FACTORS LEADING TO POOR FRUIT SET AND YIELD OF SWEET CHERRIES IN SOUTH AFRICA

By

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Thesis is submitted in partial fulfilment of the requirements for the degree Master of Science in Agriculture in the Department of Horticultural Science, University of Stellenbosch.

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DECLARATION

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

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SUMMARY

Sweet cherries (*Prunus avium* L.) have a high chilling requirement and grow best in areas receiving >1 100 Utah chill units during winter. The main production areas in South Africa, and particularly the eastern Free State, frequently receive insufficient winter chilling and late spring frosts leading to problems of poor budburst, flowering, floral abnormalities and poor fruit set. Research was conducted on the cultivar 'Bing' to determine the main factors causing its low fruit set. Various trials were conducted to optimize the timing of rest breaking agents, identify suitable cross pollinizers that flower synchronously with 'Bing', and evaluate the influence of temperature and pollen-pistil interactions on fertilization and fruit set.

Pollen biology studies using 2- to 3-year-old 'Bing' sweet cherry trees were conducted near Clarens, eastern Free State, during the 2005 and 2006 seasons to determine the most suitable cross pollinizer/s for 'Bing' and to assess the influence of temperature and pollen-pistil interactions on pollen tube growth and ovule longevity. Significant differences in pollen germination ('rates' deleted) occurred between pollinizers, although differences were noted in pollen performance on the stigma and style (*in vivo*) compared to the artificial media (*in vitro*), indicating a lack of correlation between *in vitro* germination and *in vivo* pollen-pistil interactions. Pollen tube growth, following cross pollination, was influenced by pollinizer genotype, temperature, and the number of pollen grains deposited on the stigma. The highest pollen tube growth rates in 'Bing' styles were recorded for the pollinizers 'Black Tartarian' (2006), 'Lapins' and 'Rainier' sweet cherry cultivars at temperatures of approximately 21°C. Temperature had the most significant influence on ovule longevity with the lower orchard temperatures extending ovule viability compared to the higher laboratory temperatures, although pollen tube growth rates were also reduced, thus shortening the effective pollination period. Cross pollination was also shown to extend ovule viability. The results indicate that 'Black Tartarian', 'Lapins' and 'Rainier' were the most suitable pollinizers for 'Bing'. Hand-pollination with pollen from these donors resulted in a several-fold increase in seed set over naturally-pollinated control flowers. It appears that the principle factors causing poor fruit set in 'Bing' sweet cherry are premature abortion of the ovule before fertilization and inadequate transfer of sufficient viable pollen under orchard conditions.

Rest breaking trials were conducted on 4-year-old 'Bing' sweet cherry trees on 'Gisela® 5' rootstock near Clarens (28°28'S; 28°19'E, 1860m) and Reitz (28°0'S; 28°28'E; 1717m) in the

eastern Free State, South Africa, during the 2005 and 2006 seasons respectively. In 2005 five treatments were evaluated; viz. 1% and 2% Dormex® (hydrogen cyanamide, HCN); 1% Dormex® + 3% mineral oil; and 3% Lift® (thidiazuron and mineral oil) sprayed at three dates (29 July 2005, 5 August 2005 and 12 August 2005) preceding expected the "green-tip" stage of flower development, plus an unsprayed control. In 2006 four treatments were evaluated; viz. 1% Dormex®; 1% Dormex® + 3% mineral oil; 3% Lift® applied on three dates (26 July 2006, 7 August 2006 and 12 August 2006) and an unsprayed control. No interaction was observed between time of application and type of rest breaking agent (RBA). RBAs were effective at improving budburst and yield during both seasons with the time of application of RBAs having the most significant influence on budburst and production efficiency in 'Bing' sweet cherry trees. RBAs were most effective at improving vegetative budburst when applied 9 to 16 days before the ('actual' deleted) "green-tip" stage of flower development. Floral budburst and yield were increased by 1% Dormex® + 3% mineral oil and 3% Lift®, but results varied between seasons indicating that time of RBA application should be based on chilling accumulation and bud development stage and not based on calendar date.

This current research suggests that 'Bing' sweet cherry is poorly suited climatically to the current production areas of the eastern Free State and short-term research needs to identify methods of improving chilling and fruit set by means of evaporative cooling and fruit set-enhancing plant growth regulators. Longer term work requires the identification of new, lower chill cultivars with improved climatic adaptation to South African conditions.

OPSOMMING

Soet kersies (*Prunus avium* L.) het ‘n hoë koue behoefte en groei die beste in streke wat >1 100 Utah koue eenhede gedurende winter ontvang. Die hoofproduksie streke in Suid Afrika, en spesifiek die oos-Vrystaat, ondervind gereeld probleme met ontoereikende koue in winter, en ryp in die laat lente. Dit kan lei tot gebrekkige knopbreek, blom abnormaliteite en swak vrugvorming. Navorsing om die hooffaktore wat swak vrugvorming veroorsaak, is op ‘Bing’ gedoen. Proewe is gedoen, om die optimale tydsberekening vir die toediening van rusbrekende middels te bepaal, en bruikbare kruisbestuiwers wat saam met ‘Bing’ blom, te probeer identifiseer, en die invloed van tempratuur en stuifmeel-stamper interaksies op bevrugting en vrugvorming te bepaal.

