Management practices to improve quality of apple nursery trees in containers

By

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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SUMMARY

The quality of nursery trees at planting has a significant effect on productivity during the early years of newly established orchards. Trees need to grow and fill their allocated space in the orchard as quickly as possible for optimal return on investment. One of the main concerns when establishing new orchards is the quality of nursery trees as well as the prolonged establishment period commonly referred to as transplant shock. Transplant shock occurs due to damage to the root system, which is aggravated by an imbalance in root to shoot volume.

Different management practises and timing of these practises were evaluated on containerised apple nursery tree. The practises focussed on growth cessation and hardening-off, nitrogen reserve build-up, and defoliation. The effect of these management practises as well as different planting times and methods on spring bud break and new growth during the first growing season in a commercial orchard was investigated. Prohexadione calcium and abscisic acid showed no significant effect on growth cessation during the nursery phase, and no significant effect in spring bud break or new growth in the orchard. Trees planted in autumn showed earlier bud break whereas trees planted in spring had a higher bud break percentage. Foliar nitrogen (urea) during the nursery phase did not significantly affect spring bud break and new growth. At the rates applied, foliar copper, in comparison to 1-aminocyclopropane-1-carboxylic acid proved to be a more successful chemical defoliant in the nursery with no significant negative effect on the subsequent performance in the orchard.

Dormancy management practises improved bud break in spring as well as the architecture of young trees planted in a commercial orchard. It is recommended that trees receive a six-week 6 °C cold storage period and a chemical rest breaking treatment to improve establishment. Different planting methods of containerised nursery trees did not significantly influence bud break in spring, but planting containerised trees with an undisturbed growing medium or only slightly loosening the growing medium before planting improved new lateral shoot growth and apical extension growth.

Producing “feathered” single or double-leader apple nursery trees in containers proved to be difficult. Either the container was too small, thus restricting the root volume too much, or the rate and number of plant growth regulator (PGRs) applications were not enough.

No clear conclusion could be made on the use of PGRs to harden-off trees or chemical defoliants in our trials. Trees planted during autumn, in a warm winter region, did not

accumulate sufficient chilling, which resulted in reduced bud break during spring. This time of planting resulted in a basal dominant tree architecture. As trees were probably not managed ideally in the orchard, the treatment effects could have been masked by a lack of tree vigour during the first growing season in the orchard. A period of a six-week cold storage at 6 °C as well as a chemical rest breaking treatment are important dormancy management practises, regarding spring bud break and tree architecture, when trees are planted in warm regions.

Because containerised nursery tree propagation is a new concept in South Africa, further research is needed on propagation and subsequent management of these trees.

OPSOMMING

Bestuurspraktyke om appelboom produksie in sakkies te verbeter

Die kwaliteit van kwekerybome speel ‘n belangrike rol in die produktiwiteit van nuut gevestigde boorde. Vir optimale opbrengs op ‘n belegging moet bome hul geallokeerde spasie in die boord so gou as moontlik vul. Een van die grootste bekommernisse tydens die vestiging van nuwe boorde is die kwaliteit van kwekerybome sowel as die uitgerekte vestigingsperiode wat as uitplantskok bekendstaan. Uitplantskok vind plaas as gevolg van ‘n wanbalans in die wortel tot loot volume, wat vererger word deur beskadiging van die wortelsisteem tydens hantering en vervoer van die bome.

Die effek van verskillende bestuurspraktyke sowel as tydsberekeing van die praktyke was geëvalueer op appelkwekerybome wat in sakke geproduseer is. Die bestuurspraktyke het op groeistaking en afharding, opbou van stikstofreserwes en blaarval gefokus. Ondersoek was ingestel na die effek van hierdie bestuurspraktyke op knopbreek tydens die lente sowel as op nuwe groei in ‘n kommersieële boord. Proheksadioon-kalsium sowel as absisiensuur het geen betekenisvolle effek op groeistaking tydens die kwekeryproses getoon nie, asook geen betekenisvolle effek op knopbreek en nuwe groei nie. Bome wat in die herfs geplant is se knopbreek was vroeër, maar ‘n hoër knopbreekpersentasie was verkry as bome in die lente geplant is. Toediening van stikstofblaarvoeding (ureum) gedurende die kwekeryfase het geen betekenisvolle effek op knopbreek en nuwe groei gehad nie. Koperblaartoedienings was meer suksesvol in vergelyking met 1-aminosiklopropaan-1-karboksielsuur om blaarval te induseer met geen betekenisvolle negatiewe effek op die daaropvolgende produktiwitiet in die boord nie.

Die persentasie knopbreek in die lente sowel as boomargitektuur was verbeter met die implimentering van dormansie bestuurspraktyke. Daar word aanbeveel dat bome ‘n ses-week periode van koelopberging (6° C), sowel as ‘n opvolg toediening van ‘n chemiese rusbreker behandeling ontvang om vestiging in die boord te verbeter. Verskillende plantmetodes het geen betekenisvolle effek op knopbreek in die lente gehad nie, maar om die bome met hul onversteurde groeimedium te plant, of om die groeimedium effens los te maak voor plant, was voordelig ten opsigte van nuwe laterale lootgroei asook apikale verlengingsgroei.

Die produksie van geveërde enkel-leier bome sowel as dubbel-leier bome in sakkies was problematies. Die beperkte wortelvolume, as gevolg van die sakkie, of die lae dosis en aantal toedienings van plantgroei reguleerders (PGRs) was moontlik hiervoor verantwoordelik.

Geen duidelike afleidings kon gemaak word rakende die gebruik van PGRs ten opsigte van afharding sowel as oor chemieseontblaringsmiddels nie. Verlaagde knopbreek was waargeneem gedurende die lente op bome wat in die herfs geplant is in ‘n warm winter klimaat waar onvoldoende koue geakkumuleer het. Boonop het die bome ‘n basaal dominante argitektuur getoon. Omdat bome nie optimaal in die boord bestuur was nie, kon ‘n tekort aan groeikrag gedurende die eerste groeiseisoen moontlik die effek van die verskillende behandelings maskeer het. Die opberg van bome in ‘n koelkamer vir ses weke by 6 °C, sowel as ‘n chemiese rusbreker behandeling was belangrike bestuurspraktyke met betrekking tot die dormansie periode. Hierdie praktyke was veral belangrik rakende knopbreek in die lente sowel as die boomargitektuur.

Omdat kwekerybome wat in sakkies geproduseer word nog ‘n nuwe konsep in Suid-Afrika is, moet toekomstige navorsing fokus op die produksie sowel as die bestuurspraktyke van hierdie bome.

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.

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GENERAL INTRODUCTION

The South African pome fruit industry consists of approximately 36 000 hectare (ha), of which apples are planted over an area of approximately 24 000 ha (Hortgro, 2017). The majority of deciduous fruit in South Africa is produced in marginal climates especially in terms of lack of winter chilling (Costa et al., 2004). South Africa, a net exporter of apples, is currently the 18th largest apple producer in the world, but the second largest exporter of apples in the Southern Hemisphere (WAPA, 2016).

Currently 9% of apple orchards in South Africa are between zero and three years old (DAFF, 2016). To have a sustainable and competitive apple industry, the percentage of orchards between zero and three years old needs to be 10% (DAFF, 2016). With the high cost of establishing new apple orchards, to which plant material contributes significantly, it is important to produce good quality nursery trees which will improve the potential of newly planted orchards. The quality of nursery trees is influenced by various physical and physiological factors (Theron and Steyn 2015, 2016). Physical factors include shoot to root ratio, root quality and quantity, tree size, bud quality and the absence of physical injury, whereas time of growth cessation and hardening-off, nitrogen reserve status and dormancy induction contribute to the physiological quality.

The quality of nursery trees at planting has a significant effect on productivity during the early years of a newly established orchard (Van Oosten, 1978). Trees need to grow and fill their allocated space in the orchard as quickly as possible for optimal return on investment. One of the main concerns when establishing a new orchard is the prolonged establishment period of young trees. This is characterised by a period of slow growth after planting trees in an orchard (Struve, 1990) known as transplant shock (Watson, 1986). According to Watson (1986), transplant shock occurs due to an imbalance in root to shoot volume, which can be aggravated when the root system is damaged during transplanting operations (Dong et al., 2003).

The purpose of this study was to evaluate the use of containerised apple nursery trees, although bare rooted trees are still predominantly produced, to overcome some of the problems currently experienced with nursery tree production in South Africa and to evaluate the effect of management, handling and storage practises on the physical and physiological quality of such nursery trees. In addition, dormancy management strategies as well as different planting methods were evaluated in establishing new orchards.

In Paper 1 we report on the efficacy of treatments during important horticultural processes in the nursery, viz. growth cessation, hardening-off, nitrogen reserves build-up and defoliation, on the quality and performance of containerised 'Golden Delicious'/M.9 (Nic.29) apple nursery trees subsequently in a commercial orchard. In addition, the effect of two planting times, viz. autumn and spring, is also evaluated.

In Paper 2 we report on the efficacy of dormancy management strategies on containerised 'Golden Delicious'/M.9 (Nic.29) nursery trees. The effect of a cold storage period, a chemical rest breaking treatment or the combination of the two were evaluated regarding regrowth in spring and establishment during the first growing season in a commercial orchard. A second trial was conducted to evaluate the effect of different ways of handling the root volume during planting on bud break in spring and establishment during the first growing season.

In Paper 3 we report on using plant growth regulators in the production of branched (feathered) and two-leader containerised 'Golden Delicious'/M.9 (Nic. 29) apple nursery trees.

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LITERATURE REVIEW: APPLE NURSERY TREE PROPAGATION STRATEGIES

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Introduction

Global apple production

China produced 42.6 million tons of apples in 2015/2016, the most by any country in the world (USDA, 2015). China is followed by the European Union, United States, Turkey, India, Iran, Chile, Russia, Ukraine and Brazil to complete the top apple producing regions and countries (USDA, 2015). When focussing on the Southern hemisphere, Chile is the country producing the most apples with 1.678 million tons in the 2014/2015 season followed by Argentina, Brazil and South Africa (WAPA, 2016). South Africa, a net exporter of apples, is currently the 18th largest apple producer in the world, but the second largest exporter of apples in the Southern Hemisphere (WAPA, 2016). During the 2015/2016 season, South Africa exported 511 000 tons of apples compared to the 765 000 tons exported by Chile (USDA, 2015). In 2017, 33 423 558 cartons of 12.5kg, were passed for export (Hortgro, 2017) with the majority exported to the Far East and Asia (31%), Africa (30%) followed by the United Kingdom (18%), Middle East (7%) and continental Europe (6%) (Hortgro, 2017).

South African apple production

Over the last decade (2005/2006 – 2014/2015) the gross value, in Rand, of the South African apple industry increased by 257% with apple production (in tons) increasing by 31% (DAFF, 2016). The increase of gross value can be ascribed to the increase in value of production over the last decade (Hortgro, 2017).

South Africa’s main apple cultivars are ‘Golden Delicious’, ‘Granny Smith’, ‘Fuji’, ‘Royal Gala’, ‘Cripps Pink’ and ‘Topred’ (DAFF, 2016). In 2015 ‘Golden Delicious’ apples accounted for 25% of the total area planted followed by ‘Granny Smith’ (18%), ‘Royal Gala’ (16%), ‘Topred’ (13%), ‘Cripps Pink’ (10%) and ‘Fuji’ (9%) (DAFF, 2016). The main apple producing areas in South Africa are Ceres, Groenland, Villiersdorp and Langkloof East (DAFF, 2016). Approximately 90% of the apples produced and exported from South Africa are produced in the Western Cape which has a Mediterranean-type climate with winter rainfall (Hortgro, 2015). The total production area in 2015 was 22 923 hectares (ha) of which Ceres accounted for 29%. According to DAFF (2016), 34% of the apple orchards in South Africa were older than 34 years in 2015, while 9% of the apple orchards were between zero and three years. To have a sustainable and competitive apple industry, the percentage of orchards between zero and three years old needs to be 10% (DAFF, 2016).

Establishing new orchards

To ensure sustainable apple production in South Africa, new orchards with high potential, need to be established on a regular basis. According to Hortgro (2017), establishing a new apple orchard, in South Africa, cost ca. R 400 000 per ha. Due to the high cost of establishing new orchards, it is important that the producer receives early and good return on investment. Plant material contributes approximately 26% to the establishment cost (1667 trees per ha) and the potential of this plant material is influenced by various physical and physiological factors (Theron and Steyn 2015, 2016).

Physical and physiological qualities of nursery trees

Trees need to grow and fill their allocated space in the orchard as quickly as possible for optimal return on investment. The quality of nursery trees at planting has a significant effect on productivity during the early years of a newly established orchard (Van Oosten, 1978). Characteristics such as shoot to root ratio, root quality and quantity, tree size, bud quality and lack of physical injury contribute to the quality of nursery trees (Theron and Steyn, 2015). One of the main characteristics used to indicate the quality of a nursery tree, is the diameter of the trunk above the graft union (Fazio and Robinson, 2008).

One of the main concerns when establishing a new orchard is the prolonged establishment period of the young trees. This is characterised by a period of slow growth after planting trees in an orchard (Struve, 1990) and can be characterised as transplant shock (Watson, 1986). This transplant shock occurs due to an imbalance in root volume, compared to shoot volume (Watson, 1986). During lifting, storage and transplanting operations the root system can be damaged and this damage contributes to the retardation of shoot growth (Dong et al., 2003). Larger trees are particularly more prone to transplant shock due to the imbalance in the shoot to root ratio (Flemming, 1991). During propagation and handling of nursery trees, physical damage can occur, affecting the performance of the tree in the orchard and providing entry sites for pests and pathogens (Theron and Steyn, 2015). These pests and pathogens also degrade the quality of nursery trees.

Physiological factors that contribute to the quality of nursery trees, are influenced by time of growth cessation and hardening off, dormancy, defoliation and reserve status (Theron and Steyn, 2016). All these factors are discussed in more detail later in this review.

To improve and maintain the quality of nursery trees (physical and physiological) the management and handling of the nursery trees need to be of a high and correct standard. In addition, new ideas and management practices need to be implemented to improve propagation and handling of nursery trees.

Conventional nursery process

The nursery process currently utilized in many countries including South Africa, begins when rootstock liners are harvested from the mother block during winter, planted out and allowed to grow for the following season until autumn when the buds of the scion variety will be inserted on the rootstock through chip budding. The inserted bud will overwinter and at the beginning of spring, the rootstocks are cut back above the scion. Scion growth will be allowed until the end of the next growing season (Hartmann et al., 2011). At the end of the growing season, these trees are ready to be lifted. During the growing season there are important horticultural processes that require attention. Before the trees are lifted, vegetative growth must stop. The trees harden off to minimise cold damage and dehydration during storage as well as provide protection against chemical damage during rest breaking treatment. The internal cycling of nitrogen within the trees is also important. Before leaves abscise from the tree, nitrogen is translocated from the leaves to the storage organs (Millard, 1995). These nitrogen reserves will be allocated to regrowth in spring when nitrogen uptake by the roots are limited (Millard and Neilsen, 1989). Before handling of nursery trees, nurserymen will allow leaves to defoliate naturally or remove the leaves mechanically or chemically (Guak and Fuchigami, 2002). Handling of bare-rooted nursery trees without leaves, will minimise stress (Theron and Steyn, 2016). After lifting the trees in the nursery, they are placed in cold storage to ensure that trees grown in moderate climates will accumulate sufficient chilling for successful and synchronised bud break in spring when planted in the orchard (Steyn et al., 2016).

Growth cessation and hardening off

At the end of summer and beginning of autumn after growth cessation, the buds of the grafted tree enter a state called paradormancy (Lang et al., 1987). During paradormancy, terminal and lateral bud development takes place and the trees start to harden-off, which is important for the overall quality of the tree and will therefore influence the regrowth potential of the tree in spring (Theron and Steyn, 2015). This is followed with the initiation of endodormancy that enables the trees to survive unfavourable conditions during winter (Faust et al., 1997).

Certain factors such as the soil fertility, soil moisture, growth regulating chemicals and temperatures in autumn can influence when growth cessation occurs (Faust, 1989). Early growth cessation can lead to earlier onset of hardening off and resources can be diverted from extension growth to the secondary growth and increase in the trunk diameter of the nursery trees, a parameter that is used for grading of nursery trees. Guak and Fuchigami (2001) reported that earlier growth cessation can lead to an earlier and gradual onset of dormancy and decrease the risk of cold injury. During the growth cycle of woody perennial plants, the cessation of shoot growth is associated with the formation of terminal buds (Powell, 1978). The formation of terminal buds is followed by endodormancy (Powell, 1978) and there is a close relationship between the development of endodormancy and growth cessation (Guak and Fuchigami, 2001).

The role of ABA

ABA is a soluble plant hormone commonly found in above ground parts of apple trees such as fruit, leaves, shoot tips, buds and stem tissue (Powell, 1975) and has several important physiological roles during the growing season. This hormone is usually synthesised under stress conditions such as leaf dehydration caused by dry soil conditions (Wilkinson and Davies, 2002). ABA is best known to act during stress responses (Ton et al., 2009) and also to regulate developmental processes such as seed dormancy, shoot and root growth as well as bud dormancy (Wasilewska et al., 2008). It was proposed that older leaves had more ABA per leaf area compared to the shoot tip (Powell, 1975). This was supported by Weinbaum and Powell (1975) who found that ABA diffused at a higher rate from older leaves compared to younger leaves when these leaves were placed in an aqueous solution. This led to the conclusion that more mature leaves, which are progressively present during the growing season, will lead to an accumulation of ABA that would force shoot elongation to stop.

Guak and Fuchigami (2001) evaluated the ability of 1000 $mg \cdot L^{-1}$ ABA to inhibit or slow down extension growth of ‘Fuji’/M26 nursery trees. They also studied the development of bud dormancy, cold acclimation, leaf senescence and translocation of nitrogen after applying ABA. Shoot growth was reduced by 3 cm compared to the untreated control trees, while no difference in stem diameter occurred. They explained that this was due to a possible inhibitory effect of ABA on photosynthesis, but the photosynthetic rate was not measured. When the growth cessation of the trees was accelerated, dormancy development was initiated earlier in the growing season, which could be beneficial due to reduced cold damage and easier handling of the trees. Leaf senescence of the trees was accelerated after ABA application, with the basal leaves the most responsive. Translocation of nitrogen was promoted by the application of ABA, as indicated by the increase in nitrogen in the bark of the trees (Guak and Fuchigami, 2001). This is important, as nitrogen needs to be translocated to the bark before the onset of defoliation as spring regrowth of nursery trees depends on reserve nitrogen (Cheng and Fuchigami, 2002).

In another study, Guak and Fuchigami (2002) investigated the effect of ABA on the cessation of growth, senescence of leaves and cold acclimation on containerised 'Gala'/M26 apple nursery trees, which received differential nitrogen applications. Shoot growth cessation was monitored and leaf abscission was determined as percentage defoliation. An electrolyte leakage method was used to determine cold acclimation. Three different nitrogen fertigation treatments were evaluated: Early nitrogen cut-off (21 August), mid nitrogen cut-off (18 September), and late (9 October) nitrogen cut-off. In total, 840, 1080 and 1260 mg nitrogen was applied per tree for the three treatments, respectively. The authors established that delaying the end of nitrogen fertigation, delayed shoot growth cessation. ABA application slightly advanced growth cessation and leaf abscission in both the mid – and the late nitrogen cut off plants. This improved the nitrogen translocation from the leaves to the storage organs and resulted in increased regrowth potential in spring. The authors established that ABA had no significant effect on the cold acclimation of trees when nitrogen fertigation continued to the mid or late cut-off dates.

The foliar application of ABA was evaluated on greenhouse-grown grapevines by Zhang et al. (2011). During this study, foliar ABA was used to determine the optimal application rate and which morphological and physiological changes were induced. The authors established that the growth cessation was optimally induced without phytotoxic effects on the leaves at rates between 400 $mg \cdot L^{-1}$ and 600 $mg \cdot L^{-1}$ ABA. Applying these rates of ABA also advanced leaf abscission. By advancing growth cessation and leaf senescence, the induction of endodormancy occurred earlier under favorable growing conditions.

Larsen and Higgins (1998) evaluated ABA as a chemical defoliant on 'Bartlett' pears as well as 'Imperial Gala', 'Gibson Golden Delicious', 'Scarlet Spur Delicious', 'Law Red Rome, 'Granny Smith', 'Braeburn' and 'Red Fuji' apple nursery trees. Single and multiple ABA applications (500, 1000 and 2000 $mg \cdot L^{-1}$) applied until run-off were compared and defoliation was monitored on a weekly basis for a four-week period. Generally, two 2000 $mg \cdot L^{-1}$ ABA applications, a week apart, resulted in the fastest defoliation, but was not optimal due to preventing reserve translocation from the leaves to the storage organs. All of the cultivars in this study were effectively defoliated (more than 80%) after four weeks when it was treated with two 1000 $mg \cdot L^{-1}$ ABA applications, a week apart, or a single application of 2000 $mg \cdot L^{-1}$

ABA. Defoliation of the ‘Gibson Golden Delicious’ was achieved with two applications at 500 mg·L^{-1} ABA. Larsen and Higgins (1998) concluded that nurserymen need to consider a wide range of concentrations and application dates depending on the season and cultivar.

