Manipulation of the Chilling Requirement of Sweet Cherry Trees

By

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Thesis presented in partial fulfilment of the requirements for the Degree of Master of Science in Agriculture at the University of Stellenbosch

March 2008

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature: ..... Date: .....

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Summary

Commercial production of sweet cherries has recently increased in South Africa, with more than 400 ha planted by 2006. Cherry, a high chilling fruit variety, is however not suited for the mild winter climate of South Africa. This was recognizable through common observed symptoms of delayed foliation and poor fruit set. In addition, cherry is exposed to long and hot summers in the postharvest period. The objective of this study was to evaluate cherry cultural practices that can manipulate (reduce) the trees chilling requirement under South African conditions. Cultural practices where aimed at increasing reserves (nitrogen, cytokinin and carbohydrates) in the tree. In addition, bud dormancy progression of cherry buds was quantified to determine the bud dormancy progression pattern under mild winter conditions. This was achieved through sampling of cherry shoots from different cherry production areas which was then forced in the growth cabinets. A model was developed to identify possible factors and groupings that can explain the cherry bud dormancy pattern.

A model, comprising two joined straight lines, was fitted in order to characterize bud dormancy behaviour for sweet cherry cultivars under mild winter conditions. All cherry cultivars followed the expected pattern of entrance and exit from dormancy. Factor analysis showed that factors related to the entrance into dormancy primarily characterize bud dormancy behaviour. Bud dormancy patterns were also a function of environmental conditions within a year as shown by cluster analysis. In addition, buds entered dormancy in mid-summer and remained dormant until chilling accumulation commenced. Bud dormancy release was generally extended over a three to five-month period for all cultivars. Prior to spring budburst exit of both lateral and terminal buds occurred rapidly. Data indicate that there is no ecodormant phase for cherry under the prevalent climatic conditions in South Africa.

Further experimentation was aimed at increasing reserves within the trees through cultural practices. In the nitrogen trials, fertilization in the postharvest period had no significant effect on field budburst or bud dormancy progression in one-year-old shoots. Time of flowering was advanced in N treatments during 2007 only. Yield was not significantly increased. Therefore, in this trial, N fertilization in the postharvest period did not significantly reduce the chilling requirement of mature sweet cherry trees under mild winter conditions.

Application of particle films (Surround® and Raynox®) or ethylene inhibitors (Retain®) in the summer did not reduce the heat stress the trees experienced. Treatments had no significant effect on carbon assimilation, stomatal conductance, leaf surface temperature, fluorescence, bud dormancy, budburst, flowering and fruit set.

Cytokinins sprays (benzyladenine) in autumn did not affect bud dormancy progression, spring budburst or flowering.

Hydrogen cyanamide application in spring significantly advanced budburst, time to full bloom and increased yield. Promalin® and Retain®, however, had no significant effect on budburst, flowering or yield.

It is therefore evident that cherry, due to its unexpected bud dormancy behaviour and its inability to be significantly influenced by several cultural practices, adapts poorly to South African climatic conditions through not reducing its chilling requirement significantly.

Manipulasie van die Kouebehoefte van Soetkersiebome

Opsomming

Kommersiële produksie van soet kersies het onlangs toegeneem in Suid Afrika, met meer as 400 ha geplant teen 2006. Kersie is ‘n hoë-kouebehoefte gewas wat nie aangepas is vir die gematigde winters van Suid-Afrika nie. Dit is duidelik uit die voorkoms van vertraagde bot en swak vrugset. Kersie is ook blootgestel aan lang en warm somers in die na-oes periode. Die doel van die studie was om bestuurspraktyke te evalueer wat hulle kouebehoefte kan manipuleer onder Suid-Afrikaanse klimaatsomstandighede. Bestuurspraktyke was gerig om die reserwe status (stikstof, koolhidrate en sitokiniene) te verhoog. Knopdormansie verloop was gekwantifiseer om die patroon van knopdormansiegedrag vas te stel onder die heersende gematigde klimaats kondisies. Dit is bereik deur kersie lote te monster en te forseer in groeikabinette. ‘n Model is ontwikkel om moontlike faktore of groeperings te identifiseer wat die knopdormansie gedrag van kersie kon verklaar.

‘n Model bestaande uit twee reguitlyne was gepas om knopdormansiegedrag vir soetkersiekultivars te karakteriseer onder Suid-Afrikaanse kondisies. Alle kersiekultivars het die verwagte patroon gevolg deur in en uit dormansie te gaan. Faktoranalise het getoon dat faktore verwant aan die ingaan in dormansie, primêr knopgedrag karakteriseer. Knopgedrag is ook ‘n funksie van omgewingstoestande in ‘n jaar soos aangedui deur die “cluster” analise. Knoppe het dormant geraak in die middel van die somer en dormant gebly tot die koue-eenhede begin akkumuleer het. Vir alle kultivars het die knoppe uit dormansie gekom oor ‘n periode van drie tot vyf maande. Voor lenteknopbreek het terminale en laterale knoppe vinnig uit dormansie gekom. By alle kultivars het die laterale knoppe gedomineer en voor die terminale knoppe gebreek. Data wys dat daar geen ekodormante fase in die heersende klimaat van Suid-Afrika is nie.

Verdere eksperimentering was gerig op bestuurspraktyke wat tot die verbetering van die reserwestatus van die boom sou lei. In die stiktstofproewe, het bemesting in die na-oes periode geen betekenisvolle effek gehad op veld knopbreek of op knopdormansie verloop in eenjaar oue lote. Slegs in 2007 was volblom vroeër in die stikstofbehandelings. Opbrengs het nie betekenisvol verbeter nie. Dus in hierdie eksperiment het stikstof bemesting in die na-oes periode nie die kouebehoefte van volwasse soetkersie bome betekenisvol verminder onder die gematigde klimaats kondisies nie.

Toediening van dekfilms (Surround® en Raynox®) of etileeninhibeerder (Retain®) in die somer het nie die hittestres wat die bome ervaar verminder nie. Behandelings het geen effek gehad op koolstofassimilasie, huidmondjiegeleiding, blaaroppervlaktemperatuur, flouressensie, knopdormansie, bot, blom of vrugset nie.

Sitokiniene (bensieladenien) wat gespuit is in die herfs het nie knopdormansie, bot of blom geaffekteer nie.

Waterstofsianamied-toediening in die lente het bot, tyd van volblom en opbrengs betekenisvol verhoog. Promalin® en Retain® het geen betekenisvolle effek op bot, blom en opbrengs gehad nie.

Kersiebome is dus swak aangepas vir Suid-Afrikaanse toestande soos blyk uit die onverwagte knopgedrag en die klein verbetering in aanpasbaarheid wat bestuurspraktyke gelewer het deur nie die kouebehoete betekenisvol te verminder nie.

For Mom, Hayley and my family

Acknowledgements

I would like to express my sincere gratitude to the following people:

To my mother and my sisters for their unconditional love and support whenever I needed it.

To Hayley, for all her constant love and support.

To Dr. Nigel Cook, for his guidance and Dr. E Lötze, my co-supervisor, for her assistance with my studies.

To Laura Allderman for her technical and administrative support and the technical assistants of the Horticultural Department.

To God who supplied in all my needs.

CONTENTS

CHAPTER 1

1. 1 General Introduction

At present a proportion of the world's deciduous fruit is produced in low chill regions or countries not suitable for their cultivation. In addition, global temperatures are increasing (global warming) and consequently areas that were suitable once, may now depend on adjusted cultural practices to maintain optimal yields. Sweet cherry (*Prunus avium*) was recently introduced on a commercial scale in South Africa with more than 400 ha planted by 2006. Cherry is a high chilling fruit variety, originating from Europe (Faust and Surányi, 1997). Here dormancy and the development of cold hardiness was an important adaptation to survive the harsh winters; with the resumption of bud growth in spring if the prevailing environmental conditions favoured growth. In South Africa, climatic conditions do not favour the cultivation of high chilling fruit varieties due to the lack of sufficient chilling (mild winters) and high summer temperatures. Symptoms of delayed foliation (Strydom *et al.* 1971; Jacobs *et al.*, 1981), an associated basitonic growth tendency (Cook *et al.*, 1998), deformed flowers or abortion of flowers (Crabbé, 1994; Oukabli and Mahhou, 2007) and subsequent poor fruit set i.e. a reduction in potential yield (Mahmood *et al.*, 2000) have been widely observed. This led to the adoption of cultural practices to alleviate the symptoms of insufficient chilling.

The most feasible solution to this problem of insufficient chilling, however, would be to breed new low chill varieties that will be suited for this climate. However, no new low chill varieties are currently available. Conventional breeding methods are also time consuming and may take several years to develop a new variety. Therefore, cultural practices that would address the problem of insufficient winter chilling were considered worth investigation.

Deciduous fruit trees require a period of chilling (low temperatures) to obtain a good and quality crop (Crabbé, 1994). To quantify this chilling requirement, chill models were developed where the chilling requirement was defined as the number of effective chilling hours needed to restore bud growth potential in spring (Richardson *et al.*, 1974). This typically follows a optimum curve where temperatures above or below 0°C-7°C are not believed to contribute to chill unit accumulation (Arora *et al.*, 2003).

Manipulation of the chilling requirement by cultural practices was approached in different ways depending on the desired outcome (Saure, 1985). For example, in the tropics, apple and peach trees were defoliated either mechanically or chemically, or deprived of water to induce leaf senescence to prevent the trees from entering endodormancy. The need for effective chilling temperatures was therefore avoided by preventing the trees to enter endodormancy. Other approaches were to induce earlier budburst when buds entered endodormancy through increasing chilling by evaporative cooling (Gilreath and Buchanan, 1981; Erez and Couvillon, 1983), exposing trees or cuttings to 35°C-50°C (Chandler, 1960; Chaudhry *et al.* 1970), applying late autumn N fertilizer (Terblanche *et al.*, 1973; Terblanche *et al.*, 1979), or the application of rest breaking agents (Küden *et al.*, 1997; Martinez *et al.*, 1999; Palasciano *et al.*, 2005; Papa, 2001; Salvador and Tommaso, 2003). With the aid of cultural practices, (without considering the tropics) fruit varieties were able to be cultivated under low chill conditions by decreasing the amount of chilling required to resume bud growth in spring i.e. the chilling requirement was manipulated. The tree's adaptability under low chill conditions was therefore improved.

Available literature does cover various aspects of dormancy in general, but bud dormancy is poorly documented for cherry under low chill conditions (Erez, 2000; Faust, *et al.*, 1997; Saure, 1985). It is known that temperature and to a lesser extent photoperiod mainly determines bud dormancy behaviour, but other factors were shown to affect bud dormancy (Lang, 1987). These include water potential, nutrient status, oxygen levels, photoperiod and endogenous signals. Of interest was the role that reserves (nitrogen, carbohydrates and cytokinins) play in bud dormancy progression. The possibility that optimal reserves may improve the adaptability of high chill fruit varieties under low chill conditions was therefore considered worth investigating.

In this study, a background study was conducted on cherry bud dormancy progression under the low chill conditions of South Africa to understand when bud dormancy was induced and when the exit from dormancy occurred. Further experimentation was aimed at manipulating the carbohydrate, cytokinin and nitrogen reserve in the tree in order to establish if these reserves can reduce the chilling requirement of cherry by inducing earlier flowering and budburst and improving its adaptability under low chill conditions. Lastly, a rest breaking agent was applied with plant growth regulators to establish if these chemicals can induce earlier and a more even budburst pattern in cherry under low chill conditions of South Africa. In the following section the reader is referred to several

dormancy reviews in literature due to the extensive coverage on the dormancy topic and to cultural practices or methods employed in the past to manipulate the chilling requirement. In addition, nitrogen, cytokinin and carbohydrate distribution in the plant was reviewed to understand how these reserves affect tree performance. A summary is provided at the end of the literature review.

1.2 LITERATURE REVIEW

1.2.1 Dormancy

Bud dormancy progression of cherry is poorly documented, but plant dormancy in general has been extensively reviewed. The reader is referred to the following reviews on dormancy: Anderson and Chao, 2001; Arora *et al.*, 2003; Crabbé, 1994; Crabbé and Barnola, 1996; Dennis, 1994; Erez, 2000; Faust, *et al.*, 1997; Fuchigami and Nee, 1987; Lang, 1987; Saure, 1985; Vegis, 1964. General aspects of bud dormancy in cherry are discussed here.

Dormancy has been reviewed and studied for a number of years, although the exact mechanism of control of bud dormancy and how chilling overcomes dormancy are not well understood (Arora *et al.*, 2003; Erez, 2000). Erez (2000) noted that the mechanisms of bud dormancy control proposed by researchers did not offer convincing explanations. Attempts focused on relating the effect of chilling on dormancy to growth inhibitors and promoters. Growth retardants did indeed induce dormancy and the phytohormones, cytokinins and gibberellins induce dormancy release, but this has not been proved by research. Others related bud dormancy control to the exchange of sink power between the bud and neighbouring tissues or to a change in their growth potential, but did not clarify the mechanism of control. Lastly, the change in water status of the buds was proposed as a mechanism, but appeared to be more closely related to cold resistance than bud dormancy. Erez (2000), however, proposed that the change in fatty acids (linoleic acid to linolenate) in the cell membranes, which was temperature driven process, provide a possible link between cold accumulation and the restoration of bud growth potential in spring.

Sweet cherry is a high chilling fruit variety that requires between ca. 733-1344 CU (hours below 7°C) to restore bud growth potential in spring which is cultivar dependent (Seif and Gruppe, 1985). Bud dormancy progression in cherry can be classed in three phases that

often overlap *viz*. paradormancy, endodormancy and ecodormancy (Saure, 1985). Under conditions of adequate chilling buds would progress through these stages i.e. it follows this expected pattern of bud dormancy progression. Paradormancy involves the inhibition of the lateral buds by the terminal bud, i.e., the suspension of growth is due to correlative inhibition (Crabbé, 1994). The next phase, endodormancy, originates within the affected structure where growth is controlled by an environmental or endogenous signal within the bud. The third phase, ecodormancy, entails the suspension of growth due to one or more environmental factors that are unsuitable for growth (Lang, 1987).

The exact environmental factor/s that induce dormancy are unclear, but were generally believed to be associated with the occurrence of short days (reduced photoperiod) and low temperatures. However, Hauagge and Cummins (1991) suggested that bud dormancy induction was a two-step process where higher temperatures (>18°C) were required for bud dormancy induction, with lower temperatures only intensifying bud dormancy. The exact environmental cue/s for dormancy induction remain unclear. Temperature effects on bud set and bud dormancy induction is, however, not clear-cut, and may depend on the physiological state of the bud (Crabbé and Barnola, 1996). After dormancy induction, deciduous fruit trees enter a stage of endodormancy essential for the survival of freezing temperature injury.

Temperature optima during the endodormant phase differ between and within species. For peach, constant low temperatures (6-8°C) are required to break dormancy (Richardson, 1974). Mahmood *et al.* (2000) determined that temperatures as low as 3°C are ideal for cherry. Upon the fulfilment of the chilling requirement, buds exit dormancy rapidly provided that the environmental conditions are favourable. If the chilling requirement is only partially satisfied, as in a mild winter climate, apple buds exit dormancy poorly resulting in an associated basitonic growth tendency (Cook *et al.*, 1998). Furthermore, the endodormant phase becomes extended not allowing for the intervention of an ecodormant phase before spring budburst. It is apparent that the same low temperatures required for dormancy induction are also required to exit dormancy (Crabbé, 1994; Heide and Prestrud, 2005).

1.2.1.1 Methods to manipulate the chilling requirement of deciduous fruit trees

Evaporative cooling

Erez *et al.* (1979) and Erez and Lavee (1971) showed in a study on peach that a diurnal cycle of eight hours at a maximum of 21°C and 16 hours at 4°C negate chilling accumulated. At a maximum of 18°C however, no effect was observed. At a maximum of 15°C budburst was advanced. This resulted in employing methods (evaporative cooling) that would reduce bud temperature under conditions mild winter conditions and therefore increase the number of chill hours that the buds experience (Gilreath and Buchanan, 1979). In peach flowering was advanced with 11 days when overhead sprinklers were used when temperatures rise above 10°C (Gilreath and Buchanan, 1981). Here with evaporative cooling, average weekly maximum scaffold temperature was reduced by up to 4.3°C. For nectarine, evaporative cooling enhanced floral and vegetative budburst, when overhead sprinkling was used when day temperature exceeded 16°C (Erez and Couvillon, 1983). Here bud temperatures were lowered by 3°C to 5°C. Gilreath and Buchanan (1981) did not ascribed obtained results entirely to temperature, because the rest completion models failed to predict when dormancy would be terminated and suggested that other factors may be involved.

Heat treatment

Chandler (1960) showed that budburst can be induced in apple trees exposed to 44°C-45°C for six hours of a single or on two consecutive days in July, October, or November (Northern hemisphere). For pear, heat treatment with water at 45°C for three hours also induced budburst (Chaudhry *et al.* 1970). This was compared with a treatment of 8-12 weeks of chilling at 3°C. It was found that the chilling treatment was more effective, because buds showed higher activity and sprouted more vigorously.

Chemical rest breaking

Chemical rest breaking has been commonly used to induce earlier budburst in deciduous fruit in South Africa (Costa *et al.* 2004). In cherry, budburst and flowering was advanced and yield increased with the application of hydrogen cyanamide (Dormex®) prior to field budburst (Küden *et al.*, 1997; Martinez *et al.*, 1999; Palasciano *et al.*, 2005; Papa, 2001; Salvador and Tommaso, 2003). Erez (1995) listed chemical rest breaking agents which includes: Mineral oils, cyanamide, thiourea, potassium nitrate, growth regulators (Gibberellic acid, cytokinins) and Armobreak.

Nitrogen reserves

The role that N reserves in dormancy is review in the next section (Section 1.1.2)

Although temperature and to a lesser extent photoperiod mainly determine bud dormancy behaviour other factors were shown to affect bud dormancy (Lang, 1987). These include water potential, nutrient status, oxygen levels, photoperiod and endogenous signals. Of particular interest was nutrient status or N. In the following discussion N allocation patterns were reviewed to understand how N affects tree performance and dormancy. This is followed by a discussion of what aspects of tree performance are affected by N and how this is related to carbohydrate and cytokinin reserves. The macro elements phosphorus and potassium were also reviewed, but no reports showed that these elements influence bud dormancy.

1.2.2 Nitrogen Dynamics in *Prunus* Species and Uptake and Partitioning of Phosphate and Potassium

1.2.2.1 Introduction

The deciduous nature of fruit trees necessitates the differential uptake, allocation and partitioning of elements throughout the tree's annual cycle. Allocation is a function of metabolic need that includes storage, utilization and transport of elements. The differential distribution of products within the plant, termed partitioning, is primarily a function of phenological stage (Taiz and Zeiger, 2002). As a result, elements are needed in different quantities at different times according to tree demand. For deciduous trees, including cherries, 16 elements are essential to complete their life cycle (Neilsen and Neilsen, 2003). Only the macro-elements, nitrogen (N), potassium (K) and phosphate (P) are reviewed here.

N, P and K are needed in different quantities at different times and each fulfils different functions in the plant. N, together with K and calcium, are the macro elements that are required in the greatest quantity for optimal growth in apple trees (Batjer *et al.*, 1952). For cherries, it is estimated that 110 kg/ha N and 80 kg/ha K annually are sufficient for normal production (Ystaas, 1990). N forms an integral part of carbon compounds, and therefore cell components, that include proteins, amino acids, amides, nucleic acids, etc. N, therefore, plays an important role in various metabolic, hormonal and other plant processes. K plays an important role in the regulation of osmotic potential in plant cells, but also activates approximately 40 enzymes involved in photosynthesis and respiration (Taiz and Zeiger, 2002). Compared to other macro-elements, P is needed in smaller

quantities (ca. 9-18 kg/ha for apple) (Batjer *et al.*, 1952; Stassen *et al.*, 1983). P, however, plays an important role in energy transfer reactions and forms part of phospholipids, nucleic acids, etc. (Taiz and Zeiger, 2002). Although differences are apparent regarding the macro-elements, each still plays a vital role in completion of the deciduous tree annual cycle.

Deciduous trees demonstrate a very aggressive annual growth cycle accompanied by distinct uptake and partitioning patterns for various elements. From literature, it is well established that during spring, initial growth of deciduous trees depends on internal reserves mobilized mainly from the roots and other perennial parts. Subsequent enrichment of N, P and K for *Prunus* depends on root uptake to support further development of young fruits, leaves and shoots. (Muñoz *et al.*, 1993; Policarpo *et al.*, 2002; Stassen *et al.*, 1981a; Stassen *et al.*, 1981b; Stassen *et al.*, 1983; Stassen and Stadler, 1988; Taylor and van den Ende, 1969b; Weinbaum *et al.*, 1978; Weinbaum *et al.*, 1984; Zavalloni, 2004). With bud set through to leaf senescence, trees remobilize various elements from the leaves and accumulate more through post-harvest root uptake to increase their reserve status. (Muñoz *et al.*, 1993; Policarpo *et al.*, 2002; Stassen *et al.*, 1981a; Stassen *et al.*, 1981b; Stassen *et al.*, 1983; Stassen and Stadler, 1988; Tagliavini *et al.*, 1999; Taylor and van den Ende, 1969a; Taylor and van den Ende, 1970; Weinbaum *et al.*, 1978; Weinbaum *et al.*, 1984; Zavalloni, 2004).

In the following section, only the uptake periods and partitioning of N, P and K are discussed for *Prunus* species. Each is discussed according to the general phenological development, i.e. budburst, bud set, harvest and leaf senescence of the deciduous trees.

