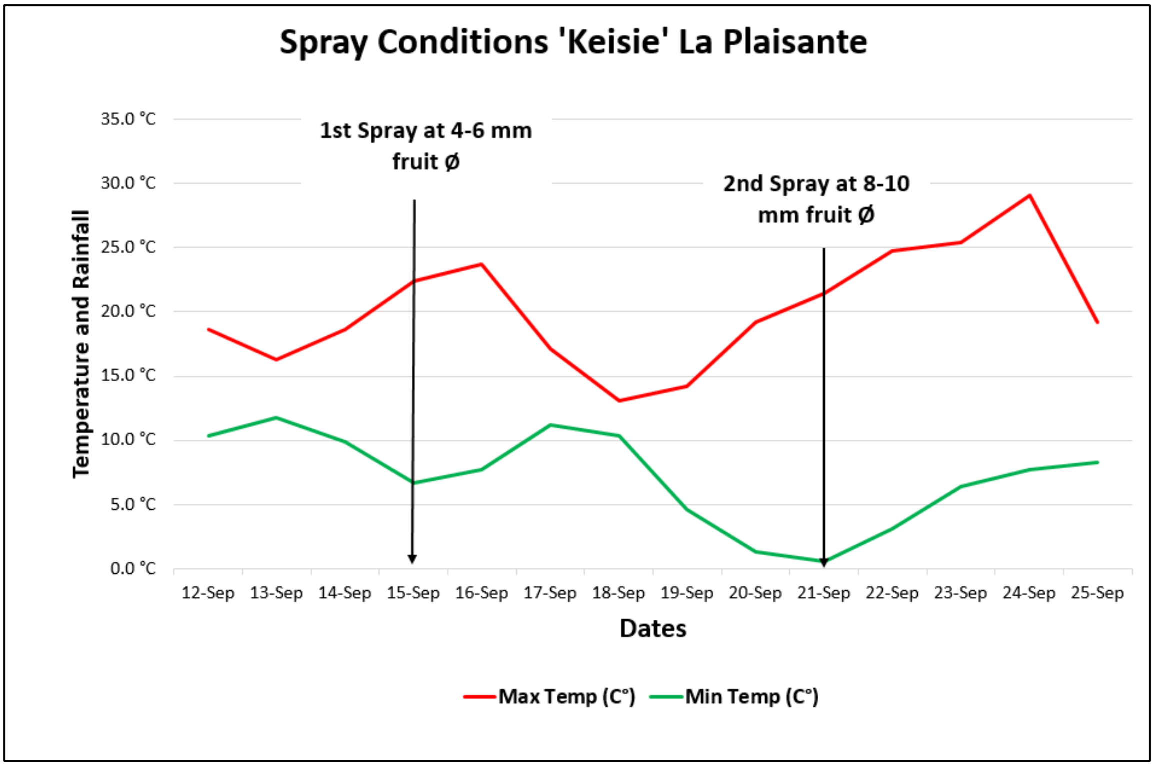

Fig. 5. Temperature and rainfall during 1-aminocyclopropane carboxylic acid (ACC) application 'Keisie' peaches at La Plaisante, Wolseley district, South Africa (2018/2019).

Fig. 6. Effect of 1-aminocyclopropane carboxylic acid (ACC) at 800 $\mu l \cdot L^{-1}$ applied at 4 - 6 mm fruitlet diameter compared to the control on leaf drop for 'Fortune' plums at Goedemoed, Ashton-Robertson region, Western Cape (2017/2018).

Table 1. Treatment specifications for trials done with 1-aminocyclopropane carboxylic acid (ACC) on ‘Laetitia’ and ‘Fortune’ plums in the season of 2017/2018.

Treatments
Untreated
ACC 200 $\mu l \cdot L^{-1}$ at 4-6 mm fruit diameter
ACC 400 $\mu l \cdot L^{-1}$ at 4-6 mm fruit diameter
ACC 800 $\mu l \cdot L^{-1}$ at 4-6 mm fruit diameter
ACC 200 $\mu l \cdot L^{-1}$ at 8-10 mm fruit diameter
ACC 400 $\mu l \cdot L^{-1}$ at 8-10 mm fruit diameter
ACC 800 $\mu l \cdot L^{-1}$ at 8-10 mm fruit diameter
ACC 400* $\mu l \cdot L^{-1}$ at 8-10 mm fruit diameter

*Old formulation of ACC

Table 2. Summary of dates of treatment application, follow up hand thinning and harvest dates for ‘Laetitia' and ‘Fortune’ plums at Goedemoed, Ashton-Robertson region, Western Cape (2017/2018).

Phenological stage	‘Laetitia'	‘Fortune'
1st spray	5 Oct. 2017	26 Sep. 2017
2nd spray	13 Oct. 2017	2 Oct. 2017
Hand thinning	26 Oct. 2017	17 Oct. 2017
1st Harvest	22 Jan. 2018	03 Jan. 2018*
2nd Harvest	5 Feb. 2018	-

* All fruit harvested on one day

Table 3. Treatment specifications for trials done with 1-aminocyclopropane carboxylic acid (ACC) on ‘August Red’ nectarines at Bo-Bokfontein, Koue Bokkeveld district, South Africa (2017/2018).

Treatments
Untreated
ACC 400 $\mu l \cdot L^{-1}$ at 4 - 6 mm fruit diameter
ACC 600 $\mu l \cdot L^{-1}$ at 4 - 6 mm fruit diameter
ACC 800 $\mu l \cdot L^{-1}$ at 4 - 6 mm fruit diameter
ACC 400 $\mu l \cdot L^{-1}$ at 8 - 10 mm fruit diameter
ACC 600 $\mu l \cdot L^{-1}$ at 8 - 10 mm fruit diameter
ACC 800 $\mu l \cdot L^{-1}$ at 8 - 10 mm fruit diameter

Table 4. Summary of dates of treatment application, follow up hand thinning and harvest dates for 'August Red' nectarines at Bo-Bokfontein, Koue Bokkeveld district, South Africa (2017/2018).

Phenological stage	'August Red'
1st spray	22 Sep. 2017
2nd spray	28 Sep. 2017
Hand thinning	27 Oct. 2017
1st Harvest	20 Feb. 2018
2nd Harvest	27 Feb. 2018
3rd Harvest	2 Mar. 2018

Table 5. treatment specifications for trials done with 1-aminocyclopropane carboxylic acid (ACC) on 'Keisie' peaches at Lucerne, Robertson district, South Africa (2018/2019).

Treatments
Untreated
ACC 200 µl·L^{-1} at 4 - 6 mm fruit diameter
ACC 400 µl·L^{-1} at 4 - 6 mm fruit diameter
ACC 800 µl·L^{-1} at 4 - 6 mm fruit diameter
ACC 200 µl·L^{-1} at 8 - 10 mm fruit diameter
ACC 400 µl·L^{-1} at 8 - 10 mm fruit diameter
ACC 800 µl·L^{-1} at 8 - 10 mm fruit diameter

Table 6. Summary of treatment dates and harvest dates of 'Keisie' peaches at Lucerne, Robertson district and at La Plaisante, Wolseley South Africa (2018/2019).

Phenological stage	Keisie	Keisie
1st chemical application	21 Sep. 2018	15 Sep. 2018
2nd chemical application	27 Sep. 2018	21 Sep. 2018
1st harvest	17 Jan. 2019	3 Jan. 2019
2nd harvest	23 Jan. 2019	9 Jan. 2019
3rd harvest	-	18 Jan. 2019

Table 7. Effect of 1-aminocyclopropane carboxylic acid (ACC) on hand thinning requirement, yield and yield efficiency of 'Laetitia' plums at Goedemoed, Ashton-Robertson region, Western Cape (2017/2018).

Treatment	Time of application	Average number of fruitlets thinned by hand		Average total yield per tree (kg)		Average total yield efficiency (kg.cm^{-2})	
Untreated	No application	374	ns	21.8	a	0.39	a
ACC 200	4-6 mm fruit diam.	393		16.9	cd	0.31	bc
ACC 400	4-6 mm fruit diam.	493		20.5	ab	0.40	a
ACC 800	4-6 mm fruit diam.	329		15.8	d	0.27	c
ACC 200	8-10 mm fruit diam.	381		20.4	ab	0.38	a
ACC 400	8-10 mm fruit diam.	344		21.1	a	0.36	ab
ACC 800	8-10 mm fruit diam.	343		17.9	bcd	0.31	bc
ACC 400*	8-10 mm fruit diam.	289		19.5	abc	0.34	ab
Significance level		*0.3116*		***<.0001***		***<.0001***	
LSD %		-		*2.7*		*0.06*	
ACC vs Control		*0.9415*		***0.0057***		***0.0338***	
1st vs 2nd application		*0.4641*		***0.0088***		*0.1555*	
ACC 1st Linear		*0.4320*		*0.1316*		***0.0216***	
ACC 1st Quadratic		*0.2395*		***0.0012***		***0.0004***	
ACC 2nd Linear		*0.7698*		***0.0353***		***0.0336***	
ACC 2nd Quadratic		*0.8158*		*0.2064*		*0.7237*	

*Old formulation of ACC (VBC 30160)

Table 8. Effect of 1-aminocyclopropane carboxylic acid (ACC) on percentage fruit picked at the first harvest and average fruit weight of over both harvests for 'Laetitia' plums at Goedemoed, Ashton-Robertson region, Western Cape (2017/2018).Treatment

	Time of application	% Fruit picked at first harvest		Average fruit weight over both harvests (g)	
Untreated	No application	42.4	b	95.7	bcd
ACC 200	4-6 mm fruit diam.	44.9	b	91.1	d
ACC 400	4-6 mm fruit diam.	41.2	b	93.6	cd
ACC 800	4-6 mm fruit diam.	66.2	a	92.1	d
ACC 200	8-10 mm fruit diam.	41.0	b	94.4	cd
ACC 400	8-10 mm fruit diam.	48.0	b	100.0	abc
ACC 800	8-10 mm fruit diam.	49.2	b	102.4	ab
ACC 400*	8-10 mm fruit diam.	48.6	b	103.7	a
Significance level		***<.0001***		***0.0333***	
LSD 5%		*8.5*		*7.7*	
ACC vs Control		*0.0692*		*0.9787*	
1st vs 2nd application		*0.0588*		***0.0040***	
ACC 1st Linear		***<.0001***		*0.8630*	
ACC 1st Quadratic		***0.0055***		*0.5279*	
ACC 2nd Linear		*0.0830*		*0.0571*	
ACC 2nd Quadratic		***0.0055***		*0.5279*	

*Old formulation of ACC (VBC 30160)

Table 9. Effect of 1-aminocyclopropane carboxylic acid (ACC) on fruit size at the first harvest of 'Laetitia' plums at Goedemoed, Ashton-Robertson region, Western Cape (2017/2018).

Treatment	Time of application	Average weight of fruit at 1st harvest (g)		Average diameter of fruit at 1st harvest (mm)		Average length of fruit at 1st harvest (mm)	
Untreated	No application	83.5	b	51.9	ns	56.0	ns
ACC 200	4-6 mm fruit diam.	75.5	c	53.9		51.9	
ACC 400	4-6 mm fruit diam.	85.0	b	52.3		55.4	
ACC 800	4-6 mm fruit diam.	86.4	b	52.5		54.0	
ACC 200	8-10 mm fruit diam.	82.3	bc	52.8		55.5	
ACC 400	8-10 mm fruit diam.	95.1	a	54.6		56.7	
ACC 800	8-10 mm fruit diam.	96.5	a	54.9		56.0	
ACC 400*	8-10 mm fruit diam.	98.3	a	54.9		56.2	
Significance level		***<.0001***		*0.5769*		*0.8309*	
LSD 5%		*8.0*		-		-	
ACC vs Control		*0.1101*		*0.1756*		*0.6674*	
1st vs 2nd application		***0.0003***		*0.2407*		*0.1439*	
ACC 1st Linear		***0.0165***		*0.5342*		*0.5773*	
ACC 1st Quadratic		*0.0984*		*0.4560*		*0.2433*	
ACC 2nd Linear		***0.0023***		*0.2794*		*0.9170*	
ACC 2nd Quadratic		***0.0259***		*0.4722*		*0.6780*	

*Old formulation of ACC (VBC 30160)

Table 10. Effect of 1-aminocyclopropane carboxylic acid (ACC) on fruit size at the second harvest of 'Laetitia' plums at Goedemoed, Ashton-Robertson region, Western Cape (2017/2018).

Treatment	Time of application	Average weight of fruit at 2nd harvest (g)		Average diameter of fruit at 2nd harvest (mm)		Average length of fruit at 2nd harvest (mm)	
Untreated	No application	107.9	ns	56.3	ns	61.1	ns
ACC 200	4-6 mm fruit diam.	106.6		56.0		60.2	
ACC 400	4-6 mm fruit diam.	102.2		55.2		59.2	
ACC 800	4-6 mm fruit diam.	97.9		54.0		57.7	
ACC 200	8-10 mm fruit diam.	106.6		55.9		60.3	
ACC 400	8-10 mm fruit diam.	105.0		55.7		59.8	
ACC 800	8-10 mm fruit diam.	108.4		56.3		60.2	
ACC 400*	8-10 mm fruit diam.	109.2		56.5		59.7	
Significance level		*0.4133*		*0.3938*		*0.2190*	
LSD 5%		-		-		-	
ACC vs Control		*0.4520*		*0.5929*		*0.0758*	
1st vs 2nd application		*0.2004*		*0.1820*		*0.1051*	
ACC 1st Linear		*0.1539*		*0.1006*		*0.0267*	
ACC 1st Quadratic		*0.7620*		*0.8837*		*0.8121*	
ACC 2nd Linear		*0.6987*		*0.7223*		*0.9303*	
ACC 2nd Quadratic		*0.7620*		*0.8837*		*0.8121*	

*Old formulation of ACC (VBC 30160)

Table 11. Effect of 1-aminocyclopropane carboxylic acid (ACC) on fruit firmness and color at the first harvest of 'Laetitia' plums at Goedemoed, Ashton-Robertson region, Western Cape (2017/2018).

Treatment	Time of application	Average firmness of fruit at 1st harvest	Average color score of fruit at 1st harvest**
Untreated	No application	6.2 ns	10.1 ns
ACC 200	4-6 mm fruit diam.	6.5	9.3
ACC 400	4-6 mm fruit diam.	5.3	10.4
ACC 800	4-6 mm fruit diam.	7.4	9.8
ACC 200	8-10 mm fruit diam.	6.3	10.2
ACC 400	8-10 mm fruit diam.	6.0	10.3
ACC 800	8-10 mm fruit diam.	5.1	10.6
ACC 400*	8-10 mm fruit diam.	5.3	10.8
Significance level		*0.1832*	*0.0648*
LSD 5%		-	-
ACC vs Control		*0.6287*	*0.8679*
1st vs 2nd application		*0.0768*	*0.1186*
ACC 1st Linear		*0.4532*	*0.6177*
ACC 1st Quadratic		***0.0219***	*0.0957*
ACC 2nd Linear		*0.4836*	*0.5283*
ACC 2nd Quadratic		*0.3670*	*0.8908*

*Old formulation of ACC (VBC 30160); **Color was scored using chart PL. 25 (Deciduous Fruit Board) with values ranging from 1 to 12 where 12 = dark red

Table 12. Effect of 1-aminocyclopropane carboxylic acid (ACC) on fruit firmness and color at the second harvest of ‘Laetitia’ plums at Goedemoed, Ashton-Robertson region, Western Cape (2017/2018).

Treatment	Time of application	Average firmness of fruit at 2nd harvest	Average color score of fruit at 2nd harvest**
Untreated	No application	5.2 ns	10.0 ns
ACC 200	4-6 mm fruit diam.	5.2	9.5
ACC 400	4-6 mm fruit diam.	4.9	9.8
ACC 800	4-6 mm fruit diam.	5.7	9.6
ACC 200	8-10 mm fruit diam.	5.2	9.8
ACC 400	8-10 mm fruit diam.	5.4	9.0
ACC 800	8-10 mm fruit diam.	4.9	9.4
ACC 400*	8-10 mm fruit diam.	4.8	9.0
Significance level		*0.6550*	*0.1974*
LSD 5%		-	-
ACC vs Control		*0.8597*	*0.1163*
1st vs 2nd application		*0.7211*	*0.3022*
ACC 1st Linear		*0.3118*	*0.8698*
ACC 1st Quadratic		*0.3317*	*0.4863*
ACC 2nd Linear		*0.5323*	*0.4728*
ACC 2nd Quadratic		*0.3317*	*0.4863*

*Old formulation of ACC (VBC 30160); **Color was scored using chart PL. 25 (Deciduous Fruit Board) with values ranging from 1 to 12 where 12 = dark red

Table 13. Effect of 1-aminocyclopropane carboxylic acid (ACC) on hand thinning requirement, yield and yield efficiency of 'Fortune' plums at Goedemoed, Ashton-Robertson region, Western Cape (2017/2018).

Treatment	Time of application	Average number of fruitlets thinned by hand		Average total yield per tree (kg)		Average total yield efficiency (kg.cm^{-2})	
Untreated	No application	1055	a	15.9	ns	0.26	ns
ACC 200	4-6 mm fruit diam.	1087	a	17.0		0.29	
ACC 400	4-6 mm fruit diam.	931	ab	16.3		0.28	
ACC 800	4-6 mm fruit diam.	726	cd	15.5		0.26	
ACC 200	8-10 mm fruit diam.	984	ab	16.1		0.27	
ACC 400	8-10 mm fruit diam.	912	abc	15.9		0.26	
ACC 800	8-10 mm fruit diam.	668	d	14.5		0.24	
ACC 400*	8-10 mm fruit diam.	808	bcd	17.1		0.29	
Significance level		***<.0001***		*0.0914*		*0.1160*	
LSD 5%		*186.5*		-		-	
ACC vs Control		***0.0123***		*0.9021*		*0.7392*	
1st vs 2nd application		*0.2707*		*0.2471*		*0.1794*	
ACC 1st Linear		***0.0003***		*0.2366*		*0.4138*	
ACC 1st Quadratic		*0.6703*		*0.8482*		*0.8636*	
ACC 2nd Linear		***0.0008***		*0.1807*		*0.2043*	
ACC 2nd Quadratic		*0.6863*		*0.7584*		*0.8765*	

*Old formulation of ACC (VBC 30160)

Table 14. Effect of 1-aminocyclopropane carboxylic acid (ACC) on fruit size of 'Fortune' plums at Goedemoed, Ashton-Robertson region, Western Cape (2017/2018).

Treatment	Time of application	Average weight of fruit (g)		Average diameter of fruit (mm)		Average length of fruit (mm)	
Untreated	No application	107.9	ns	56.0	ns	61.1	ns
ACC 200	4-6 mm fruit diam.	106.6		56.0		60.2	
ACC 400	4-6 mm fruit diam.	102.2		55.2		59.2	
ACC 800	4-6 mm fruit diam.	97.9		54.0		57.7	
ACC 200	8-10 mm fruit diam.	106.1		55.9		60.4	
ACC 400	8-10 mm fruit diam.	105.0		55.7		59.8	
ACC 800	8-10 mm fruit diam.	108.4		56.3		60.2	
ACC 400*	8-10 mm fruit diam.	109.2		56.5		60.1	
Significance level		*0.4479*		*0.3938*		*0.2351*	
LSD 5%		-		-		-	
ACC vs Control		*0.5324*		*0.7087*		*0.0891*	
1st vs 2nd application		*0.2186*		*0.1820*		*0.1068*	
ACC 1st Linear		*0.1550*		*0.1006*		***0.0274***	
ACC 1st Quadratic		*0.7626*		*0.8837*		*0.8130*	
ACC 2nd Linear		*0.6470*		*0.7223*		*0.9306*	
ACC 2nd Quadratic		*0.7157*		*0.7208*		*0.5785*	

*Old formulation of ACC (VBC 30160)

Table 15. Effect of 1-aminocyclopropane carboxylic acid (ACC) on fruit firmness of 'Fortune' plums at Goedemoed, Ashton-Robertson region, Western Cape (2017/2018).

Treatment	Time of application	Average firmness of fruit	
Untreated	No application	5.2	ns
ACC 200	4-6 mm fruit diam.	5.2	
ACC 400	4-6 mm fruit diam.	4.9	
ACC 800	4-6 mm fruit diam.	5.7	
ACC 200	8-10 mm fruit diam.	5.2	
ACC 400	8-10 mm fruit diam.	5.4	
ACC 800	8-10 mm fruit diam.	4.9	
ACC 400*	8-10 mm fruit diam.	4.8	
Significance level		*0.6552*	
LSD 5%		-	
ACC vs Control		*0.9654*	
1st vs 2nd application		*0.7398*	
ACC 1st Linear		*0.3110*	
ACC 1st Quadratic		*0.3309*	
ACC 2nd Linear		*0.5067*	
ACC 2nd Quadratic		*0.6098*	

*Old formulation of ACC (VBC 30160)

Table 16. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average fruit set percentage and hand thinning requirement of 'August Red' nectarines at Bo-Bokfontein, Koue Bokkeveld district, South Africa (2017/2018).

Treatment	Time of application	Average fruit set %		Average number of fruitlets thinned by hand	
Untreated	No application	93.53	ab	1191	ns
ACC 400	4-6 mm fruit diam.	89.22	bc	1094	
ACC 600	4-6 mm fruit diam.	94.73	a	1114	
ACC 800	4-6 mm fruit diam.	88.30	c	1279	
ACC 400	8-10 mm fruit diam.	93.07	abc	1277	
ACC 600	8-10 mm fruit diam.	88.65	bc	1269	
ACC 800	8-10 mm fruit diam.	90.73	abc	1119	
Significance level		***0.0074***		*0.0956*	
LSD 5%		*5.19*		-	
Control vs ACC		*0.1701*		*0.9925*	
1st ACC Linear		*0.7240*		*0.1583*	
1st ACC Quadratic		***0.0101***		*0.5226*	
2nd ACC Linear		*0.3707*		*0.2262*	
2nd ACC Quadratic		*0.1525*		*0.5319*	

Table 17. Effect of 1-aminocyclopropane carboxylic acid (ACC) on harvest distribution of 'August Red' nectarines at Bo-Bokfontein, Koue Bokkeveld district, South Africa (2017/2018).

Treatment	Time of application	% Fruit picked at 1st harvest		% Fruit picked at 2nd harvest		% Fruit picked at 3rd harvest	
Untreated	No application	3.80	c	41.32	ab	54.88	ab
ACC 400	4-6 mm fruit diam.	6.30	bc	43.73	a	50.00	bc
ACC 600	4-6 mm fruit diam.	11.9	b	41.61	ab	46.50	bc
ACC 800	4-6 mm fruit diam.	19.7	a	43.22	ab	37.03	c
ACC 400	8-10 mm fruit diam.	9.90	bc	50.81	a	39.26	c
ACC 600	8-10 mm fruit diam.	6.50	bc	30.27	b	63.20	a
ACC 800	8-10 mm fruit diam.	8.90	bc	46.78	a	44.21	ab
Significance level		***<.0001***		***0.0237***		***0.0007***	
LSD 5%		*6.90*		*13.00*		*13.12*	
Control vs ACC		***0.0122***		*0.7749*		*0.1072*	
1st ACC Linear		***0.0002***		*0.9386*		*0.0526*	
1st ACC Quadratic		*0.7086*		*0.7403*		*0.6012*	
2nd ACC Linear		*0.7844*		*0.5382*		*0.4526*	
2nd ACC Quadratic		*0.3278*		***0.0017***		***0.0004***	

Table 18. Effect of 1-aminocyclopropane carboxylic acid (ACC) on yield, yield efficiency and average fruit weight over both harvests of 'August Red' nectarines at Bo-Bokfontein, Koue Bokkeveld district, South Africa (2017/2018).

Treatment		Total yield per tree (kg)		Total yield efficiency ($kg.cm^{-2}$)		Average fruit weight over both harvests (g)	
Untreated	No application	41.5	b	0.70	ab	132.19	ns
ACC 400	4 - 6 mm fruit diam.	47.3	ab	0.72	ab	135.56	
ACC 600	4 - 6 mm fruit diam.	45.6	ab	0.62	b	138.11	
ACC 800	4 - 6 mm fruit diam.	50.8	ab	0.79	ab	130.86	
ACC 400	8 - 10 mm fruit diam.	53.6	a	0.73	ab	124.51	
ACC 600	8 - 10 mm fruit diam.	51.0	ab	0.82	a	132.19	
ACC 800	8 - 10 mm fruit diam.	44.2	ab	0.71	ab	130.76	
Significance level		***0.0021***		***0.0286***		*0.7303*	
LSD 5%		*11.8*		*0.20*		-	
Control vs ACC		*0.1123*		*0.6749*		*0.9769*	
1ST ACC Linear		*0.5494*		*0.5155*		*0.5495*	
1ST ACC Quadratic		*0.5043*		*0.1183*		*0.4709*	
2nd ACC Linear		*0.1134*		*0.8094*		*0.4266*	
2nd ACC Quadratic		*0.6765*		*0.2540*		*0.4901*	

Table 19. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average fruit size at the second harvest of August Red' nectarines at Bo-Bokfontein, Koue Bokkeveld district, South Africa (2017/2018).

Treatment	Time of application	Average weight (g) of 2nd harvest		Average diameter (mm) of 2nd harvest		Average length (mm) of 2nd harvest	
Untreated	No application	132.5	ns	61.8	ab	63.4	ab
ACC 400	4-6 mm fruit diam.	135.8		63.0	a	63.8	a
ACC 600	4-6 mm fruit diam.	137.1		62.5	ab	62.2	ab
ACC 800	4-6 mm fruit diam.	129.6		60.5	ab	61.2	b
ACC 400	8-10 mm fruit diam.	124.2		60.0	b	61.1	b
ACC 600	8-10 mm fruit diam.	132.5		61.5	ab	62.0	ab
ACC 800	8-10 mm fruit diam.	130.7		61.5	ab	61.6	ab
Significance level		*0.7449*		***0.0035***		***0.0014***	
LSD 5%		-		*2.7*		*2.3*	
Control vs ACC		*0.8830*		*0.772*		*0.1333*	
1ST ACC Linear		*0.4411*		*0.0681*		***0.0311***	
1ST ACC Quadratic		*0.5230*		*0.5129*		*0.7752*	
2nd ACC Linear		*0.4064*		*0.2798*		*0.6732*	
2nd ACC Quadratic		*0.4654*		*0.4917*		*0.5581*	

Table 20. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average fruit size at the third harvest of August Red' nectarines at Bo-Bokfontein, Koue Bokkeveld district, South Africa (2017/2018).

Treatment	Time of application	Average fruit weight at third harvest (g)	Average fruit diameter at third harvest (mm)	Average fruit length at third harvest (mm)
Untreated	No application	131.8 ns	61.7 ns	63.3 ns
ACC 400	4 - 6 mm fruit diam.	135.4	62.5	63.7
ACC 600	4 - 6 mm fruit diam.	139.1	63.2	62.4
ACC 800	4 - 6 mm fruit diam.	132.1	61.3	61.7
ACC 400	8 - 10 mm fruit diam.	124.9	60.7	61.2
ACC 600	8 - 10 mm fruit diam.	132.1	61.8	62.0
ACC 800	8 - 10 mm fruit diam.	130.1	61.4	61.9
Significance level		*0.6975*	*0.6094*	*0.2591*
LSD 5%		-	-	-
Control vs Rest		*0.9261*	*0.8712*	*0.1626*
1st ACC Linear		*0.6754*	*0.3811*	*0.0768*
1st ACC Quadratic		*0.4223*	*0.2682*	*0.7708*
2nd ACC Linear		*0.4499*	*0.6070*	*0.5196*
2nd ACC Quadratic		*0.5181*	*0.5282*	*0.6208*

Table 21. Effect of 1-aminocyclopropane carboxylic acid (ACC) on fruit firmness at the second and third harvest dates of 'August Red' nectarines at Bo-Bokfontein, Koue Bokkeveld district, South Africa (2017/2018).

Treatment	Time of application	Average fruit firmness at 2nd harvest		Average fruit firmness at 3rd harvest	
Untreated	No application	8.5	ns	8.6	ns
ACC 400	4-6 mm fruit diam.	8.6		8.6	
ACC 600	4-6 mm fruit diam.	9.4		9.4	
ACC 800	4-6 mm fruit diam.	9.4		9.6	
ACC 400	8-10 mm fruit diam.	9.0		8.8	
ACC 600	8-10 mm fruit diam.	9.3		9.5	
ACC 800	8-10 mm fruit diam.	8.8		8.8	
Significance level		*0.3923*		*0.4744*	
LSD 5%		-		-	
Control vs Rest		*0.1606*		*0.2590*	
1st ACC Linear		*0.1553*		*0.1078*	
1stACC Quadratic		*0.3977*		*0.5679*	
2nd ACC Linear		*0.8143*		*0.9352*	
2nd ACC Quadratic		*0.3759*		*0.1875*	

Table 22. Effect of 1-aminocyclopropane carboxylic acid (ACC) on percentage split pit at the second and third harvest dates of 'August Red' nectarines at Bo-Bokfontein, Koue Bokkeveld district, South Africa (2017/2018).

