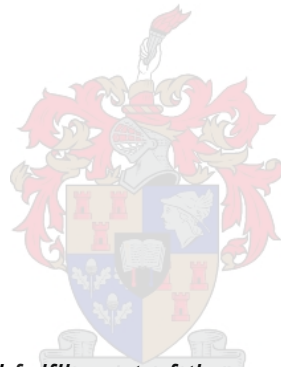


Investigating the vegetative development and yield of pecan

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

Like other deciduous trees grown in regions that experience warm winters and low winter chilling, pecans suffer from low and sporadic bud break. This often has negative implications for vegetative growth and development of the trees, especially in younger orchards that are still filling space. Furthermore, the yield will often be reduced as a result of reduced vegetative development as well as a lack of synchronisation in the flower development.

Application of hydrogen cyanamide (HC) formulated as Dormex® was evaluated on two pecan cultivars, 'Wichita' and 'Navaho', grown in the Prieska region, Northern Cape, South Africa. Three different concentrations were evaluated on two application dates. Trees were assessed for time of bud break, final percentage of total bud break, shoot growth and yield. Additionally, the effect of HC on the synchronisation of the flowering periods of 'Wichita' as the main cultivar and 'Navaho' as the cross pollinator, was assessed. During 2018, after a relatively cold winter, Dormex® had a relatively small effect on the bud break parameters of 'Wichita' trees. On 'Navaho' trees, all treatments were effective at increasing the percentage bud break. During 2019, a warmer winter was experienced. On 'Wichita', an early application four weeks before expected bud break (4WBBB) was more effective at advancing bud break than a later treatment, two weeks before expected bud break (2WBBB). Dormex® at 4% resulted in a significantly higher initial bud break percentage than the control on both cultivars and Dormex® at 4% and 2%, resulted in a significant increase in the final percentage bud break during the warmer year. These results indicate the total bud break (vegetative and reproductive buds) and do not indicate improved vegetative development or improved flowering *per se*.

No increase was seen in vegetative bud break during the 2019 season on either cultivar and this should be evaluated further. Dormex® at 4% 4WBBB resulted in a significantly higher yield on 'Wichita' compared to the control during the first season and this needs to be followed up during 2020. No increase in yield was seen on 'Navaho'.

The best result for improving the synchronisation of the flowering periods was achieved by treating 'Wichita' with 4% Dormex® 4WBBB to significantly advance bud

break, while leaving 'Navaho' trees untreated. No signs of phytotoxicity on leaves or shoots were observed for any of the treatments.

In conclusion, the effect of HC on improving the vegetative development of these cultivars could not be established, as no distinction was made between vegetative and reproductive buds during bud break and more research is required. HC applications did however influence time and percentage bud break. It is possible that the effect could be extended towards yield through better synchronisation of the flowering periods, but this effect can only be quantified during 2020 and thus could not be included in this thesis.

The effect of notching shortly before bud break was investigated on young, non-bearing 'Wichita' pecans grown in the Villiersdorp region, Western Cape, South Africa. The effect on bud break, shoot number, shoot length and branch angle was evaluated. On two-year-old wood, notching was unable to increase bud break or shoot number and was influenced to a certain extent by the size of the bud. No increases were seen in shoot length on two-year-old wood. On one-year-old wood, notching significantly increased the number of.

In conclusion, notching can increase lateral vegetative development of young trees if carried out on younger wood and could be used as a valuable tool for improving vegetative development and ensuring more successful orchard establishment.

OPSOMMING

Pekanneutbome wat gevestig word in areas met gematigde winters is geneig om lae en wisselvallige knopbreek te toon, soortgelyk aan ander bladwisselende gewasse wat onder soortgelyke toestande ontwikkel. Hierdie onvoorspelbare knopbreek het tot gevolg dat die ontwikkeling van jong bome benadeel word wat dus die vestigingsperiode van boorde onnodig verleng. Die verminderde vegetatiewe ontwikkeling beperk neutopbrengs deurdat drahout beperk word en verder beïnvloed die wisselvallige knopbreek blomontwikkeling, wat opbrengste direk beperk.

Toediening van waterstofsianamied (geformuleer as Dormex®) is op twee pekanneutkultivars, 'Wichita' en 'Navaho', geëvalueer in Prieska in die Noord-Kaap provinsie van Suid-Afrika. Drie verskillende konsentrasies is op twee toedieningstadiums geëvalueer. Die datum van knopbreek, finale persentasie knoppe wat gebreek het, totale lootgroei en die opbrengs per boom is bepaal. Die effek van waterstofsianamied op die sinchronisasie van die blomtye van 'Wichita' (die hoofkultivar) en 'Navaho' (die kruisbestuier) is ook bepaal.

Gedurende 2018, na 'n koue winter, het waterstofsianamied 'n relatiewe klein effek op die knopbreek parameters van 'Wichita' gehad. Op 'Navaho' bome het al die behandelings finale knopbreek verhoog. Gedurende 2019 is minder koue-eenhede geakkumuleer en in 'Wichita' was 'n vroeë toediening vier weke voor verwagte knopbreek (4WBBB) meer effektief om knopbreek te bevorder as 'n later behandeling, twee weke voor verwagte knopbreek (2WBBB). Dormex® teen 4% het aanvanklik 'n hoër persentasie knopbreek getoon teenoor die kontrole vir beide kultivars, en 4% en 2% Dormex® het 'n betekenisvolle toename getoon in die finale hoeveelheid knopbreek in die warmer jaar. Hierdie resultate wys net die totale knopbreek en dus is die verhoogte knopbreek nie 'n aanduiding van 'n verhoogde vegetatiewe groei of 'n verhoogte aantal blomme *per se* nie.

In die 2019 seisoen, is geen toename in vegetatiewe knopbreek waargeneem nie op beide kultivars nie en die resultaat moet verder evalueer word. Op 'Wichita' het die toediening van 4% Dormex® 4WBBB gelei tot 'n betekenisvolle hoër opbrengs in vergelyking met die kontrole gedurende die eerste seisoen en die effek moet opgevolg word gedurende 2020. Op 'Navaho' was daar egter geen toename in opbrengs nie.

Die beste resultaat in verbetering van die blomtyd sinchronisasie is bereik met 'n toediening van 4% Dormex® 4WBBB op 'Wichita', terwyl 'Navaho' bome onbehandeld gelaat word. Geen tekens van fitotoksisiteit is waargeneem in enige behandeling nie.

Ten slotte kon die effek van waterstofsianamied op die verbetering van die vegetatiewe ontwikkeling op die kultivars nie bepaal word nie, aangesien daar nie onderskeid gemaak is tussen vegetatiewe en reprodktiewe knoppe gedurende knopbreek nie en meer navorsing word benodig. Waterstofsianamied het wel die tyd en persentasie knopbreek beïnvloed. Dit is moontlik dat die effek uitgebrei kan word na opbrengs deur beter sinchronisasie van die blomperiodes, maar die effek kan eers gedurende 2020 gekwantifiseer word en kon dus nie ingesluit word in die tesis nie.

Die effek van kerfies kort voor knopbreek is op jong, nie-draende 'Wichita' pekanneut bome ondersoek in die Villiersdorp area, Wes-Kaap, Suid-Afrika . Die effek op die knopbreek, aantal lote, loot lengte en takhoeke is geëvalueer. Op twee-jaar-oue hout kon die kerfies nie knopbreek of die aantal lote verhoog nie en dit is moontlik dat knopbreek tot 'n mate beïnvloed is deur die grootte van die knop. Geen verhogings is gesien in lootlengte op twee-jaar-oue hout nie. Op een-jaar-oue hout kon kerfies egter die aantal lote betekenisvol verhoog. Ter afsluiting, kerfies op jong bome kon laterale vegetatiewe ontwikkeling verhoog. Dit kan gebruik word as 'n waardevolle hulpmiddel om vegetatiewe ontwikkeling te verbeter en om 'n meer suksesvolle boordontwikkeling te verseker.

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This thesis is presented as a compilation of four chapters, starting with a literature review followed by three research papers. Each paper was therefore introduced separately and prepared as a journal article. Therefore, repetition and overlap between chapters will be present.

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GENERAL INTRODUCTION

The Pecan industry in South Africa has recently seen significant growth with increasing export to China. In the last decade, pecan plantings have almost tripled (SAPPA, 2019). Therefore, many pecan orchards in South Africa are still very young.

Vegetative development of young orchards is very important as the trees must still fill their allocated space and develop the optimal structure, which will impact on the yield and profitability of the orchard. On pecans, pistillate flowers are borne on the tips of new shoots growing from one-year-old wood (Woodroof & Chapman-Woodroof, 1926, Wetzstein *et al.*, 1996). This emphasises the importance of vegetative development, as an increase in vegetative growth should theoretically increase the number of bearing units thereby increasing the yield.

As pecans exhibit a deciduous nature, they, like other deciduous trees, have a chilling requirement that must be fulfilled during winter to ensure optimal growth in spring (Romberger, 1963). Pecans, however typically have a much lower chilling requirement than other deciduous fruit crops (Wood, 1993; Küden *et al.*, 2013). This makes them ideal to be grown in areas that are too warm for other deciduous crops such as pome and stone fruit. However, pecans may still suffer poor bud break after the dormant period, characterized by many buds staying dormant and bud break happening over an extended period (Lagarda, 1987). With global temperatures increasing as a result of climate change (Luedeling *et al.*, 2009) this problem is expected to become worse. As a result of poor bud break in spring, several manipulations have been evaluated and are being used extensively in the deciduous fruit industry in the Western Cape. These techniques have, however, not yet been investigated in pecans under local environmental conditions. They offer the potential to improve the spring growth of pecans, thereby ensuring that the tree can fill its allocated space and develop the optimal structure as quickly as possible and come into bearing sooner and increase the profitability of pecans.

Hydrogen cyanamide, formulated as Dormex®, is one such manipulation shown to increase bud break and increase growth in many deciduous crops (Ghrab & Mimoun, 2014; Fayek *et al.*, 2008). Additionally, it has the potential to advance the bud break period (Bound & Jones, 2004; Wood, 1993), thus having the potential to manipulate the synchronisation of the flowering periods of the cross pollinator and

main cultivar and possibly lead to improved yields through better pollination. Another manipulation that can be applied at the end of the dormant period is 'notching'. A small cut is made above dormant buds, stimulating them to grow through hormonal mechanisms (Teichmann & Muhr, 2015). Notching on apples increased the development of lateral shoots (Greene & Autio, 1994; Clements et al., 2010). Notching also has the potential to be a powerful tool in developing the ideal structure of non-bearing pecans.

In Paper 1, we determined the effect of concentration and application time of Hydrogen cyanamide on bud break, vegetative development and yield of 'Wichita' pecans grown in the Prieska region, Northern Cape, South Africa, a region that receives marginal chilling and experiences relatively high maximum temperatures during winter.

In Paper 2, the effect of Hydrogen cyanamide on the bud break parameters and yield of 'Navaho' pecans is discussed. In the marginal climate of Prieska, the effect of Hydrogen cyanamide on the synchronisation of the flowering periods of 'Wichita' as the main cultivar and 'Navaho', as the cross pollinator, and how this impact on yield, is discussed.

In Paper 3, we determined the effect of notching on bud break and vegetative development of one- and two-year-old wood of non-bearing pecans grown in the Villiersdorp region, Western Cape, South Africa and discussed factors that played a role in determining whether a bud will grow out.

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LITERATURE REVIEW: BIOTIC AND ABIOTIC FACTORS INFLUENCING BUD BREAK IN DECIDUOUS FRUIT AND NUT CROPS.

Introduction

Universally, across genera, woody perennial trees exhibit rhythmic growth patterns with developmental processes such as shoot elongation, root growth and flowering, occurring in flushes, either concurrently or non-concurrently, during the growing season (Romberger, 1963). Growth flushes usually only occur during periods when environmental conditions are conducive to growth. Mature trees tend to exhibit timeous growth flushes throughout the growing season, whereas young trees often grow continuously throughout the growing season (Borchert, 1991).

These periodic growth cycles are more pronounced in deciduous tree species that lose their leaves and form tight buds during the winter months. This phenomenon is referred to as dormancy and is broadly defined as a temporary suspension of visible growth of any plant structure containing a meristem (Lang, 1987). Dormancy in temperate tree species enables them to survive the extreme low temperatures commonly experienced in the regions where these trees originate (Erez, 1995). The bud must undergo morphological and physiological changes at the end of the warmer growing season to both protect the buds from sub-zero temperatures that may be experienced during winter, and to synchronise their growth and flowering with the warmer temperatures experienced in spring (Erez, 2000). Bud dormancy is induced by a number of environmental signals, but is mainly caused by low temperature and, to a lesser extent, a short photoperiod (Campoy *et al.* 2011). After shoot growth cessation, the buds of many deciduous trees become enclosed by modified, hardy foliage leaves, known as bud scales. Following winter, the more favourable growing conditions during spring stimulates the opening of bud scales, enabling the bud to develop into a shoot or a flower (Borchert, 1991). This ultimately determines the potential fruit yield.

Lang (1987) defined dormancy as a progression between three phases: paradormancy, endodormancy and ecodormancy. Paradormancy is often referred to as correlative inhibition and is the phenomenon where the growth of axillary buds is

inhibited by a signal that originates from another plant part, such as the apical meristem, exerting dominance over lateral buds on the same branch (also known as apical dominance). Endodormancy is the state of deep dormancy, when buds are prevented from growing out because of a signal that originates from within the bud itself. This type of dormancy is under genetic control and endodormant buds will not grow out, even if placed under favourable environmental conditions. This prevents the buds from growing out in mid-winter, when the low temperatures could damage new growth. The last stage of dormancy is ecodormancy, where buds do not grow out because of unfavourable environmental conditions. If ecodormant buds are placed in warm conditions, growth will commence immediately (Lang, 1987).

During the last 30 years, dormancy research has changed drastically as research techniques and analytical technology improved. A shift in focus from environmental and hormonal cues to molecular and genetic aspects of dormancy ensued, with the aim to uncover the secrets of dormancy on a biochemical level (Arora *et al.*, 2003). This led to the following discoveries. During dormancy, vascular as well as plasmodesmatal connections in the plant become disrupted. Communication and signalling between cells in the plant are thereby reduced. Consequently, the buds respond independently of one another to cues and regulatory signals of dormancy. This can lead to variations in bud break responses, especially under conditions that cause incomplete dormancy, such as insufficient winter chilling (Erez, 2000). This variation in bud break responses has serious horticultural implications for temperate fruit crops grown in warmer regions. There are three main problems associated with variable bud break caused by incomplete dormancy – as it impacts on yield. These include: late bud break, a low bud break percentage and protracted, non-uniform bud break. Late bud break is problematic in fruit and nut crops as it leads to a later harvest time and potential lower economic return. It also creates a problem if the specific crop needs cross pollination and the main cultivar and cross pollinator's bloom are not synchronised (Dennis, 2000). A low bud break percentage in spring not only directly influences the number of flowers available to set fruit, but also the number of vegetative buds that break and develop into future bearing positions. This often results in a loss of vigour and lower yields (Arora *et al.*, 2003). Furthermore, a tree with a lower bud break will have fewer leaves, reduced photosynthetic potential and smaller fruit, with a higher chance of sunscald due to reduced shading (Erez, 2000). Non-uniform bud break results in fruit of different maturities on the tree. This requires

multiple harvests to maintain quality – posing logistic and economical challenges in practice (Dennis, 2000). In pecan, the main challenge is the effect on yield – as influenced by number of bearing positions in the current season, as well as the potential photosynthetic capacity of the leaves – all affected by bud break percentage.

This review will explore the biotic and abiotic factors that influence natural bud break in deciduous fruit and nut crops.

Environmental factors

As dormancy is a means for the plant to survive extreme climatic conditions, it is logical that the environment plays a major role in regulating the start and end of dormancy. There are two main environmental factors that play a role in regulating dormancy: photoperiod and temperature (Arora *et al.*, 2003). Of these two factors, low temperature is the main regulating signal involved in dormancy (Borchert, 1991; Heide & Prestrud, 2005).

Effect of photoperiod

Many plant species are sensitive to changes in photoperiod and use seasonal changes to synchronise their growth and developmental processes with the correct time of year (Romberger, 1963). Depending on the species and cultivar, trees have a variety of responses to photoperiod (Borchert, 1991). Li *et al.* (2003) showed that silver birch seedlings (*Betula pendula*) receiving a shorter photoperiod experience cessation of growth and leaf formation. In their study, three different ecotypes of silver birch from different latitudes were evaluated. Ecotypes from the higher latitudes were significantly more sensitive to changes in photoperiod and showed a much quicker response to short day lengths. In contrast, Heide & Prestrud (2005) found that photoperiod has no regulating effect on dormancy onset or completion in apple and pear trees. At low temperatures, the trees entered endodormancy regardless of photoperiod. They also found no growth cessation in plants kept at a high temperature and short days. It was concluded that the main environmental factor responsible for controlling bud dormancy in apple and pear trees is low temperature. The same experiment was conducted on *Prunus* species, which included crops such as almonds and cherries. In this trial, growth cessation in cherry trees kept at a low temperature of 9 °C was only observed when the plants were also kept under short day length. This is in agreement with Heide

(2008) for plums kept at 12 °C. Therefore, in the *Prunus* genus, dormancy is controlled by both temperature and photoperiod. These studies clearly show that different tree species vary in their sensitivity to photoperiod for entrance into dormancy.

Effect of temperature

Low temperatures

In temperate fruit and nut trees, the onset, maintenance and completion of dormancy is largely dependent on low temperatures (Campoy *et al.*, 2011; Heide & Prestrud, 2005). Most deciduous plants have a “chilling requirement” which must be satisfied during the winter months to ensure proper regrowth in spring (Erez, 1995). For most species, exposure to temperatures between 0 and 7 °C is optimal, but variation exists between species (Arora *et al.*, 2003). Generally, temperatures below 0 °C and above 13 °C have no effect on dormancy (Erez, 1995). Furthermore, different tissues in the plant have varying sensitivity to chilling temperatures. Compared to vegetative buds, flower buds typically have the lowest chilling requirement (Aslamarz *et al.*, 2009; Campoy *et al.*, 2011). Bud position also plays a role in the amount of chilling a bud requires, with apical buds typically having lower chilling requirements than lateral buds (Campoy *et al.*, 2011).

The chilling requirements of most deciduous fruit and nut tree species have been quantified. This knowledge is critical for growers to identify crops that are climatically suitable for specific growing regions and are profitable to grow. Chilling requirement can be calculated by placing rooted cuttings under controlled temperature conditions and measuring the bud break response (Sparks, 1993). Another method entails selecting excised shoots from the orchard after having recorded the chilling they acquired, and placing them at higher temperatures that force the buds to grow out. Alternatively, excised shoots can be collected at the beginning of the dormant season and exposed to chilling temperatures for a specific amount of time before placing them into forcing conditions at 25 °C. Bud break-counts are then conducted to determine if the chilling requirement was satisfied and what the specific chilling requirement is (Egea *et al.*, 2003; Küden *et al.*, 2013).

Aslamarz *et al.* (2009) determined that the chilling requirement of vegetative buds of Persian walnut cultivars ranges from 400 to 1000 hours below 7 °C. The chilling requirement of reproductive buds is lower, ranging from 400 to 1000 hours below 7 °C, and terminal buds require less chilling than lateral buds. Egea *et al.* (2003)

quantified the chilling requirement for 10 almond cultivars in an orchard in a Mediterranean climatic region in Spain as 266 to 1000 Richardson chilling units (RCU). Moreover, Küden *et al.* (1995) found that the chilling requirement of pistachio nuts ranges between 500 and 1000 hours below 7 °C. Elloumi *et al.* (2013) conducted a study on pistachio nuts grown in a warm and dry climate. The date of flowering was dependant on the chilling that accumulated, i.e. years with more chilling had earlier flowering periods. There was also a positive correlation between chilling and yield. Increased chilling resulted in a higher percentage of flowers following bud break and better fruit set. Sparks (1993) found that bud break still occurred in pecan with limited chilling, suggesting that pecans do not have an obligatory chilling requirement and concluding that the low chilling requirement of pecans explains the successful cultivation in warmer climatic regions, like South Africa, Brazil and Israel. However, chilling still results in more uniform flowering. In support, Kuden *et al.* (2013) reported that pecans require between 250 and 400 RCU chilling units to flower – a relatively lower chilling requirement compared to that of other deciduous crops.