1.2.2.2 Nitrogen uptake and partitioning in *Prunus* species

Compared to the other macro-elements, N is annually required in the greatest quantity for normal growth and development of peach (Stassen, 1987). It is necessary during active growth and acts as an important reserve during spring growth. N, furthermore, determines bud growth potential in spring (Cheng *et al.*, 2004). Subsequently, the earliness of flowering and leaf development is a function of N reserve levels (Terblanche and Strydom, 1973; Terblanche *et al.*, 1979). Temperature and chilling period, however, are the primary factors that control time of budburst (Jacobs *et al.*, 2002). In addition, optimal N reserves improve fruit set for various deciduous crops (Hill-Cottingham, 1967; Stassen *et al.*, 1981b; Taylor and van den Ende, 1969a). N availability and subsequent levels in the plant are,

therefore, closely correlated with bud growth and development (Geßler *et al.*, 2004; Sakakibara, 2006). Hence, it is important to assess N uptake and partitioning for *Prunus* species.

Nitrogen remobilization during budburst, and uptake and partitioning during active shoot growth

It is well established that initially, with budburst, new growth (leaves, fruit and shoots) is dependent on internal N reserves remobilized from permanent structures, especially the roots, accumulated in the previous fall or season (Tromp, 1983). N is primarily stored as amides: asparagine and arginine (Malaguti *et al.*, 2001, Stassen *et al.*, 1981a). This N is transported in the soluble form via the xylem from the roots and other perennial parts to new developing fruit, leaves and shoots (Millard *et al.*, 2006; Stassen *et al.*, 1981a). As reserves decline in the woody organs, trees depend more on root uptake (Bi *et al.*, 2003).

A large percentage of N or other reserve, utilized for new spring growth, originates primarily from the roots. This N is mainly stored in the fine and coarse roots, but also in the bark and wood of the tree (Policarpo *et al.*, 2002; Stassen *et al.*, 1981a; Tagliavini *et al.*, 1999). Furthermore, N uptake is a function of reserve status (Bi *et al.*, 2003; Grassi *et al.*, 2003). Trees with a higher reserve N will initially have lower N uptake compared with trees that have a low reserve N. In one-year-old *P. avium* trees, remobilization of internal reserves for trees low and high in N reserve status occurred until 42 and 56 days after budburst (DAB), respectively (Grassi *et al.*, 2003). For 10-year old cherry trees (*P. avium*) initial uptake of spring applied N started 21 DAB (Millard *et al.*, 2006), evidence that internal N reserves do affect time of N uptake.

Tagliavini *et al.* (1999) assessed N partitioning for two-year-old ‘Starkredgold’ nectarine by N application during active shoot growth (early) and after active shoot growth ceased (late). Although similar N quantities were absorbed during the early and late period, N partitioning was different. Early application resulted in more N partitioned to aerial parts, with 25% more N retained in the leaves with senescence. In addition, N found in the perennial parts was lower during dormancy. N content decreased by 50% in the fine and coarse roots during spring, which was incorporated into new growth. N content also decreased in other perennial parts, but their relative contribution was smaller.

Stassen *et al.* (1981a) found that the total N reserves in the bark, wood and roots decreased for two-year-old 'Kakamas' peach trees from three weeks before, up to twelve weeks after budburst. Redistribution of N reserves to new growth was estimated at 65% N. Both young, developing fruits and leaves are strong sinks for N, but initially leaves showed the largest increase in soluble N from reserves (Stassen *et al.*, 1981a). A higher percentage was estimated by Stassen *et al.* (1983) who found that 80% of the N for new growth came from reserves in the first 56 DAB. Furthermore, Stassen *et al.* (1983) showed that root N uptake occurred in the period from 56 DAB until harvest. A small N percentage (24%) found in new growth during this period, still originated from internal reserves. Muñoz *et al.* (1993) observed a similar trend by studying the N uptake and distribution pattern for three-year-old 'Maycrest' peach trees. Little N uptake (7%) occurred in the flowering and fruit set period, while 93% originated from reserves to support new growth. Similarly, Tagliavini *et al.* (1999) estimated that 72-80% of the total N in new growth came from remobilization.

Taylor and van den Ende (1969b) noted that the N concentration during spring was proportional to the N supply the previous fall. Different rates of N, however, did not affect flowering and fruit set. Flower N analyses, revealed that N concentrations were the same across all treatments. Taylor and van den Ende (1969b) therefore hypothesised that N is preferentially allocated to developing flowers and that this N originated from the tree's large storage pool. With regard to N partitioning to peach fruit, N was preferentially allocated to the seed while excess N accumulated in the fruit (Taylor and van den Ende, 1970). However, for mature almond trees (*P. dulcis*), Weinbaum *et al.* (1984) found that, during flowering, reproductive tissues are highly dependent on reserve N, whereas vegetative growth is more dependent on root uptake. For non-bearing prune, Weinbaum *et al.* (1978) found that 98% of the N for expanding buds was derived from N reserve.

N uptake was examined or assessed for prune (Weinbaum *et al.*, 1978), peach (Muñoz, 1993; Policarpo *et al.* 2002; Stassen *et al.*, 1981a; Stassen and Stadler, 1983) and cherry (Zavalloni, 2004). Grassi *et al.* (2003) suggested that N uptake by roots is regulated by N recycling in the xylem, which is inversely related to N status of the tree. Therefore, N uptake in trees with low N reserves will start earlier. The presence of white roots is important for nutrient uptake. The persistence of white roots is affected by plant N concentration, time of appearance and rooting depth for cherry (Mackie-Dawson *et al.*, 1995). High N concentrations resulted in a lower persistence of white roots, estimated at a

week less. White roots produced in the beginning of the growing season lasted for up to six weeks whereas white roots only lasts for up to two weeks later in the season. Surface roots died twice as fast as deeper roots (>20cm).

Policarpo *et al.* (2002) tabulated the percentage of root uptake partitioned to different tree parts according to phenological development throughout the growing season for two-year-old peach trees. During budburst, flowering and fruit set, uptake of applied N fertilizer was estimated at 28% to leaves and 4% to fruit, for late fruiting 'Tudia' peach. During this period, 40% of the whole tree N was still retained in the roots and the contribution of N uptake was very limited. Similarly, Zavalloni (2004) and Weinbaum *et al.* (1978) found for young cherry trees and for non-bearing prune, respectively, that uptake of applied N was low during the period of bud swell. After fruit set, Policarpo *et al.* (2002) estimated that 75-80% of N in new growth originated from internal reserves. With rapid shoot growth and fruit growth during stage I, 50% N and 30% N of the N fertilizer was partitioned to leaves and fruit, respectively, while little N was retained in the roots. Uptake supplied the most N for new growth during the active shoot growth period. Furthermore, Policarpo *et al.* (2002) and Muñoz *et al.* (1993) estimated that 60%-70% of the total applied N during the season was absorbed during the active shoot growth stage. Early fruiting 'Flordastar' peach followed a similar pattern in partitioning and uptake, but was more advanced in its phenological development. Therefore, uptake and partitioning were different when compared according to calendar date, but similar when compared according to phenological development. Furthermore, Policarpo *et al.* (2002) provided evidence that uptake occurred during leaf senescence and that roots and other perennial parts are the main N sinks.

Nitrogen uptake and partitioning with bud set until leaf senescence

Bud set usually marks the period in which N demands of the developed fruits, shoots and leaves are met. Thereafter N starts to accumulate in perennial tissues. Furthermore, N is redistributed from the leaves to the wood and roots.

Stassen *et al.* (1981a) found that three weeks before bud set, N status of new growth declined and increased in the bark, wood and roots. This was mainly N being redistributed within the tree. Similarly for non-bearing prune, Weinbaum *et al.* (1978) found that 37% of N (from fertilizer) was partitioned to current growth while 45% was partitioned to the roots during this period. Furthermore, Muñoz *et al.* (1993) showed that leaves contained 59% of the N absorbed with bud set. Allocation of fertilizer N to fruit was estimated at 17%. Similar

to this, Taylor and van den Ende (1969a) found that from the period prior to bud set until leaf senescence, leaf N estimated at 50% N relative to the total N contained in the leaves is incorporated in permanent structures through N migration out of the leaves. Zavalloni (2004) found that with terminal bud set, approximately 50% of applied fertilizer was partitioned to the roots and trunk. This trend continued as 70% of applied fertilizer was partitioned to the roots with leaf senescence. However, N-fertilizer recovery decreased to 40% with leaf senescence.

During the period of leaf senescence, migration and uptake of nutrients occurs. Nutrients are mobilized from the leaves and stored in the permanent structures, while a second root growth flush assimilates nutrients for storage in the roots (Stassen *et al.*, 1981a; Taylor and van den Ende, 1969a; Taylor and van den Ende, 1970). Here the majority of absorbed N is partitioned to the roots, trunk and branches (Stassen *et al.*, 1981a; Taylor and van den Ende, 1969a; Taylor and van den Ende, 1970).

Three weeks prior to up to three weeks after final leaf drop, a rapid increase in soluble N occurred in the roots. This is mainly due to root uptake in this period and less due to N migration from the leaves to the roots (Stassen *et al.*, 1981a). Similarly, Weinbaum *et al.* (1978) found that 66% of fertilizer N is partitioned to the roots, and 16-20% is partitioned to current year's growth. For 'Starkredgold' nectarine Tagliavini *et al.* (1999) found that of the N applied in the late autumn, ca. 90% was recovered in perennial organs, especially the roots, while only ca. 10% was retained in abscised leaves. Furthermore, Muñoz *et al.* (1993) showed that N absorbed during autumn was distributed to the bark of branches, roots and the trunk. A significant amount of N was also remobilized from the leaves. N exported out of the leaves was estimated at 50% of the leaf N being allocated to woody organs (Taylor and van den Ende, 1969a). This N is mainly stored in the roots.

1.2.2.3 Effect of nitrogen on tree performance

N is proven to play a considerable role in flower bud initiation, differentiation, flowering and ovule longelivity and subsequently fruit set in apple (Hill-Cottingham, 1967). This was demonstrated by differential N application during the growth season that coincided with clearly visible phenological stages for apple. In addition, Terblanche and Strydom (1973) and Terblanche *et al.* (1979) observed earlier active budburst for 'Golden Delicious' when supplied with a higher N concentration during autumn. Through this, Hill-Cottingham (1967), Terblanche and Strydom (1973) and Terblanche *et al.* (1979) provided evidence

that for fruit trees, reserve N prior to budburst do affect an event such as time of flowering or fruit set. It is important therefore, to review here the time of flower bud differentiation and the effect differential N application has on time of flowering and fruit set.

The role of N in flower bud development, flowering, and fruit set in deciduous fruit trees

Flower bud development

For deciduous fruit trees, several physiological processes occur simultaneously during the postharvest period. Processes include flower bud differentiation, accumulation of carbohydrates, uptake of, e.g., N and the redistribution of N, P, K, copper and boron from the leaves to the perennial parts of the tree. Each of these processes can affect other processes, e.g., flower bud differentiation will be delayed if N availability is limited (Hill-Cottingham, 1967).

Rabie (1983) and Bergh (1984) showed that flower bud differentiation for peach and apple occurred in the late summer and autumn period. Sepals, petals, stamens and pistils with loculi all developed in this period. Similar to Rabie (1983) and Bergh (1984), Stadler (1965) found that differentiation of reproductive buds starts roughly in the period coinciding with bud set for peach.

As discussed earlier, Hill-Cottingham (1967) provided evidence that postharvest/autumn N application plays an important role in flower bud differentiation and time of flowering for two-year-old ‘Lord Lambourne’ apple trees. It is important to note that prior to the experiment the trees received no N. Hill-Cottingham (1967) illustrated that depending on the time of N application, flower bud development and the partitioning of N was effected. Where trees received only N in spring, flower primordial development was delayed and buds were relatively immature entering dormancy. Trees that received no N showed a steady rate of differentiation. Relative to this, flower differentiation was accelerated in treatments receiving either a summer or an autumn N application. Prior to dormancy, however, active ovule differentiation was only evident in trees that received either summer or autumn N, while with spring N application and the control trees, ovule differentiation was absent.

Flowering and fruit set

Several authors provided evidence that postharvest or autumn N application positively or negatively affects flowering and fruit set for apple (Hill-Cottingham and Williams, 1967; Terblanche and Strydom, 1973; Terblanche *et al.*, 1979), peach (George and Nissen, 1993; Stassen *et al.*, 1981b; Taylor and van den Ende, 1969a) and cherries (Linhard and Hansen, 1997). Linhard and Hansen (1997) and George and Nissen (1993), showed that postharvest or autumn N application, whether applied through urea sprays or broadcast application, reduced and delayed flowering for sour cherries and 'Flordaprince' peach.

Terblanche and Strydom (1973) acknowledged that an apple tree with optimal N reserves exhibits an improved performance during spring budburst once rest-breaking agents are applied. The active period of budburst was delayed and extended where trees were low in N reserve. On the contrary, trees optimal in N reserves showed a peak in an active period of development and the blossoming period was advanced. Furthermore, if rest-breaking agents were not applied, flowering patterns were similar regardless of N concentration. When no N was applied, however, blossoming peak was ca. one week later.

Hill-Cottingham and Williams (1967) showed that apple trees receiving only an autumn N application had a greater ability to set fruit than trees fertilized only in summer or spring. No fruit set occurred where no N was applied in the previous season or when it was applied in a single application during spring. Dormant tree N analyses showed that autumn N application increased root N reserves significantly, while the summer N application resulted in N partitioned to all parts of the tree. Compared to the other treatments, summer N application resulted in the highest N concentration in all parts of the tree, except the roots. Similarly, Terblanche *et al.* (1979) also found that increased autumn N increased fruit set in apple. Terblanche *et al.* (1979) found that budburst was delayed by 30 days when trees did not receive autumn N compared to trees that received autumn N. Flowering was also delayed in trees receiving no N.

Stassen *et al.* (1981b) compared summer and autumn N application and its subsequent effect on blossoming pattern and final fruit set of peach. Autumn N resulted in earlier full bloom (ca. 14 days). The highest percentage of reproductive buds also developed into flowers where a full N application was given in summer or autumn. Fruit set was the highest in treatments receiving autumn N.

Taylor and van den Ende (1969a) speculated that storage N for eight-year-old 'Golden Queen' peach is preferentially used for reproductive processes rather than for vegetative growth. Flowers at full bloom had the same N content whether it received postharvest N or not. However, N content in new developing leaves were in proportion to the level of storage N. Compared to N treatments, control trees had more single and fewer double flower buds. The initial rate of flower bud development was higher in control trees. N treatments did not significantly affect rate of subsequent bud development, survival of flower and leaf buds, earliness of flowering, length of flowering period, flower size, N content per flower and fruit set per tree. Hence, Taylor and van den Ende (1970) concluded that N application over a range of 0-8 kg in mid-summer and late March (growing season and autumn) had no influence on flowering performance and fruit set in eight-year-old peach trees.

Linhard and Hansen (1997) studied the effect of differential N application throughout the season on sour cherries. Flower number, fruit set, and yield were lower in trees receiving a late fall N application either through broadcast or urea sprays. More dead buds and fewer flowers per bud were counted. The author proposed no reason for this phenomenon. In addition, George and Nissen (1992) found that time of vegetative budburst and flowering were delayed by 20 to 30 days, in peach trees that received N in the late summer or autumn period. Furthermore, time to budbreak (50%) was negatively correlated with a single N application in mid-summer at 10g N per tree. Fruit set was increased by 48% and time to harvest reduced, where N was applied during late summer and autumn.

If young deciduous fruit trees are compared to older trees, which have a larger N or assimilate storage capacity (Niederholzer *et al.*, 2001), previous seasons' N supply do not necessarily affect an event such as flowering due to the preferential allocation of N to reproductive organs (Taylor and Van Den Ende, 1969a). Fruit set, however, is largely dependent on reserve N and is affected by fall or summer applied N (George and Nissen, 1992; Hill-Cottingham and Williams, 1967; Stassen *et al.*, 1981b; Terblanche and Strydom, 1973; Terblanche *et al.*, 1979; Taylor and van den Ende, 1969a). However, yield may not always be affected by reserve N with full-grown trees (Huett and Stewart, 1999; Niederholzer *et al.*, 2001).

1.2.3 Phosphorus uptake and partitioning in *Prunus* species

Compared to N and K, P is required in the least amount (ca. 8-19 kg/ha) for normal production (Stassen et al., 1983). Stassen and Stadler (1988) found that P mainly accumulates in the shoot elongation period, although it accumulated in the whole tree with leaf senescence for two-year-old pot-grown 'Kakamas' peach trees. Earlier work by Stassen *et al.* (1983) showed a similar trend for full bearing peach trees. In the following section, P uptake and partitioning patterns for *Prunus* species are reviewed.

Initially, with budburst, P content of the roots and wood decreased, while P levels in new leaves, fruits and shoots increased. No P uptake occurred in this period. P accumulation in the new growth was due to remobilization from internal reserves, primarily from the roots (Stassen *et al.*, 1983; Stassen and Stadler, 1988). In the whole tree, P levels start to increase 3 weeks after budburst and rapidly increase roughly until harvest through root uptake (Stassen and Stadler, 1988). This was mainly incorporated in the growing leaves (33%), developing fruit (17%) and new shoots (25%). Until 8 weeks after budburst, Stassen *et al.* (1983) found that 57% of the P requirement for the initial growth came from root uptake. P accumulation in the roots only started three weeks before bud set and continued until the completion of leaf senescence. With the start of leaf senescence, P levels decline in the leaves while it continued to increase in the roots. No estimate was provided for exported P to permanent structures. Root uptake did occur during leaf senescence, evident through the continuous increase of P at whole tree level. According to calculations, 23% of the total P accumulated in the postharvest period for 'Kakamas' peach (Stassen and Stadler, 1988).

1.2.4 Potassium uptake and partitioning in *Prunus* species

Fruit trees acquire K, in the ionic form, during the active growing season, primarily through root uptake. Compared to other macro-elements, K is required in the second largest amount (80 kg/ha) for normal production in peach (Stassen, 1987). Only Stassen and Stadler (1988) and Stassen *et al.* (1983) assessed uptake and partitioning of K on two-year-old and full bearing 'Kakamas' peach trees, respectively. Uptake and partitioning of K are reviewed here.

During winter, Stassen *et al.* (1983) found that full bearing peach trees had a higher K concentration in the wood compared to other perennial parts. From budburst until eight weeks after, the K content of the permanent structures decreased and was utilized for new

growth. It was estimated that 40% of the K in the new growth came from reserves. After this period, K increased rapidly in the tree until harvest. This K came from root uptake and was incorporated into leaves, fruit, and shoots. Prior to harvest, fruit contained 34% of the whole tree K, and therefore, is the largest sink for K. A similar pattern was followed for two-year-old peach trees from budburst to harvest (Stassen and Stadler, 1988). At harvest these trees contained, 53% and 29% K in the leaves and fruits respectively. During the postharvest period, i.e. with the beginning of leaf senescence, K is redistributed from the leaves to the roots, but the author could not provide an estimate for proportion of K redistributed. In addition, Stassen and Stadler (1988) showed that no increase in K occurred for the whole tree in this period, and therefore concluded that root uptake of K was unlikely.

1.2.5 The role of nitrogen, carbohydrates and cytokinin during budburst

In the period after bud set, N and carbohydrates accumulate and is redistributed in fruit trees (Keller and Loescher, 1989; Tromp, 1983). Furthermore, N uptake is a function of carbohydrates availability and light. On molecular level, nitrate reductase, which reduces nitrate to nitrite, marks the first step in N assimilation. This enzyme, however, is activated indirectly through light and the availability of carbohydrates (Taiz and Zeiger, 2002). On assimilation, cytokinin synthesis is up regulated, which directly affects growth and development (Sakakibara *et al.*, 2006). The seasonal variation in carbohydrates for cherry, its interaction with N (apple and almond) and the relationship between N and xylem cytokinin levels (vines) are reviewed here.

Seasonal pattern of carbohydrate partitioning in sweet cherry

During early development (with budburst), reserve carbohydrates are important to support the initial reproductive and vegetative development of sweet cherry (Keller and Loescher, 1989). Sucrose, which was the main non-structural carbohydrate during dormancy, decreased rapidly while sorbitol increased with active shoot growth. During dormancy, however, carbohydrates were mainly stored as starch. Carbohydrates predominantly accumulated in the postharvest period, because of the high demand for photosynthates from flowering to harvest. Carbohydrate accumulation depends on environmental factors (irrigation light, pests and nutrition) especially after bud set (Keller and Loescher, 1989). The highest levels of total non-structural carbohydrates were found with leaf senescence. With or before budburst, total non-structural carbohydrates increased in the spurs, while reserve carbohydrates decreased in other perennial tissues. After flowering, carbohydrate

reserves were at its lowest levels in all perennial parts except the spurs, but immediately increased, as the leaves became a source of carbohydrates.

Interaction between nitrogen, cytokinin and carbohydrates

Contrary to previous belief, N reserve rather than reserve carbohydrates primarily determine initial growth or growth potential of buds during spring in young apple trees (Cheng and Fuchigami, 2002; Tromp, 1983). A negative linear relationship was found between N availability and total non-structural carbohydrates in apple (Cheng *et al.*, 2004). Furthermore, as N supply increased in the previous growing season or fall, the C/N ratio decreased. With an increased N supply, more N was incorporated in amino acids while total non-structural carbohydrates concentration decreased at the expense of N assimilation. A plant with a lower C/N ratio produced larger leaves and longer shoots (Cheng *et al.*, 2004). Similarly, Bi *et al.* (2003) showed that total non-structural carbohydrates decreased with an increase in soil or foliar applied N in autumn for young almond trees. As the N supply increased, the proportion of free amino acids and proteins increased which was associated with a decrease in total non-structural carbohydrates (fructose, sucrose and glucose). Cheng *et al.* (2004) suggested that stored N, as free amino acids, was more carbon cost effective and that N was assimilated at the expense of non-structural carbohydrates. When no N was supplied, all N is mainly in the form of proteins. With an increased N supply, more N was in free amino acids, especially arginine. Proteins, however, still remained the main storage form of N. Cheng *et al.* (2004) stated that amino acids might play a role in initial budburst due to the “easy access” of N protein. In addition, O’Kennedy and Titus (1979) showed that different proteins are hydrolysed at different rates and therefore supply different building blocks/energy at different times.