Robitaille and Carlson (1971) investigated the response of one-year-old ‘Red Prince Delicious’ apple trees on three rootstocks, viz. EM IX (dwarfing), EM VII (semi-dwarfing) and MM 111 (semi-vigorous) when they were injected with 100 mg·L^{-1} ABA by using disposable 20 mL plastic syringes. Six injections were made at regular intervals between 2 and 21 October 1969. There was no significant difference in uptake of ABA between the three different rootstocks used in the trial and also no significant differences in growth cessation. However, the ABA injection reduced the mean terminal growth compared to the control trees and the dwarfing rootstocks responded more quickly, compared to the more vigorous rootstocks, when treated with ABA. ABA therefore has the potential to inhibit shoot growth, thus promoting hardening off, of nursery trees. By inhibiting shoot growth and promoting hardening off, the onset of endodormancy can happen at an earlier stage.

Use of prohexadione-calcium

The control of vegetative growth on apple and pear trees is a challenge (Medjdoub et al., 2005). On bearing apple trees, foliar applied prohexadione-calcium (ProCa) (BASF, Carl Bosch Street 38, 67056 Ludwigshafen, Germany) is widely used to inhibit vegetative growth. ProCa inhibits the synthesis of the active gibberellin GA_1 which plays an important part in cell elongation (Rademacher and Kober, 2003). This inhibition of vegetative growth is advantageous since it leads to less summer pruning, improved tree architecture and improved fruit set – and yield (Rademacher and Kober, 2003). The efficacy of the ProCa application depends on several factors such as availability of ProCa in the target tissue, the plant physiological stage at application, number of treatments as well as the dosage (Rademacher et al., 2004).

In nursery tree production, the effect of gibberellin inhibitors has only been evaluated on citrus nursery trees (Le Roux and Barry, 2010). Potted ‘Eureka’ lemon nursery trees on X639 rootstock were treated with ProCa at 100, 200, 400 and 800 mg·L^{-1}. There were no significant differences in rootstock diameter between the treated trees and an untreated control. The shoot length of the trees treated with 800 mg·L^{-1} ProCa was significantly reduced compared to other treatments. Lemon nursery trees treated with 400 and 800 mg·L^{-1} ProCa had reduced internode length of 31% and 56% respectively, compared to the untreated control trees.

The authors concluded that ProCa has the ability to reduce the vegetative growth of 'Eureka' lemon nursery trees.

There are numerous studies that evaluated the effect of ProCa on bearing apple – and pear trees. From 1999 to 2001, Medjdoub et al. (2005) evaluated ProCa on 'Fuji'/M9 and 'Royal Gala'/M9 apple trees in commercial orchards in warm and dry areas in Spain. The control of vegetative growth and achieving good colour of red cultivars is a challenge in this region. The objective of the study was to determine the optimal concentration and timing of application to control vegetative growth. All the trees were sprayed with different concentrations, single or multiple, until runoff. The shoot length of the treated trees differed significantly from the untreated control. Multiple, instead of single, ProCa applications showed the most promising results regarding inhibition of vegetative growth. The timing of the first treatment was also important and the authors concluded that applying the first ProCa spray at a concentration of 200 mg·L^{-1} at full bloom, followed by a second spray later in the season was sufficient to inhibit vegetative growth.

Smit et al. (2002) evaluated the effect of ProCa on the control of shoot growth on three apple cultivars, in a Mediterranean climate. The response of the apple cultivars, 'Golden Delicious', 'Granny Smith' and 'Royal Gala' were evaluated over two seasons (1999/2000 and 2000/2001). Treatments ranged from three to four applications of 50 mg·L^{-1} ProCa and three applications of 67 mg·L^{-1} ProCa. These treatments were compared to untreated control trees. In both seasons, ProCa reduced shoot growth on all three cultivars except for 'Royal Gala' during the 2000/2001 season.

Unrath (1999) established that multiple, lower dosage applications of ProCa to apple trees are more effective than a single, high dosage application in inhibiting vegetative growth. Zadravec et al. (2008) confirmed this on 'Gala'/M9 trees. According to Rademacher et al. (2004), data that was recorded between 1999 and 2000 is used for ProCa application recommendations on apples and pears. During these two years 57 registration and regular trials were conducted in France, The Netherlands, Italy and Germany. A total of 14 different apple cultivars, all on M9 rootstock, were treated with the same protocol. Shoot growth, fruit yield per tree and individual fruit weight were measured. After the conclusion of the trials, a single application of 250 g·ha^{-1} or a split treatment with two 125 g·ha^{-1} applications led to an average reduction of shoot growth by 40%. The authors recommended a split application to improve the control of multiple shoot growth flushes during the season. It was also found that reducing shoot growth by applying ProCa reduced the total time spent on winter pruning by 34%.

In Europe ProCa is registered to be applied at a maximum rate of 250 $g{\cdot}ha^{-1}$ and a withholding period of 55 days. Generally, a split application with two dosages of 75 to 150 $g{\cdot}ha^{-1}$ showed the best results regarding the regulation of shoot growth and to minimalize the risk of detecting ProCa in the fruit at harvest (Rademacher and Kober, 2003). In South Africa ProCa is registered on apples to be applied at a minimum rate of 125 $g{\cdot}ha^{-1}$ with a withholding period of 56 days. ProCa is applied at full bloom, followed by a second application three to four weeks later (BASF Crop Protection).

The effect of ProCa has only been investigated on citrus nursery trees. Hence the effect of ProCa on apple nursery trees is not well understood and should be investigated.

Nitrogen reserves

Internal cycling of nitrogen

The storage and remobilization of nitrogen, through internal cycling, is a characteristic of perennial plants (Millard, 1995). Trees can use various sources of nitrogen for tree growth with the main sources being nitrogen fertilizers, mineralization of organic matter in the soil, atmospheric deposition and nitrogen reserves that is stored in the tree (Millard, 1995). The internal cycling of nitrogen in plants has two components, viz. the withdrawal of nitrogen from the leaves before senescence and abscission and using the stored nitrogen for regrowth in spring (Millard and Neilsen, 1989). In summer and early autumn, 30% to 50% of the total nitrogen in mature trees, is located in the foliage (Batjer and Rogers, 1952; Forshey, 1963). Of this nitrogen, between 50% and 70% can be translocated back to the rest of the plant and stored as reserves before defoliation (Batjer and Rogers, 1952). Usually, nitrogen is stored in the bark of shoots and trunk (Titus and Kang, 1982) as well in the roots (Taylor and May, 1967).

In fruit nursery trees there are two sources that contribute to nitrogen reserves. The roots can take up nitrogen from the growing medium or the soil, or nitrogen can be translocated from the leaves, via the phloem, to the storage organs (Cheng, 2002). In addition to this natural mobilization of nitrogen, leaves can absorb foliar applied nitrogen in autumn (Cheng et al., 2002). This foliar applied nitrogen is broken down to amino acids, and mobilized to storage organs (Dong et al., 2002). Previous work by Cheng et al. (2002) showed that 80% to 90% of the absorbed nitrogen, after a foliar urea application, can be translocated to the storage organs of apple nursery trees (Cheng et al., 2002).

Advantages of foliar applied nitrogen

The main advantage of foliar applied nitrogen is that this application is effective under conditions where root uptake is limited due to unfavourable conditions like root damage, dry weather or waterlogging (Murtic et al., 2012). Urea is usually the preferred choice of nitrogen because it is relatively inexpensive (Bowman and Paul, 1992), has a high solubility (Bowman and Paul, 1992) and has a low risk of phytotoxicity (Johnson et al., 2001). The rapid absorption of urea by leaves has been documented. Shim et al. (1972) established that 80% of foliar urea was absorbed 48 hours after application. This rapid uptake after 48 hours was also found in young apple trees (Dong et al., 2002) as well as citrus nursery trees (Lea-Cox and Syvertsen, 1995). It has been established that the maximum absorption of urea occurs at 16 °C and in full sunlight conditions (Shim et al., 1972).

By applying urea in a foliar form, the amount of nitrates that could leach from the soil into the groundwater is reduced (Del Amor et al., 2009). In addition the percentage recovery of foliar applied nitrogen on young apple trees was approximately 30% higher compared to soil applied nitrogen (Hill-Cottingham and Lloyd-Jones, 1975). Moreover, Shim et al. (1972) also established that the efficiency of utilizing foliar applied nitrogen was four times greater compared to soil applied nitrogen.

Importance of nitrogen reserves.

The role of reserves on regrowth potential on pome fruit trees was studied extensively. Cheng and Fuchigami (2002) demonstrated that nitrogen, rather than carbohydrates, is the deciding factor regarding regrowth of two-year-old ‘Fuji’/M26 apple trees in spring. Due to root damage which may occur during lifting, transport and transplanting of nursery trees as well as low soil temperatures in spring, transplanted nursery trees are more dependent on reserve nitrogen for initial growth in spring (Cheng et al., 2001). Regardless of nitrogen application in spring, new shoot and leaf growth is directly related to the amount of nitrogen reserves (Cheng et al., 2001). This relationship between reserve nitrogen and shoot – and leaf growth was also visible on young almond trees (Bi et al., 2003), pear nursery trees (Cheng et al., 2001) and young ‘Fuji’/M26 trees (Cheng and Fuchigami, 2002). Reserve nitrogen also supports new root growth (Dong et al., 2001) that enhances absorption of water and nutrients, synthesis of hormones as well as anchoring of the plants (Dong et al., 2001).

If the total nitrogen can be increased in the leaves, more nitrogen can be translocated from the leaves to the storage organs where it will be stored as reserve nitrogen. This increase in nitrogen reserves can aid spring regrowth and subsequently reduce the establishment time of the newly planted orchard.

Defoliation

Defoliation refers to the removal of leaves, chemically or mechanically, from trees. To minimize dehydration, bare-rooted nursery trees are usually lifted and handled after completion of leaf drop or defoliation (Theron and Steyn, 2016). Defoliation needs to be controlled in nurseries to produce good quality trees for optimal growth in the orchard when planted out (Knight, 1983). Another reason to control leaf drop is to ensure that handling, i.e. digging of the nursery trees in autumn is as efficient as possible (Dong et al., 2004). The trees in the nursery need to be leafless if the trees are to be lifted, and stored successfully as the presence of leaves on the trees will restrict air circulation and promote decay during cold storage (Larsen, 1972). Usually natural leaf drop is delayed in warmer regions and trees enter endodormancy at a later stage in the season (Mohamed, 2008). The traditional method of removing the leaves of nursery trees is by hand through manual defoliation. The biggest negative aspect of manual defoliation is that it is expensive, labour intensive and time consuming (Guak et al., 2001). Manual defoliation can also happen prior to the completion of nitrogen translocation, resulting in lower levels of nitrogen reserves. Manual defoliation can damage the buds and bark of the trees (Dozier et al., 1987) and these injuries can become sites for infection (Basak et al., 1973). According to Larsen and Abusrewil (1983) there have been efforts to find chemical treatments that are more effective than mechanical defoliation.

The commercial use of chemicals for defoliation started in the early 1950's (Basak et al., 1973) and one of the first experiments using a chelated form of copper (CuEDTA) on citrus caused an increase in ethylene production that lead to accelerated degreening, fruit abscission and excessive defoliation (Cooper et al., 1968).

Trees are usually sprayed with a chemical defoliant in autumn when the leaves are still actively photosynthesising. According to Jones et al. (1973) a defoliation percentage of 80% is required within three weeks of application for a defoliant to be regarded as successful.

Controlled defoliation plays a key role in young tree production (Knight, 1983). Premature defoliation (before growth cessation) can inhibit the development of dormancy and hardening-off in autumn before the onset of winter, resulting in a lack of cold hardiness (Fuchigami, 1970). Another effect of premature defoliation is that inadequate reserves will be translocated from the leaves to the storage organs, hence reducing the regrowth - and survival potential of the trees in spring (Abusrewil and Larsen, 1981). Previously Loescher et al. (1990) established that premature defoliation reduced the accumulation of carbohydrates in the tree. This reduction in carbohydrates can create nitrogen deficiencies due to a lack of new root growth (Loescher et al., 1990). There is a direct correlation between premature defoliation and a protracted bud break period as well as a reduction in vegetative shoot growth during the following spring (Lloyd and Couvillon, 1974).

Jones et al. (1973) investigated the effect of timing of chemical defoliation treatments on peach seedlings. Defoliation treatments that were applied at the end of autumn achieved better results compared to defoliation treatments that were applied at the beginning of autumn.

Numerous studies were conducted to find a dependable and safe chemical defoliant for a wide variety of fruit trees, amongst others CuEDTA. These chemical defoliants need the ability to abscise the leaves from the trees, without the disadvantages of manual defoliation (Knight, 1979). Larsen and Fritts (1986) determined the level of defoliation on ‘D’Anjou’ and ‘Bartlett’ pear, ‘Bing’ cherry, ‘Golden Delicious’, ‘Redchief Delicious’, ‘Redspur Delicious’ and ‘Nured Rome Beauty’ apples with single or double applications, applied at four day intervals until runoff, at concentrations of 0.25%, 0.50% and 0.75% CuEDTA. They found that 80% of the leaves were defoliated by 0.50% and 0.75% CuEDTA with minimal damage to the trees during the time that lifting took place in the nursery.

CuEDTA-induced defoliation affects the nitrogen reserve status of nursery trees. CuEDTA resulted in early leaf abscission when applied while the leaves were still green (Laywisadkul et al., 2010). Previously Guak et al. (2001) found that CuEDTA-induced defoliation was rapid in ‘Fuji’/M.26 apple nursery trees to such an extent that reserves were not translocated back to the storage organs resulting in poor regrowth performance in the following spring. Gauk et al. (2001) evaluated the effect of applying foliar urea (3%) twice, five - and 13 days before applying CuEDTA (1%). The urea and CuEDTA application improved the reserve nitrogen levels in the roots by 145% and in the bark by 219% compared to hand defoliated trees that did not receive any urea. The urea-treated trees grew better in spring irrespective of the two different defoliation methods (hand – and chemical defoliation) and no injury to the trees and the buds occurred. The percentage spring bud break when the trees were treated with urea and CuEDTA was similar compared to natural defoliation. They concluded that a urea spray in combination with CuEDTA improved the nitrogen reserves without compromising the defoliation effect of CuEDTA. Bi et al. (2005) showed that leaves of almond nursery trees treated with a combination of urea and CuEDTA as well as urea and $ZnSO_4$ abscised earlier compared to the control trees that did not receive any treatments.

CuEDTA resulted in earlier defoliation compared to $ZnSO_4$ and combining urea with CuEDTA resulted in more effective defoliation compared to combining urea with $ZnSO_4$ (Bi et al., 2005). The authors established that a combination of 3% urea and 1% CuEDTA was the most effective regarding leaf abscission. However, the authors concluded that trees spayed with urea, alone or in combination with the defoliants, had more total nitrogen compared to the untreated control.

Dong et al. (2004) evaluated the effects of applying urea and CuEDTA in a single or mixed application in the autumn on nitrogen reserves and regrowth performance on young 'Fuji'/M26 trees. Both CuEDTA and CuEDTA plus urea treatments stimulated more than 80% defoliation within two weeks after treatment, compared to only 12 - 14% defoliation in untreated control trees and trees only treated with urea. Knight (1983) compared CuEDTA, FeEDTA, $CuSO_4$, and $FeSO_4$ as defoliants on 'Cox Orange Pippin' apple nursery trees. Of these, CuEDTA was the most effective regarding defoliation and less mature, terminal leaves on the tree abscised at a slower rate compared to older, basal leaves.

It is important to remember that environmental factors such as temperature, humidity, precipitation as well as the phenological stage of the tree can influence the efficacy of chemical defoliants and these are important to keep in mind before applying chemical defoliants (Larsen, 1972). Temperatures between 20 °C and 25 °C are recommended for effective defoliation. Low relative humidity and precipitation after treatment can be detrimental due to lower absorption caused by low humidity and precipitation that washes the defoliants off (Larsen, 1972). The timing of defoliant application should be close to the time of natural leaf abscission. When nursery trees are well fertigated (particularly with nitrogen), essential elements are abundantly supplied and trees are still growing vigorously, the defoliant will be less effective (Larsen, 1972). The age of the tree also plays an important role in the efficacy of the defoliant (Larsen, 1972). Younger (one - and two-year-old) nursery trees are more difficult to defoliate compared to older, bearing trees in the orchard due to higher vigour (Larsen, 1972). The physiology of the tree also plays a role. Cultivars that tend to grow late into the season were more difficult to defoliate compared to cultivars that already developed a terminal bud earlier in the season (Jones et al., 1973). Finally, the nature of the cuticle also plays a role. Most of the foliar applied chemicals gain entry through the cuticle of the leaves, and the cuticle offers the greatest resistance to foliar applied chemicals to gain entry. Trichomes can inhibit the entry of the chemical defoliants, and subsequently delay the defoliation response of trees (Oosterhuis, 2009).

Dormancy

Dormancy is a process that allows fruit trees to survive cold temperatures during winter, to avoid bud burst during warm weather in winter and to synchronise bud break in spring (Faust et al., 1997; Louw et al., 2016). One of the biggest challenges in countries with warm winters is overcoming the endodormancy period (Erez, 2000). The dormancy process can be classified into three stages: Paradormancy, endodormancy and ecodormancy (Lang et al., 1987).

Paradormancy

Paradormancy is usually regulated by physiological factors that originate outside the affected structure i.e. apical dominance (Lang et al., 1987). During apical dominance, the growth of lateral buds is inhibited by the shoot apex (Cline and Deppong, 1998).

Endodormancy

Endodormancy is controlled by environmental or physiological signals within the affected structure (Lang et al., 1987). Factors such as chilling, photoperiod and genotype influence the progression of endodormancy (Campoy et al., 2011; Lang et al., 1987).

Ecodormancy

After endodormancy is completed, environmental factors are responsible for the dormant buds to break. The stage were environmental factors regulate dormancy is called ecodormancy. Environmental factors such as low temperatures and drought stress inhibit the opening of the dormant buds (Lang et al., 1987; Louw et al., 2016). Once temperatures rise in spring, these buds will open and growth will resume if the chill requirement for endodormancy alleviation has been met (Louw et al., 2016).

Factors that influence dormancy

For the onset of paradormancy of the terminal bud, the cessation of vegetative growth is necessary. Certain factors such as cold temperatures, drought, and light quality can contribute to the cessation of growth (Allona et al., 2008). Following vegetative growth cessation, paradormancy and then endodormancy needs to start before the exposure to damaging cold temperatures. Trees planted in warmer regions show delayed leaf abscission and the entry into endodormancy is delayed (Mohamed, 2008).

The hormone ABA is currently viewed as one of the most important hormones involved in dormancy. The increased sensitivity to ABA, rather than increase in ABA concentration, play a role during the dormancy process (Chen et al., 2002) through the action on dehydrins (Jacobsen and Shaw, 1989), which are associated with cold hardiness and the endodormant state.

A quantitative amount of cold, referred to as chill requirement, during winter is required to complete the dormancy cycle (Louw et al., 2016). One of the biggest challenges in successfully growing apple trees under insufficient chilling conditions is poor and protracted bud break during spring (Subhadrabandhu, 1995). For buds to exit dormancy, a fixed amount of cold, depending on the cultivar or fruit type, needs to accumulate during the endodormant period. The progression of endodormancy differs between regions with sufficient chilling in winter, compared to areas that did not receive adequate chilling (Cook and Jacobs, 2000). The authors evaluated the progression of bud endodormancy in two different climates, the Elgin region that does not receive sufficient chilling in the winter, compared to the Koue Bokkeveld region that receives more chilling in winter. One-year-old branches of ‘Golden Delicious’ and ‘Granny Smith’ apples from both regions were sampled, and forced at 25 °C to evaluate bud burst. The entrance of buds into endodormancy was much quicker for trees grown in the Koue Bokkeveld compared to trees grown in Elgin. Buds from the Koue Bokkeveld trees also reached maximum endodormancy and exited dormancy much quicker than buds from trees in Elgin. It is evident that sufficient chilling in winter is an important factor that influences the progression of endodormancy and decreases the occurrence of delayed foliation. This supports the practise of cold storage of nursery trees grown in nurseries in areas that do not receive sufficient chilling during winter, or prior to planting in areas without sufficient winter chilling.

Effect of inadequate chilling

On average, apple trees need between 1000 and 1200 chill units (Utah model) to alleviate the chill requirement of apple buds (Cook, 2010). If these chill units are not obtained it can lead to reduced budburst, flowering and fruiting (Cook, 2010; Costa et al., 2004; Petri and Leite, 2004). Due to inadequate average chilling units in regions such as Elgin (650 – 850 chilling units according to the Daily positive chill unit model, Linsley-Noakes et al., 1994) the rate at which the trees enter dormancy is retarded. This extended dormant period will influence the architecture of the trees (Cook, 2010). More specific, the development of acrotony and apical control will be impeded (Cook, 2010). An acrotonic bursting tendency occurs when bud burst develops from the most distal buds, downwards to the proximal buds. Hence, the reduction in chilling units and extended endodormant period will be associated with a basitonic growth pattern of the tree (Cook et al., 1998). A basitonic growth pattern develops when the proximal buds open before the distal buds and leading to basal dominant trees.

Dormancy management practises

When nursery trees are planted out in spring, uniform bud break is necessary to create an adequate framework for good development, in terms of tree architecture, of the trees in the orchard (Petri and Stuker, 1988). To overcome the challenges of mild winter climates, growers make use of cold storage. For the cold storage to be effective and not damaging to the nursery trees, the trees need to be hardened-off before being stored in a cold room. If nursery trees are not left to overwinter in cold storage (4 °C) the visible signs of insufficient chilling will be evident during the first spring after planting (Louw et al., 2016). These signs include unsynchronised bud break and basal dominance (Louw et al., 2016).