High temperatures

While the effect of low temperatures on the entry and release of dormancy in deciduous trees is well documented, studies on the effect of high temperatures during dormancy are limited. Erez *et al.* (1979) used rooted peach cuttings to determine the effect of varying high temperature cycles during the dormant period on bud break. Cycle lengths of one, three, six and nine days were used, where the first two thirds of each cycle comprised chilling temperatures of 4 to 6 °C, and the last third of each cycle, a high temperature of 24 °C. Plants were exposed to this cycle until the required number of chilling hours accumulated. Plants exposed to the one-day cycle had no bud break, while plants exposed to the three-day cycle had very low bud break. This was in stark contrast to the plants under a six- and nine-day cycle, where normal bud break occurred. It was concluded that chilling temperatures that accumulate 20-40 hours before high temperature exposure, are ineffective and do not contribute to chilling accumulation due to negation (Erez *et al.* 1979).

This effect of chilling negation has serious implications for deciduous fruit and nut crops in regions that commonly experience high temperatures during the winter. Chilling negation leads to severe problems with spring bud break. Insufficient chilling leads to a protracted, non-uniform and often late bud break, as well as a low

percentage bud break. Poor spring regrowth has serious negative implications for crop management and production (Küden *et al.*, 1995; Erez, 2000). For instance, in pistachio nuts, Elloumi *et al.* (2013) found a positive correlation between nut yield and chilling accumulation under dry and warm climatic conditions.

Increasing temperatures as a result of climate change have serious implications for the future of the deciduous fruit and nut industry. A study in California, USA, showed that some areas of California experienced a 30 % loss in winter chilling from 1950 to 2000 (Luedeling *et al.*, 2009). The same study used climate projection models to show that this decline would reach 60 % by the end of the 21st century and it was concluded that, by the end of the century, crop production will no longer be viable for any crop with a chilling requirement higher than 700 chilling hours. A similar study was conducted in the Western Cape region of South Africa, the main production region for deciduous crops. Between 1967 and 2007, the region experienced a 26 % decline in seasonal chilling (Midgley & Lötze, 2011). This means that crops such as pecan which have a fairly low chilling requirement (Küden *et al.*, 2013) are an alternative option in areas where climate change threatens the production of high chilling crops, such as apples.

However, for buds to break in spring, a certain amount of warmth is required after the chilling requirement has been satisfied. For example, late flowering walnut cultivars typically require more chilling and have higher heat requirements than that of early flowering cultivars (Aslamarz *et al.*, 2009). The same study found that exposure to chilling temperatures after the chilling requirement has been satisfied leads to a reduction in the amount of heat required for a satisfactory bud break. Egea *et al.* (2003) confirmed this for almonds and walnuts. They speculated that more heat was required by the late flowering cultivars because they were not exposed to low temperatures for as long as the early flowering cultivars after their chilling requirement was satisfied. Therefore, the flowering date is mainly influenced by the chilling requirement and heat during spring only plays a secondary role. In contrast, in pecans, Sparks (1993) found that bud break is under the interactive control of both chilling and heating. Once the chilling requirement is satisfied, the date of bud break is highly dependent on heat accumulation, indicating differences between deciduous crops.

Plant growth regulators

Endogenous plant hormones act as signal molecules between various plant organs and play an important role in the regulation of a large number of plant processes that influence the growth and development of the plant (Osborne, 1965). One such process is apical dominance and the correlative growth inhibition of lateral buds (Romberger, 1963). The main growth regulator involved in apical dominance is auxin (IAA) (Bangerth *et al.*, 2000). IAA is usually produced in young shoots and leaves and then transported basipetally down the shoots towards the roots (Peer *et al.*, 2011). Unidirectional polar auxin transport originating in the apical buds prevents the outgrowth of lateral buds further down the shoot. When this dominant shoot tip is removed, the previously dominated shoot's IAA export levels increase to the same levels as the previously dominant shoot tip as the bud enlarges and starts to grow (Bangerth *et al.* 2000). Chatfield *et al.* (2000) also showed that in a decapitated *Arabidopsis* plant, IAA applied to the decapitated tip is still capable of inhibiting the outgrowth of lateral buds. However, most of these studies concluded that, while IAA does play an important role in the inhibition of laterals, it is most likely only the primary signal molecule, as IAA moving down the shoot is not translocated into the dominated buds (Bangerth, 1994; Chatfield *et al.*, 2000). This means that a secondary messenger must be responsible for translocating this inhibition signal into the lateral buds. Endogenous plant hormones are good candidates for this because of their involvement in so many plant signalling and regulatory processes.

Ethylene (ETH) and abscisic acid (ABA) have inhibitory effects on plant growth (Chatfield, 2000). However, neither ETH nor ABA act as the secondary signal molecule in the inhibition of lateral buds. An application of cytokinin (CK) to dominated buds leads to an increase in the rate of polar IAA transport out of the buds, subsequently releasing them from apical dominance (Bangerth *et al.*, 2000). Furthermore, it was also illustrated that when a dominant shoot is decapitated, there is a sharp increase in root derived CK in the xylem sap. In a similar study on *Arabidopsis* plants, Chatfield *et al.* (2000) found that the application of CK to the basal end of the plants stimulates the growth of lateral buds. Both authors concluded that IAA causes a suppression of CK synthesis or transport in the stem and roots where CK is produced. This prevents CK from moving into the lateral buds and stimulating growth. Another group of molecules that play a role in the inhibition of lateral buds are

strigolactones (SL). SLs inhibit the movement of IAA out of axillary buds, which subsequently suppresses lateral outgrowth and development (Peer *et al.*, 2011).

The process of lateral outgrowth is well described by Teichmann & Muhr (2015). Basipetal IAA transport decreases the expression of the CK biosynthesis gene, *IPT*, and induces the activity of CK oxidase, an enzyme responsible for the breakdown of CK. If the shoot apex is removed and there is a reduction in basipetal IAA transport, the CK concentration in the xylem sap increases, as IAA is responsible for downregulating CK synthesis. Increased CK levels in the xylem sap allows it to move into the dominated lateral bud. The increase in bud CK concentration, in turn, leads to the stimulation of the IAA synthesis capacity of the bud tissue, which allows the bud to export more IAA into the main stem. The establishment of an IAA export stream results in the stimulation of the formation of vascular tissues into the dominated bud, which allows a greater amount of water and nutrients to be transported into the bud, thereby, facilitating growth. Apical dominance or correlative inhibition plays a very important role in the later stages of apple bud dormancy (Faust *et al.* 1995). Decapitated apple shoots had 80 % lateral bud break, whereas shoots that did not have their terminal buds removed had only 30 % lateral bud break. This shows that a low lateral bud break percentage in spring is not necessarily only caused by insufficient winter chilling but is also, to a large extent, caused by the effect of apical dominance. Thus, manipulations that reduce apical dominance can also be applied when insufficient bud break occurs. Cook & Jacobs, (1999) investigated the effect that a mild winter had on bud break and found that when a branch received less chilling it exhibited a basitonic tendency with a low and sporadic bud break occurring more on the proximal end while branches that received sufficient chilling had a more even bud break with an acrotonic tendency with buds breaking on the distal end. This research shows that there is most likely an interaction between apical dominance and winter chilling.

Reserve status

Carbohydrate reserves

In deciduous trees, early spring growth must occur in the absence of photosynthesising leaves to supply carbohydrates to the new growth. This means that the plant must use stored carbohydrates that accumulated as reserves during the

previous growing season (Loescher *et al.* 1990). Carbohydrates supply carbon skeletons for a number of different molecules needed by the plant (Cheng & Fuchigami, 2002), act as building blocks for new growth and are the fuel for respiration (Loescher *et al.*, 1990). Lacoïnte *et al.* (1993) showed that, at the end of winter and in early spring, a sharp decrease occurs in reserve carbohydrate content in the perennial tissues of walnut trees, which is similar to pecans (Smith *et al.*, 2007) and pistachios (Spann *et al.*, 2008). Most of the reserve carbohydrates are recovered in the new growth. In pecans, the level of stored carbohydrates in winter has no effect on the production of flowers in spring (Kim & Wetzstein, 2005) as pecan flowers are produced after considerable leaf growth has already occurred (Smith *et al.* 2007), thus reducing their dependence on reserves compared to that of other crops.

Nutrient reserves

In addition to carbohydrates, plants must also store nutrient reserves. Bud break often occurs during a period where there is little or no uptake of nutrients by the roots and the plant must therefore make use of stored nutrient reserves until root uptake starts again (Millard, 1989). Stassen *et al.* (1981) showed that trees that received a higher concentration of nitrogen fertiliser in autumn grow better than trees supplied with lower nitrogen concentrations. Moreover, a study on Norway spruce showed that trees supplied with higher nitrogen concentration before winter have higher percentage bud break in spring (Fløistad & Kohmann, 2004). In hazelnuts, a very low percentage of the nitrogen taken up during spring is recovered in the new growth. This indicates that the plant primarily uses reserve nitrogen during the first part of the growing season (Braun *et al.*, 2009).

All the macro elements (N, P, K, Mg, Ca & S) decline in leaves between the maturation of pecan nuts and leaf drop (Kim & Wetzstein, 2005). This indicates that these nutrients are most likely translocated back into the permanent structures of the plant for storage over winter. Rosecrance *et al.* (1998) performed a similar study on pistachio. They concluded that the accumulation patterns of nitrogen by the leaves, nuts and new shoots do not correlate with the primary uptake period for nitrogen, which was later determined to occur during the nut fill period (Rosecrance *et al.*, 1998) and these results are similar to those of Acuna-Maldonado *et al.* (2003) for pecans.

It is clear that the storage of both carbohydrate and nutrient reserves are very important for bud break in deciduous tree species and must be addressed with

cultivation practices. An extended leaf drop period is beneficial because the tree has more time to produce carbohydrates for storage (Bennet *et al.*, 2005). This can be addressed with nitrogen applications. In peach trees, nitrogen application in autumn causes delayed defoliation and results in an earlier bud break and more uniform flowering the following spring (Stassen *et al.*, 1981). However, in cold climates, late growth of deciduous crops may pose a threat to plant survival and should be managed accordingly.

Physiology of dormancy and bud break

In recent years, advances in research techniques have allowed researchers to take a closer look at what happens in the bud on a molecular and cellular level and how this is influenced by the external environmental conditions. On a molecular level, there are three main factors to consider: the state of water in the bud, the membrane structure during dormancy and the respiratory potential of the buds (Faust *et al.*, 1997).

State of water in the bud

Faust *et al.* (1991) used magnetic resonance imaging to determine how much water occurred in the bud during dormancy. No image was generated for buds that had not completed their chilling requirement, however, buds that had received their complete chilling requirement generated a clear image indicating large amounts of free water. They concluded that water is bound or structured in the bud until chilling is satisfied. This led to the discovery of dehydrins. Dehydrins are proteins that show hydrophilic properties that allows them to bind water in the bud tissues. The concentration of dehydrins increases during dormancy and then decreases again as chilling accumulates and water is freed up (Erez *et al.*, 1997). In addition to apple buds, dehydrins have also been isolated from numerous other deciduous species. Almond floral buds have a higher concentration of dehydrins during dormancy than during other months (Barros *et al.*, 2012). The dormant inflorescence buds of pistachio trees showed that isolated glycoproteins are highly hydrophilic. Compositional and structural studies showed that these proteins are very similar to dehydrins and have a very high concentration throughout winter, which then rapidly declines at the start of spring (Golan-Gondhirsch, 1998).

Respiratory changes in the bud

As the plant exits dormancy and resumes growth, it has a very high energy requirement that must be satisfied by high rates of respiration. The respiration rate of a dormant apple tree begins to increase toward the end of the dormant period (Young *et al.*, 1995). Trees that receive more chilling during the dormant period have a higher respiration rate the following spring. Moreover, plants kept at higher temperatures in spring, have higher respiration rates. Respiration rate seems to be very dependent on temperature. In addition, there is a sharp rise in ATPase activity in the plasma membrane and an increase in the pH of meristem cells around spring (Aue *et al.*, 1998), which increases the sink strength of the bud.

Membrane changes

The cell membrane is an important constituent of the cell as it controls what molecules the cell takes up and therefore the membrane has an influence over the metabolism of the cell. Wang & Faust (1990) analysed the changes in membrane composition throughout dormancy and bud break in apples. They found that, as apple buds are exposed to cold, their membrane lipids become more unsaturated. When the membrane is in an unsaturated state it becomes less susceptible to freezing damage and also makes the membrane more permeable to molecules such as proteins, hormones, nutrients and other signal molecules that play an important role in bud break (Wang & Faust, 1990). A similar study by Erez *et al.* (1997) showed that sufficient chilling of peach buds leads to a rise in the concentration of the phospholipid, linoleate. As conditions then begin to warm during spring, the large amount of linoleate becomes desaturated to linolenate, which leads to a rise in membrane fluidity, making the bud more susceptible to signalling molecules and other compounds and therefore creating the perfect conditions for growth resumption (Erez *et al.*, 1997). Furthermore, the plasmodesmata become blocked during dormancy, which prevents efficient cell to cell communication. Once dormancy is released, the plasmodesmata become unblocked and intercellular communication can continue, which is very important if growth resumption is to occur (Arora *et al.*, 2003).

Conclusions

This review shows that spring bud break of deciduous crops is under the control of a diverse set of abiotic and biotic factors. Temperature is the most important factor in controlling bud break and will be the primary focus with changing climatic conditions, but other seasonal environmental factors, such as photoperiod, also play a role in some species. These environmental factors also interact with signals within the plant to determine the optimum time to resume growth. The distribution of hormones plays a major role in determining where growth will occur and can be manipulated with management practises. However, these hormonal signals are also influenced by other internal factors, such as the availability of carbohydrate and nutrient reserves. Thus, cultivation practices may have an impact on the expression of the hormonal signals and therefore affect bud break.

Although extensive research has been conducted on progression and manipulation of bud break in deciduous fruit, limited information is available for pecan trees, especially under South African conditions. With the predicted climate change for the Western Cape, pecans have the potential as an alternative crop for areas with increasing winter temperatures, based on their relatively low chilling requirement. However, the lack of reliable information regarding the requirements for sufficient bud break and resulting high yield under variable local conditions limit expansion of commercial production as a profitable alternative crop in these areas. Thus, an investigation into the factors influencing vegetative bud break under local conditions was initiated.

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PAPER 1: The effect of hydrogen cyanamide on bud break, shoot length and yield of ‘Wichita’ pecans [*Carya illinoensis* (Wangenh.) K. Koch] in the Prieska region, Northern Cape, South Africa.

Abstract

Pecans grown in regions where insufficient winter chilling accumulates suffer low and sporadic bud break and a protracted flowering period like many other deciduous crops. This study evaluated the effect of hydrogen cyanamide (HC) formulated as Dormex® applied at three concentrations (1 %, 2 % and 4 %) and two application times (four and two weeks before expected bud break) on total bud break percentage, vegetative bud break percentage, percentage of female flower bud break, shoot length and yield of ‘Wichita’ pecans in the Prieska region, Northern Cape, South Africa. Treatment at four weeks before expected bud break advanced bud break more than that of the control and treatments applied two weeks before expected bud break. However, the application time did not have an influence on the final percentage total bud break. Lower concentrations (1 % and 2 %) of Dormex® gave the highest final total bud break percentages. Increased total bud break percentages do not indicate an improved vegetative development and HC was shown to be ineffective at increasing vegetative bud break or pistillate flower bud break during the 2019 season. A treatment with 4 % Dormex® 4WBBB increased the final shoot length. Treatment with 4 % Dormex® 2WBBB resulted in a higher yield than the control in the 2018 season, but this needs to be confirmed with additional yield data for 2020. These results could not indicate changes in vegetative development in ‘Wichita’ through HC applications due to the limited evaluation of vegetative development. Bud break data showed that HC advanced bud break and this suggests that the increase in yield was due to a change in the flowering time. This observation requires further investigation.

Key words: Hydrogen cyanamide, bud break, ‘Wichita’ pecans, dormancy, vegetative growth

Introduction

Pecan nut production in South Africa has almost doubled in the past five years (SAPPA, 2019). This means that pecans are quickly becoming an economically important crop. Pecan trees originate from the temperate zone of North America and therefore have a deciduous nature, where they lose their leaves and go dormant during the cold winter period. Like many other deciduous crops grown in areas with relatively warm winters, pecan trees suffer from a relatively low and sporadic bud break that can result in a protracted flowering period and poor vegetative development (Lagarda, 1987).

In many deciduous tree species from temperate regions, the dormant period is controlled by the amount of winter chill that the tree receives during winter (Romberger, 1963). Research to elucidate the exact effect that winter chill has on pecan tree bud break is limited, especially under South African conditions. However, the literature suggests that chilling plays a less essential role in pecans than in other deciduous crops, such as apples. Sparks (1993) found that bud break occurred if no chilling temperatures below 7.2°C were received, but only if conditions were warm enough. Trees that received no chilling had a protracted bud break. Thus, a lack of winter chilling does not influence the percentage bud break, but only the uniformity. Similar results were found by Smith *et al.* (1992), showing that the more chilling 'Dodd' pecan seedlings received, the earlier and more uniform the bud break was. Küden *et al.* (2013) determined that female and male flower buds of 'Wichita' pecans have a chilling requirement of 250-300 and 300-400 Richardson chill units (RCU) (Richardson *et al.*, 1974), respectively. Vegetative buds are expected to have a slightly higher chilling requirement, as this is the case for most deciduous tree species (Campoy *et al.*, 2011). Female flower clusters (pistillate flowers) are borne on terminal points on the new shoot growth (Wetzstein *et al.*, 1996). In the Prieska growing region, these bearing shoots emerged primarily from the apical buds of one-year-old wood (Figure 1; personal observations). It will therefore be beneficial for trees to have a more one-year-old shoots with the potential to produce flower clusters.

In South Africa, there are currently no practices available that producers can use to enhance the spring bud break and manipulate the flowering period of pecans. Hydrogen cyanamide (HC), registered under the product name Dormex®, is one example of a chemical treatment that can be used to manipulate the bud break of deciduous fruit and nut crops, and is being extensively used on a number of crops in

the South African fruit industry. Hydrogen cyanamide has been evaluated on pecan trees grown in climates with insufficient chilling (Wood, 1993; Fayek *et al.*, 2008). However, none of these studies have been done under South African conditions and the product is not registered for use on pecan trees in South Africa. Fayek *et al.* (2008) found that in Egypt, pecan trees treated with hydrogen cyanamide have a higher percentage total bud break and a higher percentage reproductive bud break than control trees. Furthermore, bud break occurs two weeks earlier in treated trees than in untreated trees. Wood (1993) found similar results except that bud break on treated trees occurred three to four weeks earlier. These trials show that hydrogen cyanamide could be a valuable tool for pecan growers in warm winter regions, where a low bud break may retard the development of the tree, resulting in fewer bearing units being produced and a potential lower yield.

In this paper, we aimed to improve the total and vegetative bud break percentages, time of bud break, yield and shoot length of 'Wichita' pecans through treatment with hydrogen cyanamide. Additionally, the most effective application time and concentration of hydrogen cyanamide was determined.

Materials and Methods

Trial site and experimental design

The trial was conducted on the commercial pecan farm, Green Valley Nuts, near Prieska, Northern Cape (25° 35' 14" S; 22° 55' 06" E). Trials were conducted during the 2018/19 and 2019/20 seasons. The orchard selected as experimental site was planted in 2012 and the same trees were used in both seasons. The main cultivar is 'Wichita', with one row of 'Navaho' planted after every four rows as a cross pollinator. The trees were planted at a spacing of 10 x 10 m on seedling 'Ukulinga' rootstock, in sandy soil. Other than the treatment with hydrogen cyanamide, standard commercial management practices, such as irrigation and fertilisation, were followed for the duration of the trial.