Recently the importance and relationship between N, cytokinins and carbon metabolism have been discussed (Geβler *et al.*, 2004; Nikolaou *et al.*, 2000; Sakakibara *et al.*, 2006). Cytokinin synthesis was up regulated in the roots in response to nitrate exposure and regulated a wide variety of genes involved in metabolism, development and macronutrient acquisition (Sakakibara *et al.*, 2006). Nikolaou *et al.* (2000) showed a positive correlation between spring xylem cytokinin content and dormant N concentration of ‘Thompson seedless’ vines. Nikolaou *et al.* (2002) found that no other correlation exists with cytokinin and other minerals in the xylem exudate. In addition, relative budburst number was positively correlated with xylem cytokinin levels. The relationship of budburst patterns with cytokinin levels was also previously reported (Belding and Young, 1989; Cook *et al.*,

2001a; Cook *et al.*, 2001b; Cutting *et al.*, 1991; Hewett and Wareing, 1973; Lombard, *et al.*, 2006; Tromp and Ova, 1990; Young, 1989). Acrotonic development of apple was associated with higher cytokinin levels in the distal shoots halves (Cook *et al.* 2001b). A peak in xylem cytokinin content was found prior to budburst and declined sharply after budburst. It is generally accepted that this spring cytokinin peak is the trigger for budburst (Cook *et al.*, 2001b). This cytokinin was suggested to be mobilized from reserves in the bark or cambium and probably not from root supply (Skene, 1972 and Hewett and Wareing, 1973).

1.3 General Summary

Bud dormancy behaviour in deciduous fruit trees was shown to be mainly controlled by temperature. Under conditions of sufficient chilling buds would progress through a paradormant, endodormant and ecodormant phase. However, under conditions of insufficient chilling buds would exit dormancy without the intervention of an ecodormant phase. To restore bud growth potential in spring, a chilling requirement must be satisfied. Other factors that influence bud dormancy behaviour and the chilling requirement are water potential, nutrient status, oxygen levels, photoperiod and endogenous signals. Of particular interest was how reserves affect dormancy. N was shown to affect bud dormancy behaviour and was therefore reviewed.

Cultural practices in manipulating the chilling requirement of deciduous fruit trees involved decreasing bud temperature through evaporative cooling, heat treatment of 35°C-50°C, late autumn application and the application of rest breaking agents. The chemical rest breaking agents are the most effective in advancing budburst. Heat treatments may induce budburst but are not very effective and other factors such the effect on floral development were not evaluated. Evaporative cooling did reduce the time to budburst, but this system is subjected to the availability of water and efficient management.

By understanding the allocation patterns of N within the tree, it becomes clearer how N affects tree performance and dormancy. Initial growth and development of *Prunus* species are dependent on internal N reserves, stored in the perennial parts of the tree. The roots, however, store N in significantly bigger quantities in the thick and fine roots. Root uptake is limited during the initial development of flowers, shoots and leaves when conditions are generally unfavourable for root growth. As reserves decline after fruit set, trees depend on root uptake to supplement additional N for new growth during active shoot growth. Fruits

are a sink for N during the initial stages of fruit growth, but leaves are the largest sink for absorbed N. Most of the annual N requirement is absorbed during the active shoot growth period.

Most N is retained in the leaves during harvest. At this time, roots generally contribute the least to the total N content of the tree. Bud set generally marks the stage where trees invest more assimilates in storage than in growth. Roots are the main sink for N from uptake or from migration out of the leaves after bud set, but N increases are apparent in perennial parts as leaf senescence approaches. In addition, N is hydrolyzed, and exported from the leaves to the roots and woody tissues. It is estimated that 50% of N contained in the leaves will be exported to perennial organs. Prior to commencement of leaf senescence, a second uptake period supplies N that is stored as reserve in the roots. This period varies in duration, but may last up to six weeks. The efficiency of uptake, however, is lower during leaf senescence compared to uptake during active shoot growth.

From literature it is apparent that N in the postharvest period does influence dormancy and flowering. The authors generally deal with young trees that are largely dependent on the current year and autumn N supply of which the N storage pool can easily be manipulated. Compared to older trees, which have a larger N or assimilate storage capacity, previous seasons' N supplies do not necessarily affect an event such as flowering, due to the preferential allocation of N to reproductive organs. Fruit set, however, is largely dependent on reserve N, and is affected by N applied in autumn or summer. However, yield may not always be affected by reserve N with full-grown trees. N applied in the postharvest period was assimilated at the expense of carbohydrates and is considered as more carbon cost effective. Even though N is quantitatively stored less, it still plays a significant role during initial growth. Furthermore, it is shown that a close correlation exists between nitrate availability or N reserve level and cytokinin levels in plants, and that budburst number is related to cytokinin levels in the xylem sap.

No reports found that P and K affect bud dormancy or an event such as flowering. It is still reviewed here, because P and K form part of the macro elements and therefore included in the review. P is stored as a reserve in perennial parts and subsequently incorporated into leaves, fruits and shoots during spring. Active root uptake occurs three weeks after budburst and continues until leaf senescence. P is incorporated in new growth prior to bud

set. However, during the postharvest period, P accumulates predominantly in the roots through both root uptake and redistribution from the leaves.

During budburst, K is incorporated in new growth, mobilized from the permanent parts (especially the wood) of the tree. K accumulates in leaves, fruit and shoots during the active growing season, with the fruit containing the largest proportion of absorbed K. Fruit is therefore the largest sink for K during the active growing season. During the postharvest period, no K uptake occurs, with the leaves containing the most K. With the beginning of leaf senescence, K is redistributed from the leaves, through remobilization, predominantly to the wood and other permanent parts of the tree.

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CHAPTER 2

2. Overall Objective

Cherry, a high chilling fruit variety, is not suited for the mild winter climate of South Africa. This was recognizable through common observed symptoms of delayed foliation and poor fruit set as a result of inadequate chilling. Its poor adaptability necessitated the research into its bud dormancy behaviour under South African conditions, and cultural practices that can improve its adaptability under conditions of inadequate winter chilling. The suitability of a cultivar is estimated according to chilling requirement, i.e., the number of effective chill hours needed to restore bud growth potential in spring. Therefore, research was aimed at reducing the chilling requirement of cherry and thereby improving its adaptability under South African conditions.

Bud dormancy behaviour of cherry is poorly documented under low chill conditions. In order to determine bud dormancy behaviour of cherry, shoots of various sweet cherry cultivars were sampled under different climatic conditions within South Africa. These shoots were forced from bud set until spring budburst with days to budburst recorded in order to characterize bud dormancy behaviour under mild winter conditions. It was not certain when endodormancy starts, but was expected to start when chilling starts to accumulate. It was not certain when cherry buds would exit from endodormancy and if there are an ecodormant phase, but it was expected to be during late winter or early spring. Further experimentation was aimed at improving reserve levels (nitrogen, carbohydrates and cytokinin) within a tree through various cultural practices. Through previous studies, it is apparent that a tree optimal in reserves showed improved performance under conditions of inadequate winter chilling. It is, however, not clear which reserve primarily determines bud growth potential in spring. Both nitrogen and carbohydrate reserves accumulate postharvest in the perennial parts for cherry. Therefore efforts was focused in improving nitrogen reserve status through postharvest nitrogen fertilization, and alleviate heat or light stress with Surround®, Raynox® and Retain® during the postharvest period. It is proposed that if nitrogen or carbohydrate reserves are increased, that budburst and flowering would be advanced. Cytokinins are known to precede budburst in spring and are positively correlated with budburst. Therefore, in an attempt to increase cytokinin reserves, cherry trees were sprayed with Maxcel® in autumn. Therefore, three separate experiments were designed to evaluate the role of each reserve, nitrogen, cytokinin, or carbohydrates in bud dormancy behaviour and its effect on flowering

and fruit set. It was expected that if these reserves was at optimal concentrations and if they do influence bud dormancy, budburst would be advanced. In addition, rest-breaking agents were applied with plant growth regulators to hasten budburst and assist flower buds in their final development and thereby counteract the symptoms of inadequate winter chilling.

CHAPTER 3

Bud Dormancy Progression of Sweet Cherry (*P. avium)* Cultivars in South Africa under Conditions of Inadequate Winter Chilling

Abstract

Bud dormancy progression was quantified for five sweet cherry cultivars in South Africa during 2005, 2006 and 2007 with the objective to characterize bud dormancy behaviour for sweet cherry under the mild winter conditions of South Africa. Shoots were sampled from trees in the Koue Bokkeveld, Piketberg, Underberg, Clarens, Reitz and Belfast. Due to the complexity in obtaining shoots from different areas, sampling in a season was subjected to availability of shoots (Section 3.2.1). Twenty one-year-old shoots of 'Bing', 'Lapins', 'Royal Lee', 'Royal Dawn' and 'Sweet Heart' on Mazzard or Mahaleb rootstock were randomly collected in commercial orchards in these areas at three-week intervals from bud set in summer until spring budburst. Shoots were then forced at a constant temperature (25°C) with continuous illumination until budburst in six of the 20 shoots occurred, i.e. 30% budburst. A model, comprising two joined straight lines, was fitted in order to characterize bud dormancy behaviour for sweet cherry cultivars under mild winter conditions. All cherry cultivars entered and exit from dormancy. Factor analysis showed that factors related to the entrance into dormancy primarily characterize bud dormancy behaviour. Bud dormancy patterns were also a function of environmental conditions within a season, as shown by cluster analysis. Buds entered dormancy in mid-summer and remained dormant until chilling accumulation commenced. Bud dormancy exit was generally extended over a three to five-month period for all cultivars. Prior to spring budburst, exit of both lateral and terminal buds occurred rapidly. Data indicate that there is no ecodormant phase for cherry under the prevalent climatic conditions in South Africa. This study shows that bud dormancy of cherry does not follow the expected pattern of entering dormancy when chilling accumulates as predicted by the Utah model.

Key words: bud dormancy induction and release, chilling requirement

3.1 Introduction

South Africa is characterized by a mild winter climate (short winters, with warm daily temperatures) with long, hot and dry summers generally not suited to the cultivation of high chilling fruit varieties (Strydom *et al.*, 1971). The suitability of a cultivar is estimated

according to chilling requirement, i.e., the number of effective chill hours needed to restore bud growth potential in spring (Richardson *et al.*, 1974). Under mild winter conditions, the chilling requirement is not satisfied leading to delayed foliation (Strydom *et al.* 1971; Jacobs *et al.*, 1981), a basitonic growth tendency (Cook *et al.*, 1998), deformed flowers or abortion of flowers (Crabbé, 1994; Oukabli and Mahhou, 2007) and subsequent poor fruit set (Mahmood *et al.*, 2000). Rest-breaking agents are applied to alleviate the symptoms of delayed foliation. This results in a more even budburst and flowering pattern.

Bud dormancy progression can be classed in three phases that often overlap viz. paradormancy, endodormancy and ecodormancy (Lang, 1987). Paradormancy involves the inhibition of the lateral buds by the terminal bud or leaves, i.e., the suspension of growth are due to correlative inhibition (A type of growth control where a biochemical signal from one structure inhibits the outgrowth of another structure). The next phase, endodormancy, originates within the affected structure where growth is controlled by an endogenous signal within the bud. The third phase, ecodormancy, entails the suspension of growth due to one or more environmental factors (e.g. low temperatures, frost etc.) that are unsuitable for growth (Lang, 1987). All these phases have different but overlapping temperature requirements, for example, higher temperatures during endodormancy would partially counteract accumulated chilling, resulting in a decreased budburst rate in spring. However, during ecodormancy higher temperatures would also promote budburst. Bud dormancy progression is complex, but some factors are known to control its progression.

Bud dormancy is mainly controlled by the chilling period and temperature in both apple and pear (Hauagge and Cummins, 1991; Heide and Prestrud, 2005; Jacobs *et al.*, 2002), but other factors also influence bud dormancy progression (Lang, 1987). These include water potential, nutrient status, oxygen levels, photoperiod and endogenous signals. In a mild winter climate, however, bud dormancy progression is altered (Cook and Jacobs, 2000). The exact environmental factor/s that results in bud set remains elusive, but was generally believed to be associated with the occurrence of short days (reduced photoperiod) and low temperatures. Cook *et al.* (2005) and Cook and Jacobs (2000) suggested that the occurrence of freezing temperatures or frost might also induce bud set in apple. Furthermore, for some temperate woody plants, it was shown that short photoperiods induced bud set (Heide, 1974; Li *et al.*, 2003), but for apple and pear trees, low temperatures induced bud set irrespective of the photoperiod (Heide and Prestrud, 2005). However, Hauagge and Cummins (1991) suggested that for bud set to occur, a

two-step process was necessary where higher temperatures (>18°C) were required at first with lower temperatures only intensifying bud dormancy. Both Heide and Prestrud (2005) and Junttila *et al.* (2003) showed that the rate at which bud set occurs is a function of temperature. In birch, fastest rate of development occurred at 15-18°C whereas higher or lower temperatures reduced the rate at which bud set occurred (Junttila *et al.*, 2003). For apple 'M9' low temperatures (9-12 °C), induced dormancy whereas in 'A2' higher temperature (15°C) resulted in bud set (Heide and Prestrud, 2005). The exact environmental cue/s for bud set to occur remain unclear. Temperature effects on bud set is, however, not clear-cut, and may depend on the physiological state of the bud (Crabbé and Barnola, 1996). After bud set occurred, deciduous fruit trees enter a stage of endodormancy essential for the survival of freezing temperature injury.

Temperature optima during the endodormant phase differ between and within species. For peach, constant low chilling temperatures (6-8°C) are required to break dormancy (Richardson, 1974). Mahmood *et al.* (2000) determined that temperatures as low as 3°C are ideal for cherry. Upon the fulfilment of the chilling requirement, buds exit dormancy rapidly provided that the environmental conditions are favourable. If the chilling requirement is only partially satisfied, as in a mild winter climate, apple buds exit dormancy poorly resulting in an associated basitonic growth tendency (Cook *et al.*, 1998). Furthermore, the endodormant phase becomes extended not allowing for the intervention of an ecodormant phase before spring budburst. It is apparent that the same low temperatures required for bud set to occur are also required to exit from endodormancy (Crabbé, 1994; Heide and Prestrud, 2005). Previously, bud dormancy progression was not quantified for cherry, although some reports dealt with its adaptation to mild winter climates (Anderson *et al.*, 1990; Küden *et al.*, 1997; Martínez et al, 1999; Oukabli and Mahhou, 2007; Snir and Erez, 1998). Furthermore, Mahmood *et al.* (2000) reported on the effect of chilling and post-chilling temperatures on flowering and frequency of budburst in sweet cherry.

The objective of this study was to quantify and characterize bud dormancy progression behaviour for sweet cherry cultivars under mild winter conditions.

3.2 Material and Methods

3.2.1 Plant material and locations

Twenty, unbranched, one-year-old cherry shoots, ca. 30 cm long, were collected per cultivar in commercial orchards from bud set in summer until spring budburst. All cold units were calculated and presented as Utah model chill units. Shoots were collected at 3-week intervals in the winter rainfall region of the Western Cape (Koue Bokkeveld, 33°S, 945m, ca. 1799 CU; Piketberg, 32°S, 750m, ca. 1378 CU) and the summer rainfall region of the Highveld (Reitz, 27°S, 1560m, ca. 841 CU; Belfast, 24°S, 340m, ca. 1024 CU; Underberg, 29°S, 1580m, ca. 1069 CU) of South Africa. Shoots were delivered to the laboratories in Stellenbosch by overnight courier. Delivery of shoots was subjected to availability of trees and reliability of farm managers. Therefore, during 2007, shoots could not be sampled from Belfast or Piketberg for 'Bing'. 'Bing' shoots were collected from the Koue Bokkeveld (2005, 2006 and 2007), Piketberg (2006), Underberg (2005, 2006 and 2007), Reitz (2006 and 2007) and Belfast (2005 and 2006); 'Royal Dawn' (2006 and 2007) and 'Royal Lee' (2006 and 2007) from Reitz, 'Lapins' (2006 and 2007) form the Underberg and 'Sweet Heart' (2007) from Piketberg. These cultivars were grown on Mazzard or Mahaleb rootstock. No rest-breaking agent was applied prior to spring budburst. During 2005, shoots were collected from April until spring budburst (August) and in 2006 and 2007 from December until spring budburst (August).

3.2.2 Forcing and data collection

Number of sampled dates did not exceed 14 times per year. The shoots were bundled and placed in 5 L plastic buckets with 1L of water and forced to determine the depth of dormancy. The shoots were forced in a growth chamber with constant illumination (ca. 200 $\mu molm^{-2}s^{-1}$ photosynthetically active radiation) at a constant 25°C (Jacobs *et al.*, 2002). The water was changed every 2-3 days dipping the distal shoot end in 0.25% (v/v) sodium hypochlorite solution (3.5%) for ca. 10 minutes. Approximately 1 cm of the bottom of the shoot was cut off weekly. Lateral and terminal budburst was recorded every 2-3 days until ten of the twenty shoots (50% budburst) had reached green tip. After 100 days, the bundles were discarded. Occasionally, budburst occurred on less than 10 of the 20 shoots. As a result, to reduce the number of missing values, the time to budburst was calculated as the time taken for budburst to occur on six shoots per bundle, i.e. days to 30% budburst (Cook and Jacobs, 2000).

3.2.3 Model development and statistical analysis

A model comprised of two joined straight lines was fitted to create nine variables that described bud dormancy progression for sweet cherry cultivars under mild winter

conditions (Fig. 3.1). This is a new model and has no previous history. It is a simple approach in an attempt to characterize cherry bud dormancy behaviour. The model was only fitted to terminal buds, because terminal bud dormancy is not under correlative inhibition (Crabbé and Barnola, 1996).

3.2.3.1 Plotting

Days to budburst was plotted on the y-axis with the corresponding day of the year when the shoots were cut on the x-axis for each cultivar in a specific area for each year. A trend line is fitted to the entrance and exit data with the inclusion of two data points (100) to accurately represent observed behaviour. The additional data point, chosen at 100 days (y-axis), was also included on the first date on which no budburst occurred (entered endodormancy). This data point represents the first date when buds were deeply endodormant. The same 100 days to budburst data point was used with the exit data. This represented the last day where buds were deeply endodormant.

To understand the addition of the constant (100) it is necessary to explain the complexity in the forcing of cherry shoots. The time at which bud set occurs varies each year which cannot be accurately predicted. Thereafter, the entrance into endodormancy occurs rapidly which results in a small window period in which entrance data can be generated. In some cases it occurred rapidly, so that data was generated on one date only (M, S, H and I). Therefore, a straight line cannot be fitted without the addition of a constant which would need to fulfil the following criteria: 1) the line must have a steep slope i.e. goes rapidly endodormant 2) Must be a number of days after which no budburst would occur in the growth cabinet i.e. 100 days. In other cases budburst occurs rapidly on two or three dates (e.g. 5 and 6 days) and if a straight line is fitted the line would not accurately represent the quick entrance into endodormancy. For example, the straight line would cross the x-axis e.g. in early December whereas it should have crossed the x-axis in early January. Endodormancy cannot be measured; it can only be shown when the shoots were not endodormant i.e. the beginning and the end of endodormancy. To be consistent the data point was also added to the exit data.

3.2.3.2 Model variables

From these two joined straight lines the following variables were created: ‘Maximum’ represents the level of maximum bud dormancy for a specific cultivar. ‘Slope in’ and ‘slope out’ represents the rate as in days to budburst per day (of bud dormancy entrance and exit

respectively). ‘Entrance’ and ‘exit’ represents day of the year (DOY) when buds entered and exit dormancy, respectively. The turning point represents the DOY when buds were at maximum dormancy. ‘Time in’ and ‘time out’ represents the time (days) required to reach maximum dormancy and the time (days) required to be released from maximum dormancy, respectively. ‘Total time’ represents the total time (days) that buds were dormant.

3.2.3.3 Statistical analysis

Factor analysis was performed on variables created to isolate potential relationships between variables (Johnson, 1998). Furthermore, cluster analysis was performed by K-means clustering to enable the classification of similar experimental units. Mean separation was conducted using the Bonferroni test at the 5% level. Statistical analysis was performed using the Statistica software (Statistica Release 7, StatSoft®, Tulsa, US).

3.3 Results

Traditionally, with forcing, chlorine based solutions are used to prevent fungal or bacterial growth. Cherry shoots, however, were found to be particularly sensitive to the addition of chloride (in the form of household bleach) to the water solution. Symptoms included inter-venial yellowing with subsequent scorching of the leaf tips, and ultimately, the total browning and death of the leaves and shoots. This was problematic if the shoots were not well hardened off. Leaves also appeared smaller compared to shoots that were forced in tap water. For cherry, death of shoots was also associated with an increase in pH >7 in the water solution. Water sanitation, however, was still important. The occurrence of slime blocked water uptake by the shoots. Therefore, the bottom 10 cm of shoots were submerged in a water solution (0.25% sodium hypochlorite) for ca. 10 minutes every second day between water change to prevent the development of slime and bacterial growth. This proved to be sufficient. In addition, water buckets were scrubbed with a concentrated bleach solution and rinsed with water during each water change.

During 2005, a straight line could only be fitted to exit data for ‘Bing’ (A, B, C, and D) and, in 2006, no data could be generated for the entrance into dormancy with a straight line only fitted to the exit data for ‘Bing’ (J) from Belfast (Fig. 3.2 & 3.3). Therefore, this exit data was not included in the statistical analysis, because it is not possible to create nine variables from this data and would therefore result in an incomplete data set. In 2006 ‘Bing’ from the other regions (E, H, K and L) buds entered dormancy in summer (Fig. 3.3).