Stuifmeelbiologieproewe is onderneem naby Clarens in die oos-Vrystaat gedurende die 2005 en 2006 seisoene op 3-jaar-oue ‘Bing’ soetkersie bome. Noemenswaardige verskille in stuifmeel onkieming is waargeneem tussen bestuiwers. Verskille in stuifmeel prestasie op stigma en styl (*in vivo*) teenoor dit in die kunsmatige medium (*in vitro*) toon op ‘n swak korrelasie tussen *in vitro* ontkieming en *in vivo* stuifmeel-stamper interaksies. Stuifmeelbuis groei is beinvloed deur die genotipe van die bestuiwer, die temperatuur en die getal stuifmeel korrels wat op die stigma geland het. Die hoogste stuifmeelbuis groeitempo’s is waargeneem vir ‘Black Tartarian’ (2006), ‘Lapins’ en ‘Rainier’ soet kersie kultivars by temperature van ongeveer 21°C. ‘n Stuifmeelbevolkingseffek is waargeneem gedurende die 2006 seisoen wat waarskynlik ‘n invloed gehad het op stuifmeelbuisgroei. Temperatuur het die mees noemenswaardigste invloed gehad op ovule lewensvatbaarheid. Laer boord temperature het ovule lewensvatbaarheid verleng waneer vergelyk word met hoër laboratorium temperature hoewel stuifmeelbuis groeitempo’s vertraag is wat gelei het tot ‘n verkorte effektiewe bestuiwings periode. Kruisbestuiwing het ook ovule lewensvatbaarheid verleng. Die resultate dui daarop dat ‘Black Tartarian’, ‘Lapins’ en ‘Rainier’ die mees geskikste bestuiwers vir ‘Bing’ is. Dit wil voorkom asof die primêre faktor wat swak vrug vorming in ‘Bing’ soetkersies beinvloed met die vroë aborsie van die ovule, en die gebrekkige oordrag van genoeg lewensvatbare stuifmeel in boordomstandighede, verband hou.

Proewe is onderneem op 4-jaar-oue ‘Bing’ soetkersiebome wat ge-ent is op ‘Gisela® 5’ wortelstok naby Clarens (28°28’S; 28°19’E, 1860m) en Reitz (28°0’S; 28°28’E; 1717m) in die oos-Vrystaat gedurende die 2005 and 2006 seisoene respektiewelik. In 2005 is vyf

behandelings beoordeel; nl. 1% en 2% Dormex® (waterstof sianoamied, HCN); 1% Dormex® + 3% minerale olie; en 3% Lift® (thidiazuron and minerale olie) besproei op drie datums (29 Julie 2005, 5 Augustus 2005 en 12 Augustus 2005) voorafgegaan deur verwagte "groen-punt", plus 'n onbesproeide kontrole. In 2006 is vier behandelings beoordeel; nl. 1% Dormex®; 1% Dormex® + 3% minerale olie; 3% Lift® en 'n onbesproeide kontrole toegepas op drie datums (26 Julie 2006, 7 Augustus 2006 en 12 Augustus 2006). Geen interaksie was waargeneem tussen die tydstip waarop die behandeling toegedien is en die tipe RBM nie. RBM's was effektief om knopbreek en opbrengs gedurende beide seisoene te verbeter. Die tydstip waarop die RBM toegedien is het die mees noemenswaardige invloed gehad op knopbreek en produksie effektiviteit in 'Bing' soet kersiebome. RBM's was mees die effektiefste om vegetatiewe knopbreek te verbeter as dit 9 tot 16 dae voor die 'groen-punt' toegedien is. Blom knopbreek en opbrengs het 'n toename getoon by 1% Dormex® + 3% minerale olie en 3% Lift® maar die resultate het verskil tussen seisoene wat 'n aanduiding is dat die tydstip waarop die RBM toegedien word gebaseer moet wees op koue akkumulasie en knopontwikkelingsstadium en nie op kalender datum nie.

Die huidige navorsing impliseer dat 'Bing' soet kersies uit 'n klimatologiese oogpunt marginaal geskik is vir die huidige produksie areas van die Oos Vrystaat. Dit word voorgestel dat toekomstige kort termyn navorsing klem moet lê op die identifiseering van nuwe metodes om verbeterde koeling en vrugvorming deur middel van 'oorhoofse afkoeling' en verbeterende vrugset plant groei reguleerders te weeg te bring. Lang termyn werk moet konsentreer op die identifiseering van nuwe, lae koue-behoefte kultivars wat beter aangepas is by Suid-Afrikaanse klimaatstoestande.

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INTRODUCTION

The sweet cherry, *Prunus avium* L., is believed to have originated from the temperate areas around the Caspian and Black Seas (Webster, 1996). The first cherries thought to have been introduced into South Africa was by Jan van Riebeeck shortly after 1652 with the first small orchard planted in 1890 in the Ceres area of the Western Cape. The first commercial orchard was planted by Harry Pickstone in the Clocolan area of the eastern Free State in 1904 (Zwahlen et al., 1989).

The South African cherry industry is centred around the Ficksburg/Fouriesburg/Reitz areas (28°S) of the eastern Free State (EFS) and the Koue Bokkeveld region (33°S) of the Western Cape, producing roughly 90% (<1000 t annually) of the South African cherry crop with 'Bing' making up approximately 38% of current plantings (Deciduous Fruit Producers Trust, 2005). Most production was historically extensive in nature, but recently orchards have been planted at high densities, with many orchards under hail netting.

The sweet cherry is known to grow best in areas with cold winters receiving over 1 100 Utah chill units (Mahmood et al., 2000) and mild conditions during the growing season. The production of high-chill sweet cherries and other stone fruit in areas with mild winters, which frequently receive insufficient winter chilling, often result in poor flowering, fruit set (Iezzoni et al., 1990; Küden et al., 1997) and floral abortion (Alburquerque et al., 2003; Legave et al., 2006). Rest breaking agents such as hydrogen cyanamide (HCN) have allowed growers to partially compensate for this lack of chilling, resulting in the expansion of production into more marginal areas (Erez, 1987).