Treatment	Time of application	Average % split pit at 2nd harvest		Average % split pit at 3rd harvest	
Untreated	No application	0.3	ns	0.4	c
ACC 400	4-6 mm fruit diam.	0.5		0.5	bc
ACC 600	4-6 mm fruit diam.	0.7		0.8	ab
ACC 800	4-6 mm fruit diam.	0.6		0.7	abc
ACC 400	8-10 mm fruit diam.	1.0		1.0	a
ACC 600	8-10 mm fruit diam.	0.6		1.0	a
ACC 800	8-10 mm fruit diam.	0.6		0.7	abc
Significance level		*0.0919*		***0.0048***	
LSD 5%		-		*0.3*	
Control vs ACC		***0.0123***		***0.0052***	
1st ACC Linear		*0.6228*		*0.2504*	
1st ACC Quadratic		*0.3952*		*0.1853*	
2nd ACC linear		*0.0529*		*0.0871*	
2nd ACC Quadratic		*0.9000*		*0.3188*	

Table 23. Effect of 1-aminocyclopropane carboxylic acid (ACC) on hand thinning requirement and average leaf drop of ‘Keisie’ peaches at Lucerne, Robertson district, South Africa (2018/2019).

Treatment	Time of application	Average number of fruitlets thinned by hand		Average leaf drop score*	
Untreated	No application	363	ab	1.0	c
ACC 200	4-6 mm fruit diam.	330	b	1.0	c
ACC 400	4-6 mm fruit diam.	416	a	1.2	c
ACC 800	4-6 mm fruit diam.	239	c	1.9	b
ACC 200	8-10 mm fruit diam.	214	c	2.0	b
ACC 400	8-10 mm fruit diam.	169	c	2.9	a
ACC 800	8-10 mm fruit diam.	82	d	3.0	a
Significance level		***<.0001***		***<.0001***	
LSD 5%		*81.5*		*0.3*	
ACC vs Control		***0.0003***		***<.0001***	
1st vs 2nd		***<.0001***		***<.0001***	
ACC 1ST Linear		***0.0053***		***<.0001***	
ACC 1st Quadratic		***0.0020***		*0.4002*	
ACC 2nd Linear		***0.0016***		***<.0001***	
ACC 2nd Quadratic		*0.9749*		***<.0001***	

*Leaf drop score between 1 and 3, with 1 being no leaf drop and 3 severe leaf drop

Table 24. Effect of 1-aminocyclopropane carboxylic acid (ACC) on yield and yield efficiency of 'Keisie' peaches at Lucerne, Robertson district, South Africa (2018/2019).

Treatment	Time of application	Total yield per tree (kg)		Total yield efficiency (kg.cm-^{2})	
Untreated	No application	45.7	abc	0.19	ab
ACC 200	4-6 mm fruit diam.	43.6	bc	0.17	b
ACC 400	4-6 mm fruit diam.	57.8	a	0.23	a
ACC 800	4-6 mm fruit diam.	51.0	ab	0.21	ab
ACC 200	8-10 mm fruit diam.	55.7	ab	0.22	a
ACC 400	8-10 mm fruit diam.	37.6	c	0.17	b
ACC 800	8-10 mm fruit diam.	20.7	d	0.09	c
Significance level		***0.0002***		***0.0013***	
LSD 5%		*13.3*		*0.06*	
ACC vs Control		*0.7968*		*0.8419*	
1st vs 2nd		***0.0015***		***0.0109***	
ACC 1st Linear		*0.4527*		*0.2382*	
ACC 1st Quadratic		*0.0521*		***0.0457***	
ACC 2nd Linear		***<.0001***		***<.0001***	
ACC 2nd Quadratic		*0.2775*		*0.5574*	

Table 25. Effect of 1-aminocyclopropane carboxylic acid (ACC) on harvest distribution and average fruit weight over both harvests of 'Keisie' peaches at Lucerne, Robertson district, South Africa (2018/2019).

Treatment	Time of application	Percentage fruit picked at 1st harvest		Average fruit weight over both harvests (g)	
Untreated	No application	46.0	ab	206.8	a
ACC 200	4-6 mm fruit diam.	46.0	ab	218.2	a
ACC 400	4-6 mm fruit diam.	50.5	ab	213.8	a
ACC 800	4-6 mm fruit diam.	42.4	b	212.7	a
ACC 200	8-10 mm fruit diam.	62.2	a	213.8	a
ACC 400	8-10 mm fruit diam.	33.3	bc	204.3	a
ACC 800	8-10 mm fruit diam.	21.2	c	182.4	b
Significance level		***0.0054***		***0.0463***	
LSD 5%		*18.6*		*19.5*	
ACC vs Control		*0.6333*		*0.9392*	
1st vs 2nd		*0.1710*		***0.0099***	
ACC 1st Linear		*0.6102*		*0.6107*	
ACC 1st Quadratic		*0.4882*		*0.7707*	
ACC 2nd Linear		***0.0001***		***0.0020***	
ACC 2nd Quadratic		*0.0678*		*0.8609*	

Table 26. Effect of 1-aminocyclopropane carboxylic acid (ACC) on fruit size at first harvest of 'Keisie' peaches at Lucerne, Robertson district, South Africa (2018/2019).

Treatments	Time of application	Average fruit weight at 1st harvest (g)		Average fruit diameter at 1st harvest (mm)		Average fruit length at 1st harvest (mm)	
Untreated	No application	222.2	ab	77.3	c	62.2	a
ACC 200	4-6 mm fruit diam.	233.9	a	78.8	abc	62.0	a
ACC 400	4-6 mm fruit diam.	234.4	a	80.0	ab	62.1	a
ACC 800	4-6 mm fruit diam.	231.8	a	80.0	ab	61.4	ab
ACC 200	8-10 mm fruit diam.	230.0	a	80.5	a	60.8	ab
ACC 400	8-10 mm fruit diam.	230.0	a	80.6	a	60.3	bc
ACC 800	8-10 mm fruit diam.	208.2	b	78.1	bc	59.0	c
Significance level		***0.0011***		***0.0033***		***<.0001***	
LSD 5%		*18.6*		*2.1*		*1.7*	
ACC vs Control		*0.4208*		***0.0106***		*0.0664*	
1st vs 2nd		***0.0492***		*0.7798*		***0.0006***	
ACC 1st Linear		*0.8030*		*0.3367*		*0.4497*	
ACC 1st Quadratic		*0.8788*		*0.5795*		*0.6829*	
ACC 2nd Linear		***0.0138***		***0.0307***		***0.0421***	
ACC 2nd Quadratic		*0.4027*		*0.3822*		*0.9540*	

Table 27. Effect of 1-aminocyclopropane carboxylic acid (ACC) on fruit size at second harvest of 'Keisie' peaches at Lucerne, Robertson district, South Africa (2018/2019).

Treatments	Time of application	Average fruit weight of 2nd harvest (g)		Average fruit diameter of fruit at 2nd harvest (mm)		Average length of fruit at 2nd harvest (mm)	
Untreated	No application	191.5	ns	76.7	ns	61.6	ab
ACC 200	4-6 mm fruit diam.	202.5		78.5		63.2	a
ACC 400	4-6 mm fruit diam.	193.2		75.9		60.3	bc
ACC 800	4-6 mm fruit diam.	193.6		75.0		58.3	cde
ACC 200	8-10 mm fruit diam.	196.1		75.6		59.1	bcd
ACC 400	8-10 mm fruit diam.	179.0		73.8		57.0	de
ACC 800	8-10 mm fruit diam.	174.0		71.9		55.4	e
Significance level		*0.3251*		*0.3398*		***0.0010***	
LSD 5%		-		-		*2.0*	
ACC vs Control		*0.8400*		*0.3060*		***0.0167***	
1st vs 2nd		*0.0608*		***0.0245***		***0.0002***	
ACC 1st Linear		*0.5396*		*0.1213*		***0.0022***	
ACC 1st Quadratic		*0.5668*		*0.4178*		*0.3180*	
ACC 2nd Linear		*0.0957*		*0.0714*		***0.0172***	
ACC 2nd Quadratic		*0.3993*		*0.7774*		*0.5262*	

Table 28. Effect of 1-aminocyclopropane carboxylic acid (ACC) on percentage split pit at first and second harvest of ‘Keisie’ peaches at Lucerne, Robertson district, South Africa (2018/2019).

Treatment	Time of application	% split pit at 1st harvest		% split pit at 2nd harvest	
Untreated	No application	0.0	b	0.0	ns
ACC 200	4-6 mm fruit diam.	2.0	a	0.5	
ACC 400	4-6 mm fruit diam.	0.0	b	0.0	
ACC 800	4-6 mm fruit diam.	2.5	a	0.0	
ACC 200	8-10 mm fruit diam.	1.0	ab	1.0	
ACC 400	8-10 mm fruit diam.	0.0	b	0.5	
ACC 800	8-10 mm fruit diam.	0.0	b	0.5	
Significance level		***0.0451***		*0.8421*	
LSD 5%		*1.8*		-	
ACC vs Control		*0.1857*		*0.3723*	
1st vs 2nd		***0.0281***		*0.1608*	
ACC Linear		*0.2776*		*0.4773*	
ACC Quadratic		***0.0082***		*0.5380*	
ACC 2nd Linear		*0.3339*		*0.5136*	
ACC 2nd Quadratic		*0.4022*		*0.5325*	

Table 29. Effect of 1-aminocyclopropane carboxylic acid (ACC) on hand thinning requirement and average leaf drop of ‘Keisie’ peaches at La Plaisante, Wolseley district, South Africa (2018/2019).

Treatment	Time of application	Average number of fruitlets thinned by hand		Average leaf drop score*	
Untreated	No application	96	a	1.0	d
ACC 200	4-6 mm fruit diam.	94	a	1.0	d
ACC 400	4-6 mm fruit diam.	51	b	1.9	c
ACC 800	4-6 mm fruit diam.	12	d	2.0	c
ACC 200	8-10 mm fruit diam.	91	a	2.0	c
ACC 400	8-10 mm fruit diam.	38	bc	2.4	b
ACC 800	8-10 mm fruit diam.	23	cd	3.0	a
Significance level		***<.0001***		***<.0001***	
LSD 5%		*23.0*		*0.2*	
ACC vs Control		***<.0001***		***<.0001***	
1st vs 2nd		*0.8099*		***<.0001***	
ACC 1ST Linear		***<.0001***		***<.0001***	
ACC 1ST Quadratic		*0.1286*		***<.0001***	
ACC 2nd Linear		***<.0001***		***<.0001***	
ACC 2nd Quadratic		***0.0037***		*0.5298*	

*Leaf drop score between 1 and 3, with 1 being no leaf drop and 3 severe leaf drop

Table 30. Effect of 1-aminocyclopropane carboxylic acid (ACC) on yield and yield efficiency of 'Keisie' peaches at La Plaisante, Wolseley district, South Africa (2018/2019).

Treatment	Time of application	Total yield per tree (kg)		Total yield Efficiency (kg.cm^{-2})	
Untreated	No application	16.1	ab	0.27	ab
ACC 200	4-6 mm fruit diam.	19.9	a	0.34	a
ACC 400	4-6 mm fruit diam.	14.9	b	0.25	b
ACC 800	4-6 mm fruit diam.	6.2	c	0.11	c
ACC 200	8-10 mm fruit diam.	20.2	a	0.34	a
ACC 400	8-10 mm fruit diam.	13.2	b	0.22	b
ACC 800	8-10 mm fruit diam.	3.7	c	0.06	c
Significance level		***<.0001***		***<.0001***	
LSD 5%		*4.6*		*0.08*	
ACC vs Control		*0.0875*		*0.0875*	
1st vs 2nd		*0.3093*		*0.3093*	
ACC 1ST Linear		***<.0001***		***<.0001***	
ACC 1ST Quadratic		*0.7832*		*0.7832*	
ACC 2nd Linear		***<.0001***		***<.0001***	
ACC 2nd Quadratic		*0.4766*		*0.4766*	

Table 31. Effect of 1-aminocyclopropane carboxylic acid (ACC) on harvest distribution and average fruit weight over all harvests of ‘Keisie’ peaches at La Plaisante, Wolseley district, South Africa (2018/2019).

Treatment	Time of application	% fruit picked at 1st harvest		% fruit picked at 2nd harvest		% fruit picked at 3rd harvest		Average fruit weight over all harvests (g)	
Untreated	No application	3.56	ns	21.01	bcd	75.44	ab	209.2	a
ACC 200	4-6 mm fruit diam.	4.82		15.18	d	80.00	a	191.2	ab
ACC 400	4-6 mm fruit diam.	7.06		28.62	abc	64.31	abc	204.0	ab
ACC 800	4-6 mm fruit diam.	12.81		40.46	a	46.73	cd	211.0	a
ACC 200	8-10 mm fruit diam.	4.84		18.72	cd	76.44	ab	196.1	ab
ACC 400	8-10 mm fruit diam.	6.01		32.90	ab	61.09	bc	215.1	a
ACC 800	8-10 mm fruit diam.	9.53		37.88	a	42.59	d	180.9	b
Significance level		*0.1578*		***0.0004***		***<.0001***		***0.0306***	
LSD 5%		-		*12.77*		*16.45*		*27.3*	
ACC vs Control		*0.1002*		*0.1080*		*0.0347*		*0.3665*	
1st vs 2nd		*0.4250*		*0.6365*		*0.4450*		*0.5537*	
ACC 1st Linear		***0.0104***		***0.0003***		***0.0002***		*0.1715*	
ACC 1st Quadratic		*0.8781*		*0.3756*		*0.5276*		*0.6100*	
ACC 2nd Linear		*0.1225*		***0.0072***		***0.0001***		*0.1345*	
ACC 2nd Quadratic		*0.8866*		*0.1731*		*0.5765*		*0.0507*	

Table 32. Effect of 1-aminocyclopropane carboxylic acid (ACC) on fruit size, at the second harvest of 'Keisie' peaches at La Plaisante, Wolseley district, South Africa (2018/2019).

Treatment	Time of application	Average weight (g) of fruit 2nd harvest	Average diameter (mm) of fruit 2nd harvest	Average length of fruit (mm) 2nd harvest
Untreated	No application	216.2 ns	80.8 ns	62.9 ns
ACC 200	4-6 mm fruit diam.	191.8	78.4	61.2
ACC 400	4-6 mm fruit diam.	203.5	79.5	61.2
ACC 800	4-6 mm fruit diam.	218.7	82.1	64.6
ACC 200	8-10 mm fruit diam.	198.3	78.5	60.5
ACC 400	8-10 mm fruit diam.	218.3	81.9	63.9
ACC 800	8-10 mm fruit diam.	197.9	77.8	60.7
Significance level		*0.0551*	*0.2131*	*0.3322*
LSD 5%		-	-	-
ACC vs Control		*0.4053*	*0.0530*	*0.0604*
1st vs 2nd		*0.8588*	*0.9722*	*0.9036*
ACC 1st Linear		***0.0409***	*0.2765*	*0.2150*
ACC 1st Quadratic		*0.6803*	*0.8420*	*0.8850*
ACC 2nd Linear		*0.7562*	*0.7585*	*0.9089*
ACC 2nd Quadratic		*0.1131*	*0.3550*	*0.3012*

Table 33. Effect of 1-aminocyclopropane carboxylic acid (ACC) on fruit size at third harvest of 'Keisie' peaches at La Plaisante, Wolseley district, South Africa (2018/2019).

Treatment	Time of application	Average weight (g) of fruit 3rd harvest	Average diameter (mm) of fruit 3rd harvest	Average length of fruit (mm) 3rd harvest
Untreated	No application	207.5 ns	74.8 ns	63.8 ns
ACC 200	4-6 mm fruit diam.	195.7	73.6	63.0
ACC 400	4-6 mm fruit diam.	202.9	74.9	63.2
ACC 800	4-6 mm fruit diam.	195.5	72.4	62.4
ACC 200	8-10 mm fruit diam.	192.1	73.6	61.9
ACC 400	8-10 mm fruit diam.	208.9	75.4	63.9
ACC 800	8-10 mm fruit diam.	207.1	74.9	63.9
Significance level		*0.5513*	*0.3744*	*0.6262*
LSD 5%		-	-	-
ACC vs Control		*0.3007*	*0.4793*	*0.2944*
1st vs 2nd		*0.4037*	*0.1822*	*0.5553*
ACC 1st Linear		*0.8374*	*0.1867*	*0.4925*
ACC 1st Quadratic		*0.3639*	*0.2157*	*0.6833*
ACC 2nd Linear		*0.2188*	*0.4426*	*0.1463*
ACC 2nd Quadratic		*0.1405*	*0.2129*	*0.1560*

Table 34. Effect of 1-aminocyclopropane carboxylic acid (ACC) on percentage split pit at second and third harvest of 'Keisie' peaches at La Plaisante, Wolseley district, South Africa (2018/2019).

Treatment	Time of application	% split pit at 2nd harvest		% split pit at 3rd harvest	
Untreated	No application	3.33	ns	0.50	ns
ACC 200	4-6 mm fruit diam.	4.26		0.00	
ACC 400	4-6 mm fruit diam.	2.70		1.00	
ACC 800	4-6 mm fruit diam.	6.33		2.86	
ACC 200	8-10 mm fruit diam.	4.67		0.00	
ACC 400	8-10 mm fruit diam.	3.25		0.00	
ACC 800	8-10 mm fruit diam.	1.11		0.00	
Significance level		*0.6377*		*0.2813*	
LSD 5%		-		-	
ACC vs Control		*0.7274*		*0.7703*	
1st vs 2nd		*0.3799*		*0.0605*	
ACC 1ST Linear		*0.2302*		***0.0059***	
ACC 1ST Quadratic		*0.3676*		*0.9588*	
ACC 2nd Linear		*0.1717*		*0.9146*	
ACC 2nd Quadratic		*0.9103*		*0.5777*	

PAPER 2: The Efficacy of 1-Aminocyclopropane-1-carboxylic acid (ACC) as a Chemical Thinner on Apples

Abstract. **The purpose of this study was to evaluate a new chemical thinning molecule, 1-aminocyclopropane-1-carboxylic acid (ACC), on 'Fuji', 'Cripps' Red' and 'Royal Gala' apples. ACC was evaluated over two seasons at a range of concentrations from 125 to 500 µl·L^{-1}, applied at different phenological stages from petal fall to 15 – 20 mm fruitlet diameter. The fruit industry needs a chemical thinning agent that can successfully be applied during the later thinning window (15 – 20 mm fruitlet diameter) on apples. ACC was compared to industry standard applications, 6-benzyladenine (6-BA) and 1-napthaleneacetic acid (NAA), applied as a tank-mix or individually. In all the trials, ACC proved to be a stronger thinner than the industry standard applications. During both seasons, trials on 'Fuji' were disappointing as neither ACC nor the industry standard applications caused satisfactory thinning. In the 2018/2019 season, ACC induced leaf drop, but this was observed following high maximum temperature after treatment. ACC application in the 2017/2018 season to young 'Cripps' Red' trees was not successful.**

However, ACC proved to be very promising when applied to a mature 'Cripps' Red' orchard during the 2018/2019 season. ACC applied at the 15 – 20 mm fruitlet diameter, thinned successfully, and increased fruit size without decreasing yield. It was concluded that the rate of ACC should be between 250 and 500 µl·L^{-1}. No negative effects on stem-end russet, fruit maturity and leaf drop were observed. ACC at 250 µl·L^{-1}proved to be a mild thinner on 'Royal Gala' when applied at the 8 – 10 mm fruitlet diameter. No negative effects were found on stem-end russet and fruit maturity at harvest. At 250 µl·L^{-1} ACC, leaf drop was not a concern on 'Royal Gala'. Further research, however, is needed before final recommendations can be made.

Keywords: 1-aminocyclopropane-1-carboxylic acid (ACC), 6-benzyladenine (6-BA) and 1-napthaleneacetic acid (NAA), thinning, yield, leaf drop, fruit size.

Fruit thinning is an important cultural practice in commercial apple production. Thinning is done in order to increase fruit size, improve fruit quality and optimize return bloom the following season (Green and Costa, 2012). Most apple trees set more flowers than needed for commercial apple production. If all the fruit that set are allowed to develop until maturity, there will most likely not be enough resources to support the growth of these fruit to commercially acceptable size (Green and Costa, 2012). Pome fruit have self-regulatory mechanisms that reduce crop load, primarily by shedding fruit that are weak, small or contain few seeds. However, these self-regulatory mechanisms are often not severe enough to ensure that enough fruit reach a commercially acceptable size (Costa et al., 2005). Thinning apple trees solely by hand is not a viable option due to labor cost and availability of labor in certain parts of the world, as well as the delay in thinning (Wertheim, 1997). For this reason, chemical thinning has become a standard practice, often followed by hand thinning if required (Wertheim, 1997).

Recently, problems have emerged with certain chemical thinning agents. Some do not fit in sustainable fruit production such as 1-napthyl methylcarbamate (carbaryl) which is harmful to beneficial insects and aquatic organisms and is already banned in certain countries (Wertheim, 1997). Another problem is the high costs of reregistration. Renewal of the registration of chemical thinning agents may be too expensive and not justifiable by the returns, which could lead to manufacturers discontinuing the production of certain chemical thinning agents. This is especially relevant in Europe with the synthetic auxin thinners 1-napthaleneacetic acid (NAA) and its amide (NAAm) (Wertheim, 1997).

Two of the most frequently used post bloom thinners in apple production is the synthetic cytokinin, 6-benzyladenine (6-BA), and NAA (Schupp et al., 2012). These chemicals successfully thin a wide range of apple cultivars, e.g. McIntosh, Red Delicious, Golden Delicious, Empire, Royal Gala, Idared, Campbell Redchief Delicious and Gala, when applied from petal fall up until 15 to 16 mm fruitlet diameter (Greene, 1992; Marini, 1996; Basak, 2006). Marini (1996) evaluated NAA on ‘Campbell Redchief Delicious’ apples at rates of 2.5 to 10 $mg{\cdot}L^{-1}$, applied from petal fall to 10 mm fruitlet diameter. NAA applied at 5 and 10 $mg{\cdot}L^{-1}$ was an effective thinner when applied from petal fall to 9 mm fruitlet diameter. When NAA was applied when ‘Campbell Redchief Delicious’ fruit were larger than 9 mm, the number of pigmy fruit increased substantially. Basak (2006) evaluated the efficacy of 10 $mg{\cdot}L^{-1}$ NAA on ‘Gala’ apples over a range of phenological stages and found that NAA was an effective thinner that increased fruit size and the number of marketable fruit when applied from petal fall up to 10 mm fruitlet diameter.

Greene (1992) investigated 6-BA as a fruit thinner on ‘McIntosh’, ‘Red Delicious’, ‘Golden Delicious’, ‘Empire’, and ‘Idared’ apples at rates of 50 to 100 $\mu l{\cdot}L^{-1}$. 6-BA caused a thinning effect when applied from full bloom to 3 weeks after full bloom (w.a.f.b.), but was most effective at the 10 - 12 mm fruitlet diameter range, which was about 14 to 18 days after full bloom (d.a.f.b.). When 6-BA was applied at rates higher than 150 $\mu l \cdot L^{-1}$, over thinning occurred, spur elongation increased as well as an increase in the number of asymmetric fruit was found. Green (1992) concluded that 6-BA between 50 to 100 $\mu l \cdot L^{-1}$ applied at 10 mm fruit diameter is an effective thinner for 'McIntosh', 'Red Delicious', 'Golden Delicious', 'Empire', and 'Idared' apples.

There is substantial evidence that one of the earliest responses induced by chemical or environmental stimuli when causing young fruitlets to abscise, is a carbohydrate deficit within the fruit (McArtney and Obermiller, 2012). Young fruit generally become insensitive to chemical thinners at a fruit diameter of ca.16 mm, which coincides with an increase in carbohydrate availability within the tree (Lakso et al., 1999). It is not always possible to apply thinning agents during this sensitive phase due to unfavorable environmental conditions, uncertainty about the number of fruit that had set and/or failure of these compounds to adequately thin when previously used (Schupp et al., 2012).

Thus, there is a need for chemical thinning agents that could be applied during a later application window. Currently there are two chemical thinning agents registered for use in the later thinning window (17 to 25 mm fruit diameter), viz. ethephon and carbaryl (Schupp et al., 2012). Ethephon application results in erratic thinning responses and is highly temperature dependent - high ambient temperatures especially in the days following application result in excessive thinning (Jones and Koen, 1985). Ethephon sometimes also negatively affects fruit shape in some cultivars resulting in flat fruit (Basak, 2006). Carbaryl is considered to be a mild thinner and is mostly used in combination with other chemical thinners to increase the thinning effect (Schupp et al., 2012), but as previously mentioned, is under pressure and needs to be replaced by an alternative, more environmentally friendly product. There is thus a need to find a predictable chemical thinner that could be used as a rescue-thinning agent (16 - 30 mm fruit diameter) in years when thinning agents used in the earlier window inadequately reduced fruit set.

The ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), effectively thins apples (Schupp et al., 2012; McArtney and Obermiller, 2012). The conversion of s-adenosyl methionine to ACC is the rate-limiting step in the ethylene biosynthesis pathway. Adams and Yang (1979) found that applied ACC is effectively converted to ethylene in apple tissue and Bradford and Yang (1980) found that ACC is readily translocated in plants. Schupp et al. (2012) evaluated ACC on ‘Golden Delicious’/Bud.9 apple trees and concluded that 300 and 500 mg·L^{-1} ACC applied at the 20 mm fruit diameter stage had potential as a chemical thinner. McArtney and Obermiller (2012) evaluated ACC on ‘Royal Gala’ apples and found that an application of 400 mg·L^{-1} at the 20 mm fruit diameter stage significantly thinned ‘Royal Gala’ apples and thus showed potential as a rescue chemical thinner.

The aim of the paper was to determine the efficacy of ACC as a chemical thinning agent on ‘Royal Gala’, ‘Fuji’ and ‘Cripps’ Red’ at various concentrations and phenological stages, in order to develop commercial recommendations.

Materials and Methods

Plant material and site description for the 2017/2018 season. Trials were conducted on the apple cultivars Fuji and Cripps’ Red. The ‘Fuji’ trial was conducted on Oak Valley Estate (34°10'02"S 19°03'33"E) in the Elgin region in the Western Cape, South Africa. ‘Fuji’ on the rootstock MM109 was planted in 2013 together with 10% ‘Granny Smith’ as cross pollinator, included in every third row. ‘Fuji’ was planted at a 4.0 x 1.5 m spacing. The ‘Cripps’ Red’ on MM109 was planted at a 4.0 x 2.0 m spacing in 2013 on Dennebos farm (34°04'00.0"S 19°07'00.0"E) in the Vyeboom region in the Western Cape, South Africa. The cross pollinator was also 10% ‘Granny Smith’ in alternative rows.

Plant material and site description for the 2018/2019 season. Trials were conducted on the apple cultivars ‘Fuji’, ‘Cripps’ Red’ and ‘Royal Gala’. The ‘Fuji’ and ‘Cripps’ Red’ trials were conducted on Oak Valley Estate. The experiment was done in the same ‘Fuji’ orchard as the previous season, but different trees were used. The ‘Cripps’ Red’ on the rootstock M7 was planted in 2003 at a 4 m × 1.5 m spacing, with 30% ‘Fuji’ as cross pollinator in alternative rows. The ‘Royal Gala’ trial was conducted on the farm Applegarth (34°08'02"S 19°01'48"E) in the Grabouw area in the Western Cape, South Africa. The ‘Royal Gala’ on M793 rootstock was planted in 1995 at a 4 m × 1.5 m spacing. The cross pollinators were 20% ‘Golden Delicious’ and 10% ‘Granny Smith’ in alternative rows.

Treatments. ACC (Valent BioSciences Corporation, Libertyville, Illinois 60048, USA) was evaluated as summarized in Table 1 and 2. Treatment two was a second control, viz. the grower standard, which was determined by the producer and therefore varied between orchards. No surfactants were added to the ACC treatments in any of the trials. Dates of application, hand thinning and harvest for 'Fuji' and 'Cripps' Red' in the 2017/2018 season can be found in Table 3 and for 'Fuji', 'Cripps' Red' and 'Royal Gala' in 2018/2019 season in Table 4.