Here terminal buds tended to dominated the lateral buds. Shortly thereafter, budburst did not occur in lateral or terminal buds. For ‘Bing’, maximum dormancy was reached during February long before any chilling accumulated. Buds start to exit dormancy as chilling start to accumulate. Thereafter, both lateral and terminal buds exit slowly, but more rapidly before spring budburst. Here, lateral buds tended to dominate terminal buds before spring budburst. The same trend was observed for ‘Royal Dawn’ (F) in 2006 although it entered dormancy and reached its maximum depth of dormancy earlier than ‘Bing’. ‘Lapins’ (I), however, entered dormancy rapidly and reached its maximum depth of dormancy by the end of January. Here lateral buds dominated terminal buds before no budburst occurred. With bud dormancy exit, lateral buds dominated terminal buds. Again, exit from dormancy started with chilling accumulation. ‘Royal Lee’ (G) exhibited the same pattern as ‘Lapins’, but reached maximum level of dormancy later.

During 2007, buds entered dormancy more rapidly than in 2006 (Fig. 3.4). ‘Bing’ (M, P and R) entered dormancy during summer. Here too, terminals dominated lateral buds before no budburst occurred. The maximum level of dormancy was reached in February. This occurred before any chilling accumulated. ‘Royal Dawn’ (N and S), ‘Royal Lee’ (O) and ‘Lapins’ (Q) all followed the same pattern as ‘Bing’ in 2007. Again laterals dominated terminal buds before spring budburst. Buds also started to exit before chilling accumulation commenced.

Chill units were calculated in 2006 and 2007 using the Utah model (Richardson, 1974). Accumulated chilling is shown in Fig. 3.3 & 3.4. Chilling accumulation was the highest in the Koue Bokkeveld and Piketberg in both years (Table 3.1). Reitz was regarded as a warmer area with the lowest chill unit accumulation. Similarly, Belfast can be classified as warm even though only one year’s data were presented. Chilling varied in Underberg with more chilling accumulated in 2006.

Cluster analysis identified two groups in which bud dormancy patterns of the various sweet cherry cultivars were similar (Table 3.2; Table 3.3 & Fig. 3.5). Further cluster grouping could not yield any logical groupings. In Cluster 1 (E, G, H, K, L), buds entered dormancy in summer (December) and reached their maximum depth of dormancy in April. Buds exit dormancy in August. In Cluster 2 (F, I, M, N, O, P, Q, R, S and T), buds also entered dormancy in summer (December), but reached the maximum level of dormancy significantly earlier in late February. Depth of dormancy was deeper, but non-significant in

Cluster 1. Cluster 2 exited dormancy in August. Bud dormancy release was generally extended over a 3 month period for cluster 1 and 5-month period for cluster 2. Total time that buds were dormant was 225 days for Cluster 2, significantly longer compared to 207 days in Cluster 1.

Factor analysis showed that factor 1 described 52% of the variation in the data, i.e., 'Slope in', 'Entrance', Turning point', 'Time in' and 'Time out' (Table 3.4 & 3.5). Factor 2 showed that depth of dormancy, rate of bud dormancy exit described 22% of the variation in the data. Time of exit and total time that buds were dormant explained 20% of the bud dormancy behaviour as shown by factor 3.

3.4 Discussion

Sampling of 20 shoots per cultivar per date was sufficient, although sampling at 3-week intervals did not generate sufficient data for the entrance into dormancy. The variation in time of induction further complicated data collection. In some instances, the lateral buds were immature and data could not be generated in the summer period. It is suggested that shoots should be sampled at weekly intervals from bud set until 30% of the shoots do not reach green tip. After this date, shoots can be sampled at three-week intervals, to determine time where buds start to exit dormancy. Closer to spring budburst, one week intervals will provide more accurate data.

The two cluster groups identified, shows that bud dormancy pattern was most probably a function of environmental conditions during a specific year. Cluster 1 consisted only of orchards sampled in 2006. Cluster 2 mainly consisted of orchards sampled in 2007, although 'Royal Dawn' (I) and 'Lapins' (F) were included in Cluster 2. Note, however, that these two cultivars are lower chilling varieties (compared to 'Bing') which demonstrated similar behaviour by entering dormancy earlier and rapidly. Although 'Royal Lee' (G) is also a low chill variety, it did not respond in the same manner to the same environmental cues as the other varieties. Similarly, Hauagge and Cummins (1991) found that low chill apple varieties responded in a different way to the same environmental cues. Both clusters showed that buds enter dormancy in summer (December). Sampling of shoots started as soon as shoot growth ceased (after bud set). Shortly thereafter, while conditions were still optimal for growth, no budburst occurred, i.e., buds were dormant. This contrasts with the common belief that buds go dormant with the onset of winter. Similarly, in Tromp's (2005) review of dormancy, buds enter endodormancy in mid-summer when conditions are

optimal for growth. Furthermore, bud set (growth cessation) preceded the entrance into endodormancy which is in turn influenced by low temperatures, short days or plant factors. In this study, endodormancy set in shortly after bud set. In addition, factor analysis showed that bud dormancy behaviour was primarily a function of factors related to the entrance into dormancy. Although Cluster 2 reaches its maximum level of dormancy earlier than Cluster 1, both reached their turning point in summer before the accumulation of any chilling. Chilling accumulation can however not entirely explain these groupings. This may be ascribed to shortcomings in the Utah chill unit model under conditions of inadequate chilling (Cook and Jacobs, 2000; Linsley-Noakes, 1994). Furthermore, chilling only accumulated after buds started to exit dormancy. The chilling period only consisted less than 40% of the total time of bud dormancy which further underlines the limitations of the Utah model under low chill conditions.

Bud dormancy release was generally extended over a three month period for Cluster 1 and a five month period for Cluster 2. This was clearly visible in the time required to exit from maximum dormancy. Prior to spring budburst, exit of both lateral and terminal buds occurred rapidly. Lateral buds, however, tended to acquire dominance and exit before terminal buds prior to spring budburst for all cultivars. According to Cook *et al.* (1998), Crabbé (1994), and De Wit *et al.* (2000) under conditions of sufficient chilling, an acrotonic budburst pattern was observed in shoots. This was not the case for the sweet cherries under the mild winter conditions where lateral buds tended to dominated prior to spring budburst. Cook and Jacobs (1998) showed that in apple this basitonic development was a result of inadequate winter chilling. Furthermore, data indicate that there is no ecodormant phase for cherry under the prevalent climatic conditions in South Africa. This was in accordance with Saure (1985) where deciduous fruit trees showed a prolonged phase of endodormancy and exit dormancy without an intervention of an ecodormant phase under mild winter conditions.

3.5 Conclusion

Cherry buds in South Africa enter endodormancy in mid-summer shortly after bud set for all cherry cultivars. Maximum depth of dormancy was reached in summer before any chilling started to accumulate. Lateral and terminal buds exited dormancy only after chilling accumulation commenced. As a result, bud dormancy induction and subsequent progression appears not to be related to chilling accumulation, at least as quantified by the Utah model. In addition, bud dormancy behaviour was primarily determined by factors

related to entrance into dormancy and environmental conditions within a season determine bud dormancy progression patterns. These findings contradict common belief that buds go dormant with the onset of winter as predicted by chill unit models. This raises the question if bud dormancy, at least in cherry, is in fact initiated with terminal bud set?

3.6 References

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Table 3.1. Accumulated Utah model chill units for each cherry production area in 2006 and 2007.

Year	Area	Utah model CU			
		May	June	July	August
2006	Reitz	254	437	654	938
	Underberg	350	661	975	1310
	Belfast	279	553	754	1024
	Koue Bokkeveld	421	807	1261	1702
	Piketberg	366	593	975	1381
2007	Reitz	149	424	569	743
	Underberg	235	452	656	828
	Koue Bokkeveld	366	855	1356	1895
	Piketberg	441	780	1203	1375

Table 3.2 Variables created for the bud dormancy data for cluster analysis

Year	Cluster	Area	Cultivar	Maximum[a]	Slope in[b]	Slope out[c]	Entrance[d]	Turning point[e]	Exit[f]	Time in[g]	Time out[h]	Total time[i]
2006	1	Reitz	Bing	129	1.52	-1.00	-18	67	196	85	129	214
2006	2	Reitz	Royal Dawn	88	1.52	-0.57	-17	41	194	58	153	211
2006	1	Reitz	Royal Lee	101	0.97	-1.02	-14	90	189	105	99	204
2006	1	Underberg	Bing	243	2.76	-1.92	-19	69	196	88	127	215
2006	2	Underberg	Lapins	109	2.64	-0.64	-21	21	192	41	171	213
2006	1	Bokkeveld	Bing	103	1.05	-0.89	4	102	217	98	115	213
2006	1	Piketberg	Bing	133	1.16	-1.71	8	122	200	114	78	191
2007	2	Reitz	Bing	144	1.71	-0.96	-14	70	221	84	150	234
2007	2	Reitz	Royal Dawn	115	1.76	-0.72	-18	48	207	65	160	225
2007	2	Reitz	Royal Lee	123	1.66	-0.84	-13	61	207	74	146	220
2007	2	Underberg	Bing	138	2.68	-0.77	-12	39	219	52	179	231
2007	2	Underberg	Lapins	87	1.69	-0.59	-8	43	191	51	147	199
2007	2	Bokkeveld	Bing	123	1.78	-0.70	-9	60	235	69	176	245
2007	2	Bokkeveld	Royal Dawn	133	3.09	-0.71	-17	26	212	43	187	230
2007	2	Piketberg	Sweet Heart	135	1.86	-0.81	-7	65	231	73	166	238

[a] 'Maximum' represents level of maximum bud dormancy.
[b] 'Slope in' represents rate of bud dormancy entrance (days to budburst per day).
[c] 'Slope out' represents rate of bud dormancy exit (days to budburst per day).
[d] 'Entrance' represents time of entrance into bud dormancy (DOY).
[e] 'Turning point' represents time when bud reached maximum dormancy (DOY).
[f] 'Exit' represents time of bud dormancy exit (DOY).
[g] 'Time in' represents time required to reach maximum bud dormancy (days).
[h] 'Time out' represents time required to exit dormancy once maximum dormancy was reached (days).
[I] 'Total time' represent the total time that bud was dormant (days).

Table 3.3 Means for each variable describing bud dormancy progression of sweet cherry for Clusters 1 and 2.

Cluster	Maximum	Slope in	Slope out	Entrance	Turning point	Exit	Time in	Time out	Total time
1	141	1.49	-1.31 a[z]	-8	90 a	200	98 a	110 a	207 a
2	119	2.03	-0.73 b	-14	47 b	211	61 b	164 b	225 b
Pr > F	*0.2843*	*0.1264*	*0.0023*	*0.2132*	*0.0012*	*0.1796*	*0.0002*	*<0.0001*	*0.0313*

[z] Mean separation within columns followed by different letters according to Bonferroni (5% level).

Table 3.4 The Scree test of Catell was used to determine the number of factors to be extracted during the factor analysis produce performed on variables created from the bud dormancy data.

Factor	Eigenvalue	Percentage Total Variance	Cumulative Percentage Total variance
1	4.64	51.59	51.59
2	1.99	22.12	73.70
3	1.81	20.08	93.78

Table 3.5 Varimax normalized factor loadings were computed for each extracted factor to determine the variable-factor association for the bud dormancy data. Loadings greater than 0.70 represent variables defining the nature of the respective factors (loadings marked).

Variable	Factor 1	Factor 2	Factor 3
Maximum	0.128819	***0.974165***	0.142405
Slope in	**0.825893**	0.386495	0.215987
Slope out	0.411160	**-0.868386**	0.249873
Entrance in	**-0.838333**	-0.104636	0.137063
Turning point	**-0.958628**	0.262239	-0.089205
Exit	-0.138115	-0.021820	**0.989385**
Time in	**-0.878689**	0.367530	-0.163657
Time out	**0.791067**	-0.245087	0.555971
Total time	0.334807	0.037233	**0.906705**

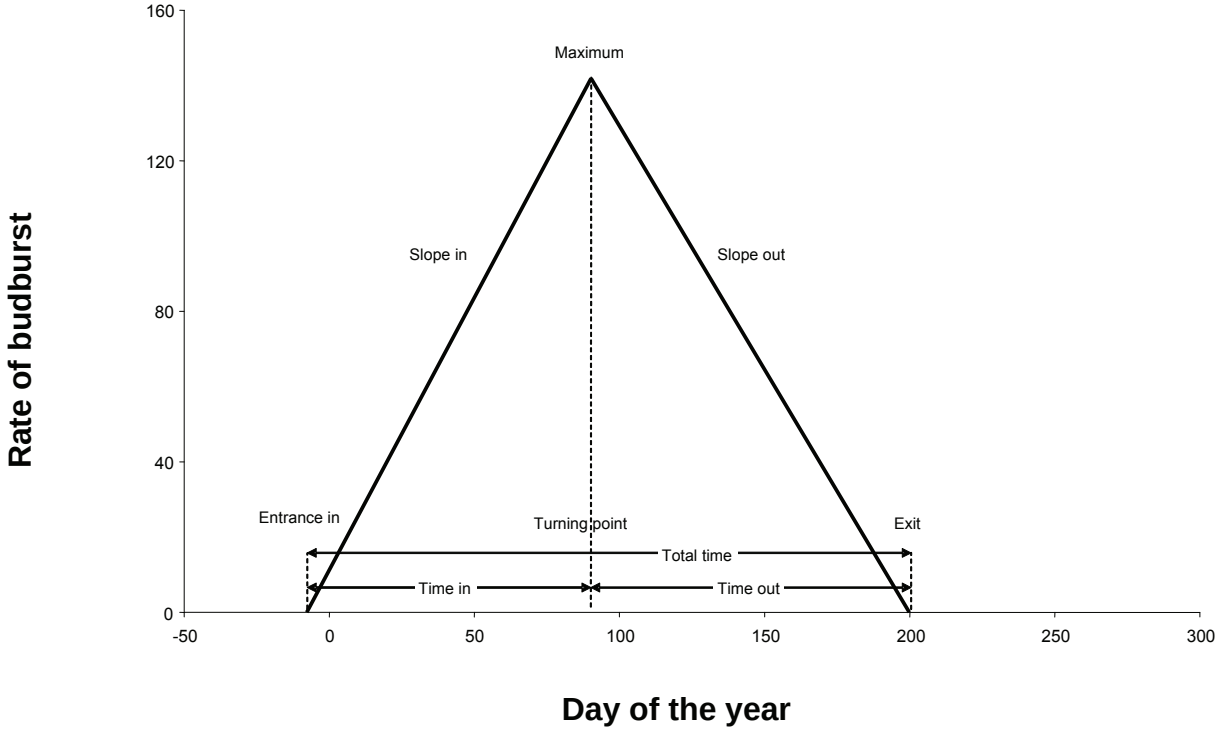

Fig. 3.1. Diagrammatic representation of the model fitted to bud dormancy data to generate the nine dormancy descriptive variables.

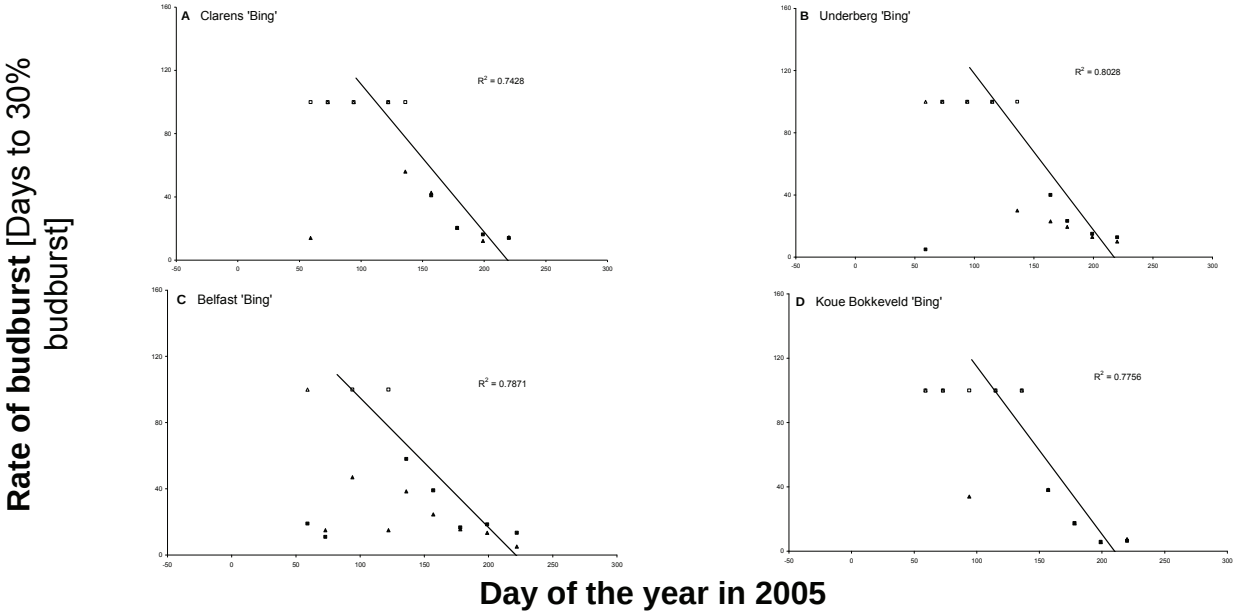

Fig. 3.2. Terminal and lateral bud dormancy progression of one-year-old 'Bing' cherry shoots in different areas of South Africa in 2005 (Lateral ▲; Terminal ■). Open data points represents dates were data could not be generated.

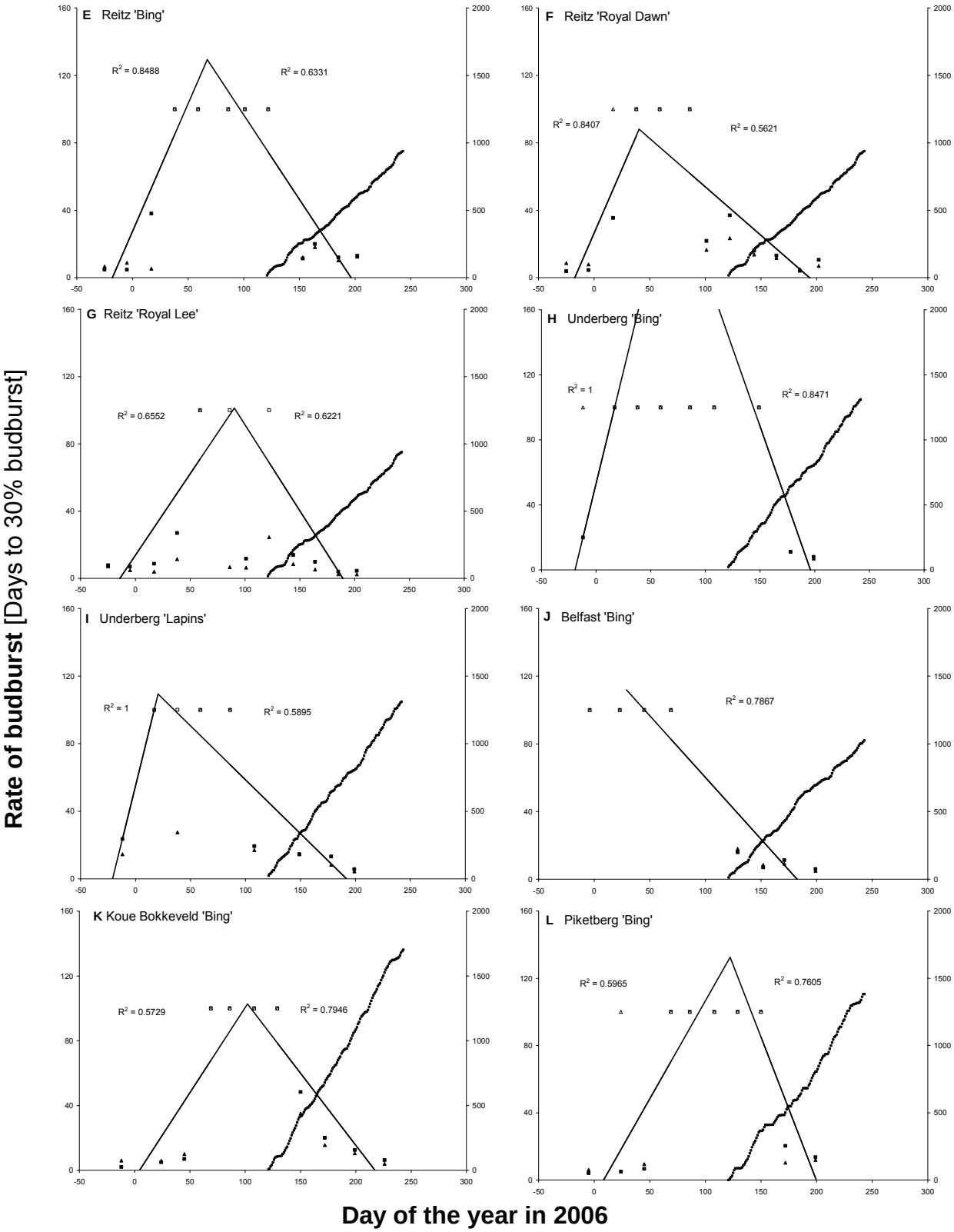

Fig. 3.3. Terminal and lateral bud dormancy progression of one-year-old cherry shoots in different areas of South Africa in 2006 (Lateral ▲; Terminal ■). Open data points represents dates were data could not be generated. Dotted lines indicate accumulated of Utah model chill units.