Prieska receives relatively few chill units in winter. Daily positive chill units (DPCU) (Linsley-Noakes *et al.*, 1994) from 1 May to the end of August for the 2016 and 2017 seasons were 461 and 337 units, respectively (356 and 161 RCU, respectively). Relatively High maximum temperatures are experienced throughout summer and winter. The annual summer rainfall for the region is approximately 200 mm. Furthermore, the region receives approximately 70 days of frost a year during

winter and spring, which ranges in severity and may cause damage to young growing shoots (personal communication, R. Botha, consultant). Prieska is included in one of the pecan producing regions by the South African pecan nut producer's association (SAPPA, 2019).

A randomised complete block design was used as the experimental layout. Buffer rows were left between treatment rows to avoid spray drift. Seven treatments were applied to 10 single tree replicates (Table 1).

Treatments

Date of expected bud break was estimated based on historical records (personal communication, R Botha, consultant). Hydrogen cyanamide (Dormex®) (Philagro South Africa (Pty) Ltd. PO Box 36213 Menlopark 0102)) was applied using a Rovic Leers "Cima 2000 L" sprayer. Spray mixture was applied at 500 L/ha. Approximately 5 L of water was applied per tree, per application.

Treatments were applied in the early morning, during a period of low wind speed to ensure proper spray coverage and avoid drift onto neighbouring trees. Three concentrations of Dormex® were applied at two times, two weeks before expected bud break (2WBBB) and again four weeks before expected bud break (4WBBB). Treatment details for 2018 and 2019 are summarised in Table 1. After application of the treatments, temperatures were monitored to ensure that conditions were warm enough to ensure the efficacy of the product.

Data collection 2018/19 season

Two representative branch units were selected, per tree, before growth resumption. Each branch unit consisted of a two-year-old section with four to five one-year-old shoots growing from this section (Figure 2). To quantify the growth response after treatment, bud break was visually quantified as a percentage of the total buds (vegetative and reproductive) for all one-year-old shoots on the branch unit. During this early developmental stage, it was impossible to distinguish between them. Total bud break percentages were determined separately on the two-year-old section of the branch unit. A bud was counted as having burst if the bud scales had split and the first sign of green growth was visible. Two evaluations were carried out to determine the bud break reaction. The first evaluation was done on the 16th and 17th October 2018 and the second, one week later, on the 23rd and 24th October 2018. On these early

observation dates it was not possible to distinguish between vegetative and reproductive growth on some trees and therefore only the total bud break (vegetative and reproductive together) per unit was determined. Unfortunately, no further evaluation to quantify the percentage of buds that developed into vegetative organs was carried out. The trees were observed visually for signs of phytotoxicity during the course of the trial by observing for signs of burning on the treated shoots and new growth. Yield was recorded for individual trees at harvest, on 29 May 2019. Additionally, stem circumference was also measured and yield efficiency was calculated as the yield in grams divided by the cross-sectional stem area. Shoot growth for the 2018/19 growing season was recorded at the end of the season (11/09/2019) on five randomly selected new shoots per tree that grew during 2018/19. Hourly climate data was obtained from an automatic weather station on the farm to determine conditions following application. Chill units were also determined DPCU and RCU.

Data collection 2019/20 season

In the second season, five uniform, one-year-old shoots were selected on each tree. Selected shoots were at least 30 cm long and 1 cm in diameter. In contrast to the 2018 season, bud break was only quantified on one-year-old wood, as the results in 2018 showed that the older wood was insensitive to hydrogen cyanamide and very little bud break took place on older wood. Bud break was quantified as a percentage of the total number of buds on each shoot. Evaluations were carried out at bud break and at two-week intervals thereafter, evaluation dates are summarised in Table 2. Total bud break (vegetative and reproductive were quantified together) was counted on the first and second evaluation dates to determine whether there was an effect of the treatments on the time of bud break and the final percentage bud break. For the 2019 season, a third evaluation date was included to quantify the percentage of buds that developed vegetatively. This was done to determine whether HC could increase the number of potential bearing units for the following season. Trees were observed for signs of phytotoxicity throughout the course of the trial. Due to the time scope of the study yield and shoot length could not be quantified after the 2019/20 growing season.

Statistical analysis

Data were analysed using the GLM (General Linear Models) procedure in Statistical analysis software (SAS), Enterprise Guide 7.1. An ANOVA for a randomised complete block design was performed on the bud break, shoot length and yield data. Means were separated at a 5 % significance level using Fischer's LSD test.

Results

Bud break reaction 2018

Significant differences in bud break percentages between treatments were seen on one year-old 'Wichita' shoots, on both evaluation dates (Table 3). On the first evaluation date (16/10/2018) 4 % Dormex® 4WBBB reduced bud break percentage compared to all other treatments. However, this treatment did not result in a significantly lower bud break percentage than the control. The control did not differ significantly from any HC treatment. One week later (23/10/2018), treatment with 4 % Dormex® 4WBBB still resulted in a significantly lower bud break percentage than all other treatments, but it did not differ significantly from the control. In contrast to the first count, the second count showed that treatment with 1 % Dormex® 2WBBB resulted in a significantly higher bud break percentage than the control. None of the other treatments differed significantly from either the control or the 1 % Dormex® 2WBBB treatment (Table 3).

On the two-year-old wood, there were no significant differences between any treatments at either evaluation date. Bud break percentages remained very low across all treatments on both evaluation dates. No signs of phytotoxicity were observed at any point during the trial.

Yield efficiency 2018/19

Dormex® at 4% applied 4WBBB resulted in a significantly higher yield than that of control trees (Table 4). Moreover, this treatment also had a significantly higher yield than that of all other hydrogen cyanamide treatments, except the 4 % Dormex® 2WBBB treatment. None of the other HC treatments differed from each other. Yield efficiency showed the same significant results as yield.

Shoot length 2018/2019

Trees treated with 4 % Dormex® 2WBBB had a significantly longer total shoot length than that of control trees (Table 5). This was the only treatment to differ significantly from the control and none of the HC treatments differed from each other.

Bud break reaction 2019

There were significant differences in total bud break percentages between treatments in the 2019 season on the first evaluation date (initial bud break) (Table 6). All Dormex® 4WBBB treatments induced a significantly higher initial bud break percentage, compared to the control, but did not differ from each other. Of the later treatments (2WBBB) only the 4 % Dormex® treatment resulted in a significantly higher bud break percentage than the untreated control. However, this high rate also did not differ significantly from the two lower rates (1 % Dormex® and 2 % Dormex®) applied at the same time. The initial bud break following all 4WBBB treatments was significantly higher than that of the later treatments except for the 2% Dormex® 4 WBBB not differing from 4% Dormex® 2 WBBB (Table 6). On the final evaluation date, the bud break percentages of the two application times did not differ significantly anymore, except for 2%Dormex® 4 WBBB resulting in a higher bud break percentage than 1% Dormex® 2 WBBB. However, they both differed from that of the control. This indicates that the 4WBBB treatment advanced bud break, but did not affect the final percentage bud break. In terms of the final bud break, only two treatments did not result in a significantly higher bud break percentage than that of the control. These were the two high rate treatments (4 % Dormex® 4WBBB and 4% Dormex® 2WBBB). The 2 % Dormex® 4WBBB, caused the highest bud break percentage this treatment also had a significantly higher bud break percentage than that of the 4 % Dormex® 2WBBB treatment. Other than this, there were no other significant differences between the other HC treatments (Table 6).

Vegetative and Female flower cluster reaction 2019

HC treatment did not have an effect on the percentage buds that developed into vegetative shoots, regardless of the timing of the application or the concentration applied (Table 7).

The percentage buds that developed into shoots with female flower clusters remained fairly constant regardless of the treatment (Table 7).

Discussion

Bud break

In the 2018 season, a low concentration of HC applied close to bud break (1% Dormex® 2WBBB) resulted in a significantly higher bud break percentage than that of untreated trees. However, this significant increase was only seen on the second evaluation date, indicating that Hydrogen cyanamide improved the final percentage bud break when a lower concentration was applied closer to natural bud break. Yet this same treatment did not improve the bud break percentage on the first evaluation date, a week earlier. This indicates that Hydrogen cyanamide did not have an effect on advancing the bud break. Both evaluations were fairly late and no bud break was counted at initial bud break, but rather ca.10 days after the onset of bud break. This means that there is a possibility that an advancement in bud break was not successfully recorded.

Despite these results not being conclusive, they are still indicative that treatment with Hydrogen cyanamide did not advance bud break in 2018. These results are a contrast to the findings in numerous studies done on other deciduous crops, which suggest that application of Hydrogen cyanamide results in earlier bud break. This includes pistachios (Ghrab & Mimoun, 2014), apples (Jackson & Bepete, 1994), cherries (Godini *et al.*, 2008) and grape vines (Dokoozilan *et al.*, 1995). More relevant to this study are the results of Wood (1993), who found that a 4 % HC induces earlier bud break on 'Cheyenne' pecan trees. In some cases, bud break in treated trees occurred up to 30 days earlier. This contrasting result could possibly be explained by the application times used by Wood (1993). Their trial tested a wider range of application times and found that a treatment 60 days before expected bud break was most successful in advancing bud break, and that treatments later than 30 days before expected bud break had no effect on advancing bud break. Our trial included two application dates, one at four weeks and another at two weeks before expected bud break. It is therefore possible that no advancement in bud break was experienced because application times were too close to bud break to have a significant effect on forcing bud break. Before application of Hydrogen cyanamide, Prieska had accumulated 354 RCU (452 DPCU). Küden *et al.* (2013) showed that male and female 'Wichita' flowers have a chilling requirement of 300-400 RCU and 250-300 RCU, respectively, therefore enough chilling should have accumulated in the winter of 2018 to ensure endodormancy completion by the time of application, resulting in a timely

and uniform bud break. Sparks (1993) found that pecan trees resumed growth and bud break even when they did not receive any chilling below 7.2°C suggesting that they do not have an obligatory chilling requirement. This research points to the possibility that pecans do not go the same depth of endodormancy as other deciduous trees. Despite this lack of an obligatory chilling requirement suggested by Sparks (1993) they concluded that when pecan trees did receive chilling, the uniformity of bud break was improved. Therefore, the relatively cold winter experienced in Prieska in 2018 would have caused fewer issues with the timing and uniformity of bud break than would be prevalent in a year with a warmer winter. This could make it more difficult to see the effect that HC had on the time of bud break. Even though these bud break counts did not show a measurable increase in early bud break for most treatments, it was observed that many HC treated trees seemed to be developmentally advanced. This can be seen in Figure 3, where both the tree and the branch of the tree that was treated with 4 % Dormex® 2WBBB, showed a clear difference in the development of the buds compared to that of the control tree and branch. Despite this clear difference in the developmental stage of the buds, this treatment did not result in a significantly higher bud break percentage than that of the control trees. This is likely due to the initial evaluation in 2018 being slightly too late, allowing the control trees to undergo significant bud break and “catch up” to the treated trees in terms of bud break percentage. Therefore, our results for the timing of bud break in the 2018 season are inconclusive.

Even though the results show that HC did not have an effect on the time of bud break during the 2018 season, there was an effect on the final percentage bud break. One percent HC applied two weeks before expected bud break resulted in 10.4 % higher bud break than the untreated control. Our results indicate total bud break percentage and are not necessarily an indication of an increase in percentage shoots following HC applications. This need to be confirmed with additional quantification of the development of vegetative organs. Fayek *et al.* (2008) applied a 3 % Dormex® treatment to five different pecan cultivars in Egypt and managed to successfully increase the percentage bud break. These findings confirmed our results, even though a different concentration of HC was shown to be effective and the effect of the timing of the application was not investigated. Other than this study, information on the effect of HC on the percentage bud break in pecan trees is scarce. On other deciduous fruit and nut crops grown in regions with warm winters, HC generally increases the final

percentage bud break. Müller (2008) reported that 4% Dormex® increased of bud break of pistachio trees in the Prieska region. Ghrab & Mimoun (2014) also evaluated Dormex® on pistachios and found similar results; a 2 % and 4 % Dormex® treatment applied 45 days before bud break increased bud break. This again suggests that a higher HC concentration is more effective at improving bud break. However, our results from the 2018 season contradicts these findings and indicated that a low concentration had a similar effect as a higher concentration in this region. This is reiterated by the fact that, in our study, no significant differences were seen between treatments with different HC concentrations, except for one treatment with a high concentration on an early application date, which resulted in a lower bud break than that of other treatments.

Hydrogen cyanamide treatment seemed to consistently result in a higher bud break percentage than that of control trees, regardless of concentration or application time, even if these increased percentages were not always significant (Table 3). This was true for all treatments except 4 % Dormex® 4WBBB, which had a non-significantly lower bud break percentage (54.70 %) than that of the control (61.64 %). However, this bud break percentage was significantly lower than that of all other treatments on both evaluation dates. When a high concentration of HC is applied and results in a reduced amount of bud break, the initial theory is that there was a phytotoxic effect. However, this is unlikely, as no symptoms of phytotoxicity were observed on the new growth. Additionally, temperatures following the earlier application in 2018 were fairly low (Figure 4), which would have reduced the possibility of phytotoxicity. Furthermore, the second treatment, at the same concentration, was applied even closer to bud break and also showed no symptoms of phytotoxicity. This second treatment did not suffer a reduced percentage bud break. This is supported by the findings of Wood (1993) that phytotoxicity only occurs with applications of 10 % HyC or higher on pecans.

The bud break reaction on two-year-old wood was very different from that of one-year-old wood. Bud break percentages were very low on both evaluation dates and no significant increase in bud break percentage was seen for any HC treatment. Wood (1993) suggested that there was no difference in the sensitivity to HC of wood of different ages after treatments applied to both Eighty and seven-year-old trees reacted similarly. Our findings clearly indicate that one-year-old wood is not only more sensitive to treatment with HC, but that the vast majority of new growth occurs on it.

Linsley-Noakes (1988) evaluated HC on kiwis and discovered that older vines are less sensitive and do not have the same increase in flowering and bud break that is seen in younger vines. This shows that pecans react to HC similarly to kiwis with regards to the age of the wood. These results illustrate that it is very difficult to stimulate growth inside the tree and that improving the structure inside the canopy will be very difficult. Therefore, it is very important to determine the structure of the tree from very early in the establishment of the orchard.

In the 2018 season, HC treatments had a relatively weak effect on the bud break parameters. Only one treatment increased the final level of bud break significantly and no treatment was successful in promoting earlier bud break. This weak reaction to HC is most likely due to accumulation of sufficient chilling in the 2018 winter (354 RCU and 452 DPCU), which ensured a successful bud break period without application of a rest breaker. In contrast, during the winter of 2019, 331 DPCU accumulated (117 RCU), which is substantially less than that of the previous season. This difference in the amount of chilling can clearly be seen in the reaction of the trees to treatment with HC. More treatments resulted in bud break percentages that were significantly higher than that of untreated trees and differences between treatments were more pronounced.

In 2019, earlier application of HC seemed more effective and resulted in higher bud break on the first evaluation date than that of trees that received later or no applications (Table 6). This indicates that bud break was advanced by an earlier application in a year where insufficient chilling was received. These results are similar to what was found by Wood (1993), who reported that HC applied more than 30 days before bud break resulted in advanced bud break, while any treatments applied later than 30 days before bud break, do not have an effect. While these application times are much earlier than our applications, the same trend can be seen, where an earlier treatment had a stronger effect in bringing the bud break period forward than a later treatment. Ghrab & Mimoun (2014), despite only testing one application time, found that a very early application at 45 days before expected bud break resulted in a significant advancement of bud break in pistachios. This same trend was seen in apples by Bound & Jones (2004), who reported that treatments at 40 and 30 days before expected bud break were more successful than later treatments. Siller-Cepeda *et al.* (1992) found that treatments applied to peach trees earlier, during endodormancy, were more successful at advancing bud break than later applications

during ecodormancy. They postulated that when HC was applied before sufficient chilling had accumulated to release the tree from endodormancy, the chemical was able to compensate for the lack of chilling and improve the bud break period. In contrast, when it was applied after sufficient chilling had accumulated and the tree was in ecodormancy, the HC caused a phytotoxic effect on some of the buds, reducing the efficacy of the product and delaying bud break. Erez (1995) supported this. Therefore, it is possible that the earlier application in this study was applied before chilling was completed and the HC therefore had more of a forcing effect than that of later applications, which were possibly applied after a greater amount of chilling had accumulated, allowing the buds to be in an ecodormant state and resulting in a low level of phytotoxicity that caused a slight delay in bud break. Despite this, the highest concentration (4 %), applied on the later application date, resulted in a significantly higher bud break percentage than that of the control. If the reduction in bud break in the later treatment was caused by a low level of phytotoxicity, it should have been more apparent on the high dosage instead of the lower dosages (1 % Dormex® and 2 % Dormex®), however, this was not the case in our study. Furthermore, during 2019, only 331 DPCU (117 RCU) accumulated during winter. Findings by Küden *et al.* (2013) suggest that this was not sufficient to satisfy the chilling requirement of 'Wichita' pecan buds, which would mean that both treatment times occurred before sufficient chilling could accumulate. The specific concentration of HC used on the earlier application date did not have a significant effect on the bud break percentage and all three rates increased the bud break when compared to that of the control. On the second evaluation date, which specifies a final bud break percentage, the effect of the application time was less pronounced. In terms of the final bud break percentage there did not seem to be a difference between the early and late application times however both application times were effective at increasing the final percentage of bud break (Table 6). This indicates that HC application time has an effect on the time of bud break and can bring the bud break period forward, but it does not influence the final percentage bud break. In terms of the effect of the HC concentration on the final bud break percentage, only the two treatments using a high concentration of HC (4 % Dormex®) resulted in bud break percentages that were not significantly higher than that of the control (Table 6).

All four other treatments using lower rates (1 % and 2 % Dormex®) caused a significant increase in the final percentage bud break compared to that of the control.

The two 4 % Dormex® treatments were slightly more effective at advancing bud break than the two lower concentrations (first evaluation date, Table 6), however, this slight improvement in terms of advancing bud break resulted in a trade-off with the final bud break percentage. The higher concentration may have had a stronger stimulating effect, but also had a negative effect on some buds that were more sensitive to HC. A similar effect was found on pistachios by Ghrab & Mimoun (2014) who found that a 4 % Dormex® treatment also initially had a higher bud break than that of a 2 % Dormex® treatment, but that the final percentage bud break did not differ. Furthermore, 2 % Dormex® applied four weeks before expected bud break resulted in the highest bud break percentage and this was significantly higher than that of trees treated with 4 % Dormex®, also applied four weeks before expected bud break. This was the same treatment that resulted in a bud break percentage that was significantly lower than all other treatments in the 2018 season. The fact that this result was replicated between the seasons points to a definite negative effect of this treatment on the final percentage bud break.

Temperature following the completion of chilling can influence bud break development. Warm weather is desirable to ensure a good rate of phenological development (Sparks, 1993). Additionally, Erez (1979) reported that temperatures after a rest breaking agent was applied also influences the efficacy of the rest breaker, suggesting that too low temperatures after application reduces the effectivity of the product and too high temperatures can result in phytotoxicity. Figure 4 shows that temperatures after the first application in 2018 were slightly lower than those in 2019. This could offer an explanation as to why the earlier application was not effective in 2018, but had a large effect on bud break during the 2019 season. Figure 4 also indicates that, during 2018, conditions following application of HC were slightly cooler compared to those during 2019. This could have resulted into a slower phenological development and efficacy of HC and therefore a less pronounced result compared to the 2019 season. During the latter season, temperatures after treatment application remained relatively high and could have therefore resulted in a more advanced phenological development and efficacy of HC.

Our results (Table 3 & Table 6) only refer to total bud break percentages. No distinction was made between vegetative and reproductive buds and all bud break percentages are a combination of all buds. Therefore, where an increase in the percentage of bud break was reported, it does not indicate that the treatment improved

the vegetative development or the number of reproductive structures. The methodology of the evaluation limits the practical implementation of these results. To re-iterate, total bud break data showing an increase in the percentage of bud break does not indicate that HC improved the vegetative development. Results show that HC had a significant effect on the timing of bud break and could potentially advance bud break. This has potential implications for the manipulation of the flowering period and could possibly result in advanced nut maturity and a potential early harvest as indicated by Wood (1993). Furthermore, our results confirmed that the efficacy of a HC treatment is influenced by winter chilling and application may not be equally efficient in a year with high chilling.