Successful fruit set and productivity in sweet cherry and other fruit crops is dependent on a number of successful reproductive processes occurring during the progamic phase (Hedhly et al., 2004). Pollen-pistil interactions have been shown to play an important role in these events by regulating both pollen tube dynamics and ovule viability in the pistil which influence fertilization and fruit set (Sanzol and Hererro, 2001). Pollen competition in the style, and variations in the number of pollen grains deposited on the stigma, affect pollen performance (Hormaza and Hererro, 1994). Temperature is an important environmental factor influencing pollen germination and pollen tube kinetics in the style with the response to temperature often being genotype-dependent (Hedhly et al., 2005; Hedhly et al., 2004).

Most commercial sweet cherry cultivars are gametophytically self-incompatible resulting in most modern orchards being planted with two or more compatible pollinizers whose bloom dates overlap and flower synchronously with the main cultivar (Nyéki et al., 2003; Tehrani and Brown, 1992; Thompson, 1996). The lack of knowledge of suitable cross compatible varieties has resulted in new orchards in South Africa being planted with four or more pollinizers, spaced evenly throughout the orchard. The prolonged flowering periods and lack of bloom synchrony further complicates orchard design, as differences in chilling requirement of the pollinizers often result in considerable variation in bloom date from year to year.

Various experiments were conducted on ‘Bing’ sweet cherry to firstly, to identify the most suitable cross pollinizer/s for ‘Bing’ sweet cherry based on cross compatibility and bloom synchronicity, secondly, to assess the influence of temperature and pollen-pistil interactions on pollen tube growth and ovule longevity, and thirdly, to optimize the timing of low concentrations of rest breaking agents to enhance budburst and yield of ‘Bing’ when grown under conditions of insufficient winter chilling,

The research presented here will help the cherry industry make more effective decisions on the timeous application of rest breaking agents and to improve the selection of suitable cross pollinizers for ‘Bing’, which is currently the main cultivar grown in South Africa.

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1. LITERATURE REVIEW

INTRODUCTION

Flowering is the single most important physiological event to occur in fruit trees as flowers are the initial step in the production of fruit. Flowering in deciduous fruit trees can be divided into two major developmental processes which are the initiation and morphological development of the floral bud in summer and autumn, and the actual flowering process in early spring of the following season (Faust, 1989).

Flower initiation in many plants is controlled and triggered by a specific environmental stimulus such as photoperiod or vernalization (Jackson and Sweet, 1972), while the process of flower bud formation in fruit trees is a far more complex and complicated sequence of events influenced by a number of environmental factors, management techniques and plant growth regulators (PGR's) which may modify the flowering response (Greene, 1996). Flower bud initiation and differentiation in sweet cherry (*Prunus avium* L.) occurs during early summer and autumn respectively (Guimond et al., 1998a), and is followed by a period of dormancy during the winter. The stage at which the bud enters dormancy is determined by the prevailing climatic conditions (Dennis, 2000).

Dormancy in deciduous fruit trees and woody perennials of the temperate zones is a phase of development that occurs annually, allowing plants to survive unfavourable winter conditions (Erez, 2000; Saure, 1985). The tree is able to resist low temperatures in the dormant state but once buds start to break in spring, resistance is lost (Martin, 1991). The characteristic of dormancy is that it is released by a quantitative accumulation of a certain amount of chilling and only part of this chilling requirement can be substituted for by other means. High winter day temperatures are known to have a detrimental effect on chilling accumulation of deciduous fruit trees, and especially stone fruit species (*Prunus* spp.) which are highly sensitive to insufficient chilling (Faust, 2000; Faust et al., 1997). This leads not only to delayed bud break, but also various floral bud anomalies and bud necrosis (Legave, 1978a; Oukabli and Mahhou, 2007; Viti et al., 2006).

Reproductive success in plants depends on the co-ordinated, synchronous development of male and female reproductive organs (Sedgley and Griffin, 1989). The timing of the pollen-

pistil interaction is vital as the pistil, which is in a constant state of development, is only receptive to pollen for a relatively short period of time. Besides pollen adhesion and hydration by the stigma, the pistil also plays an important role in controlling pollen germination, pollen tube entry into and growth in the style, and pollen tube guidance in to the ovary and ovule (Herrero, 2003).

1.1 FLOWER BUD DEVELOPMENT IN *PRUNUS*

The sweet cherry, *Prunus avium* L., is member of the family *Rosaceae* and is classified under the genus *Prunus* which includes almond (*P. amygdalus* Batsch), apricot (*P. armeniaca* L.), sour cherry (*P. cerasus* L.), peach (*P. persicae* L. Batsch), nectarine (*P. persicae* L. Batsch), plum (*P. domestica* L.) and many species of rootstocks (Flore, 1994). Flowers are botanically perfect consisting of male (stamens) and female (pistil) reproductive parts. The pistil consists of a stigma, style and ovary containing two ovules. The secondary ovule degenerates soon after pollination, leaving only the primary ovule available for fertilization (Pimienta and Polito, 1982; Postweiler et al., 1985; Thompson, 1996).

Flowers are borne on long-lived (10-12 year) two-year or older branches, or as axillary buds at the base of one-year-old shoots (Thompson, 1996). The flowers are not borne on the two-year-old wood, but rather on one-year-old spurs which form on branches which are at least two-years old. A high percentage of floral buds on shorter 1-year-old shoots of trees on dwarfing, precocious rootstocks may have a negative impact on the following seasons spur shoot development, resulting in a high percentage of blind wood on one-year-old lateral shoots (Schaumberg and Gruppe, 1985; Thompson, 1996). A number of flower buds develop laterally on each spur with each bud, containing two to five flowers depending on apex size (Diaz et al., 1981). These flowers form condensed clusters or fascicles. Cherries produce simple buds with separate floral and vegetative buds being borne on the same spur or shoot (Brewer and Azarenko, 2005). Other *Prunus* species can be distinguished by the arrangement of their floral buds. Peach buds are initiated in leaf axils of the current seasons growth only (Raseira, 1986) while sour cherries produce buds both laterally on 1-year-old shoots and 2-year-old spurs (Thompson, 1996).