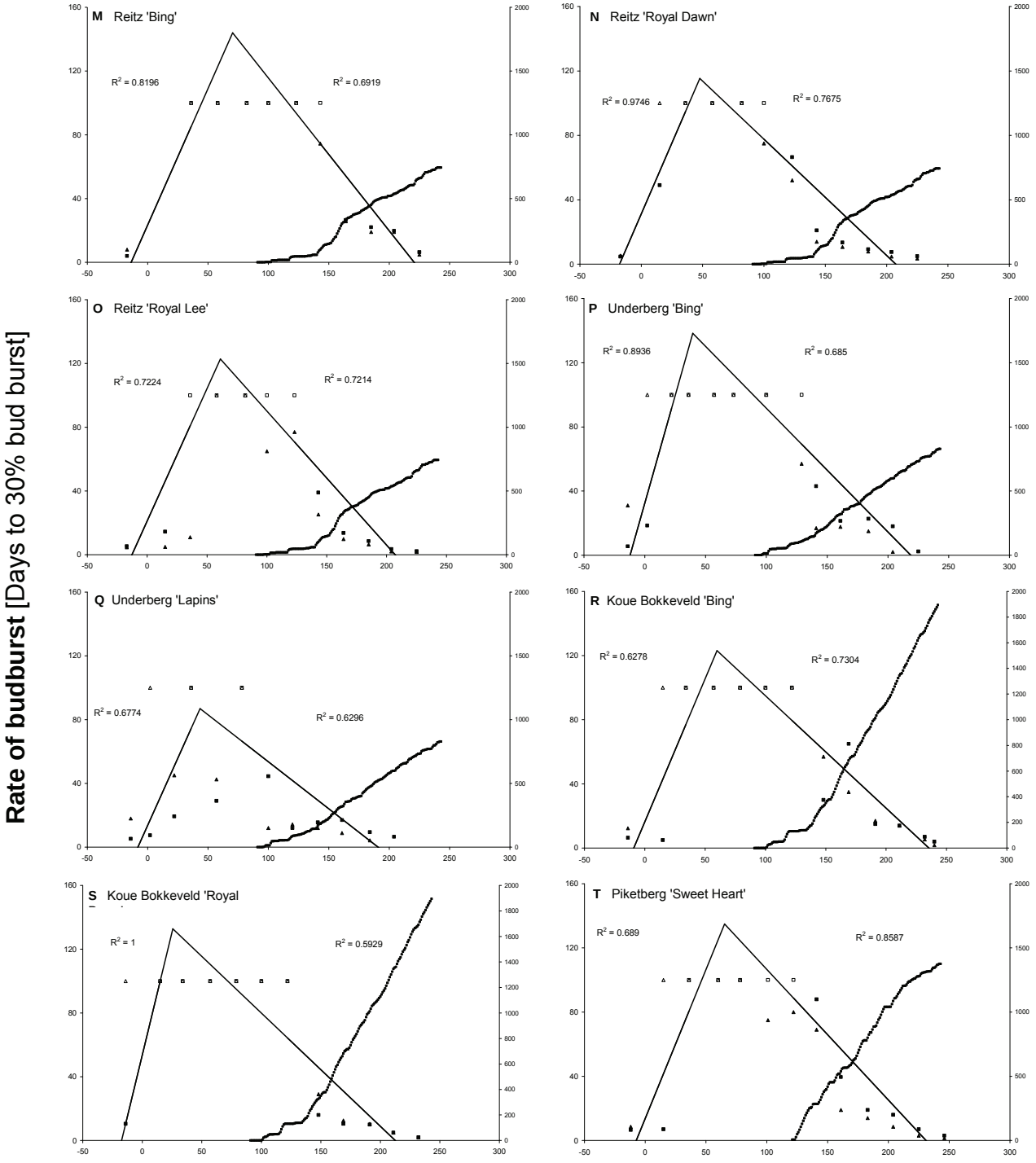

Fig. 3.4. Terminal and lateral bud dormancy progression of one-year-old cherry shoots in different areas of South Africa in 2007 (Lateral ▲; Terminal ■). Open data points represents dates were data could not be generated. Dotted lines indicate accumulated Utah model chill units.

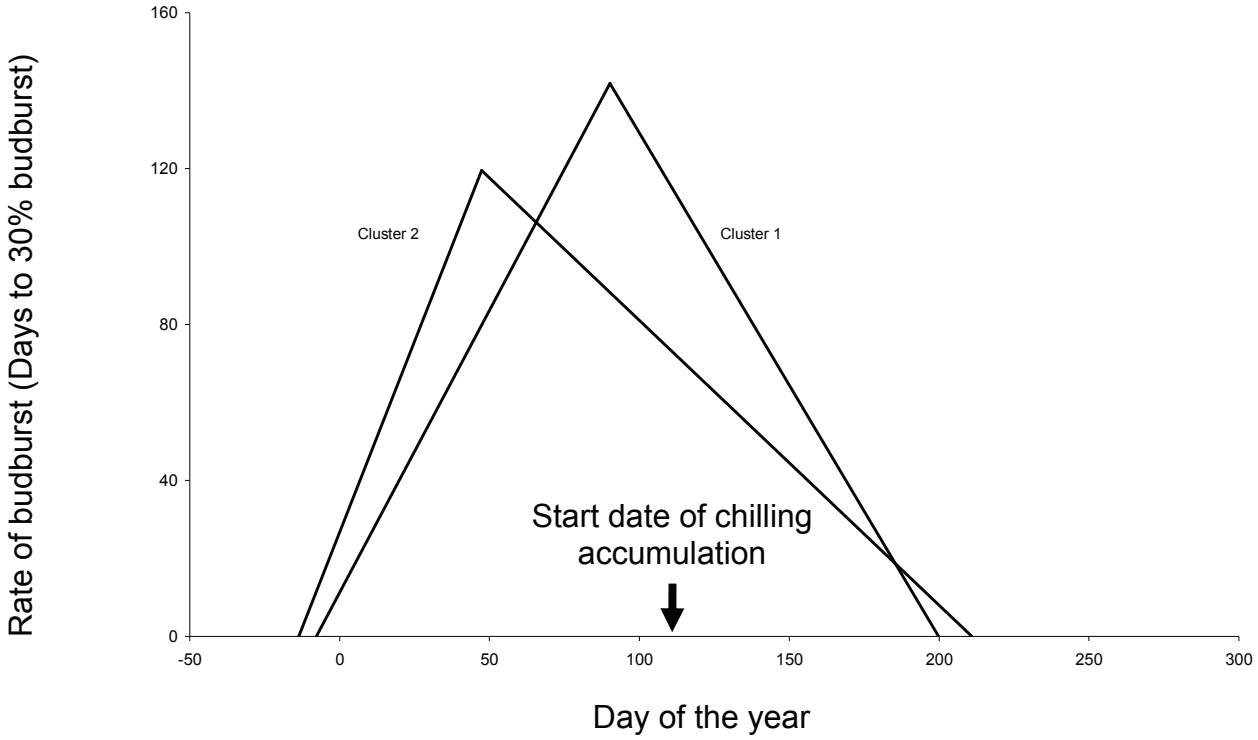

Fig. 3.5. Fitted bud dormancy progression model for Clusters 1 and 2 for sweet cherry cultivars grown under low chill conditions in South Africa.

CHAPTER 4

Optimization of Postharvest Nitrogen Fertilization of 'Bing' Sweet Cherry for Adaptation to Inadequate Winter Chilling

Abstract

Experimentation was aimed at manipulating the nitrogen (N) reserve level of mature, high-chill sweet cherry trees in order to determine if a mature cherry tree with optimal N reserves would require less winter chilling under conditions of inadequate chilling, as experienced in South Africa. Furthermore, the optimal time for postharvest N fertilization had to be determined. N was applied in equal amounts as a single or a split application in 2006 and 2007. N was applied either after bud set until/or with leaf senescence. Timing of N fertilization in the postharvest period did not improve leaf N concentration in 2007. Nitrogen analyses of dormant two-year-old branches showed no significant differences between treatments; except for N fertilization on 14 April 2006 where N concentration was significantly higher in the two-year old wood and the two-year-old unit. Bud dormancy progression, as monitored by forcing in the growth cabinets, was not significantly affected. The timing of N application had no significant effect on time of flowering in mature field-grown cherry in 2006. During 2007, however, flowering was advanced in N treatments. Fruit density was higher in 2006 when N was applied on 14 April 2006 although the number of cherry fruit per cluster was unaffected in both years. Timing of N fertilization or the application of N in the postharvest period did not result in a significant reduction in the chilling requirement of cherry under mild winter conditions of South Africa.

Keywords: bud dormancy progression, nitrogen reserves

4.1 Introduction

Commercial production of sweet cherries has recently increased in South Africa, with more than 400 ha planted by 2006. Cherry trees are grown under mild winter conditions (short winters, with warm daily temperatures) with long, hot summers not optimally suited for the cultivation of high chilling fruit varieties (Strydom *et al.*, 1971). Inadequate winter chilling results in symptoms of delayed foliation and poor fruit set. Therefore, common practice involves the application of rest-breaking agents to alleviate these symptoms. Fruit set,

however, remains poor. Furthermore, the optimal time of postharvest N application for the improvement of N reserve status needs to be established. The poor performance of cherry trees is associated with insufficient chilling accumulation and an unfavourable temperature regime. Management practices that can assist the cherry tree to tolerate these unfavourable conditions, and still produce a profitable crop, are urgently required.

Several factors are known to affect bud dormancy, of which temperature conditions during the annual cycle of the tree are considered the most important in deciduous fruit trees. Pre-chilling conditions, chilling temperatures, duration of chilling, post-chilling temperatures and reserve N are known to affect time of budburst, flowering and fruit set in deciduous fruit trees (Hill-Cottingham, 1967; Jonkers, 1979; Mahmood *et al.*, 2000; Stassen *et al.*, 1981b; Taylor and van den Ende, 1969a; Terblanche and Strydom 1973; Terblanche *et al.* 1979). Higher temperatures (>18°C) during the growing season delay bud development in spring in apple and pear (Jonkers, 1979). Furthermore, insufficient winter chilling together with poor (> 19°C) spring temperatures were shown to adversely affect time to budburst, flowering and fruit set in cherry (Mahmood *et al.*, 2000). With increased chilling, however, the rate of vegetative growth, flower size and fruit set were improved for 'Stella' cherry (Mahmood *et al.*, 2000). Temperature, therefore, primarily controls bud dormancy progression and plays a critical role in the time of budburst. Although chilling or temperature conditions cannot be fully manipulated under field conditions, several authors showed that chilling requirement can be manipulated in young fruit trees through the manipulation of N reserve level. Time to budburst and flowering was reduced for young peach and apple trees optimal in N reserves (Stassen *et al.*, 1981b; Terblanche and Strydom, 1973; Terblanche *et al.*, 1979). This was achieved through N fertilization in late summer or autumn which improved N content of the perennial parts. For young 'Kakamas' peach trees, full bloom was advanced by ca. 14 days and the highest percentage of reproductive buds developing into flowers was related to higher dormant total N (Stassen *et al.*, 1981a). For young 'Golden Delicious' apple trees, budburst started approximately 30 days earlier relative to the control (Terblanche and Strydom, 1973). Hill-Cottingham (1967) showed that N content were higher in apple trees that showed improved flowering and fruit set. However, some inconsistencies occurred once the effects of postharvest N fertilization were assessed on mature field-grown fruit trees. Autumn N application did not improve earliness of flowering, flowering and fruit set in mature peach trees (Huett and Stewart, 1999; Niederholzer *et al.*, 2001; Taylor and van den Ende, 1969a).

Literature mostly reports on the positive effects of N application during the summer or autumn period, and the positive effects of N reserve on flowering and fruit set. However, these reports generally deal with young trees, which largely depend on the current year and autumn N supply of which the N storage pool can easily be manipulated. Compared to older trees, which have a larger N or assimilate storage capacity (Niederholzer *et al.*, 2001), the previous season's N supply does not necessarily affect an event such as flowering.

The mechanism through which N reserve levels affect budburst in young deciduous fruit trees was less clear. However, the relationship between N, cytokinins and carbon metabolism provided some basis for a possible mechanism through which N can affect budburst (Geßler *et al.*, 2004; Nikolaou *et al.*, 2000; Sakakibara *et al.*, 2006). Nikolaou *et al.* (2000) showed a direct link between dormant total N concentration and spring endogenous cytokinin levels. Furthermore, it is generally accepted that endogenous cytokinin triggers spring budburst. Therefore, literature provides evidence that bud dormancy can indeed be affected by reserve N. It should however be stressed that chilling primarily controls bud growth potential in spring (Jacobs *et al.*, 2002), with N only affecting bud dormancy on a secondary level.

The objective of this study was to determine if N, applied during the postharvest period, could reduce the chilling requirement of cherry. The hypothesis was that this effect would be brought about by an increased tree N reserve status.

4.2 Materials and Methods

4.2.1 Plant material and statistical layout (2006)

N was differentially applied during the postharvest period in four treatments for seven-year-old 'Bing' sweet cherry on Mazzard rootstock grown in the Koue Bokkeveld, Ceres (33°S, 945m, ca. 1799 Utah model chill units). A randomized complete block design was used with eight replications and six trees per replication. The two centre trees served as sample trees while the four centre trees were fertilized. Tree spacing was 4.5 m x 1.5 m. Fertilizer (Limestone ammonium nitrate, LAN 28%N) was applied around the micro sprinkler for fertilized trees. No spring N was applied.

Treatments:

1. “Early”: Nitrogen was applied in late summer/autumn after bud set (3 March) at a rate of 45 kg/ha.
2. “Split (x3)”: A split application of 15kg/ha was applied in late summer/autumn (3 March), inbetween (26 March) and in autumn when leaf senescence commenced (14 April).
3. “Late”: In autumn when leaf senescence commenced at a rate of 45 kg/ha.
4. “Control”: No N fertilization.

4.2.2 Plant material and statistical layout (2007)

N was differentially applied during the postharvest period in five treatments for eight-year-old ‘Bing’ sweet cherry on Mazzard rootstock grown in the Koue Bokkeveld, Ceres (33°S, 945m, ca. 1799 CU). A new orchard was used, due to the change in experimental layout. A randomized complete block design was used with nine replications and ten trees per replication. The six centre trees served as sample trees while the eight centre trees were fertilized. Tree spacing was 4.5 m x 1.5 m. Fertilizer (Limestone ammonium nitrate, LAN 28%N) was applied around the micro sprinkler for fertilized trees. No spring N was applied.

Treatments:

1. “Early”: N was applied in late summer (3 Feb.) a rate of 51 kg/ha.
2. Split (x3)”: A split application of 17 kg/ha was applied in late summer (3 Feb.), inbetween (3 March) and in autumn when leaf senescence commenced (13 April).
3. “Late”: In autumn when leaf senescence commenced at a rate of 51 kg/ha.
4. “Control”: No N fertilization.
5. “Split (x2)”: A split application of 25.5 kg/ha was applied in late summer (3 Feb.) and when leaf senescence commenced (13 April).

4.2.3 Forcing

During 2006, two shoots per replication and in 2007, six shoots per replication were collected at five dates (8 May 2006, 26 May 2006, 19 June 2006, 12 July 2006, 28 July 2006, 28 May 2007, 18 June 2007, 10 July 2007, 30 July 2007, and 20 Aug. 2007) prior to spring budburst. No rest-breaking agent was applied. Shoots were bundled and placed in 5 L plastic buckets with 1L of water and forced to determine the depth of dormancy. The shoots were forced in a growth chamber with constant illumination (200 $\mu molm^{-2}s^{-1}$) and at a constant temperature of 25°C (Jacobs *et al.*, 2002). The water was changed every 2-3 days with submerged shoot

sections being dipped in 0.25% (v/v) sodium hypochlorite solution (3.5%) for ca. 10 minutes between each water change. Approximately 1 cm of the bottom of the shoot was cut off weekly. Lateral and terminal budburst was recorded every 2-3 days until 50% of the shoots in the bundle had reached green tip. “Total” was recorded if a lateral or a terminal bud on half of the shoots in a bundle had reached green tip. After 100 days, the bundles were discarded. Data were graphically represented as the inverse of days to 50% budburst (the rate of budburst), because bud dormancy exit was evaluated.

4.2.4 Flowering and budburst

Flowering was measured on two-year-old and older branches. Sections of ca. 70cm in length were used. Bud stage (1-9) was recorded for each spur on that section at full bloom (http://www.ncw.wsu.edu/treefruit/bdch.htm). Budburst was monitored on two one-year-old shoots per replication in 2006 and on four one-year-old shoots per replication in 2007. In 2006 two branches per plot and in 2007 four branches per plot was used to asses the flowering pattern.

4.2.5 Fruit set

Cherry fruit per cluster were counted on the same 70 cm branch sections used for flowering. Yield or “fruit density” was expressed as total fruit per branch divided by branch cross sectional area (πr^2) (Byers, 1997). During 2006, fruit density was determined on 6 November after pit hardening and in 2007 prior to and after pit hardening on 29 October and 22 November, respectively.

4.2.6 Chemical N analysis

The LECO FP-528L (LECO Corporation, St. Joseph, MI) was used to determine N concentration, as a dry weight percentage, of the branch and leaf samples according to the Dumas method (Horwitz, 2000). Leaf and branch samples were washed, dried, weighed and milled to a powder for N analyses.

4.2.7 Branch and leaf analysis

In the past, whole tree excavation and tree analysis proved to be expensive and labour intensive. Therefore, a simpler method for estimating tree reserve status was necessary. Johnson *et al.* (2006) found good correlations with N, P, Ca, B and Zn in dormant shoots and

tree performance as measured in flower density, fruit set, early shoot growth, fruit size and vegetative growth in *Prunus persica*. Therefore, branch N analysis was performed on 12 and 10 July in 2006 and 2007 respectively on two-year-old flowering branches during dormancy to assess N reserve status. Branches were divided into shoot, spur (flower cluster) and wood sections for N analyses. In addition, 15 shoot leaves per replication were analyzed in 2007, sampled at two week intervals from 3 Feb. until leaf senescence commenced (13 April) to assess N uptake. It was expressed as N percentage (g/100g) of dry weight.

4.2.8 Statistical analysis

Data were analyzed using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) program (SAS Institute Inc., 2003, Cary, NC, USA). In cases where data was not normally distributed, the analysis was performed on the logit transformed data.

4.3 Results

During the postharvest period, active white roots were visibly present at all fertilization dates. Therefore, root activity was sufficient for mineral uptake by roots. Dormant branch N analysis showed that flower spurs contained the highest N concentration in 2006 (Table. 4.1). Treatments did not significantly increase N concentration in spurs and shoots although N concentration of the 2-year-old unit tended to be higher in treatments which received N. A significant increase in N for two-year-old wood and for the total two-year-old branch was observed in 2006 when trees received N prior to leaf senescence (45kg N/ha). Cluster leaf development was not advanced in N treatments compared to the control (Table 4.2). Budburst on one-year-old shoots was unaffected in 2006 (Table 4.2). Between treatments, no significant effect on lateral and terminal bud dormancy was found during 2006 (Fig. 4.1). Full bloom was on 28 September in 2006, flowering, however, was not significantly affected (Table 4.2). Fruit density was significantly higher where N was applied prior to leaf senescence, but the number of cherry fruit per cluster was not affected (Table 4.3).

Leaf N analysis showed that N fertilization on 3 Feb. 2007 (‘Early’, ‘Split [x3]’ and ‘Split [x2]’ did not significantly increase leaf N concentration of treatments ‘Early’, ‘Split [x3]’ and ‘Split [x2]’ (Table 4.4). Fertilization on the 3 March 2007 (‘Split [x3]’) did not increase leaf N concentration. Leaves were not sampled after the last fertilization date (14 April), because leaf senescence started after 14 April 2007. During this period N was exported out of the

leaves to the perennial parts of the tree, and therefore no leaves were sampled (Stassen *et al.*, 1981). Dormant branch N analysis showed that flower spurs contained the highest N concentration in 2007 (Table. 4.5). Treatments did not significantly improve N concentration in spurs, shoots or wood although N concentration in the 2-year-old unit in treatments that received N tended to be higher in 2007. Budburst on one-year-old shoots was unaffected in 2007 (Table 4.6). Full bloom was on 6 October in 2007. Flowering was significantly advanced in all the N treatments in 2007 (Table 4.6). During 2007, no significant differences were found in fruit density and in cherry fruit per cluster as measured on 29 Oct. 2007 and 22 Nov. 2007 (Table 4.7). Between treatments, no significant effect on lateral and terminal bud dormancy was found during 2007 (Fig 4.2, A and B). Total bud dormancy progression was, however, affected in 2007 (Fig. 4.2, C). There was, however a time x treatment interaction, which prohibits the assessment of main effects.

4.4 Discussion

The present experiment was designed to determine if N reserves affects bud dormancy progression in mature sweet cherry trees. Therefore, the experimental design was aimed at manipulating the N reserve status of the tree through varied N application in the postharvest period. Reports showed that postharvest N fertilization improves tree N reserves and therefore trees that receive no N should be lower in N reserve (Hill-Cottingham, 1967). Furthermore, allocation patterns of N within the tree depend on time of application relative to the phenological stage of the tree. Generally, with autumn allocation, N starts to accumulate in perennial organs of cherry with a smaller portion being allocated to new growth (Zavalloni, 2004). However, efficiency of uptake and allocation of N to aerial parts reduces as leaf senescence approaches (Zavalloni, 2004). With leaf senescence a second uptake period absorbs N (Stassen *et al.*, 1981a; Zavalloni, 2004) and thereby improves N reserve levels predominantly in the perennial parts of the tree (Stassen *et al.*, 1981 a). Therefore, timing of N application in the postharvest period may affect N reserve levels in different parts of the tree.