Vegetative Bud break reaction and pistillate flowers

Due to the timing of evaluations in the 2018 season, vegetative and reproductive bud break was not quantified separately. During 2019 an additional evaluation date was included to record the percentage of buds that grew out into shoots (vegetative), therefore determining the effect that HC has on creating new bearing units. The lack of distinguishing between vegetative and reproductive bud break during the first season, limits conclusive evidence for the effect of HC on increasing vegetative development of pecans to increase yield.

On the third evaluation date in 2019 (Table 2), the percentage of buds that developed into vegetative shoots on one-year-old wood, was recorded and indicates the vegetative bud break percentage. Table 7 shows that no significant differences occurred between treatments in terms of vegetative bud break. This is in contrast with the findings of Fayek *et al.* (2008) who reported that HC increases the number of new shoots in Egypt. An increased vegetative bud break is desirable as nuts are borne on the terminal points of new shoots growing from one-year-old wood. Therefore, if HC can be applied to increase the number of vegetative shoots, there would more bearing units and a higher yield the following season (Figure 5). The effect of HC on vegetative bud break should be investigated further, as trends suggested a possible HC effect. The 2020 yield may indicate possible effects more clearly.

No increase in the percentage of pistillate flower buds was recorded with HC treatment (Table 7). As no increases were seen in terms of vegetative or pistillate flower bud break the increased level of bud break that was recorded for several treatments in 2019 (Table 6) could only be caused by an increase in the number of

catkins. Unfortunately, this was not recorded in 'Wichita' trees. However, observations during the 2019 season suggested that HC treatment increased the number of buds in axillary positions on one-year-old wood (Figure 4) where the majority of catkins are found (Woodroof & Chapman-Woodroof, 1926). Furthermore, vegetative shoots usually develop from the apical buds and these positions usually have sufficient bud break, as they are not inhibited by apical dominance.

Shoot Length

Kubota *et al.* (2000) evaluated the effect of HC application on the shoot growth of grapevines with a 2.5 % Dormex® treatment. Their results suggested that HC causes significant elongation in new shoots and postulated that this was a result of the earlier bud break that the HC induced, which gave the treated buds a "head start". A similar result in terms of the increase in shoot length with HC treatment was also reported by El-Sabagh *et al.* (2012) in apples. Due to the time scope of this study, shoot length was only measured during the first season of the trial. Treatment with 4 % Dormex® 2WBBB produced new shoots that were significantly longer than those of control trees. Even though this treatment did not increase the bud break percentage on the first evaluation date in 2018 (Table 3), the evaluation time did not accurately represent the timing of bud break. The developmental stage of trees treated with 4 % Dormex® 2WBBB showed a clear difference to that of the control (Figure 3) Therefore, we can postulate that this treatment improved shoot elongation due to possibly earlier bud break, thereby lengthening the growing season and allowing longer shoots to develop. Shoot length should be measured again at the end of the 2019/20 growing season to determine whether the earlier application time, which had a positive effect on advancing bud break, resulted in increased shoot length. This would confirm the theory that shoot length increases if the growing season is extended by an earlier bud break.

Yield

The effect of HC on the yield of deciduous fruit and nut trees is well documented and increases in yield have been reported in pistachios (Rahemi & Asghari, 2004) and apples (Jackson & Bepete, 1989). However, in pecans, Wood (1993) found that HC did not have an impact on the size of the yield, but impacted on the time of harvest. A treatment with 4 % Dormex® 4WBBB resulted in a significantly higher yield than that

of control trees, as well as all other treatments where a lower concentration of HC was applied. Later application of a 4 % Dormex® treatment resulted in a slight increase in the yield that was not significant from the earlier treatment with 4% Dormex® or from the control. This proves that treatment with a high concentration of HC has the potential to improve yield. This result cannot be due to higher bud break, resulting in the production of more flowers, as pecan flower initiation occurs at the end of the previous growing season and the flowers are therefore pre-formed before bud break (Amling & Amling, 1983). Additionally, pecan flowers are produced on the terminal ends of one-year-old shoots (Woodroof & Chapman-Woodroof, 1926) where bud break occurred regardless of treatment with HC. It is therefore unlikely that HC would be capable of increasing the number of flowers (as shown during the 2019 season, Table 7). Therefore, the increase in yield can only be the result of improved fruit set. Fruit set could have been significantly higher due to a lack of competition for CHO and nutrients during the fruit set period. An early treatment with 4 % Dormex® (4WBBB) resulted in a lower bud break than that of all other treatments (Table 3). Therefore, it is possible that this lower bud break had an inverse effect on the yield, as there was less competition for resources between the different bud types Kubota *et al.* (2000) reported similar results, showing that a high bud break percentage and vigorous shoot growth of grapevines has a negative impact on flower development due to competition between the vegetative and reproductive growth. In our study, earlier bud break in 'Wichita' should theoretically have resulted in a higher yield as the flowering period of the trees would better overlap with the development of the catkins on the cross pollinator 'Navaho' trees, which are planted alongside the trial rows and bud break is approximately a week earlier than in the 'Wichita' trees. However, according to the bud break evaluations performed in the 2018 season, no advancement of bud break was recorded. Therefore, the effect on yield due to a better overlap of the flowering period should be investigated after a year where insufficient chilling accumulated during winter, leading to a more protracted bud break, where the effect of HC could be seen more clearly. A further potential effect of HC to consider is the effect that it might have on the bearing positions produced, this effect would only be seen in the second season after treatment. Theoretically, if HC improved the vegetative development in the 2018 season there should be more bearing positions available during the 2019 season and the harvest in the second year after application should therefore be improved (Figure 5). However, it is important to consider that pecan trees bear nuts

on the terminal points of one-year-old shoots. Therefore, even if shoot length was increased during the 2018 season, it would not have resulted in an increased number of bearing positions. For more bearing positions to be produced, the number of new vegetative shoots would have to be increased. This was unfortunately not assessed during the 2018 season, but counts during the 2019 season showed that HC did not have a significant effect on the number of vegetative shoots developing (Table 7). Therefore, it is unlikely that HC can increase the yield. However further investigations should be carried out as we cannot draw conclusions from one season's results. Although the time scope of this study does not include assessing the yield after the 2019 growing season (2020 harvest) due to logistics, it will be valuable to assess the yield to see whether the more pronounced bud break in 2019, after the warm winter, had an influence on the yield.

Conclusion

HC treatment has a stronger effect when applied during a warm winter, with the insufficient accumulation of chilling. Therefore, application in a commercial setting, focus should be on years where the chilling requirement was not satisfied. This shows the importance of monitoring the chilling accumulation of pecans.

Earlier applications seemed more effective at advancing bud break. The exact concentration of HC being used does not appear to play a large role on the time of bud break, however, lower concentrations appear to have a better effect on the final percentage of total bud break, as higher concentrations may have a negative effect on the breaking of some buds.

However, the total bud break percentage cannot be interpreted in terms of the effect of HC on vegetative or flower development, but can be used to indicate an advancement in bud break between treatments. In 2019, HC treatment resulted in a slight increase in shoot length, but did not increase the percentage of buds developing into vegetative shoots. This was however only measured in one season and trends suggest that this should be further investigated as our results are inconclusive.

HC affected the yield, however, the exact mechanism of this remains elusive. The significant increase in yield (2018) in the 4 % Dormex treatments, cannot be a direct result of higher bud break in 2018, as HC did not increase the number of flower clusters on a branch. It is most likely that the increase in yield was a result of earlier bud break, resulting in a better synchronisation of the flowering period of the 'Wichita'

trees with the cross pollinator, resulting in a higher fruit set percentage. Our results therefore indicate that HC affected some aspects of 'Wichita' bud development, but its effect on the vegetative development needs to be further investigated. HC has the potential to manipulate the time of bud break and this could potentially be a valuable management tool in manipulating the flowering periods and improving the synchronisation of the flowering periods, resulting in a better fruit set and yield.

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Tables and Figures

Table 1: Dormex® concentrations and application times on ‘Wichita’ pecan trees on Green Valley Nuts, Prieska, during the 2018/19 and 2019/20 seasons.

Rate	Concentration hydrogen cyanamide (g/L)	Application timing	Application Date.	
			2018	2019
Untreated control	0	N/A	N/A	N/A
1% Dormex®	5.2	2 Weeks Before Expected Bud Break	18/09/2018	24/09/2019
2% Dormex®	10.4	2 Weeks Before Expected Bud Break	18/09/2018	24/09/2019
4% Dormex®	20.8	2 Weeks Before Expected Bud Break	18/09/2018	24/09/2019
1% Dormex®	5.2	4 Weeks Before Expected Bud Break	04/09/2018	11/09/2019
2% Dormex®	10.4	4 Weeks Before Expected Bud Break	04/09/2018	11/09/2019
4% Dormex®	20.8	4 Weeks Before Expected Bud Break	04/09/2018	11/09/2019

Table 2: The three evaluation dates in the 2019/20 season.

Observations	Evaluation date
1 st count	02/10/2019
2 nd count	16/10/2018
3 rd count	30/10/2019

Table 3: Effect of various concentrations and application times with *Dormex*® on 'Wichita' pecans total bud break percentages for both one and two-year-old branches on two dates in the 2018 season.

Treatment	Bud break percentage			
	One-year-old shoots		Two-year-old shoots	
	16/10/2018	23/10/2018	16/10/2018	23/10/2018
Control	56.1 ab ^y	61.6 bc	4.0 ns	4.9 ns
1 % Dormex® 2 WBBB ^z	63.0 a	72.0 a	5.0	6.6
2 % Dormex® 2 WBBB	57.2 a	65.3 ab	4.6	6.2
4 % Dormex® 2WBBB	60.5 a	64.9 ab	5.2	6.1
1 % Dormex® 4 WBBB	59.7 a	64.7 ab	0.00	0.7
2 % Dormex® 4 WBBB	58.6 a	66.0 ab	2.6	4.8
4 % Dormex® 4 WBBB	48.2 b	54.7 c	4.2	7.4
Pr > F	0.0160	0.0120	0.1789	0.1215

^z Weeks Before Expected Bud Break

^y Means followed by the same letter within a column are not significantly different

Table 4: Effect of various concentrations and application times with *Dormex*® on yield and yield efficiency of 'Wichita' pecan trees after the 2018/2019 growing season.

Treatment	Yield (kg)	Yield efficiency(g/cm ²)
Control	12.342 b ^y	108.8 b
1 % Dormex® 2 WBBB ^z	10.352 b	90.7 b
2 % Dormex® 2 WBBB	11.291 b	108.9 b
4 % Dormex® 2 WBBB	15.085 ab	133.1 ab
1 % Dormex® 4 WBBB	12.770 b	119.3 b
2 % Dormex® 4 WBBB	13.212 b	121.4 b
4 % Dormex® 4 WBBB	18.336 a	165.8 a
Pr > F	0.0410	0.0440

^z Weeks Before Expected Bud Break.

^y Means followed by the same letter are not significantly different.

Table 5: Effect of various concentrations and application times with Dormex® on the average shoot length of five one-year-old shoots per tree.

Treatment	Average shoot length 2018 (cm)
Control	64.15 ns
1% Dormex® 2WBBB ^z	65.37
2% Dormex® 2WBBB	67.03
4% Dormex® 2WBBB	64.16
1% Dormex® 4WBBB	70.98
2% Dormex® 4WBBB	69.3
4% Dormex® 4WBBB	66.98
Pr > F	0.725

^z Weeks Before Expected Bud Break.

Table 6: Effect of various concentrations and application times with Dormex® on 'Wichita' pecans total bud break percentages on one-year-old shoots for two evaluation dates in the 2019 season.

Treatment	Bud break (%)	
	02/10/2019	16/10/2019
Control	24.1 d ^y	59.0 c
1 % Dormex® 2 WBBB ^z	31.6 cd	65.7 ab
2 % Dormex® 2 WBBB	31.3 cd	66.0 ab
4 % Dormex® 2WBBB	34.9 bc	64.4 abc
1 % Dormex® 4 WBBB	47.1 a	65.2 ab
2 % Dormex® 4 WBBB	45.0 ab	69.8 a
4 % Dormex® 4 WBBB	47.6 a	62.9 bc
Pr>F	<0.0001	0.014

^z Weeks Before Expected Bud Break

^y Means followed by the same letter within a column are not significantly different

Table 7: Effect of various concentrations and application times with hydrogen cyanamide on the percentage of buds that develop into vegetative shoots and pistillate flower clusters on one-year-old wood of 'Wichita' during the 2019 season.

Treatment	Vegetative shoots (%)	Pistillate flower clusters (%)
Control	31.1 ns	9.9 ns
1 % Dormex® 4 WBBB ^z	32.8	11.9
2 % Dormex® 4 WBBB	36.7	11.3
4 % Dormex® 4 WBBB	32.0	12.7
1 % Dormex® 2 WBBB	36.2	12.1
2 % Dormex® 2 WBBB	30.7	13.6
4 % Dormex® 2WBBB	32.2	11.3
Pr>F	0.053	0.380

^z Weeks Before Expected Bud Break

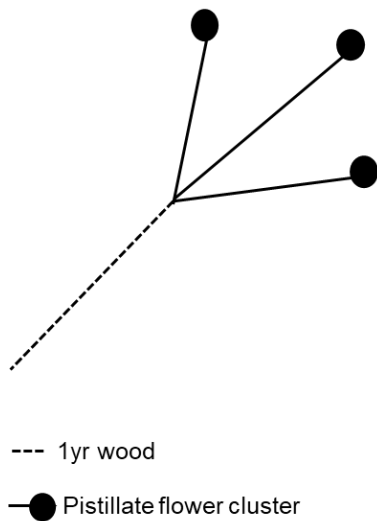


Figure 1: A schematic indicating the bearing habit of pecan. Pistillate flower clusters are borne terminally from new shoots growing from terminal buds on one-year-old wood

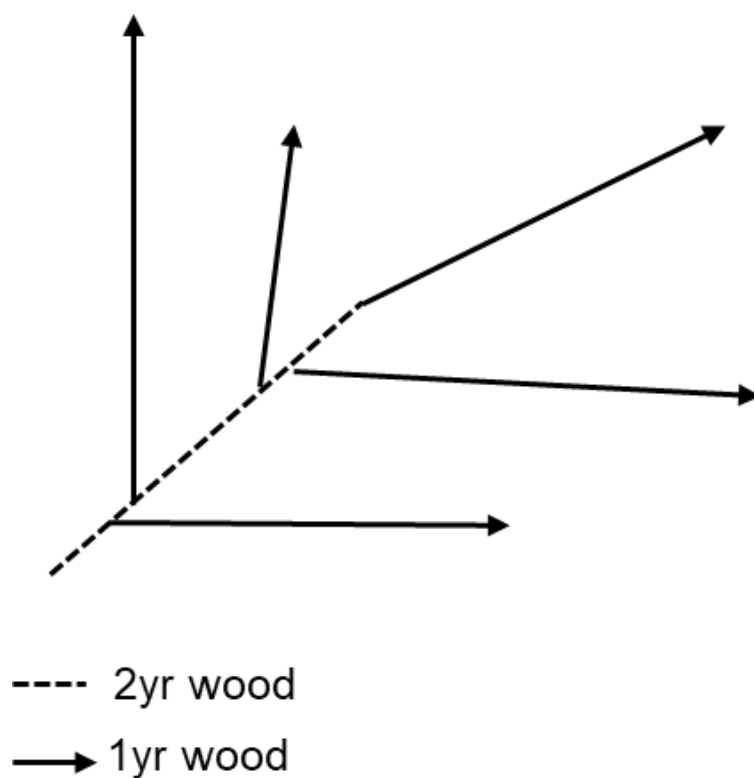


Figure 2: A schematic of an average branch unit that was chosen to do evaluation on for the 2018 season. A section of two-year-old wood with 4-5 one-year-old shoots.

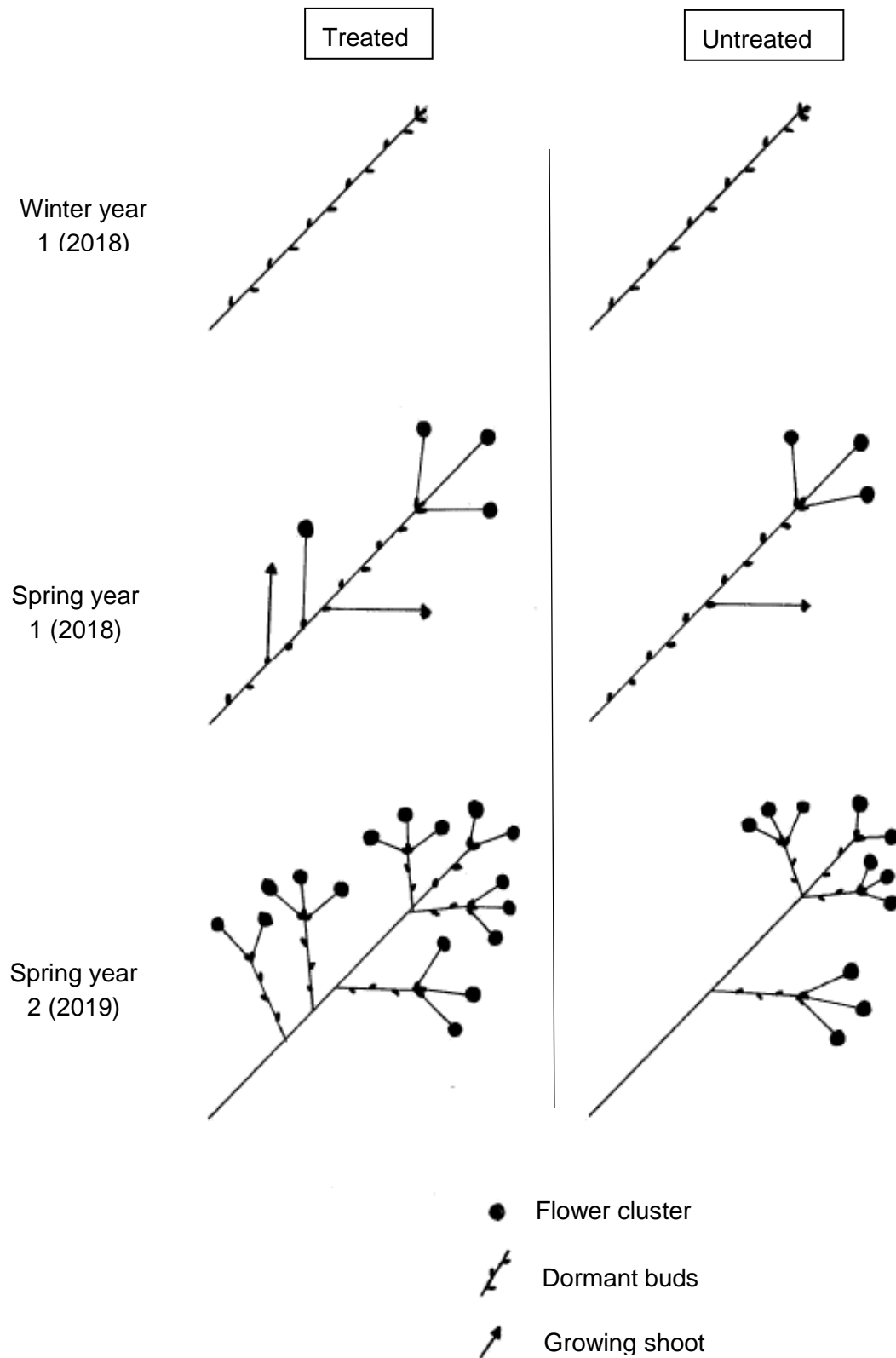


Figure 3: Images showing the bud break reaction of 'Wichita' trees and branches from one treatment and a control on the first evaluation date in 2018. A) A tree treated with 4% Dormex® 2 weeks before bud break; B) A branch treated with 4% Dormex® 2 weeks before bud break; C) An untreated control tree; D) A branch from an untreated control tree.