Trial lay-out and treatment application. For all trials, a randomized complete block design was used with 10 single-tree replications. Applications were made using a motorized knap sack sprayer (STIHL, Pietermaritzburg, South Africa) and product was applied at approximately 1000 L per ha. Treatments were applied early morning when the temperature did not exceed 20 C° and when the wind speed did not exceed 3 ms^{-1}. Weather data during and after application is presented in Fig. 1 – 5. At least one tree was left untreated between the treated trees and a buffer row was left in between treated rows in order to prevent any drift effects.

Data collection. For all the trials, the following procedure was followed. Fruit set was determined in the lower half of the tree canopy by tagging two scaffold branches on each tree. At full bloom, the number of flower clusters were counted and approximately 14 days after treatment application the number of fruitlets that set were counted to calculate the average fruit set. Hand thinning was done according to the standard commercial practice of the specific farm. All the fruitlets that were thinned by hand were collected and brought back to the laboratory where the number of fruitlets thinned per tree was counted. Leaf drop was recorded on a scale of 0 - 5, with 0 being no leaf drop and 5 being severe drop. At each commercial harvest date, the yield of fruit (kg) per tree was recorded. After harvest, the tree trunk circumference was measured in order to calculate the trunk cross sectional area. This was done in order to calculate the yield efficiency expressed as kg fruit per trunk cross sectional area ($kg \cdot cm^{-2}$). In the 2017/2018 season a sample of 30 fruit per replicate per harvest was collected and brought back to the laboratory for destructive and non-destructive analysis and in the 2018/2019 season the sample size was 20. For all fruit in the sample, fruit length, diameter and weight were recorded. In the 2018/2019 season, ground color, full seed number, number of aborted seeds as well as stem-end russet was recorded. The color chart A.45 was used for 'Fuji' and A.42 for 'Royal Gala' with values ranging from 1 to 12, with 12 the least color development. The Pink Lady® color chart was used for 'Cripps' Red' with values ranging from 1 until 12, with 12 being the best color development. The stem-end russet chart A.43 was used for the 'Cripps' Red' and A.31 for 'Fuji' and 'Royal Gala' with a score of 0 indicating no russet and a score of 12 indicating severe russet. Return bloom was calculated the following spring by counting all the reproductive and vegetative buds that broke, on the same two branches used to determine fruit set. Return bloom was then calculated as the percentage of buds that where reproductive.

Statistical analyses. The data were analyzed using SAS Enterprise guide 7.1 (SAS Institute Inc., Cary, North Carolina, USA) using the linear model procedure and the pairwise t-test to determine the Least Significant Difference (LSD) when the F-statistic indicated significance at $P<0.05$. Single degree of freedom, orthogonal, polynomial contrast were fitted where applicable.

Results

'Fuji' 2017/2018 season. No significant differences were found between the thinning treatments in fruit set per flower cluster compared to the untreated control (Table 5). On average, the early ACC applications at petal drop reduced fruit set compared to the late applications at 15 - 20 mm fruitlet diameter (p=0.0394). The grower application (NAA tank mixed with 6-BA) significantly increased the number of fruitlets that had to be hand thinned (Table 5). None of the ACC treatments significantly affected the number of fruitlets thinned compared to the untreated control (Table 5). On average, ACC at 500 $\mu l \cdot L^{-1}$ required significantly less hand thinning of fruitlets than the 250 $\mu l \cdot L^{-1}$ ACC (p=0.0184). ACC applied at 8 - 10 mm fruitlet diameter resulted in a slightly lower hand thinning requirement than ACC applied at petal drop (p=0.0453), but did not differ from application at 15 - 20 mm fruitlet diameter (Table 5).

The grower application as well as the 500 $\mu l \cdot L^{-1}$ ACC applied at all three timings significantly decreased total yield compared to the untreated control (Table 6). ACC at 500 $\mu l \cdot L^{-1}$ significantly decreased yield and yield efficiency compared to the 250 $\mu l \cdot L^{-1}$ application (p<.0001). The applications at 8 - 10 mm fruitlet diameter reduced yield efficiency compared to the 15 - 20 mm fruitlet diameter stage, but not compared to the petal drop stage (Table 6). On average, ACC decreased yield (p <.0001) and yield efficiency (p<.0001) compared to the untreated control (Table 6). The only treatment that significantly influenced fruit size was ACC 500 $\mu l \cdot L^{-1}$ applied at 15 - 20 mm fruitlet diameter, which significantly reduced fruit length compared to the untreated control (Table 7). The 6-BA and NAA tank-mix application significantly decreased fruit diameter and length compared to the untreated control (Table 7). ACC applied at petal drop did increase fruit weight (p=0.0111) and length (p=0.0170) compared to the same applications at the 15 - 20 mm stage; however, this increase was not significant compared to the untreated control (Table 7).

ACC 500 µl·L^{-1} applied at petal drop and 8 - 10 mm fruitlet diameter stage significantly increased return bloom percentage the following season compared to the untreated control. The higher concentration of ACC (500 µl·L^{-1}) significantly improved return bloom percentage compared to the lower concentration (250 µl·L^{-1}) (Table 8).

'Cripps' Red' 2017/2018 season. The highest concentration of ACC (500 µl·L^{-1}) applied at petal drop and 8 - 10 mm fruitlet diameter stage significantly decreased the percentage fruit set per cluster compared to the untreated control (Table 9). The higher concentration of ACC (500 µl·L^{-1}) significantly decreased fruit set compared to the lower concentration (250 µl·L^{-1}) (p=0.0002). The petal drop (p=0.0203) and 8 - 10 mm fruitlet diameter (p=0.0030) applications reduced fruit set compared to the 15 - 20 mm fruitlet applications. None of the treatments had a significant effect on number of fruitlets that had to be hand thinned at commercial hand thinning (Table 9). None of the ACC treatments had a significant effect on yield, but 500 µl·L^{-1} ACC significantly decreased yield efficiency compared to the untreated control (Table 10). On average, ACC significantly lowered yield efficiency compared to the untreated control (p=0.0144). 6-BA did not significantly affect yield or yield efficiency compared to the untreated control (Table 10).

ACC on average decreased fruit weight (p= 0.0008), diameter (p=0.0130) and length (p=0.0002) compared to the untreated control (Table 11). Application in both later windows (8 - 10 and 15 - 20 mm fruitlet diameter) significantly decreased fruit size compared to the untreated control (Table 11). ACC applied at petal fall caused a slight increase in fruit weight (p<.0001), diameter (p<.0001) and length (p<.0001) compared to the 8 - 10 fruitlet diameter application (Table 11). ACC applied at petal fall also caused a slight increase in fruit weight (p=0.0012), diameter (p=0.0003) and length (p=0.0172) compared to the 15 - 20 mm fruitlet diameter applications (Table 11). However, these increases at petal fall were not significant compared to the untreated control (Table 11). The 250 and 500 µl·L^{-1} ACC applications did not differ significantly from one another in terms of fruit size (Table 11). All of the ACC treatments significantly increased return bloom percentage compared to the untreated control (p=<.0001). The grower application (6-BA) also significantly increased the return bloom percentage (Table 11). ACC treatments and 6-BA did not differ significantly from one another (Table 12).

'Fuji' 2018/2019 season. None of the treatments had a significant effect on fruit set per flower cluster nor the hand-thinning requirement compared to the untreated control (Table 13). On average, ACC significantly increased leaf drop compared to the untreated control (*p*<.0001) (Table 13). Leaf drop increased linearly with increasing ACC rate, with ACC 500 µl·L^{-1} at 15 – 20 mm fruitlet diameter having the most severe effect on leaf drop (Table 13). ACC applied during the later application window (15 – 20 mm fruitlet diameter) had a more significant effect on leaf drop than ACC applied at the early application window (8 - 10 mm fruitlet diameter) (*p*<.0001). The 6-BA treatment did not induce leaf drop (Table 13). None of the treatments significantly affected total yield or yield efficiency compared to the untreated control (Table 14). There was, however, a linear decrease in yield with an increase in ACC rate when applied at 8 - 10 mm fruitlet diameter (*p*=0.0125) (Table 14). Only the 6-BA application significantly increased fruit size (weight and diameter) compared to the untreated control (Table 15). Fruit weight (*p*=0.0271) and diameter (*p*=0.0292) was significantly larger following the 15 - 20 mm applications compared to the 8 - 10 mm applications. None of the treatments significantly affected fruit length (Table 15).

None of the treatments had a significant effect on ground color and aborted seed number compared to the untreated control (Table 16). A small, but linear increase in ground color score (more yellow) was found with increasing rate of ACC applied during the early window (*p*= 0.0500). On average, ACC caused an increase in full seed number compared to the untreated control (*p*=0.0009). All the treatments, except ACC 250 µl·L^{-1} applied at the early application window, significantly increased the average number of full seeds per fruit compared to the untreated control. During the early application window, the effect of rate of ACC on full seed number was a quadratic increase, with ACC 250 and 500 µl·L^{-1} not differing from one another (Table 16). However, these differences in seed number were very small with average seed number ranging from 7.4 to 8.3 seeds per fruit. The ACC treatments on average increased the stem-end russet score compared to the untreated control (*p*=0.0004), but russet scores were very low. None of the treatments significantly affected return bloom percentage compared to the untreated control (Table 17). There was a linear increase in percentage return bloom with increasing rate of ACC applied at 8 - 10 mm fruitlet diameter (Table 17).

'Cripps' Red' 2018/2019 season. The middle (250 µl·L^{-1}) and highest (500 µl·L^{-1}) rates of ACC applied at the late timing (15 - 20 mm fruitlet diameter) significantly reduced average

fruit set per flower cluster compared to the untreated control (Table 18). The late applications decreased fruit set (*p*=0.0013) and hand thinning requirement (*p*=0.0029) more than the earlier applications (8 - 10 mm fruitlet diameter). The highest concentration applied at the late stage significantly decreased the hand thinning requirement during commercial hand thinning (Table 18). No leaf drop was observed in this trial. None of the treatments significantly affected yield compared to the untreated control, but the highest concentration applied at the late stage significantly reduced yield efficiency (Table 19). During the later application window, an increase in ACC rate resulted in a linear decrease in yield (*p*=0.0078) and yield efficiency (*p*=0.0008) (Table 19).

The 6-BA application caused the most significant increase in fruit size compared to the untreated control (Table 20). ACC applied at the lowest (125 $\mu l \cdot L^{-1}$) and highest (500 $\mu l \cdot L^{-1}$) rate during the late application window (15 – 20 mm fruitlet diameter) increased fruit weight significantly compared to the untreated control. The later applications of ACC increased fruit weight (*p*=0.0003) and diameter (*p*=0.0007) more than the earlier applications (Table 20). A quadratic increase in fruit size (weight and diameter) due to slightly lesser effect at 250 $\mu l \cdot L^{-1}$ occurred with an increase in ACC rate when treatments were applied at the later stage, while a linear decrease in length occurred with an increase in ACC rate applied at 8 - 10 mm fruitlet diameter (Table 20).

No significant differences were found following thinning treatments in the average number of full seeds per fruit compared to the untreated control (Table 21). The highest rate of ACC applied at the later stage caused a small increase in aborted seed number per fruit. However, this treatment only increased the number of aborted seeds to 0.36 per fruit, which is not of horticultural significance. ACC applied during the first application window resulted in fewer aborted seeds than application during the later application window. During the later application window, an increase in ACC rate resulted in a linear increase in aborted seed number, but as mentioned before these small effects were horticulturally insignificant (Table 21). ACC applied during the later applications timing caused a higher incidence of stem-end russet compared to the earlier applications (*p*=0.0045). The differences in stem-end russet were very small and not of horticultural significance with scores ranging from 1.8 to 2.3. ACC caused an increase in ground color yellowing compared to the untreated control (*p*<.0001). Yellowing increased linearly with an increase in ACC rate. However, average ground color scores ranged from 3.0 - 3.3 only and the differences were therefore not of horticultural significance (Table 21). On average, ACC treatments caused an increase in return bloom compared to the untreated control (p=0.0108). ACC 500 µl·L^{-1} applied at both application timings, as well as 6-BA, significantly increased return bloom percentage compared to the untreated control (Table 22). An increase in rate of ACC resulted in a linear increase in return bloom percentage when treatments were applied early (p=0.0002), while a significant increase in return bloom at 500 µl·L^{-1} gave rise to a quadratic effect on return bloom at later application (p=0.0036) (Table 22).

'Royal Gala' 2018/2019 season. None of the treatments had a significant effect on fruit set per flower cluster (Table 23) or on the number of fruitlets that were hand thinned at commercial hand thinning compared to the untreated control (Table 23). An increase in rate of ACC applied during the later window resulted in a quadratic increase in the number of fruitlets thinned with the 250 and 500 µl·L^{-1} applications not differing from one another (Table 23). NAA was the only treatment that did not significantly increase leaf drop compared to the untreated control. ACC induced leaf drop compared to the untreated control (p<.0001) and drop increased linearly with increasing ACC rate. The later applications had a more severe effect on leaf drop (p<.0001) (Table 23). There was no significant differences in yield and yield efficiency compared to the untreated control, but both decreased linearly with an increase in ACC rate applied early (8 - 10 mm fruitlet diameter) (Table 24).

The NAA application at petal drop and the highest ACC concentration applied at 8 - 10 mm fruitlet diameter increased the percentage of fruit picked during the first harvest compared to the untreated control (Table 25). The early applications increased the percentage of the crop picked at the first harvest compared to the later applications (p=0.0201). A decrease at 250 and 500 µl·L^{-1} ACC applied in the later window gave rise to a quadratic response in the percentage of fruit picked at the first harvest (p=0.0256) (Table 25). When the average fruit weights of both harvests were combined, only ACC at 250 µl·L^{-1} applied early significantly increased fruit weight compared to the untreated control. The average fruit weight decreased linearly with increasing ACC rate during the first applications timing (Table 25). For the first harvest, the 250 µl·L^{-1} ACC treatment applied early resulted in significantly larger fruit (weight, diameter and length) than the untreated control (Table 26). On average, ACC treatments increased fruit diameter and length compared to the untreated control for the first harvest (p=0.0339 and 0.0001, respectively). The earlier ACC applications increased fruit length more than the later applications (Table 26). For the second harvest, the 500 µl·L^{-1} treatment applied at the earlier application time significantly reduced the average fruit weight and diameter compared to the untreated control, while the 500 μl·L^{-1} treatment applied at the later stage significantly increased the average fruit weight. A linear decrease in fruit weight, diameter and length was observed following an increase in ACC rate at the 8 - 10 mm fruitlet diameter stage, with the 500 μl·L^{-1} treatment significantly reducing fruit weight and diameter compared to the untreated control (Table 27). This was reversed in terms of fruit weight following the 15 - 20 mm fruitlet diameter applications where the 500 μl·L^{-1} treatment significantly increased fruit weight compared to the untreated control. On average, the later applications resulted in bigger fruit than the earlier applications (Table 27). However, none of the treatments had a significant effect on the average length of fruit at second harvest compared to the untreated control (Table 27).

No significant differences were found in full seed number, stem-end russet and ground color at the first harvest compared to the untreated control. The ground color score was slightly higher following the earlier ACC applications compared to the later applications (Table 28). For the first harvest, the number of aborted seeds per fruit decreased linearly with increasing rate of the early applications, but increased linearly with increasing rate of the later applications (Table 28). There were very small significant differences between treatments in the number of aborted seeds per fruit ranging from 0.0 to 0.3 per fruit with the ACC 500 μl·L^{-1} treatment applied later significantly increasing the number of aborted seeds per fruit compared to the untreated control (Table 28). No significant differences were found in full seed number at the second harvest date (Table 29). None of the treatments had a significant effect on the aborted seed number compared to the untreated control (Table 29). The early applications causing a quadratic decrease in aborted seed number with the lowest extent of abortion at 250 μl·L^{-1}. The NAA application at petal drop significantly decreased stem-end russet compared to the untreated control, but differences were very small ranging from 1.8 to 2.1 (Table 29). All of the treatments, including the NAA application, increased the ground color. These increases were also minor with the untreated control scoring an average of 2.6, and the highest ground color score being 3.4. (Table 29). Later ACC application significantly increased ground color development compared to the earlier applications timing (p=0.0492). There was a quadratic response of ground color to later ACC application with the highest score at 500 μl·L^{-1}. None of the treatments had a significant effect on average return bloom percentage compared to the untreated control (Table 30). However, a slight linear increase in return bloom percentage occurred with an increase in ACC rate, when treatments were applied at a 15 – 20 mm fruitlet diameter (p=0.0477) (Table 30).

Discussion

'Fuji'. Although none of the ACC treatments significantly reduced the fruit set and hand thinning requirement compared to the untreated control during both seasons, there were some marginal thinning effects during the 2017/2018 season. ACC applications at petal drop reduced fruit set more than the applications at 15 - 20 mm fruitlet diameter, and the 500 $\mu l \cdot L^{-1}$ ACC applications reduced hand thinning requirement more than 250 $\mu l \cdot L^{-1}$ ACC. In contrast, Schupp et al. (2012) found a significant thinning effect on 'Golden Delicious' apples with 300 and 500 $\mu l \cdot L^{-1}$ ACC applied at 20 mm fruitlet diameter. 'Fuji' is, however, particularly unresponsive to chemical thinning agents (Stopar, 2006). In the 2017/2018 season, the 500 $\mu l \cdot L^{-1}$ application at all three phenological stages significantly decreased the total yield and yield efficiency compared to the untreated control and 250 $\mu l \cdot L^{-1}$ ACC. During this season, hand thinning was performed 11 days after the ACC application at the 15 - 20 mm fruitlet diameter, possibly before all the fruitlets had a chance to drop, which could be the reason why there were no significant differences in the hand-thinning requirement. In addition, it is also possible that the team of laborers performing the commercial hand thinning, thinned too severely. Usually the laborers thin to two fruit per cluster. If ACC thins whole clusters rather than fruitlets within a cluster, this could have resulted in over-thinning. In the 2018/2019 season, yield and yield efficiency was not significantly affected by any of the ACC treatments and was in line with the observations in fruit set and hand thinning. There was, however, a small linear decrease in yield with an increase in ACC rate from 125 – 500 $\mu l \cdot L^{-1}$ when treatments were applied at 8 - 10 mm fruitlet diameter. The decrease in yield and yield efficiency during the first season and the linear decrease in yield in the second season could be interpreted as mild thinning. Therefore, from these results it seems that ACC at 500 $\mu l \cdot L^{-1}$ thinned too severely. Application at the 8 – 10 mm fruitlet diameter stage had the most significant thinning effect. Temperatures following thinner application is an important factor influencing the efficacy of a chemical thinner (Forshey, 1976). Warm temperatures intensify competition among sinks at a time when metabolic demand is high in the tree as a whole (Forshey, 1976). During both seasons, however, temperatures were high enough following the

different applications (Fig. 1 and 2) and therefore cannot explain the relative lack of response to ACC.

A common reason for thinning is an increase in fruit size, thus an increase in fruit size could be expected with a decrease in yield (Costa and Vizzotto, 2000). In the 2017/2018 season, there were small increases in fruit size following the 500 $\mu l \cdot L^{-1}$ applications at petal fall and 8 - 10 mm fruitlet diameter compared to the 15 - 20 mm fruitlet diameter applications, although as mentioned, these were not significant. ACC caused a significant decrease in fruit length when applied at 15 - 20 mm fruitlet diameter, which is difficult to explain. In the 2018/2019 season, fruit weight and diameter was increased following the 15 - 20 mm fruitlet diameter application compared to the 8 - 10 mm fruit diameter application. This was not expected, as there was a linear decrease in yield at the 8 - 10 mm fruitlet diameter stage and therefore one would expect fruit to be larger following this application compared to the 15 - 20 mm fruitlet diameter application. This unexpected effect has previously been reported for 'Empire' apples when a thinning program that included endothall decreased crop load and yield, but did not increase fruit size (Stover et al., 2002). Schupp et al. (2012) found a linear decrease in yield and a linear increase in fruit size with an increase in ACC rate when applied at 20 mm fruitlet diameter. Yield reductions often lead to improved return bloom (Wertheim, 1997), and this was the case in the 2017/2018 season as return bloom was significantly increased compared to the untreated control when ACC 500 $\mu l \cdot L^{-1}$ was applied at petal drop and at 8 -10 mm fruit diameter. In the 2018/2019 season, however, when yield was not reduced significantly, none of the ACC treatments significantly affected return bloom compared to the untreated control. However, there was a linear increase in return bloom with an increase in ACC rate, when treatments were applied at 8 – 10 mm fruitlet diameter. Schupp et al. (2012) also noted an increase in return bloom when ACC was applied at 300 and 500 $\mu l \cdot L^{-1}$ when applied at 20 mm fruitlet diameter.

Significant leaf drop was observed in the second season with a linear increase with an increase in ACC rate, during both application windows, with ACC 500 $\mu l \cdot L^{-1}$ resulting in the highest leaf drop compared to the untreated control. Treatments applied at the later application timing caused more severe leaf drop than the treatments applied at the early applications timing. This is expected, as the total leaf surface area would be considerably higher at the 15 - 20 mm fruitlet application, compared to the 8 - 10 mm fruitlet diameter stage. Temperatures were higher during treatment application and up to three days after treatment application in the 2018/2019 season, with maximum temperatures ranging from 18 to 36 °C, compared to the

2017/2018 season where little leaf drop occurred, and where maximum temperatures ranged from 14 to 31 °C (Fig. 1 and 2). In the 2018/2019 season, the linear increase in leaf drop could explain the slight linear decrease in yield, as a certain percentage of the fruit may have abscised due to increased competition for carbohydrates as the trees' photosynthetic capacity decreased.

During the 2018/2019 season, the grower applied 70 g Regalis® (prohexadione-calcium 100 g Kg^{-1}) (ProCa) per 100 L water to the orchard. ProCa was applied 4 days after the ACC application at the 8 - 10 mm fruit diameter stage and 13 days after the ACC application at the 15 - 20 mm fruit diameter stage. ProCa primarily inhibits the formation of highly active GAs from inactive precursors, and is used commercially to control vegetative growth (Rademacher, 2000), but this retardant also reduces ethylene biosynthesis (Rademacher et al., 2005). This reduction in endogenous ethylene therefore probably diminishes the ethylene levels induced by ACC application making ACC less effective when applied in close proximity to a ProCa application (Miller, 2002). Thus it is possible that ProCa could have affected the efficacy of ACC in this trial. This could be the reason why ACC was not more effective during the 8 - 10 mm fruit diameter application timing compared to the other application timings even though temperatures were more favorable (Fig. 2). In addition, less fruit abscission in ProCa treated trees could be due to diminished competition for assimilates from developing shoots (Basak and Krzewinska, 2005).

The grower applications in the 2017/2018 season (6-BA tank-mixed with NAA) caused an increase in fruit set on the two tagged branches in the lower canopy compared to the untreated control, but did not significantly affect the hand thinning requirement. Fruit set is determined on two tagged branches, whereas the hand-thinning requirement gives an indication of fruit set throughout the whole tree canopy, and is a better indication of the efficacy of chemical thinning applications. In the 2018/2019 season, 6-BA alone also did not significantly affect fruit set or the hand-thinning requirement compared to the untreated control. As these treatments are registered as effective thinning treatments, the lack of response seen in our trials indicate that both seasons were not ideal for chemical thinning and therefore the relatively small thinning effect obtained by ACC should be interpreted in this light. 6-BA is a synthetic cytokinin, and can not only increase fruit size through its thinning action, but also can increase fruit size by increasing cell division (Stopar, 2006). Apples thinned with 6-BA are larger than would be expected based on the thinning effect alone (Greene, 1992). This could explain why 6-BA increased fruit size without having any obvious thinning effect in our trial. 6-BA tank mixed with and without NAA, had no significant effect on return bloom in either season.

During both seasons, temperatures were high enough during and after 6-BA applications. Therefore, temperature cannot explain the lack of efficacy of 6-BA and NAA (Fig. 1 and 2).

The reason why chemical thinning was ineffective on 'Fuji' during both seasons might be more complicated than just the efficacy of the chemical thinning applications. Bangerth (2000) attributed a significant part of the inconsistent results found with chemical thinners to environmental and intrinsic plant factors. Warm winters result in protracted endodormancy, resulting in various symptoms of delayed foliation, i.e. delayed, protracted, and very weak leafing, delayed and protracted flowering, uneven bloom with full bloom reached earlier in the lower part of the canopy, variation in bloom stage between trees in an orchard and a shortage of spurs capable of forming flowers (Theron, 2012; Sagredo, 2008). The 'Fuji' orchard used in our trials suffered from delayed foliation quite badly (personal observation). Under such conditions, the timing of chemical applications is difficult, potentially resulting in unsatisfactory thinning.

ACC did not have a major effect on seed content. Abortion of seeds often leads to fruit drop, but this did not happen in our trials on 'Fuji'. However, we have to bear in mind that we also did not have a strong thinning effect. Stem-end russet was not induced by ACC and ground color did not differ between treatments thus indicating that fruit maturity was not affected. The small differences between treatments in these variables were negligible and would not be of concern when applying ACC on 'Fuji'.

'Cripps' Red'. In the 2017/2018 season, ACC 500 $\mu l \cdot L^{-1}$ applied at petal drop and 8 - 10 mm fruitlet diameter significantly decreased the fruit set per cluster compared to the untreated control. ACC at 500 $\mu l \cdot L^{-1}$ reduced fruit set more than ACC at 250 $\mu l \cdot L^{-1}$, and the earlier applications reduced fruit set more than the later application. Therefore, ACC did induce thinning in 'Cripps' Red' when applied at the right rate and time. In the 2017/2018 season, the reduction in fruit set was not reflected in the hand-thinning requirement. In the following season, ACC at 250 and 500 $\mu l \cdot L^{-1}$ applied at 15 - 20 mm fruitlet diameter significantly reduced average fruit set per flower cluster. In this trial, the higher concentrations of ACC were once again more effective than the lower concentrations. In the 2018/2019 trial, the 15 - 20 mm fruitlet diameter application, reduced fruit set more than the 8 - 10 mm fruitlet diameter application. In the 2018/2019 season, the reduction in fruit set was reflected in the hand-thinning requirement, as the highest concentration significantly decreased hand-thinning

requirement. As discussed before, Schupp et al. (2012) reported a significant thinning effect in 'Golden Delicious' when ACC was applied at 20 mm fruit diameter at concentrations of 300 and 500 µl·L^{-1}. McArtney (2011) also found a negative linear relationship between the concentrations of ACC applied to individual fruiting spurs of 'GoldRush' apples and the number of fruit that set on the treated spurs. Even though there was no linear effect with rate of ACC on thinning in our trials, the higher rates of ACC generally resulted in a stronger thinning efficacy. In the 2017/2018 season, none of the treatments had a significant effect on yield, but ACC at 500 µl·L^{-1} applied at all three application timings significantly decreased yield efficiency. This is an indication that some degree of over thinning had taken place. In the 2018/2019 season, none of the treatments significantly affected yield, but the 500 µl·L^{-1} treatment once again significantly reduced yield efficiency when applied during the 15 - 20 mm fruitlet diameter window. In the second season, there were linear decreases in yield and yield efficiency with an increase in ACC rate at later application timing.

Schupp et al. (2012) found an increase in fruit size following ACC application, although yields were decreased to below optimal in some instances in his trials. A well know response to a decrease in yield is an increase in fruit size (Pavel and DeJong, 1993). Surprisingly in the 2017/2018 season, applications at the 8 - 10 and 15 - 20 mm fruitlet diameter stage significantly decreased fruit size compared to the untreated control. Applications at petal fall significantly increased fruit size compared to the 8 – 10 and 15 – 20 mm fruitlets diameter applications. One possible explanation for the decrease in yield efficiency and fruit size could be that ACC thinned the early, larger fruit. The king blossom is the strongest blossom in the flower cluster and develops earlier and into the largest fruit. Thus, if the majority of fruitlets developing from the king blossoms are thinned, the crop will develop predominantly from lateral blossoms, which inherently produce smaller fruits (Forshey, 1976). In the 2018/2019 season, however, ACC applied at the highest concentration during the 15 – 20 mm fruitlet diameter thinned most and increased fruit size most, as expected. The later applications significantly increased fruit size compared to the earlier applications. ACC applied at the 15 - 20 mm fruitlet diameter caused a quadratic increase in fruit size (weight and diameter), with fruit weight and diameter not significantly influenced by the 250 µl·L^{-1} application, but ACC at 125 and 500 µl·L^{-1} did significantly increase in fruit weight and diameter.