Petri and Stuker (1988) evaluated the effect of the duration and temperature of cold storage on the development of one-year-old ‘Gala’ and ‘Fuji’ apple nursery trees grafted on MM106 rootstocks in Brazil. Trees were stored at 2 °C and 6 °C, respectively, in a cold room with relative humidity above 80% for periods of 0, 15, 30, 45 and 60 days. Half of the trees were treated with 4% mineral oil and 0.16% dinitro-butyl-phenol. The chemicals were applied by painting the trunk of the trees after they were planted. Bud break of the ‘Gala’ trees was much higher when it was stored for 45 or 60 days compared to 15 or 30 days, indicating that the duration of cold storage was significant. There was no significant difference regarding bud break when the trees were stored at 2 °C and 6 °C. The ‘Fuji’ trees showed similar results to the ‘Gala’ trees. The authors also established that a chemical treatment after the trees were planted out in spring improved bud break. The trees stored for 45 days with no chemical treatment had a higher percentage bud break compared to the trees that were stored for 30 days and received a chemical rest breaking treatment. Trees that received cold storage had a larger trunk cross sectional area after the first growing season that can be ascribed to the long side shoots as well as the higher number of leaves on trees during the first growing season.

The exit from endodormancy is just as important as the entry into endodormancy. One of the most common solutions to combat the delayed release out of endodormancy in mature, commercial orchards is the application of chemicals, as such trees cannot be placed in cold storage.

The use of chemical sprays on temperate fruit crops grown in warm climates is a common practice (Erez et al., 2008). Mineral oil was the first chemical used (Erez, 2000); alone as well as in combination with various other chemicals to enhance the rest breaking effect. One of these chemicals, dinitro-*o*-cresol, has since been banned due to health concerns. Over the last decade cyanamide has become the leading rest breaking chemical (Erez, 2000). Calcium cyanamide has long been used as a rest breaking treatment, but due to its low water solubility hydrogen cyanamide has been studied and used more extensively (Erez, 2000).

Dormex® (a.i. hydrogen cyanamide) has been found effective in breaking dormancy on a variety of stone– and pome fruit. By applying Dormex®, bud break was improved on trees that received inadequate chilling (Dozier et al., 1990; Mohamed, 2008; Subhadrabandhu, 1995). Dormex® also improved the rate, as well as the uniformity of bud break on a variety of apple cultivars that received inadequate chilling (Jackson and Bepete, 1995). Sagredo et al. (2005) evaluated the effect of combining Dormex® and mineral oil in South Africa, and established that a combination of these two chemicals were sufficient to break dormancy.

Conclusion

Apples are one of the most important deciduous crops produced in South Africa and contribute significantly to the economy (Hortgro, 2017). For sustainable apple production, new apple orchards need to be planted every year. Due to the high cost of establishing new orchards (Hortgro, 2017) it is important to produce good quality trees. The planting material accounts for a significant portion of the total cost of orchard establishment (Theron and Steyn, 2015) stressing the importance of good quality nursery trees. Good quality nursery trees are important for early return on investment due to improved productivity during the early years (Van Oosten, 1978).

New approaches regarding the propagation and handling of nursery trees are required to improve physical –and physiological tree quality. Such approaches should be investigated. Improved nursery tree quality can potentially reduce the severity of transplant shock due to a reduction in root volume loss during production. This can shorten the establishment period that is currently often characterised by a period of slow growth after planting.

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PAPER 1: EVALUATING NEW APPROACHES TO MANAGE CONTAINERISED APPLE (*MALUS DOMESTICA* BORKH.) NURSERY TREES.

Additional index words. Abscisic acid (ABA), 1-aminocyclopropane-1-carboxylic acid, defoliation, foliar copper, growth cessation, hardening off, prohexadione calcium, transplant shock.

Abstract.

One of the main challenges during the establishment of new orchards is the period of slow growth called transplant shock. In addition, the cost of plant material contributes significantly to the establishment of a new apple orchard and good quality nursery trees are important for early return on investment. Certain physical and physiological factors contribute to the quality of plant material, which is influenced by horticultural practises and propagation methods in the nursery. The purpose of this study was to evaluate containerised nursery tree management practises and timing of these management practices on e.g. growth cessation and hardening off, improving nitrogen reserves, and defoliation during propagation in the nursery. The effect of these treatments and different planting times on spring bud break and new growth during the first growing season in a commercial orchard was investigated. Prohexadione calcium and abscisic acid showed no significant effect on growth cessation during the nursery phase, and no significant effect was observed regarding regrowth in spring and new growth during the first growing season. Trees planted in autumn had earlier bud break whereas trees planted in spring had a higher bud break percentage. Foliar nitrogen (urea) during the nursery phase did not significantly affect spring bud break and new growth during the first growing season. Foliar copper, in comparison to 1-aminocyclopropane-1-carboxylic acid proved to be a more successful chemical defoliant in the nursery with no significant negative effect on the subsequent performance in the orchard. Applications of exogenous gibberellin had no effect on reproductive bud initiation. Because containerised nursery tree propagation is a new concept in South Africa, further research is needed on propagation and subsequent management of these trees.

Introduction

For sustainable apple production in South Africa, new orchards with high potential need to be established on a regular basis. Due to the increase in cost of establishing new orchards, of which the planting material contributes significantly (Hortgro, 2017), it is important that the producer receives early and good return on investment. The quality of nursery trees at planting has a significant effect on productivity during the early years of a newly established orchard (Van Oosten, 1978). In the nursery there are important horticultural processes that contribute to the physical and physiological quality of the nursery trees.

Vegetative growth cessation needs to take place before nursery trees are lifted or transplanted, and trees must be hardened-off to minimise damage during storage or transport. Early growth cessation can lead to earlier onset of hardening-off and resources can be diverted from extension growth to the secondary growth and increase in trunk diameter, a parameter that is used for grading of nursery trees (Fazio and Robinson, 2008). Previous research established that abscisic acid (ABA) (Guak and Fuchigami, 2001) and prohexadione-calcium (ProCa) (Le Roux and Barry, 2010) can induce growth cessation in apple and citrus nursery trees, respectively.

Perennial plants have the ability to store and remobilise nitrogen during the growing season. The internal cycling of nitrogen in plants has two components, viz. the withdrawal of nitrogen from the leaves before senescence and abscission and using the stored nitrogen for regrowth in spring (Millard and Neilsen, 1989). Nitrogen, rather than carbohydrate reserves, is the deciding factor regarding regrowth of apple trees in spring (Cheng and Fuchigami, 2002). An increase in nitrogen reserves of nursery trees can potentially aid with regrowth in spring and subsequently reduce the establishment time of the newly planted orchard.

Nursery trees are usually handled and transported after completion of leaf drop or defoliation (Theron and Steyn, 2016). Defoliation should be controlled in nurseries for optimal regrowth in the orchard after transplantation (Knight, 1983). Usually the natural leaf drop is delayed in warmer regions and as a result trees enter endodormancy at a later stage in the season (Mohamed, 2008). This delayed leaf drop contributes to logistical issues. Preferably, nurserymen want to lift trees after natural leaf drop, but before the onset of winter rain which will make the nursery impassable. Due to the negative aspects of mechanical defoliation (Guak et al., 2001), nurserymen are looking for chemical alternatives to aid with defoliation. CuEDTA is a successful defoliant of nursery trees, but it is still unclear how CuEDTA-induced defoliation affects the nitrogen reserve status of nursery trees. The majority of research found that chemical defoliation is negative regarding tree performance in the following spring. CuEDTA application results in early leaf abscission while leaves are still green (Laywisadkul et al., 2010) and previously Guak et al. (2001) proposed that CuEDTA-induced defoliation was rapid to such an extent that nitrogen from the leaves were not translocated back to the storage organs.

In this paper we report on the efficacy of treatments during important horticultural processes, viz. growth cessation, hardening-off, nitrogen reserves build-up and defoliation on the quality and performance of containerised apple nursery trees. In addition, we report on the effect of these treatments regarding regrowth and establishment in a commercial orchard.

During the 2017/2018 season, a high number of reproductive buds were visible during the orchard phase. To optimize growth during the first growing season, shoot growth from a vegetative bud rather than a bourse shoot from a reproductive bud, is preferred. Previous work established that gibberellin can inhibit the initiation of reproductive buds on apple nursery trees (Lordan et al., 2017). The effect of exogenous gibberellin applications on the percentage reproductive buds during the 2017/2018 season is reported.

Materials and Methods

Plant material and site description. All trials were conducted at Witzenberg Range Nursery, a commercial nursery, situated in Simondium, Western Cape region (33°50'14.1"S 18°57'43.6"E, altitude 150 m, Mediterranean-type climate, 664 chilling units (Daily positive chill unit model (DPCU), Linsley-Noakes et al., 1994) and ca. 856 mm rainfall annually). In the 2016/2017 season, four trials were conducted on one-year-old containerised 'Golden Delicious' apple nursery trees on Nic 29 Malling 9 (M.9) rootstock. Rootstocks, harvested from stool beds on 15 August 2016 and planted in 8 or 10 L black plastic bags containing coir medium, were chip-budded on 1 October 2016 and placed under a 40% Black/White shade net with a shade factor of 32% (Knittex (Pty) Ltd, Brackenfell, Western Cape). Trees used for trials were on average in excess of 1.8 m tall by the end of the growing season (Fig.1). The nursery trees were trained to a single stem and irrigation scheduling varied during the growing season according to continuous logging soil moisture probes (DFM Technologies, Eersterivier, South Africa) in the planting medium. Standard commercial irrigation, plant protection and fertilizer practices were followed. All the foliar applications in the trials were applied until runoff with a motorized knapsack sprayer (STIHL, Pietermaritzburg, South Africa) unless stated otherwise. A randomized complete block design with ten single tree replications was used in all trials, unless stated otherwise.

In the 2017/2018 season, a trial was conducted on one-year-old containerised ‘Bigbucks Gala’ apple nursery trees on Nic 29 Malling 9 (M.9) rootstock. The nursery trees were propagated in the same nursery and in the same way as described above. Rootstocks were chip-budded on 30 October 2017. Trees used for the trial had an average height in excess of 1.5 m by the end of the growing season.

Following the nursery phase, trial trees were planted in a new orchard on Glen Elgin, a commercial farm, situated in Elgin, Western Cape Region (34° 9'18.89"S 19° 2'6.32"E, Altitude 332 m, Mediterranean-type climate, 880 chilling units (DPCU) and ca. 949 mm rainfall annually). During the 2017/2018 season’s trials, trees planted in autumn (23 May 2017) were transported from Witzenberg Range Nursery on 23 May 2017 to Glen Elgin Farm, while those planted in spring were transported from Witzenberg Range Nursery on 24 July 2017 (Fig. 2). Spring planted trees were stored in commercial fruit cold stores set at 6 °C, from 1 August 2017 to 12 September 2017 and then planted on 12 September 2017. A 1.5% hydrogen cyanamide [3% Dormex®, Philagro (Pty) Ltd, Somerset West, South Africa] rest breaking treatment was applied to trees planted in autumn on 1 September 2017 and to trees planted in spring on 16 September 2017. The distal ±2 cm of the central leader was removed with a heading cut on 21 September 2017. After the bud break period, all pre-existing sylleptic lateral shoots were cut back to ±5 cm from the trunk as is the commercial practice in this region.

The trees for the trial conducted in the 2017/2018 season was transported from the Witzenberg Range Nursery to Glen Elgin on 1 August 2018, placed in cold storage from 1 August 2018 to 3 September 2018 and planted in a new orchard on Glen Elgin Farm, as described before, on 3 September 2018. Before transport trees received ca. 390 chilling units in Simondium, and an additional ca. 780 chilling units (DPCU) during cold storage. Therefore, accumulating a total of ca. 1170 chilling units (DPCU).

Growth inhibition, hardening-off and time of planting trial - 2016/2017 season. A split-plot design was used to evaluate the effect of growth inhibition and hardening-off of trees in the nursery as well as the effect of different planting times (autumn versus spring) in a commercial orchard. Growth inhibition was induced by ProCa (125 mg·L^{-1}) [Regalis®, BASF, Ludwigshafen, Germany] while abscisic acid (ABA) (500 µl·L^{-1}) [Protone SL™, Valent BioSciences Corporation, Long Grove, IL, USA] was applied to induce hardening-off. ProCa and ABA were applied as described in Table 1. Time of planting was the main factor with plant growth regulator (PGR) treatment the sub-plot factor.

Nitrogen nutrition trial - 2016/2017 season. Foliar nitrogen (N) (13.8 g·L^{-1}) [46% Low-Biuret Urea, Nulandis (Pty) Ltd, Kempton Park, South Africa] was applied to increase the tree nitrogen level. Foliar nitrogen was applied four weeks (1 June 2017), six weeks (14 June 2017) or both four- and six weeks after shoot growth cessation.

Defoliation trial - 2016/2017 season. The effect of different timings of chemical– and mechanical defoliation was evaluated. Foliar copper (Cu) (1.5 g·L^{-1}) [ethylenediaminetetraacetic acid copper salt (CuEDTA), LGAGRO International Holdings Limited, Beijing, China] and 1-aminocyclopropane-1-carboxylic acid (ACC) (1000 µl·L^{-1}) [Valent BioSciences Corporation, Long Grove, IL, USA] were evaluated. Treatments are summarised in Table 2. Mechanical defoliation entailed careful hand removal of all leaves, without damaging axillary buds on the dates as indicated in Table 2.

Nitrogen nutrition and defoliation trial - 2016/2017 season. The effect of foliar Cu with a follow-up application of foliar nitrogen was evaluated as summarised in Table 3.

Inhibition of reproductive bud formation – 2017/2018 season. The effect of foliar gibberellin (GA) application on the number of lateral reproductive buds was evaluated. Foliar GA_3 (100 and 250 mg·L^{-1}) and GA_{4+7} (250 mg·L^{-1}) [ProGibb® 40% and Regulex®, respectively, Valent BioSciences Corporation, Long Grove, IL, USA] were applied with a manual hand pressure sprayer. Ten treatments were used as summarized in Table 4.

Data collection for all trials in the nursery. During the 2016/2017 season, the following data were recorded before the onset of each trial: (1) trunk diameter 5 cm above the graft union with an electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan) and (2) tree height from the surface of the growing medium to the terminal growing point or bud. From March 2017 until May 2017, the onset of growth cessation was monitored by measuring apical extension growth. In addition, defoliation was monitored for all trials from 11 April 2017 until 17 July 2017. To determine the rate of defoliation, the number of leaves per tree was counted weekly. In addition, the following data were recorded during the 2017/2018 season: (1) trunk diameter

(9 February 2018) 5 cm above the graft union as described before and (2) initial (9 February 2018) and final (22 June 2018) height of each tree.

Data collection for all trials following planting in the orchard - 2017/2018 season. The following data were collected after the trees were planted in a commercial orchard: (1) initial (29 September 2017) and final (25 May 2018) trunk diameter 5 cm above graft union as described before, (2) initial (29 September 2017) and final (25 May 2018) height of each tree, (3) percentage and rate of total bud break, (4) percentage and rate of vegetative bud break, (5) percentage reproductive buds (6) total new growth of lateral shoots.

The percentage and rate of total bud break was determined as follow: The number of buds (vegetative and reproductive) were counted over 34 days from the “mouse ear stage” or Biologische Bundesanstalt, Bundessortenamt and CHemical industry (BBCH) stage 10 (Meier, 2001) from 5 October 2017 to 8 November 2017. On 8 November the number of dormant buds, were counted. From this a curve of the total bud break percentage was constructed. The number of days until 40% bud break was determined from the graph. The percentage total bud break in the tree was calculated as follow:

$$\left(\frac{\text{Total number of sprouted buds}_{\text{ At a specific date}}}{\text{Total number of buds per tree}} \times 100\right)$$

The percentage and rate of vegetative bud break was determined as described above for total bud break. In addition, trees were divided into two sections upward from the graft union (lower two-thirds and top third). On 8 November, the number of dormant buds, in the two different sections was counted. During the bud break period, the number of reproductive buds on each tree was also counted to calculate the percentage reproductive buds that sprouted.

After the growing season, on 25 May 2018, the number and length of new one-year-old lateral shoots were counted and measured in the two sections of the tree (described above). In addition the total new growth of these shoots were calculated. One-year-old shoots were divided into two categories, viz. short (0-15 cm) and long shoots (15 cm +).

Data collection following planting in the orchard – 2018/2019 season. The number of reproductive, vegetative and dormant buds was counted on the tree on 29 October 2018, and bud break percentages as well as percentage reproductive buds calculated as described before.

Statistical analysis. The data were analysed using SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, North Carolina, USA) using a linear model procedure and the pairwise t-test to determine Least Significant Difference (LSD) when the F-statistic indicated significance at $P<0.05$. Where applicable, an analysis of covariance (ANCOVA) was used to determine Least Significant Difference (LSD) when the F-statistic indicated significance at $P<0.05$.

Results and Discussion

Nursery phase

Growth inhibition and hardening-off trial. The primary aim of this trial was to induce growth cessation and hardening-off of the trees in autumn in order to improve bud break and tree growth in the orchard after planting. ProCa on its own or in combination with ABA did not significantly induced growth cessation compared to the untreated control trees (Table 5). The first application of ProCa and ABA was applied at 11 April and an earlier application, when vegetative growth was more active, could have been more effective to induce growth cessation. However, earlier growth cessation could reduce the length of the nursery trees, which in itself could lead to an increase in the diameter of the trees which is a parameter used to grade nursery trees and seen as a positive quality characteristic (Guak and Fuchigami, 2001).

The rate and number of applications of ABA and ProCa used in our study could also explain the lack of response. In previous studies, multiple applications of up to 800 $mg \cdot L^{-1}$ ProCa on citrus nursery trees (Le Roux and Barry, 2010) and multiple applications of 1000 $mg \cdot L^{-1}$ ABA on apple nursery trees (Guak and Fuchigami, 2001) were used to induce growth cessation and/or hardening. Furthermore, multiple instead of single applications of ProCa showed the most promising results on commercial bearing apple (Medjdoub et al., 2005) and pear trees (Smit et al., 2005).

In addition, the trees in our study were growing under favourable conditions under shade netting. In the study by Guak and Fuchigami (2001) the trees were grown in a lath house and placed in direct sunlight before the onset of the trial. Raveh et al. (2003) evaluated the effect of shading on two-year-old potted citrus trees. Shading was induced by using woven aluminized plastic nets. They evaluated the effect of horizontal- and tunnel shading. Horizontal shading entailed a 60% shade net, hung horizontally above the rows, while tunnel shade was induced by hanging a 30% or 60% shade net, above and on the side of the rows. Both shading methods increased the plant biomass and vegetative growth and were associated with an increase in leaf conductance and a higher CO_2 assimilation rate. Therefore, by propagating and treating our trial trees under a shade, which was conducive for vegetative growth, the rate of ABA or ProCa should have been higher in order to induce growth cessation.

A possible secondary effect of an ABA treatment could be earlier defoliation, but ABA did not significantly enhance defoliation by 16 May 2017 compared to the untreated control (Table 5). A wide range of pear and apple nursery trees were successfully defoliated (>80% defoliation) with single (2000 $mg \cdot L^{-1}$) or multiple autumn applications of 1000 $mg \cdot L^{-1}$ ABA (Larsen and Higgins, 1998) or two applications of 1000 $mg \cdot L^{-1}$ ABA (Guak and Fuchigami, 2002). Hence, in our trial the inability of ABA to successfully defoliate nursery trees can be ascribed to the concentration used as well as the timing of the application. In this specific trail, ABA was applied to primarily evaluate growth cessation and hardening-off. Hence, the timing of application was earlier than conventional applications that induce leaf defoliation. Leaves are more sensitive to chemical defoliants later in the season (Jones et al., 1973) and the ABA was potentially applied at a stage where the abscission zone was not sensitive to ABA.

Defoliation trial. Foliar Cu and ACC application induced defoliation at a faster rate (data not presented) and removed significantly more leaves from the nursery trees compared to the untreated control (Table 6). Both a single application at the end of June and multiple applications of foliar Cu removed 100% of the leaves, whereas 91% of the leaves were removed with a single application beginning of June. ACC, compared to Cu, was less effective in defoliation but still induced significant leaf-drop compared to the untreated control. Application of both defoliants at the end of June induced a significantly higher percentage defoliation compared to the applications at the beginning of June. Jones et al. (1973) also established that applications of a chemical defoliant later in the season were more successful compared to earlier applications. However, multiple applications (1 June 2017 and 29 June 2017) did not remove significantly more leaves compared to a single application at the end of June. In other words, nurserymen can induce defoliation on containerised nursery trees with half the cost by a single application of a chemical defoliant later in the season. However, a late application will not be feasible in conventional production, because trees needs to be lifted before the onset of winter rains which make the site impassable for heavy machinery.

ACC is a naturally occurring compound and a precursor of ethylene (Greene and Costa, 2013). ACC is oxidised enzymatically, by ACC oxidase, to produce ethylene, CO_2 and cyanide

(Wang et al., 2002). Once converted to ethylene, it accelerates leaf abscission when the cells in the abscission layer of the leaves become sensitive to ethylene (Brown, 1997; McArtney and Obermiller, 2012). This process can occur over a longer time, compared to ethylene production induced by CuEDTA. Cu is a heavy metal and high concentrations cause the breakdown of chlorophyll and carotenoids and increase membrane permeability and lipid peroxidation (Luna et al., 1994). This contributes to rapid senescence of leaves. Cu in a chelated form with ethylenediaminetetraacetic acid (EDTA) also induces defoliation through direct production of ethylene (Cooper et al., 1968). In addition, CuEDTA produces three times more ethylene compared to FeEDTA (Cooper et al., 1968). These differences in modes of action between ACC and CuEDTA, could explain the higher efficacy of CuEDTA compared to ACC.