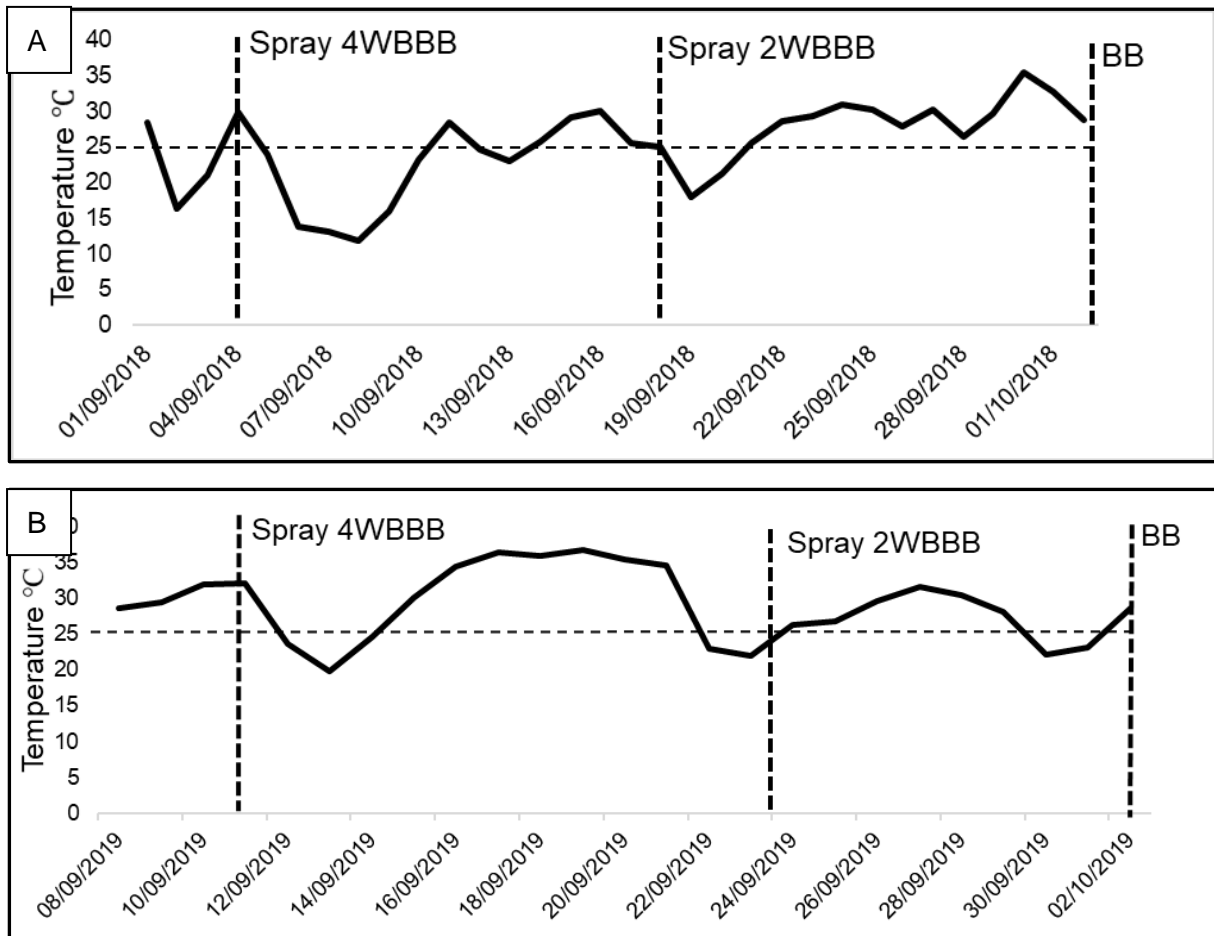


Figure 4: Daily maximum temperatures (°C) following application with Hydrogen cyanamide and leading up to bud break for A) 2018 and B) 2019.

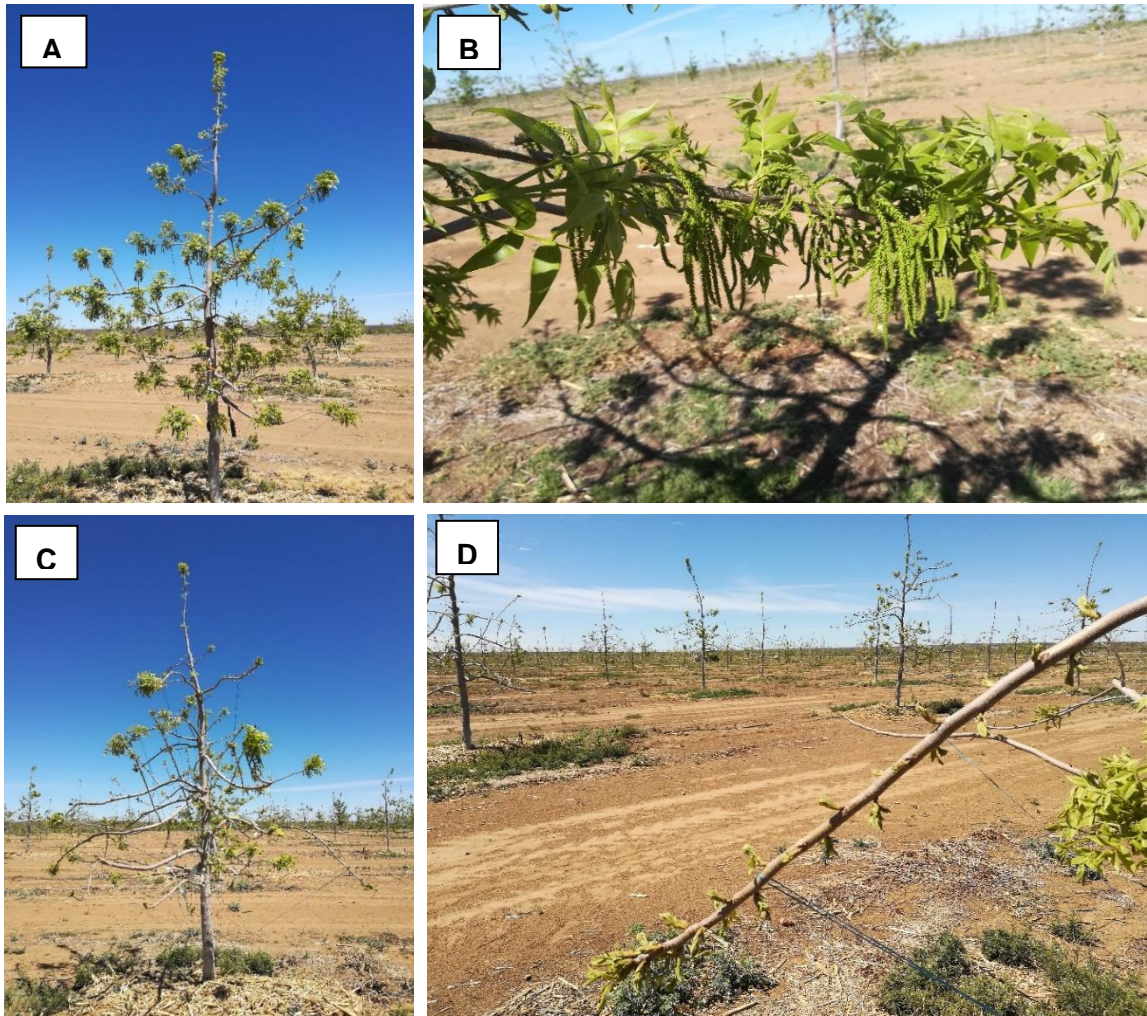


Figure 5: A schematic illustration of the theoretical development two one-year-old pecan shoots. One treated with Dormex® and one untreated branch.

PAPER 2: The effect of hydrogen cyanamide on bud break, shoot length and yield of ‘Navaho’ pecans [*Carya illinoensis* (Wangenh.) K. Koch] and the synchronisation of the reproductive development of ‘Navaho’ and ‘Wichita’ pecans in the Prieska region, Northern Cape, South Africa.

Abstract

Pecan can experience poor bud break, protracted bloom and poor vegetative growth when grown in an area that experiences relatively warm winters. Hydrogen cyanamide (HC), formulated as Dormex®, is used in many deciduous crop industries to improve bud break. Three concentrations (1 %, 2 % and 4%) and two application times (four and two weeks before expected bud break (WBBB)) of Dormex® were evaluated to determine its effect on the time and percentage bud break, vegetative development and yield of ‘Navaho’ pecans. Additionally, the effect on synchronisation of the flowering periods of ‘Navaho’ as cross-pollinator and main cultivar, ‘Wichita’, was determined. In 2018, all HC treatments significantly improved total bud break percentage compared to the control on one-year-old shoots. In 2019, 4 % Dormex® caused a significantly higher initial bud break, and therefore advanced bud break, compared to 1% Dormex® and the control. Both 4 % and 2 % Dormex® had a significant influence on the final percentage of bud break compared to the control. These total bud break counts does not provide evidence about the effect of HC on vegetative development, as no distinction was made between reproductive and vegetative buds on evaluation dates in 2018. Vegetative bud break percentages during the 2019 season were not improved significantly by HC treatments. It was concluded that the increased bud break is due to an increase in the percentage of catkins developing mainly on lateral positions. HC treatments did not impact significantly on yield during 2019, however results suggest that HC may have had a negative influence on the yield of ‘Navaho’. The best synchronisation between cross-pollinator and production cultivar was achieved by leaving ‘Navaho’ untreated and applying 4 % Dormex® 4WBBB on ‘Wichita’ in 2019. No visible signs of phytotoxicity were observed at any of the concentrations or application times in this trial.

Key words: catkins, Hydrogen cyanamide, flowering period, synchronization, bud break

Introduction

Pecan production in South Africa has recently seen substantial expansion from 200 000 trees planted in 2010 to an estimated 580 000 during 2018 (SAPPA, 2019). This is due to increase in export potential to China. Previously, the majority of these orchards were established in the Vaalharts and Northern Cape regions, however, new orchards have also been established in the Western Cape (personal communication, A Coetzee, Hortgro). Despite this growth in the industry, little information is available on basic phenological development under South African climatic conditions.

Pecan is a deciduous tree crop that experiences a dormant period during winter. Winter conditions differ between the major cultivation areas in South Africa and this may impact on the profitability of the crop. Similar to other deciduous crops, pecan trees suffer from a low and sporadic bud break during spring when grown in regions with relatively warm winters, which can result in poor vegetative development and a protracted flowering period, resulting in poor pollination and a smaller crop (Lagarda, 1987).

In deciduous crops, the dormant period is mainly controlled by the cold temperatures that a tree receives during winter (Romberger, 1963). The exact effect of winter chilling on pecans is still unknown. Sparks (1993) found that bud break occurs regardless of the amount of chilling received by a tree, assuming it received a sufficient amount of heat in spring. They also found that trees that received chilling have a less protracted bud break. Thus, they concluded that winter chilling does not have an influence on the percentage bud break, but that it improves the uniformity of the bud break. This was confirmed for 'Dodd' pecan seedlings that showed an earlier and more uniform bud break after receiving more chilling (Smith *et al.*, 1992). Küden *et al.* (2013) calculated the exact chilling requirements of several pecan cultivars. Female buds of 'Wichita' have a chilling requirement of 250-300 Richardson Chill Units (RCU), but no information is available for 'Navaho'. Vegetative buds are expected to have a slightly higher chilling requirement than reproductive buds, as this is the case for most deciduous tree species (Campoy *et al.*, 2011). Pecans produce pistillate flowers on terminal points of new shoots produced mainly from the apical buds of one-year-old wood (Figure 1) (Wetzstein *et al.*, 1996). Thus it is highly important to have many one-year-old shoots to act as bearing units for the following season's crop.

A lower bud break percentage, as well as a protracted flowering period over an extended period of time, can impact on the synchronisation of the flowering period

(Lagarda, 1987; Sparks, 1993; Dennis, 2000). Pecans exhibit heterodichogamy (male and female gametophytes are found on separate structures on the tree). Male flowers (catkins) and female flowers (pistillate flowers) also mature separately, rendering self-pollination less likely to take place (Fayek *et al.*, 2008) and as a result, pecans require cross-pollination for successful commercial production (Thompson & Connor, 2012). Therefore, to ensure successful pollination, it is important for commercial producers to plant a cross-pollinator, where the catkins mature at the same time as the pistillate flowers of the primary cultivar. One combination that is commonly planted in South Africa, is 'Wichita' as a protogynous cultivar (pistillate flowers mature first) and 'Navaho' as a protandrous cultivar (catkins mature first), with 'Navaho' as the cross-pollinator (Thompson *et al.*, 1995; Thompson & Connor, 2012). Thus, as the catkins of 'Navaho' are maturing, the pistillate flowers of 'Wichita' are also reaching maturity. However, in a marginal winter climate that shows a low and protracted bud break, these two periods may not synchronise sufficiently, resulting in a reduced yield. Furthermore, low bud break is often followed by poor vegetative development of the tree.

Hydrogen cyanamide (HC), is widely applied in the deciduous fruit industry to manipulate the time and percentage bud break to obtain an increased, earlier and more uniform bud break under conditions of insufficient winter chilling (Erez, 1995). HC has been evaluated on pecans in climates with insufficient winter chilling in the USA and Egypt (Wood, 1993; Fayek *et al.*, 2008). Wood (1993) reported three to four week earlier bud break on treated trees and Fayek *et al.* (2008) found that treatments resulted in a higher percentage total bud break, as well as a two-week advancement of bud break. Müller (2008) found that 4% Dormex® had a positive effect on the bud break of pistachios grown in a marginal climate in South Africa a similar result was found by Rahemi & Asghari (2004). Their study found that Dormex® at 2 % and 4 % applied to pistachio trees, significantly increased the percentage bud break when compared to the control. Their overall efficacy, however, did not differ, despite the 4 % treatment having a slightly higher bud break percentage. The same effect was reported by Veloso *et al.* (2003) on kiwis, where both 4 % and 6 % HC resulted in a significant increase in bud break, but 6 % HC only resulted in a slight increase in bud break in comparison to the 4 % treatment.

Jackson & Bepete (1995) evaluated HC on several apple cultivars in a warm region and found that HC is effective at synchronising full bloom dates between

different cultivars. Thus, HC applications improve cross-pollination and increased yield potential. In pecans, the flowering period can be advanced by up to 30 days with HC (Wood, 1993). However, little information on the application of HC on pecan under South African conditions has been reported and chilling requirements of the cultivars under local conditions have not been quantified.

In this trial we aimed to i) advance and increase the bud break, ii) increase the percentage vegetative bud break iii) shoot length, iv) yield of 'Navaho' pecans and v), determine the protocol to synchronise the flowering period of 'Navaho' and 'Wichita' pecans by applying different concentrations of HC at different application times.

Materials and Methods

Trial site and experimental design

The experiment was conducted on the commercial pecan farm Green Valley Nuts, near Prieska, Northern Cape (25° 35' 14" S; 22° 55' 06" E). Trials were conducted during the 2018/19 and 2019/20 seasons. The orchard selected as experimental site was planted in 2012 and the same trees were used for both seasons. The main cultivar is Wichita and for every four rows of 'Wichita', one row of Navaho is planted as cross-pollinator. The trees are planted at a spacing of 10 x 10 m on 'Ukulinga', a seedling rootstock, in sandy soil. Standard commercial management practices such as irrigation, pest and disease management and fertilisation were followed throughout the duration of the trial.

A randomized complete block design was used as the experimental layout. Buffer rows were left between treatments to avoid spray drift. Seven treatments were applied to 10 single tree replicates per cultivar (Table 1).

Prieska receives relatively few chilling units in winter. From May to the end of August in 2016 and 2017, Prieska received 461 and 337 Daily Positive Chill Units (DPCU) (Linsley-Noakes *et al.*, 1994) respectively (356 and 161 RCU) (Richardson *et al.*, 1974). High maximum and minimum temperatures are experienced in winter and summer. The annual rainfall for the region is approximately 200 mm. Furthermore, the region receives approximately 70 days of frost a year, which ranges in severity and may cause damage to young shoots (personal communication, R Botha consultant). Prieska is one of the established pecan producing regions by the South African Pecan Nut Producers Association (SAPPA, 2019).

Treatments

Expected bud break was estimated based on historical records (personal communication, R Botha consultant). HC formulated as Dormex® (Philagro South Africa (Pty) Ltd. PO Box 36213 Menlopark 0102) was applied using a Rovic Leers “Cima 2000 L” sprayer. Approximately 5 L of water was applied per tree, per application. Treatments were applied in the early morning, during a period of low wind speed, to ensure proper spray coverage. After applications, temperatures were recorded to determine whether conditions were conducive to the proper efficacy of the product.

Treatment details and application dates for both years are summarised in Table 1. Three concentrations of Dormex® (1%, 2% and 4%) were applied at four weeks before expected bud break (4WBBB) and again at two weeks before expected bud break (2WBBB).

In the 2019 season, treatments on ‘Navaho’ were only applied once, as the bud break period started earlier than anticipated and the possibility of phytotoxicity causing serious damage to trees was too high. Our one application time that was carried out was aimed at four weeks before expected bud break but more accurately represents a treatment at two weeks before expected bud break.

Data collection

In 2018, two representative branch units (Figure 2 Paper 1) were selected on each tree, based on uniformity, a branch unit consisted of a two-year section with four to five one-year old shoots. To quantify the bud break response, the total number of buds on all one-year old shoots per branch unit was quantified and bud break was then determined as a percentage. The same was done on the two-year section of the branch unit. All buds (vegetative and reproductive) were quantified together. This was done as the different types of buds could not be differentiated from one another on the chosen evaluation dates. Therefore, results do not allow us to determine whether HC increased the number of vegetative breaks and therefore possible bearing units in the first season. A bud was counted as having burst if the bud scales had split and green growth was visible (Figure 1). Evaluation dates are summarised in Table 2. The trees were also observed visually for phytotoxicity throughout the course of the trial by looking for signs of burning on treated shoots and young growth. Final shoot length was recorded on five randomly selected shoots per tree at the end of the growing

season (11/09/2019). Yield was recorded for individual trees at harvest, on the 29 May 2019. Hourly weather data were obtained from an automatic weather station on the farm and chill units were calculated.

In 2019, five uniform, one-year-old shoots were selected on each tree. Selected branches were at least 30 cm long and 1 cm in diameter. In contrast to the 2018/2019 season, bud break was only quantified on one-year-old wood as two-year-old wood was shown to be insensitive to HC. Bud break was quantified as a percentage of total number of buds per shoot. Total bud break was assessed on two occasions, once at 10 days after the final spray (Start of BB) and then again two weeks later. As it was not possible to distinguish between vegetative and reproductive growth on the first two evaluation dates (as also observed in 2018), a third evaluation date was included during the second season in order to assess the vegetative bud break, and determine whether HC could improve the amounts of bearing wood.

After observing an effect for HC on the timing of bud break during 2018 it was decided that the synchronisation of the flowering periods of our two cultivars would be assessed. On 'Navaho', the percentage of mature catkin clusters was determined per shoot on each evaluation date. Catkin clusters were counted as mature if they were at the pollen shed stage, when the cluster changes from a green to a golden yellow colour. On 'Navaho' only catkin clusters were counted. On the 'Wichita' trees, only pistillate flowers were counted and a percentage of mature flower clusters was determined for each shoot on each of the evaluation dates. A female flower was determined as receptive if the tips of the flowers were a shiny, pinkish colour. Trees were visually evaluated for phytotoxicity throughout the course of the trial.

Statistical analysis

Data were analysed using the GLM (General Linear Models) procedure in Statistical analysis software (SAS), Enterprise Guide 7.1. A one-way ANOVA was performed on all the bud break as well as shoot length data. Means were separated using Fischer's LSD at a 5 % significance level.

Results

Bud break reaction 2018

Bud break percentages on control trees were significantly lower than that of all HC treatments on both evaluation dates, but did not differ between HC treatments. All

HC treatments reached average bud break percentages above 70 % and were similar on both evaluation dates. Higher concentrations of HC seemed to cause a slightly higher bud break percentage than that of the lower concentrations (Table 3).