Leaf N analysis showed that postharvest N fertilization did not significantly increased leaf N concentration for ‘Bing’ sweet cherry. Fertilizing on 3 February 2007 did not result in a higher leaf N concentration on 17 February 2007. Similarly, fertilization on 3 March 2007 did not result in a higher leaf N concentration in the ‘Split [x3]’ treatment on 25 March 2007. Previous studies determined that N was preferentially allocated to the roots after bud set occurred,

which could partially explain the non-significant increase in leaf N concentration due to N fertilization (Stassen *et al.*, 1981a; Zavalloni, 2004). To determine total reserve N in a dormant tree necessitates whole tree excavation and analysis. Whole tree excavation and subsequent analysis is expensive and labour intensive. Therefore, a simpler method for estimating tree reserve status was employed. Johnson *et al.* (2006) found good correlations with N, P, Ca, B and Zn in dormant shoots and tree performance as measured in flower density, fruit set, early shoot growth, fruit size and vegetative growth for *Prunus persica*. Therefore, branch N analysis was performed on two-year-old flowering branches during dormancy to estimate N reserve status. No significant differences were found between treatments in shoots and spurs in both 2006 and 2007. Spurs tended to have the highest N concentration. Similarly, Taylor and van den Ende (1969a) found that reproductive organs contained higher N concentrations. Only in 2006, N concentration of the wood was significantly increased when N was applied on the 14 April 2006. Furthermore, whole branch N concentration was significantly higher in 2006 when N was applied on 14 April 2006. This showed that, if branch analyses give an accurate estimate of the N reserve status in cherry, that the differential postharvest N fertilization does not significantly improve N reserve status in cherry. However, it does not explain the higher number of flowering clusters in 2007 in all N treatments or the significant increase in fruit density in 2006.

No significant treatment effects on dormancy release were present in both years, however, the following tendencies were observed. Trees that did not received N (control) in 2007 exit dormancy slower than trees that received N ('Split [x3]'), however, the time and treatment interaction prohibits the isolation of the treatment effects. Depth of bud dormancy was, however, not primarily controlled by N. Depth of bud dormancy and its progression was shown to be primarily controlled by chilling temperatures, duration of chilling and post-chilling temperatures in cherry and peach (Gariglio *et al.*, 2006; Mahmood *et al.*, 2000). However, N shortened time to budburst in young peach and apple trees (Hill-Cottingham, 1967; Stassen *et al.*, 1981b). Furthermore, a tree optimal in N reserves showed improved performance through earlier flowering and improved fruit set (Hill-Cottingham, 1967; Stassen *et al.*, 1981b; Taylor and van den Ende, 1969a; Terblanche and Strydom 1973; Terblanche *et al.* 1979). Gauk *et al.* (2005) reduced time to budburst by one day with the application of urea and Promalin® in the postharvest period in cherry. In this trial, it was expected that cherry buds would be significantly less dormant; however, treatments effects were not present. Results

indicate that dormant branch analysis could not entirely explain observed trends in bud dormancy, flowering and fruit set. This may be attributed to several reasons: N treatments could have been equally effective in improving N reserve levels to an extent that no significant differences could be obtained in parameters measured, or the method does not provide an accurate estimate of the N reserve status of cherry. A third reason may be that significant manipulation of N reserve level in mature tree was not possible by manipulating time of postharvest N fertilization within one year or, fourthly, chilling was the primary factor that controlled budburst, flowering and fruit set in mature trees which masked the effect of N on observed effects.

In the past the effect of N on budburst and flowering was assessed using young pot-grown fruit trees of which the storage pool can easily be manipulated. Several authors warned that extrapolating results for young potted trees to field-grown trees may yield ambiguous results (Millard, 1996; Niederholzer *et al.*, 2001; Oland, 1959; Taylor and van den Ende, 1969a). Niederholzer *et al.* (2001) and Taylor and Van den Ende (1969a) found that autumn N fertilization did not shorten the time to budburst or improve flowering and fruit set in peach. This was ascribed to the difficulty in manipulating the storage pool of a field-grown tree. As trees mature, the influence of external inputs was reduced through internal cycling of nutrients (Millard and Nielsen, 1989; Miller, 1986). Therefore, the lack in response in bud dormancy progression may be attributed in the difficulty in manipulation of the reserve level in mature trees. Chilling effects, however, still primarily control bud dormancy progression. Mahmood *et al.* (2000) showed that frequency of budburst, flowering and fruit set were improved as more chilling accumulated in cherry.

The possible mechanism through which N affects budburst may be mediated by endogenous cytokinin levels. It is important to note that no cytokinin levels were measured in these experiments to verify this hypothesis, but literature provides some supporting evidence. However, to understand this, it is important to view this in the light of N dynamics in the tree. Under natural conditions, deciduous fruit trees absorbed N in the postharvest period through root uptake to build N reserve (Stassen *et al.*, 1981a). Upon absorption, cytokinin synthesis was up regulated in the roots in response to nitrate exposure and regulated a wide variety of genes involved in metabolism, development and macronutrient acquisition (Sakakibara *et al.*, 2006). For deciduous fruit trees, N was predominately allocated to the roots and perennial

parts in the postharvest period, where it is stored during tree dormancy (Millard, 1996). Trees remained dormant until their chilling requirement was met, and cell communication was partially or fully re-established. Growth would resume, provided that the environmental conditions were favourable for budburst to occur. Prior to spring budburst, however, a peak in xylem cytokinin levels is observed (Belding and Young, 1989; Cook *et al.*, 2001a; Cook *et al.*, 2001b; Cutting *et al.*, 1991; Hewett and Wareing, 1973; Lombard, *et al.*, 2006; Tromp and Ova, 1990; Young, 1989). Cook *et al.* (2001b) showed in apple that this increase in cytokinin levels triggers spring budburst, and shoot-derived rather than root-derived cytokinin triggers spring budburst. Furthermore, a positive correlation exists between dormant total N and spring xylem sap cytokinin content of 'Thompson seedless' vines (Nikolaou *et al.*, 2000) and therefore provided evidence that the effect of N on budburst was possibly mediated through cytokinin. In addition, relative budburst number is positively correlated with xylem cytokinin levels (Nikolaou *et al.*, 2000). No other correlations exist with cytokinin and other minerals in the xylem sap. The relationship between budburst patterns with cytokinin levels was also previously reported (Cook *et al.*, 2001b). Acrotonic development of apple is associated with higher cytokinin levels in the distal shoots halves. Therefore, postharvest N fertilization may affect cytokinin levels through improvement of N reserves, and thereby tree dormancy. However, the sensitivity of buds to cytokinins depends on the dormancy stage of the buds (Lang, 1987). This coincides with the latter stages of endodormancy and ecodormancy were buds were shown to be receptive to these stimuli. This required that buds already accumulated a considerable amount of chilling and that cell communication was partially re-established. This period was associated with increase in membrane permeability and an increase in free water in the buds (Faust *et al.*, 1997). Therefore, increase in reserve N will have the potential to reduce the chilling requirement through higher cytokinin levels, but will depend on the amount of chilling accumulated and whether conditions favour growth. N effects will therefore be modified by field temperatures.

4.5 Conclusion

Results indicate, under the conditions at which the trial was conducted, that the chilling requirement cannot be significantly reduced through postharvest N fertilization under mild winter conditions for mature cherry trees. Timing of N fertilization in the postharvest period was ineffective in reducing the chilling requirement of mature cherry trees. Mature trees depend less on external inputs due to internal cycling of nutrients. It is important to note that

N effects on bud dormancy are primarily controlled by winter chilling. N only affects bud dormancy on a secondary level.

4.6 References

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Table 4.1. N concentration (g/100g) of two-year-old wood, spurs and one-year-old shoots sampled on 2-year-old units of 'Bing' sweet cherry grown in the Koue Bokkeveld on 12 July 2006 during tree dormancy. N was applied on 3 March (Early [45kg N/ha]; Split [15kg N/ha]), 26 March (Split [15kg N/ha]) and 14 April (Late [45kg N/ha]; Split [15kg N/ha]).

Treatment	2-year-old wood	Spurs	Shoots	2-year-old unit
Early	0.94 b[z]	1.23[NS]	1.09[NS]	1.00 b
Split	0.96 b	1.32	1.09	1.02 b
Late	1.10 a	1.40	1.20	1.15 a
Control	0.90 b	1.25	1.04	0.97 b
Significance (Pr > F)	*0.0137*	*0.1811*	*0.2301*	*0.0200*

[z]Means separation within columns by LSD at the 5% level.

[NS] Non-significant

Table 4.2 Effect of differential N fertilization in the postharvest period on flowering spurs and budburst of one-year-old shoots for 7-year-old 'Bing' sweet cherry trees grown in the Koue Bokkeveld on 28 September 2006. N was applied on 3 March. (Early [45kg N/ha]; Split [15kg N/ha]), 26 March (Split [15kg N/ha]) and 14 April (Late [45kg N/ha]; Split [15kg N/ha]).

Treatments	Number of spurs at and/or post full bloom on 2-year-old sections	Reproductive clusters with leaf development	Total reproductive spurs	Number of buds open	Total
Early	14[NS]	9[NS]	18[NS]	11[NS]	11[NS]
Split (x3)	16	9	19	11	11
Late	17	10	20	10	11
Control	16	9	20	11	11
Significance (Pr > F)	*0.3339*	*0.7263*	*0.3057*	*0.6931*	*0.8240*

[NS] Non-significant

Table 4.3 Effect of differential N fertilization in the postharvest period on yield of 7-year-old 'Bing' sweet cherry trees grown in the Koue Bokkeveld during 2006. N was applied on 3 March (Early [45kg N/ha]; Split [15kg N/ha]), 26 March (Split [15kg N/ha]) and 14 April (Late [45kg N/ha]; Split [15kg N/ha]).

Treatments	Fruit density[y] (6 Nov)	Fruit per cluster (6 Nov)
Early	1.29 b[z]	0.29[NS]
Split (x3)	1.33 b	0.27
Late	2.99 a	0.21
Control	0.88 b	0.23
Significance (Pr > F)		
Treatment	*0.0451*	*0.8331*
Contrast		
N vs Control	*0.1201*	*0.7401*

[y] Total fruit per branch divided by branch cross sectional area (πr^2)
[z]Means separation within columns by LSD at the 5% level
[NS] Non-significant

Table 4.4. Effect of differential N fertilization in the postharvest period on leaf N concentration (g/100g) of 'Bing' sweet cherry grown in the Koue Bokkeveld in 2007. N was applied on 3 February (Early [51kg N/ha]; Split [x3; 17kg N/ha]; Split [x2; 25.5kg N/ha]), 3 March (Split [x3; 17kg N/ha]) and 13 April (Late [51kg N/ha]; Split [x3; 17kg N/ha]; Split [x2; 25.5 kg N/ha]).

	Date				
Treatment	3 Feb	17 Feb	3 March	25 March	13 April
Early	1.76^{NS}	1.82^{NS}	1.83^{NS}	1.97 a^{z}	2.14 a
Split (x3)	1.75	1.78	1.71	1.90 ab	2.09 ab
Late	1.75	1.71	1.66	1.71 c	1.86 c
Split (x2)	1.76	1.78	1.79	1.88 ab	2.08 ab
Control	1.79	1.77	1.71	1.78 bc	1.95 bc
Significance (P r > F)	*0.9712*	*0.8380*	*0.2327*	*0.0042*	*0.0051*

^{z}Means separation within columns by LSD at the 5% level.

^{NS} Non-significant

Table 4.5. N concentration (g/100g) of two-year-old wood, spurs and one-year-old shoots sampled on 2-year-old units of 'Bing' sweet cherry in the Koue Bokkeveld on 10 July 2007 during tree dormancy. N was applied on 3 February (Early [51kg N/ha]; Split [x3; 17kg N/ha]; Split [x2; 25.5kg N/ha]), 3 March (Split [x3; 17kg N/ha]) and 13 April (Late [51kg N/ha]; Split [x3; 17kg N/ha]; Split [x2; 25.5 kg N/ha]).

Treatment	2-year-old wood	Spurs	Shoots	2-year-old unit
Early	1.26^{NS}	1.61^{NS}	1.44^{NS}	1.35^{NS}
Split (x3)	1.19	1.64	1.41	1.29
Late	1.23	1.60	1.40	1.31
Split (x2)	1.20	1.65	1.41	1.30
Control	1.19	1.51	1.37	1.27
Significance (Pr > F)	*0.4933*	*0.2211*	*0.8591*	*0.5101*

^{NS} Non-significant

Table 4.6 Effect of differential N fertilization in the postharvest period on flowering and budburst of one-year-old shoots for 8-year-old 'Bing' sweet cherry trees in the Koue Bokkeveld on 6 October 2007. N was applied on 3 February (Early [51kg N/ha]; Split [x3; 17kg N/ha]; Split [x2; 25.5kg N/ha]), 3 March (Split [x3; 17kg N/ha]) and 13 April (Late [51kg N/ha]; Split [x3; 17kg N/ha]; Split [x2; 25.5 kg N/ha]).

Treatments	Number of spurs at and/or post full bloom on 2-year-old sections	Reproductive clusters with leaf development	Total reproductive spurs	Number of buds open on shoot	Total buds on shoot
Early	18 a[z]	22[NS]	22[NS]	13[NS]	13[NS]
Split (x3)	21 a	23	23	12	12
Late	19 a	21	21	12	12
Split (x2)	19 a	21	21	12	12
Control	14 b	20	20	12	12
Significance (Pr > F)					
Treatment	*0.0204*	*0.3181*	*0.6490*	*0.2454*	*0.2454*
Contrast					
N vs Control	*0.0016*	*0.2139*	*0.2139*	*0.4386*	*0.4386*

[z]Means separation within columns by LSD at the 5% level.

[NS] Non-significant

Table 4.7 Effect of differential N fertilization in the postharvest period on yield of 8-year-old 'Bing' sweet cherry trees grown in the Koue Bokkeveld during 2007. N was applied on 3 February (Early [51kg N/ha]; Split [x3; 17kg N/ha]; Split [x2; 25.5kg N/ha]), 3 March (Split [x3; 17kg N/ha]) and 13 April (Late [51kg N/ha]; Split [x3; 17kg N/ha]; Split [x2; 25.5 kg N/ha]).

Treatments	Fruit density[y] (29 Oct)	Fruit per cluster (29 Oct)	Fruit density[y] (22 Nov)	Fruit per cluster (22 Nov)
Early	41[NS]	2.6[NS]	23[NS]	1.6[NS]
Split (x3)	51	2.9	31	1.6
Late	49	2.8	30	1.6
Split (x2)	38	2.7	24	1.7
Control	41	2.6	26	1.4
Significance (Pr > F)	*0.8327*	*0.9869*	*0.8722*	*0.9853*

[y] Total fruit per branch divided by branch cross sectional area (πr^2)
[NS] Non-significant

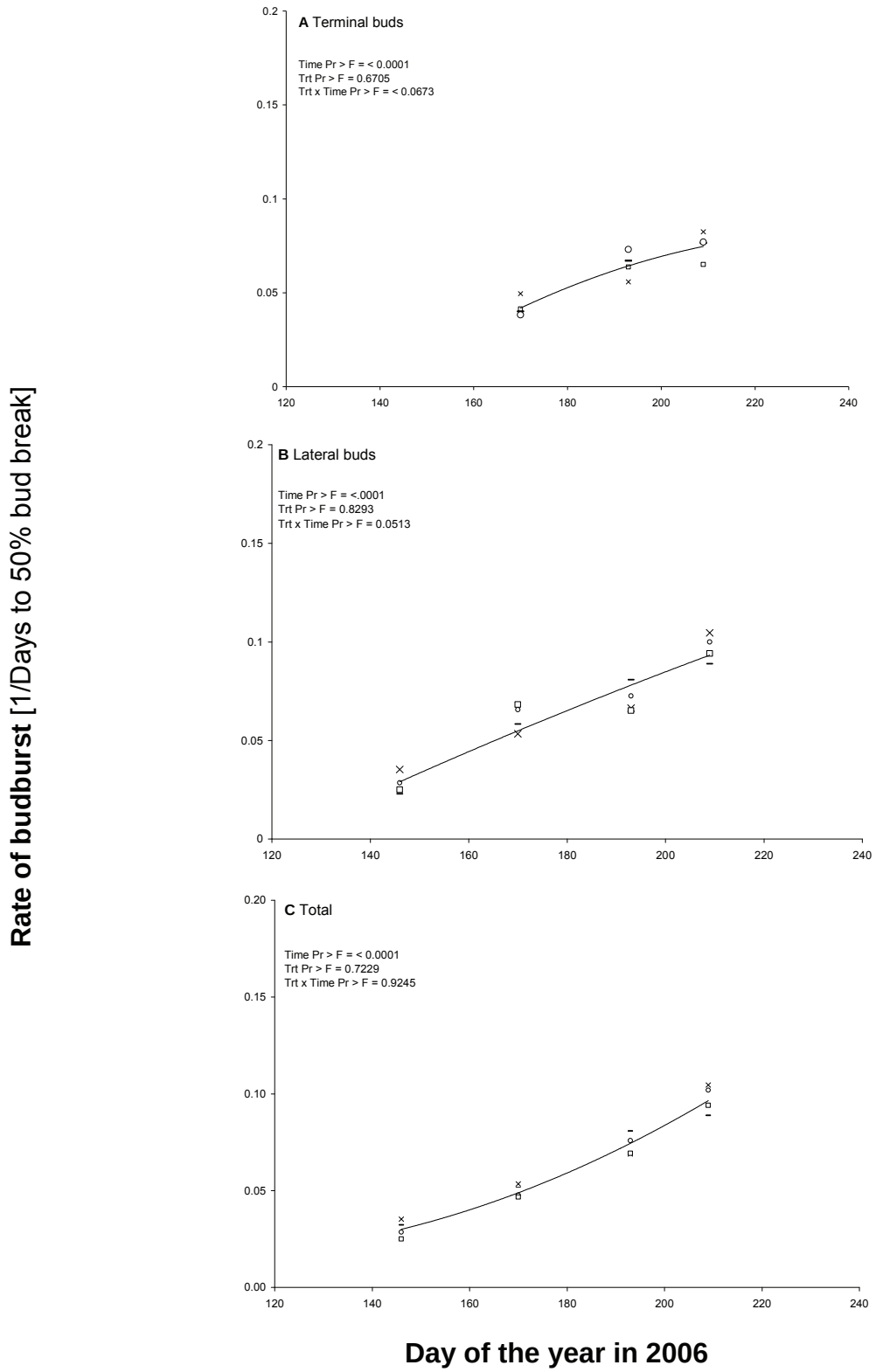

Fig. 4.1. Effect of differential postharvest N application on terminal [**A**] and lateral [**B**] and total [**C**] budburst on one-year-old shoots during bud dormancy of 'Bing' sweet cherry grown in the Koue Bokkeveld(Early -; Split x; Late ○; Control □).

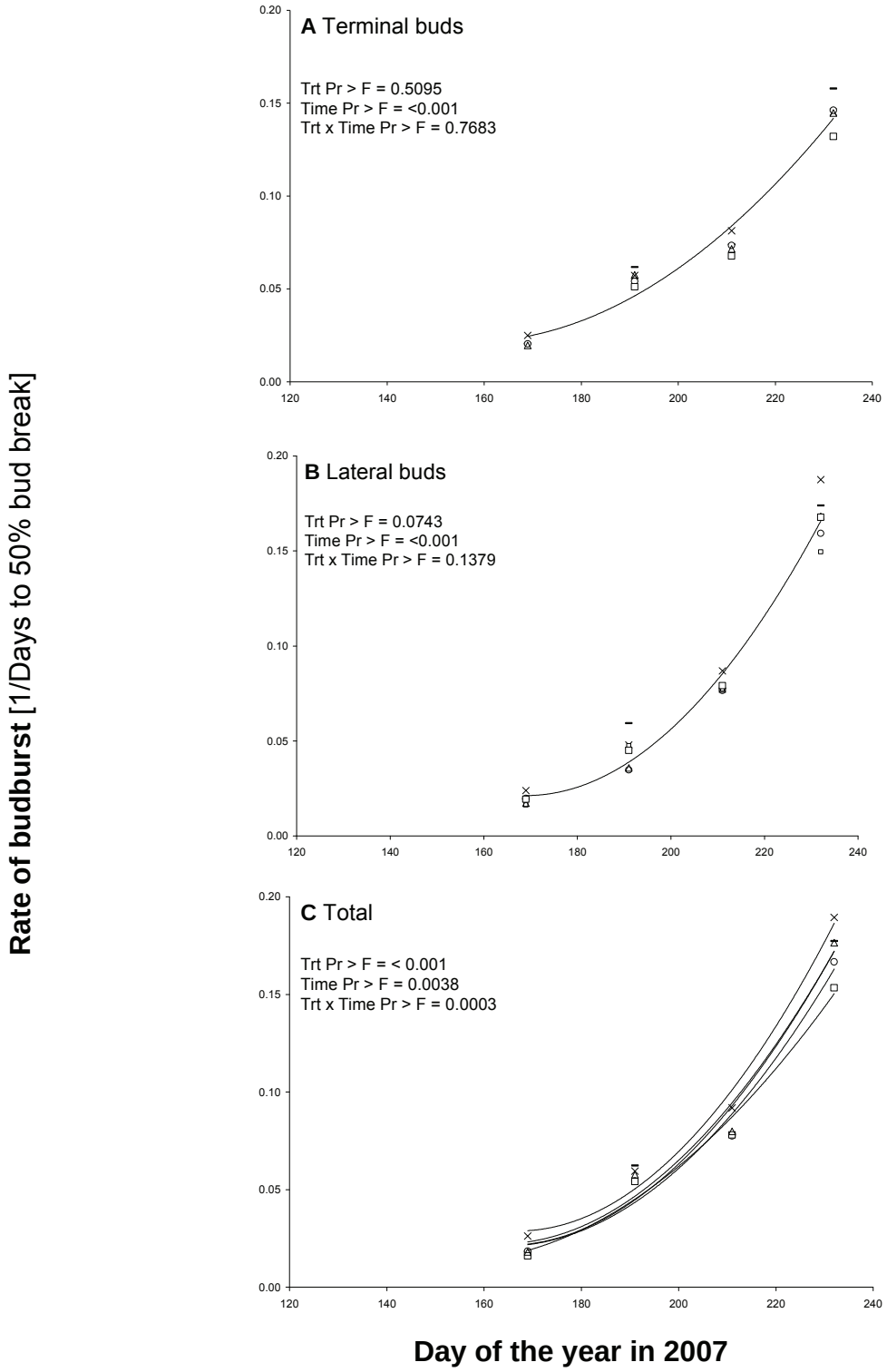

Fig. 4.2. Effect of differential postharvest N application on terminal [**A**] and lateral [**B**] and total [**C**] budburst on one-year-old shoots during bud dormancy of 'Bing' sweet cherry grown in the Koue Bokkeveld(Early -; Split [x3] x; Late ○; Control □; Split [x2] △).