ACC at the beginning of June was the only treatment that defoliated the trees by less than 80% (Table 6). Previously, Jones et al. (1973) proposed that for a chemical defoliant to be effective, the number of leaves on the tree needs to be reduced by 80% three weeks after application. Hence, an application of ACC at the beginning of June cannot be classified as an effective chemical defoliant treatment. CuEDTA has been shown to be an effective chemical defoliant on apple nursery trees (Guak et al., 2001), almond nursery trees (Bi et al., 2005) and on one-year-old apple trees (Dong et al., 2004).

Nitrogen nutrition and defoliation trial. The reason for conducting this study was to increase nitrogen levels in the foliage before applying a chemical defoliant. Gauk et al. (2001) proposed that a chemical defoliant, CuEDTA, causes rapid defoliation to an extent that nitrogen translocation is impeded. Due to the rapid rate of defoliation after consecutive foliar Cu and nitrogen application in our trial, the rate of defoliation could not be monitored. Forty-eight hours after applying these two chemicals separately, trees were completely defoliated (data not shown). Previous research established that two applications of 3% urea, with a follow-up application of 0.5% CuEDTA caused more defoliation than CUEDTA alone on almond nursery trees (Bi et al., 2005). The authors speculated that this effect can be ascribed to urea being a surfactant and enhanced the uptake of the chemical defoliant. It was also established by Khemira et al. (2000) that foliar applied urea has the ability to cause leaf damage as well as chlorosis. We applied foliar Cu and urea on the same day, and this can explain the rapid defoliation within 48 hours.

If nurserymen want to combine a chemical defoliant with a foliar nitrogen application to increase the nitrogen reserves, it would be recommended to evaluate the effect of combining foliar nitrogen with ACC, a less potent chemical defoliant, which will potentially allow translocation of nitrogen before defoliation of trees take place. In addition, a lower concentration of Cu in combination with urea can be evaluated. The effect of a temporal split, of a few days, between the urea and Cu application can be evaluated.

Spring bud break and new growth in the orchard

Growth inhibition, hardening-off and planting time trial. ProCa and ABA had no significant effect on the percentage total bud break, the percentage vegetative bud break or the percentage reproductive buds (Table 7). The total percentage bud break for all the treatments was above 80% which left little room for improvement. Neither was new growth, i.e. increase in trunk diameter, apical extension growth (Table 8) or number and length of lateral growth (Table 9 and 10), influenced by the ProCa or ABA nursery treatments. Due to the lack of significant effect of ProCa and ABA on growth cessation in the nursery, it is not surprising that there was no significant difference regarding the percentage bud break or new growth in the orchard.

Our hypothesis was that autumn planting would allow root development in the mild Elgin winter therefore resulting in a better developed, larger root system compared to spring planted trees and therefore enhanced bud break in spring. Van Zyl (2016) established that there is a peak in root growth during late autumn and winter in South Africa. However, the spring planted trees had a higher percentage total bud break, as well as a higher percentage vegetative bud break in the top third and lower two-thirds (Table 7). This can be explained by the difference in the amount of winter chilling for the different planting times. Autumn-planted trees received approximately 25 chilling units (DPCU) from 1 May 2017 at Witzenberg Range Nursery before being transported to Elgin, where they received approximately 856 chilling units from June 2017 to August 2017. Therefore, accumulating a total of ca. 880 (DPCU). In contrast, spring planted trees received approximately 517 CU from May 2017 to July 2017 at Witzenberg Range Nursery before being transported to Elgin. In addition, these trees were stored at a set temperature of 6 °C for six weeks accumulating approximately ca. 1000 chilling units (DPCU) during this period. Therefore, accumulating a total of ca. 1500 chilling units (DPCU), which could account for the difference in bud-break.

However, bud swell occurred approximately two weeks earlier on trees that were planted in autumn, compared to trees planted in spring. Due to this, the timing of the rest breaking treatment for the autumn planted trees (1 September 2017) was earlier compared to the trees planted in spring (16 September 2017). This contributed to earlier bud break for the autumn-planted trees. After endodormancy is completed, environmental factors are responsible for the ecodormant buds to break. Environmental factors such as low temperatures and drought stress inhibit the sprouting of dormant buds (Lang et al., 1987; Louw et al., 2016). Up to the point where trees were planted in spring, autumn-planted trees were exposed to higher temperatures during the day in the orchard compared to the spring planted trees in cold storage (6°C). Hence, this warm temperatures and the fact that the rest breaking treatment was applied two weeks earlier, can be the reasons why trees planted in autumn achieved earlier bud break.

The different planting times did not significantly influence the new growth, i.e. the apical extension growth (Table 8) and number of lateral shoots and length of lateral growth (Table 9, 10), but trees planted in autumn had a significantly greater increase in trunk diameter (Table 8). In a Mediterranean-type climate, continuous root growth takes place from flowering until late autumn on young apple trees (Cripps, 1970). In addition as mentioned earlier, root growth during winter occurs in South Africa (van Zyl, 2016). Autumn-planted trees could have developed a larger root system, which aids in the uptake of water and nutrients that could have contributed to this significant increase in diameter. In addition, the two planting times had an influence on the tree architecture. Trees planted in autumn produced significantly more new growth in the lower two-thirds of the tree (Table 9). The opposite is true in the top third of the trees where spring planted trees produced significantly more new growth (Table 9). This difference in architecture can be ascribed to the difference in winter chilling between the two planting times as described previously. The percentage reproductive buds were influenced by the different planting times. Autumn-planted trees had a significantly higher percentage reproductive buds (Table 7). Planting in autumn could have induced a stress response in the trees resulting in an increase in ethylene levels and a decrease in polar auxin transport, while cytokinin levels increased and thus the percentage of floral buds (Sanyal and Bangerth, 1998).

Nitrogen nutrition trial. Single or multiple applications of foliar nitrogen in the nursery had no significant effect on the percentage and rate of total bud break, the percentage- and rate of vegetative bud break as well as the percentage reproductive buds (Table 11). The percentage total bud break for all the treatments was more than 90%, which left little room for improvement. In addition new growth, i.e. increase in trunk diameter, apical extension growth (Table 12) or number of lateral shoots and length of lateral growth (Table 13, 14), was not influenced. One would have expected that applications of foliar nitrogen in autumn during the

2016/2017 season would have increased the nitrogen levels in the leaves and subsequently the nitrogen reserves in storage organs. These nitrogen reserves should then have contributed to regrowth in spring, because reserve nitrogen is the main contributing factor for spring regrowth (Cheng and Fuchigami, 2002). Minimal root damage occurred during handling and planting, so the lack of significance from the nitrogen application cannot be attributed to physical damage of the roots. We sampled leaves after N application in the nursery, but did not analyse the nitrogen levels in the trees, as we did not find any differences in tree performance. In retrospect this can be seen as wrong, as we might have found that the N levels did not differ between treatments therefore explaining the lack of response in the orchard. Nitrogen analysis is however very expensive and we believe that the overriding reason for the lack of response is due to the orchard management practices.

Defoliation trial. Defoliation techniques in the nursery, mechanical and chemical, had no significant effect on the total bud break percentage, vegetative bud break percentage (Table 15) as well as the new growth, i.e. increase in trunk diameter, apical extension growth (Table 16) or number and length of lateral growth (Table 17, 18). However, there was a trend ($p = 0.0735$) that late mechanical defoliation as well as late applications of CUEDTA improved the total new growth in the top third of the trees (Table 17). CuEDTA, as a chemical defoliant, has been shown to decrease growth of apple trees after planting due to the rapid rate of defoliation and insufficient translocation of nitrogen from the leaves (Guak et al., 2001). However, this was not visible in our trial. Early mechanical defoliation as well as early and multiple applications of CuEDTA increased the rate (as seen in days to 40% bud break) of total bud break as well as the rate of vegetative bud break in the top third and the lower two-thirds (Table 15). Jones et al. (1973) found that treatments that caused earlier leaf defoliation promoted earlier bud break in spring on one-year-old peach seedlings. However, Fuchigami (1977) found the opposite on red-osier dogwood where earlier leaf defoliation treatments delayed spring bud break. Early or late defoliation is however, a relative statement and it is difficult to compare between these trials.

Manual, as well as chemical defoliation did not significantly decrease the percentage reproductive buds on the tree compared to the untreated control. Single applications (early and late) of foliar Cu reduced the percentage reproductive buds compared to ACC treatments on the same date. However, multiple applications of foliar Cu did not significantly decrease the percentage reproductive buds compared to any of the ACC treatments (Table 15). The reason why multiple applications of Cu did not differ significantly from any ACC treatment is unclear.

Previously Bennet et al. (2005) suggested that subsequent flowering in grapevines was reduced due to restricted carbohydrate accumulation as a consequence of defoliation.

Nitrogen nutrition and defoliation trial. The combination of nitrogen with a chemical defoliant had no significant effect on the percentage and rate of total bud break, the percentage- and rate of vegetative bud break as well as the percentage reproductive buds (Table 19). In addition new growth, i.e. increase in trunk diameter, apical extension growth (Table 20) or number and length of lateral growth (Table 21, 22), were not influenced by combinations of foliar copper and nitrogen treatments in the nursery. Due to rapid defoliation in the nursery, one would have expected that the regrowth potential would be reduced due to the insufficient translocation of nitrogen from the leaves to the storage organs. From the results it can be concluded that this vigorous defoliation had no significant negative influence regarding growth during the first growing season.

Inhibition of reproductive buds trial. The application of exogenous gibberellin had no significant effect on the percentage reproductive buds during the 2017/2018 season (Table 23). It is well known that gibberellin has the ability to inhibit the initiation of flower buds. Gibberellins such as $GA_{3,}$ GA_4 and GA_7 are used to suppress flowering in apple trees (Unrath and Whitworth, 1991). To date there are limited studies that focus on the inhibition of flower buds in nursery trees (Lordan et al., 2017). Lordan et al, (2017) found that multiple applications of gibberellins during the nursery phase reduced the number of reproductive buds in the following season when ‘Gala’ and ‘Cripps Pink’ were planted in three different orchards. The effect on ‘Gala’ trees was temperature dependant. Multiple (two to four) applications of GA_3 (100 $mg \cdot L^{-1}$ and 250 $mg \cdot L^{-1}$) as well as three applications of GA_{4+7} (250 $mg \cdot L^{-1}$) reduced the number of reproductive buds on ‘Gala’ trees planted in a colder region, whereas only four applications of GA_3 (250 $mg \cdot L^{-1}$) and three applications of GA_{4+7} (250 $mg \cdot L^{-1}$) reduced the number of reproductive buds on ‘Gala’ trees planted in a warmer region. This difference was ascribed to damage, caused by low temperatures, to the reproductive buds. In addition, four applications of GA_3 (100 $mg \cdot L^{-1}$ and 250 $mg \cdot L^{-1}$) and three applications of GA_{4+7} (250 $mg \cdot L^{-1}$) reduced the number of reproductive buds on ‘Cripps Pink’ trees. This effect was not visible in our trial during the 2017/2018 season.

Conclusion

No clear conclusion could be made on the use of PGRs to harden-off trees or chemical defoliants in our trials. Trees planted in autumn in a warm region did not accumulate sufficient chilling that resulted in reduced bud break during spring. In addition, a basal dominant tree architecture was visible for autumn planted trees due to a lack of chilling during winter. Because containerised apple nursery trees are a new concept in South Africa, further research needs to be conducted to evaluate and determine management strategies for containerised nursery trees. As trees were probably not managed ideally in the orchard, the treatment effects could have been masked by a lack of tree vigour during the first growing season in the orchard. Hence, this could have been the overriding factor to the lack of treatment responses. Therefore, research is also needed to determine management strategies in the orchard when planting containerised, instead of bare rooted nursery trees.

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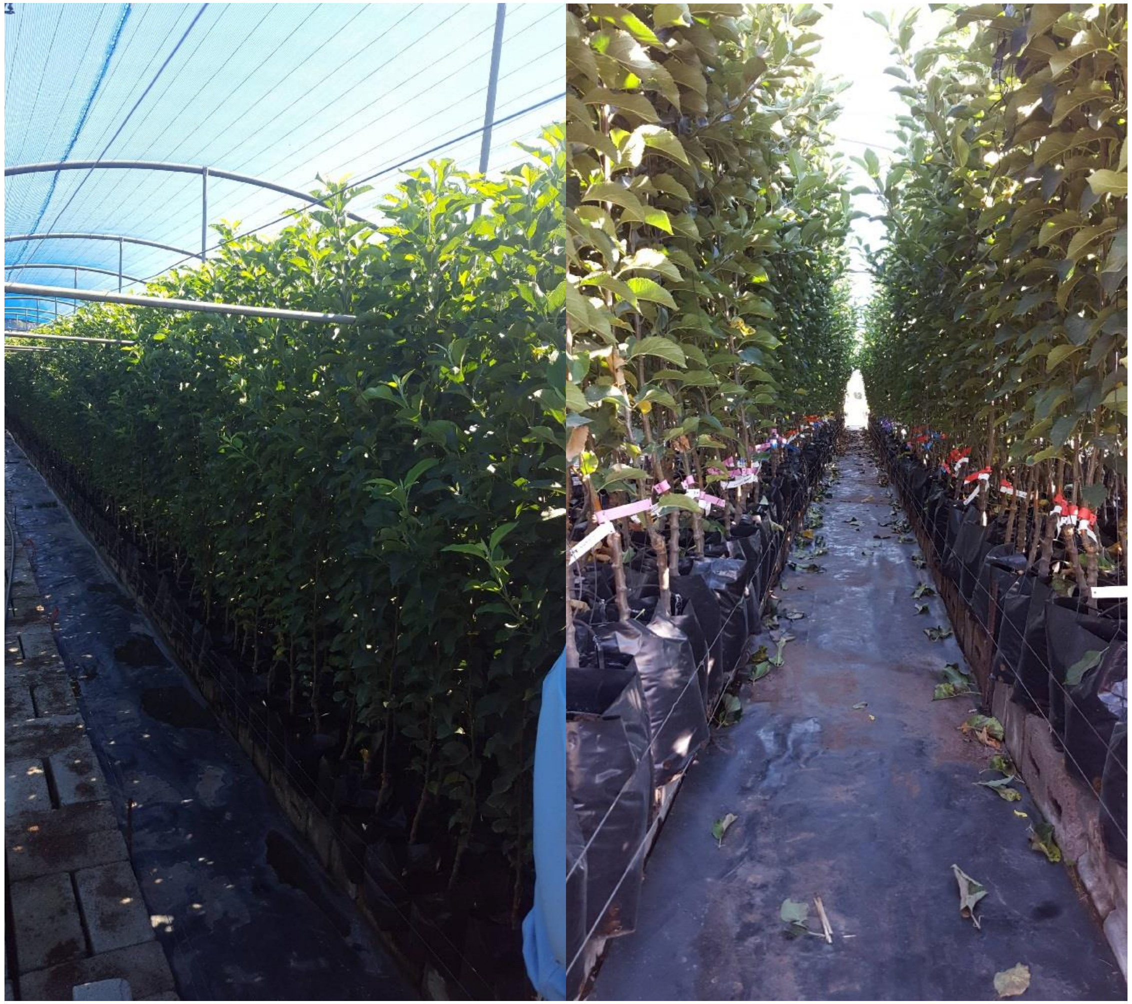

Fig 1. One-year-old containerised ‘Golden Delicious’/M.9 nursery trees at Witzenberg Range Nursery, Simondium, South Africa (2016/2017).

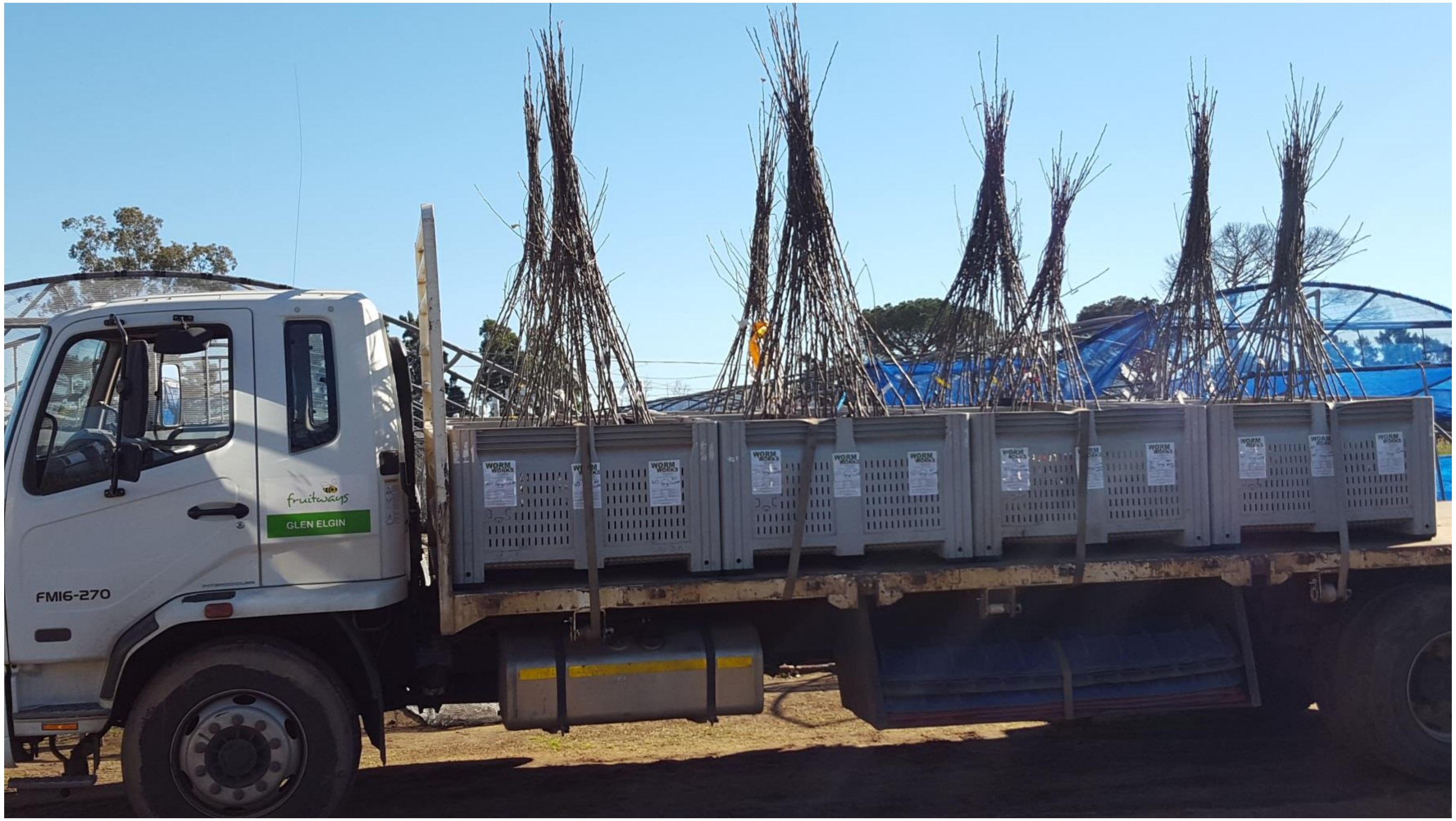

Fig. 2 Containerised ‘Golden Delicious’/M.9 trees transported from Witzenberg Range Nursery to Glen Elgin Farm on 24 July 2017.

Table 1. Treatment specifications and application dates of treatments for trials done with prohexadione calcium (ProCa) and abscisic acid (ABA) on containerised 'Golden Delicious'/M.9 nursery trees in the season of 2016/2017.

Plant growth regulator	Application date (ProCa)	Application date (ABA)
Untreated control		
ProCa (125 mg·L^{-1})	11 Apr. 2017	
ProCa (125 mg·L^{-1}) + ABA (500 µl·L^{-1}) early	11 Apr. 2017	11 Apr. 2017
ProCa (125 mg·L^{-1}) + ABA (500 µl·L^{-1}) late	11 Apr. 2017	2 May 2017
ProCa (125 mg·L^{-1}) + ABA (500 µl·L^{-1}) twice	11 Apr. 2017	11 Apr. 2017 + 2 May 2017

Table 2. Treatment specifications and application dates of treatments for trials done with mechanical defoliation, foliar copper (CuEDTA) and 1-aminocyclopropane-1-carboxylic acid (ACC) on containerised 'Golden Delicious'/M.9 nursery trees in the season of 2016/2017.

Treatments	Application date
Untreated Control	
Mechanical defoliation	1 Jun. 2017
Mechanical defoliation	29 Jun. 2017
Copper (1.5 g·L^{-1})	1 Jun. 2017
Copper (1.5 g·L^{-1})	29 Jun. 2017
Copper (1.5 g·L^{-1})	1 Jun. 2017 + 29 Jun. 2017
ACC (1000 µl·L^{-1})	1 Jun. 2017
ACC (1000 µl·L^{-1})	29 Jun. 2017
ACC (1000 µl·L^{-1})	1 Jun. 2017 + 29 Jun. 2017

Table 3. Treatment specifications and application dates of treatments for trials done with foliar copper (CuEDTA) and foliar nitrogen (N; urea) on 'Golden Delicious'/M.9 nursery trees in the season of 2016/2017.

Treatments	Application date (Nitrogen)	Application date (CuEDTA)
Untreated Control		
CuEDTA (1.5 g·L^{-1})		1 Jun. 2017
CuEDTA (1.5 g·L^{-1})		29 Jun. 2017
N + CuEDTA*	1 Jun. 2017	1 Jun. 2017
N + CuEDTA*	1 Jun. 2017	29 Jun. 2017
N + CuEDTA*	1 Jun. 2017 + 14 Jun. 2017	1 Jun. 2017 + 29 Jun. 2017

*N (13.8 g·L^{-1}) + CuEDTA (1.5 g·L^{-1})

Table 4. Treatment specifications and application dates of treatments for trials done with foliar gibberellin (GA_3 and GA_{4+7}) on containerised 'Bigbucks Gala'/M.9 nursery trees in the season of 2017/2018.