1.1.1 Juvenility

Most woody plants are unable to flower until they have reached a specific stage or condition of growth. Thus the growth of fruit trees can be divided into two different phases. The first,

the juvenile or seedling phase, where the tree grows vegetatively, and second, the reproductive or adult phase when floral buds are produced (Jackson and Sweet, 1972). During the juvenile phase, which is genetically controlled, the seedling is unable to induce floral buds by any means (Goldschmidt and Samach, 2004; Zimmerman, 1972).

The transition from juvenile to mature or reproductive phase is not clearly defined and occurs when the tree has the ability to flower but is not actually flowering (Zimmerman, 1973). This may be modified by the environment or various cultural practices (nitrogen, dwarfing rootstocks, irrigation, plant growth regulators) (Faust, 1989; Oliviera and Browning, 1993). Practically, the end of the juvenile phase is indicated by the first production of flowers (Raseira and Moore, 1987).

The tree fruit industry and fruit breeders have for many years tried to shorten the length of the juvenile period to reduce the time trees are unproductive. Fruit trees are generally vegetatively propagated by grafting scionwood from mature trees onto specific rootstocks thus eliminating the problem of juvenility. The two to three years following grafting when the tree does not flower should not be confused with juvenility and is referred to as the vegetative period (Visser, 1965). Seedling trees can enter the flowering phase without passing through the vegetative phase. Once a tree has reached the adult flowering stage, it alternates between the flowering and vegetative phases, but will not make the transition back to the juvenile phase (Faust, 1989; Hackett, 1985).

1.1.2 Floral induction, initiation and differentiation

Although flowering in spring is a visible manifestation of the reproductive process in deciduous fruit crops, induction and initiation of the vegetative apical meristem to a reproductive bud commences the previous summer (Diaz et al., 1981; Guimond et al., 1998a; Thompson, 1996). This induction and initiation involves a shift in the apical meristems pattern of growth and development from a vegetative to a floral meristem. Gasser (1991) distinguished three main phases of flower development: (1) induction and evocation; (2) organ initiation and specification at floral apex; and (3) differentiation (or development) of tissues within organs. Flower differentiation ultimately culminates in flowering in the spring (Bubán, 1996).

Floral initiation is preceded by an unknown inductive stimulus, perceived by the vegetative meristem, leading to floral induction (Bernier, 1988; Wellensiek, 1977). This process in

deciduous fruit trees is considered to be endogenously controlled by changes in plant hormones (Bangerth, 2006; Bernier, 1988) and/or changes in the distribution of nutrients within apical meristems (Sachs, 1977) as a result of correlative (inter-organ) signals. Plant hormones appear to be the only endogenous substances that consistently have the ability to directly influence floral induction, either by inhibiting (GA's and IAA) (Bangerth, 2006) or promoting (cytokinins) (Ramírez, 2000) the process.

Floral initiation is defined as the "first visible morphological alteration of the shoot apex that denotes the onset of flower development" (Raseira and Moore, 1987). This begins as a broadening and flattening of the rounded meristem on which the floral organs differentiate (Tufts and Morrow, 1925) and is followed by the development of two to four lateral protuberances, representing primordial bracts, which subtend each flower. During the summer, the floral primordia differentiate acropetally within the axils of these bracts with sepal primordial appearing first. This is followed by differentiation of the petal, stamen and pistil primordia respectively. The floral bud thus enters dormancy with all floral parts in a visible, immature stage (Diaz et al., 1981; Thompson, 1996). The ovules and pollen sacs differentiate particularly late, with them only being observable by late autumn or even early spring at the latest (Faust, 1989), while connecting xylem in buds of some *Prunus* species does not appear until just before bloom (Bartoloni and Giorgelli, 1994; Hanson and Breen, 1985).

Floral initiation and differentiation in stone fruit, using the scanning electron microscope, has been observed in almond (Lamp et al., 2001), peach (Engin and Iqbal, 2004), sweet cherry (Guimond et al., 1998a; Kappel et al., 1990) and sour cherry (Diaz et al., 1981). The first visible sign of flower initiation in 'Bing' sweet cherry in Washington State was observed ≈119 days after anthesis (Guimond et al., 1998a), whereas in Japan, the first signs of floral initiation were observed 49 days after anthesis (Watanabe, 1983). In sour cherry, initiation occurred between four weeks after anthesis (Diaz et al., 1981). Time of floral initiation under low chilling conditions, as experienced in South Africa, is unknown.

Some stone fruit are known to develop a high percentage of functional male flowers with underdeveloped pistillate parts. This has an important influence on fruit set resulting in flowers having under-developed pistils, prematurely abscising from the tree before fertilization has occurred (Faust, 1989; Nyéki, 1974; Nyéki et al., 2003a). Field collections of

samples from some eastern Free State ‘Bing’ orchards revealed a relatively high percentage (up to 20%) of functional male flowers with underdeveloped pistillate parts (sterile flowers) (personal observation).