CHAPTER 5

Postharvest Stress Management in 'Bing' Sweet Cherry under Mediterranean Conditions

Abstract

Cherry trees are supposedly exposed to high light and temperature conditions during the summer period in South Africa. Therefore, research was conducted in order to establish if cherry trees experience stress in the postharvest period. Furthermore, management practices were aimed at improving carbohydrate reserves through alleviating this supposed stress. Stress was monitored through measurement of leaf surface temperature, CO_2 assimilation, stomatal conductance, fluorescence, flowering and fruit set. In addition, sweet cherry trees were sprayed in summer with Surround®, Retain® and Raynox®. Bud dormancy progression was quantified to determine if a tree with higher carbohydrate reserves would require less chilling. Treatments had no significant effect on carbon assimilation, stomatal conductance, leaf surface temperature, fluorescence, bud dormancy, budburst, flowering and fruit set. Fluorescence measurements (Fv/Fm: 0.84) indicated that the photosystems in the leaves were not permanently damaged and were able to function under prevailing climatic conditions of South Africa. Leaves experienced high temperatures (41°C) on days with air temperatures > 30°C. Carbon assimilation (9 $\mu molm^{-2}s^{-1}$) was lower prior to leaf senescence. Treatments had no significant effect on bud dormancy of mature sweet cherry trees. Application of Surround®, Retain® and Raynox® in summer did not alleviate the heat stress.

Keywords: Bud dormancy, stress, carbohydrates, carbon assimilation

5.1 Introduction

South Africa is characterized by a mild winter climate (short winters, with warm daily temperatures) with long, hot summers not suited for the cultivation of high chilling fruit varieties (Strydom *et al.*, 1971). For cherry trees, the postharvest period is roughly five months under South African conditions. Therefore, high light and temperature conditions, water stress or non-optimal nutrition during this period can affect physiological processes such as photosynthesis. Trees low in reserves is known to perform poorly in spring and management practices should aim to reduce the level of stress these trees may experience.

Under light stress conditions, plants have developed several methods to dissipate excess light energy. This includes non-photochemical quenching or diversion of energy towards heat production (Taiz and Zeiger, 2002). Heat can either be removed from the leaf surfaces through sensible heat loss or evaporative heat loss, cooling the leaf down. Photoinhibition occurs if excess energy cannot be effectively dissipated through non-photochemical quenching or as heat. In this case, the reaction centre of PSII is inactivated or damaged. One can distinguish between dynamic or chronic photoinhibition. Dynamic photoinhibition occurs under moderate levels of excess light, where energy is diverted to heat production causing a decrease in quantum efficiency. However, if the photon flux decreases below the saturation point, quantum efficiency returns to normal levels. This generally occurs during midday. With chronic photoinhibition, excess light energy causes irreversible damage to the photosystem which has long-lasting effects (Taiz and Zeiger, 2002). Current methods to reduce sunburn on fruit involves the application of particle films (Surround® and Raynox®) which either reflect light or reduce absorption of UV rays (Wand and Gindaba, 2005). Other strategies include evaporative cooling to cool fruit or the use of shade netting to reduce the total incoming radiation and increase the proportion of scattered light (Wand and Gindaba, 2005). Retain® was mainly used to extend the harvest period of fruit by applying it 5-10 days before harvest (Singh *et al.* 2003). Retain® was not previously used for reducing stress in fruit trees.

Cherry trees accumulate carbohydrates predominantly in the postharvest period after shoot growth has ceased (Webster and Looney, 1996). With leaf senescence, cherry trees contain the highest concentration of non-structural carbohydrates (Keller and Loescher, 1989). Therefore, any stress experienced by the tree in this period may negatively affect carbon assimilation. These non-structural carbohydrates are stored predominantly as starch in the perennial parts and are utilized for spring growth until newly formed leaves become a source for assimilates. This generally occurs after flowering (Keller and Loescher, 1989). Non-structural carbohydrates utilized include starch and soluble sugars (sucrose, glucose, fructose and sorbitol). With or before budburst, total non-structural carbohydrates accumulate in spurs while decreasing in other perennial parts. After flowering, carbohydrate reserves were generally at their lowest level in the tree (Keller and Loescher, 1989). Reserve carbohydrates levels do not, however, determine spring bud growth potential. Cheng *et al.* (2004) and Cheng and Fuchigami (2002) showed that total reserve N and not reserve carbohydrate, improve bud growth potential in spring.

Furthermore, temperature conditions during the annual cycle of the tree are considered the most important factor determining bud growth potential in deciduous fruit trees. Pre-chilling conditions, chilling temperatures, duration of chilling and post-chilling temperatures were shown to affect time of budburst, flowering and fruit set in deciduous fruit trees (Jonkers, 1979; Mahmood *et al.*, 2000).

The objective of this experiment was to determine if heat or light stress during the postharvest period affects cherry tree dormancy. The hypothesis was that the chilling requirement of cherry could be reduce by increasing the carbohydrate reserve status through alleviating the postharvest stress by the use of Surround®, Raynox® and Retain® and thereby cause an increase in carbon assimilation and storage.

5.2 Materials and Methods

5.2.1 Plant material and statistical layout

The experimental design was aimed at improving carbon assimilation through alleviating the supposed light or heat stress that leaves may experience under high light intensities or warm days. Surround® is a kaolinitic clay which reflects radiation primarily in the UV and IR range and therefore reduces the light intensity in these ranges (Wand and Gindaba, 2005). Raynox® is a wax-based formula that reduces absorption of UV at the surface of the tissue protecting epidermal and underlying cell layers (Wand and Gindaba, 2005). Retain® is a compound that inhibits ethylene synthesis and was used to block the synthesis of ethylene commonly produced under stress conditions (Mittler, 2006).

The trial was carried out in the Koue Bokkeveld (33°S, 945m), Ceres on mature, seven-year-old 'Bing' (*Prunus avium L.*) sweet cherry trees on Mazzard rootstock. This trial was only conducted in 2007. The trees were spaced at 4.5 m x 1.5 m. Raynox® (Carnauba wax, Pace Int. LLC., USA), Retain® (15g/100g [S]-trans-2-amino-4-(2-aminoethoxy)-3-butenoic acid hydrochloride) and Surround® (95g/100g kaolin) were applied in summer on cherry trees. A control treatment was left unsprayed. A randomized complete block design was used with four treatments, ten replications and five trees per plot. The three centre trees were used for data capturing. Each treatment was applied with a motorised spray gun at a fixed rate (ca. 0.65 L/tree) until run off. Surround® was applied at 6 kg/100L water (16 Jan.) with two re-applications of 3kg/100L of water (7 Feb.; 27Feb.). Raynox® was applied at 2.5L/100L (16 Feb.) and also with follow-up applications at 2.5L/100L of water (7 Feb.; 27 Feb.). Break-Thru® (100%, Polyether-polymethylsiloxane-copolymer) was used

as a wetting agent at 50ml/100L of water for Surround® and Retain®. Retain® (1000L/ha) was applied three times (16 Jan.; 17 Feb.; 3 Mar.) which coincided with the warmest months of summer.

5.2.2 Stress monitoring

Stress was monitored using several techniques. Three leaves per replication on distal halves of one-year-old shoots on exposed branches were chosen for all measurements. Carbon assimilation (A) and stomatal conductance were measured (20 March 2006) between 9am and 12pm on a clear sky day with the LI-6400 Photosynthesis System (LiCor, Lincoln, NE, USA) two weeks prior to the beginning of leaf senescence. Leaf surface temperature was measured on two contrasting dates of 20°C and 32°C, 3 March 2006 and 17 February 2006, respectively. Leaf surface temperature was measured with a hand held infra-red thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, CA). Fluorescence was measured (3 March) on mature shoot leaves *in situ* with a pulse-modulated flourimeter (FMS2, Hansetech Instruments, Kings Lynn, UK) at ambient air temperature. Leaves were dark adapted for 30 min. using the FMS2 leaf clips. The Fm/Fv ratio (maximum quantum efficiency of PSII) was calculated from the parameters measured, Fo (fluorescence level when sample is dark adapted and Q_A fully oxidised) and Fm (maximum fluorescence yield).

5.2.3 Forcing

During 2006, six shoots per replication were collected at five dates (8 May, 26 May, 19 June, 12 July, and 28 July) prior to spring budburst. Shoots were bundled and placed in 5L plastic buckets with 1L of water and forced to determine the depth of dormancy. The shoots were forced in a growth chamber with constant illumination (200 $\mu molm^{-2}s^{-1}$) and at a constant temperature of 25°C (Jacobs *et al.*, 2002). The water was changed every 2-3 days with submerged shoot sections being dipped in 0.25% (v/v) sodium hypochlorite solution (3.5 %) for ca. 10 minutes between each water change. Approximately 1 cm of the bottom of the shoot was cut off weekly. Lateral and terminal budburst was recorded every 2-3 days until three of the six shoots (50% budburst) had reached green tip. “Total” budburst was recorded if a lateral or a terminal bud on ten of the twenty shoots (50% budburst) had reached green tip. After 100 days, the bundles were discarded.

5.2.4 Flowering and budburst

Flowering was measured on 2-year and older branches. Sections of ca. 70 cm in length were used. Bud stage (1-9) was recorded for each spur on that section at full bloom (http://www.ncw.wsu.edu/treefruit/bdch.htm). Budburst was monitored on two one-year-old shoots per replication. Flowering was monitored on two branches per replication.

5.2.5 Fruit set

Cherry fruit per cluster were counted on the same 70 cm branch sections used for flowering. It was expressed as a total fruit per branch divided by branch cross sectional area (πr^2) to yield "fruit density" (Byers, 1997). Fruit set was measured after pit hardening on 6 November 2006.

5.2.6 Statistical analysis

Data were analyzed using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) program (SAS Institute Inc., 2003, Cary, NC, USA).

5.3 Results and Discussion

Carbon assimilation, internal CO_2 concentration and stomatal conductance were not significantly affected by the treatments (Table 5.1). On average, carbon assimilation was 9.3 $\mu molm^{-2}s^{-1}$. This was lower than the optimal 17.9 $\mu molm^{-2}s^{-1}$ estimate for sweet cherry (Flore and Layne, 1999). No significant differences were found for fluorescence measurements with values ranging from 0.8412 – 0.8466 (Table 5.1) that is in agreement with values for healthy leaves (Björkmann and Demmig, 1987; Buwalda and Noga, 1994). Therefore, leaves appear healthy and were able to adapt to prevailing light (ca. 1700 $\mu molm^{-2}s^{-}1$) conditions. Surface leaf temperatures were however high on days with air temperatures > 30°C (Table 5.2). Mean leaf temperature was ca. 9°C higher than ambient air (32°C) conditions. Treatments, however, proved ineffective to reduce mean leaf temperature, even on days with an ambient temperature of 20°C (Table 5.2). These higher temperatures (> 30°C) on 17 February 2006 would reduce carbon assimilation and therefore carbohydrate accumulation. It was shown in *Salvia splendens* that export of sugars from the leaves were reduced under ambient air CO_2 and O_2 when air temperatures rise above 25°C (Jiao and Grodzinski, 1996). Lateral and terminal bud dormancy was not significantly affected (Fig. 5.1). Full bloom was on 26 September 2006. Flowering and budburst on one-year-old shoots did not differ significantly (Table 5.3). Fruit density and number of cherry fruit per cluster were low, but similar to the control (Table

5.4). No treatment effects were obtained in this trial. This was probably due to the inefficiency of these treatments to improve or reduce carbon assimilation and therefore resulted in no significant differences between treatments.

In conclusion, cherry leaves appear healthy, but does experience heat stress, because leaf temperatures were 9°C than ambient air temperature. Furthermore, Raynox®, Retain® and Surround® were ineffective in affecting carbon assimilation and stomatal conductance or in reducing heat stress in the postharvest period for 'Bing' sweet cherry. It is questionable whether carbohydrate levels were affected by treatments, which would explain the non-significant differences obtain in bud dormancy, flowering and yield. Therefore the question remains whether or not carbohydrate reserves determine bud growth potential in spring.

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Table 5.1. The effect of Raynox®, Surround® and Retain® on carbon assimilation, stomatal conductance, C_i, and fluorescence of 'Bing' sweet cherry grown in the Koue Bokkeveld in 2006.

Treatment	Carbon assimilation, A ($\mu molm^{-2}s^{-1}$)	Stomatal conductance ($\mu molm^{-2}s^{-1}$)	Internal CO_2 concentration (C_i) ($\mu mol\ CO_2\ mol\ air^{-1}$)	Fluorescence (Fv/Fm)
Surround®	9.25	0.127	213.61	0.8466
Retain®	9.01	0.117	210.90	0.8460
Raynox®	9.08	0.113	198.98	0.8463
Control	9.00	0.120	212.56	0.8412
Pr > F	*0.9790*	*0.8006*	*0.2691*	*0.7764*

Table 5.2. Effect of Surround®, Retain® and Raynox® on leaf temperature during mid day on shoot leaves on two contrasting dates of 'Bing' sweet cherry grown in the Koue Bokkeveld in 2006.

Treatment	**Mean air temperatures**	
	32°C	20°C
	Mean leaf temperatures	
Surround®	40.88	26.79
Retain®	41.76	27.58
Raynox®	41.20	27.84
Control	41.38	27.48
Pr > F	*0.4713*	*0.1377*

Table 5.3. The effect of Raynox®, Surround®, and Retain® on flowering and budburst of 'Bing' sweet cherry grown in the Koue Bokkeveld in 2006.

Treatment	Number of spurs at and/or post full bloom on 2-year-old sections	Total reproductive spurs	Budburst (Buds open)	Total number of buds (30cm)
Raynox®	13	20	11	11
Surround®	13	20	11	11
Retain®	12	19	11	11
Control	12	18	11	11
Pr > F	*0.6006*	*0.4892*	*0.8242*	*0.8242*

Table 5.4. The effect of Raynox®, Surround®, and Retain® on fruit density and cherry fruit per cluster of 'Bing' sweet cherry grown in the Koue Bokkeveld in 2006.

Treatment	Fruit density	Cherry fruit per cluster
Surround®	0.75	0.15
Retain®	1.77	0.21
Raynox®	1.40	0.19
Control	1.76	0.26
Pr > F	*0.5466*	*0.4456*

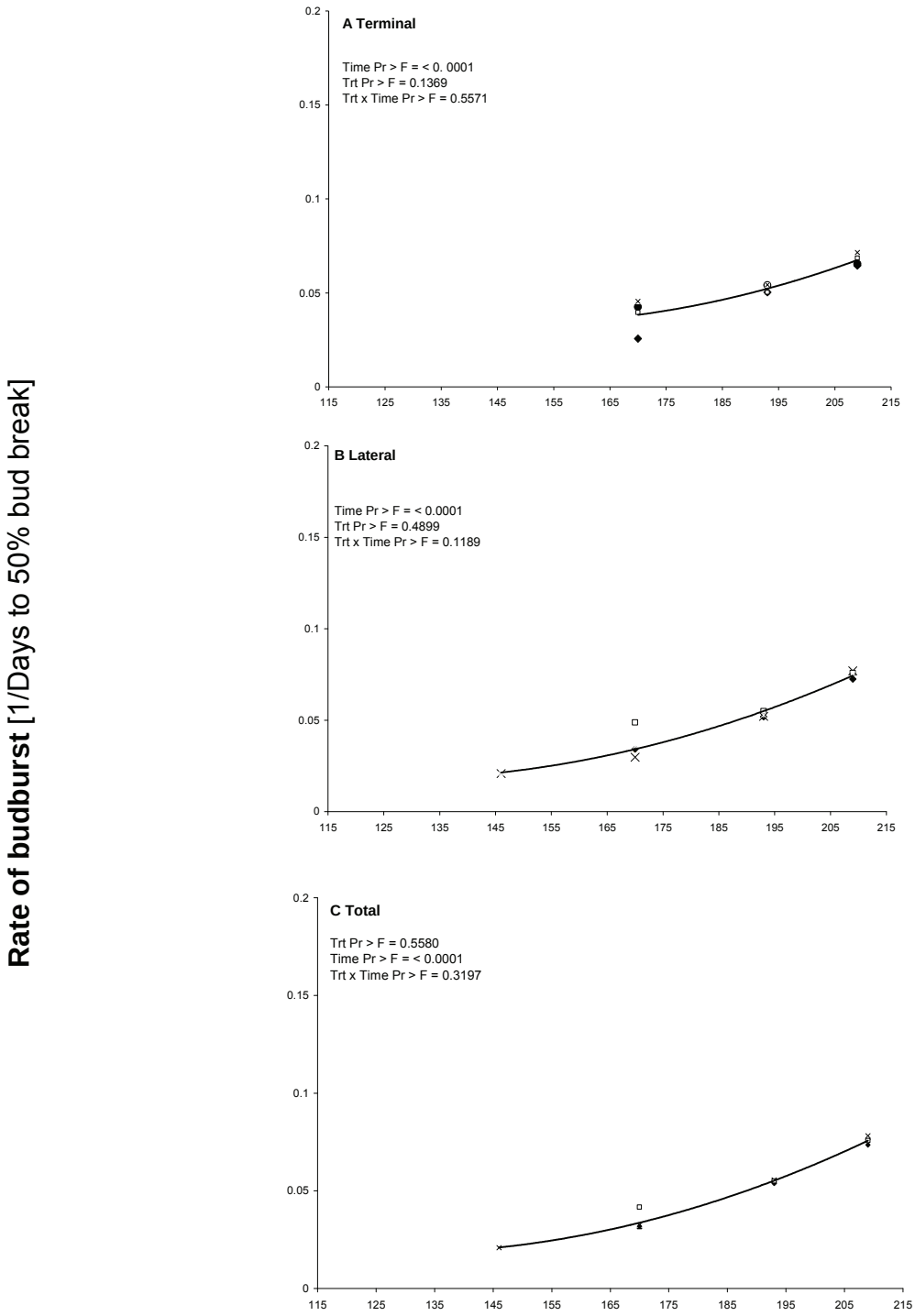

Fig. 5.1. Effect of Raynox®, Surround® and Retain® on terminal [**A**], lateral [**B**] and total [**C**] budburst during bud dormancy of 'Bing' sweet cherry grown in the Koue Bokkeveld. (Surround ●; Raynox ♦; Retain x; Control □).

CHAPTER 6

The effect of hydrogen cyanamide and growth regulators (Promalin® and Retain®) on flowering and fruit set of 'Bing' sweet cherry under mild winter conditions

Abstract

Cherry growing under South African climatic conditions is subjected to various temperature related problems. In South Africa delayed foliation is commonly observed in high chilling fruit varieties such as cherries. Retain® and Promalin® were applied with or without hydrogen cyanamide (Dormex®) prior to full bloom to determine if symptoms of insufficient winter chilling can be alleviated and flowering and fruit set improved for cherry. Hydrogen cyanamide significantly improved budburst, time to full bloom and fruit density. Promalin® and Retain®, however, had no significant effect on budburst, flowering or fruit density.

Keywords: dormancy, rest-breaking agents

6.1 Introduction

South Africa is characterized by a mild winter climate (short, with warm daily temperatures) with long, hot and dry summers generally not suited to the cultivation of high chilling fruit varieties (Strydom *et al.*, 1971). Under mild winter conditions, the chilling requirement is not satisfied leading to delayed foliation (Jacobs *et al.*, 1981; Strydom *et al.* 1971), an associated basitonic growth tendency (Cook *et al.*, 1998), deformed flowers or abortion of flowers (Crabbé, 1994; Oukabli and Mahhou, 2007) and subsequent poor fruit set (Mahmood *et al.*, 2000). Rest-breaking agents are therefore applied to alleviate the symptoms of delayed foliation resulting in a more even budburst pattern and earlier flowering.

Hydrogen cyanamide (Dormex®) proved to be effective in reducing time to flowering in deciduous fruit trees under the mild winter conditions of South Africa (Costa *et al.*, 2004). For different cherry cultivars, hydrogen cyanamide application resulted in 6 to 13 day advancement in flowering (Küden *et al.*, 1997; Martinez *et al.*, 1999; Palasciano *et al.*, 2005; Papa, 2001; Salvador and Tommaso, 2003). Fruit set was also improved with hydrogen cyanamide and fruit harvest was earlier in apricot and plum (Küden and Son, 2005; Papa, 2001) and peach (George and Nissen, 1993). However, not all cherry cultivars adapt to mild winter climates. 'Hedelfingen', 'Emperor Francis' and 'Sam'

exhibited poor adaptability even with the application of hydrogen cyanamide to break dormancy in Northwest Mexico (Martinez *et al.*, 1999).