Plant growth regulator	Application date
Untreated control	
GA_3 (100 mg·L^{-1})	16 Feb. and 2 Mar. 2018
GA_3 (100 mg·L^{-1})	16 Feb., 2 and 16 Mar. 2018
GA_3 (100 mg·L^{-1})	16 Feb., 2, 16 and 28 Mar. 2018
GA_3 (250 mg·L^{-1})	16 Feb. and 2 Mar. 2018
GA_3 (250 mg·L^{-1})	16 Feb., 2 and 16 Mar. 2018
GA_3 (250 mg·L^{-1})	16 Feb., 2, 16 and 28 Mar. 2018
GA_{4+7} (250 mg·L^{-1})	16 Feb. and 2 Mar. 2018
GA_{4+7} (250 mg·L^{-1})	16 Feb., 2 and 16 Mar. 2018
GA_{4+7} (250 mg·L^{-1})	16 Feb., 2, 16 and 28 Mar. 2018

Table 5. Effect of prohexadione calcium (ProCa) and abscisic acid (ABA) on apical extension growth and defoliation of containerised 'Golden Delicious'/M.9 nursery trees at Witzenberg Range Nursery, Simondium, South Africa (2016/2017).

Plant growth regulator	Apical extension growth (cm)	% Defoliation
Untreated control	2.5 ns	6.8 ns
ProCa[a]	1.9	12.3
ProCa + ABA*[b]	1.5	6.5
ProCa + ABA*[c]	2.0	9.6
ProCa + ABA*[d]	1.8	12.4
Significance level		
Treatment	*0.5071*	*0.0794*
LSD 5%	-	-
Initial height (covariant)	*0.4230*	-

* ProCa (125 mg·L^{-1})

** ProCa (125 mg·L^{-1}), ABA (500 µl·L^{-1})

[a] ProCa applied: 11 Apr. 2017

[b] ProCa applied: 11 Apr. 2017, ABA applied: 11 Apr. 2017

[c] ProCa applied: 11 Apr. 2017, ABA applied: 2 May 2017

[d] ProCa applied: 11 Apr. 2017, ABA applied: 11 Apr. 2017 + 2 May 2017

Table 6. Effect of foliar copper (CuEDTA) and 1-aminocyclopropane-1-carboxylic acid (ACC) on the rate of defoliation of containerised 'Golden Delicious'/M.9 nursery trees at Witzenberg Range Nursery, Simondium, South Africa (2016/2017).

Treatment	Application date	% Defoliation	
Untreated Control	-	53.4	e
Copper (1.5 $g \cdot L^{-1}$)	1 Jun. 2017	91.2	b
Copper (1.5 $g \cdot L^{-1}$)	29 Jun. 2017	100.0	a
Copper (1.5 $g \cdot L^{-1}$)	1 + 29 Jun. 2017	100.0	a
ACC (1000 $\mu l \cdot L^{-1}$)	1 Jun. 2017	68.8	d
ACC (1000 $\mu l \cdot L^{-1}$)	29 Jun. 2017	82.6	c
ACC (1000 $\mu l \cdot L^{-1}$)	1 + 29 Jun. 2017	85.5	bc
Significance level			
Treatment		*<0.0001*	
LSD 5%		*6.7*	

Table 7. Effect of prohexadione calcium (ProCa) and abscisic acid (ABA), with different planting times, on spring bud break of containerised 'Golden Delicious'/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree				Top third		Lower two-thirds	
Plant growth regulator	Final % total bud break		% Reproductive buds		Final % vegetative bud break		Final % vegetative bud break	
Untreated control	87.8	ns	16.9	ns	86.0	ns	85.7	ns
ProCa*[a]	86.7		18.5		85.8		84.8	
ProCa + ABA**[b]	83.6		20.9		78.2		82.8	
ProCa + ABA**[c]	82.8		14.5		86.4		77.7	
ProCa + ABA**[d]	85.4		17.1		86.1		85.9	
<u>Time of planting:</u>								
Autumn	81.1	b	19.7	a	74.8	b	81.0	b
Spring	89.4	a	15.5	b	94.2	a	85.7	a
Significance level								
Treatment	0.3894		*0.1785*		*0.1870*		*0.2079*	
LSD 5%	-		-		-		-	
Time of planting	<0.0001		*0.0140*		*<0.0001*		*0.0084*	
*Time of planting*treatment*	0.0749		*0.4339*		*0.3364*		*0.1527*	

* ProCa (125 mg·L^{-1})
** ProCa (125 mg·L^{-1}), ABA (500 µl·L^{-1})
[a] ProCa applied: 11 Apr. 2017
[b] ProCa applied: 11 Apr. 2017, ABA applied: 11 Apr. 2017
[c] ProCa applied: 11 Apr. 2017, ABA applied: 2 May 2017
[d] ProCa applied: 11 Apr. 2017, ABA applied: 11 Apr. 2017 + 2 May 2017

Table 8. Effect of prohexadione calcium (ProCa) and abscisic acid (ABA), with different planting times, on the increase in diameter and apical extension growth of containerised 'Golden Delicious'/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

Plant growth regulator	Increase in diameter (mm)	Apical extension growth (cm)
Untreated control	6.4 ns	34.9 ns
ProCa*[a]	6.5	36.2
ProCa + ABA**[b]	5.1	34.3
ProCa + ABA**[c]	6.9	43.1
ProCa + ABA**[d]	6.4	40.2
Time of planting:		
Autumn	6.7 a	37.6 ns
Spring	5.8 b	38.0
Significance level		
Treatment	*0.1237*	*0.1702*
LSD 5%	-	-
Time of planting	*0.0090*	*0.7316*
*Time of planting*treatment*	*0.2592*	*0.8556*
Initial diameter and leader length (covariant)	*0.1141*	*0.4364*

* ProCa (125 mg·L^{-1})
** ProCa (125 mg·L^{-1}), ABA (500 µl·L^{-1})
[a] ProCa applied: 11 Apr. 2017
[b] ProCa applied: 11 Apr. 2017, ABA applied: 11 Apr. 2017
[c] ProCa applied: 11 Apr. 2017, ABA applied: 2 May 2017
[d] ProCa applied: 11 Apr. 2017, ABA applied: 11 Apr. 2017 + 2 May 2017

Table 9. Effect of prohexadione calcium (ProCa) and abscisic acid (ABA), with different planting times, on the new growth of one-year-old lateral shoots in one season of containerised 'Golden Delicious'/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree			Top third			Lower two-thirds		
Plant growth regulator	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)
Untreated control	264.1 ns	16.5 ns	16.5 ns	83.1 ns	5.9 ns	13.6 ns	181.0 ns	10.8 ns	17.8 ns
ProCa*[a]	236.4	15.5	16.1	70.9	6.2	11.3	165.5	9.3	19.1
ProCa + ABA**[b]	201.6	15.1	14.3	63.8	5.5	11.9	137.8	9.7	15.4
ProCa + ABA**[c]	232.0	15.7	16.9	71.0	6.4	11.5	161.1	9.3	18.9
ProCa + ABA**[d]	229.1	16.9	14.2	81.2	7.2	11.6	147.9	9.8	14.9
<u>Time of planting:</u>									
Autumn	247.7 ns	13.3 b	19.1 a	62.0 b	4.7 b	13.2 ns	185.8 a	8.5 b	22.3 a
Spring	217.0	18.6 a	12.1 b	85.6 a	7.7 a	10.8	131.3 b	10.9 a	12.2 b
Significance level									
Treatment	*0.0930*	*0.6754*	*0.1918*	*0.6123*	*0.7228*	*0.5318*	*0.2997*	*0.7393*	*0.0540*
LSD 5%	-	-	-	-	-	-	-	-	-
Time of planting	*0.0646*	*<0.0001*	*<0.0001*	*0.0077*	*<0.001*	*0.0779*	*<0.0001*	*0.0006*	*<0.0001*
*Time of planting*treatment*	*0.4893*	*0.3149*	*0.9585*	*0.7563*	*0.6093*	*0.4207*	*0.1495*	*0.0618*	*0.8851*

* ProCa (125 mg·L^{-1}); ** ProCa (125 mg·L^{-1}), ABA (500 µl·L^{-1})

[a] ProCa applied: 11 Apr. 2017

[b] ProCa applied: 11 Apr. 2017, ABA applied: 11 Apr. 2017

[c] ProCa applied: 11 Apr. 2017, ABA applied: 2 May 2017

[d] ProCa applied: 11 Apr. 2017, ABA applied: 11 Apr. 2017 + 2 May 2017

Table 10. Effect of prohexadione calcium (ProCa) and abscisic acid (ABA), with different planting times, on the number of short - and long shoots of containerised 'Golden Delicious'/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree		Top third		Lower two-thirds	
Plant growth regulator	Number of short shoots	Number of long shoots	Number of short shoots	Number of long shoots	Number of short shoots	Number of long shoots
Untreated control	9.4 ns	7.1 ns	3.4 ns	2.5 ns	6.1 ns	4.5 ns
ProCa*[a]	9.3	6.2	4.4	1.8	4.9	4.4
ProCa + ABA**[b]	10.3	4.8	4.0	1.5	6.4	3.3
ProCa + ABA**[c]	10.4	5.5	4.8	2.1	5.6	3.5
ProCa + ABA**[d]	11.5	5.5	5.5	1.7	5.9	3.8
<u>Time of planting:</u>						
Autumn	6.6 b	6.7 a	3.0 b	1.8 ns	*3.6* b	4.9 a
Spring	13.7 a	4.9 b	5.8 a	2.1	*7.9* a	2.9 b
Significance level						
Treatment	*0.6898*	*0.0783*	*0.3169*	*0.3912*	*0.6778*	*0.1709*
LSD 5%	-	-	-	-	-	-
Time of planting	*<0.0001*	*0.0149*	*<0.0001*	*0.2979*	*<0.0001*	*0.0002*
*Time of planting*treatment*	*0.6832*	*0.6262*	*0.5860*	*0.8439*	*0.2425*	*0.4463*

* ProCa (125 mg·L^{-1})
** ProCa (125 mg·L^{-1}), ABA (500 µl·L^{-1})
[a] ProCa applied: 11 Apr. 2017
[b] ProCa applied: 11 Apr. 2017, ABA applied 11 Apr. 2017
[c] ProCa applied: 11 Apr. 2017, ABA applied 2 May 2017
[d] ProCa applied: 11 Apr. 2017, ABA applied 11 Apr. 2017 + 2 May 2017

Table 11. Effect of foliar nitrogen (N; urea) application on spring bud break of containerised ‘Golden Delicious’/M.9 apple trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree			Top third		Lower two-thirds	
Treatment	Final % total bud break	% Reproductive buds	Number of days to 40% bud break	Final % vegetative bud break	Number of days to 40% vegetative bud break	Final % vegetative bud break	Number of days to 40% vegetative bud break
Untreated control	93.3 ns	12.8 ns	13.9 ns	92.2 ns	12.1 ns	91.9 ns	13.2 ns
Nitrogen*[a]	93.6	9.0	12.4	94.8	10.2	91.5	13.3
Nitrogen*[b]	91.0	12.0	12.3	94.4	10.7	87.4	12.8
Nitrogen*[c]	95.0	17.3	11.5	96.8	9.7	92.7	11.3
Significance level							
Treatment	0.4309	*0.2222*	*0.1221*	*0.5813*	*0.3658*	*0.3660*	*0.2157*
LSD 5%	-	-		-	-	-	-

* Nitrogen (13.8 g·L^{-1})

[a] Nitrogen application: 1 Jun. 2017

[b] Nitrogen application: 14 Jun. 2017

[c] Nitrogen application: 1 Jun. 2017 + 14 Jun. 2017

Table 12. Effect of foliar nitrogen (N; urea) on the increase in diameter and apical extension growth of containerised ‘Golden Delicious’/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

Treatment	Increase in diameter (mm)	Apical extension growth (cm)
Untreated control	6.2 ns	45.0 ns
Nitrogen*^{a}	7.1	47.1
Nitrogen*^{b}	6.6	44.9
Nitrogen*^{c}	6.1	44.7
Significance level		
Treatment	*0.7550*	*0.8823*
LSD 5%	-	-
Initial diameter and leader length (covariant)	*0.0191*	*0.9730*

* Nitrogen (13.8 g·L^{-1})

[a] Nitrogen application: 1 Jun. 2017

[b] Nitrogen application: 14 Jun. 2017

[c] Nitrogen application: 1 Jun. 2017 + 14 Jun. 2017

Table 13. Effect of foliar nitrogen (N; urea) on the new growth of one-year-old lateral shoots in one season of containerised 'Golden Delicious'/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree						Top third						Lower two-thirds					
Treatment	Total new growth (cm)		Number of lateral shoots		Average length of lateral shoot (cm)		Total new growth (cm)		Number of lateral shoots		Average length of lateral shoot (cm)		Total new growth (cm)		Number of lateral shoots		Average length of lateral shoot (cm)	
Untreated control	215.4	ns	14.7	ns	14.6	ns	57.7	ns	5.2	ns	11.1	ns	157.8	ns	9.4	ns	17.2	ns
Nitrogen*[a]	195.0		14.2		13.9		65.5		6.4		10.1		129.5		7.8		16.9	
Nitrogen*[b]	187.1		13.0		14.9		51.2		4.8		7.6		135.9		8.2		18.8	
Nitrogen*[c]	171.8		13.7		12.8		78.3		7.2		10.8		93.5		6.5		15.6	
Significance level																		
Treatment	*0.6254*		*0.8062*		*0.6682*		*0.4680*		*0.2744*		*0.2793*		*0.0622*		*0.1484*		*0.8236*	
LSD 5%	-		-		-		-		-		-		-		-		-	

* Nitrogen (13.8 g·L^{-1})

[a] Nitrogen application: 1 Jun. 2017

[b] Nitrogen application: 14 Jun. 2017

[c] Nitrogen application: 1 Jun. 2017 + 14 Jun. 2017

Table 14. Effect of foliar nitrogen (N; urea) on the number of short - and long shoots of containerised 'Golden Delicious'/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree		Top third		Lower two-thirds	
Treatment	Number of short shoots	Number of long shoots	Number of short shoots	Number of long shoots	Number of short shoots	Number of long shoots
Untreated control	10.3 ns	4.3 ns	4.4 ns	0.8 ns	5.9 ns	3.6 ns
Nitrogen*[a]	9.9	4.3	5.4	1.0	2.5	3.3
Nitrogen*[b]	8.6	4.4	3.8	1.0	4.8	3.4
Nitrogen*[c]	10.6	3.1	6.2	1.0	4.4	2.1
Significance level						
Treatment	*0.6268*	*0.5149*	*0.1644*	*0.9640*	*0.6231*	*0.0878*
LSD 5%	-	-	-	-	-	-

* Nitrogen (13.8 $g \cdot L^{-1}$)

[a] Nitrogen application: 1 Jun. 2017

[b] Nitrogen application: 14 Jun. 2017

[c] Nitrogen application: 1 Jun. 2017 + 14 Jun. 2017

Table 15. Effect of mechanical defoliation, foliar copper (CuEDTA) and 1-aminocyclopropane-1-carboxylic acid (ACC) application on spring bud break of containerised ‘Golden Delicious’/M.9 apple trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree			Top third		Lower two-thirds	
Treatment	Final % total bud break	% Reproductive buds	Number of days to 40% bud break	Final % vegetative bud break	Number of days to 40% vegetative bud break	Final % vegetative bud break	Number of days to 40% vegetative bud break
Untreated control	92.4 ns	14.2 abcd	13.4 a	100.0 ns	10.0 ab	83.4 ns	14.4 a
Mechanical[a]	92.0	19.2 ab	7.4 e	94.3	5.3 d	88.1	7.8 b
Mechanical[b]	89.7	12.3 bcd	10.8 bc	96.2	8.8 abc	83.0	13.1 a
Copper*[a]	92.4	9.1 cd	9.5 cd	96.3	8.3 bc	89.2	8.9 b
Copper*[b]	87.1	6.3 d	11.8 ab	96.2	11.3 a	80.8	13.0 a
Copper*[c]	90.6	19.4 ab	8.7 de	90.1	7.0 cd	90.2	7.5 b
ACC*[a]	90.3	22.0 a	11.8 ab	97.2	8.7 abc	82.5	12.1 a
ACC*[b]	91.6	16.4 abc	11.9 ab	95.7	9.6 abc	86.1	13.0 a
ACC*[c]	92.9	11.9 bcd	11.8 ab	95.5	9.0 abc	90.2	12.3 a
Significance level							
Treatment	0.8923	*0.0034*	*<0.0001*	*0.1386*	*0.0059*	*0.5157*	<0.0001
LSD 5%	-	*8.1*	2.0	-	2.9	-	2.7

* Copper (1.5 $g \cdot L^{-1}$), ACC (1000 $\mu l \cdot L^{-1}$)

[a] Applied 1 Jun. 2017

[b] Applied 29 Jun. 2017

[c] Applied 1 Jun. 2017 + 29 Jun. 2017

Table 16. Effect of mechanical defoliation, foliar copper (CuEDTA) and 1-aminocyclopropane-1-carboxylic acid (ACC) application on the increase in diameter and apical extension growth of containerised 'Golden Delicious'/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

Treatment	Increase in diameter (mm)	Apical extension growth (cm)
Untreated control	5.3 ns	50.0 ns
Mechanical[a]	4.9	42.7
Mechanical[b]	4.0	40.4
Copper*[a]	5.3	43.5
Copper*[b]	5.7	41.8
Copper*[c]	5.2	40.8
ACC*[a]	3.5	38.9
ACC*[b]	4.8	47.5
ACC*[c]	5.2	43.5
Significance level		
Treatment	*0.6349*	*0.5933*
LSD 5%	-	-
Initial diameter and leader length (covariant)	*0.0565*	*0.2908*

* Copper (1.5 $g \cdot L^{-1}$), ACC (1000 $\mu l \cdot L^{-1}$)

[a] Applied 1 Jun. 2017

[b] Applied 29 Jun. 2017

[c] Applied 1 Jun. 2017 + 29 Jun. 2017

Table 17. Effect of mechanical defoliation, foliar copper (CuEDTA) and 1-aminocyclopropane-1-carboxylic acid (ACC) application on the new growth of one-year-old lateral shoots in one season of containerised 'Golden Delicious'/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree			Top third			Lower two-thirds		
Treatment	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)
Untreated control	202.2 ns	14.8 ns	14.1 ns	91.2 ns	7.7 ns	11.9 ns	111.0 ns	7.1 ns	17.0 ns
Mechanical[a]	147.2	11.2	12.5	50.0	4.9	10.4	97.2	6.3	14.6
Mechanical[b]	192.9	13.6	14.4	106.3	7.9	14.1	86.6	5.8	15.5
Copper*[a]	191.2	14.1	13.9	83.3	6.2	14.1	107.9	7.9	15.2
Copper*[b]	226.4	16.1	14.2	116.1	8.6	14.0	110.3	7.6	14.3
Copper*[c]	192.6	13.9	14.0	86.9	6.5	14.1	105.7	7.4	15.0
ACC*[a]	177.9	14.6	12.0	70.2	5.6	13.0	107.7	9.0	11.6
ACC*[b]	220.8	15.2	15.2	81.5	5.7	14.3	139.3	9.5	16.1
ACC*[c]	205.6	14.6	14.1	80.1	6.7	13.5	125.5	7.9	15.5
Significance level									
Treatment	*0.5179*	*0.5207*	*0.9092*	*0.0735*	*0.2586*	*0.7174*	*0.7864*	*0.4657*	*0.8832*
LSD 5%	-	-	-	-	-	-	-	-	-

* Copper (1.5 $g \cdot L^{-1}$), ACC (1000 $\mu l \cdot L^{-1}$)

[a] Applied 1 Jun. 2017

[b] Applied 29 Jun. 2017

[c] Applied 1 Jun. 2017 + 29 Jun. 2017

Table 18. Effect of foliar copper (CuEDTA) and 1-aminocyclopropane-1-carboxylic acid (ACC) application on the number of short - and long shoots on containerised ‘Golden Delicious’/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree		Top third		Lower two-thirds	
Treatment	Number of short shoots	Number of long shoots	Number of short shoots	Number of long shoots	Number of short shoots	Number of long shoots
Untreated control	10.7 ns	4.1 ns	6.1 ns	1.6 ns	4.6 ns	2.5 ns
Mechanical[a]	8.2	3.0	4.5	0.4	3.7	2.6
Mechanical[b]	9.1	4.5	5.0	2.9	4.1	1.6
Copper*[a]	9.4	4.7	4.2	2.0	5.2	2.7
Copper*[b]	10.3	5.7	5.7	2.9	4.7	2.8
Copper*[c]	9.6	4.3	4.6	1.9	5.0	2.4
ACC*[a]	10.1	4.4	3.6	2.0	6.6	2.4
ACC*[b]	10.4	4.8	3.8	1.9	6.6	2.9
ACC*[c]	9.8	4.8	5.3	1.4	4.5	3.4
Significance level						
Treatment	*0.9788*	*0.8342*	*0.8017*	*0.1113*	*0.5063*	*0.9118*
LSD 5%	-	-	-	-	-	-

* Copper (1.5 $g\cdot L^{-1}$), ACC (1000 $\mu l\cdot L^{-1}$)