1.1.2.1 Factors influencing flower induction, initiation and differentiation

Flower bud initiation in stone fruit is considered to be endogenously controlled by balances in plant hormones, and this may be particularly important during the early phases of bud development, whereas later development may be more dependent on carbohydrate availability and nitrogen (Faust, 1989). A number of factors, such as the environment, plant growth regulators, tree age and cultural practices (rootstock, plant nutrition, pruning), affect the physiological status of the tree and hence influence floral initiation.

a) Environmental factors

High summer temperatures

Temperature has been shown to play an important role in both flower bud initiation and differentiation in various stone fruit crops. Exposure of cherry trees to high temperatures during the critical early stages of floral initiation may increase their susceptibility to pistil doubling (Thompson, 1996). Pistil doubling appears to be most severe when buds are exposed to high temperatures during the formation of sepal and petal primordia and less susceptible once the pistil and stamen primordia have differentiated (Beppu et al., 2001a). Beppu and Kataoka (1999) showed that approximately 80 percent of ‘Satonishiki’ sweet cherry flowers produced double pistils when exposed to 35°C/25°C (day/night) temperatures during late July to early September (N.H.), while work by Whiting and Martin at Prosser, Washington State University (WSU) in 2006, showed that exposure of buds to temperatures of 37.2°C, for a two-week period in late July, increased doubling to 10 percent (Hansen, 2007). ‘Bing’ and ‘Napoleon’ appear to be particularly susceptible to heat-induced doubling (Thompson, 1996). High temperatures during rapid summer growth has been associated with the failure to develop axillary flower buds in peach (Richards et al., 1994).

Inadequate winter chilling and heat requirement

Insufficient winter chilling is a major limiting factor to the production of deciduous fruit under warm climatic conditions. Most areas in South Africa are climatically marginal for the production of high-chill requiring stonefruit such as sweet cherries which have a chilling requirement of between 500 and 1300 hours below 7°C (Faust, 1989) or greater than 1100

Utah chill units (Mahmood et al., 2000). The chilling requirement of the buds is seldom fully satisfied leading to poor bud break, delayed foliation, extended bloom period (Erez, 1987), and floral abortion or bud drop (Alburquerque et al., 2003; Brown, 1958; Legave et al., 2006; Weinberger, 1967).

Time of flower bud initiation in the peach has been shown to be relatively independent of the chilling requirement of the cultivar (Raseira and Moore, 1987; Stadler and Strydom, 1967). This results in low and high chill cultivars, initiating flowers at the same time when grown under the same environmental conditions, but differing in the stage of flower bud development, at the onset of dormancy.

Once the chilling requirement has been satisfied, the buds enter an eco-dormant period where cessation of growth is due to unfavourable environmental factors (Lang et al., 1987). Flower bud growth and development through the various phenological stages resumes once temperatures exceed a minimum, species-dependent threshold (4.5°C) and after a certain number of heat units or growing degree-days (GDD) have accumulated. This method has successfully been used to predict bloom date in almond (Alonso et al., 2005) and sour cherry (Zavalloni et al., 2006).

Light intensity

Most deciduous fruit trees are insensitive to photoperiod but light intensity is known to play an important role in flower bud initiation (Sedgley, 1990). Reduced light levels, due to within-canopy shading, have been shown to inhibit or reduce flower bud initiation in apricots (Jackson, 1969) and kiwifruit (*Actinidia chinensis* Planch) (Grant and Ryugo, 1984). Flower bud formation in 'Montmorency' sour cherry required a minimum of 15-20% of full sun with fruit set being adversely affected by light levels of less than 20% (Flore, 1980).

b) Plant growth regulators

Endogenous plant growth substances play an important regulatory role in the control of floral initiation. The specific influence of each on the initiation process, being dependent on the level of hormones, tissue sensitivity, time of the season and the availability of nutrients (Faust, 1989).

The stimulus for floral initiation involves the interaction of a number of hormones, in particular, cytokinins (CKs) and gibberellins (GAs) (Bernier, 1988). Applications of GAs to fruit trees have been shown to inhibit floral initiation. The application of GA_3 to mature sweet cherry spurs before floral initiation inhibited floral initiation (Oliveira and Browning, 1993), while applications during floral induction reduced return bloom (Bradley and Crane, 1960; Lenahan et al., 2006). Similar results have been reported for apricots (Byers et al., 1990), peaches and nectarines (González-Rossia et al., 2007).

Cytokinins are known to be involved in the process of cell division and have been shown to promote floral initiation in apples (*Malus domestica* Borkh.) (Ramírez et al., 2004; Ramírez and Hoad, 1981), litchi (*Litchi chinensis* L.) (Chen, 1991) and grapevines (*Vitis vinifera* L.) (Srinivasan and Mullins, 1978). Bangerth (2006) suggests a major role for CKs as a correlative signal involved in the regulation of floral induction in perennial fruit trees, although the source of these CKs is still unknown.

Polyamines have been shown to be involved in cell division, induction and differentiation of floral organs (Evans and Malmberg, 1989; Zhu et al., 1997). Pritsa and Voyiatzis (2004) showed a relationship between polyamine fluctuations and floral differentiation in olives (*Olea europaea* L.) with spermine levels peaking in the buds during differentiation.

c) Cultural practices

Certain orchard management practices are known to influence both the time of floral initiation and the number of buds initiated (Thompson, 1996). Active vegetative growth reduces bud formation, thus any cultural practices which reduce vigour will enhance floral bud initiation (Forshey and Elfving, 1989). Summer pruning of apples was shown to encourage flower bud formation while dormant pruning stimulates more vigorous growth and reduced bud formation. In sweet cherries, summer pruning was shown to positively influence the number of flower buds (Guimond et al., 1998b) as well as the timing of floral bud initiation on the current season's shoots (Guimond et al., 1998a).

Practices such as growth restriction, topping, shoot twisting, and limb spreading which position branches in a more horizontal position, are used to reduce terminal growth and promote flower bud formation (Faust, 2000; Jackson and Sweet, 1972).

Rootstocks are known to have an influence on precocity, tree size, yield efficiency and flowering in fruit trees (Thompson, 1996). The Giessen hybrid rootstocks such as Gisela® 5 and 6 have been shown to increase precocity over the traditional vigorous Mazzard rootstock (Whiting et al., 2005), with the amount of dwarfing having little influence on precocity (Gruppe, 1985). Time of morphological differentiation of sour cherry flower buds during autumn and winter appears to be independent of rootstock (Kühn and Callesen, 2001).