A reduction in fruit set should lead to an increase in return bloom due to fewer fruit, with seeds producing GAs, being present during the flower induction phase (Wünsche and Ferguson, 2005). Surprisingly, all of the treatments significantly increased return bloom percentage compared to the untreated control in the 2017/2018 season, not only the treatments that successfully reduced fruit set. In the 2018/2019 season 500 $\mu l \cdot L^{-1}$ ACC applied during both application timings significantly increased return bloom percentage compared to the untreated control. In the 2018/2019 season, a linear increase in return bloom percentage occurred with an increase in ACC rate when treatments were applied at 8 – 10 mm fruitlet diameter, while there was a quadratic increase in return bloom with increase in ACC rate, at the 15 – 20 mm fruitlet diameter application.

In both seasons, the industry standard application was 6-BA, which did not have an effect on fruit set, hand thinning requirement, nor yield and yield efficiency. Thus, 6-BA did not have any thinning affect in either season, but did cause the largest increase in fruit size in the 2018/2019 season. As previously mentioned, 6-BA stimulates cell division, which might have been the reason for the increase in fruit size rather than the thinning effect of 6-BA (Stopar, 2006). In the 2017/2018 season, 6-BA along with all of the ACC treatments increased return bloom, but 6-BA did not differ significantly from any of the ACC treatments. In the 2018/2019 season however, the 6-BA application caused an increase in return bloom percentage compared to the untreated control. During both seasons, temperatures were sufficient for 6-BA to be effective (Fig. 3 and 4).

ACC at 500 $\mu l \cdot L^{-1}$ caused a significant increase in the number of aborted seeds, and abortion of seeds often leads to fruit drop. Hence, this treatment also caused the most significant thinning. However, since these increases were small, it is unlikely that the increase in aborted seed number caused the fruit drop. Stem-end russet was not induced by any of the ACC treatments and ground color did not differ between any of treatments, thus indicating that fruit maturity at harvest was not affected. The small differences in these variables were not of horticultural significance, and would not be of concern when using ACC on 'Cripps' Red'. No leaf drop occurred during either season. Maximum temperatures were not excessively high during and shortly after treatment applications during both seasons and ranged from 18 to 32 °C (Fig. 3 and 4). In the second season, ACC performed better than in the 2017/2018 season. During treatment application at the 8 - 10 mm fruit diameter stage, maximum temperatures were around 32 °C and dropped to 25 °C three days after application in the first season (Fig. 3) while in the second season, maximum temperatures at the 8 - 10 mm fruit diameter application were around 18 °C rising to 30 °C three days after treatment application (Fig. 4). During the 15 - 20 mm fruit diameter application, where ACC was the most successful in the 2018/2019 season, temperatures were very similar during both seasons (Fig. 3 and 4) with maximum temperatures ranging from around 26 to 27 °C during and just after treatment application in the 2017/2018 season, and 26 to 30 °C during and three days after treatment application in the 2018/2019 season. Therefore, the reason why ACC performed better in the 2018/2019 season does not appear to be the differences in temperature.

The growers did not apply ProCa to the orchards used in either season, and therefore ProCa could not have affected the thinner efficiency. The trials on ‘Cripps’ Red’ in the 2018/2019 season were much more promising than the trials in the 2017/2018 season. As mentioned earlier, environmental conditions and intrinsic plant factors could be the biggest problem with the inconsistent results found when using chemical thinners (Bangerth, 2000). Warm winters result in uneven bloom accompanied by prolonged and irregular fruit set, which as mentioned earlier could decrease the thinner efficiency. However, in this case, another variable which could have affected the thinner efficiency is the age of the orchard. Trees used in 2017/2018 season were 6th leaf and not full-bearing, while the orchard used in 2018/2019 trees were in their 16th leaf. The younger trees had a sparser bloom and it is well known that light flowering trees are more difficult to thin (Williams, 1979). However, usually young trees are easier to thin than mature trees (Williams, 1979), which in our case did not happen.

ACC showed promise for this cultivar as a chemical thinner that could be successfully applied during the later application window (15 - 20 mm fruitlet diameter). ACC thinned more effectively than 6-BA during both seasons. The recommended rate of ACC would be between 250 and 500 $\mu l \cdot L^{-1}$, probably closer to 500 $\mu l \cdot L^{-1}$. ACC at 500 $\mu l \cdot L^{-1}$ was effective in decreasing fruit set and the hand thinning requirement as well as increasing fruit size. However, this treatment also caused the largest reduction in yield and yield efficiency and therefore growers should be cautious to not over thin and would most likely prefer to apply ACC at slightly lower concentrations than 500 $\mu l \cdot L^{-1}$ to minimize yield reductions.

‘Royal Gala’. In this trial, none of the treatments had a significant effect on fruit set per flower cluster and hand thinning requirement compared to the untreated control. For the 15 - 20 mm fruit diameter applications, there was a quadratic decrease in the hand thinning requirement with an increase in ACC rate, with the 250 and 500 $\mu l \cdot L^{-1}$ applications not differing from one another. As mentioned earlier, the hand thinning requirement gives a better indication of the efficacy of chemical thinning applications, and therefore it seems that ACC did induce some thinning. Schupp et al. (2012) found a linear decrease in fruit set in ‘Golden Delicious’

with increasing ACC rate applied at 20 mm fruit diameter. McArtney and Obermiller (2012) evaluated the use of ACC on 'Gale Gala' apples as a "rescue thinning agent" and found that ACC at 200 mg·L^{-1} significantly reduced fruit set when applied at 11 and 20 mm fruit diameter.

Yield and yield efficiency were not significantly affected by any of the treatments. However, a linear decrease in both yield and yield efficiency occurred with an increase in ACC rate during the 8 – 10 mm fruitlet diameter applications. This could be an indication that ACC did cause thinning and that either hand thinning was done too soon or, as mentioned for 'Fuji', ACC thinned clusters rather than within clusters.

An advancement in fruit maturity could result from a decrease in yield (Wünsche et al., 2000). ACC 500 µl·L^{-1} applied at the 8 – 10 mm fruitlet diameter significantly increased the percentage of fruit picked during the first harvest, thus indicating an advancement in maturity. Due to ACC causing a linear decrease in yield and yield efficiency, although not significant compared to the untreated control, fruit size could be expected to increase, even though no significant thinning action was seen. On average, ACC did cause slight significant increases in fruit size on both harvests dates. There were slight differences in fruit size at each harvest, but when looking at the two harvests combined, ACC 250 µl·L^{-1} applied during the 8 - 10 mm fruitlet diameter stage, was the only treatment that significantly increased fruit size compared to the untreated control. Schupp et al. (2012) found a significant increase in fruit size with an increase in ACC rate when applied at 20 mm fruitlet diameter. However, these increases coincided with more significant decreases in yield, which did not happen in our trial. None of the treatments had a significant effect on average return bloom percentage, but due to fruit set not being affected, this response was not unexpected (Wertheim, 1997). There was a slight linear increase in return bloom percentage when treatments were applied at 15 – 20 mm fruitlet diameter.

All of the ACC treatments caused leaf drop to some degree, as there was a linear increase in leaf drop with an increase in ACC rate during both applications timings. The 15 – 20 mm fruitlet diameter application had a more severe effect on leaf drop compared to the 8 – 10 mm fruitlet diameter application. Temperatures where similar during both applications timings, with temperatures being slightly warmer during the 8 – 10 mm fruitlet applications (Fig. 5). The reason why ACC induced more leaf drop when applied during the 15 - 20 mm fruitlet diameter stage is most likely due to there being more leaf area on the tree during that application period compared to the 8 - 10 mm fruitlet diameter applications. Temperatures during the 15 - 20 mm fruitlet diameter stage, where the most leaf drop occurred, were not excessively high, with maximum temperatures ranging from around 27 to 24 °C. Therefore it appears that 'Royal Gala' could be more sensitive to ACC-induced leaf drop than 'Fuji' and 'Cripps' Red' (Fig. 5).

NAA was applied as the industry standard application in this trial, but NAA did not thin significantly and yield, yield efficiency, fruit size during both harvests and return bloom were not significantly affected compared to the untreated control. The percentage of fruit picked at first harvest was increased by NAA, thus NAA advanced maturity. During and three days after NAA application, temperatures were sufficiently high, with maximum temperatures ranging from 19 – 30 °C, thus temperatures cannot explain the lack of efficacy of NAA.

ACC did not have a major effect on seed content. Abortion of seeds has been known to lead to fruit drop, but this did not happen in our 'Royal Gala' trial. In the absence of a strong thinning effect, this was expected. ACC did not induce stem-end russet or influence ground color, thus indicating that ACC did not influence fruit maturity. The small differences in these variables, which did occur, were negligible and would not be of concern when using ACC on 'Royal Gala'.

The growers did not apply ProCa to this orchard during this season, thus ProCa could not have affected the thinner efficiency. ACC at 250 $\mu l \cdot L^{-1}$ applied at the 8 – 10 mm fruitlet diameter gave the most promising results, and performed better than NAA. ACC at this rate and timing acted as a mild thinner and did not cause leaf drop that would be of concern.

Conclusion

In all the trials conducted in the 2017/2018 and 2018/2019 seasons on 'Fuji', ACC did not have a significant thinning effect. ACC did, however, decrease yield and yield efficiency in the first season as well as improved return bloom, and during the second season yield decreased linearly with ACC rate, which indicates that ACC does indeed have some thinning effect on 'Fuji'. These trends should be investigated further. Since the industry standard applications also did not thin significantly during either season, it is likely that climatic and intrinsic tree factors could have caused a decrease in thinner efficiency during both seasons. Thus, the efficacy of ACC on 'Fuji' is still undetermined. In the 2017/2018 season, ACC over thinned on the young 'Cripps' Red' trees. In the 2018/2019 season, ACC was applied to mature

'Cripps' Red' trees and showed great promise as a chemical thinner at the 15 - 20 mm fruitlet diameter stage. The recommended rate of ACC would be between 250 and 500 µl·L^{-1}. ACC at 250 µl·L^{-1} applied at the 8 - 10 mm fruitlet diameter stage showed promise on 'Royal Gala' as a thinner and performed better than the industry standard application (NAA). McArtney and Obermiller (2012) evaluated ACC alone or in combination with metamitron on apples, and generally found that the thinning effect was additive when thinners were combined. Thus, ACC could possibly be combined with other thinning agents, like metamitron for example, during this application window in order to increase the overall thinning action. This could be evaluated in future research.

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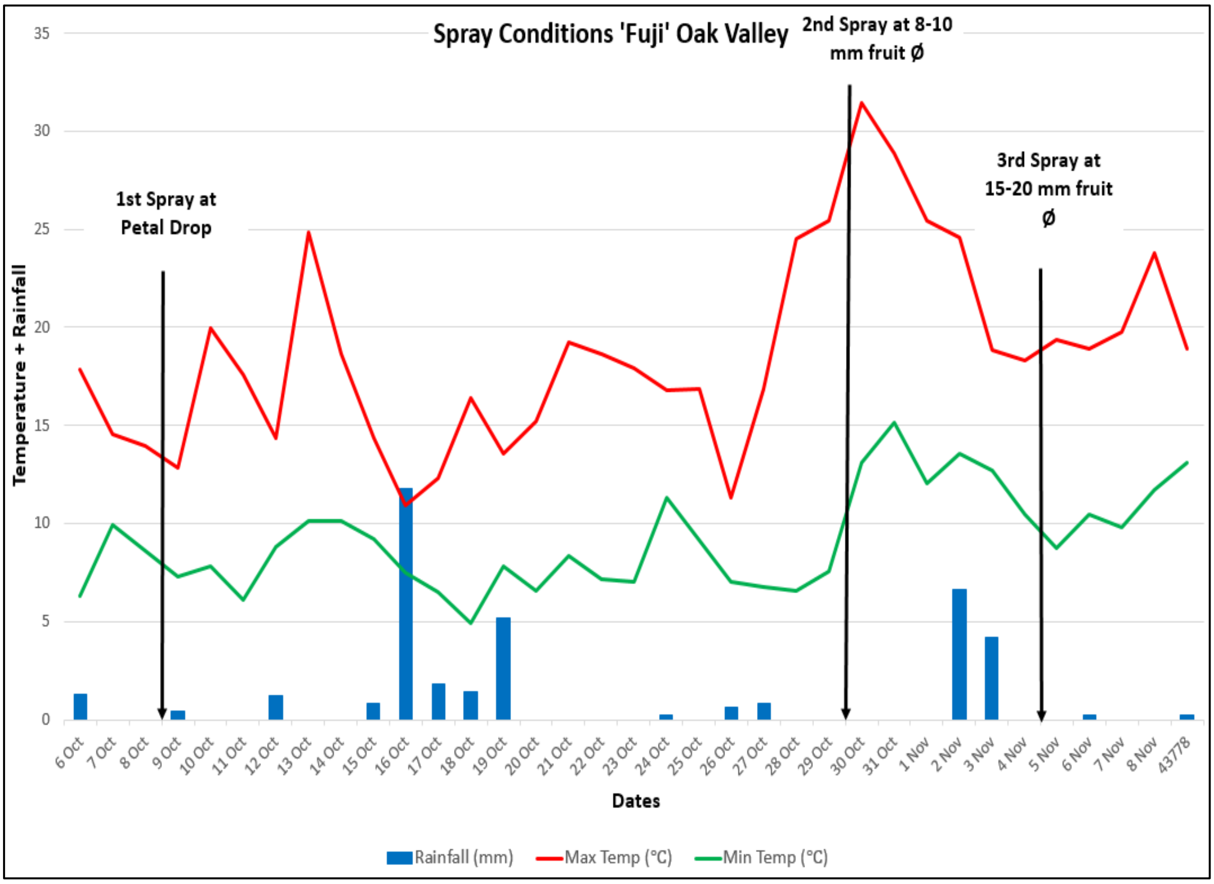

Fig. 1. Temperature and rainfall during treatment applications for ‘Fuji’ apples at Oak Valley Estate, Grabouw area, South Africa (2017/2018).

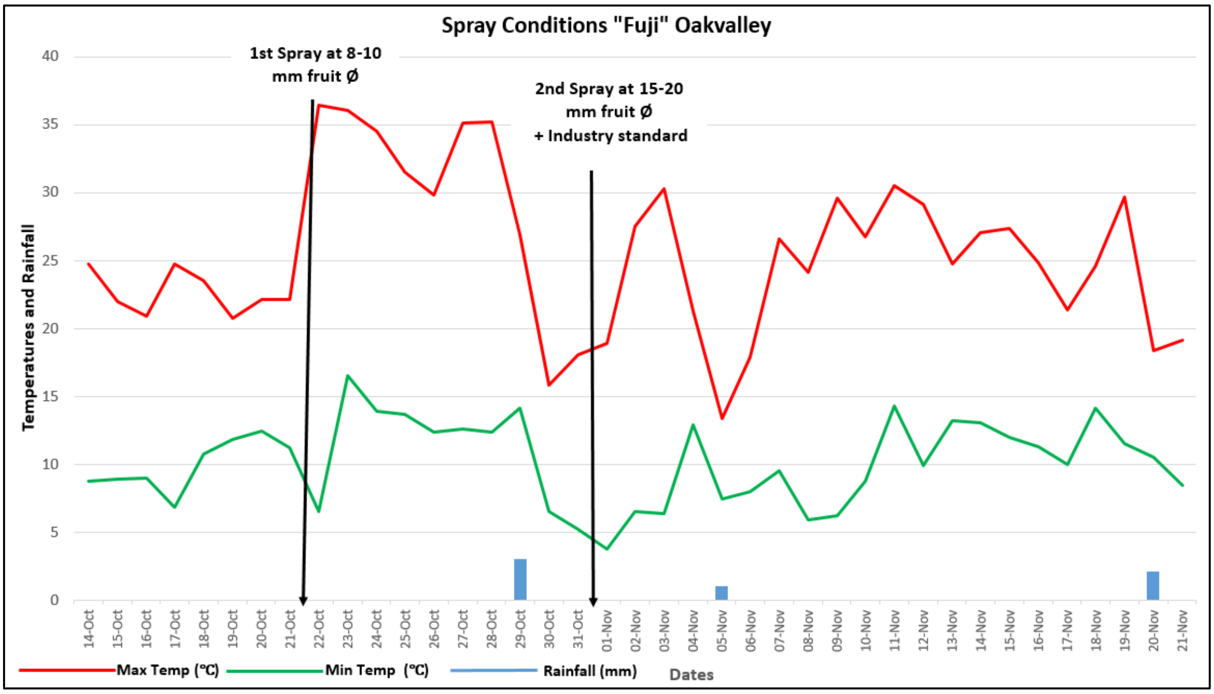

Fig. 2. Temperature and rainfall during treatment application for ‘Fuji’ apples at Oak Valley Estate, Grabouw area, South Africa (2018/2019).

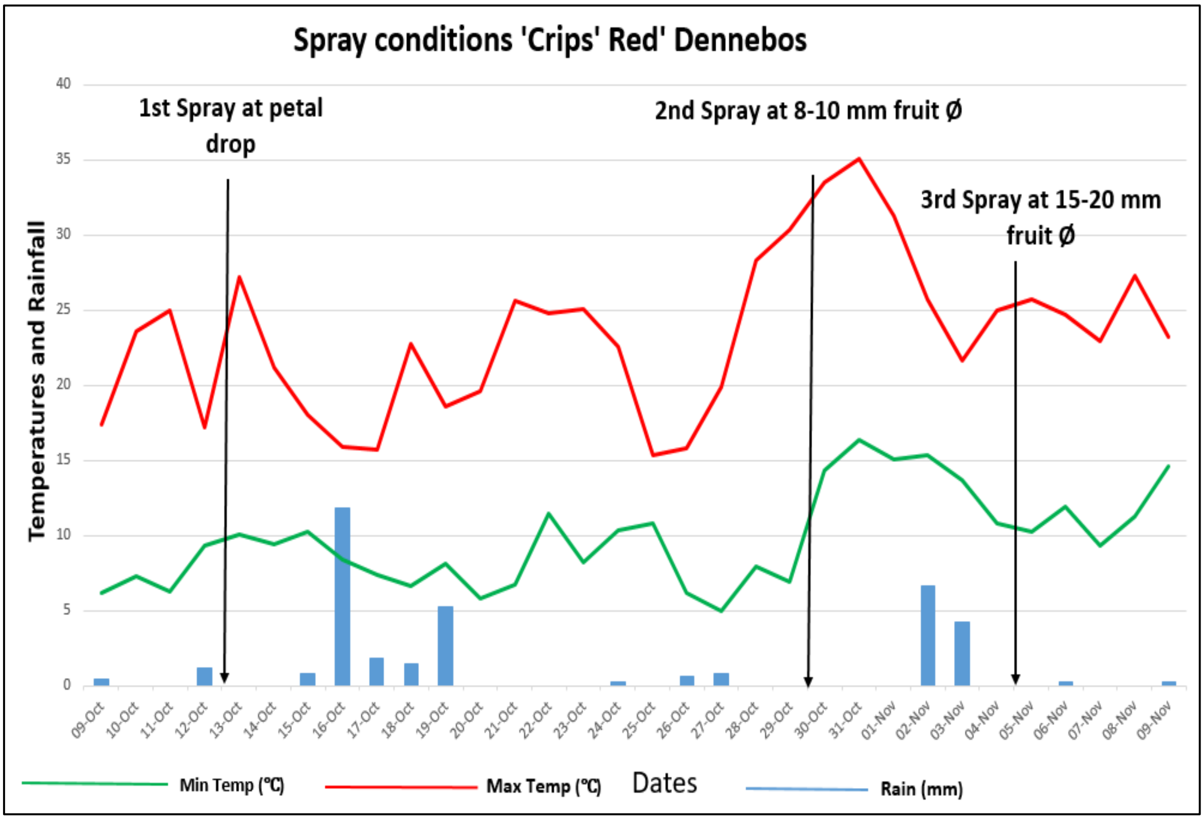

Fig. 3. Temperature and rainfall during treatment application for 'Cripps' Red' apples at Dennebos, Vyeboom area, South Africa (2017/2018).

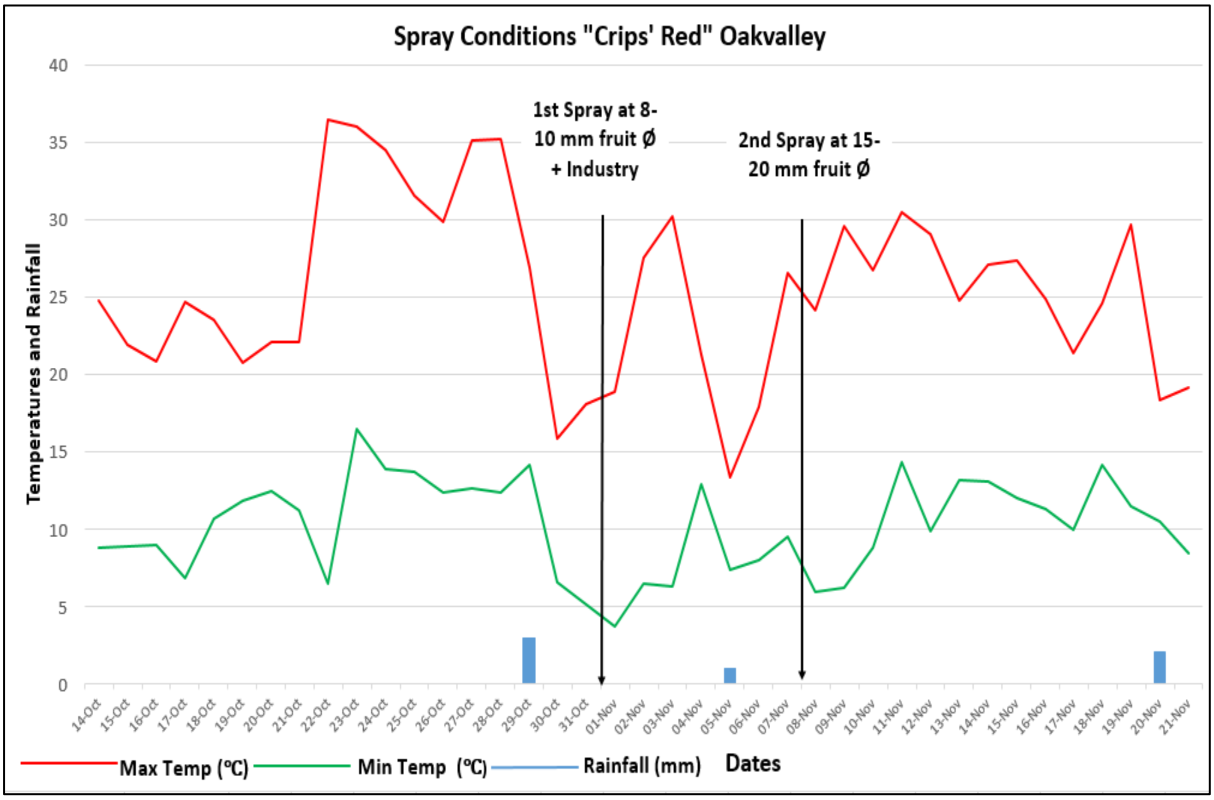

Fig. 4. Temperature and rainfall during treatment application for 'Cripps' Red' apples at Oak Valley Estate, Grabouw area, South Africa (2018/2019).

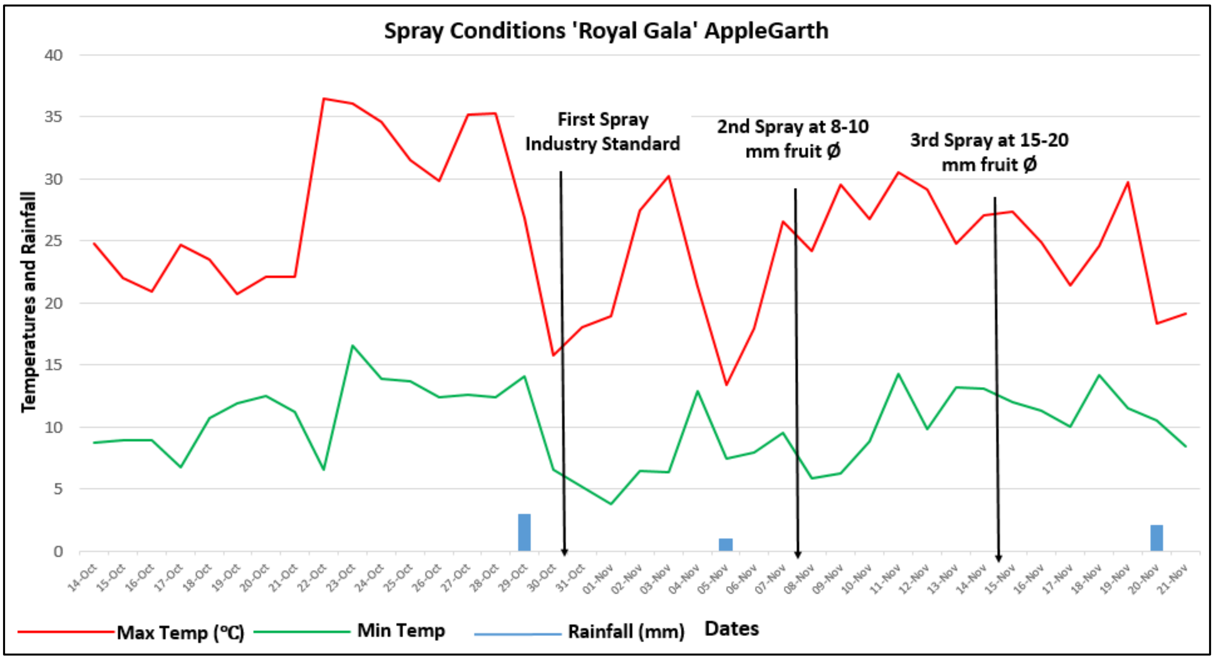

Fig. 5. Temperature and rainfall during treatment application for 'Royal Gala' apples at Applegarth, Grabouw area, South Africa (2018/2019).

Table 1. Treatment detail for trials done with 1-aminocyclopropane carboxylic acid (ACC) on 'Fuji' and 'Cripps' Red' apples in the season of 2017/2018.

Treatment
Untreated control (UTC)
Industry standard application*
ACC 250 µl·L^{-1} at petal drop
ACC 500 µl·L^{-1} at petal drop
ACC 250 µl·L^{-1} at 8- 10 mm fruit diameter
ACC 500 µl·L^{-1} at 8- 10 mm fruit diameter
ACC 250 µl·L^{-1} at 15- 20 mm fruit diameter
ACC 500 µl·L^{-1} at 15- 20 mm fruit diameter

*6-BA (100 mg·L^{-1}) tank mix with NAA (5 mg·L^{-1}) was industry standard application for 'Fuji'
*6-BA (100 mg·L^{-1}) was industry standard application for 'Cripps' Red'

Table 2. Treatment details for trials done with 1-aminocyclopropane carboxylic acid (ACC) on 'Fuji', 'Cripps' Red' and 'Royal Gala' apples in the season of 2018/2019.

Treatments
Untreated control (UTC)
Industry standard application*
ACC 125 µl·L^{-1} at 8- 10 mm fruit diameter
ACC 250 µl·L^{-1} at 8-1 0 mm fruit diameter
ACC 500 µl·L^{-1} at 8- 10 mm fruit diameter
ACC 125 µl·L^{-1} at 15- 20 mm fruit diameter
ACC 250 µl·L^{-1} at 15- 20 mm fruit diameter
ACC 500 µl·L^{-1} at 15- 20 mm fruit diameter

* 6-BA at 150 mg·L^{-1} was industry standard application for 'Cripps' Red' and 'Fuji'
* NAA at 5 mg·L^{-1} was industry standard application for 'Royal Gala'

Table 3. Summary of dates of treatment application, follow up hand thinning and harvests for 'Fuji' and 'Cripps' Red' in the season of 2017/2018.

Phenological stage	'Fuji'	'Cripps' Red'
Industry stand. application	9 Oct. 2017	13 Oct. 2017
1st ACC spray	9 Oct. 2017	13 Oct. 2017
2nd ACC spray	30 Oct. 2017	30 Oct. 2017
3rd ACC spray	5 Nov. 2017	5 Nov. 2017
Hand thinning	16 Nov. 2017	29 Nov. 2017
Harvest	22 March 2018*	14 May 2018*

*All fruit harvested on one day

Table 4. Summary of dates of treatment application, follow up hand thinning and harvest for ‘Fuji’, ‘Cripps’ Red’ and ‘Royal Gala’ in the season of 2018/2019.