[a] Applied 1 Jun. 2017

[b] Applied 29 Jun. 2017

[c] Applied 1 Jun. 2017 + 29 Jun. 2017

Table 19. Effect of foliar nitrogen (N; urea) in combination with foliar copper (CuEDTA) on spring bud break of containerised 'Golden Delicious'/M.9 apple trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree			Top third		Lower two-thirds	
Treatment	Final % total bud break	% Reproductive buds	Number of days to 40% bud break	Final % vegetative bud break	Number of days to 40% vegetative bud break	Final % vegetative bud break	Number of days to 40% vegetative bud break
Untreated control	85.1 ns	14.5 ns	14.3 ns	94.5 ns	10.8 ns	88.7 ns	12.1 ns
CuEDTA*[a]	90.6	25.5	10.7	99.3	6.9	94.8	9.1
CuEDTA*[b]	92.0	20.9	12.3	96.9	8.2	95.3	11.7
N + CuEDTA**[c]	87.4	17.5	12.1	96.3	8.5	92.1	11.4
N + CuEDTA**[d]	93.2	22.1	11.5	98.6	8.4	95.1	10.4
N + CuEDTA**[e]	84.6	13.9	10.3	96.9	6.5	86.9	8.7
Significance level							
Treatment	*0.4327*	*0.3711*	*0.2465*	*0.5949*	*0.0834*	*0.7397*	*0.4308*
LSD 5%	-	-	-	-	-	-	-

*CuEDTA (1.5 $g \cdot L^{-1}$)

**Nitrogen (13.8 $g \cdot L^{-1}$) + CuEDTA (1.5 $g \cdot L^{-1}$)

[a] CuEDTA applied on 1 Jun. 2017

[b] CuEDTA applied on 29 Jun. 2017

[c] Nitrogen and CuEDTA applied on 1 Jun. 2017

[d] Nitrogen applied on 1 Jun. 2017, CuEDTA applied on 29 Jun. 2017

[e] Nitrogen and CuEDTA applied on 1 Jun. 2017 + 29 Jun. 2017

Table 20. Effect of foliar nitrogen (N; urea) in combination with foliar copper (CuEDTA) on the increase in diameter and apical extension growth of containerised ‘Golden Delicious’/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

Treatment	Increase in diameter (mm)	Apical extension growth (cm)
Untreated control	2.6 ns	38.3 ns
CuEDTA*[a]	3.3	36.6
CuEDTA*[b]	3.4	37.7
N + CuEDTA**[c]	3.7	37.5
N + CuEDTA**[d]	3.5	36.0
N + CuEDTA**[e]	3.1	32.1
Significance level		
Treatment	*0.8712*	*0.9685*
LSD 5%	-	-
Initial diameter and leader length (covariant)	*0.4485*	*0.9663*

*CuEDTA (1.5 $g \cdot L^{-1}$)

**Nitrogen (13.8 $g \cdot L^{-1}$) + CuEDTA (1.5 $g \cdot L^{-1}$)

[a] CuEDTA applied on 1 Jun. 2017

[b] CuEDTA applied on 29 Jun. 2017

[c] Nitrogen and CuEDTA applied on 1 Jun. 2017

[d] Nitrogen applied on 1 Jun. 2017, CuEDTA applied on 29 Jun. 2017

[e] Nitrogen and CuEDTA applied on 1 Jun. 2017 + 29 Jun. 2017

Table 21. Effect of foliar nitrogen (N; urea) in combination with foliar copper (CuEDTA) on the new growth of one-year-old lateral shoots in one season of containerised 'Golden Delicious'/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree			Top third			Lower two-thirds		
Treatment	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)
Untreated control	221.6 ns	13.4 ns	17.9 ns	35.4 ns	3.9 ns	9.0 ns	186.3 ns	9.5 ns	19.6 ns
CuEDTA*[a]	171.2	10.1	17.4	30.6	2.2	10.2	140.6	7.9	18.5
CuEDTA*[b]	193.8	11.8	16.8	27.0	2.6	10.5	166.8	9.2	18.2
N + CuEDTA**[c]	169.9	9.4	19.4	30.8	2.4	9.7	139.0	7.0	25.2
N + CuEDTA**[d]	206.5	12.3	17.3	49.6	3.5	11.1	156.9	8.8	18.4
N + CuEDTA**[e]	228.3	12.5	18.1	70.3	4.2	12.1	158.0	8.3	19.7
Significance level									
Treatment	*0.2731*	*0.0608*	*0.9098*	*0.3777*	*0.7720*	*0.9085*	*0.3431*	*0.2974*	*0.4063*
LSD 5%	-	-	-	-	-	-	-	-	-

*CuEDTA (1.5 $g\cdot L^{-1}$)

**Nitrogen (13.8 $g\cdot L^{-1}$) + CuEDTA (1.5 $g\cdot L^{-1}$)

[a] CuEDTA applied on 1 Jun. 2017

[b] CuEDTA applied on 29 Jun. 2017

[c] Nitrogen and CuEDTA applied on 1 Jun. 2017

[d] Nitrogen applied on 1 Jun. 2017, CuEDTA applied on 29 Jun. 2017

[e] Nitrogen and CuEDTA applied on 1 Jun. 2017 + 29 Jun. 2017

Table 22. Effect of foliar nitrogen (N; urea) in combination with foliar copper (CuEDTA) on the number of short - and long shoots on containerised 'Golden Delicious'/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree		Top third		Lower two-thirds	
Treatment	Number of short shoots	Number of long shoots	Number of short shoots	Number of long shoots	Number of short shoots	Number of long shoots
Untreated control	4.9 ns	7.0 ns	1.0 ns	1.4 ns	3.9 ns	5.6 ns
CuEDTA*[a]	4.8	5.3	1.4	0.8	3.4	4.5
CuEDTA*[b]	6.1	5.7	2.1	0.4	4.0	5.2
N + CuEDTA**[c]	4.1	6.0	1.9	0.5	2.3	5.5
N + CuEDTA**[d]	7.1	5.2	2.7	0.8	4.4	4.4
N + CuEDTA**[e]	*5.2*	*7.3*	2.3	1.8	*2.8*	*5.5*
Significance level						
Treatment	*0.1805*	*0.5635*	*0.2998*	*0.4767*	*0.4237*	*0.7666*
LSD 5%	-	-	-	-	-	-

*CuEDTA (1.5 g·L^{-1})
**Nitrogen (13.8 g·L^{-1}) + CuEDTA (1.5 g·L^{-1})
[a] CuEDTA applied on 1 Jun. 2017
[b] CuEDTA applied on 29 Jun. 2017
[c] Nitrogen and CuEDTA applied on 1 Jun. 2017
[d] Nitrogen applied on 1 Jun. 2017, CuEDTA applied on 29 Jun. 2017
[e] Nitrogen and CuEDTA applied on 1 Jun. 2017 + 29 Jun. 2017

Table 23. Effect of plant growth regulators on the final percentage total bud break and the reproductive buds of containerised ‘Bigbucks Gala’/M.9 nursery trees at Glen Elgin, Elgin, South Africa 2018/2019.

Plant growth regulator	Final % total bud break	% Reproductive buds
Untreated control	80.6 ns	36.6 ns
GA_3 (100 mg·L^{-1}) 16 Feb. & 2 Mar. 2018	78.7	28.5
GA_3 (100 mg·L^{-1}) 16 Feb., 2 & 16 Mar. 2018	80.5	21.8
GA_3 (100 mg·L^{-1}) 16 Feb., 2, 16 & 28 Mar. 2018	85.3	40.6
GA_3 (250 mg·L^{-1}) 16 Feb. & 2 Mar. 2018	81.7	28.3
GA_3 (250 mg·L^{-1}) 16 Feb., 2 & 16 Mar. 2018	79.0	34.9
GA_3 (250 mg·L^{-1}) 16 Feb., 2, 16 & 28 Mar. 2018	85.4	30.1
GA_{4+7} (250 mg·L^{-1}) 16 Feb. & 2 Mar. 2018	84.8	26.1
GA_{4+7} (250 mg·L^{-1}) 16 Feb., 2 & 16 Mar. 2018	82.2	25.5
GA_{4+7} (250 mg·L^{-1}) 16 Feb., 2, 16 & 28 Mar. 2018	79.6	32.0
Significance level		
Treatment	*0.5775*	*0.3860*
LSD 5%	-	-

PAPER 2: Evaluating Dormancy Management Practises and Different Planting Methods for Containerised Apple (*Malus domestica* Borkh.) Nursery Trees.

Additional index words. Bud break, winter chilling, cold storage, hydrogen cyanamide, transplant shock.

Abstract.

One of the main challenges in South Africa is growing apples under conditions of insufficient winter chilling. Reduced bud break is a characteristic of insufficient chilling, and when nursery trees are planted in the orchard, uniform bud break is essential in spring. The successful establishment of a new orchard is often further delayed by transplant shock. The purpose of this study was to evaluate dormancy management practises as well as different planting methods, viz. planting the trees while trying to not disturb the planting medium at all, washing off the planting medium before planting, loosening the planting medium in the root zone before planting and shaking off and removing most of the planting medium with minimal damage to the roots, of containerised apple nursery trees. Dormancy management practises included 6 weeks cold storage at 6 °C, a 1.5% hydrogen cyanamide rest breaking treatment or a combination of the two. The treatments significantly increased the total bud break and vegetative bud break percentages, with a combination of cold storage and chemical rest breaking the most effective dormancy management strategy. The different dormancy management practises did not significantly increase the total new growth during the first growing season, but improved the architecture of the tree. Trees that received insufficient chilling or only the chemical rest breaking treatment produced a basal dominant tree architecture. Different planting methods did not significantly influence total and vegetative bud break percentages. However, planting trees with an undisturbed growing medium, or only slightly loosening the growing medium improved new lateral shoot growth and apical extension growth.

Introduction

Apples in South Africa are produced on approximately 24 000 hectares (ha) (Hortgro, 2017), of which the majority receive inadequate chilling during winter. This leads to reduced and protracted bud break and flowering (Cook, 2010) as well as poor pollination, reduced fruit set, and variable fruit size (Costa et al., 2004). The protracted bud break, also known as delayed foliation, makes orchard practises such as spray applications (Petri and Leite, 2004), fruit thinning and harvest (Costa et al., 2004) more challenging. Poor tree architecture characterised by a basitonic growth pattern is also a characteristic of inadequate chilling and leads to a basal dominant tree architecture (Cook and Strydom, 1998; Cook, 2010; Costa et al., 2004). Basal dominance impedes apical extension growth and decreases the bearing potential of the tree (Müller and Theron, 2018).

When new orchards are established in spring, uniform bud break is necessary to create an adequate framework for the trees (Petri and Stuker, 1988). Therefore, nursery trees are placed in cold stores after lifting and prior to planting to achieve enough chilling (Petri and Stuker, 1988). Alternatively, or additionally, the application of a rest breaking treatment can promote bud break (Honeyborne, 1996). However, bud break per se is not an indication of good lateral shoot formation as bud burst could result only in spur development, which happens frequently following a rest breaking treatment (Cook, 2010).

One of the main concerns when establishing a new orchard is the prolonged establishment period of young trees which is characterised by a period of slow growth (Struve, 1990) and is attributed to transplant shock (Watson, 1986). According to Watson (1986), transplant shock occurs due to an imbalance in root to shoot volume since the root system can be damaged during transplanting operations (Dong et al., 2003). In containerised-production, trees are propagated, handled and transplanted with the root system intact, which could reduce the imbalance and thus the transplant shock (Mathers et al., 2007). According to Castle (1987), there are conflicting reports regarding the field performance of containerised citrus nursery trees following different handling and planting methods of these trees. Producing containerised apple nursery trees is novel study in South Africa and no information is available on how they respond to different planting methods.

This paper reports on the effect of dormancy management practises and different planting methods on bud break during spring as well as tree growth during the first season of young, containerised apple trees planted in a commercial orchard.

Materials and Methods

Plant material and site description. During the 2017/2018 season, two trials were conducted on one-year-old containerised ‘Golden Delicious’/M.9 (Nic 29) trees nursery trees planted in a commercial orchard. One trial was conducted before and after the trees were planted, while the second trail was conducted during planting. Trials were planted on Glen Elgin, a commercial farm, situated in Elgin, Western Cape region (34° 9'18.89"S 19° 2'6.32"E, Altitude 332 m, Mediterranean-type climate, 880 chilling units (Daily positive chill unit (DPCU), Linsley-Noakes et al., 1994) and ca. 949 mm rainfall annually) on 12 September 2017. Trees used were propagated at Witzenberg Range nursery as described in Paper 1. After the trees were planted, the distal ±2 cm of the central leader was removed with a heading cut on 21 September 2017. After the bud break period, all sylleptic lateral shoots that developed in the nursery were cut back to ±5 cm from the trunk of the tree as is the normal commercial practice in the region. Standard commercial plant protection, irrigation and fertilizer practises were followed in the orchard.

Treatments and trial design. In the first trial, the effect of six weeks cold storage set at 6 °C (1 August 2017 – 12 September 2017) was compared to a 1.5% hydrogen cyanamide [Dormex®; (AlzChem Group AG, Trostberg, Germany]chemical rest breaking treatment on 16 September 2017 or a combination of the cold storage period with the chemical rest breaking treatment.

In the second trial the effect of different planting methods was evaluated. Trees were stored in commercial fruit cold stores at 6 °C, from 1 August 2017 to 12 September 2017. The different planting methods were (1) planting the trees while trying to not disturb the planting medium at all, (2) washing off the majority of the planting medium before planting, (3) loosening the planting medium in the root zone before planting and (4) shaking off and removing most of the planting medium with minimal damage to the roots (Fig. 1). All trees in this trial received a 1.5% hydrogen cyanamide (Dormex®) rest breaking treatment on 16 September 2017. Both trials were randomized complete block designs with ten single tree replications.

In a preliminary trial in 2016/2017, ‘Rosy Glow’ on M.9 (Nic 29) trees, made according to the same procedure as described for ‘Golden Delicious’ on M.9, were planted at two different trial sites. Trials were planted at Breëvlei and Glen Fruin, two commercial farms, situated in the Elgin, Western Cape region (34°14'50.4"S 19°05'49.7"E and 34°11'11.6"S 19°03'47.9"E respectively. Treatments used were similar to (1), (2) and (4) described above at Glen Fruin and (1) and (2) at Breëvlei. Trees at both trial sites were treated with 1.5% hydrogen cyanamide (Dormex®), while trees planted at Glen Fruin also received a cold storage period of eight weeks before planting.

Data collection. The following data were recorded per tree: (1) initial (29 September 2017) and final (25 May 2018) trunk diameter 5 cm above the graft union with an electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan), (2) initial (29 September 2017) and final (25 May 2018) tree height, (3) rate and percentage total bud break of the whole tree from the graft union, (4) rate and percentage vegetative bud break, (4) percentage reproductive buds and (5) total new growth via lateral shoots.

The percentage and rate of total bud break in the whole tree was determined as follow: The number of buds (vegetative and reproductive) were counted over 34 days from the "mouse ear stage" or BBCH stage 10 (Meier, 2001) from 5 October 2017 to 8 November 2017. On 8 November the number of dormant buds along the whole length of the tree from the graft union was counted. From this a curve of the total bud break percentage was constructed. The number of days until 40% bud break was determined from the graph to determine the rate of bud break. The percentage total bud break in the whole tree was calculated as follows:

$$\left(\frac{\text{Total number of sprouted buds }_{\text{At a specific date}}}{\text{Total number of buds per tree}} \times 100 \right)$$

The percentage and rate of vegetative bud break was determined as described above for total bud break. In addition, trees were divided in two sections from the graft union (lower two-thirds and top third). On 8 November the number of dormant buds in the two sections were counted. During the bud break period, the number of sprouted reproductive buds on each tree was also counted to calculate the percentage reproductive buds that opened.

After the growing season, on 25 May 2018, the number and length of new one-year-old lateral shoots were counted in the two sections of the tree (described above). In addition the total new growth of these shoots were calculated. One-year-old shoots were divided into two categories, viz. short (0-15 cm) and long shoots (15 cm +).

In the preliminary trials during the 2016/2017 season, the new growth was measured as described above.

Statistical analysis. The data were analysed using SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, North Carolina, USA) using a linear model procedure and the pairwise t-test to determine Least Significant Difference (LSD) when the F-statistic indicated significance at P<0.05. Where applicable, an analysis of covariance (ANCOVA) was used to determine Least Significant Difference (LSD) when the F-statistic indicated significance at P<0.05.

Results and Discussion

Cold storage and rest breaking treatment trial

Spring bud break: The total percentage bud break of the tree was significantly improved by approximately 30% by all the treatments compared to the untreated control (Table 1). Trees receiving cold storage and the chemical rest breaking treatment achieved the highest total bud break percentage, but not significantly so (Table 1). Previously Petri and Stuker (1988) established that a cold storage period of both 2 °C and 6 °C improved bud break of apple trees during spring. ‘Gala’ and ‘Fuji’ trees stored for 45 and 60 days, respectively, had a significantly higher bud break percentage compared to trees stored for 15 or 30 days. Half of the trees were treated with a chemical rest breaking treatment (4% mineral oil + 0.16% dinitro-butyl-phenol) after the trees were planted. The authors concluded that trees receiving cold storage followed by the chemical rest breaking treatment had a significantly higher bud break percentage compared to trees that only received a cold storage period. In our trial the percentage reproductive buds that sprouted on the trees did not differ significantly and therefore only vegetative bud break was followed in the different sections of the tree (Table1).

Only two of the ten untreated control trees achieved a vegetative bud break percentage of 40% or higher in the top third of the tree. Hence, these trees were not included in the statistical analyses. The lower two-thirds of control trees however achieved a vegetative bud break percentage of approximately 68% (Table 1), illustrating the basal dominant tree architecture that ensues when trees receive inadequate winter chilling (Cook and Strydom, 1998). Trees that were cold stored with or without the rest breaking treatment had a significant higher vegetative bud break of nearly 100% in the top third compared to trees that only received the rest breaking treatment which had a bud break percentage of 81% (Table 1). In the lower two-thirds, both treatments that included chemical rest breaking significantly improved the percentage vegetative bud break compared to the control but not compared to cold storage on its own (Table 1).

The trees used in our trial received approximately 517 chilling units (DPCU) (Linsley-Noakes et al., 1994) in the nursery situated in Simondium before being transported to Glen Elgin. The untreated control trees received approximately an additional 296 chilling units (DPCU) in Elgin prior to planting, while trees stored in cold rooms received approximately an additional 1000 chilling units (DPCU). ‘Golden Delicious’ is a high chill cultivar (Costa et al., 2004) requiring approximately 1400 chilling units (Ogundeji and Jordaan, 2017). Therefore the ca. 800 chilling units (DPCU) the control trees received was too low. The cold storage period was therefore important in overcoming dormancy, especially to achieve a balance in bud break between the top third and the lower two-thirds of the tree, which should contribute to improved tree architecture.

One of the characteristics of inadequate winter chilling is a protracted bud break period (Costa et al., 2004). The rate of bud break of the tree (as seen in days to 40% bud break) was increased by all the treatments compared to the control trees. However, the fastest rate of total bud break was achieved by the combined cold storage and chemical rest breaking treatment. Although not significant, there was a trend that a period of cold storage resulted in faster rate of total bud break compared to trees that only received a rest breaking treatment cold storage period (Table 1). The highest rate of vegetative bud break in the top third and lower two-thirds was obtained by a combination of cold storage and the chemical rest breaking treatment.

New growth: A period of cold storage, the chemical rest breaking treatment or a combination of the two did not significantly increase the total new growth or the number of lateral shoots on the trees compared to the untreated control (Table 2). There was a trend that the treatments increased the number of laterals ($p = 0.0676$) which then resulted in a significant decrease in the average length of the laterals of the whole tree (Table 2). This trend is the same as observed in the total bud break percentage.

A period of cold storage with or without the chemical rest breaking treatment produced significantly more new growth in the top third of the trees compared to the untreated control or trees only receiving the chemical rest breaking treatment (Table 2). The number of lateral shoots in the top third was significantly more when trees received cold storage with or without the rest breaking treatment. In addition, the new growth and number of lateral shoots produced by trees receiving only a period of cold storage was two-fold greater compared to trees only receiving a rest breaking treatment (Table 2). This can be ascribed to the difference in chilling units received during winter for these two treatments. Petri and Leite (2004) established that there is a correlation between the number of chill units received and an increase in shoot growth in young apple trees. When dividing the laterals in different length categories, a clear trend ($p = 0.0570$) exists that cold storage resulted in overall more short shoots (1-15 cm), but no difference in the number of long shoots (Table 3). This trend was due to the increase in the number of short shoots in the upper third of the tree.

Although there was no significant difference in total new growth between the different treatments in the lower two-thirds of the tree, it is the ratio of the new growth in this specific part versus the total new growth in the tree as a whole that is of importance. More specifically, the untreated control trees produced approximately 85% of the total new growth in the lower two-thirds (Table 2). This is a sign of a basal dominant tree architecture, where the vigour of proximally situated branches is increased (Cook and Jacobs, 1999). In addition, the untreated control trees produced shoots in the lower two-thirds that were on average significantly longer (Table 2). Trees that only received a rest breaking treatment also had a predominant basal dominant architecture. This change in architecture is a characteristic of mild-winter climates (Cook, 2010; Müller and Theron, 2018). In contrast, trees that received a period of cold storage with or without a rest breaking treatment had a more even distribution of new growth along the axis of the tree, which can be described as a mesotonic growth tendency (Bell, 2008). The rest breaking treatment had no additional benefit regarding tree architecture.