On two-year-old wood, bud break percentages remained very low (< 15 %) and no significant differences were seen between treatments (Table 3). No signs of phytotoxicity were observed on any treatments, at any point during the trial.

Shoot length and Yield efficiency 2018/19

No significant differences occurred between HC treatments and/or the untreated control in shoot extension growth or yield of 'Navaho' (Table 4 & Table 5).

Bud break reaction 2019

Differences between HC application times could not be assessed for this season as only one application was successfully carried out due to an earlier than expected bud break. Treatment with 4% Dormex® resulted in the highest bud break on the initial evaluation date, and it was significantly higher than that of the control and 1 % Dormex® treatment, but not the 2 % Dormex® (Table 6). On the second evaluation date, both 4 % and 2 % Dormex® resulted in bud break percentages that were significantly higher than that of the untreated control, indicating an increase in the final percentage of bud break for these treatments in comparison to the control. The 4 % Dormex® treatment also differed significantly from 1 % Dormex®, which did not differ significantly from the 2 % Dormex® treatment (Table 6).

Vegetative and Catkin bud break 2019

Treatment with Dormex® did not have a significant effect on the percentage vegetative shoots that developed on one-year-old wood (Table 7). The 2 % Dormex® treatment resulted in the highest percentage catkin bud break. This was significantly higher than the response for 1% Dormex® and the untreated control. The 4 % Dormex® treatment produced a similar percentage of catkins than the 2 % Dormex® and the untreated control.

Synchronisation of cross-pollinator and production cultivar flowering period 2019

Due to logistics, only one evaluation on the percentage mature flowers could be done, as flower development occurred faster than anticipated (Table 8). The

progress in flower development on this date was determined as a percentage of the total number of flowers that opened. On this date, twice as many 'Wichita' pistillate flowers had reached maturity on the 4 % Dormex® 4WBBB treatment than in the control. This treatment also differed significantly from all other treatments except 2 % Dormex® 2WBBB. This treatment showed a significantly higher bud break percentage than that of 2 % Dormex® 2 WBBB.

Compared to the mature pistillate flowers on 'Wichita' trees, the catkins of the 'Navaho' trees were phenologically more advanced. However, no significant differences were seen between treatments.

Discussion

Bud break

During the 2018 season, the two bud break evaluations were conducted fairly late during the bud break period and only one week apart, due to logistical reasons. It is therefore difficult to determine whether HC had an effect on the time of bud break, as no increase in bud break percentage was observed between the dates, and the bud break percentage on the first date was generally already in excess of 70 % on one-year-old shoots for the Dormex® treatments. Our results therefore only represent the final percentage bud break. This was addressed in the following season with earlier evaluations after treatments were applied. Additionally, bud break data refer to total bud break and not vegetative development, therefore our results for 2018 does not implicate that Dormex® (HC) improved the vegetative development. This was addressed in the 2019 season, by adding a later evaluation date to determine if there was an increase in the vegetative development. Bud break on two-year-old shoots did not react to the treatments (< 15 % bud break).

Observations during 2018 indicated advancement of bud break in the HC treatments compared to that of the control (Figure 2). Wood (1993) reported that Dormex® treatment influences the time of bud break, while our results clearly indicate that Dormex® applications increased the final bud break percentage significantly, regardless of concentration or application time. This confirmed the findings of Fayek *et al.* (2008) in pecans and other reports in deciduous fruit crops, such as apples (Jackson, 1997; Bound & Jones, 2004), kiwis (Veloso *et al.*, (2003), peaches (Siller-Cepeda *et al.*, 1992) and pistachios (Rahemi & Asghari, 2004; Ghrab & Mimoun,

2014). The trend that we observed that higher rates of HC increased bud break, merits further investigation.

Application time did not influence the final bud break as evaluated on the two dates, as no significant differences were seen between treatments of the two application dates (Table 3). The effect of the application time on final bud break percentage in pecans is not well researched. Although Wood (1993) (pecans) and Bound & Jones (2004) (apples) reported that earlier application times result in earlier bud break, our evaluations were recorded too late to conclusively determine the effect of application time on the time of bud break. Thus, to report on this, the trial should be repeated.

In line with results for 'Wichita' (Paper 1), bud break percentages on two-year-old wood remained very low and no significant differences were seen between treatments. This indicates that buds on older wood may be less sensitive to rest breaking under these conditions and treatments, and will most likely remain dormant.

During the 2019 season, only one application of Dormex® could be conducted, as bud break occurred earlier than anticipated and the danger of phytotoxicity on the later spray date being too high. Application was aimed at 4WBBB however the treatment more accurately represents a treatment 2WBBB. Thus, the effect of application time on the timing of bud break could not be evaluated. A comparison of treatments on the same application date showed that treatments with higher HC concentrations had slightly higher bud break percentages than that of lower concentration treatments and the control, confirming results from 2018. The 4 % Dormex® (2 WBBB) treatment advanced bud break percentage most and resulted in a significantly higher bud break than that of both the control and the lowest concentration treatment (1 % Dormex® (2 WBBB)), for both evaluation dates (Table 6). This suggests that this treatment advanced the timing of bud break and increased the final percentage of bud break. The 2 % Dormex® (2 WBBB) treatment resulted in an increase of the final percentage bud break compared to that of the control, but the initial bud break of this treatment did not differ significantly from that of the control. This treatment did not differ significantly from the high concentration on either evaluation date. A low HC concentration did not significantly improve the level of bud break on either evaluation date compared to the control. Our results are in line with Wood (1993) who found that, on pecans, higher concentrations of Dormex® (up to 8 %) are more effective at advancing bud break than lower concentrations. However, this was only true if

applications were carried out earlier than 30 days before expected bud break, which contradicts our findings. Ghrab & Mimoun (2014) reported a very similar result to ours on pistachios. A 4 % Dormex® application was more effective at advancing bud break, but in terms of the final percentage bud break, it did not differ from a 2 % Dormex® application.

Our results on 'Navaho' also indicate that during 2019, 'Navaho' bud break was affected differently by late applications of HC than in 2018. Similar results were reported for 'Wichita' (Paper 1). Bud break reaction in response to Dormex® treatments for the 2018 and 2019 seasons was similar, despite the difference in chilling accumulation between seasons. Chilling units for 2018 were 453 DPCU (354 RCU) versus the substantially lower 332 DPCU (117 RCU) in 2019.

In Paper 1, this difference in chilling accumulation resulted in a stronger effect on bud break with Dormex® treatment during 2019, when fewer chilling units accumulated. This trend was however, not observed in 'Navaho'. In addition, bud break in 'Navaho' was approximately a week earlier than that of 'Wichita' trees, irrespective of treatment. Thus, although the chilling requirement of 'Navaho' has not been determined, 'Navaho' probably has a lower chilling requirement than 'Wichita'. This was reported before for other fruit types where earlier bud break was correlated with lower chill requirement (Borchert, 1991). This is further supported by the relatively high bud break percentage (70 %) on one-year-old shoots in 'Navaho', during both seasons.

The bud break percentages discussed above represent the total bud break for both vegetative and reproductive buds. Therefore, results do report on vegetative growth or reproductive development individually. Our results showed how HC treatments with various concentrations and application times differed in their efficacy to influence the bud break period of 'Navaho'. Time of bud break could be manipulated and used as a management tool to achieve an earlier harvest time or used to synchronize flowering time between cultivars.

Vegetative reaction

In young trees, vegetative growth is beneficial, as it allows the trees to fill their designated space in the orchard more quickly. Additionally, in pecans, pistillate flowers are borne terminally on new shoots produced on one-year-old shoots (Woodroof & Chapman-Woodroof, 1926, Wetzstein *et al.*, 1996). Therefore, the higher the number

of one-year-old vegetative shoots, the higher the number of potential bearing units and the higher the yield potential (Figure 3).

Our trial assessed two factors regarding vegetative development: shoot elongation and the proportion of buds that developed into shoots. Our result confirmed results from Ghrab & Mimoun (2014) on pistachios, where HC treatments had a positive impact on total bud break and flowering, but did not have an impact on shoot growth or number. Firstly, no increase in shoot elongation was observed during the 2018 season (Table 4). Secondly, in 2019, Dormex® did not affect the number of buds that developed into vegetative shoots (Table 7). Despite this, there are indications that 2 % Dormex® 4WB3B may increase the number of vegetative shoots on one-year-old wood. Observations by Fayek *et al.* (2008) showed that 3 % Dormex® increases vegetative bud break in pecans.

As no significant differences were observed between treatments in the number of vegetative shoots that developed during 2019, significant differences in total bud break can only be attributed to the level of catkin bud break. Table 7 shows that 2 % Dormex® treatments resulted in significantly more catkin clusters compared to that of the control. Typically, apical buds have a lower chilling requirement than laterals (Campoy *et al.*, 2011). Furthermore, lateral bud break is generally lower than apical bud break, because laterals are under correlative inhibition by the apex and are prevented from growing out due to apical dominance (Romberger, 1963). In pecans, vegetative shoots are typically found at the apex, while catkins make up the majority of the lateral positions (Woodroof & Chapman-Woodroof, 1926). This would explain why HC would have a stronger effect on catkins than on vegetative shoots. Petri & Stucker (1995) found that on apple HC has a greater influence on lateral bud break than on terminal bud break. As most apical buds broke regardless of HC treatment, on positions where the majority of vegetative development takes place, it is unlikely that vegetative growth of pecans can be significantly influenced through HC application. This contrasts reports on grapevines (Kubota *et al.*, 2000) and apples (El-Sabagh *et al.*, 2012).

Unfortunately, conclusive vegetative data was only recorded in the second season of our trial. An additional season would be required to confirm the results from 2019 to provide conclusive evidence on whether HC can improve vegetative development in pecan in the Prieska region.

Yield

HC treatments during 2018 did not affect yield significantly compared to the control. No significant differences were seen between any treatments. However, a trend was observed that the HC treatments decreased yield (Table 5), which requires further investigation. This is possibly caused by a lack of synchronisation in the flowering periods. As 'Navaho' is a protandrous cultivar (Thompson *et al.*, 1995) the female flowers should mature at the same time as the catkins on 'Wichita', which is a protogynous cultivar (Thompson & Connor, 2012). 'Wichita' therefore acts as the main pollen source for the 'Navaho' pistillate flowers. Previous observations indicated that 'Navaho' bud break occurred approximately one week before that of 'Wichita' trees, resulting in poor synchronisation. If HC is applied to 'Navaho' trees only, it will probably advance the development of its female flowers, and the flowers will reach maturity before the catkins of the untreated 'Wichita' trees are mature enough to supply sufficient pollen. This will result in a lower fruit set and a smaller yield in 'Navaho'. This data also emphasises that HC cannot increase the yield by increasing the level of pistillate flower bud break as this would have clearly resulted in increased yields for the treated trees.

Synchronisation of the flowering period of 'Navaho' and 'Wichita' pecans

To determine whether HC had the potential to improve synchronisation between the pollen producing catkins of 'Navaho' and the pistillate flowers of 'Wichita', the percentage of mature flowers was determined on all three evaluation dates. On the first evaluation date, flowers had not yet reached maturity and progression was relatively slow. On the second date, flowers started to mature. By the third evaluation date, flowers were pollinated and catkins dried out, therefore, this evaluation also could not be used to accurately represent flower progression. This initial slow rate of development followed by a rapid progression through flowering left only one evaluation date for accurate representation of the degree of flower progression. On the second evaluation date, flowering had progressed relatively far, but neither cultivar had reached full bloom, therefore, this date was selected for interpretation.

In apples, HC is effective at synchronising full bloom dates between different cultivars in warm climates (Jackson & Bepete, 1995). In pecans, Wood (1993) found that the flowering period can be advanced with HC treatments. Our bud break results also indicated that Dormex® treatments can advance bud break for both 'Wichita' and

'Navaho'. By applying several different HC treatments to each cultivar to manipulate the time of bud break, we determined the preferred treatment for improved synchronisation of the pistillate flowers of 'Wichita' with the catkins of 'Navaho'. In 'Wichita' trees treated with 4 % Dormex® 4WBBB, 52.5 % of pistillate flowers had reached maturity compared to 24.9 % in untreated control trees, indicating a significant advancement of the pistillate flowering period of 'Wichita'. A 2 % Dormex® 4WBBB application also advanced pistillate flower maturity, but did not differ significantly from that of the untreated control. The 2 % Dormex® 2WBBB treatment resulted in only 17.3 % of flowers having reached maturity, further implicating that earlier application dates are more successful in advancing bud break than later treatments.

On 'Navaho' in 2019, catkins were physiologically more mature than the pistillate flowers on the 'Wichita' trees and were closer to full bloom. 'Wichita' pistillate flowers had reached only 24.9 % maturity, while 'Navaho' catkins were more advanced and already reached 52.6 % maturity. This was partly because bud break in 'Navaho' trees typically occurs approximately one week before 'Wichita' trees. None of the HC treatments resulted in a significant advancement of catkin maturity. However, control trees had the lowest percentage of mature catkins (52.6 %), while a 2 % Dormex® treatment already had 68.0 % catkins at maturity. These results indicate that, if both cultivars receive a HC application, the phenological development of both will be advanced and the flowering periods will not be synchronised (Figure 6). This will have severe commercial implications. A 4 % Dormex® 4WBBB treatment resulted in flower maturity of 52.5 % on the second evaluation date that synchronised with the untreated 'Navaho' trees (52.6 % catkin maturity). The best synchronisation between cultivars will, therefore, probably be achieved with a 4 % Dormex® 4WBBB application to 'Wichita' only, leaving the 'Navaho' trees untreated. This will advance the flowering period of the 'Wichita' trees and stimulate their pistillate flowers to reach full bloom, while the catkins of 'Navaho' trees are still available. This is illustrated in Figure 6. A similar effect was demonstrated by Ghrab & Mimoun (2014) who found that a 4 % Dormex® treatment caused the female flowers of pistachios to be better synchronised with the flowering period of the cross-pollinators. However, this treatment also resulted in a more protracted development on 'Navaho', with catkin clusters reaching maturity over an extended period. This could be an advantage, as there would be a greater chance that a pollen source would be available when pistillate flowers on 'Wichita' become receptive under unfavourable climatic conditions. The effect that this will have

on the synchronisation of 'Navaho' pistillate flowers with the 'Wichita' catkins still needs to be determined to ensure that there is no major deleterious effect on the 'Navaho' yield.

Conclusion

HC can be used on 'Navaho' pecans to advance bud break and increase the final total bud break percentage. A 4 % Dormex® application significantly advanced bud break during the 2019 season when less chilling accumulated. During the 2018 season, the effect of a high concentration on the timing of bud break was not determined. In terms of the final percentage total bud break, during 2018, all HC treatments successfully improved bud break compared to the control. In 2019, the 4 % and 2 % Dormex® treatments increased the final percentage total bud break significantly, but 1 % Dormex® did not differ from the control when applied two weeks before expected bud break.

Our results do not conclusively show the effect the timing of the application on the time of bud break and the final percentage total bud break. The increase in the final percentage total bud break could have also been attributed to an increase in lateral bud break. Our bud break data do not report on the percentage of vegetative buds only, as the totals were assessed. Only one season of vegetative bud break data was recorded, therefore we cannot draw conclusions on the vegetative development and bearing units of the tree after HC application. Results during the 2019 season indicate that there was no increase in the numbers of vegetative bud breaks. This will have to be confirmed. The increased total bud break percentages in the 2019 season could be partly ascribed to an increase in the number of catkins, as there was an increased percentage of catkin bud break recorded. In addition to no increases in the number of vegetative shoots, no significant increase in shoot elongation was reported. This further suggests that HC did not result in increased vegetative development of 'Navaho' pecans.

In terms of synchronising the pistillate flowering period of 'Wichita' with the catkin flowering period of 'Navaho', the best combination of treatments was an early application with 4 % Dormex® 4WBBB for 'Wichita', while leaving 'Navaho' untreated. This will advance development of 'Wichita', which will better synchronise them with the developmental progression of 'Navaho', which typically burst approximately one week after 'Wichita' (Figure 6). The only advantage that treating 'Navaho', where it is planted

as the cross-pollinator, would have, is that the potential exists to increase the number of catkins that grow out, thereby, creating a larger source of pollen for 'Wichita' trees. However, this will result in 'Navaho' trees no longer being synchronised with 'Wichita' trees, which will lower the yield potential of 'Wichita'.

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Tables and Figures

Table 1: Dormex® concentrations and application times on ‘Navaho’ pecan trees on Green Valley Nuts, Prieska, during the 2018/19 and 2019/20 seasons. 2WBBB treatment in 2019 was only applied to ‘Wichita’ trees.

Rate	Concentration hydrogen cyanamide (g/L)	Application timing	Application Date.	
			2018	2019
Untreated control	0	N/A	N/A	N/A
1% Dormex®	5.2	2 Weeks Before Expected Bud Break	18/09/2018	24/09/2019 (‘Wichita’)
2% Dormex®	10.4	2 Weeks Before Expected Bud Break	18/09/2018	24/09/2019 (‘Wichita’)
4% Dormex®	20.8	2 Weeks Before Expected Bud Break	18/09/2018	24/09/2019 (‘Wichita’)
1% Dormex®	5.2	4 Weeks Before Expected Bud Break	04/09/2018	11/09/2019
2% Dormex®	10.4	4 Weeks Before Expected Bud Break	04/09/2018	11/09/2019
4% Dormex®	20.8	4 Weeks Before Expected Bud Break	04/09/2018	11/09/2019

Table 2: The dates on which evaluations for bud break were carried out on Green Valley Nuts, Prieska during 2018 and 2019 on ‘Navaho’ and ‘Wichita’ pecans.

	Evaluation date	
	2018	2019
1 st count	16/10/2018	02/10/2019
2 nd count	23/10/2018	16/10/2019
3 rd count		30/10/2019

Table 3: Effect of various concentrations and application times with Dormex® on 'Navaho' pecans bud break percentages for both one- and two-year-old branches on two dates in 2018.

Treatment	Bud break percentage			
	1-year-old shoots		2-year-old shoots	
	16/10/2018	23/10/2018	16/10/2018	23/10/2018
Control	60.55 b ^y	61.23 b	9.55 ns	10.38 ns
1% Dormex® 2 WBBB ^z	72.37 a	74.19 a	8.26	8.26
2% Dormex® 2 WBBB	75.26 a	76.19 a	12.30	15.19
4% Dormex® 2 WBBB	75.55 a	76.67 a	5.11	7.11
1% Dormex® 4 WBBB	68.30 a	70.18 a	9.80	11.05
2% Dormex® 4 WBBB	70.26 a	71.80 a	6.80	8.30
4% Dormex® 4 WBBB	70.34 a	71.21 a	13.62	14.18
Pr > F	0.0016	0.0009	0.4564	0.4863

^z Weeks Before Bud Break^y Means followed by the same letter within a column are not significantly different**Table 4:** Effect of various concentrations and application times with Dormex® on average shoot length of five one-year-old shoots per tree on 'Navaho' pecan trees in 2019.

Treatment	Average shoot length(cm)
Control	64.15 ns
1% Dormex® 2 WBBB ^z	65.37
2% Dormex® 2 WBBB	67.03
4% Dormex® 2 WBBB	64.16
1% Dormex® 4 WBBB	70.98
2% Dormex® 4 WBBB	69.30
4% Dormex® 4 WBBB	66.98
Pr > F	0.725

^z Weeks Before Bud Break.

Table 5: The effect of various application times and concentrations of Dormex® on the yield and yield efficiency of 'Navaho' pecans.

Treatment	Yield(kg)	Yield efficiency(g/cm ²)
Control	13.320 ns	94.5 ns
1% Dormex® 2WBBB ^z	8.357	68.8
2% Dormex® 2WBBB	7.700	65.4
4% Dormex® 2WBBB	7.796	63.3
1% Dormex® 4WBBB	8.083	58.5
2% Dormex® 4WBBB	8.934	58.3
4% Dormex® 4WBBB	8.177	53.7
Pr > F	0.466	0.553

^z Weeks Before Expected Bud Break.