Phenological stage	‘Fuji’	‘Cripps’ Red’	‘Royal Gala’
Industry stand. application	1 Nov. 2018	1 Nov. 2018	1 Nov. 2018
1st ACC spray	22 Oct. 2018	1 Nov. 2018	8 Nov. 2018
2nd ACC spray	1 Nov. 2018	8 Nov. 2018	15 Nov. 2018
Hand thinning	28 Nov. 2018	11 Dec. 2018	5 Dec. 2018
1st harvest	25 March 2019*	16 May 2019*	14 Feb. 2019
2nd harvest	-	-	20 Feb 2019

*All fruit harvested on one day

Table 5. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average fruit set per flower cluster and hand thinning requirement of ‘Fuji’ apples at Oak Valley Estate, Grabouw area, South Africa (2017/2018).

Treatment	Time of application	Average fruit set per flower cluster		Average number of fruitlets thinned by hand	
UTC*	No application	1.61	ns	118	bc
6-BA + NAA**	8-10 mm fruit diam.	1.42		185	a
ACC 250	petal drop	1.13		135	b
ACC 500	petal drop	0.86		91	bc
ACC 250	8- 10 mm fruit diam.	1.34		98	bc
ACC 500	8- 10 mm fruit diam.	1.12		71	c
ACC 250	15- 20 mm fruit diam.	1.56		112	bc
ACC 500	15- 20 mm fruit diam.	1.28		99	bc
Significance level		*0.4546*		***<.0001***	
LSD 5%		-		*40.0*	
Control vs ACC		*0.0737*		*0.2839*	
ACC 250 vs ACC 500		*0.1237*		***0.0184***	
1st vs 2nd		*0.2470*		***0.0453***	
1st vs 3rd		***0.0394***		*0.5811*	
2nd vs 3rd		*0.3536*		*0.1411*	

*UTC = Untreated control; **6-BA (100 $mg \cdot L^{-1}$) tank mixed with NAA (5 $mg \cdot L^{-1}$)

Table 6. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average yield and yield efficiency of 'Fuji' apples at Oak Valley estate, Grabouw area, South Africa (2017/2018).

Treatment	Time of application	Average total yield per tree (kg)		Average total yield efficiency (kg.cm^{-2})	
UTC*	No application	41.0	a	0.71	a
6-BA + NAA**	8- 10 mm fruit diam.	23.8	c	0.38	c
ACC 250	petal drop	41.9	a	0.66	ab
ACC 500	petal drop	23.4	c	0.39	c
ACC 250	8- 10 mm fruit diam.	36.6	ab	0.58	ab
ACC 500	8- 10 mm fruit diam.	26.0	c	0.41	c
ACC 250	15- 20 mm fruit diam.	37.6	ab	0.69	ab
ACC 500	15- 20 mm fruit diam.	30.3	bc	0.54	bc
Significance level		***0.0003***		***<.0001***	
LSD 5%		*9.3*		*0.16*	
Control vs ACC		***0.0224***		***0.0073***	
ACC 250 vs ACC 500		***<.0001***		***<.0001***	
1st vs 2nd		*0.6822*		*0.5637*	
1st vs 3rd		*0.6870*		*0.1097*	
2nd vs 3rd		*0.4177*		***0.0315***	

*UTC = Untreated control; **6-BA (100 mg·L^{-1}) tank mixed with NAA (5 mg·L^{-1})

Table 7. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average fruit size of 'Fuji' apples at Oak Valley Estate, Grabouw area, South Africa (2017/2018).

Treatment	Time of application	Average weight of fruit (g)		Average diameter of fruit (mm)		Average length of fruit (mm)	
UTC*	No application	177.4	abc	73.5	ab	62.0	ab
BA+NAA**	8- 10 mm fruit diam.	162.9	c	70.2	c	60.0	c
ACC 250	petal drop	179.0	ab	73.3	ab	61.7	abc
ACC 500	petal drop	186.5	a	74.3	a	62.5	a
ACC 250	8- 10 mm fruit diam.	170.0	bc	72.0	bc	60.3	ab
ACC 500	8- 10 mm fruit diam.	179.3	ba	73.0	ab	61.7	abc
ACC 250	15- 20 mm fruit diam.	169.2	bc	72.1	abc	60.8	abc
ACC 500	15- 20 mm fruit diam.	169.3	bc	72.8	ab	60.1	c
Significance level		***0.0275***		***0.0154***		***0.0222***	
LSD 5%		*14.5*		*2.2*		*1.9*	
Control vs ACC		*0.7360*		*0.4797*		*0.2292*	
ACC 250 vs ACC 500		*0.1825*		*0.1626*		*0.3369*	
1st vs 2nd		*0.1217*		*0.0940*		*0.1092*	
1st vs 3rd		***0.0111***		*0.0870*		***0.0170***	
2nd vs 3rd		*0.2952*		*0.9694*		*0.4081*	

*UTC = Untreated control; **6-BA (100 mg·L^{-1}) tank mixed with NAA (5 mg·L^{-1})

Table 8. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average return bloom percentage of 'Fuji' apples at Oak Valley Estate, Grabouw area, South Africa (2017/2018).

Treatment	Time of application	Average return bloom %	
UTC*	No application	33.06	bc
6-BA + NAA**	8- 10 mm fruit diam.	39.77	ab
ACC 250	petal drop	27.68	c
ACC 500	petal drop	46.09	a
ACC 250	8- 10 mm fruit diam.	34.33	bc
ACC 500	8- 10 mm fruit diam.	44.53	a
ACC 250	15- 20 mm fruit diam.	31.59	bc
ACC 500	15- 20 mm fruit diam.	36.72	abc
Significance level		***0.0083***	
LSD 5%		*9.90*	
Control vs ACC		*0.3271*	
ACC 250 vs ACC 500		***0.0003***	
1st vs 2nd		*0.4743*	
1st vs 3rd		*0.4416*	
2nd vs 3rd		*0.1404*	

*UTC = Untreated control; **6-BA (100 mg·L^{-1}) tank mixed with NAA (5 mg·L^{-1})

Table 9. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average fruit set per flower cluster and hand thinning requirement of 'Cripps' Red' apples at Dennebos, Vyeboom area, South Africa (2017/2018).

Treatment	Time of application	Average fruit set per flower cluster		Average number of fruitlets thinned by hand	
UTC*	No application	0.99	ab	127	ns
6-BA**	8-10 mm fruit diam.	1.12	a	96	
ACC 250	petal drop	0.98	ab	92	
ACC 500	petal drop	0.29	d	80	
ACC 250	8- 10 mm fruit diam.	0.68	bc	94	
ACC 500	8- 10 mm fruit diam.	0.42	cd	91	
ACC 250	15- 20 mm fruit diam.	0.96	ab	83	
ACC 500	15- 20 mm fruit diam.	0.83	ab	107	
Significance level		***0.0001***		*0.8467*	
LSD 5%		*0.32*		-	
Control vs ACC		***0.0176***		*0.0838*	
ACC 250 vs ACC 500		***0.0002***		*0.8625*	
1^{st} vs 2^{nd}		*0.4765*		*0.7563*	
1^{st} vs 3^{rd}		***0.0203***		*0.6566*	
2^{nd} vs 3^{rd}		***0.0030***		*0.8930*	

*UTC = untreated control; **6-BA (100 mg·L^{-1})

Table 10. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average yield and yield efficiency of 'Cripps' Red' apples at Dennebos, Vyeboom area, South Africa (2017/2018).

Treatment	Time of application	Average total yield per tree (kg)		Average total yield efficiency (kg.cm^{-2})	
UTC*	No application	24.8	ns	0.67	a
6-BA**	8-10 mm fruit diam.	23.2		0.64	ab
ACC 250	petal drop	19.3		0.51	abc
ACC 500	petal drop	18.6		0.45	c
ACC 250	8- 10 mm fruit diam.	19.8		0.51	abc
ACC 500	8- 10 mm fruit diam.	18.6		0.47	bc
ACC 250	15- 20 mm fruit diam.	21.9		0.55	abc
ACC 500	15- 20 mm fruit diam.	20.0		0.47	bc
Significance level		*0.1159*		***0.0321***	
LSD 5%		-		*0.18*	
Control vs ACC		*0.0532*		***0.0144***	
ACC 250 vs ACC 500		*0.5194*		*0.2581*	
1st vs 2nd		*0.9131*		*0.9476*	
1st vs 3rd		*0.3965*		*0.6887*	
2nd vs 3rd		*0.4596*		*0.7377*	

*UTC = untreated control; **6-BA (100 mg·L^{-1})

Table 11. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average fruit size of 'Cripps' Red' apples at Dennebos, Vyeboom area, South Africa (2017/2018).

Treatment	Time of application	Average weight of fruit (g)		Average fruit diameter (mm)		Average fruit length (mm)	
UTC*	No application	146.0	a	69.7	ab	58.8	a
6-BA**	8- 10 mm fruit diam.	144.9	a	70.0	a	57.9	abc
ACC 250	petal drop	145.7	a	70.7	a	58.4	ab
ACC 500	petal drop	138.9	ab	69.1	ab	56.8	bcd
ACC 250	8- 10 mm fruit diam.	125.5	d	66.3	d	54.9	e
ACC 500	8- 10 mm fruit diam.	130.4	bcd	67.1	cd	55.3	de
ACC 250	15- 20 mm fruit diam.	134.5	bc	68.2	bc	56.6	cde
ACC 500	15- 20 mm fruit diam.	128.5	cd	67.3	cd	55.9	de
Significance level		***<.0001***		***<.0001***		***<.0001***	
LSD 5%		*9.0*		*1.6*		*1.6*	
Control vs ACC		***0.0008***		***0.0130***		***0.0002***	
ACC 250 vs ACC 500		*0.3118*		*0.2219*		*0.1881*	
1st vs 2nd		***<.0001***		***<.0001***		***<.0001***	
1st vs 3rd		***0.0012***		***0.0003***		***0.0172***	
2nd vs 3rd		*0.2694*		*0.0776*		*0.0611*	

*UTC = untreated control; **6-BA (100 mg·L^{-1})

Table 12. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average return bloom percentage of 'Cripps' Red' apples at Dennebos, Vyeboom area, South Africa (2017/2018).

Treatment	Time of application	Average return bloom %	
UTC*	No application	59.85	c
6-BA**	8- 10 mm fruit diam.	70.83	ab
ACC 250	petal drop	72.34	ab
ACC 500	petal drop	74.50	a
ACC 250	8- 10 mm fruit diam.	70.97	ab
ACC 500	8- 10 mm fruit diam.	67.20	b
ACC 250	15- 20 mm fruit diam.	70.88	ab
ACC 500	15- 20 mm fruit diam.	74.49	a
Significance level		***0.0021***	
LSD 5%		*7.05*	
Control vs ACC		***<.0001***	
ACC 250 vs ACC 500		*0.7435*	
1st vs 2nd		*0.0873*	
1st vs 3rd		*0.7695*	
2nd vs 3rd		*0.1541*	

*UTC = untreated control; **6-BA (100 mg·L^{-1})

Table 13. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average fruit set per flower cluster, hand thinning requirement and leaf drop of ‘Fuji’ apples at Oak Valley Estate, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	Average fruit set per flower cluster		Average number of fruitlets hand thinned		Average leaf drop score***	
UTC*	No application	2.53	ns	334	ns	0.0	e
6-BA**	15- 20 mm fruit diam.	2.45		313		0.0	e
ACC 125	8- 10 mm fruit diam.	2.54		331		0.8	d
ACC 250	8- 10 mm fruit diam.	2.55		337		1.2	c
ACC 500	8- 10 mm fruit diam.	2.51		307		1.7	b
ACC 125	15- 20 mm fruit diam.	3.02		337		1.6	b
ACC 250	15- 20 mm fruit diam.	2.93		318		1.9	b
ACC 500	15- 20 mm fruit diam.	2.46		370		2.9	a
Significance level		*0.9421*		*0.2818*		***<.0001***	
LSD 5%		-		-		*0.4*	
Control vs ACC		*0.5848*		*0.9723*		***<.0001***	
1^{ST} vs 2^{nd}		*0.1679*		*0.4706*		***<.0001***	
ACC Linear 1^{st}		*0.9274*		*0.5053*		***<.0001***	
ACC Quadratic 1^{st}		*0.9551*		*0.6956*		*0.5733*	
ACC Linear 2^{nd}		*0.0772*		*0.3170*		***<.0001***	
ACC Quadratic 2^{nd}		*0.7504*		*0.4083*		*0.4532*	

*UTC = untreated control; **6-BA (150 mg·L^{-1}); ***Leaf drop scored between 0 and 5 with 0 being no leaf drop and 5 severe leaf drop

Table 14. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average yield and yield efficiency of 'Fuji' apples at Oak Valley Estate, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	Average total yield per tree (kg)		Average total yield efficiency (kg.cm^{-2})	
UTC*	No application	50.9	abc	0.65	ab
6-BA**	15- 20 mm fruit diam.	59.8	a	0.72	a
ACC 125	8- 10 mm fruit diam.	54.7	ab	0.67	ab
ACC 250	8- 10 mm fruit diam.	52.6	ab	0.65	ab
ACC 500	8- 10 mm fruit diam.	41.9	c	0.55	b
ACC 125	15- 20 mm fruit diam.	50.0	abc	0.61	ab
ACC 250	15- 20 mm fruit diam.	47.3	bc	0.62	ab
ACC 500	15- 20 mm fruit diam.	54.1	ab	0.61	ab
Significance level		***0.0283***		***0.0058***	
LSD 5%		*10.5*		*0.14*	
Control vs ACC		*0.8362*		*0.5292*	
1ST vs 2nd		*0.8012*		*0.7516*	
ACC Linear 1st		***0.0125***		*0.0726*	
ACC Quadratic 1st		*0.6349*		*0.7209*	
ACC Linear 2nd		*0.3376*		*0.9582*	
ACC Quadratic 2nd		*0.3886*		*0.8338*	

*UTC = untreated control; **6-BA (150 mg·L^{-1})

Table 15. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average fruit size of 'Fuji' apples at Oak Valley Estate, Grabouw area, South Africa (2018/2019).

Treatments	Time of application	Average fruit weight (g)		Average diameter of fruit (mm)		Average length of fruit (mm)	
UTC*	No application	154.2	bc	72.8	bc	57.3	ns
6-BA**	15- 20 mm fruit diam.	168.1	a	74.9	a	59.5	
ACC 125	8- 10 mm fruit diam.	149.2	bc	72.2	bc	57.0	
ACC 250	8- 10 mm fruit diam.	151.8	bc	72.6	bc	57.2	
ACC 500	8- 10 mm fruit diam.	145.2	c	71.4	c	56.7	
ACC 125	15- 20 mm fruit diam.	154.5	bc	72.9	bc	57.8	
ACC 200	15- 20 mm fruit diam.	157.7	b	73.5	ab	57.7	
ACC 500	15- 20 mm fruit diam.	153.8	bc	72.8	bc	57.4	
Significance level		***0.0033***		***0.0032***		*0.0656*	
LSD 5%		*10.1*		*1.6*		-	
Control vs ACC		*0.5739*		*0.7177*		*0.9196*	
1ST vs 2nd		***0.0271***		***0.0292***		*0.1431*	
ACC Linear 1st		*0.3421*		*0.2317*		*0.6120*	
ACC Quadratic 1st		*0.3807*		*0.3354*		*0.6203*	
ACC Linear 2nd		*0.7815*		*0.7089*		*0.5934*	
ACC Quadratic 2nd		*0.4366*		*0.4020*		*0.9070*	

*UTC = untreated control; **6-BA (150 mg·L^{-1})

Table 16. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average ground color, full and aborted seed number and stem-end russet of 'Fuji' apples at Oak Valley Estate, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	Average ground color score***		Average full seed number per fruit		Average aborted seed number per fruit		Average stem-end russet****	
UTC*	No application	3.3	ab	7.4	d	0.2	ns	0.32	ns
6-BA**	15- 20 mm fruit diam.	3.2	ab	7.8	bc	0.4		0.61	
ACC 125	8- 10 mm fruit diam.	3.1	b	7.6	cd	0.1		0.62	
ACC 250	8- 10 mm fruit diam.	3.0	b	8.1	ab	0.1		0.69	
ACC 500	8- 10 mm fruit diam.	3.5	a	7.8	bc	0.2		0.66	
ACC 125	15- 20 mm fruit diam.	3.3	ab	7.9	abc	0.2		0.65	
ACC 250	15- 20 mm fruit diam.	3.3	ab	8.3	a	0.0		0.70	
ACC 500	15- 20 mm fruit diam.	3.3	ab	7.9	abc	0.3		0.73	
Significance level		***0.0497***		***0.0003***		*0.1824*		*0.0643*	
LSD 5%		*0.3*		*0.4*		-		-	
Control vs ACC		*0.8357*		***0.0009***		*0.4778*		***0.0004***	
1ST vs 2nd		*0.3060*		*0.1275*		*0.2683*		*0.6562*	
ACC Linear 1st		***0.0500***		*0.5684*		*0.4600*		*0.8174*	
ACC Quadratic 1st		*0.1074*		***0.0170***		*0.7485*		*0.5759*	
ACC Linear 2nd		*0.9819*		*0.8117*		*0.4905*		*0.5186*	
ACC Quadratic 2nd		*0.9064*		*0.0517*		*0.1026*		*0.8461*	

*UTC = untreated control; **6-BA (150 $mg \cdot L^{-1}$); ***Ground color was scored using chart A.45 with scores ranging from 1 to 12, with 12 being the least color development; **** Stem-end russet was scored using chart A.31 where 0 = no russet and 12 severe russet

Table 17. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average return bloom percentage of 'Fuji' apples at Oak Valley Estate, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	Average return bloom %
UTC*	No application	14.23 ns
6-BA**	15- 20 mm fruit diam.	16.32
ACC 125	8- 10 mm fruit diam.	9.44
ACC 250	8- 10 mm fruit diam.	10.63
ACC 500	8- 10 mm fruit diam.	14.82
ACC 125	15- 20 mm fruit diam.	11.61
ACC 250	15- 20 mm fruit diam.	11.73
ACC 500	15- 20 mm fruit diam.	12.77
Significance level		*0.3051*
LSD 5%		-
Control vs ACC		*0.2306*
1^{ST} vs 2^{nd}		*0.7852*
ACC Linear 1^{st}		***0.0338***
ACC Quadratic 1^{st}		*0.7900*
ACC Linear 2^{nd}		*0.6319*
ACC Quadratic 2^{nd}		*0.9084*

*UTC = untreated control; **6-BA (150 mg·L^{-1})

Table 18. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average fruit set per flower cluster and hand thinning requirement of 'Cripps' Red' apples at Oak Valley Estate, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	Average fruit set per flower cluster		Average number of fruitlets hand thinned	
UTC*	No application	1.96	a	877	ab
6-BA**	8- 10 mm fruit diam.	1.56	abc	670	bc
ACC 125	8- 10 mm fruit diam.	2.20	a	983	a
ACC 250	8- 10 mm fruit diam.	1.84	ab	945	a
ACC 500	8- 10 mm fruit diam.	1.98	a	786	abc
ACC 125	15- 20 mm fruit diam.	1.59	abc	685	bc
ACC 250	15- 20 mm fruit diam.	1.12	c	797	abc
ACC 500	15- 20 mm fruit diam.	1.21	bc	558	c
Significance level		***0.0083***		***0.0015***	
LSD 5%		*0.72*		*251.0*	
Control vs ACC		*0.2757*		*0.3775*	
1^{ST} vs 2^{nd}		***0.0013***		***0.0029***	
ACC Linear 1^{st}		*0.6516*		*0.1058*	
ACC Quadratic 1^{st}		*0.3735*		*0.8064*	
ACC Linear 2^{nd}		*0.3997*		*0.1995*	
ACC Quadratic 2^{nd}		*0.2823*		*0.1695*	

*UTC = untreated control; **6-BA (150 mg·L^{-1})

Table 19. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average yield and yield efficiency of ‘Cripps’ Red’ apples at Oak Valley Estate, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	Average total yield per tree (kg)		Average yield efficiency ($kg.cm^{-2}$)	
UTC*	No application	60.2	ab	0.72	a
6- BA**	8- 10 mm fruit diam.	63.9	ab	0.66	a
ACC 125	8- 10 mm fruit diam.	63.0	ab	0.72	a
ACC 250	8- 10 mm fruit diam.	61.5	ab	0.67	a
ACC 500	8- 10 mm fruit diam.	62.5	ab	0.66	a
ACC 125	15- 20 mm fruit diam.	68.7	a	0.68	a
ACC 250	15- 20 mm fruit diam.	63.1	ab	0.63	a
ACC 500	15- 20 mm fruit diam.	50.7	b	0.47	b
Significance level		***0.0011***		***<.0001***	
LSD 5%		*13.4*		*0.12*	
Control vs ACC		*0.7971*		*0.0832*	
1^{ST} vs 2^{nd}		*0.7052*		***0.0135***	
ACC Linear 1^{st}		*0.9784*		*0.3216*	
ACC Quadratic 1^{st}		*0.8195*		*0.5549*	
ACC Linear 2^{nd}		***0.0078***		***0.0008***	
ACC Quadratic 2^{nd}		*0.9443*		*0.7497*	

*UTC = untreated control; **6-BA (150 $mg \cdot L^{-1}$)

Table 20. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average fruit size of 'Cripps' Red' apples at Oak Valley Estate, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	Average weight of fruit (g)		Average diameter of fruit (mm)		Average length of fruit (mm)	
UTC*	No application	124.2	d	66.8	d	58.8	a
6-BA**	8- 10 mm fruit diam.	142.6	a	71.4	a	57.9	abc
ACC 125	8- 10 mm fruit diam.	123.2	d	68.6	c	58.4	ab
ACC 250	8- 10 mm fruit diam.	125.9	cd	69.3	bc	56.8	bcd
ACC 500	8- 10 mm fruit diam.	122.6	d	68.5	c	54.9	e
ACC 125	15- 20 mm fruit diam.	133.1	bc	70.3	ab	55.3	de
ACC 250	15- 20 mm fruit diam.	125.9	cd	68.9	c	56.6	cd
ACC 500	15- 20 mm fruit diam.	137.4	ab	71.2	a	55.9	de
Significance level		***<.0001***		***<.0001***		***<.0001***	
LSD 5%		*7.5*		*1.3*		*1.6*	
Control vs ACC		*0.1891*		***<.0001***		***0.0002***	
1^{ST} vs 2^{nd}		***0.0003***		***0.0007***		*0.0930*	
ACC Linear 1^{st}		*0.7296*		*0.6798*		***<.0001***	
ACC Quadratic 1^{st}		*0.3863*		*0.1882*		*0.5637*	
ACC Linear 2^{nd}		*0.1034*		*0.0565*		*0.6971*	
ACC Quadratic 2^{nd}		***0.0115***		***0.0031***		*0.1436*	

*UTC = untreated control; **6-BA (150 mg·L^{-1})

Table 21. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average full and aborted seed number, stem-end russet and ground color of 'Cripps' Red' apples at Oak Valley Estate, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	Average ground color score***		Average full seed number		Average aborted seed number		Average stem-end russet****	
UTC*	No application	3.0	e	6.5	ab	0.06	b	2.2	ab
6-BA**	8- 10 mm fruit diam.	3.2	bcd	6.5	ab	0.03	b	2.1	abc
ACC 125	8- 10 mm fruit diam.	3.3	ab	6.2	b	0.07	b	1.8	c
ACC 250	8- 10 mm fruit diam.	3.2	abc	6.6	ab	0.08	b	2.0	bc
ACC 500	8- 10 mm fruit diam.	3.1	de	6.4	ab	0.15	b	1.9	bc
ACC 125	15- 20 mm fruit diam.	3.1	cd	6.6	ab	0.13	b	2.2	ab
ACC 250	15- 20 mm fruit diam.	3.2	abc	6.5	ab	0.12	b	2.3	a
ACC 500	15- 20 mm fruit diam.	3.3	a	6.7	a	0.36	a	2.0	bc
Significance level		***0.0004***		***<.0001***		***0.0218***		***0.0002***	
LSD 5%		*0.1*		*0.5*		*0.18*		*0.3*	
Control vs ACC		***<.0001***		*0.8868*		*0.1608*		*0.2201*	
1ST vs 2nd		*0.2373*		*0.1656*		***0.0464***		***0.0045***	
ACC Linear 1st		***0.0020***		*0.5450*		*0.3692*		*0.8532*	
ACC Quadratic 1st		*0.6309*		*0.0838*		*0.8576*		*0.3876*	
ACC Linear 2nd		***0.0030***		*0.6931*		***0.0047***		*0.1266*	
ACC Quadratic 2nd		*0.6309*		*0.6222*		*0.2860*		*0.1754*	

*UTC = untreated control; **6-BA (150 mg·L^{-1}); ***Ground color was scored using the PINK LADY® color chart with scores ranging from 1 to 12, with 12 being the best color development; **** Stem-end russet was scored using chart A.43 where 0 = no russet and 12 severe russet

Table 22. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average return bloom percentage of 'Cripps' Red' apples at Oak Valley Estate, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	Average return bloom %	
UTC*	No application	44.98	c
6-BA**	8- 10 mm fruit diam.	56.07	b
ACC 125	8- 10 mm fruit diam.	44.65	c
ACC 250	8- 10 mm fruit diam.	48.27	c
ACC 500	8- 10 mm fruit diam.	57.48	ab
ACC 125	15- 20 mm fruit diam.	50.88	bc
ACC 250	15- 20 mm fruit diam.	45.98	c
ACC 500	15- 20 mm fruit diam.	63.14	a
Significance level		***<.0001***	
LSD 5%		*6.76*	
Control vs ACC		***0.0108***	
1^{ST} vs 2^{nd}		*0.1043*	
ACC Linear 1^{st}		***0.0002***	
ACC Quadratic 1^{st}		*0.8256*	
ACC Linear 2^{nd}		***<.0001***	
ACC Quadratic 2^{nd}		***0.0036***	

*UTC = untreated control; **6-BA (150 mg·L^{-1})

Table 23. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average fruit set per flower cluster, hand thinning requirement and leaf drop of 'Royal Gala' apples at Applegarth, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	Average fruit set per flower cluster		Average number of fruitlets thinned by hand		Average leaf drop score***	
UTC*	No application	0.36	ns	92	ab	0.0	f
NAA**	4- 6 mm fruit diam.	0.29		75	ab	0.0	f
ACC 125	8- 10 mm diam.	0.32		67	b	0.8	e
ACC 250	8- 10 mm fruit diam.	0.33		111	a	1.2	d
ACC 500	8- 10 mm fruit diam.	0.28		76	ab	1.7	bc
ACC 125	15- 20 mm fruit diam.	0.36		103	ab	1.5	cd
ACC 250	15- 20 mm fruit diam.	0.28		76	ab	1.9	bc
ACC 500	15- 20 mm fruit diam.	0.28		77	ab	2.9	a
Significance level		*0.8760*		***<.0001***		***<.0001***	
LSD 5%		-		*41.0*		*0.4*	
Control vs ACC		*0.3581*		*0.6555*		***<.0001***	
1^{ST} vs 2^{nd}		*0.9970*		*0.9556*		***<.0001***	
ACC Linear 1^{st}		*0.5642*		*0.9824*		***<.0001***	
ACC Quadratic 1^{st}		*0.6912*		***0.0267***		*0.5480*	
ACC Linear 2^{nd}		*0.4018*		*0.2896*		***<.0001***	
ACC Quadratic 2^{nd}		*0.4017*		*0.3142*		*0.6885*	

*UTC = untreated control; ** NAA (5 mg·L^{-1}); ***Leaf drop scored between 0 and 5 with 0 being no leaf drop and 5 severe leaf drop

Table 24. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average yield and yield efficiency of 'Royal Gala' apples at Applegarth, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	Average total yield per tree (kg)		Average total yield efficiency (kg.cm^{-2})	
UTC*	No application	39.2	ab	0.39	ab
NAA**	4- 6 mm fruit diam.	39.1	ab	0.36	ab
ACC 125	8- 10 mm fruit diam.	42.4	a	0.45	a
ACC 250	8- 10 mm fruit diam.	37.0	ab	0.33	b
ACC 500	8- 10 mm fruit diam.	30.1	b	0.32	b
ACC 125	15- 20 mm fruit diam.	45.0	a	0.33	b
ACC 250	15- 20 mm fruit diam.	42.1	ab	0.38	ab
ACC 500	15- 20 mm fruit diam.	40.3	ab	0.34	ab
Significance level		***<.0001***		***<.0001***	
LSD 5%		*12.2*		*0.11*	
Control vs ACC		*0.9471*		*0.4859*	
1ST vs 2nd		*0.0973*		*0.6147*	
ACC Linear 1st		***0.0490***		***0.0335***	
ACC Quadratic 1st		*0.8083*		*0.1427*	
ACC Linear 2nd		*0.4629*		*0.9661*	
ACC Quadratic 2nd		*0.8047*		*0.3053*	

*UTC = untreated control; **NAA (5 mg·L^{-1}) was Growers' Standard

Table 25. Effect of 1-aminocyclopropane carboxylic acid (ACC) on harvest distribution and average fruit weight of both harvest combined of 'Royal Gala' apples at Applegarth, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	% fruit picked at 1st harvest		Average fruit weight (g) of both harvest combined	
UTC*	No application	49.3	cde	96.7	bc
NAA**	4- 6 mm fruit diam.	70.3	a	98.4	bc
ACC 125	8- 10 mm fruit diam.	53.5	bcde	102.0	ab
ACC 250	8- 10 mm fruit diam.	62.8	abc	105.0	a
ACC 500	8- 10 mm fruit diam.	67.3	ab	95.0	c
ACC 125	15- 20 mm fruit diam.	62.3	abcd	99.2	abc
ACC 250	15- 20 mm fruit diam.	43.5	e	99.0	abc
ACC 500	15- 20 mm fruit diam.	48.6	de	102.6	ab
Significance level		***0.0001***		***0.0005***	
LSD 5%		*14.1*		*6.4*	
Control vs ACC		*0.1969*		*0.1258*	
1ST vs 2nd		***0.0201***		*0.8151*	
ACC Linear 1st		*0.0689*		***0.0121***	
ACC Quadratic 1st		*0.4482*		*0.0630*	
ACC Linear 2nd		*0.1298*		*0.2364*	
ACC Quadratic 2nd		***0.0256***		*0.6324*	

*UTC = untreated control; **NAA (5 mg·L^{-1})

Table 26. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average fruit size of first harvest of 'Royal Gala' apples at Applegarth, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	Average weight of fruit (g) 1st harvest		Average diameter of fruit (mm) 1st harvest		Average length of fruit (mm) 1st harvest	
UTC*	No application	98.7	b	59.2	b	51.4	c
NAA**	4- 6 mm fruit diam.	105.1	ab	60.5	ab	53.0	bc
ACC 125	8- 10 mm fruit diam.	105.2	ab	60.5	ab	53.8	b
ACC 250	8- 10 mm fruit diam.	111.8	a	61.4	a	55.7	a
ACC 500	8- 10 mm fruit diam.	104.9	ab	60.3	ab	54.6	ab
ACC 125	15- 20 mm fruit diam.	102.4	b	60.1	ab	53.6	b
ACC 250	15- 20 mm fruit diam.	103.8	ab	60.5	ab	54.2	ab
ACC 500	15- 20 mm fruit diam.	101.5	b	60.0	ab	53.2	b
Significance level		***0.0035***		***0.0062***		***0.0003***	
LSD 5%		*8.4*		*1.6*		*2.0*	
Control vs ACC		*0.0555*		***0.0339***		***0.0001***	
1ST vs 2nd		*0.0549*		*0.2536*		***0.0400***	
ACC Linear 1st		*0.6664*		*0.5866*		*0.5880*	
ACC Quadratic 1st		*0.0725*		*0.1966*		*0.4701*	
ACC Linear 2nd		*0.7685*		*0.8128*		*0.5676*	
ACC Quadratic 2nd		*0.6475*		*0.5255*		*0.4421*	

*UTC = untreated control; **NAA (5 $mg \cdot L^{-1}$)

Table 27. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average fruit size of second harvest of 'Royal Gala' apples at Applegarth, Grabouw area, South Africa (2018/2019).