The increase in diameter was not significantly influenced by cold storage or the rest breaking treatment. This finding correlates with the total new growth that did not differ significantly between these treatments (Table 4). However, there was a trend ($p = 0.0626$) that a period of cold storage with or without a rest breaking treatment increased the apical extension growth (Table 4). As apical extension growth is often not optimal under conditions of inadequate chilling, gibberellin is applied to the apical parts of trees (Müller and Theron, 2018). In this orchard gibberellin A_{4+7} [Regulex® 10%, Valent BioSciences Corporation, Long Grove, IL, USA] was applied on a weekly basis as part of the commercial practices to the upper 10 cm of the trees to increase extension growth of the leader. This probably had an overriding effect on the extension growth.

Trial on different planting methods.

Preliminary trial – 2016/2017 season: The planting method did not have any significant effect on the total new lateral shoot growth. In addition, the average length of the lateral shoots was not significantly influenced by the different planting methods. However, the undisturbed growing medium produced significantly more lateral shoots at Breëvlei compared to trees where the growing medium was washed off. The apical extension growth, at both trial sites, was not significantly influenced by the different planting methods (Table 5).

Spring bud break: In the 2017/2018 trial more planting methods were evaluated, but these did not influence the total bud break percentage per tree, or the vegetative bud break in the two different sections (Table 6). All of the trees in this trial received 517 chilling units (DPCU) (Linsley-Noakes et al., 1994) in the nursery situated in Simondium before being transported to Glen Elgin. In addition, all of the trees were stored in a cold room and received an additional ca. 1000 chilling units (DPCU). The different planting methods did not significantly influence the percentage reproductive buds on the trees (Table 6). Hence, the disturbance of the growing medium to any degree had no significant negative effect on bud break during spring. Transplanted nursery trees are more dependent on nutrient reserves for bud break and initial growth in spring and less on nutrient uptake from the growing medium or soil (Cheng et al., 2001). During the different removal techniques of the growing medium in our trial, root damage was minimized. This minimal loss of roots can explain why no significant differences in bud break were observed. However, the rate of bud break was significantly influenced by the different planting methods. The rate of bud break increased when the growing medium was washed or shaken off before planting (Table 6). This effect was evident in the top third as well as the lower two-thirds of the tree axis. The washing off of the growing medium as well as shaking off the growing medium could have reduced the contact between the roots and soil. This reduced contact could have dried out the roots, which can potentially explain the faster rate of bud break.

New growth: The different methods at planting significantly influenced total new shoot growth developing during the first growing season (Table 7 and 8). The total new growth was highest when roots were undisturbed or only slightly loosened while the new growth was drastically reduced by washing the medium off or to a lesser extent when shaken off (Table 7). As the average length of the laterals was not influenced by planting method, the reason for the differences in total growth is due to the number of laterals developing. As mentioned previously, the vegetative bud break percentage did not differ significantly between the planting methods, therefore, these significant differences in the number of lateral shoots indicate that by shaking or washing off the medium the buds did not continue to develop into shoots after bud break. Shoots were measured from a minimum length of 1 cm. Hence, this discrepancy between the bud break percentage and new lateral shoot growth can be attributed to the fact that buds that did sprout, did not produce shoots longer than 1 cm when the growing medium was washed off or shaken off.

A similar trend in new growth was observed in the top third of the tree, where the new growth was significantly reduced when the growing medium was washed off from the root system (Table 7). Although not significant, there was a trend that trees planted with an undisturbed growing medium, or by only loosening the growing medium produced more new growth as well as more laterals, especially short shoots (Table 7 and 8). The total new growth in the lower two-thirds of the trees was not affected by the planting method but the number of lateral shoots, was significantly lower when the growing medium was washed off (Table 7). Different planting methods had no significant influence on the increase in trunk diameter during the growing season (Table 9). In contrast, planting methods significantly influenced the apical extension growth during the growing season. By shaking or washing off the growing medium, the apical extension growth was significantly reduced compared to trees planted with an undisturbed growing medium (Table 9).

Marler and Davies (1987) found the opposite in ‘Hamlin’ citrus trees where removing all of the growing medium before planting significantly increased the root growth and canopy volume compared to planting the nursery trees with the growing medium intact. The authors suggested that 50% of the growing medium should be shaken off before planting to improve initial growth. In our trial more than 50% of the growing medium was washed – or shaken off, but more new growth was still produced when trees were planted with an undisturbed growing medium.

In ornamentals, different techniques are used to disturb the root system to promote new root growth and improve transplant success. These techniques include pulling the circling roots apart, slicing of the root-ball or slicing the root-ball and separating the ends although the response to these techniques is still uncertain (Cregg and Ellison, 2018). These techniques are used when roots are pot bound, but in our trials this was not the case (Fig. 2) therefore not necessitating such measures. According to Marler and Davies (1987), some citrus growers believe that containerised trees are superior regarding establishment due to the water holding capacity of the growing medium. According to Ferrini et al. (2000), water stress after planting is the main contributing factor to transplanting failure. This can be one possible reason why an undisturbed, or slightly loosened growing medium produced more new growth in our trials. As mentioned previously, the washing and shaking off of the growing medium could have reduced the contact between the roots and the soil which could have dried out some of the roots. This could have resulted in a lower effective root volume to support new growth. Because containerised apple trees are a new concept in South Africa, the management and irrigation practises in the orchard were probably not optimal this could have influenced our results. Due to the difference in root systems between bare rooted and containerised nursery trees, irrigation practises need to be adjusted.

Conclusion

Cold storage for 6 weeks at 6 °C, an application of 1.5% hydrogen cyanamide or the combination of the two significantly improved the percentage bud break of newly planted containerised ‘Golden Delicious’ apple trees. The combination of the cold storage period and the rest breaking treatment produced the highest percentage vegetative bud break while a period of cold storage with or without a rest breaking treatment produced trees with a more mesotonic tree architecture.

To improve the regrowth potential and reduce the transplant shock of containerised apple trees, it is recommended, from our results, that trees are planted with the growing medium intact and undisturbed, or alternatively only slightly loosened before planting. However, if the contact between the soil and roots can be improved after removing the growing medium, better performance can possibly be obtained.

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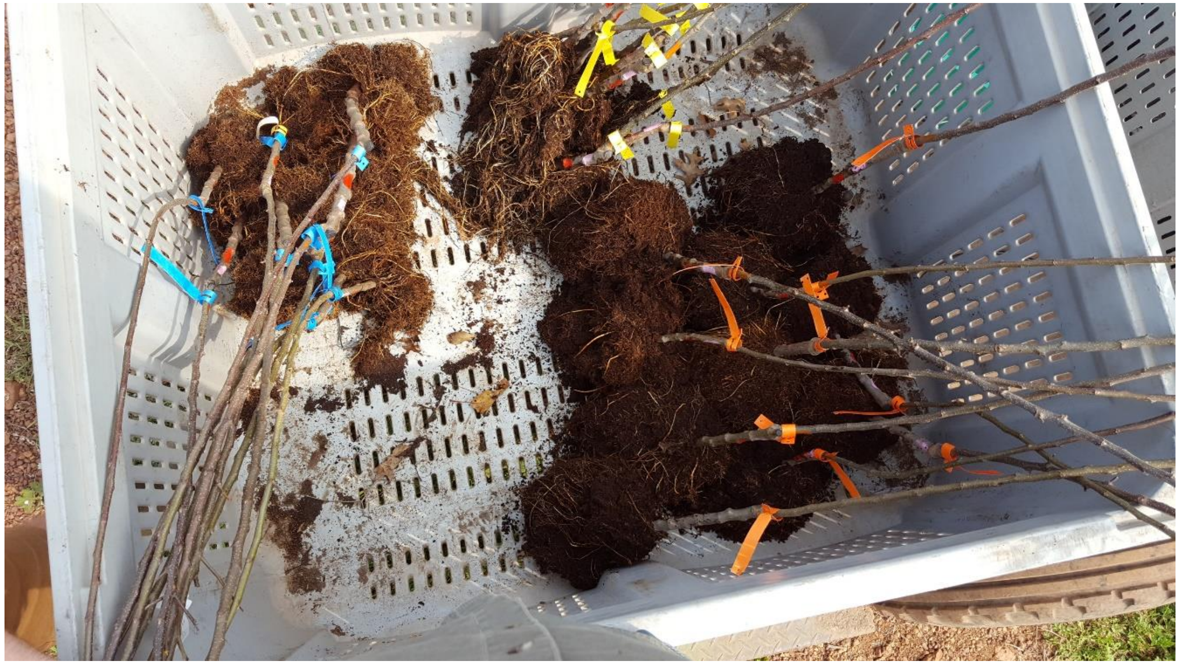

Fig.1 Treatments specifications for trials done to evaluate the effect of different planting methods on the performance of containerised ‘Golden Delicious’/M.9 nursery trees when planted in a commercial orchard during spring 2017/2018. Yellow label. Growing medium washed off; Orange label. Growing medium loosened; Blue label. Growing medium shaken off.

Table 1. Effect of cold storage and a rest breaking treatment (1.5% hydrogen cyanamide (HC)) on spring bud break of containerised ‘Golden Delicious’/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree			Top third		Lower two-thirds	
Treatment	Final % total bud break	% Reproductive buds	Number of days to 40% total bud break	Final % vegetative bud break	Number of days to 40% vegetative bud break	Final % vegetative bud break	Number of days to 40% vegetative bud break
Untreated control	62.0 b	15.7 ns	27.8 a	-	-	67.5 c	25.3 a
1.5% HC*	91.7 a	21.5	15.9 b	80.9 b	12.5 a	84.9 ab	15.2 c
Cold storage**	90.1 a	18.4	18.1 b	99.1 a	15.2 a	78.3 bc	18.9 b
Cold storage** + 1.5% HC*	95.5 a	18.7	9.3 c	99.7 a	5.1 b	90.0 a	10.6 d
Significance level							
Treatment	*<0.0001*	*0.6859*	*<0.0001*	*0.0006*	*<0.0001*	*0.0037*	*<0.0001*
LSD 5%	*7.9*	-	*2.8*	*9.3*	*3.5*	*10.9*	*2.8*

*Applied on 16 Sep. 2017

** Six weeks cold storage at 6 °C

Table 2. Effect of cold storage and a rest breaking treatment (1.5% hydrogen cyanamide (HC)) on the new growth of one-year-old lateral shoots in one season of containerised ‘Golden Delicious’/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree			Top third			Lower two-thirds		
Treatment	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)
Untreated control	207.9 ns	11.0 ns	19.6 a	30.8 b	2.3 b	9.9 ns	177.1 ns	8.7 ns	21.5 a
1.5% HC*	215.3	14.8	15.3 b	53.9 b	4.4 b	11.9	161.4	10.4	16.3 b
Cold storage**	237.2	16.5	15.0 b	125.3 a	8.5 a	16.3	111.8	8.0	14.4 b
Cold storage** + 1.5% HC*	229.1	16.8	13.8 b	103.6 a	8.4 a	12.6	125.5	8.4	14.2 b
Significance level									
Treatment	*0.9346*	*0.0676*	*0.0199*	*0.0010*	*0.0015*	*0.0730*	*0.1933*	*0.4972*	*0.0118*
LSD 5%	-	-	*3.5*	*37.3*	*2.9*	-	-	-	*4.3*

*Applied on 16 Sep. 2017

** Six weeks cold storage at 6 °C

Table 3. Effect of cold storage and a rest breaking treatment (1.5% hydrogen cyanamide (HC)) on the number of short - and long shoots of containerised 'Golden Delicious'/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree		Top third		Lower two-thirds	
Treatment	Number of short shoots	Number of long shoots	Number of short shoots	Number of long shoots	Number of short shoots	Number of long shoots
Untreated control	4.8 ns	6.2 ns	1.4 c	0.9 b	3.3 ns	5.3 ns
1.5% HC*	9.0	5.8	3.4 bc	1.0 b	5.6	4.8
Cold storage**	11.0	5.5	5.0 ab	3.5 a	6.0	2.0
Cold storage** + 1.5% HC*	11.7	5.1	6.8 a	1.6 b	4.9	3.5
Significance level						
Treatment	*0.0570*	*0.8432*	*0.0084*	*0.0334*	*0.4268*	*0.1085*
LSD 5%	-	-	*2.8*	*1.3*	-	-

*Applied on 16 Sep. 2017
** Six weeks cold storage at 6 °C

Table 4. Effect of cold storage and a rest breaking treatment (1.5% hydrogen cyanamide (HC)) on the increase in diameter and apical extension growth of containerised 'Golden Delicious'/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

Treatment	Increase in diameter (mm)	Apical extension growth (cm)
Untreated control	5.0 ns	44.8 ns
1.5% HC*	5.4	42.5
Cold storage**	4.0	45.8
Cold storage** + 1.5% HC*	5.7	47.8
Significance level		
Treatment	*0.4235*	*0.3817*
LSD 5%	-	-
Initial diameter and leader length (covariant)	*0.3602*	*0.0626*

*Applied on 16 Sep. 2017
** Six weeks cold storage at 6 °C

Table 5. Effect of different planting methods on the new growth over one season of containerised ‘Rosy Glow/M.9 trees at Breëvlei and Glen Fruin, Elgin, South Africa (2016/2017).

	Breëvlei				Glen Fruin			
Treatment	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)	Apical extension growth (cm)	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)	Apical extension growth (cm)
No removal	252.2 ns	14.9 a	17.4 ns	49.1 ns	447.8 ns	20.5 ns	25.0 ns	58.0 ns
Washed off	187.7	11.7 b	16.5	51.0	374.3	17.2	25.7	57.7
Shaken off					421.3	19.2	26.9	60.3
Significance level	*0.1857*	*0.0077*	*0.8490*	*0.5550*	*0.5014*	*0.2476*	*0.9415*	*0.9356*
LSD 5%	-	*2.0*	-	-	-	-	-	-

Table 6. Effect of different planting methods on spring bud break of containerised ‘Golden Delicious’/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree			Top third		Lower two-thirds	
Treatment	Final % total bud break	% Reproductive buds	Number of days to 40% total bud break	Final % vegetative bud break	Number of days to 40% vegetative bud break	Final % vegetative bud break	Number of days to 40% vegetative bud break
No removal	90.7 ns	14.5 ns	16.0 a	97.1 ns	13.9 a	82.2 ns	16.7 a
Washed off	89.4	10.3	7.8 c	96.6	6.1 b	79.3	9.8 b
Loosened	91.0	15.9	13.9 ab	95.6	10.2 ab	84.2	13.8 ab
Shaken off	88.1	11.8	12.0 b	93.2	9.6 ab	80.7	11.7 b
Significance level							
Treatment	*0.6586*	*0.6627*	*0.0010*	*0.7731*	*0.0154*	*0.8088*	*0.0315*
LSD 5%	-	-	*3.8*	-	*4.6*	-	*4.5*

Table 7. Effect of different planting methods on the new growth of one-year-old lateral shoots in one season of containerised ‘Golden Delicious’/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree			Top third			Lower two-thirds		
Treatment	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)	Total new growth (cm)	Number of lateral shoots	Average length of lateral shoot (cm)
No removal	192.2 a	13.5 ab	15.9 ns	91.7 a	8.2 a	10.6 ns	100.5 ns	5.3 ab	19.4 ns
Washed off	97.6 c	7.5 c	12.9	37.9 b	3.9 b	10.1	59.7	3.6 b	18.9
Loosened	176.7 ab	14.2 a	12.5	91.4 a	7.6 a	11.2	85.2	6.7 a	14.9
Shaken off	126.5 bc	10.2 bc	12.1	64.8 ab	5.6 ab	10.4	61.7	4.6 ab	12.9
Significance level									
Treatment	*0.0073*	*0.0016*	*0.2606*	*0.0218*	*0.0140*	*0.9622*	*0.1205*	*0.0464*	*0.2652*
LSD 5%	*56.4*	*3.5*	-	*37.4*	*2.7*	-	-	*2.2*	-

Table 8. Effect of different planting methods on the number of short - and long shoots of containerised ‘Golden Delicious’/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

	Whole tree		Top part		Lower two-thirds	
Treatment	Number of short shoots	Number of long shoots	Number of short shoots	Number of long shoots	Number of short shoots	Number of long shoots
No removal	9.6 a	3.9 ns	6.7 a	1.5 ns	2.9 ns	2.4 ns
Washed off	5.5 b	2.1	2.9 b	1.1	2.6	1.0
Loosened	11.0 a	3.2	5.9 a	1.7	5.1	1.6
Shaken off	7.8 ab	2.4	4.4 ab	1.2	3.4	1.2
Significance level						
Treatment	*0.0202*	*0.3016*	*0.0235*	*0.8365*	*0.0788*	*0.1241*
LSD 5%	*3.6*	-	*2.5*	-	-	-

Table 9. Effect of different planting methods on the increase in diameter and apical extension growth of containerised ‘Golden Delicious’/M.9 trees at Glen Elgin, Elgin, South Africa (2017/2018).

Treatment	Increase in diameter (mm)	Apical extension growth (cm)
No removal	6.2 ns	47.8 a
Washed off	4.7	32.4 b
Loosened	5.0	40.3 ab
Shaken off	4.8	39.1 b
Significance level		
Treatment	*0.7617*	*0.0095*
LSD 5%	-	*7.9*
Initial diameter and leader length(covariant)	*0.0133*	*0.5644*

PAPER 3: The Propagation of Multi Leader Apple (*Malus domestica* Borkh.) Nursery Trees and Sylleptic Shoot Induction

Additional index words. Cytokinin, "feathered" nursery tree, gibberellin, plant growth regulators, ProGibb®, Promalin®

Abstract.

Due to the high cost of establishing new apple orchards, growers want return on their investment as soon as possible. Early fruit production and lowering the cost of planting material can contribute to earlier return on investment. Branched or "feathered" nursery trees, as well as multi-leader nursery trees, could fill orchard space faster and thus start yielding fruit earlier. These nursery trees can be propagated in the nursery by applying cultural practices including plant growth regulators. The purpose of this study was to evaluate the potential to propagate "feathered"– and two-leader containerised nursery trees by applying plant growth regulators. Promalin® (cytokinin and gibberellin) induced lateral sylleptic shoots on nursery trees, but the number of shoots was low possibly due to the relatively small root volume associated with the containerised nursery trees. Although not significant, there was an advantage in applying ProGibb® (gibberellic acid) with Promalin® to propagate two-leader trees in terms of the balance between the two leaders of the tree. Because containerised nursery tree propagation is a new concept in South Africa, further research is needed on propagation and subsequent management of these trees in the nursery.

Introduction

Two types of nursery trees can be produced in South Africa, viz. unbranched whip trees or "feathered" (branched) trees (Theron et al., 2000), but predominantly the former is produced (Cook, 2010). Due to an increase in establishment cost of new apple orchards, it is important that trees produce fruit at an early stage to obtain early return on investment. There is a positive correlation between the number of lateral shoots on nursery trees and the yield obtained in the second and third season after planting (Van Oosten, 1978). Usually apple nursery trees do not produce enough lateral shoots due to strong apical dominance (Jaumien et al., 1993), but lateral branching can be induced in the nursery by using cultural practices including the application of plant growth regulators (PGRs) (McArtney and Obermiller, 2015).

A "feathered" nursery tree is usually produced in a three-year cycle and consists of a three-year-old root system, a two-year-old tree trunk and a one-year-old feathered crown (Nicolaï, 1998). To produce a "feathered" nursery tree, harvested rootstocks are allowed to grow for one season with a planting distance of 90 cm x 33 cm (Van den Berg, 2003). In the second growing season, a scion bud is inserted on the rootstock. The shoot that grows out of the scion bud is supported by a stick to achieve a straight stem. At the end of the growing season, trees are headed between 70 cm and 80 cm above the ground. In the following season a new shoot from the most distal bud will grow and nurserymen will induce sylleptic branching on this shoot by utilizing PGRs, while all other lateral shoots developing on the trunk are removed (Van den Berg, 2003). Sylleptic branching occurs when lateral shoots develop concurrently with the extension of the apical meristem (Bell, 2008). In addition to applying PGRs, small leaves at the growing tip are pinched off once a week, without damaging the apical meristem. Pinching of the small leaves will reduce the auxin levels by approximately 50% which results in the development of "feathers" (sylleptic shoots) that originate from the axillary meristems below the apical meristem (Van den Berg, 2003). The basipetal flow of auxin from the apical meristem inhibits growth of axillary meristems due to lowering of the cytokinin entering these meristems (Müller and Leyser, 2011). Hence the application of synthetic cytokinin (6-BA), alone or in combination with gibberellins 4+7 (GA_{4+7}) is important to produce "feathered" nursery trees (Elfving, 2010).

The row spacing in the nursery is important for the development of sylleptic shoots. This row spacing needs to be wider than when producing unbranched whip trees (Strydom and Honeyborne, 1980) because root development is impeded at narrow row spacing. The widening of row spacing increased the number of sylleptic shoots on 'Delicious' nursery trees on MM106 rootstock (Wilson and Jarassamrit, 1994). Richards and Rowe (1977) established that root restrictions reduced the plant dry weight of peach seedlings by approximately 66%. The authors postulated that a limited root system reduced the above ground growth due to lower production and supply of growth substances. In addition, at narrow row spacing the red to far red ratio decreases, and trees adapt to this shading by increasing apical dominance and rapid elongation of internodes (Salter et al., 2003). This increased apical dominance will impede the development of lateral or sylleptic shoots. In contrast, by widening the row spacing the red to far red light ratio increases, and hence the shade avoidance syndrome is reduced.