Table 6: Effect of various concentrations of Dormex® applied 2WBBB on 'Navaho' pecans total bud break percentages on one-year-old wood for two evaluation dates in the 2019 season.

Treatment	Bud break %	
	02/10/2019	16/10/2019
Control	68.44 b ^y	71.18 c
1% Dormex® 4 WBBB ^z	69.13 b	71.18 bc
2% Dormex® 4 WBBB	73.46 ab	77.51 ab
4% Dormex® 4 WBBB	77.30 a	77.84 a
Pr>F	0.007	0.038

^z Weeks Before Bud Break

^y Means followed by the same letter are not significantly different

Table 7: Effect of various concentrations of Dormex® on the percentage of buds that develop into vegetative shoots and catkin clusters on one-year-old wood of 'Navaho' pecans during the 2019 season.

Treatment	Vegetative shoots (%)	Catkin clusters (%)
Control	23.23 ns	41.05 b
1% Dormex® 2 WBBB ^z	26.80	41.25 b ^y
2% Dormex® 2 WBBB	29.02	48.75 a
4% Dormex® 2 WBBB	23.78	47.33 ab
Pr>F	0.062	0.033

^z Weeks Before Bud Break

^y Means followed by the same letter are not significantly different

Table 8: The effect of various application times and concentrations of Dormex® on the percentage of mature pistillate flowers on 'Wichita' and mature catkins on 'Navaho' on the second evaluation date.

Treatment	Mature pistillate flower % 'Wichita'	Mature catkin % 'Navaho'
Control	24.86 bc	52.63 ns
1% Dormex® 2 WBBB ^z	19.35 bc	
2% Dormex® 2 WBBB	17.30 c	
4% Dormex® 2 WBBB	25.48 bc	
1% Dormex® 4 WBBB	21.91 bc	57.96
2% Dormex® 4 WBBB	40.50 ab	67.98
4% Dormex® 4 WBBB	52.50 a	65.50
Pr>F	0.025	0.087

^z Weeks Before Bud Break

^y Means followed by the same letter are not significantly different



Figure 1: An image showing what was counted as a 'broken' bud, scales had split and green growth was visible.

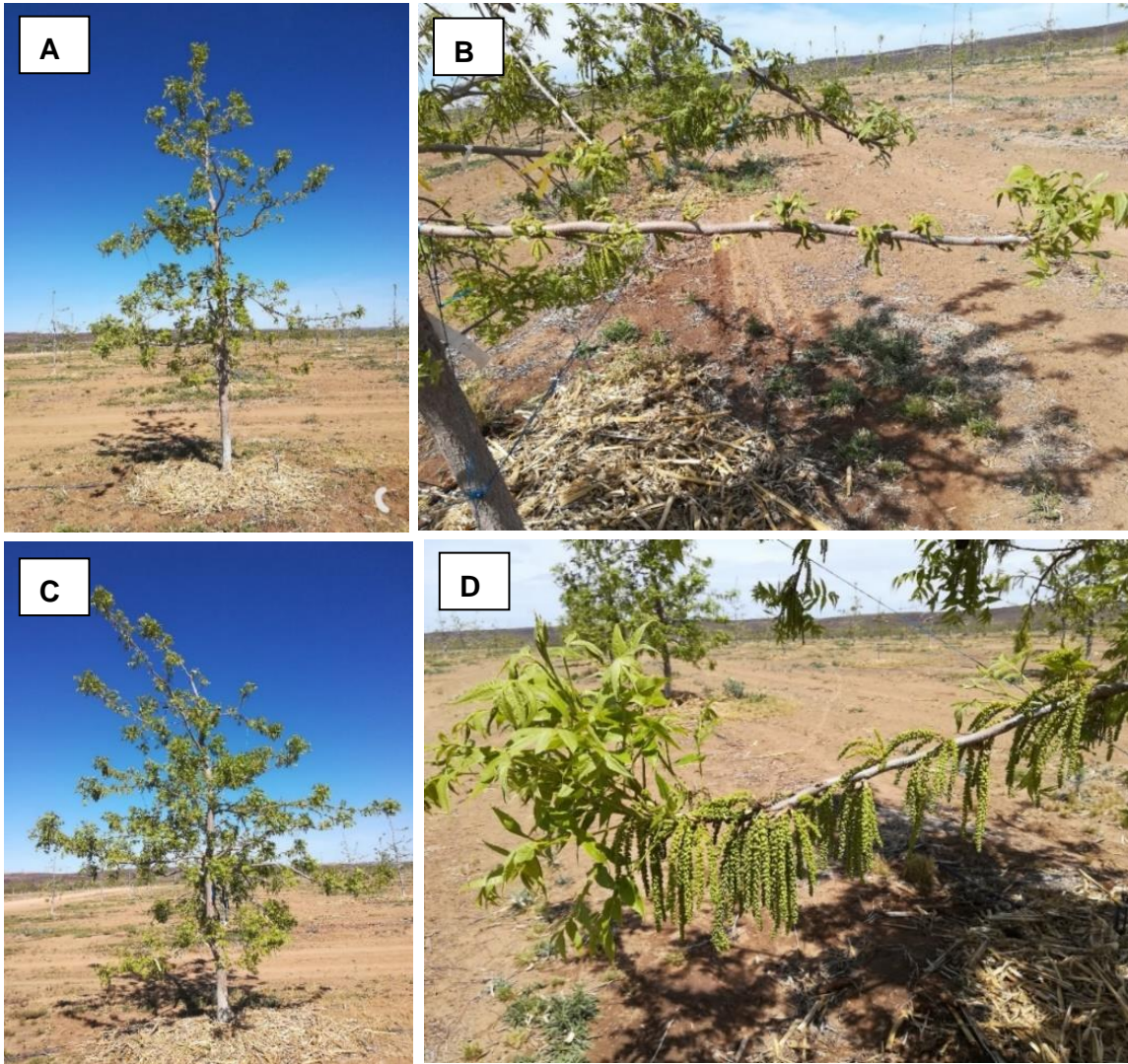


Figure 2: Images showing the bud break reaction of representative 'Navaho' trees and branches from one treatment and a control on the first evaluation date in 2018. A) Untreated control tree; B) untreated control branch; C) A tree treated with 4% Dormex® 2 weeks before bud break; D) A branch treated with 4% Dormex® 2 weeks before bud break.

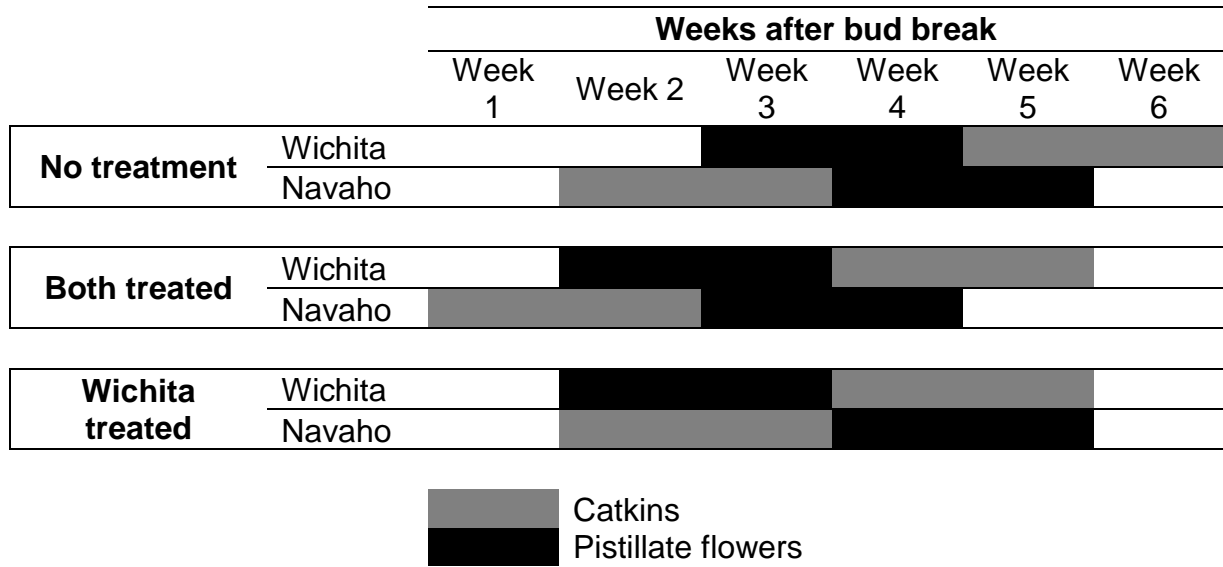


Figure 3: An illustration of the progression and synchronisation of the flowering periods of 'Wichita' and 'Navaho' pecans under different scenarios of Dormex® treatment.

PAPER 3: The effect of notching on bud break, shoot length and branch angle of ‘Wichita’ pecans [*Carya illinoensis* (Wangenh.) K. Koch] in the Villiersdorp region, Western Cape, South Africa

Abstract

When a new orchard is established, maximum vegetative growth is initially desired to develop the structure of the tree. When deciduous trees are grown in warm regions, they usually suffer from reduced lateral bud break, resulting in fewer side shoots, thus taking longer to properly develop the tree structure and ensure early bearing. In this trial, notching was used to induce lateral branching and increase shoot length. In 2018 and 2019, notching was carried out on two-year-old and one-year-old wood, respectively. One-year-old wood was more responsive to notching and produced an average of 3.9 shoots per 10 notched buds. This was significantly more than the unnotched control. Despite a larger number of shoots developing from notched buds the shoot length of these shoots was not significantly increased. Two-year-old wood was less responsive to notching, with no significant improvement observed in bud break, shoot number or shoot length. Moreover, on two-year-old wood, variation in the size of the buds influenced the branching of laterals.

Key words: Pruning, vegetative growth, notching, orchard establishment, bud break, dormancy

Introduction

In the last five years, pecan nut production in South Africa has almost doubled (SAPPA, 2019). Thus, pecans are quickly becoming an economically important crop to the agricultural sector. Establishment of a new orchard is a very important phase in determining the economic potential of a crop. In addition to tree density, vegetative growth is an important management focus to ensure that a tree fills its allocated space quickly, in order to reach the desired size and structure for maximum yield and early break-even point. According to SAPPA, a pecan orchard under local conditions starts cropping five years after planting and reaches its maximum potential in year 12 (SAPPA, 2019). To achieve this, management practices should aim to stimulate

vegetative growth of young trees and determine optimum tree density for the different cultivars. However, little information is available on manipulation of pecan trees under local conditions.

During orchard establishment, nutrition, pruning and irrigation are additional factors that must be managed optimally to ensure that the orchard reaches its potential as early as possible. For most crops, the essential element for stimulating vegetative growth and establishing young plants is nitrogen (N). Smith *et al.* (2000) found that application of high N rates to young pecan trees increases tree size. Additionally, an application of a mulch potentially has a positive impact on young tree growth.

Pruning practices, such as a heading cut during winter into one-year-old wood, stimulate branching, as it removes apical dominance by the terminal bud (Teichmann & Muhr, 2015). Wood (1996) reported that pruning young pecan trees after transplanting in spring has a positive effect on tree vigour.

With notching, a small incision is made just above a dormant bud at the end of winter, just before bud break. The aim is to remove the phloem and vascular cambium. A small saw or knife is used to make a cut just above the bud, extending slightly around the trunk (Greene & Autio, 1994). This has the same hormonal effect as decapitating the shoot, where the polar auxin stream moving down the shoot is temporarily interrupted, allowing cytokinin from the roots to move into the dormant lateral buds, inducing growth (Teichmann & Muhr, 2015). The effect of notching is well recorded in apples. Clements *et al.* (2010) evaluated several manipulations, including notching, on newly transplanted apple trees. Their results showed that notching increased the number of new shoots that develop. Greene & Autio (1994) also reported improved bud break on young apple trees. They found that larger buds have a greater potential to grow into shoots than smaller buds. This is most likely due to the fact that larger buds have more carbohydrates and nutrient reserves as well as better vascular conductivity to ensure proper growth resumption in spring.

According to our knowledge, no studies have been conducted on the effect of notching on pecan trees. This study aimed to elucidate the effect of notching on the vegetative development of young 'Wichita' pecan trees. This would allow growers to ensure that vegetative development of young trees occurs more evenly and that they are therefore able to fill the allocated tree space earlier.

Materials and Methods

Trial site and experimental design

The trial was conducted on a commercial farm, Arendskloof, in the Villiersdorp region, Western Cape (33° 50' 19" S 19° 24' 44" E). Trials were done during the 2018 and 2019 seasons on trees planted in October 2017, in between rows of grapevines, at a spacing of 4 m x 8 m. The main production cultivar is Wichita, with Navaho as cross pollinator. The trial orchard has sandy soil and trees are irrigated using micro sprinklers. Except for the treatments, standard commercial practices, such as fertilisation and irrigation were followed throughout the course of the trial. A randomised complete block design was used, with 10 single tree replicates per treatment.

Treatments 2018

A control (no manipulations) and a notching treatment were applied. Notching was done two weeks before expected bud break on 11 September 2018. Expected time of bud break was estimated based on historical data. At the end of winter in 2018, before notching, the trees were headed, by removing approximately 5-10 cm of the new growth as a standard commercial procedure on young trees. Additionally, all side shoots were removed with a clean thinning cut to enhance vegetative growth of the leader, as growth was compromised during the drought of 2017/18. In 2018, notching was only done on two-year-old wood, due to the lack of extension growth of the central leader in the 2017/18 season. The ten lateral buds on the distal section of two-year-old wood of the central leader (from the intercalation between one and two-year-old wood down), were notched by making a small incision in the bark, 5 mm above each bud, as shown in Figure 1.

Treatments 2019

In the 2019 season, notching was done on the same trees as in 2018, two weeks before expected bud break (16/09/2019). In contrast to the first season, notching was done only on one-year-old section of the central leader, as there was sufficient shoot growth the previous season. Notching was done on the basal ten buds of the one-year-old wood of the central leader, as this is where the lowest number of buds break. This results from the strong apical dominance that usually results in bud break

occurring exclusively towards the distal end of upright one-year-old shoots (Cook & Jacobs, 1999). Notches were made in the same way as in the previous season.

Data collection 2018

Final bud break was recorded two weeks after bud break on 26 October 2018. A bud was classified as growing if bud scales had opened and green leaves were visible (Figure 2). The reaction of each of the first 10 lateral buds was quantified individually. The size of the buds on two-year-old wood was variable (visual observation), therefore each bud was grouped into one of three categories (small, medium and large) based on their relative size (Figure 3). Bud size was classified to determine whether the size of the bud had an impact on the growth reaction.

After shoot growth cessation, final shoot length was measure of all shoots exceeding 5cm to determine whether notching had an effect on the shoot vigour. Shoot length was measured from the inception to the tip.

The angle of the new shoots was also recorded to determine whether the notching treatment resulted in sharper angles that those of un-notched buds. Shoot angles were separated into one of two categories (less than 45° and more than 45° from the stem) to distinguish between flat (> 45 °) and upright branches (< 45 °). The angle was measured between the stem and the new shoot that developed and was recorded after shoot growth cessation.

Data collection 2019

Final bud break for 2019 was recorded on 21 October 2019. As bud size on the one-year-old wood was more uniform, instead of quantifying the growth of individual buds, bud break was quantified as a percentage of buds that burst per tree. Shoot length and shoot angle were measured as described above. However, measurements occurred on 28 November 2019 before shoot growth cessation due to the time scale of the project.

Statistical analysis

Due to the categorical nature of the data the 2018 bud break and bud size data were analysed using a logistic regression using the bud break reaction (yes or no) as the response and the treatment and bud size as the independent variables. Contrasts between each of the bud sizes were determined to determine whether they differed in

their potential to break. Shoot length for both seasons was analysed using a Generalised Linear Model (GLM). An ANOVA was performed and means were separated using Fischer's LSD at a 5 % significance level. The 2019 bud break percentage data were analysed with a simple GLM procedure. An ANOVA for a randomised complete block design was used and means were again separated at a 5 % level using Fischer's LSD. All data were analysed in XL STAT 2019.

Results

Bud break

In 2018, notching did not increase bud break on two-year-old wood of 'Wichita' trees (Figure 4). Table 1 Shows the contrasts that were determined for the bud sizes. Large and medium buds did not differ from one another in their potential to grow, however, small buds differed from both large and medium buds in their potential to grow. The percentages of bud break for each bud size are illustrated in Figure 5. Only 28.6 % of small buds burst, regardless of whether they were notched or not. Medium and large buds were significantly more likely to grow and had a bud break of 81.8 % and 93.9 %, respectively (Figure 5). This difference in the potential to burst could have influenced the total bud break reaction calculated for 2018. Not all buds that burst developed into shoots and many of the shoots stopped growing and died (Figure 6). Therefore, the total number of shoots that developed from the 10 observed buds, was quantified, with notched trees typically having 2.4, and control trees had 1.7 shoots per ten buds (Figure 7), this difference was not significant.

In 2019, results followed a similar trend than those of 2018, despite the fact that trials were carried out on one-year-old shoots. Notching resulted in a slightly higher bud break percentage, yet not significantly more than the control (Figure 8). Despite the fact that bud break did not increase significantly, the average number of shoots (> 10 cm) developing from notched buds was significantly higher than that of shoots developing from the control buds (Figure 9).

Shoot length and branch angle

In the 2018 season, shoots from buds on notched trees did not differ significantly in length from control trees (Figure 10). This trend was similar to the trends seen in the bud break data, where notching seems to cause a slight non-significant improvement

(Figure 4). Shoot angle data were not analysed statistically, however, during 2018, 48.0 % of shoots from the notched buds grew with an angle $> 45^\circ$ from the vertical, whereas shoots from the control trees had a considerably higher average of flat shoots (88.24 %).

In 2019, notching seemed to cause a slight increase in the shoot length but was not found to be significantly longer than the controls (Figure 11) these results therefore confirmed what was found in 2018 on two-year-old wood. A similar trend to 2018 was seen with shoot angle in 2019, where control shoots tended to have a flatter ($> 45^\circ$ from the vertical) branch angle. This indicates a possible trend towards notched buds growing out as more upright shoots.

Discussion

The results obtained in this study achieved the aims and showed that it is possible to improve the vegetative development of non-bearing pecans by notching.

Bud break response

Our results contrast the findings of Clements *et al.* (2010) and Green & Autio (2014) in apples, as notching did not improve bud break. Instead, our results confirmed reports on cherries by Hoying *et al.* (2001). In our study, buds on two-year-old wood varied in size and this may have contributed to the non-significant bud break response in 2018. After categorising these buds, results confirmed that small buds typically had a much smaller chance of growing than large and medium buds (Table 1 & Figure 5) confirming the effect of size on the reaction of notching, as reported by Greene & Autio (1994) in apples. When categories were analysed separately, we confirmed that the bud break percentage of small notched buds was lower than when they were left untreated (control). Notching of large and medium buds resulted in a higher bud break percentage than in control trees. Larger buds should have a greater pool of carbohydrates to use for growth resumption in spring than smaller buds. Additionally, it has been shown that the size of an organ plays a role in sink strength (Hirota *et al.*, 1990). Therefore, larger buds will probably receive more carbohydrate reserves from the roots after winter than smaller buds. This effect, where larger buds have a higher potential to grow than smaller buds means that to successfully implement a notching treatment, it would be beneficial to only notch larger buds for shoot development to avoid additional costs.

During the 2018 season, a large number of shoots died after bud break (Figure 6). Therefore, the number of shoots that developed from the 10 apical buds on two-year-old wood was determined, but no significant differences were found. These results indicate that notching had very little impact on the two-year-old wood of young, non-bearing 'Wichita' pecans at this location.

In Paper 1 and 2 we concluded that two-year-old wood of pecans trees did not react to treatments with Hydrogen cyanamide, and bud break remained low. This supports the low response to notching in 2018 (two-year-old wood) and thus, treatments changed in 2019 and were done on one-year-old wood. Despite the change, the notching treatment resulted in only a slightly, non-significant higher bud break percentage than the control. Therefore, two-year-old wood of pecans is not necessarily less sensitive to notching than one-year-old wood, which contradicts findings on apples. Greene & Autio (1994) reported that, on two-year-old apple wood, 100 % of notched buds grew, compared to only 80 % on one-year-old wood. This indicates that two-year-old apple wood responded better to notching than two-year-old pecan wood.