Temporary water deficit or drought stress in late summer/autumn have been shown to delay the time of flower bud differentiation in apricot (Alburquerque et al., 2003; Brown, 1953) while severe water stress of cherries prevented flower bud differentiation the following season (Proebsting et al., 1981). The positive influence of mineral nutrition, mainly nitrogen and phosphorous, on flower initiation has been documented for a number of deciduous fruit species (Jackson and Sweet, 1972).

1.1.3 Development of sexual/floral organs

The period between floral initiation and anthesis in most temperate fruit crops is between 9 and 11 months. The buds are initiated in late summer or autumn, undergo dormancy during winter followed by budburst and flowering in spring (Sedgley, 1989). During this period, the floral organs continue to undergo a number of changes which vary dependent on the period of chilling, with development more rapid after a period of chilling (Chandler and Tufts, 1933). High- and low-chill peach cultivars grown under low chilling conditions showed a period of arrested bud development (Stage 8 – Pollen sacs and sporogenous tissue present) during autumn with the period of arrested development dependent on the cultivar's cold requirement (Stadler and Strydom, 1967). The stages of morphological development of the stamens and pistil will be briefly discussed.

1.1.3.1 Formation of stamens and pollen grains

The stamens of *Prunus* differentiate into an anther and stalk or filament. The appearance of the anther primordium signals the beginning of stamen development and consists of the epidermis surrounding the primary archesporium (Bubán, 1996). The anther differentiates into four groups of archesporial tissue which in turn differentiate into pollen mother cells (PMC). At the initiation of meiosis, individual PMCs become enclosed in a callose wall. Following meiotic divisions, each PMC divides into four microspores or tetrads, each surrounded by a callose wall (Guerriero and Bartolini, 1995; Sedgley and Griffin, 1989; Shivanna et al., 1997).

It was initially considered that tetrad formation was the stage of termination of endodormancy in fruit crops (Dracynski, 1958) but various authors have shown that tetrad formation, in stone fruit, and endodormancy completion are not closely coupled (Citadin et al., 2002; Weinbaum et al., 1989). Weinbaum et al. (1989) showed that tetrad formation occurred 7 to 14 days prior to endodormancy completion in almond and that higher temperatures experienced during mild winters may favour the earlier appearance of tetrads. In sweet cherry, low temperatures did not affect meiosis but temperatures >18°C resulted in an increased frequency of meiotic abnormalities (Whelan et al., 1968). Citaden et. al. (2002) showed a heat unit requirement for the transition from PMC to tetrad formation. In apricots, prolonged development of the PMC stage under conditions of insufficient chilling, suggested a varying depth of endodormancy at successive flower bud developmental stages, with the deepest dormancy at or just prior to PMC formation (Brown and Abi-Fadel, 1953).

At maturity, the pollen grain wall consists of four components, namely an outer layer or exine, an inner layer or intine, an outer coating of tryphine and one or more germination apertures. Proteins and enzymes making up the pollen wall are rapidly released upon hydration on the stigmatic surface and are vital for pollen-pistil recognition and pollen germination (Sedgley and Griffin, 1989). During wall formation the nucleus undergoes a mitotic division giving rise to a large vegetative cell and a small generative cell separated by a thin wall (Shivanna et al., 1997).

1.1.3.2 Pistil structure and physiology

The pistil is the female floral organ involved in sexual reproduction and consists of the stigma, which receives pollen grains and provides a favourable environment for germination, the style, which supports pollen tube growth and the ovary, containing the ovules (Shivanna, 2003b).

a) Stigma

The stigma is important as it provides a surface for three different process namely, pollen hydration, germination and initial pollen tube growth (Sanzol et al., 2003). The stigma is classified as either wet or dry based on the presence or absence of stigmatic exudate at the time of pollination. It is further divided on the presence or absence of papillae (Shivanna et al., 1997). The stigmatic surface of *Prunus avium* consists of numerous papillae contributing to a wet stigma surface (Uwate and Lin, 1981).

b) Style

The style provides a pathway for the intercellular growth of pollen tubes from the stigma surface to the top of the ovary and is particularly important during the progamic phase of fertilization (stage between pollen deposition on stigma and gamete fusion in the ovule)(Knox et al., 1986). It is either open, termed the stylar canal, or closed/solid which is typical of apple (Cresti et al., 1980) and sweet cherry styles (Stösser and Anvari, 1983). The sweet cherry style consists of mucilaginous transmitting or conducting tissue made up of elongated cells with large intercellular spaces through which the pollen tubes grow. Degradation and collapse of the transmitting cells 9 to 10 days after anthesis had little or no effect on pollen tube growth (Stösser and Anvari, 1983).

The style is an important site for gametophytic self-incompatibility in temperate fruit crops and especially *Prunus avium* resulting in the arrest of pollen tube growth within the top third of the stylar tissue. Self incompatibility (SI) is genetically controlled by multiple S-alleles (Tehrani and Brown, 1992). Glycoproteins associated with SI alleles have been detected in the styles of sweet cherry (Mau et al., 1982).

c) Ovule and embryo sac development

Stone fruit species always contain two ovules with only one being capable of fertilization. One ovule (primary or functional ovule) has the ability to develop into a seed while the secondary ovule is mostly underdeveloped and aborts prematurely (Eaton, 1959; Pimienta and Polito, 1982; Postweiler et al., 1985).

The ovule, situated in the ovary, is the seat of the female gametophyte, containing the embryo sac and egg cell. The ovule is attached to the placenta via the funiculus, and consists of two outer layers of tissue (integuments), the nucellus and the embryo (Knox et al., 1986). The integuments wrap around the nucellus leaving a narrow opening, called the micropyle, through which pollen tube entry occurs. The obturator (placental protuberance), situated between the base of the style and the micropyle, may play an important role in the fertilization process in peach (Arbeloa and Hererro, 1987), almond (Cousin and El Maataoui, 1998) and sour cherry (Anvari and Stösser, 1978b). In peach, pollen tube growth was shown to be delayed by up to 5 days but no delays were noted in almond and sour cherry.