	Time of application	Average weight of fruit (g) 2nd harvest		Average diameter of fruit (mm) 2nd harvest		Average length of fruit (mm) 2nd harvest	
UTC*	No application	94.6	b	59.0	ab	53.0	abc
NAA**	4- 6 mm fruit diam.	91.7	bc	58.3	b	52.1	bc
ACC 125	8- 10 mm fruit diam.	98.8	ab	59.7	ab	53.8	a
ACC 250	8- 10 mm fruit diam.	98.3	ab	59.3	ab	54.1	a
ACC 500	8- 10 mm fruit diam.	85.2	c	56.3	c	51.5	c
ACC 125	15- 20 mm fruit diam.	95.9	b	59.1	ab	54.3	a
ACC 250	15- 20 mm fruit diam.	94.1	b	58.8	ab	53.6	ab
ACC 500	15- 20 mm fruit diam.	103.7	a	60.0	a	54.4	a
Significance level		***0.0009***		***0.0012***		***0.0035***	
LSD 5%		*7.8*		*1.5*		*1.7*	
Control vs ACC		*0.6422*		*0.8376*		*0.3446*	
1ST vs 2nd		*0.0908*		***0.0476***		***0.0470***	
ACC Linear 1st		***0.0003***		***<.0001***		***0.0035***	
ACC Quadratic 1st		*0.2483*		*0.2659*		*0.1574*	
ACC Linear 2nd		***0.0250***		*0.2029*		*0.8026*	
ACC Quadratic 2nd		*0.2017*		*0.4090*		*0.3339*	

*UTC = untreated control; **NAA (5 mg·L^{-1})

Table 28. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average full seed and aborted seed number, stem-end russet and ground color of first harvest of 'Royal Gala' apples at Applegarth, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	Average ground color score 1st harvest***	Average full seed number 1st harvest	Average aborted seed number 1st harvest	Average stem-end russet 1st harvest****
UTC*	No application	4.0 ab	5.8 ns	0.2 bcd	3.0 ns
NAA**	4- 6 mm fruit diam.	4.0 b	5.7	0.3 ab	2.8
ACC 125	8- 10 mm fruit diam.	4.3 a	5.1	0.3 ab	2.9
ACC 250	8- 10 mm fruit diam.	4.0 ab	5.8	0.2 abc	3.3
ACC 500	8- 10 mm fruit diam.	4.1 ab	5.6	0.0 d	3.2
ACC 125	15- 20 mm fruit diam.	3.9 b	5.6	0.2 bc	3.2
ACC 250	15- 20 mm fruit diam.	4.1 ab	5.5	0.1 cd	3.1
ACC 500	15- 20 mm fruit diam.	3.9 b	5.4	0.3 a	3.1
Significance level		***0.0194***	*0.2621*	***0.0032***	*0.5070*
LSD 5%		*0.3*	-	*0.1*	-
Control vs ACC		*0.7791*	*0.1407*	*0.7445*	*0.3215*
1ST vs 2nd		***0.0357***	*0.8530*	*0.3026*	*0.8495*
ACC Linear 1st		*0.2237*	*0.1523*	***0.0012***	*0.2648*
ACC Quadratic 1st		*0.1343*	*0.0567*	*0.8213*	*0.2594*
ACC Linear 2nd		*0.9676*	*0.7235*	***0.0120***	*0.6254*
ACC Quadratic 2nd		*0.2682*	*0.8136*	*0.0584*	*0.8583*

*UTC = untreated control; **NAA (5 mg·L^{-1}); ***Ground color was scored using the A.42 color chart with scores ranging from 1 to 12, with 12 being the least color development; **** Stem-end russet was scored using chart A.31 where 0 = no russet and 12 severe russet

Table 29. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average full seed and aborted seed number, stem-end russet and ground color of second harvest of 'Royal Gala' apples at Applegarth, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	Average ground color score 2nd harvest***		Average full seed number 2nd harvest		Average aborted seed number 2nd harvest		Average stem-end russet 2nd harvest****	
UTC*	No application	2.6	d	5.5	ns	0.4	ns	4.1	ab
NAA**	4- 6 mm fruit diam.	3.0	abcd	5.2		0.5		3.8	c
ACC 125	8- 10 mm fruit diam.	2.8	cd	5.6		0.3		4.1	abc
ACC 250	8- 10 mm fruit diam.	3.3	ab	5.4		0.1		4.1	abc
ACC 500	8- 10 mm fruit diam.	2.9	bcd	5.3		0.5		4.1	ab
ACC 125	15- 20 mm fruit diam.	3.2	abc	5.6		0.3		3.9	bc
ACC 250	15- 20 mm fruit diam.	3.2	abc	5.4		0.4		4.1	abc
ACC 500	15- 20 mm fruit diam.	3.4	a	5.4		0.5		4.2	a
Significance level		***0.0005***		*0.7900*		*0.0856*		***0.0004***	
LSD 5%		*0.5*		-		-		*0.3*	
Control vs ACC		***0.0028***		*0.9758*		*0.5934*		*0.5105*	
1ST vs 2nd		***0.0492***		*0.9308*		*0.2927*		*0.7196*	
ACC Linear 1st		*0.9962*		*0.3285*		***0.0218***		*0.8392*	
ACC Quadratic 1st		***0.0196***		*0.7325*		***0.0278***		*0.8634*	
ACC Linear 2nd		*0.3213*		*0.5947*		*0.0725*		*0.0566*	
ACC Quadratic 2nd		*0.4852*		*0.5511*		*0.6551*		*0.5909*	

*UTC = untreated control; **NAA (5 mg·L^{-1}); ***Ground color was scored using the A.42 color chart with scores ranging from 1 to 12, with 12 being the least color development; **** Stem-end russet was scored using chart A.31 where 0 = no russet and 12 severe russet

Table 30. Effect of 1-aminocyclopropane carboxylic acid (ACC) on average return bloom percentage of 'Royal Gala' apples at Applegarth, Grabouw area, South Africa (2018/2019).

Treatment	Time of application	Average return bloom %	
UTC*	No application	47.82	ab
NAA**	4- 6 mm fruit diam.	45.47	ab
ACC 125	8- 10 mm fruit diam.	43.74	ab
ACC 250	8- 10 mm fruit diam.	51.70	a
ACC 500	8- 10 mm fruit diam.	46.98	ab
ACC 125	15- 20 mm fruit diam.	40.14	b
ACC 250	15- 20 mm fruit diam.	44.77	ab
ACC 500	15- 20 mm fruit diam.	50.80	a
Significance level		*0.0247*	
LSD 5%		*10.54*	
Control vs ACC		*0.7162*	
1^{ST} vs 2^{nd}		*0.4633*	
ACC Linear 1^{st}		*0.7344*	
ACC Quadratic 1^{st}		*0.1440*	
ACC Linear 2^{nd}		***0.0477***	
ACC Quadratic 2^{nd}		*0.8152*	

*UTC = untreated control; **NAA (5 $mg \cdot L^{-1}$)

PAPER 3: The Efficacy of Various Strategies Using Plant Growth Regulators to Manipulate Crop Load on Pears

Abstract:

Plant growth regulators were evaluated to manipulate crop load in 'Forelle' and 'Packham's Triumph' pears. The efficacy of s-abscisic acid (S-ABA) as a chemical fruit thinner was evaluated on 'Forelle' at two trial sites during the 2017/2018 season. S-ABA was applied at a range of concentrations from 100 to 600 mg·L^{-1} at 8 - 10 mm fruitlet diameter. At one of the trial sites, very low natural fruit set was experienced, thus S-ABA induced over thinning. Chemical thinning would not have been advisable under such conditions. At the other trial site S-ABA decreased fruit set and increased fruit size without negatively affecting yield. Leaf phytotoxicity was also not a concern when S-ABA was applied at 600 mg·L^{-1}. Incorporating previous trials, 300 to 400 mg·L^{-1} S-ABA applied at 8 – 10 mm fruitlet diameter might be the chemical thinning solution for 'Forelle'. In the 2018/2019 season, one fruit set trial was conducted on a young 'Packham's Triumph' orchard to switch it from low- to full-bearing. To realize this aim, a mixture of gibberellin A_{4+7} and 6-benzyladenine (GA_{4+7} plus 6-BA) at 85% flowering, as well as aminoethoxyvinylglycine (AVG), and AVG tank mixed with prohexadione-calcium (ProCa), applied 2 weeks after full bloom (w.a.f.b), were evaluated. The GA_{4+7} plus 6-BA and AVG tank mixed with ProCa treatments did not significantly increase fruit set nor yield. AVG at 125 and 250 mg·L^{-1}, however, increased yield. Lastly, fruit drop control trials were conducted on 'Forelle' using 1-naphthaleneacetic acid (NAA). NAA was applied at three different concentrations at either 7 days before harvest (d.b.h.) or 7 and 3 d.b.h. Unfortunately, due to strong winds, a high incidence of fruit drop occurred before the trials commenced. The fruit drop trials of NAA on 'Forelle' were thus disappointing, and inconclusive.

Keywords: s-Abscisic Acid (S-ABA), prohexadione-calcium (ProCa), 1-naphthaleneacetic acid (NAA), aminoethoxyvinylglycine (AVG), thinning, fruit set, fruit drop

Pear growers benefit from regular, annual crops of high internal and external fruit quality. Sufficient flower bud formation must take place in the previous season in order to ensure that there is an adequate number of flower buds that could set fruit (Wertheim, 2000). However, alternate bearing could result if the crop load is too high. Fruit, and particularly the seeds inside the fruit, negatively affect flower bud formation (Wertheim, 2000). Seed-produced hormones, like gibberellins inhibit flower bud induction and are responsible for this alternation (Callejas and Bangerth, 1997). Thus, it is essential to manage the number of fruit that mature on a tree by either decreasing crop load (thinning) or improving set. In addition crop load can be improved by limiting pre-harvest fruit drop.

When too many flowers set, the number of fruit must be reduced through thinning, otherwise inter fruitlet competition for the available resources may be high, which would result in a decrease in fruit size at harvest (Webster, 2000) and return bloom the following season (Wertheim, 2000). The earlier this thinning takes place, the more pronounced the effect will be (Bergh, 1990). Most growers prefer not to thin during bloom and first evaluate tree health and fruit set (Byers et al., 1990) as poor natural fruit set could result in over thinning. Therefore, growers tend to thin fruit rather than flowers (Wertheim, 2000). Chemical thinning has become standard practice in pome fruit production (Forshey, 1976), due to the time consumption and expenses of large scale hand thinning (Stern and Ben-Arie, 2009).

There are a number of chemical thinning agents available on the market, however, some have recently come under pressure due to negative environmental impact, e.g. carbaryl and dinitro-orthocresol (DNOC) (Wertheim, 2000). In Europe, the high cost of re-registration has caused manufacturers to discontinue the production of the synthetic auxins, 1-naphthalene acetic acid (NAA) and its amide (NAAm) (Wertheim, 2000). There is thus a constant search for new chemical thinning agents that are reliable, occur naturally in plants and are environmentally friendly (Dennis, 2000).

Abscisic acid (ABA) is a naturally occurring plant hormone (Osborne and Morgan, 1989) involved in amongst others, regulating stomatal opening and closing, which regulates water loss through transpiration (Runkle et al., 2007). When stomata are closed, photosynthesis is decreased, thus ABA influences the carbohydrate status of a plant (Horton, 1971). Due to the effect on carbohydrate status, ABA was investigated as a potential fruit thinner in apples and was found to be quite effective (Greene et al., 2011). Greene (2012) then evaluated S-ABA on ‘Bartlett’ pears over a range of rates from 50 to 500 $mg{\cdot}L^{-1}$ at the 10 mm fruit diameter stage. Greene (2012) found an increase in thinning response with an increase in S-ABA concentration up until 250 $mg{\cdot}L^{-1}$ S-ABA where after it flattened off. Thinning with S-ABA generally resulted in increased return bloom, fruit size, flesh firmness and higher soluble solids. However, excessive leaf yellowing and abscission was noted, especially at 250 and 500 $mg{\cdot}L^{-}$

^{1} S-ABA and these rates were therefore deemed unacceptable. Even when S-ABA was combined with 6-benzyladenine (6-BA), leaf yellowing and abscission was not decreased (Greene, 2012). S-ABA was also evaluated at two sites over two seasons on ‘Forelle’ pears in South Africa (Theron et al., 2017). S-ABA at rates of 200 - 300 mg·L^{-1}, significantly reduced fruit set and increased fruit size in three of the four trials sites. Theron et al. (2017) concluded that S-ABA at a rate of 300 mg·L^{-1} has potential as a chemical thinner on ‘Forelle’ pears, but that this should be verified by further trials.

Pear trees frequently fail to produce adequate crops since fruitlet abscission may be severe, especially in young trees, (Webster, 2000). Therefore, improving fruit set may be beneficial in young trees and especially in orchards that have a history of low crop loads. ‘Packham's Triumph' pear trees produce abundant blossoms from an early age, but fruit set is often unsatisfactory (Van Zyl and Strydom, 1982). Saunders et al. (1991) looked at the effect of pruning and shoot growth on fruit set of ‘Packham's Triumph' pears and concluded that fruit set of 'Packham's Triumph' pears is inhibited by new shoot growth. The position of this growth had a stronger influence than the number of new shoots that developed. Since fruit set inhibition by new shoots occurred during a short period around anthesis, it appears that correlative inhibition of the fruit by shoots is more important than competition between fruits and shoots for nutrients (Saunders et al., 1991). However, implementing pruning alone does not always improve fruit set in young trees enough to switch them from low crop loads to full cropping. On young ‘Conference’ pear trees, Regulex 90 mg·L^{-1} GA_{4+7} (75% GA_4 and 18% GA_7) reduced fruitlet abscission and improved the yield without negatively impacting on fruit shape and return bloom (Deckers and Schoofs, 2000). However, the treatment often leads to malformation in ‘Packham’s Triumph’ pears (S. Reynolds, personal communication).

Ethylene is an abscission promoting hormone (Wertheim, 1997) that decreases the auxin concentration across the abscission zone (AZ) by inhibiting auxin biosynthesis and auxin transport (Valdovinos et al., 1967) and activates abscission (Wertheim, 1997). Aminoethoxyvinylglycine (AVG) is an ethylene biosynthesis inhibitor that can be used to increase fruit set by decreasing ethylene levels (Webster, 2000). AVG, at 150 to 600 mg·L^{-1}, was evaluated on seven-year-old ‘Comice’ pear trees with applications at full bloom and two and four weeks after full bloom (w.a.f.b.) (Lombard and Richardson, 1982). Lombard and Richardson (1982) found that the optimal rate of AVG on ‘Comice’ is between 25 and 100 mg·L^{-1}, as high concentrations of AVG resulted in leaf injury and fruit russet development.

They suggested that further trials should be conducted two w.a.f.b. as the most benefit in yield from the fruit set response occurred at this stage.

Some pear cultivars are particularly susceptible to pre-harvest fruit drop, thus also reducing yield (Amarante et al., 2002). Auxin reduces mature fruit and leaf drop through *de novo* synthesis of cellulose by inhibiting ethylene-enhanced expression of the cellulase gene (Yuan and Carbaugh, 2007). Cellulase is an important hydrolytic enzyme involved in cell wall degradation and subsequent loosening or abscission of fruit and leaves. Exogenous application of a synthetic auxin, e.g. 1-naphthaleneacetic acid (NAA), causes a delay in fruit abscission and pre-harvest fruit drop (Villalobos-Acuña et al., 2010). NAA was applied at 1000 and 2000 $\mu g \cdot L^{-1}$ on 'Cure' pears and reduced pre-harvest fruit drop significantly (up to an 85 % reduction in fruit drop) (Stan et al., 1984). Stan et al. (1984) found that a single application was as effective as a two applications, and the reduction in fruit drop was directly proportional to the rate of NAA.

In this paper, we report on various trials to regulate pear crop load. We further investigated the thinning efficacy of S-ABA on 'Forelle' pears, we evaluated the use of NAA on 'Forelle' pears to decrease pre-harvest drop and various plant growth regulators (PGRs) on young 'Packham's Triumph' pear trees to increase fruit set.

Materials and Methods

Plant material, site description and treatments of the 2017/2018 season: S-ABA thinning. In the 2017/2018 season, two fruit thinning trials were conducted on 'Forelle' pear trees. One trial was conducted on the farm Glen Elgin (34°08'21"S 19°02'44"E) in the Elgin area in the Western Cape, South Africa. 'Forelle' on BP1 rootstock was planted in 1984 at a spacing of 4.5 m x 2 m. The other second trial was also conducted in the Elgin area, but on the farm Glen Fruin (34°10'52.4"S 19°03'52.4"E). 'Forelle', also on BP1, was planted in 1994 at 4.5 x 2 m. S-ABA (ProTone™; Valent BioSciences Corporation, Libertyville, Illinois 60048, USA) was evaluated as summarized in Table 1. Dates of treatment application, hand thinning and harvest can be found in Table 2.

Plant material, site description and treatments of the 2018/2019 season: Fruit set. One fruit set trial was conducted on 'Packham's Triumph' pear trees. This trial was conducted on the farm Buchuland (33°21'31"S 19°20'08"E) in the Ceres region in the Western Cape, South

Africa. ‘Packham’s Triumph’ on Calleryana rootstock was planted in 2013 with a tree spacing of 4.5 x 2 m. Three products were evaluated, viz. 6-BA plus GA_{4+7} (Promalin®; Valent BioSciences Corporation, Libertyville, Illinois 60048, USA), AVG (ReTain®; Valent BioSciences Corporation, Libertyville, Illinois 60048, USA) and prohexadione-calcium (Regalis; BASF (Pty) Ltd, Mahogany Ridge, South Africa) as summarized in Table 3. Dates of treatment application and harvest can be found in Table 4.

Plant material, site description and treatments of the 2018/2019: Pre-harvest drop. Two pre-harvest fruit drop prevention trials were conducted on ‘Forelle’ pear trees. One trial was conducted on the farm Lushof (34°17'56"S 19°20'16"E) in the Ceres area in the Western Cape, South Africa. ‘Forelle’ on the rootstock BP1 was planted in 1993 at a 4 x 1 m spacing. The other ‘Forelle’ trial was conducted on the farm Glen Elgin (34°08'21"S 19°02'44"E) in the Elgin area in the Western Cape region, South Africa. ‘Forelle’ on BP1 rootstock was planted in 1986 at a spacing of 4.5 m x 2 m. In both ‘Forelle’ trials, NAA (PoMaxa™; Valent BioSciences Corporation, Libertyville, Illinois 60048, USA) was evaluated as summarized in Table 5. Dates of treatment application and harvest can be found in Table 6.

Trial lay-out and treatment application. Randomized complete block designs with 10 single tree replications were used in all trials. Applications were made using a motorized knap-sack sprayer (STIHL, Pietermaritzburg, South Africa) and product was applied at an equivalent of approximately 1000 L per ha. Treatments where applied early morning before the temperature exceeded 20 C° and when the wind speed did not exceed 3 $m \cdot s^{-1}$. Weather data during and after application is presented in Fig. 1 - 3. Unfortunately, no weather data was collected for the NAA pre-harvest drop trials. At least one tree was left untreated between the treated trees and at least one buffer row was left in between treated rows in order to prevent any spray drift effects.

Data collection. After the treatment applications, a period of at least three weeks were allowed for the fruitlets to drop in the thinning and fruit set trials. Fruit set was determined in the lower half of the tree canopy by tagging two scaffold branches on each tree. At full bloom, the number of flower clusters were counted and the number of fruitlets that set counted approximately 14 days after treatment application in order to calculate the average fruit set. In the ‘Packham’s Triumph’ trial, the grower decided not to hand thin as fruit set was poor. Hand thinning was thus only done in the ‘Forelle’ thinning trials according to standard commercial practice. All the fruitlets that were thinned by hand were collected and brought back to the laboratory where the number of fruitlets thinned per tree were counted. Phytotoxicity on foliage leaves was recorded on a scale of 0-3, with 0 being no phytotoxicity and 3 severe phytotoxicity (±20 leaves per tree affected) (Fig. 4). At each commercial harvest, the yield of fruit per tree was recorded. After harvest the tree trunk circumference was measured in order to calculate the tree cross sectional area. This was done in order to calculate the yield efficiency expressed as kg fruit per trunk cross sectional area ($kg \cdot cm^{-2}$). For the 'Forelle' trials in the 2017/2018 season, a sample of 30 fruit per replicate per harvest were collected and brought to the laboratory for destructive and non-destructive analysis. In the 'Forelle' and 'Packham's Triumph' trials in the 2018/2019 season, a sample of 20 fruit per replicate per harvest was collected. For all the fruit in the sample, fruit length, diameter and weight were recorded. Red blush color was only recorded in the 'Forelle' trial in the 2017/2018 season using the Forelle pear P-25 color chart, ranging from 1 to 6, where 6 is no blush and 1 is high blush. The fruit in the sample that had a score lower than 4 was deemed as Class 1 quality and expressed as a percentage Class 1 fruit. Fruit firmness was measured in the 2018/2019 season using the GÜSS texture analyzer with a 7.9 mm probe (Güss electronic model GS 20, Strand, South Africa). Return bloom was calculated the following spring by counting all the reproductive and vegetative buds that broke, on the same two branches used to determine fruit set. Return bloom was then calculated as the percentage of buds that where reproductive. Fruit drop was determined in the NAA pre-harvest drop prevention trials by removing all the fallen fruit under the trees before the first spray. Just prior to harvest, the fruit that had fallen between the time of the first NAA application and harvest were counted in order to determine pre-harvest fruit drop. No malformation was observed in any of the trials.

Statistical analysis. The data were analyzed using SAS Enterprise guide 7.1 (SAS Institute Inc., Cary, North Carolina, USA) using the linear model procedure and the pairwise t-test to determine the Least Significant Difference (LSD) when the F-statistic indicated significance at $P<0.05$. Single degree of freedom, orthogonal, polynomial contrast were fitted where applicable.

Results

Results for the 2017/2018 season: S-ABA thinning of 'Forelle', Glen Elgin. S-ABA significantly reduced fruit set linearly with an increase in S-ABA rate, with the 600 $mg \cdot L^{-1}$ S-

ABA reducing fruit set the most compared to the untreated control (Table 7). Only 100 mg·L^{-1} S-ABA did not reduce fruit set compared to the untreated control. The same was found for the number of fruitlets that were hand thinned during commercial hand thinning except that 300 mg·L^{-1} S-ABA also did not differ from the untreated control. All S-ABA treatments induced small black spots on leaves (Fig. 4) and these increased linearly with rate of S-ABA, with the highest concentration of S-ABA (600 mg·L^{-1}) causing the most severe phytotoxicity (Table 7).

No significant differences were found in the total yield or yield efficiency (Table 8). S-ABA increased fruit size (weight, diameter and length) on average compared to the control. With an increase in S-ABA rate a quadratic increase in fruit weight (p=0.0343) and length (p=0.0242) was observed with an increase compared to the control only occurring at 600 mg·L^{-1} S-ABA. The increase in fruit diameter was linear with increase in S-ABA rate (p<.0001), with S-ABA at 300, 400 and 600 mg·L^{-1} significantly increasing fruit diameter compared to the untreated control (Table 9). The return bloom percentage did not differ significantly between treatments, but increased linearly with increasing rate of S-ABA (p=0.0042) (Table 10). None of the treatments significantly affected the percentage fruit classed as Class 1 based on red blush development (Table 10).

Results for the 2017/2018 season: S-ABA thinning of 'Forelle', Glen Fruin. No significant differences were found in the average fruit set per cluster or in the number of fruitlets thinned during commercial hand thinning compared to the untreated control (Table 11). However, S-ABA did, on average, decrease fruit set compared to the control and fruit set decreased linearly with an increase in S-ABA rate (Table 11). All S-ABA treatments increased the occurrence of leaf phytotoxicity compared to the untreated control. Leaf phytotoxicity increased quadratically (p=0.0396) with an increase in S-ABA rate, with a gradual increase from 100 to 400 mg·L^{-1} after which the phytotoxicity increased sharply at 600 mg·L^{-1} S-ABA (Table 11).

All S-ABA treatments decreased the yield compared to the control (Table 12). The highest concentration of S-ABA caused the most significant decrease in total yield compared to the untreated control. There was a quadratic decrease in total yield with an increase in S-ABA rate with the 100 and 600 mg·L^{-1} applications reducing yield most (p=0.0496). The lowest and highest concentrations of S-ABA significantly decreased yield efficiency compared to the untreated control. However none of the S-ABA treatments differed significantly from one another with regards to yield efficiency (Table 12). A linear increase in fruit size (weight, diameter and length) occurred with an increase in S-ABA rate, with only the 600 mg·L^{-1} S-ABA treatment significantly increasing fruit weight and diameter compared to the untreated control (Table 13). None of the treatments significantly affected fruit length compared to the untreated control (Table 13). There was no significant effect on the return bloom percentage or the percentage of fruit that met the red blush requirements of Class 1 fruit (Table 14).