Around the world apple growers are planting high density orchards, but there is still a difference in opinion regarding the optimum planting density or tree shape (Robinson, 2007). In addition, there is a debate of the best-fit training system which includes Slender Spindle, Vertical Axis system or some version of the Vertical Axis System (Robinson, 2007). There are extreme planting densities; viz. 5000 trees per hectare (ha) or 500 trees per ha (Robinson, 2007). Due to the number of trees (4 000 – 6000 trees per ha) in a high density orchard, it requires high establishment costs, precision management practises and the extensive use of PGRs to balance reproductive and vegetative growth (Dorigoni, 2011). One alternative to reduce the cost of high density orchards is to plant fewer, multi-leader trees. Originally producers induced two-leader trees by heading newly planting trees in the orchard and choosing two leaders (Dorigoni, 2011). More recently, these two-leader trees are produced in the nursery with two equally strong branches that serve as the leaders, originating 25 cm above the ground. To achieve uniform axes, bench grafting has been replaced by double chip budding (Dorigoni, 2011). This system has been patented as Bibaum® (Musacchi, 2008). These preformed double axis nursery trees reduce the need for heading newly planting trees thereby allowing more shoot formation during the first growing season in the orchard (Musacchi, 2008).

In this paper, we evaluate the potential of propagating containerised “feathered” and two-leader apple nursery trees.

Materials and Methods

Plant material and site description. Trials were conducted on two-year-old containerised ‘Golden Delicious’ apple nursery trees on Nic 29 Malling 9 (M.9) rootstock. Rootstocks, harvested from stool beds (15 August 2016) and planted in black plastic bags containing coir medium, were chip-budded on 1 October 2016 and placed under a 40% Black/White shade net with a shade factor of 32% (Knittex (Pty) Ltd, Brackenfell, Western Cape) and grown as a whip for one season. Trials were conducted at Witzenberg Range Nursery, a commercial nursery, situated in Simondium, Western Cape region (33°50'14.1"S 18°57'43.6"E, Altitude of 150 m, Mediterranean-type climate, 664 chilling units (Daily positive chill unit model, Linsley-Noakes et al., 1994) and 856 mm annual rainfall). Irrigation scheduling varied during the growing season according to continuous logging soil moisture probes (DFM Technologies, Eersterivier, South Africa) in the planting medium. Standard commercial plant protection and fertilizer practices were followed.

Propagation of "feathered" nursery trees – 2017/2018 season. Originally, the idea was to compare proleptic branching with sylleptic branching, but bud break was inadequate following two 1.5% hydrogen cyanamide (3% Dormex®) applications. Hence, proleptic branching was not evaluated. The whip trees were approximately 1.5 m tall before the onset of the trial. Trees were headed (27 October 2017) at 65 cm above the medium surface. Trees were spaced 33 x 85 cm. After the new growth reached approximately 10 cm, sylleptic branching was induced by applying cytokinins and gibberellins (6-BA + GA_{4+7}) at 475 $\mu l \cdot L^{-1}$ [Promalin® 10SG, Valent BioSciences Corporation, Long Grove, IL, USA] three times (10 November 2017, 24 November and 8 December 2017) to the apical part of the tree. Every week after application, small leaves immediately below the apical meristem were pinched by hand. All the foliar applications were applied with a manual hand pressure sprayer.

Propagation of two-leader "feathered" nursery trees – 2017/2018 season. Two-leader "feathered" tree propagation consisted of heading trees (27 October 2017) at 25 cm above the growing medium (Fig. 1). Trees were spaced 33 x 85 cm. The two strongest shoots immediately below the heading cut served as the two new leaders. During this trial three different treatments were evaluated. The effect of PGRs on the apical extension growth of both leaders and the sylleptic shoot induction were evaluated. Foliar gibberellic acid (GA_3, 400 $mg \cdot L^{-1}$) [ProGibb® 40%, Valent BioSciences Corporation, Long Grove, IL, USA] was applied to the apical part of both leaders once a week (27 October 2017 until 22 December 2017, nine applications) to evaluate the effect on apical extension growth. Promalin® at 475 $\mu l \cdot L^{-1}$ was applied three times (10 November 2017, 24 November and 8 December 2017) to the apical part of both leaders to evaluate the induction of sylleptic shoots. A combination of the nine GA_3 applications and three applications of Promalin® at 475 $\mu l \cdot L^{-1}$ was applied to the apical meristem of both leaders to evaluate the combined effect of these two PGRs on the apical extension growth and sylleptic shoot induction. Every week after an application of Promalin®, small leaves immediately below the apical meristem were pinched by hand. This was not done on trees that only received nine GA_3 applications. All the foliar applications were applied with a manual hand pressure sprayer. A randomized complete block design with ten single tree replications was used.

Data collection: The following data were recorded on “feathered” trees: Number and length of sylleptic shoots and total new growth. On the two-leader trees the extension growth of the two leaders was measured as well as the number and length of the sylleptic shoots determined.

Results and Discussion

Propagation of “feathered” nursery trees. The application of 6-BA + GA_{4+7} induced on average four (± 0.7) sylleptic shoots with an average length of 33.1± 5.2 cm. On average, the total new growth produced by the sylleptic shoots was 132.5 ± 18.4 cm. Dorić et al. (2015) established that BA + GA_{4+7} significantly increased the number of lateral shoots produced on two-year-old “feathered” apple trees. The authors established that three applications of 400 $\mu l \cdot L^{-1}$ BA + GA_{4+7} (Progerbalin LG®) produced, on average, more than ten sylleptic shoots on three different apple cultivars, viz. ‘Jonagold Red Jonaprince’, ‘Golden Delicious Reinders’ and ‘Granny Smith’, grafted on M.9 rootstocks. The smaller number of sylleptic shoot produced in our containerised trees is probably due to a relatively small root volume in the 8 L bag. In addition, nursery trees grafted on more vigorous rootstocks compared to M.9, such as M.26 and MM.106, have a greater tendency to produce lateral shoots (Jaumien et al., 1993). Hence, to induce sufficient lateral branching on trees grafted on a dwarfing rootstock with a limited root volume, possibly requires more than three applications of 6-BA + GA_{4+7}, or trees should be propagated in larger containers.

Propagation of “feathered” two-leader trees. There was no significant difference between PGR treatments in the combined apical extension growth of the two leaders (Table 1). One would have expected that the GA_3 treatment without 6-BA+ GA_{4+7} would have increased the apical extension growth. Without 6-BA+ GA_{4+7}, the sylleptic shoot competition would be less and one would have expected the stimulation of apical extension growth. GAs promote apical dominance (Pharis et al., 1970) and regulates cellular processes such as cell elongation and cell division (Bulley et al., 2005). Multiple applications of GA_3 (400 $mg \cdot L^{-1}$, 600 $mg \cdot L^{-1}$ and 800 $mg \cdot L^{-1}$), GA_{4+7} (400 $mg \cdot L^{-1}$, 600 $mg \cdot L^{-1}$ and 800 $mg \cdot L^{-1}$) as well as 6-BA + GA_{4+7} (800 $mg \cdot L^{-1}$) promoted apical extension growth on first leaf ‘Rosy Glow’/MM.109 apple trees in a commercial orchard (Müller and Theron, 2018). The authors recommended that ten applications of GA_{4+7} at 400 $mg \cdot L^{-1}$ should be applied weekly from green tip to increase apical extension growth.

Although not significant, there was a trend ($p = 0.0536$) that the difference in the length of the two leaders was influenced by the PGR treatments (Table 1). Applications of GA_3 and 6-BA + GA_{4+7} produced trees with the best balance and uniformity of the two leaders with an average difference of only 7 cm, whereas applications of 6-BA + GA_{4+7} resulted in the greatest imbalance.

Promalin® (6-BA + GA_{4+7}) is the most common plant growth regulator used for sylleptic branching (Wertheim and Estabrooks, 1994). There was no significant difference between our treatments in the number of sylleptic shoots induced. The number of sylleptic shoots were however very low (≤1.3 per tree) in all treatments (Table 1). The fewer sylleptic shoots developing compared to the "feathering" trial could be due to the two leaders developing and competing with each other. Axillary meristems can be released from apical dominance by repeated applications of exogenous cytokinins (Li and Bangerth, 2003). The mechanism is still unclear, but the authors suggested that exogenous cytokinin applications increase the auxin (IAA) synthesis, as well as the IAA export from the axillary meristem which then is responsible for the growth of these meristems (Li and Bangerth, 2003). In essence, 6-BA is responsible for the release of axillary meristem inhibition and GA_{4+7} stimulates growth of the sylleptic shoot (Wertheim and Webster, 2005). Every week after an application of 6-BA + GA_{4+7}, the young leaves directly below the apical meristem were pinched off by hand, although not on trees only receiving nine GA_3 applications. The primary correlative inhibition signal of axillary meristems by the apical meristem is derived from the young leaves around the apex (Webster, 2005). Wertheim (1978) established that the number of sylleptic shoots was significantly increased when small leaves were removed. Thus, one would have expected that the lack of pinching with the repeated applications of GA_3 would produce fewer sylleptic shoots compared to 6-BA + GA_{4+7}. Three applications of 400 $\mu l \cdot L^{-1}$ BA + GA_{4+7} (Progerbalin LG®) significantly increased the length of sylleptic shoots on 'Jonagold Red Jonaprince' and 'Golden Delicious Reinders' grafted on M.9 rootstocks. However, the length of sylleptic shoots was not significantly increased by these applications on 'Granny Smith'/M.9 trees. In our trial, there was no significant difference regarding sylleptic shoot length between the treatments. This effect can possible be ascribed to the small root volume of containerised trees.

Conclusion

The application of 6-BA + GA_{4+7} induced sylleptic shoots to produce "feathered" apple nursery trees. However, the number of sylleptic shoots was probably restricted due to a limiting root volume. This can potentially be negated in future by a higher number of applications, higher rates of PGRs or larger containers allowing for a bigger root volume. The application of GA_3 with 6-BA + GA_{4+7} showed promise in propagating two-leader trees with two leaders of similar length, but the number and/or rate of applications should be increased and possibly a larger container used. Alternatively, the effect of ten applications of GA_{4+7} should be investigated.

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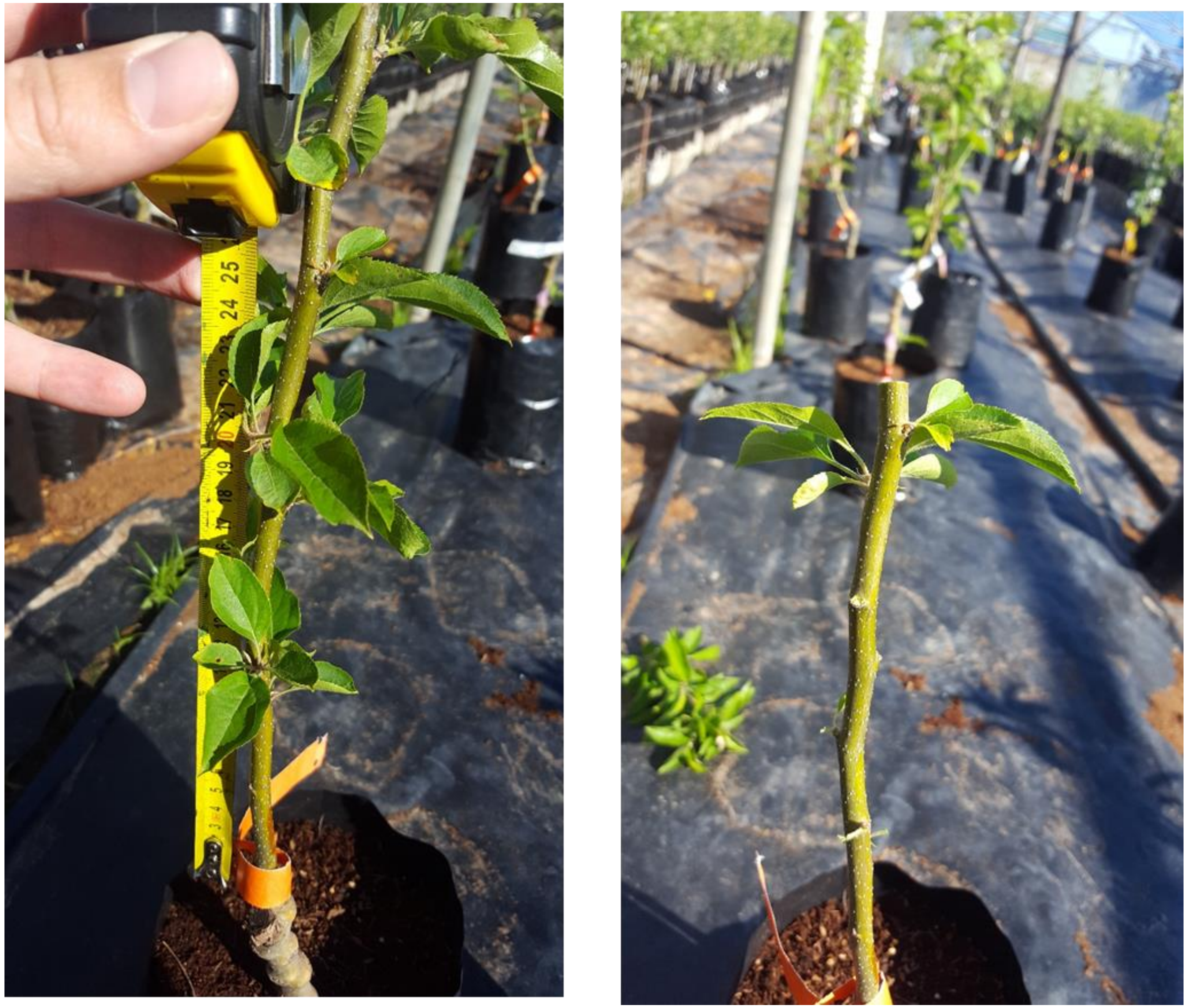

Fig. 1 Containerised ‘Golden Delicious’ apple nursery trees on M.9 rootstock headed (27 October 2017) at 25 cm from the planting medium.

Table 1. Effect of GA_3 (ProGibb®), 6-BA + GA_{4+7} (Promalin®) and a combination of GA_3 (ProGibb®) with 6-BA + GA_{4+7} (Promalin®) on new growth of containerised two-leader 'Golden Delicious'/M.9 nursery trees at Simondium, Western Cape (2017/2018).

Plant growth regulator	Combined apical extension growth (cm)	Difference in length between two leaders (cm)	Number of sylleptic shoots	Average length of sylleptic shoots (cm)
GA_3*	167.1 ns	10.5 ns	0.8 ns	31.8 ns
GA_{4+7} + 6-BA**	153.8	15.8	1.3	44.5
GA_3* and GA_{4+7} + 6-BA**	151.7	7.0	1.3	52.5
Significance level				
Treatment	*0.2408*	*0.0536*	*0.4809*	*0.6165*
LSD 5%	-	-	-	-

*400 mg·L^{-1}
**475 µl·L^{-1}

GENERAL DISCUSSION AND CONCLUSIONS

The quality of nursery trees at planting has a significant effect on productivity during the early years of a newly established orchard (Van Oosten, 1978). One of the main concerns when establishing a new orchard is the prolonged establishment period of young trees. This is characterised by a period of slow growth after planting trees in the orchard (Struve, 1990) and is commonly referred to as transplant shock (Watson, 1986). There is a need to optimise management, handling and storage practises to improve the physical and physiological characteristics of nursery trees and to overcome problems that are currently experienced.

Prohexadione calcium (ProCa) and abscisic acid (ABA) was applied during autumn to induce growth cessation and hardening-off in order to improve bud break and tree growth in the orchard after planting. ProCa (125 $mg \cdot L^{-1}$) and ABA (500 $\mu l \cdot L^{-1}$) had no significant effect on growth cessation during the nursery phase as well as on bud break and tree growth in the orchard. Previously it was established that multiple applications of 800 $mg \cdot L^{-1}$ ProCa on citrus nursery trees (Le Roux and Barry, 2010) and multiple applications of 1000 $mg \cdot L^{-1}$ ABA on apple nursery trees (Guak and Fuchigami, 2001) induced growth cessation and/or hardening-off. In addition, the trees in our study were growing under favourable conditions under shade netting. Shade netting increases plant biomass and vegetative growth that is associated with an increase in leaf conductance and a higher CO_2 assimilation rate (Raveh et al., 2003). Hence, the low concentrations of plant growth regulators (PGRs) used in our trial as well as die favourable growing conditions could be the reason for the lack of effect of ProCa and ABA. In future, the rate and number of applications can be increased to induce growth cessation and hardening-off and new orchard management strategies need to be evaluated. The lack of response in the orchard might be ascribed to orchard management practises that did not optimise the vigour of the trees.

Van Zyl (2016) established that there is a peak in root growth during late autumn and winter in South Africa. We postulated that planting trees in autumn would allow root development in the mild Elgin winter therefore resulting in a better developed, larger root system compared to spring planted trees and thereby enhanced bud break in spring. However, trees planted in autumn in the mild winter region did not accumulate sufficient chilling and this resulted in reduced bud break during spring. In addition, a basal dominant tree architecture was visible in autumn-planted trees due to a lack of winter chilling. Future research can focus on root growth when trees are planted in autumn. In addition, new management practises specifically for autumn-planted trees need to be evaluated.

Foliar nitrogen (urea) was applied to improve the nitrogen (N) reserve build-up in nursery trees and subsequently bud break and tree growth in the orchard. Foliar N did not significantly improve the percentage bud break in spring or new growth. The bud break percentage for all the treatments in this trial was more than 90%, which left little room for improvement. The analysis of N levels is very expensive and due to the absence of an effect of foliar treatments on tree performance, N reserves were not analysed. We believe that the lack of response can be ascribed to an overriding effect of unsuitable orchard management practises. In future research, the N levels of each treatment needs to be analysed to determine if the application of foliar N increased the build-up of reserves.

Usually natural leaf drop is delayed in warmer regions and contributes to logistical issues. Preferably, nurserymen want to lift trees after natural leaf drop, but before the onset of winter rain which will make the nursery impassable. Foliar copper (Cu) (1.5 $g\cdot L^{-1}$) and 1-aminocyclopropane-1-carboxylic acid (1000 $\mu l\cdot L^{-1}$) were applied to induce defoliation. Both chemical defoliants were successful in defoliating the trees, with foliar Cu being the more effective of the two. The difference in modes of action of the two chemical defoliants can explain the higher efficacy of Cu. Previous research showed that CuEDTA decreases the growth of apple trees after planting due to the rapid defoliation and insufficient translocation of N from the leaves (Guak et al., 2001). This negative effect was not visible in our trial.

A combination of foliar Cu and urea was applied to increase the N levels in the foliage before inducing chemical defoliation. Because both Cu and urea can be used as defoliants, consecutive applications of these two defoliants during our trial contributed to immediate defoliation. Hence, the rate of defoliation could not be monitored. In future, the effect of combining foliar N with ACC, a less potent chemical defoliant, which will potentially allow translocation of N before defoliation of trees take place. In addition, a lower concentration of Cu in combination with urea can be evaluated.

Dormancy management practises were evaluated to negate the negative effects of insufficient chilling. All of the dormancy management practises improved the percentage and rate of bud break. Trees receiving six-weeks cold storage set at 6 °C with or without a 1.5% hydrogen cyanamide (3% Dormex®) rest breaking treatment had a more even distribution of new growth along the axis of the tree, which can be described as a mesotonic growth tendency

(Bell, 2008). In contrast, the untreated control trees as well as trees that only received the chemical rest breaking treatment had a basitonic growth tendency. The chemical rest breaking treatment had no additional benefit regarding tree architecture. Therefore, the current practice used for bare-rooted trees viz. chemical rest breaking following cold storage should also be used for containerised apple nursery trees.

Different planting methods were evaluated to determine what the preferred method of planting containerised nursery trees should be. According to Castle (1987), there are conflicting reports regarding the field performance of containerised citrus nursery trees following different handling and planting methods. During our trial, the disturbance of the growing medium had no significant influence on bud break in spring. However, the rate of bud break was increased when the growing medium was washed off or shaken off. The total new shoot growth was the highest when trees were planted with an undisturbed growing medium or after slight loosening of the growing medium. In addition, the apical extension growth of the trees were significantly increased when trees were planted with the growing medium undisturbed. When the growing medium was washed off or shaken off before planting, roots could have possibly died or dried out due to reduced contact between the roots and soil. However, if the contact between the roots and soil can be improved when the growing medium is washed off or shaken off, better performances can be expected.

PGRs were applied to produce “feathered” nursery trees as well as two-leader nursery trees. Branched or “feathered” nursery trees, as well as multi-leader nursery trees, may fill orchard space faster and thus start yielding fruit earlier. Cytokinin + gibberellic acid (GA_{4+7}, 475 $\mu l \cdot L^{-1}$) induced lateral sylleptic shoots on nursery trees, but the number of shoots was low possibly due to the relatively small root volume associated with the containerised nursery trees. Although not significant, there was an advantage in applying gibberellic acid (GA_3, 400 $mg \cdot L^{-1}$) with cytokinins + gibberellic acid (6-BA + GA_{4+7}) (475 $\mu l \cdot L^{-1}$) to propagate two-leader trees in terms of the balance between the two leaders. Due to the limited root volume of containerised nursery trees, the number and/or rate of applications should be increased as well as the size of the container.

Conclusion

No clear conclusion could be made on the use of PGRs to harden-off trees or the use of chemical defoliants in our trials. Trees planted in autumn in a warm region did not accumulate sufficient chilling which resulted in reduced bud break and a basal dominant tree architecture. In future, the performance of autumn-planted trees can be evaluated in a colder region. The negative effects of a lack of winter chilling could not be overcome by only a chemical rest breaking application. As trees were not ideally managed in the orchard, the treatment effects could have been masked by a lack of tree vigour during the first growing season in the orchard. To negate the effect of insufficient chilling, dormancy management practises are beneficial for spring bud break and tree architecture. Because containerised apple trees are a new concept in South Africa, further research is needed to evaluate and determine management, handling and storage strategies.

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