Development of the embryo sac or megagametophyte is described in detail by a number of authors (Knox et al., 1986; Shivanna, 2003b; Shivanna et al., 1997; Thompson, 1996) and thus a brief description will be given here. The embryo sac differentiates within the nucellus at the micropylar end of the ovule. The mature embryo sac consists of the egg apparatus (egg cell and two synergid cells), at the micropylar end, two central polar cells and three antipodal situated at the chalazal end. The antipodal cells degenerate before fertilization takes place while the two central polar nuclei fuse to give rise to the secondary nucleus. One of the synergids frequently degenerates prior to pollen tube arrival while the other plays a role in the penetration of the pollen tube into the embryo sac. During double fertilization, one sperm cell from the pollen tube fuses with the egg cell (bound by the synergids) to form the embryo, while the second sperm cell fuses with the secondary nucleus to form endosperm (Thompson, 1996).

1.2 POLLINATION BIOLOGY OF *PRUNUS*

Reproductive success in plants depends on the co-ordinated, synchronous development of male and female reproductive organs (Sedgley and Griffin, 1989). The timing of the pollen-pistil interaction is vital as the pistil, which is in a constant state of development, is only receptive to pollen for a relatively short period of time. Besides pollen adhesion and hydration by the stigma, the pistil also plays an important role in controlling pollen germination, pollen tube entry into and growth in the style, and pollen tube guidance into the ovary and ovule (Herrero, 2003).

Incompatibility is the inability of functional male and female gametes of a fertile seed plant to effect fertilization and produce zygotes after either self-pollination (self-incompatibility) or cross pollination (cross-incompatibility) (Burgos and Pérez-Tornero, 1999; Shivanna and Johri, 1989). Pistil tissues are typically able to discriminate between pollen grains in a specific population by recognizing pollen from the same species while rejecting pollen from unrelated species (Edlund et al., 2004). This phenomenon of cross-incompatibility among plant species prevents outbreeding depression which arises when paternal and maternal sets of chromosomes are too different for growth and development, as well as meiotic functioning (Kao and McCubbin, 1996). However, cross-incompatibility is also common among cultivars within a species which share a common *S*-allele (Crossa-Raynaud and Grasselly, 1985), S_3S4, which are identical in ‘Bing’ and ‘Napoleon’ (syn. ‘Royal Anne’) sweet cherry (Iezzoni et al., 2005). Thus their compatibility behaviour, in terms of pollen tube growth dynamics, would be

identical when pollinated with their own pollen or when used as pollinizers for cultivars in the same incompatibility group (Socias i Company and Alonso, 2004).

In addition, about 50% of plants have genetic self-incompatibility systems (Kao and McCubbin, 1996) which prevent fertilization by a plant's own pollen and thus reduce inbreeding depression. Many fruit crops, such as *Prunus* and *Malus*, are self-incompatible (SI) and thus require cross pollination to ensure proper fertilization and fruit set (Thompson, 1996; van Marrewijk, 1989). Most sweet cherry varieties are SI resulting in modern orchards being planted with compatible varieties or pollinizers whose bloom dates overlap and flower synchronously with the main crop cultivar (Nyéki et al., 2003b; Tehrani and Brown, 1992; Thompson, 1996).

1.2.1 Self-Incompatibility (SI)

Self-incompatibility is one of the most important mechanisms used by plants to prevent self-pollination, and is genetically controlled by a single S-locus which has multiple S-alleles, allowing for the recognition or rejection of "self" or genetically identical pollen (Franklin-Tong and Franklin, 2003b; Tehrani and Brown, 1992). This recognition or rejection of pollen before fertilization is important to promote out crossing and improve genetic variability (van Marrewijk, 1989), but is a major limiting factor for successful fruit set in orchard fruit crops (Thompson, 1996).

Most commercial sweet cherry (Nyéki et al., 2003b; Tehrani and Brown, 1992), almond (Ortega et al., 2006; Socias i Company et al., 2004) and apricot (Burgos and Pérez-Tornero, 1999) cultivars are gametophytically SI and cross-incompatible while the European plum (*P. domestica*) is self-fertile, partially self-fertile, and a few SI cultivars exist (Szabó, 2003). Peach and sour cherry cultivars are generally considered self-fertile (Nyéki and Szabó, 1997; Nyéki et al., 2003a), although some SI and partially SI varieties exist (Lansari and Iezzoni, 1990).

Gametophytic SI takes place during the progamic phase of fertilization (stage between pollination and fertilization), with inhibition of pollen tube growth occuring at three different levels: on the stigma surface, in the stylar tissue or in the ovary (Newbigin et al., 1993; Shivanna, 2003a). The stylar tissue is an important site for gametophytic SI in *Prunus* spp., and especially sweet cherry, resulting in the arrest of pollen tube growth within the top third

of the stylar tissue (Tehrani and Brown, 1992). Typical signs of pollen incompatibility in the upper third of the style of *Prunus* species include swollen pollen tube ends and thick pollen tubes which fluoresce intensely (Cerović and Ružić, 1992), as a result of the deposition of callose (1,3-β-glucan) (Tehrani and Brown, 1992; Wilhelmi and Preuss, 1997).