Results from the 2018/2019 season: Fruit set of 'Packham's Triumph', Buchuland. None of the treatments significantly affected the percentage fruit set per flower cluster on two tagged scaffold branches in the lower tree canopy (Table 15). The producer did not implement commercial hand thinning in the season of 2018/2019 as the crop load was too low. The middle (125 mg·L^{-1}) and highest (250 mg·L^{-1}) concentrations of AVG significantly increased yield and yield efficiency compared to the untreated control (Table 15). In the case of yield efficiency, this gave rise to a significant quadratic increase in yield efficiency with an increase in AVG rate with the 125 mg·L^{-1} rate resulting in a higher yield efficiency compared to 62.25 mg·L^{-1} and 250 mg·L^{-1} (Table 15). A linear decrease in fruit size (weight, diameter and length) occurred with an increase in AVG concentration, with the highest concentration of AVG causing a significant decrease in fruit weight and length compared to the untreated control (Table 16). AVG tank-mixed with ProCa and the GA_{4+7} + 6-BA application at 85% bloom, also caused a significant reduction in fruit weight compared to the untreated control (Table 16). None of the treatments affected fruit diameter compared the untreated control (Table 16), and did not affect fruit firmness at harvest or return bloom percentage the following spring (Table 17).

Results from the 2018/2019 season: Pre-harvest drop, 'Forelle'. At Lushof farm, none of the treatments affected pre-harvest fruit drop (Table 18). NAA 11.2 at mg·L^{-1} applied once at 7 d.b.h. increased average yield per tree compared to the untreated control and 22.4 mg·L^{-1} NAA applied twice (7 and 3 d.b.h.) (Table 18). None of the treatments significantly affected yield efficiency (Table 18). The highest concentration NAA (22.4 mg·L^{-1}) applied once at 7 d.b.h. significantly increased fruit weight compared to the untreated control, but this treatment did not differ significantly from any of the other chemical treatments (Table 19). None of the treatments had a significant effect on fruit diameter, while the lowest concentration NAA (5.6 mg·L^{-1}) applied once at 7 d.b.h. significantly increased fruit length compared to the untreated control (Table 19). None of the treatments had a significant effect in fruit firmness at harvest compared to the untreated control (Table 20). At Glen Elgin farm, none of the treatments had a significant effect on average pre-harvest fruit drop, nor on yield and yield efficiency, fruit size or fruit firmness at harvest (Table 23).

Discussion

'Forelle' fruit thinning. All the S-ABA treatments were applied at the 8 - 10 mm fruitlet diameter stage as this stage is the most sensitive to chemical thinners (Wertheim, 2000). This is due to the carbohydrates deficit being at a maximum, strong competition exists between sinks, which include other fruitlets as well as rapidly growing shoots (Lakso et al., 2006). Einhorn and Arrington (2018) evaluated the combined effect of S-ABA and shading on gas exchange and fruit set of 10-year-old 'Bartlett' pears and concluded that the mechanism of thinning of S-ABA is through creating carbohydrate shortages by limiting carbohydrate supply. Thus when S-ABA is applied during periods when fruitlets are already under elevated carbohydrate stress (Wertheim, 2000), S-ABA causes thinning. This was the case at the Glen Elgin trial site as there were linear decreases in fruit set and hand thinning requirement with an increase in S-ABA rate. At this site, no significant effects on yield and yield efficiency were found, suggesting that hand thinning successfully evened out the yield per tree. At the Glen Fruin site, however, natural fruit set was very low and fruit thinning would not have been implemented commercially. Thus no significant affects were found on fruit set or the hand thinning requirement by any S-ABA treatments as it is known that trees with a low crop load are difficult to thin chemically (Williams, 1979). At the Glen Fruin trial site, all the S-ABA treatments significantly reduced yield, except the 400 $mg \cdot L^{-1}$ application. Yield efficiency was significantly reduced by the 100 and 600 $mg \cdot L^{-1}$ applications, although none of the applications differed significantly from one another. Thus, even though set on the two tagged scaffold branches was not affected, it does appear that some thinning was induced by S-ABA.

Theron et al. (2017) evaluated S-ABA on 'Forelle' pears at rates of 100 to 600 $mg \cdot L^{-1}$ also applied at the 8 – 10 mm fruitlet diameter, during two seasons at two sites. Theron et al. (2017) reported a significant thinning effect in the 2015/2016 season when S-ABA was applied at 300 $mg \cdot L^{-1}$ at both trial sites and temperatures were in the lower 20 °C - range during and after treatment application. During the next season, S-ABA only significantly thinned at one site where maximum temperatures were around 20 °C during treatment application, but then rose into the high 20 °C - range after treatment application. Temperatures were considerably lower (around 15 - 20 °C) in the days following treatment application at the site where no significant thinning action occurred (Theron et al., 2017). Even though no significant thinning occurred, fruit size was increased following the 400 and 600 mg·L^{-1} S-ABA applications (Theron et al., 2017). Theron et al. (2017) attributed the increased thinner efficacy of S-ABA at the one trial site to the higher temperatures, but also to slightly more vigorous tree growth.

A significant thinning effect should translate into an increase in fruit size (Costa and Vizzotto, 2000) and at the Glen Elgin trial site, S-ABA generally increased fruit size compared to the untreated control. With an increase in S-ABA rate causing a quadratic increase in fruit weight and diameter, and a linear increase in fruit length, with minimal increases at lower concentrations of S-ABA and then rapid increase after 600 mg·L^{-1}, with this treatment causing the largest increase in fruit size. As yield and yield efficiency was not affected, this increase in fruit size was due to the early thinning response. At the Glen Fruin trial site where fruit set and hand thinning was not affected, an increase in fruit size would also be expected as yield was decreased. The effect of rate of S-ABA was a linear increase in fruit size; however, only the treatment that caused the highest reduction in yield (S-ABA at 600 mg·L^{-1}) increased fruit size (weight, diameter and length) compared to the untreated control. At both trial sites, 600 mg·L^{-1} S-ABA caused a considerable increase in fruit size (weight, length and diameter) compared to the untreated control. Theron et al. (2017) accredited the additional increase in fruit size, above the effect expected from crop load reduction, to the stomatal closure in cluster and spur leaves induced by S-ABA. This might strengthen the transpiration stream to the fruit, thereby enhancing fruit growth. The results from our trials support this theory.

A well-known response to thinning is an increase in return bloom (Wertheim, 1997). At the Glen Elgin trial site, a linear reduction in fruit set and hand thinning requirement led to a linear increase in return bloom. However, at the Glen Fruin site where fruit set and the hand thinning requirement was not affected, but yield and yield efficiency was reduced, no significant differences were observed in return bloom, indicating that crop load was not excessive with any treatment.

Treatments that stimulate an increase in the carbohydrate status of a plant are often associated with an increase in anthocyanin synthesis, locally or as a whole (Steyn, 2008). The formation of anthocyanins (which occur as glycosylated aglycones) could be expected to indicate the availability of carbon substrates (Hussey, 1963). A reduction in crop load as caused by a thinning would increase the overall carbohydrate status of the plant and could increase anthocyanin production, which would lead to improved red blush development in ‘Forelle’. However, the major factors which influences color development is light and temperatures, and the S-ABA treatment did not improve fruit color development at either site.

Theron et al. (2017) found very little leaf phytotoxicity of S-ABA in their trials. In our trials, S-ABA did cause significant leaf phytotoxicity, with a linear increase with increasing rate of S-ABA at the Glen Elgin and quadratic increase at the Glen Fruin trial site due to a sharp increase in phytotoxicity from 400 to 600 mg·L^{-1}. The 600 mg·L^{-1} application caused the most severe leaf phytotoxicity at both trial sites. The high rate of 600 mg·L^{-1} was only included as a double rate of 300 mg·L^{-1} S-ABA for the possible registration of 300 mg·L^{-1} S-ABA on 'Forelle' and will not be included in the label. Greene (2012) evaluated S-ABA alone and in combination with 6-benzyladenine at a range of concentrations at the 10 mm fruit diameter stage on 'Bartlett' pears. Greene (2012) found commercially unacceptable leaf yellowing at 500 mg·L^{-1}S-ABA, and adding 6-BA to S-ABA did not reduce leaf yellowing, unlike previously reported when 6-BA reduced leaf drop on susceptible apple cultivars (Greene, 2012). It is important to note that Greene (2012) reported temperatures in excess of 30 °C, which could enhance leaf yellowing and abscission. Thus, the negative effect of leaf phytotoxicity is of some concern, but only when concentrations in excess of 300 mg·L^{-1} are applied during high ambient temperatures. In our trials maximum temperatures peaked three days after treatment application between 25 and 27 °C (Fig. 1 and 2). It should be noted that the threshold for scoring the highest level of phytotoxicity was quite low at ±20 leaves per tree. In addition, leaf phytotoxicity did not negatively affect fruit size or yield at the Glen Elgin trial site. Hence, we believe that phytotoxicity, if present, should not cause any risk to yield or fruit quality.

'Forelle' does not respond as easily to thinning agents as other cultivars and is known as "more difficult to thin" (Chabikwa, 2008). Our trials, as well as those performed by Theron et al. (2017), indicate that S-ABA at 300 to 400 mg·L^{-1} applied at 8 – 10 mm fruitlet diameter might be the solution for 'Forelle'.

'Packham's Triumph' fruit set. Young 'Packham's Triumph' trees flower profusely, but do not set enough fruit during the first few years of production (Dussi et al., 2000). The Nov. /June drop can be so severe that by harvest not enough fruit are left on the tree (Vercammen and Gomand, 2008). The Nov/June drop starts 2 to 4 weeks after full bloom (w.a.f.b) and the severity depends on the amount of stress the trees is under during these two weeks (Vercammen and Gomand, 2008). When stress occurs, ethylene production is stimulated and it is thought to be the signal for fruit drop (Vercammen and Gomand, 2008). Attempts can be made to reduce drop with an anti-ethylene agent; however, the timing of the treatment should be before the occurrence of stress (Vercammen and Gomand, 2008). In an attempt to improve

fruit set of young 'Packham's Triumph' trees, GA_{4+7} plus 6-BA, AVG was applied as well as a combination of AVG and ProCa. AVG has previously been reported to increase fruit set when applied 2 w.a.f.b. in 'Comice' (Lombard and Richardson, 1982), 'Packham's Triumph' (Dussi et al., 2000; Sánchez et al., 2011) and 'Abate Fetel' (Sánchez et al., 2011). Lombard and Richardson (1982) found that AVG applied 2 and 4 w.a.f.b. significantly increased fruit set in 'Comice' pears. However, in their trials concentrations as high as 600 $mg \cdot L^{-1}$ were used, and leaf injury and an increased incidence of stem-end russet was observed. Dussi et al. (2000) evaluated slightly lower concentrations (200 and 400 $mg \cdot L^{-1}$) than Lombard and Richardson (1982) applied 2 w.a.f.b. on 'Packham's Triumph'. Dussi et al. (2000) found an increase in fruit set with an increase in AVG rate, with a considerable increase in fruit set occurring at 400 $mg \cdot L^{-1}$. Like Lombard and Richardson (1982), Dussi et al. (2000) noted considerable leaf injury at 400 $mg \cdot L^{-1}$, although no russeting occurred. Sánchez et al. (2011) found that AVG at 250 $mg \cdot L^{-1}$ applied 2 w.a.f.b. increased fruit set and yield of 'Packham's Triumph' and 'Abate Fetel'. In our trial, natural fruit set was so low that the grower decided not to thin fruit, illustrating the problems with fruit set during the initial years of 'Packham's Triumph' production. In contrast to trials by Dussi et al. (2000), Lombard and Richardson (1982) and Sánchez et al. (2011), in our trial none of the AVG treatments significantly affected fruit set on two tagged scaffold branches in the lower tree canopy. However, AVG at 125 and 250 $mg \cdot L^{-1}$ increased yield and yield efficiency compared to the untreated control. A rapid increase in yield and yield efficiency followed the two highest AVG treatments with AVG at 125 and AVG at 250 $mg \cdot L^{-1}$ not differing significantly from one other, resulting in a quadratic increase in yield efficiency with an increase in AVG rate. Therefore, AVG did increase fruit set. Fruit set was determined on two scaffold branches in the lower part of the tree canopy, and were probably not representative of the fruit set in the whole tree. Dussi et al. (2000), Lombard and Richardson (1982) and Sánchez et al. (2011) also reported that AVG significantly increased yield. Reduced fruit size was reported with an increase in AVG concentration in previous trials (Lombard and Richardson, 1982; Dussi et al., 2000; Sánchez et al., 2011). This is in agreement with what was found in our trials as a linear decrease in fruit size (weight, diameter and length) with an increase in the rate of AVG.

In this trial, a combination of AVG tank-mixed with ProCa was included to determine if a low concentration ProCa tank-mixed with AVG would cause an additional increase in fruit set. This is a treatment used commercially by some growers. Smit et al. (2005) evaluated ProCa at rates from 50 to 250 $mg \cdot L^{-1}$ applied between one and three times per season, on a variety of pear cultivars. These trials were conducted in order to retard shoot growth. The first treatment was applied when 4 – 5 leaves were unfolded. Smit et al. (2005) found that ProCa application increased fruit set significantly in 'Rosemarie', but no affect was found in 'Packham's Triumph'. High rates of ProCa reduce ethylene levels (Rademacher et al., 2005). Rates of ProCa used to retard growth are much higher than the concentration of ProCa used in this trial. The reason why we included ProCa to the AVG application is to see if ProCa would increase fruit set by further reducing ethylene levels (Rademacher et al., 2005) and by reducing competition of shoots by reducing active shoot growth. The tank-mix of AVG and ProCa did not significantly affect fruit set or yield compared to the control or the same rate of AVG alone. The AVG tank mixed with ProCa treatment significantly decreased fruit weight compared to the control, which was surprising as fruit set and yield was not effected by either of these treatments. Sachs et al. (1959) conducted trials in order to explain the action of gibberellic acid in stem elongation in rosette plants. Sachs et al. (1959) concluded that GA increases the number of cells undergoing division per unit time, and that the action of GA or effect of its action acts as a cell-division "regulator". Thus this could explain why AVG and ProCa decreased fruit size, as ProCa reduced GAs during a time when cell enlargement is taking place.

Fruit set is increased by the combination of gibberellins (GAs) and the cytokinin, 6-benzyladenine (GA_{4+7} plus 6-BA) (Dreyer, 2013). Dreyer (2013) evaluated GA_{4+7} plus 6-BA both at 11 $mg{\cdot}L^{-1}$ on 'Forelle' and 'Abate Fetel' pears with an application at 30 % flowering and a second at full bloom and found an increase in fruit set (Dreyer, 2013). However, GA_{4+7} plus 6-BA also did not significantly affect fruit set, yield and yield efficiency in our trial. As was the case with the AVG tank-mixed with ProCa treatment, GA_{4+7} plus 6-BA decreased fruit size, which was surprising. The GA_{4+7} plus 6-BA treatment was applied at 85% flowering, and during application and three days after the treatment application, maximum temperatures ranged from 32 to 36 ℃ (Fig. 3). These high temperatures during flowering could have contributed to natural fruit set being very low in the orchard and possibly affected the efficacy of the GA_{4+7} plus 6-BA application as well. Surprisingly, also no malformation occurred after the GA_{4+7} plus 6-BA application.

Return bloom was not significantly affected by any of the treatments, unlike previous trials where return bloom was decreased (Lombard and Richardson, 1982). None of the treatments affected fruit firmness compared to the untreated control in our trial, therefore fruit maturity at harvest was not affected.

'Forelle' pre-harvest fruit drop. Reducing pre-harvest fruit drop with NAA has been successfully used in 'Cure' pears at 1000 – 2000 µg·L^{-1} (Stan et al., 1984). Marini et al. (1993) evaluated NAA on 'Delicious' apples at rates up to 20 mg·L^{-1} applied up to five times before harvest. Two applications were generally found to be as effective as three or more. Marini et al. (1993) concluded that 10 mg·L^{-1} applied 21 and 7 days before harvest (d.b.h.) is effective in controlling pre harvest fruit drop in 'Delicious' apples. In our trial, however, none of the treatments significantly affected pre-harvest fruit drop at either trial site. Before the treatments were applied, a considerable number of fruit had already dropped, which was accredited to very strong winds at both trial sites, shortly before the trials commenced. Thus, it is likely that a large percentage of fruit that were destined to drop pre-harvest, had already fallen prior to treatment application. NAA at 11.2 mg·L^{-1}, applied once, significantly increased yield at the Lushof site. However, this treatment did not affect yield efficiency and did not differ significantly from all of the other treatments, except NAA at 22.4 mg·L^{-1} applied twice. At the Elgin trial site, none of the treatments had a significant effect on yield and yield efficiency. There were some unexpected increases in fruit size at the Lushof site, as NAA at 22.4 mg·L^{-1} applied once significantly increased fruit weight and NAA at 5.6 mg·L^{-1} also applied once significantly increased fruit length compared to the untreated control. Overall these treatments did not differ significantly from the other NAA treatments. NAA also did not differ significantly from the untreated control with regards to fruit weight (p=0.1032) and fruit diameter (p=0.2465). At the Glen Elgin site, no significant differences were found in fruit size. It has been reported before that NAA counteracts the stimulation of abscission while allowing ethylene to accelerate maturity and color (Edgerton, 1972). NAA applications of 20 mg·L^{-1} applied 1 and 3 weeks before harvest, have stimulated fruit ripening as NAA enhanced loss of flesh firmness on 'Golden Delicious' apples (Yuan and Carbaugh, 2007). However, in our trials fruit firmness was not negatively affected at either trial site. The only explanation for NAA not affecting pre-harvest fruit drop and thus yield in our trials is therefore that susceptible fruit had dropped prior to the NAA applications or that NAA has no efficacy on 'Forelle' pear.

Conclusion

Our thinning trials on 'Forelle', as well as those performed by Theron et al. (2017), indicate that S-ABA at 300 to 400 mg·L^{-1} applied at 8 – 10 mm fruitlet diameter might be the solution to chemically thin the 'difficult-to-thin' 'Forelle' pear. In the fruit set trials on young

'Packham's Triumph' trees, GA_{4+7} plus 6-BA and AVG tank mixed with ProCa were not effective in increasing fruit set. Even though AVG did not increase fruit set, yield and yield efficiency was increased with AVG treatments. As expected, a decrease in fruit size did occur with this increase in crop load, therefore a commercial balance in yield and fruit size needs to be found. Further trials are needed to determine whether 250 $mg{\cdot}L^{-1}$ is the optimum rate of AVG in order to maximize the positive effect on crop load, without significantly compromising fruit size. The fruit drop trials with NAA on 'Forelle' were disappointing. Due to a large number of fruit falling before these fruit drop trials commenced, we could not confidently report on the efficacy of NAA in controlling pre-harvest fruit drop of 'Forelle'. These trials would have to be repeated, possibly adding even earlier applications.

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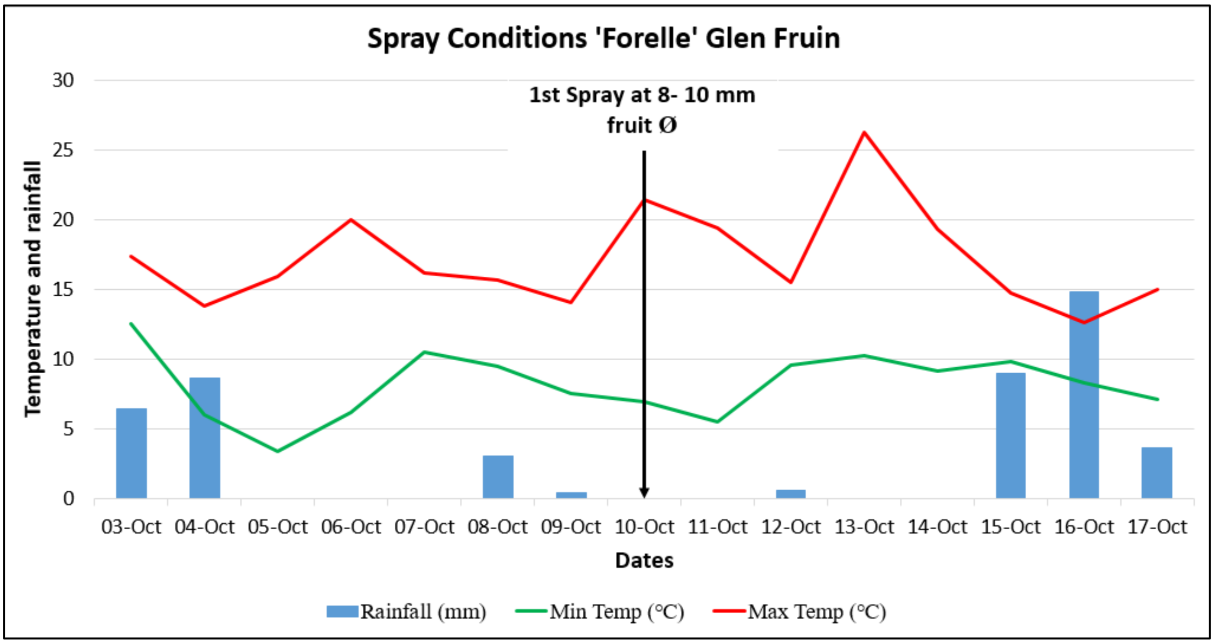

Fig. 1. Temperature and rainfall during and after s-abscisic acid (S-ABA) application on ‘Forelle’ pears at Glen Fruin, Elgin area, Western Cape (2017/2018).

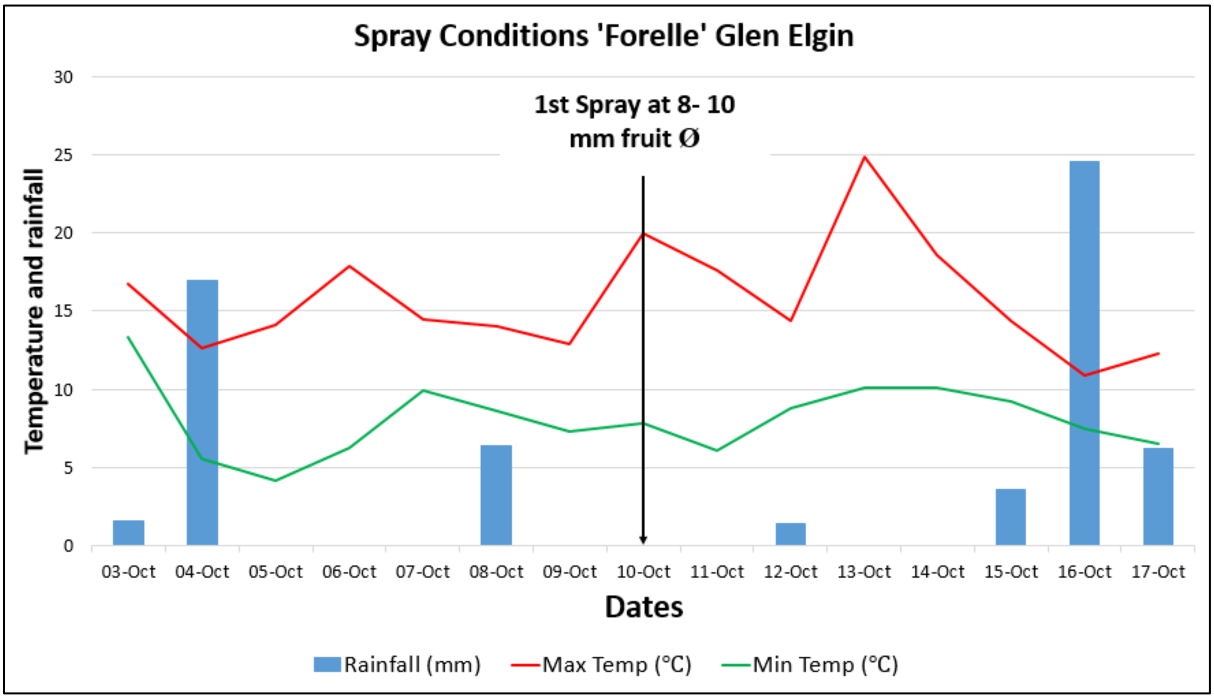

Fig. 2. Temperature and rainfall during and after s-abscisic acid (S-ABA) application on ‘Forelle’ pears at Glen Elgin, Elgin area, Western Cape (2017/2018).

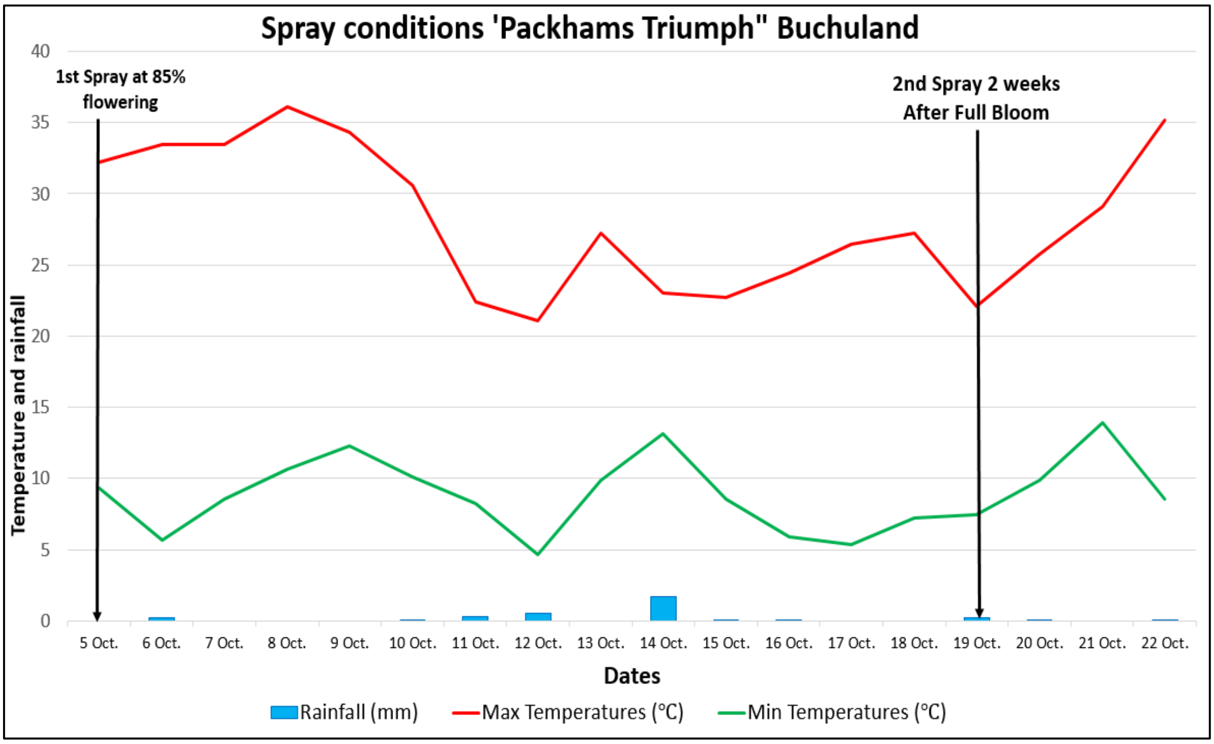

Fig. 3. Temperature and rainfall during and after the application of a variety of plant growth regulators (PGR) on ‘Packham’s Triumph’ pears at Buchuland, Ceres area, South Africa (2018/2019).

Fig. 4. Symptoms of severe phytotoxicity caused by s-abscisic acid on ‘Forelle’ leaves.

Table 1. Treatment specifications for trials done with s-abscisic acid (S-ABA) on 'Forelle' pears in the season of 2017/2018.

Treatments
Untreated
S-ABA 100 mg·L^{-1} at 8 - 10 mm fruit diameter
S-ABA 200 mg·L^{-1} at 8 - 10 mm fruit diameter
S-ABA 300 mg·L^{-1} at 8 - 10 mm fruit diameter
S-ABA 400 mg·L^{-1} at 8 - 10 mm fruit diameter
S-ABA 600 mg·L^{-1} at 8 - 10 mm fruit diameter

Table 2. Summary of dates of treatment application, follow up hand thinning and harvest of 'Forelle' pears in the season of (2017/2018).

Phenological stage	'Forelle'	
	Glen Elgin	Glen Fruin
1st spray	10 Oct. 2017	10 Oct. 2017
Hand thinning	31 Oct. 2017	2 Nov. 2017
Harvest	19 Feb. 2018*	9 Mar 2018*

*All fruit harvested on one day

Table 3. Treatment specifications for trials done with a variety of plant growth regulators (PGR) on 'Packham's Triumph' pears at Buchuland, Ceres area, South Africa (2018/2019).