Flower and fruit abortion can be ascribed to many factors, where high temperatures during flower bud development, insufficient winter chilling, too high or low temperatures during flowering etc. can promote flower and fruit abortion (Stephenson, 1981; Mahmood *et al.*, 2000). In addition, Blanpied (1972) showed that flower and fruit abortion in cherry was associated with higher endogenous ethylene levels in these tissues compared to adjacent non-aborting organs. The growth regulators, Retain® ([S]-trans-2-amino-4-(2-aminoethoxy)-3-butenoic acid hydrochloride) and Promalin® (N-(phenylmethyl)-1H-purine 6-amine and 3000mg/l Gibberellins A_4+A_7), have several uses. Retain® is used for delaying fruit maturity by inhibiting ethylene synthesis (Singh *et al.*, 2003). Promalin®, a source of cytokinin, was shown to improve branching in nursery trees (Elving and Visser, 2006) and improved flowering, budburst and fruit set in apricot and plum (Küden and Son, 2005).

The objective of the study was to determine if the symptoms of inadequate winter chilling can be alleviated for cherry and flowering and fruit set can be increased by the application of Retain® and Promalin® prior to full bloom applied in combination with hydrogen cyanamide. The hypothesis was that Dormex® would advance budburst and flowering and increase potential yield if applied at bud swell. Furthermore, Retain® could inhibit ethylene synthesis and prevent excessive flower and fruit abortion and that Promalin® could assist in bud development through exogenous applied cytokinins and gibberellins.

6.2 Materials and Methods

6.2.1 Plant material and statistical layout

A split plot design was followed with 10 replications. The main plot consisted of a Dormex® treatment and a control. This plot was subdivided into subplots: Retain®, Promalin® and a control. Subplots consisted out of single tree plots with one guard tree on each side. Dormex® (1%) and oil (3%) was applied with a motorised pump at bud swell (11 September 2006) at a rate of 1000L/ha and a control treatment was left unsprayed. Promalin® (3000mg/l N-(phenylmethyl)-1H-purine 6-amine and 3000mg/l Gibberellins A_4+A_7) was applied at 60ml/100L water at a rate of 1000L/ha and Retain® (15g/100g [S]-trans-2-amino-4-(2-aminoethoxy)-3-butenoic acid hydrochloride) was applied at 83g/100L water at a rate of 1000L/ha. Promalin® and Retain® were applied on 19 September 2006 at

the open cluster stage. Break-Thru® (100%, Polyether-polymethylsiloxane-copolymer) was used as a wetting agent at 50ml/100L of water for Retain®.

6.2.2 Flowering and budburst

Flowering was monitored on two-year and older branches. Sections of ca. 70 cm in length were used. Bud stage (1-9) was recorded for each spur on that section at full bloom (http://www.ncw.wsu.edu/treefruit/bdch.htm). Budburst was monitored on two one-year-old shoots. Flowering was monitored on two branches per replication. Full bloom was on 25 September 2006.

6.2.3 Fruit set

Cherry fruit per cluster were counted on the same 70 cm branch sections used for flowering. It was expressed as a total fruit per branch divided by branch cross sectional area (πr^2) to yield "fruit density" (Byers, 1997).

6.2.4 Statistical analysis

Data were analyzed using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) program (SAS Institute Inc., 2003, Cary, NC, USA).

6.3 Results and Discussion

Hydrogen cyanamide significantly advanced budburst, flowering and fruit set in 'Bing' sweet cherry (Table 6.1). With the control only seven buds out of the 17 was open compared to the 10 for the hydrogen cyanamide on one-year-old cherry shoots. Flowering was more advanced in the hydrogen cyanamide treatment (67%) compared to the control (41%). Fruit set was significantly improved but remained very low in 2006. Poor fruit set was a particular problem in 2006 in the Western Cape. These results are in agreement with other researchers who found that flowering, budburst and fruit set was improved with the application of hydrogen cyanamide (George and Nissen, 1993; Küden *et al.*, 1997; Son and Küden, 2005; Martínez *et al.*, 1999, Palasciano *et al.*, 2005; Papa, 2001; Salvador and Tommaso, 2003). No significant differences were found with the Promalin® and Retain® treatments. These results disagree with Küden and Son (1997) who found that Promalin® improved budburst, flowering and fruit set in apricot and plum.

In conclusion, hydrogen cyanamide did advance budburst, flowering and increased fruit set. Promalin® and Retain® did not improve budburst, flowering and fruit set.

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Table 6.1 Effect of Promalin® and Retain® as well as Dormex® on budburst (on one-year-old shoots), flowering and fruit density of 'Bing' sweet cherry grown in Piketberg in 2006.

Treatments		Budburst (buds open on shoot)	Total buds on shoot	Number of spurs at and/or post full bloom on 2-year-old sections	Total spurs	Fruit density
Main effect[a]						
Main treatments						
Control		7 a[z]	17	9 a	21	0.1 a
Dormex		10 b	17	14 b	22	0.6 b
Sub treatments						
Control		9.5	17.5	11.5	21.5	0.55
Retain		8	17	11.5	22.5	0.20
Promalin		8.5	17.5	10	21.5	0.21
Split effect[b]						
Main	Sub					
Control	Control	7 a	17	9 a	23	0.1 a
	Retain	6 a	16	9 a	22	0.1 a
	Promalin	7 a	18	7 a	22	0.02 a
Dormex	Control	12 b	18	14 b	20	1.0 b
	Retain	10 b	18	14 b	23	0.3 b
	Promalin	10 b	17	13 b	21	0.4 b
		Significance (Pr>F)				
Main treatment		*0.0003*	*0.8576*	*< 0.0001*	*0.1308*	*0.0011*
Sub treatment		*0.2450*	*0.3733*	*0.3273*	*0.5026*	*0.1236*
Interaction		*0.5970*	*0.0138*	*0.8239*	*0.0080*	*0.1898*

[z]Means separation within column by LSD at the 5% level.

[a]Average of pooled values from individual treatments

[b]Individual treatment effects

CHAPTER 7

The Effect of Exogenous Applied Cytokinin in Autumn on Bud Dormancy Progression of 'Sweet Heart' Sweet Cherry under Mild Winter Conditions

Abstract

Mature 'Sweet Heart' cherry trees were sprayed with benzyladenine (Maxcel®) in late autumn to determine if exogenously applied cytokinin can reduce the chilling requirement of cherry under mild winter conditions. In addition, flowering and spring budburst were measured. Bud dormancy progression, spring budburst and flowering were not significantly different in any treatment. It is therefore doubtful whether exogenous applied cytokinins in late autumn can reduce the chilling requirement of 'Sweet Heart' cherry.

Keywords: dormancy, cytokinin, cherry

7.1 Introduction

Budburst patterns, flowering and fruit set are generally negatively affected under conditions of inadequate winter chilling, as experienced in South Africa. Symptoms of delayed foliation (Jacobs *et al.*, 1981; Strydom *et al.* 1971), an associated basitonic growth tendency (Cook *et al.*, 1998), deformed flowers or abortion of flowers (Crabbé, 1994; Oukabli and Mahhou, 2007) and subsequent poor fruit set (Mahmood *et al.*, 2000) are associated with inadequate winter chilling. Rest-breaking agents are therefore applied to alleviate the symptoms of delayed foliation, resulting in a more even budburst and flowering pattern (Costa *et al.*, 2004).

It is generally accepted that a peak in xylem cytokinin precedes spring budburst (Belding and Young, 1989; Cook and Bellstedt, 2001; Cook *et al.*, 2001a; Cutting *et al.*, 1991; Hewett and Wareing, 1973; Lombard, *et al.*, 2006; Tromp and Ova, 1990; Young, 1989). Cook *et al.* (2001b) showed that this increase in cytokinin levels triggers spring budburst and shoot-derived rather than root-derived cytokinin trigger spring budburst. In vines and apple, budburst was advanced with the application of rest-breaking agents (Cutting *et al.*, 1991; Lombard *et al.*, 2006). This was associated with an earlier peak in xylary cytokinins prior to budburst. Furthermore, Nikolaou *et al.* (2000) showed that relative budburst number was positively correlated with xylem cytokinin levels in vines. This close relationship between xylary cytokinins and budburst pattern was previously reported (Cook

and Bellstedt, 2001; Cook *et al.*, 2001; Lombard *et al.*, 2006). It is important to note that these cytokinins originate from reserves within the plant. Tromp and Ova (1990), who studied whole season xylary cytokinins, found that cytokinins only reached high levels prior to spring budburst until leafing. Spring xylary cytokinin levels were shown to be positively correlated with reserve N in vines (Nikolaou *et al.*, 2000). Therefore it is hypothesized that higher reserve cytokinin levels would improve bud growth potential and thereby reduce the chilling requirement of cherry.

The objective of this study was to determine if cytokinins applied exogenously (sprayed) on mature trees in late fall can influence bud dormancy progression of high chill sweet cherry. The hypothesis was that exogenous application of cytokinin in autumn could possibly reduce the chilling requirement of cherry through increasing reserve cytokinin levels.

7.2 Materials and Methods

7.2.1Plant material and experimental design

Maxcel® (6-Benzyladenine, 1.9% w/w, Philagro South Africa, Pty. Ltd.) was applied at a single concentration of 1500 ml Maxcel®/100 L water on five-year-old 'Sweat Heart' cherries on Mahaleb rootstock in Piketberg. A control treatment was left unsprayed. A randomized complete block design was used with two treatments, two replications and ten trees per block. No commercial rest-breaking agent was applied in the spring and no winter pruning was done. Maxcel® was applied repeatedly to the same trees at three dates, i.e. 20 April, 26 April, and 7 May, 2006.

7.2.2 Forcing

Twenty one-year-old shoots, 30 cm in length, per replication were collected on five dates (15 May, 5 June, 26 June, 17 July, and 5 Aug.) during dormancy. Shoots were bundled (10 per bundle) and placed in 5 L plastic buckets with water. The shoots were forced in a growth chamber with constant illumination (200µmol$m^{-2}s^{-1}$) and at a constant temperature of 25°C (Jacobs *et al.*, 2002). The water was changed every 2-3 days with submerged shoot sections being dipped in 0.25% (v/v) sodium hypochlorite solution (3.5%) for ca. 10 minutes between each water change. Approximately 1 cm of the bottom of the shoot was cut off weekly. Lateral and terminal budburst was recorded every 2-3 days until ten of the twenty shoots (50% budburst) had reached green tip. "Total" budburst was recorded if a lateral or a terminal bud on ten of the twenty shoots (50% budburst) had reached green tip.

After 100 days, the bundles were discarded. Data were graphically represented as the inverse of days to 50% budburst, because bud dormancy exit was evaluated.

7.2.3 Flowering and budburst

Flowering was monitored on two-year and older branches. Sections of ca. 70 cm in length were used. Bud stage (1-9) was recorded for each spur on that section at full bloom (http://www.ncw.wsu.edu/treefruit/bdch.htm). Budburst was monitored on two one-year-old shoots per replication. Full bloom was on 25 September 2006. Four branches per plot were used to asses flowering.

7.2.4 Statistical analysis

Data were analyzed using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) program (SAS Institute Inc., 2003, Cary, NC, USA).

7.3 Results and Discussion

Terminal (A), lateral (B) and total (C) bud dormancy progression was not significantly affected (Fig. 7.1). Flowering and budburst was not significantly affected (Table 7.1). No endogenous cytokinins were measured, but it is doubtful that reserve cytokinin levels were increased judging from no response in parameters measured. This may be ascribed to the difficulty in movement of cytokinin from the site of application (Taiz and Zeiger, 2002). Therefore, xylary cytokinin levels may have not been sufficiently increased to affect bud dormancy progression. It is concluded that exogenously applied cytokinins are ineffective in reducing the chilling requirement of high chill sweet cherry trees under mild winter conditions.

7.4 References

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Table 7.1. Effect of Maxcel® applied at 1500ml/1000L on 20 April, 26 April, and 7 May on budburst and flowering of 'Sweet Heart' cherry grown in Piketberg during 2006.

Treatment	Number of spurs at and/or post full bloom on 2-year-old sections	Total spurs	Buds open on 1-year-old shoot	Total buds on 1-year-old shoot
Maxcel®	3	19	16	19
Control	4	18	18	20
Significance (Pr < F)	*0.1189*	*0.1755*	*0.3015*	*0.7368*

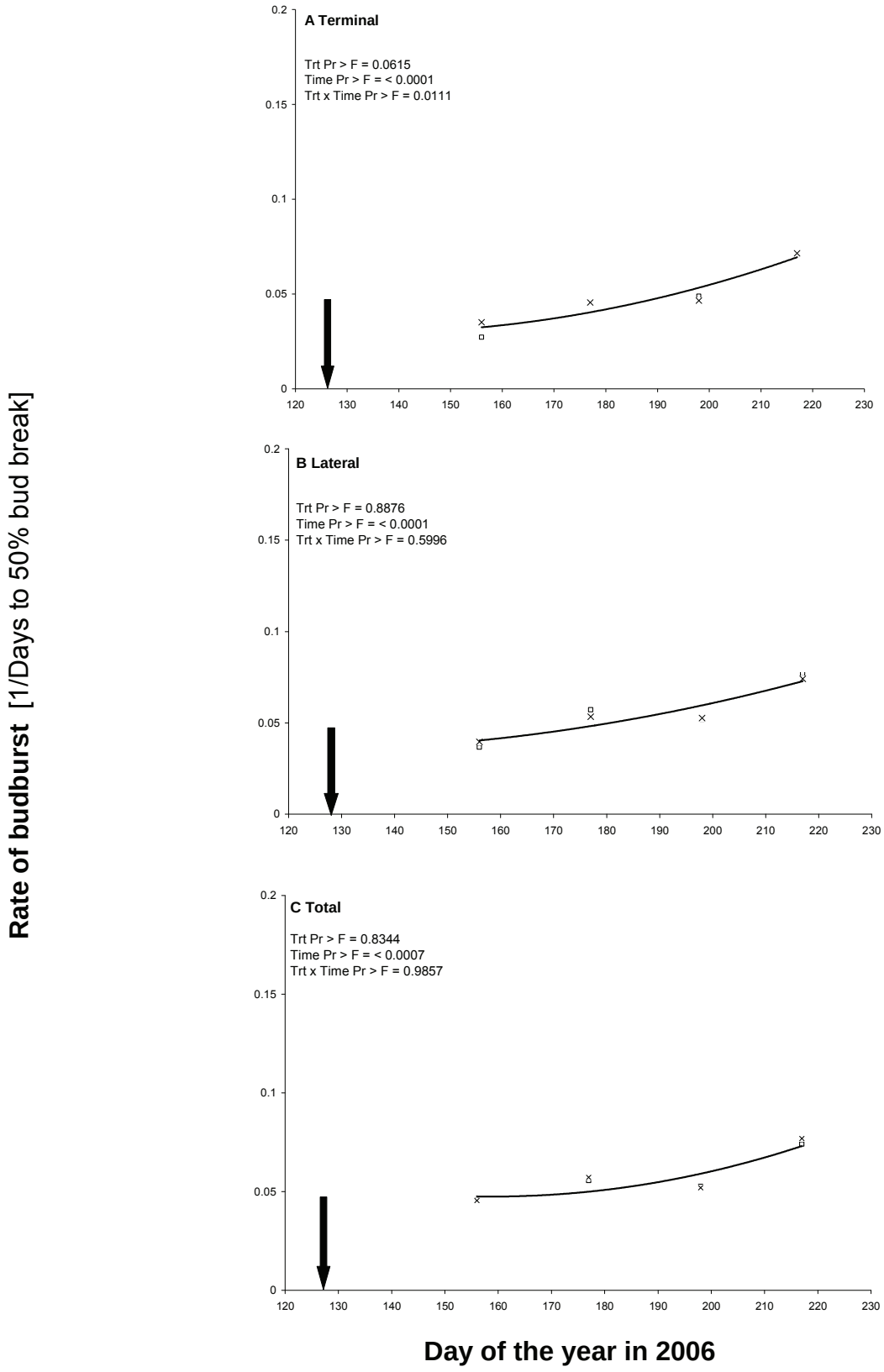

Fig. 7.1 Effect of Maxcel® on terminal [**A**], lateral [**B**] and total [**C**] budburst during bud dormancy of one-year-old 'Sweat Heart' cherry shoots (Control □; Maxcel x; arrow indicates last Maxcel® application date).

CHAPTER 8

8. General conclusion

Experimentation was aimed at characterizing bud dormancy behaviour of sweet cherry and cultural practices which can improve the cherry tree's adaptability under the mild winter climate of South Africa. Data obtained from forcing shoots was used to characterize bud dormancy behaviour and cultural practices were aimed at increasing tree reserves to reduce the chilling requirement of cherry and thereby improve its adaptability. Rest-breaking agents were applied in spring with plant growth regulators, to reduce the chilling requirement of cherry and improve budburst, flowering and yield. Here a general conclusion is drawn and its implication regarding cherry production under South African conditions is discussed.

The cherry bud dormancy pattern was characterized by sampling shoots at three week intervals throughout the season. To increase accuracy, it is suggested that shoots should be sampled at weekly intervals. Cherry buds entered and exit dormancy under South African conditions. Buds enter dormancy in mid- summer and reach their maximum level of dormancy in late summer. What made these findings significant was that buds entered dormancy and reached maximum dormancy before any chilling accumulation. This contradicts the common belief that buds of fruit trees enter dormancy once chilling accumulates as predicted by the Utah model. In addition, buds exit dormancy as chilling accumulates. This showed that the chilling period was less than 40% of the total time that the buds were dormant. Chill unit models have clear limitations under low-chill conditions and new models should be developed for South African conditions. Furthermore, bud dormancy behaviour was shown to be most probably linked to factors related to the entrance to dormancy (summer) and by climatic conditions within a year. It needs to be determined to what extent these environmental conditions, prior to chilling accumulation, influence bud dormancy behaviour and if environmental conditions before chilling accumulation influences the chilling requirement. This, together with the fact that buds enter dormancy in summer, needs to be taken into account once new chill unit models are developed under South African conditions.

Apart from the application of rest-breaking agents and growth regulators in spring, other experiments aimed at increasing the reserve status (nitrogen, cytokinin, or carbohydrates), because a tree optimal in reserves is known to perform better under low chill conditions. N

fertilization in the postharvest period aimed at improving N reserves in the tree. Fertilizing with N in the postharvest period did not reduce the chilling requirement of mature sweet cherry trees. The non-significant results can be ascribed to the limitations of the experimental design. First, the mature tree depends less on external inputs compared with one or two-year-old trees. Therefore, reserve N could not be sufficiently manipulated in order to obtain a significant reduction in the chilling requirement in mature trees. Secondly, N reserves affect bud dormancy on a secondary level, and the response to improved reserves may be subjected to environmental conditions during exit from dormancy. This showed that postharvest N offers marginal aid in improving mature cherry trees adaptability under low chill conditions. The method for estimating N reserve status could not adequately explain observed trends in bud dormancy behaviour. Therefore, whole tree excavation may be necessary, but is expensive and labour intensive.

The application of Retain®, Raynox® or Surround® did not reduce leaf temperature and did not significantly affect parameters measured. It is therefore doubtful whether carbohydrate reserves were affected and it was evident that the objective of this experiment was not realized. It was shown that trees experience heat stress, but is a typical response for fruit trees exposed to those conditions (midday depression). Future research should focus on the reducing the heat stress and therefore the effect of shade netting (to reduce incoming radiation and heat) or evaporative cooling (to cool the leaves), which may, however, not be viable due to installation cost. A more viable option may be to improve the water use efficiency of cherry. This will allow the tree to effectively use water and cool the leaves through transpiration and in turn optimize photosynthesis. In this case carbohydrate reserves may be improved allowing for the evaluation of the role of carbohydrates in bud growth potential.

Exogenously applied cytokinins did not affect bud dormancy progression of cherry buds. Xylary cytokinins were not measured, but judging from the non-significant differences, it is uncertain whether reserve cytokinin was increased. Furthermore, cytokinins do not easily move from the site of application, therefore preventing them from reaching the bud. It is suggested that the synthetic cytokinins be applied with a wetting agent that would allow the penetration of cytokinins into the plant, thereby improving endogenous cytokinin levels. However, it was shown that there exists a close relationship between nitrogen, cytokinins and carbohydrates. To evaluate the role of each of these reserves and how they interact, it would have been more appropriate to use young trees instead of mature trees. Results

may not be extrapolated to mature trees, because they depend less on external inputs and may be ambiguous. Young trees can be housed in controlled conditions and are easy to manipulate and analysed compared to mature trees. In addition, due to cytokinin's close relationship with nitrogen it would be advisable to improve nitrogen reserves and thereby cytokinin reserves and report on the interaction between nitrogen and cytokinins.

Hydrogen cyanamide was effective in improving flowering, budburst and yield. The application of Retain® and Promalin® did not result in improved flowering and fruit set. Treatments may be ineffective or the timing of the application might have played a role. Therefore, the growth regulators (Retain® and Promalin®) did not prove to be effective in improving the cherry tree's adaptability under low chill conditions.

Concluding Statement

In this study it was shown that high chill sweet cherry cultivars adaptability can be improved by applying Dormex® under South African conditions. Other attempts (cultural practices) did not yield any positive results. It is therefore evident that cultural practices provide little aid in improving adaptability of cherry trees under South African conditions. For the industry to be viable, future research efforts should therefore focus on breeding new low-chill cherry cultivars under South African conditions because cultural practices only marginally reduce the risk in cherry production. Little is known on how environmental conditions prior to chilling accumulation affect bud dormancy and how cherry buds were able to enter dormancy in summer. Once the mechanism for bud dormancy induction is understood, research efforts can focus on manipulating dormancy under low chill conditions. Currently, global temperatures are also rising due to the excessive emission of greenhouse gasses (global warming) which would affect the deciduous fruit industry globally. Higher temperatures would result in lower chill unit accumulation, which could induce chilling related problems similar to those experienced in low chill regions. Therefore, dormancy research and how to manipulate bud dormancy may become of great importance in the future.