Another possible explanation for the lack of response during 2019 is the location (basal) on the one-year-old wood where notching was done. Observations during the 2018 season suggest that young pecan shoots have a strong acrotonic branching habit, where only a few shoots near the distal end of shoots grow and very little lateral growth occurs after bud break. Therefore, it was decided that the most basal end of the shoot (10 buds on one-year-old wood) would be notched, as this would theoretically be the area of the shoot with the lowest bud break percentage. However, on the young trees grown in the Villiersdorp region, a large number of buds grew at the basal as well as distal end (Figure 12). Typically, under insufficient chilling conditions, trees have a stronger tendency toward a basitonic growth habit rather than the acrotonic growth habit expected with sufficient chilling (Cook & Jacobs, 1999). However, our trees exhibited a combination of the two: a large number of buds breaking on the distal and basal portion, but very little bud break in the middle section of the shoot. It is possible that the trees do, in fact exhibit a predominantly basitonic growth habit and that the strong growth reaction observed distally was caused by the heading cut made to the trees in winter. This strong basitonic growth meant that bud break occurred regardless of notching treatment. It would therefore be more sensible

to notch buds in the middle region of one-year-old shoots, as this is where bud break seems to be most deficient in pecans. This needs to be investigated further.

The aim of a notching treatment is to increase the number of shoots that develop, and therefore, in addition to bud break, the number of shoots growing after treatment was quantified, as buds often die or do not grow beyond the bud scale split stage. Results from 2019 contradicted those of 2018. Notched trees had a significantly higher number of shoots than that of control trees. This indicates that notching is effective at increasing branching on one-year-old wood. The increased number of shoots is of importance to the grower, as it provides more branches that will allow the trees to fill their allocated space more quickly. In terms of building the desired structure of the trees, it is also an advantage, as more branches gives the grower the option to select the branches in the best positions and additional undesired branches can then simply be removed.

Shoot elongation and branch angle

Studies on the effect of notching on the resultant shoot length of apples have shown contrasting results. Greene & Autio (1994) showed that notching not only increases the number of new shoots, but also results in the formation of longer shoots. This contradicts Clements *et al.* (2010), who found the same effect in terms of shoot number, but did not observe any increase in apple shoot length. In 2018 as well as 2019 we did not find a significant increase in the shoot length. A trend was, however, observed that suggested notching might have some small effect on the shoot length and this should be further investigated. Shoots on one-year-old wood were far more vigorous than those on two-year-old wood and were generally far longer. Hoying *et al.* (2001) notched every third bud on the central leader of cherry trees and found that this resulted in an even distribution of shoots across the leader. This should also be evaluated on pecans.

Producers commonly desire a tree structure with wider crotch angles, because more horizontal branches are more productive, intercept more sunlight and have a larger number of flowers and better fruit set than branches growing more upright (Dann *et al.*, 1990). Therefore, if young trees can be manipulated to have more horizontal crotch angles after filling the allocated space, they will be more precocious at an earlier stage. This is very prominent in current commercial apple and pear orchards worldwide, where bending increases the branch angle to more than 90° in the case of

apples on a spindle training system (Robinson *et al.*, 2006). Notching does not influence branch angle directly, but as notching influences the hormonal regulation of branch development, it is possible that it could indirectly influence branch angle. No statistical analysis was performed on the branch angle during our study. However, average percentages showed that in both seasons, shoots on control trees tended to have wider crotch angles while shoots growing from notched buds tended to have more upright angles. It is possible that notched trees had a stronger growing potential due to higher hormone activity. This could perhaps induce them to grow in a more upright manner than buds that were not notched. Additionally, shoots that are notched will not be under the hormonal regulation of shoots developing above them which may also encourage them to develop with a sharper angle.

If this crotch angle reaction to notching is indeed inherent to pecans, and pecans react similarly to other deciduous fruits in terms of precocity of flat branches, then notching could have a negative impact on increasing the time to commercial harvest. This would not be beneficial to producers. However, it is possible that a greater number of shoots can also result in increased yields on young trees by increasing the number of bearing units. This should be investigated, along with the effect of branch angle, to determine whether notching is a valuable tool that producers can use to improve the precocity of young orchards.

Alternative manipulations

Various practices effectively induce lateral development. One commonly used practice in deciduous fruit is applying cytokinin to lateral buds just before bud break at bud swell, to induce growth. Cytokinin induces cell division (Zhang *et al.*, 2013). Jackson (1997) applied a combination of gibberellin A 4 and 7 (GA₄₊₇) with 6-benzyl adenine (6-BA), formulated as Promalin® to apple trees in a warm region and found a significant increase in shoot development on the central leader. Similar results were found by Clements *et al.* (2010) and Greene & Autio (1994), who concluded that GA₄₊₇ plus 6-BA is more effective at inducing branching than notching. The effect of GA₄₊₇ plus 6-BA on the resulting shoot length is unclear, with Clements *et al.* (2010) reporting an increase in shoot length and Greene & Autio (1994), an opposite effect. The combination of GA₄₊₇ plus 6-BA should still be evaluated for pecans if an increase in shoot number is required.

Conclusion

Notching of buds on two-year-old wood was not effective in increasing bud break and seems to be influenced to some extent by the size of the bud. Therefore, if notching needs to be done on two-year-old wood, our results suggest that only larger buds should be notched. On one-year-old wood, notching seems to be more effective and resulted in a greater number of shoots growing out from these buds. On one-year-old wood, very little lateral growth was observed in the middle section of the one-year-old shoot. Thus, we suggest that the efficacy of notching should be evaluated in this area of low bud break in future. This would also increase the potential to obtain an even distribution of shoots across the whole section of the leader and not only at the basal end of the shoot. This would allow the producer more choice in selecting future scaffold branches and would allow them to build the optimum tree structure. The effect that notching has on the crotch angle and the precocity of the tree must still be determined in more detail.

Notching cuts did not have an adverse effect on the trees and did not cause any permanent damage, as is possible in other crops. Additionally, other manipulations such as GA₄₊₇ plus 6-BA application should be evaluated to inducing bud break, but the cost implications will also need to be considered.

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Tables and figures

Table 1: Contrasts between bud sizes indicating the likelihood of each bud size growing out compared to each other bud size for 'Wichita' in Villiersdorp.

Contrast	Pr > Chi²
Large vs Medium	0.070
Large vs Small	< 0.0001
Medium vs Small	< 0.0001



Figure 1: An image showing how the 'notching' treatments were carried out in the 2018 season.



Figure 2: An image showing at what stage buds were counted as having "broken".

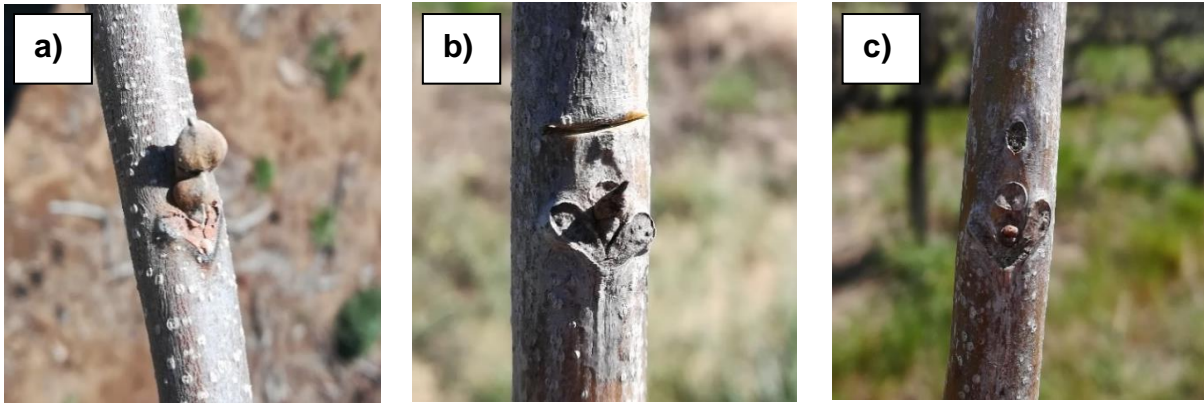


Figure 3: An image showing the three categories that buds on two-year-old wood were separated into in 2018. A) Large bud; B) Medium bud; C) Small bud

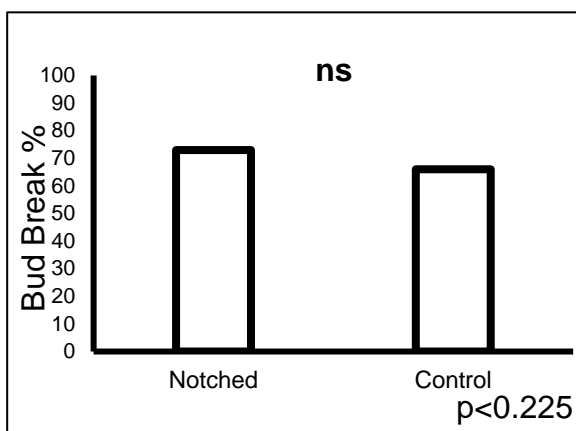


Figure 4: Final bud break percentages of notched two-year-old wood of young 'Wichita' trees in 2018 compared to a control

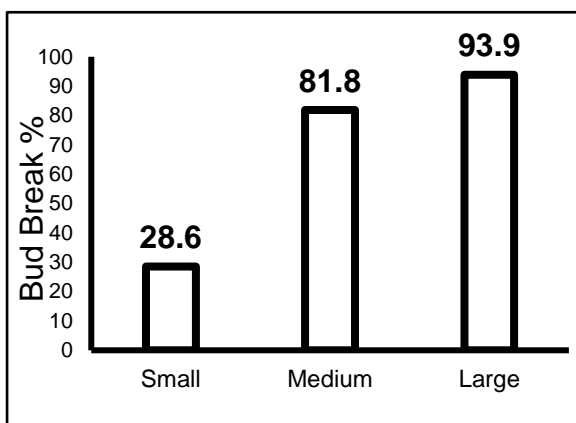


Figure 5: Final bud break percentages for each bud size, regardless of treatment, on two-year-old wood of young 'Wichita' trees in 2018.



Figure 6: An image of a bud which had broken, stopped growing and was aborted.

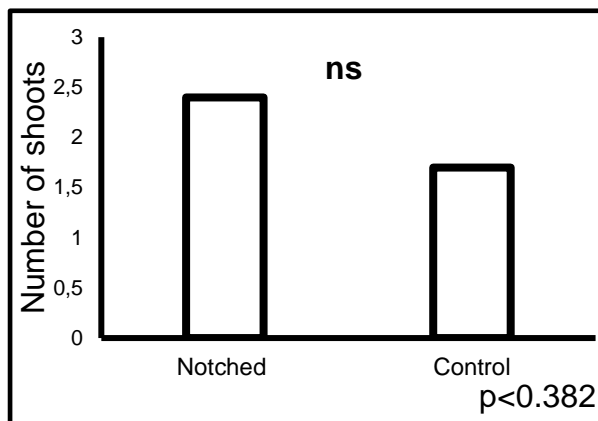


Figure 7: The effect of notching on the number of shoots produced from the 10 buds on the apical section of two-year-old wood of young 'Wichita' trees during the 2018 season.

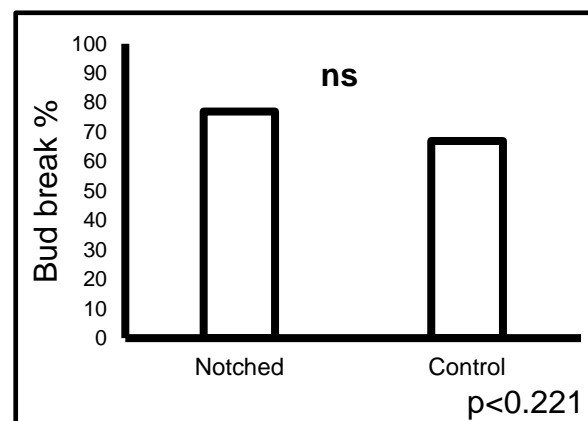


Figure 8: Effect of notching on the final bud break percentage of one-year-old wood of 'Wichita' pecans during the 2019 season.

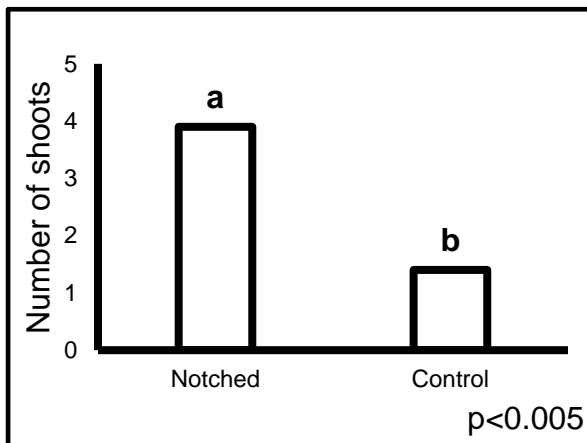


Figure 9: The effect of notching on the number of shoots produced from the 10 buds on the proximal portion of one-year-old wood of young 'Wichita' trees during the 2019 season.

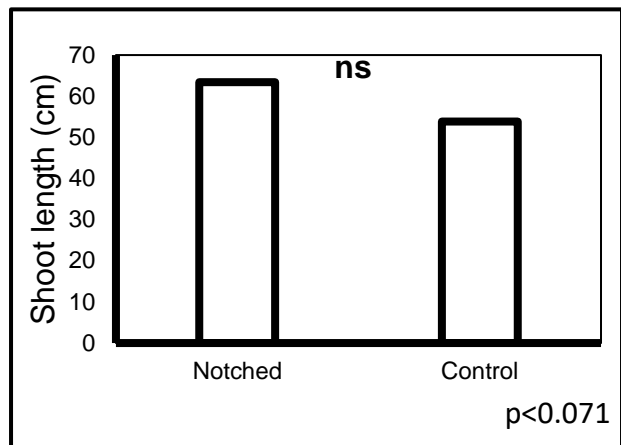


Figure 10: The effect of notching on the average shoot length (cm) at the end of the 2018/2019 growing season.

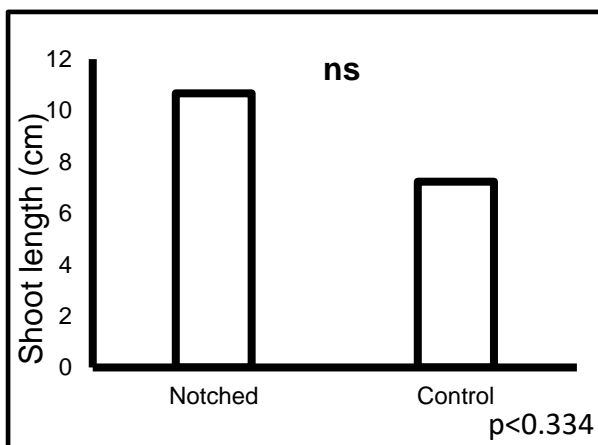


Figure 11: The effect of notching on the resultant average shoot length (cm) during the 2019 growing season.



Figure 12: An image of a young 'Wichita' pecan tree showing the bud break patterns in 2019. A combination of an actrotonic and basitonic growth habit were observed.

GENERAL CONCLUSION

The poor spring bud break experienced by pecan trees caused by relatively warm winters can, to a certain extent, be mitigated by the management practices trialled in this study.

In terms of the vegetative development, hydrogen cyanamide (HC) did not have a significant effect in improving growth. During the 2018/19 growing season, HC caused a significant increase in the shoot elongation growth of 'Wichita'. Other than this effect, no significant improvements were seen in any of the vegetative growth parameters that were measured. However, more research should be conducted on the effect of HC on vegetative buds to confirm observed trends that suggested a potential increase in the vegetative bud break percentage. Unfortunately, vegetative bud break was only measured in season 2 (2019) and therefore we cannot comment on the effect of HC on increasing vegetative growth of pecans.

Notching of buds on one-year-old shoots seemed to be more effective at increasing lateral development on young trees as significant improvements were observed in lateral branching. This was however not observed on two-year-old wood. Practically, however, notching can only be used on smaller trees as there will be significant implications in terms of labour if bearing trees were notched. Other manipulations such as application of plant growth regulators should also still be evaluated for their efficacy on improving vegetative development and lateral branching, such as the combination of gibberellin A₄ plus A₇ with 6-benzyl adenine, which is used on many other deciduous crops (Greene & Autio, 1994).

HC was more effective at manipulating the time and percentage of total bud break. Its effect on total bud break seems to be influenced to a large extent by the amount of chilling that accumulates. In 2018, a relatively cold winter was experienced and sufficient chilling probably accumulated. On 'Wichita' only one HC treatment significantly increased bud break. On 'Navaho', however, all HC treatments improved the final percentage bud break significantly compared to the control. Our data for 2018 do not show any improvements in terms of an advancing bud break.

During 2019, fewer chilling units accumulated during winter and HC application seemed to have a more pronounced effect. On 'Wichita', earlier applications (four weeks before expected bud break) advancing bud break compared to the control and later applications, confirming what is seen in literature (Wood, 1993; Ghrab & Mimoun,

2014). The effect of application time on bud break was not accurately determined on 'Navaho' and further research should be conducted on this cultivar. Higher concentrations (4% Dormex®) were more effective at advancing bud break on both cultivars. Timing of the application did not influence the final bud break percentage. Treatments with 4% and 2% Dormex® both resulted in a significant increase in the final bud break. This indicates the increased efficacy of higher concentrations of HC in inducing bud break in pecan trees. This same phenomenon was shown in other crops (Rahemi & Asghari, 2004). The increase in the bud break discussed in this thesis always refers to total bud break and therefore these results do not indicate that HC increased the percentage of vegetative bud break or pistillate flower bud break. There are therefore no direct practical implications for these results.

The end goal for any manipulation performed on a tree is an increased productivity. HC had a positive effect on the yield of 'Wichita' in the 2018/19 growing season. As HC could not increase the pistillate flower bud break or the vegetative bud break this increase in yield could not have been a result of an increased number of flowers or bearing units and must have been caused by an improvement in the synchronisation of the flowering periods of 'Wichita' as a main cultivar and 'Navaho' as cross pollinator. This is further evidenced by the fact that a trend was seen in 'Navaho' where treated trees seemed to have a lower yield. This reduced yield could be a result of an earlier bud break on treated trees causing the pistillate flowers on 'Navaho' to mature before the catkins on 'Wichita' were ready to pollinate them. This improved synchronisation in flowering has been reported on other crops (Jackson & Bepete, 1995; Ghrab & Mimoun, 2014).

During the 2019 season the synchronisation of the flowering periods of the two cultivars was investigated. The best synchronisation and therefore production potential resulted when treating only 'Wichita' trees with 4% Dormex® 4WBBB, while leaving 'Navaho' trees untreated. This will advance the bloom of 'Wichita' and allow it to better synchronise with the earlier breaking 'Navaho' and therefore improve the pollination potential. This would most likely be more effective during years where insufficient chilling accumulates and bud break is more protracted, however this method of improving synchronisation of the flowering periods should be further investigated during a range of warm and cold years.

In both the notching and HC trials, results suggested that two-year-old wood is not responsive to manipulations to induce bud break. Further trials using application of

plant growth regulators such as gibberellin A₄ plus A₇ with 6-benzyl adenine (Promalin®) should follow to determine whether bud break can be induced on older wood as this could provide a useful management tool in developing tree structure. It is also suggested that shoot length and yield be determined at the end of the 2019/20 growing season to confirm results from the 2018/19 growing season.

No phytotoxicity was observed throughout the course of the trial. Based on what was found by Wood (1993), higher concentrations can be evaluated to determine what effect they will have under local conditions. In addition, a wider range of application times should also be investigated to determine whether even earlier applications can further influence bud break as was found by Wood (1993).

In addition, general studies into the physiological and phenological development of pecan trees through HC and bud break should be investigated under South African conditions to draw more accurate comparisons between pecans and other deciduous crops that behave in a similar fashion.

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