Treatment
Untreated
GA_{4+7} + 6-BA* at 85% flowering
AVG 62.25 mg·L^{-1} 2 w.a.f.b.**
AVG 125 mg·L^{-1} 2 w.a.f.b.
AVG 250 mg·L^{-1} 2 w.a.f.b.
AVG + ProCa*** 2 w.a.f.b.

*GA_{4+7} (11.4 mg·L^{-1}) + 6-BA (11.4 mg·L^{-1}); **Weeks after full bloom (w.a.f.b.); ***AVG (125 mg·L^{-1}) tank-mixed with Prohexadione-calcium (35 mg·L^{-1})

Table 4. Summary of dates of treatment application and harvest for 'Packham's Triumph' pears at Buchuland, Ceres area, South Africa (2018/2019).

Phenological stage	Packham's Triumph
1st Spray	5 Oct. 2018
2nd spray	19 Oct. 2018
Harvest	8 Feb. 2019*

*All fruit harvested on one day

Table 5. Treatment specifications for trials done with 1-naphthaleneacetic acid (NAA) on 'Forelle' pears in the season of 2018/2019.

Treatments
Untreated
NAA 5.6 mg·L^{-1} 7 d.b.h.*
NAA 11.2 mg·L^{-1} 7 d.b.h.
NAA 22.4 mg·L^{-1} 7 d.b.h.
NAA 5.6 mg·L^{-1} 7 d.b.h. and 3 d.b.h.
NAA 11.2 mg·L^{-1} 7 d.b.h. and 3 d.b.h.
NAA 22.4 mg·L^{-1} 7 d.b.h. and 3 d.b.h.

*Days before harvest (d.b.h.)

Table 6. Summary for the dates of treatment application and harvest dates for 'Forelle' pears in the season of 2018/2019.

Phenological stage	Forelle	
	Glen Elgin	Lushof
1st chemical application	21 Feb. 2019	25 Feb. 2019
2nd chemical application	25 Feb. 2019	1 Mar. 2019
Harvest	28 Feb. 2019*	4 Mar. 2019*

*All fruit harvested on one day

Table 7. Effect of s-abscisic acid (S-ABA) on average fruit set per flower cluster, hand thinning requirement and leaf phytotoxicity of 'Forelle' pears at Glen Elgin, Grabouw area, Western Cape (2017/2018).

Treatment	Time of application	Average fruit set per flower cluster	Average number of fruitlets thinned by hand	Average leaf phytotoxicity score*
Untreated	No application	1.91 a	534 a	1.0 a
S-ABA 100	8- 10 mm fruit diam.	1.54 ab	440 ab	1.8 b
S-ABA 200	8- 10 mm fruit diam.	1.28 bc	376 bc	1.9 b
S-ABA 300	8- 10 mm fruit diam.	1.47 b	441 ab	2.1 c
S-ABA 400	8- 10 mm fruit diam.	1.35 b	282 cd	2.5 d
S-ABA 600	8- 10 mm fruit diam.	0.92 c	223 d	2.9 e
Significance level		***0.0026***	***<.0001***	***<.0001***
LSD 5%		-	*118.24*	*0.23*
Control vs S-ABA		***0.0001***	***0.0007***	***<.0001***
S-ABA Linear		***0.0036***	***0.0002***	***<.0001***
S-ABA Quadratic		*0.2412*	*0.5569*	*0.6439*

*Leaf phytotoxicity scored between 0 and 3 with 0 being no phytotoxicity and 3 severe phytotoxicity (20 leaves per tree affected)

Table 8. Effect of s-abscisic acid (S-ABA) on average yield and yield efficiency of 'Forelle' pears at Glen Elgin, Grabouw area, Western Cape (2017/2018).

Treatment	Time of application	Total yield per tree (kg)		Total yield efficiency (kg.cm^{-2})	
Untreated	No application	91.9	ns	0.17	ns
S-ABA 100	8- 10 mm fruit diam.	92.3		0.17	
S-ABA 200	8- 10 mm fruit diam.	95.2		0.18	
S-ABA 300	8- 10 mm fruit diam.	100.6		0.18	
S-ABA 400	8- 10 mm fruit diam.	84.7		0.15	
S-ABA 600	8- 10 mm fruit diam.	86.3		0.16	
Significance level		*0.5196*		*0.1919*	
LSD 5%		-		-	
Control vs S-ABA		*0.9889*		*7.997*	
S-ABA Linear		*0.2523*		*0.2815*	
S-ABA Quadratic		*0.5857*		*0.9461*	

Table 9. Effect of s-abscisic acid (S-ABA) on average fruit size of 'Forelle' pears at Glen Elgin, Grabouw area, Western Cape (2017/2018).

Treatment	Time of application	Average weight of fruit (g)		Average diameter of fruit (mm)		Average length of fruit (mm)	
Untreated	No application	121.8	c	56.6	c	79.3	b
S-ABA 100	8- 10 mm fruit diam.	130.2	bc	57.4	bc	81.4	b
S-ABA 200	8- 10 mm fruit diam.	129.3	bc	57.6	bc	80.3	b
S-ABA 300	8- 10 mm fruit diam.	138.7	b	59.2	b	82.0	b
S-ABA 400	8-10 mm fruit diam.	135.5	bc	58.7	b	81.0	b
S-ABA 600	8- 10 mm fruit diam.	165.8	a	63.2	a	86.6	a
Significance level		***<.0001***		***<.0001***		***0.0024***	
LSD 5%		*14.47*		*2.06*		*3.23*	
Control vs S-ABA		***0.0022***		***0.0017***		***0.0209***	
S-ABA Linear		***<.0001***		***<.0001***		***0.0009***	
S-ABA Quadratic		***0.0343***		*0.0580*		***0.0242***	

Table 10. Effect of s-abscisic acid (S-ABA) on average return bloom percentage and percentage of fruit which meets the color requirements of class 1 fruit of 'Forelle' pears at Glen Elgin, Grabouw area, Western Cape (2017/2018).

Treatment	Time of application	Average return bloom %	% Fruit which meets class 1 color requirements
Untreated	No application	14.93 ns	63.7 ns
S-ABA 100	8- 10 mm fruit diam.	13.20	59.3
S-ABA 200	8- 10 mm fruit diam.	15.67	69.0
S-ABA 300	8-10 mm fruit diam.	13.84	64.7
S-ABA 400	8- 10 mm fruit diam.	17.74	70.0
S-ABA 600	8- 10 mm fruit diam.	22.62	69.3
Significance level		*0.0781*	*0.2928*
LSD 5%		-	-
Control vs S-ABA		*0.5113*	*0.4990*
S-ABA Linear		***0.0042***	*0.1298*
S-ABA Quadratic		*0.4181*	*0.8869*

Table 11. Effect of s-abscisic acid (S-ABA) on average fruit set per flower cluster, hand thinning requirement and leaf phytotoxicity of 'Forelle' pears at Glen Fruin, Elgin area, Western Cape (2017/2018).

Treatment	Time of application	Average fruit set per flower cluster	Average number of fruitlets thinned by hand	Average phytotoxicity score*
Untreated	No application	0.83 ns	24 ns	1.0 d
S-ABA 100	8- 10 mm fruit diam.	0.79	20	1.9 c
S-ABA 200	8- 10 mm fruit diam.	0.69	22	2.0 c
S-ABA 300	8- 10 mm fruit diam.	0.55	16	2.1 c
S-ABA 400	8- 10 mm fruit diam.	0.68	19	2.4 b
S-ABA 600	8- 10 mm fruit diam.	0.45	17	2.9 a
Significance level		*0.2017*	*0.1497*	***<.0001***
LSD 5%			-	
Control vs S-ABA		***0.0495***	*0.2116*	***<.0001***
S-ABA Linear		***0.0160***	*0.4822*	***<.0001***
S-ABA Quadratic		*0.9458*	*0.8820*	***0.0396***

*Leaf phytotoxicity scored between 0 and 3 with 0 being no phytotoxicity and 3 severe phytotoxicity (20 leaves per tree affected

Table 12. Effect of s-abscisic acid (S-ABA) on average yield and yield efficiency of 'Forelle' pears at Glen Fruin, Elgin area, South Africa (2017/2018).

Treatment	Time of application	Total yield per tree (kg)		Total yield efficiency (kg.cm^{-2})	
Untreated	No application	87.1	a	0.22	a
S-ABA 100	8- 10 mm fruit diam.	63.2	bc	0.17	b
S-ABA 200	8- 10 mm fruit diam.	70.0	bc	0.20	ba
S-ABA 300	8- 10 mm fruit diam.	69.3	bc	0.19	ba
S-ABA 400	8- 10 mm fruit diam.	76.3	ab	0.20	ba
S-ABA 600	8- 10 mm fruit diam.	60.4	c	0.16	b
Significance level		***0.0125***		***0.0288***	
LSD 5%		*15.72*		*0.04*	
Control vs S-ABA		***0.0026***		*0.0566*	
S-ABA Linear		*0.7487*		*0.4476*	
S-ABA Quadratic		***0.0496***		*0.0739*	

Table 13. Effect of s-abscisic acid (S-ABA) on average fruit size of 'Forelle' pears at Glen Fruin, Elgin area, Western Cape (2017/2018).

Treatment	Time of application	Average weight of fruit (g)		Average diameter of fruit (mm)		Average length of fruit (mm)	
Untreated	No application	161.2	b	62.8	b	87.5	ab
S-ABA 100	8- 10 mm fruit diam.	162.5	b	62.6	b	84.9	b
S-ABA 200	8- 10 mm fruit diam.	160.8	b	62.8	b	87.0	b
S-ABA 300	8- 10 mm fruit diam.	165.1	b	63.0	b	88.0	ba
S-ABA 400	8- 10 mm fruit diam.	165.4	b	63.9	ba	86.5	b
S-ABA 600	8- 10 mm fruit diam.	185.6	a	65.9	a	91.6	a
Significance level		***0.0475***		***0.0358***		***0.0337***	
LSD 5%		*18.53*		*2.36*		*4.37*	
Control vs S-ABA		*0.3533*		*0.3715*		*0.9222*	
S-ABA Linear		***0.0089***		***0.0033***		***0.0060***	
S-ABA Quadratic		*0.1737*		*0.3160*		*0.5805*	

Table 14. Effect of s-abscisic acid (S-ABA) on average return bloom percentage and percentage fruit which meets class 1 color requirements of 'Forelle' pears at Glen Fruin, Elgin area, Western Cape (2017/2018).

Treatment	Time of application	Return bloom %	% Fruit which meets class 1 color requirements
Untreated	No application	30.7 ns	40.0 ns
S-ABA 100	8- 10 mm fruit diam.	25.7	31.0
S-ABA 200	8- 10 mm fruit diam.	28.6	44.3
S-ABA 300	8- 10 mm fruit diam.	26.2	43.7
S-ABA 400	8- 10 mm fruit diam.	22.4	42.3
S-ABA 600	8- 10 mm fruit diam.	26.0	44.7
Significance level		*0.0580*	*0.1912*
LSD 5%		-	-
Control vs S-ABA		*0.1412*	*0.8039*
S-ABA Linear		*0.6355*	*0.2562*
S-ABA Quadratic		*0.6988*	*0.7277*

Table 15. Effect of plant growth regulators (PGR) on average fruit set per flower cluster, yield and yield efficiency of 'Packham's Triumph' pears at Buchuland, Ceres area, Western Cape (2018/2019).

Treatment	Time of application	Average fruit set per flower cluster	Average total yield per tree (kg)	Average total yield efficiency ($kg.cm^{-2}$)
Untreated	No application	0.17 ns	21.9 c	0.32 c
GA_{4+7} + 6-BA*	85% flowering	0.17	24.1 bc	0.40 bc
AVG 62.25	2 w.a.f.b.* *	0.19	26.3 abc	0.41 bc
AVG 125	2 w.a.f.b.	0.27	29.1 ab	0.52 a
AVG 250	2 w.a.f.b.	0.25	31.0 a	0.47 ab
AVG + ProCa***	2 w.a.f.b.	0.25	25.3 bc	0.42 bc
Significance level		*0.4724*	***0.0118***	***0.0002***
LSD 5%		-	*4.94*	*0.08*
AVG Linear		*0.3356*	*0.0686*	*0.4110*
AVG Quadratic		*0.2162*	*0.5706*	***0.0184***

*GA_{4+7} (11.4 $mg{\cdot}L^{-1}$) + 6-BA (11.4 $mg{\cdot}L^{-1}$); **Weeks after full bloom (w.a.f.b.); ***AVG (125 $mg{\cdot}L^{-1}$) tank-mixed with prohexadione-calcium (35 $mg{\cdot}L^{-1}$)

Table 16. Effect of a variety of plant growth regulators (PGR) on average fruit size of 'Packham's Triumph' pears at Buchuland, Ceres area, Western Cape (2018/2019).

Treatment	Time of application	Average weight of fruit (g)	Average diameter of fruit (mm)	Average length of fruit (mm)
Untreated	No application	252.5 a	78.3 ns	86.4 a
GA_{4+7} + 6-BA*	85% flowering	228.9 bc	76.4	86.1 ab
AVG 62.25	2 w.a.f.b.**	240.0 ab	77.1	84.8 abc
AVG 125	2 w.a.f.b.	225.5 bc	75.5	83.4 bc
AVG 250	2 w.a.f.b.	211.9 c	74.0	82.1 c
AVG + ProCa***	2 w.a.f.b.	221.2 bc	75.7	84.5 abc
Significance level		***0.0026***	***0.0122***	***0.0092***
LSD 5%		*20.49*	-	*2.02*
AVG Linear		***0.0097***	***0.0184***	***0.0423***
AVG Quadratic		*0.5704*	*0.6260*	*0.7251*

*GA_{4+7} (11.4 mg·L^{-1}) + 6-BA (11.4 mg·L^{-1}); **Weeks after full bloom (w.a.f.b.); ***AVG (125 mg·L^{-1}) tank-mixed with Prohexadione-calcium (35 mg·L^{-1})

Table 17. Effect of a variety of plant growth regulators (PGR) on average fruit firmness and return bloom percentage of 'Packham's Triumph' pears at Buchuland, Ceres area, South Africa (2018/2019).

Treatment	Time of application	Average fruit firmness	Average return bloom %
Untreated	No application	7.86 ns	72.76 ns
GA_{4+7} + 6-BA*	85% flowering	8.04	58.26
AVG 62.25	2 w.a.f.b.**	7.80	65.09
AVG 125	2 w.a.f.b.	7.65	65.03
AVG 250	2 w.a.f.b.	7.92	45.09
AVG + ProCa***	2 w.a.f.b.	7.59	47.02
Significance level		*0.9880*	*0.3768*
LSD 5%		-	-
AVG Linear		*0.2301*	*0.1440*
AVG Quadratic		*0.1001*	*0.6118*

*GA_{4+7} (11.4 mg·L^{-1}) + 6-BA (11.4 mg·L^{-1}); **Weeks after full bloom (w.a.f.b.); ***AVG (125 µg·L^{-1}) tank-mixed with prohexadione-calcium (35 mg·L^{-1})

Table 18. Effect of 1-naphthaleneacetic acid (NAA) on average number of fruit drop, total yield and yield efficiency of 'Forelle' pears at Lushof, Ceres area, Western Cape (2018/2019).

Treatment	Time of application	Average number of fruit drop	Average total yield per tree (kg)	Average total yield efficiency harvest (kg.cm^{-2})
Untreated	No application	0.90 ns	18.6 b	0.08 ns
NAA 5.6	7 d.b.h.*	0.50	22.0 ba	0.08
NAA 11.2	7 d.b.h.	0.70	27.0 a	0.10
NAA 22.4	7 d.b.h.	0.20	23.2 ba	0.09
NAA 5.6	7 d.b.h. and 3 d.b.h.	0.40	21.0 ba	0.09
NAA 11.2	7 d.b.h. and 3 d.b.h.	0.80	21.5 ba	0.09
NAA 22.4	7 d.b.h. and 3 d.b.h.	0.80	21.8 b	0.07
Significance level		*0.5467*	***0.0056***	*0.2322*
LSD 5%		-	*6.292*	-
CONTROL vs NAA		*0.4294*	*0.1489*	*0.4367*
Once vs Twice		*0.3706*	*0.0863*	*0.6805*
Once NAA Linear		*0.3379*	*0.9456*	*0.8453*
Once NAA Quadratic		*0.3792*	*0.1000*	*0.1938*
Twice NAA Linear		*0.3668*	*0.8152*	*0.4440*
Twice NAA Quadratic		*0.4341*	*0.9343*	*0.4108*

*Days before harvest (d.b.h.)

Table 19. Effect of 1-naphthaleneacetic acid (NAA) on average fruit size of 'Forelle' pears at Lushof, Ceres area, Western Cape (2018/2019).

Treatment	Time of application	Average fruit weight (g)	Average fruit diameter (mm)	Average fruit length (mm)
Untreated	No application	151.1 b	66.1 ns	83.7 b
NAA 5.6	7 d.b.h.*	162.5 ba	66.9	87.3 a
NAA 11.2	7 d.b.h.	157.6 ba	66.3	85.3 ba
NAA 22.4	7 d.b.h.	166.1 a	67.5	85.5 ba
NAA 5.6	7 d.b.h. and 3 d.b.h.	162.0 ba	67.6	84.6 ba
NAA 11.2	7 d.b.h. and 3 d.b.h.	158.2 ba	66.8	84.7 ba
NAA 22.4	7 d.b.h. and 3 d.b.h.	154.3 ba	66.3	84.3 ba
Significance level		***0.0015***	*0.4638*	***<0.0001***
LSD 5%		*14.22*	-	*3.52*
CONTROL vs NAA		*0.1032*	*0.3142*	*0.2465*
Once vs Twice		*0.3433*	*0.9564*	*0.1473*
Once NAA Linear		*0.4839*	*0.4133*	*0.4139*
Once NAA Quadratic		*0.3370*	*0.3279*	*0.3725*
Twice NAA Linear		*0.2887*	*0.2112*	*0.8240*
Twice NAA Quadratic		*0.8478*	*0.6653*	*0.8921*

*Days before harvest (d.b.h.)

Table 20. Effect of 1-naphthaleneacetic acid (NAA) on average fruit firmness of 'Forelle' pears at Lushof, Ceres area, Western Cape (2018/2019).

Treatment	Time of application	Average fruit firmness
Untreated	No application	7.0 ns
NAA 5.6	7 d.b.h.*	6.9
NAA 11.2	7 d.b.h.	7.0
NAA 22.4	7 d.b.h.	7.0
NAA 5.6	7 d.b.h.and 3 d.b.h.	6.9
NAA 11.2	7 d.b.h.and 3 d.b.h.	6.9
NAA 22.4	7 d.b.h.and 3 d.b.h.	6.9
Significance level		*0.0636*
LSD 5%		-
CONTROL vs NAA		*0.4754*
Once vs Twice		*0.6469*
Once NAA Linear		*0.7323*
Once NAA Quadratic		*0.4059*
Twice NAA Linear		*0.8259*
Twice NAA Quadratic		*0.8551*

*Days before harvest (d.b.h.)

Table 21. Effect of 1-naphthaleneacetic acid (NAA) on average number of fruit drop, total yield and yield efficiency of 'Forelle' pears at Glen Elgin, Grabouw area, Western Cape (2018/2019).

Treatment	Time of application	Average number of fruit drop	Average total yield per tree (kg)	Average total yield efficiency harvest (kg.cm^{-2})
Untreated	No application	2.1 ns	93.9 ns	0.26 ns
NAA 5.6	7 d.b.h.*	3.1	91.4	0.22
NAA 11.2	7 d.b.h.	2.5	86.7	0.21
NAA 22.4	7 d.b.h.	1.5	84.3	0.22
NAA 5.6	7 d.b.h. and 3 d.b.h.	2.9	96.5	0.25
NAA 11.2	7 d.b.h. and 3 d.b.h.	1.7	81.3	0.22
NAA 22.4	7 d.b.h. and 3 d.b.h.	2.9	81.6	0.22
Significance level		*0.5603*	*0.7522*	*0.6677*
LSD 5%		-	-	-
CONTROL vs NAA		*0.6792*	*0.3616*	*0.1007*
Once vs Twice		*0.8267*	*0.8665*	*0.5946*
Once NAA Linear		*0.1298*	*0.4976*	*0.9572*
Once NAA Quadratic		*0.9429*	*0.7922*	*0.5901*
Twice NAA Linear		*0.8040*	*0.1981*	*0.3592*
Twice NAA Quadratic		*0.2004*	*0.2470*	*0.6399*

*Days before harvest (d.b.h.)

Table 22. Effect of 1-naphthaleneacetic acid (NAA) on average fruit size of 'Forelle' pears at Glen Elgin, Grabouw area, Western Cape (2018/2019).

Treatment	Time of application	Average fruit mass (g)	Average fruit diameter (mm)	Average fruit length (mm)
Untreated	No application	132.5 ns	63.4 ns	80.6 ns
NAA 5.6	7 d.b.h.*	135.2	64.2	78.9
NAA 11.2	7 d.b.h.	135.9	64.3	81.2
NAA 22.4	7 d.b.h.	134.4	64.0	80.9
NAA 5.6	7 d.b.h. and 3 d.b.h.	142.9	65.2	82.8
NAA 11.2	7 d.b.h. and 3 d.b.h.	139.5	64.8	81.6
NAA 22.4	7 d.b.h. and 3 d.b.h.	138.8	64.5	81.8
Significance level		*0.3596*	*0.1831*	*0.3678*
LSD 5%		-	-	-
CONTROL vs NAA		*0.2419*	*0.0939*	*0.6109*
Once vs Twice		*0.1261*	*0.1771*	*0.0687*
Once NAA Linear		*0.8737*	*0.7140*	*0.2881*
Once NAA Quadratic		*0.8562*	*0.8931*	*0.2670*
Twice NAA Linear		*0.5211*	*0.4150*	*0.6193*
Twice NAA Quadratic		*0.6976*	*0.8130*	*0.5432*

*Days before harvest (d.b.h.)

Table 23. Effect of 1-naphthaleneacetic acid (NAA) on average fruit firmness of 'Forelle' at Glen Elgin, Grabouw area, Western Cape (2018/2019).

Treatment	Time of application	Average fruit firmness
Untreated	No application	6.1 ns
NAA 5.6	7 d.b.h.*	6.1
NAA 11.2	7 d.b.h.	6.1
NAA 22.4	7 d.b.h.	6.1
NAA 5.6	7 d.b.h. and 3 d.b.h.	6.2
NAA 11.2	7 d.b.h. and 3 d.b.h.	6.1
NAA 22.4	7 d.b.h. and 3 d.b.h.	6.2
Significance level		*0.2941*
LSD 5%		-
CONTROL vs NAA		*0.4803*
Once vs Twice		*0.0992*
Once NAA Linear		*0.9361*
Once NAA Quadratic		*0.9044*
Twice NAA Linear		*0.2605*
Twice NAA Quadratic		*0.2401*

*Days before harvest (d.b.h.)

GENERAL DISCUSSION AND CONCLUSION

Results from our trials on the effectiveness of 1-aminocyclopropane-1-carboxylic acid (ACC) in thinning the Japanese plums 'Laetitia' and 'Fortune' were promising, and supports results previously reported (Theron et al., 2017a). In 'Laetitia', ACC caused an increase in fruit size and in 'Fortune' a significant thinning effect without significantly affecting yield. We concluded that ACC at 400 $\mu l \cdot L^{-1}$ applied at the 8 – 10 mm fruit diameter stage would be the recommended rate and application timing for both 'Laetitia' and 'Fortune'. ACC did not appear to be an effective thinning agent for 'August Red' nectarines. This in agreement with Steenkamp (2015) who found that ACC was not an effective thinner on 'Alpine' nectarine at 8 – 10 mm fruitlet diameter. He, however, found some thinning on 'Turquoise' nectarine and therefore we cannot conclude that ACC is not an effective thinner of nectarines. ACC at 400 $\mu l \cdot L^{-1}$ consistently gave promising results and could be the recommended rate for 'Keisie' cling peaches. As for the optimal timing, ACC applied at the 4 – 6 mm fruitlet diameter stage had a less severe thinning effect than when applied at the 8 – 10 mm fruitlet diameter stage. However, notable leaf drop was observed following the 8 – 10 mm fruitlet diameter application. A heat wave during and shortly after this application could have accounted for the increased severity in leaf drop and weather forecasts should be considered prior to ACC application. Temperatures should be considered when applying ACC. One possible option is to combine ACC with another thinning agent, with a different mode of action, to increase the thinning effect at 4 – 6 mm fruitlet diameter. McArtney and Obermiller (2012) evaluated ACC alone or in combination with metamitron on apples and generally found that the thinning effect was additive when these thinners were combined. Although metamitron has not thinned effectively on its own on stone fruit (Greene and Costa. 2012), it might be worthwhile evaluating this combination in future research. ACC applications at petal drop could also be investigated. Some preliminary results obtained in demonstration trials have shown efficacy of ACC at this early stage (S. Reynolds, personal communication). Such early application should also result in less leaf drop as ACC application will be made before leafs have developed.

Neither ACC, nor the industry standard 1-naphthaleneacetic acid (NAA) tank-mixed with 6-benzyladenine (6-BA) in 2017/2018 and 6-BA alone in 2018/2019 season, thinned the "difficult-to-thin" 'Fuji' (Stopar, 2006). The lack of thinner efficacy during both seasons was ascribed to climatic and intrinsic tree factors that could have decreased thinner efficacy. The efficacy of ACC on 'Fuji' must be further evaluated. In the 2017/2018 season, ACC over-thinned a young 'Cripps' Red' orchard. In the 2018/2019 season, a ten year older 'Cripps' Red' orchard was used and ACC showed great promise as a chemical thinner at 15 - 20 mm fruitlet diameter. Taking into account the results of the 2018/2019 season, the recommended rate of ACC on 'Cripps' Red' would be between 250 and 500 $\mu l \cdot L^{-1}$, but further trials are recommended. ACC proved to be a promising thinner on 'Royal Gala' when applied at 250 $\mu l \cdot L^{-1}$ at 8 - 10 mm fruitlet diameter in the one trial we conducted. ACC performed better than the industry standard NAA applied at 5 $mg \cdot L^{-1}$ at 4 – 6 mm fruitlet diameter. ACC could possibly be combined with other thinning agents during this application window in order to increase the overall thinning action. On apples, ACC and metamitron had an additive effect and the combination of thinning agents were more effective than either treatments on its own (McArtney and Obermiller, 2012). ACC is a promising new molecule that could potentially increase the chemical thinning arsenal available to growers, but additional research is needed.

Results from our chemical thinning trials, as well as results from trials performed by Theron et al. (2017b), indicate that S-abscisic acid (S-ABA) at 300 to 400 $mg \cdot L^{-1}$ applied at 8 – 10 mm fruitlet diameter might be the solution to thin the "difficult-to-thin" 'Forelle' pears. In the fruit set trials on young 'Packham's Triumph' trees, aminoethoxyvinylglycine (AVG) was successful in increasing yield and yield efficiency. Neither a mixture of gibberellin A_{4+7} nor 6-benzyladenine (GA_{4+7} plus 6-BA), nor AVG tank-mixed with prohexadione-calcium **(**ProCa), were effective in increasing fruit set. Currently, the latter treatment is used commercially in the Elgin-Grabouw-Vyeboom-Villiersdorp region in South Africa and from our trials does not support its use. As expected, a decrease in fruit size did occur with an increase in crop load following AVG application. Further trials are needed to determine whether 250 $mg \cdot L^{-1}$ is the optimum rate of AVG in order to maximize the positive effect on crop load, without significantly compromising fruit size. The fruit drop trials with NAA on 'Forelle' did not yield a result. Strong winds prior to treatment applications caused a large number of fruit to drop before these trials could commence. Thus we cannot confidently report on the efficacy of NAA in controlling pre-harvest fruit drop of 'Forelle'. Either NAA did not have any effect on controlling fruit drop of 'Forelle' or all the fruit that could have been affected by an NAA application had dropped due to the wind before treatment application. These trials would have to be repeated, possibly adding even earlier applications.

During the course of two seasons, we found promising effects of various plant growth regulators on regulating crop load. It is clear that environmental conditions as well as intrinsic plant factors affect the efficacy of plant growth regulators. This must be taken into account before applying plant growth regulators. The efficacy of plant growth regulators appears to be cultivar dependent. Therefore, on cultivars that did not respond as well to plant growth regulators, combinations of plant growth regulators could be evaluated to see whether an additive effect could be obtained.

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