Gametophytic SI has been studied in Campanulaceae, Solanaceae, Scrophulariaceae and Rosaceae, and have been shown to have a similar *S-RNase*-based SI system where SI is controlled by a single *S*-locus that has multiple *S*-alleles (Franklin-Tong and Franklin, 2003b). *S*-alleles are expressed in the style as *S-RNases* (ribonucleases), which specifically reject those pollen tubes with the same S genotype (Kao and McCubbin, 1996). Incompatible pollen is able to germinate normally on the stigma, and is only inhibited by the stylar tissue, once the pollen tube has reached approximately a third of the way down the style (Franklin-Tong and Franklin, 2003a). For example, S_1 pollen and S_2 pollen are inhibited in the style of S_1S_2 varieties (cross-incompatibility) or of the same variety (self-incompatibility) but the S_2 pollen will successfully grow in an S_1S_3 style (partial cross-incompatibility). When no alleles match, both S_1 and S_2 pollen will grow through the style of S_3S_4 varieties (fully compatible).

The pollen component in the SI system has until recently, been unknown in *Prunus*. This newly identified, pollen S-gene, *S* haplotype-specific F-Box (*SLF*/*SFB*) protein in *P. avium* and *P. cerasus* controls pollination specificity in the pollen (Yamane et al., 2003). Thus pollen RNA is degraded by stigma *S-RNase* in incompatible crosses and not in compatible ones, e.g. *S1-RNase* degrades of S_1-pollen RNA but not $S_2..S_n$ genotypes (McClure, 2006). In *Prunus*, pollen is rejected if even one functional pollen *S*-haplotype is matched in the pistil (Hauck et al., 2006). A study of the two self-compatible mutants, *JI 2420* and *JI 2434*, has confirmed the role of the *SLF*/*SFB* gene in self-incompatibility in *Prunus* (Sonneveld et al., 2005).

Sweet cherry cultivars have been classified into various compatibility groups with cultivars having the same two S-alleles being assigned to the same group (Tehrani and Brown, 1992). Cultivars within the same group are cross-incompatible but are compatible with cultivars from all other groups (Thompson, 1996). There are currently 22 compatibility groups plus Group O, which are universal donors and are compatible with all cultivars in Groups I-XXII (Iezzoni et al., 2005).

Self-compatibility in sweet cherry is as a result of an induced mutation of the S-allele, denoted *S´* (Lewis and Crowe, 1954). All self fertile sweet cherry cultivars released so far contain the S_4´ allele from *JI 2420* (S_4S_4´), which is attributed to a mutation of the S_4 allele, denoted S_4´, where the prime symbol indicates the loss of pollen S-allele function (Iezzoni et al., 2005). Similarly, self-fertility in *JI 2434* (S_3S_3´), is attributed to a mutation of the S_3 allele (S_3´) (Bošković et al., 2000). Thus pollen from self-fertile varieties is able to function on its own pistil and is compatible with all other cultivars, i.e. universal pollen donors (Thompson, 1996). The first self-compatible sweet cherry cultivar, 'Stella' (S_3S_4´) was released from Summerland Research Station, Canada, in 1971 (Lapins, 1971).

1.2.1.1 Factors affecting incompatibility

The breakdown of SI has been reported for various crops (Arasu, 1968; Lewis, 1942) and besides genotype, is influenced by temperature, stage of bud development, chemicals, and quantity and viability of pollen (van Marrewijk, 1989).

a) Temperature and genotype

The exposure of flowering *Lilium* and tomato plants (both gametophytically incompatible) to high temperatures (32-40°C) resulted in the destruction of the incompatibility mechanism (van Marrewijk, 1989) while low temperatures reduced the expression of SI in *P. avium* (Lewis, 1942). Choi and Andersen (2005) showed that the frequency of SI breakdown in various sweet cherry cultivars was dependent on both temperature and the cultivars *S*-genotype. The frequency of breakdown increased from 10 to 25°C and was highest in S_3S_4 ('Bing') genotypes. They postulated that high temperatures may inactivate or denature specific incompatibility-determining *S*-locus proteins or the level of the proteins responsible for self-incompatibility may be lower at high temperatures.

Temperature has also been shown to influence the rate of incompatible pollen tube growth in 'Sundrop' apricot. Self pollen tube growth was highest between 10 and 15°C, but lower at higher temperatures, although no fertilization took place (Austin et al., 1998).

b) Pioneer and mentor effect

The 'pioneer effect' is described as the effect of a double pollination, where the action of the second application of pollen is stimulated by the first or pioneer pollen (Visser, 1981). In apples and pears, the application of compatible pioneer pollen, followed 1-2 days later by self pollen, promoted self pollen tube growth and fruit set, though not seed set (Visser, 1983). The mechanism of the pioneer effect is unknown but may be due to positive recognition of compatible pollen which overcomes the inhibitory influence of the incompatible pollen (Sedgley and Griffin, 1989). The slowly growing pioneer pollen tubes may also modify the stylar response which allows the later arriving self-pollen tubes to penetrate the ovary (Knox et al., 1986).

The 'mentor pollen effect' involves the mixing of incompatible pollen with compatible pollen from a compatible pollen source which results in the style accepting the incompatible pollen (Sedgley and Griffin, 1989; Visser, 1981). Both the mentor and pioneer effects increase the number of pollen grains on the stigma resulting in a population effect at germination. This population effect has been shown to overcome SI in *P. avium* (Arasu, 1968) and increased pollen tube growth rates in various fruit crops (Dogterom et al., 2000; Herrero, 1992; Tonutti et al., 1991) although the effects are not always positive.

1.2.2 Pollination and pollen-pistil interactions

Satisfactory fruit set and final yield of stone fruit, and especially sweet cherry, is dependent on the successful completion of a sequence of reproductive events. These can be divided into a number of stages: (1) availability of an adequate source of viable, compatible pollen, (2) effective transfer of pollen to receptive stigmas of a compatible cultivar, (3) pollen hydration and germination on the stigma, (4) pollen tube growth down the style and ovary, (5) pollen tube guidance into the ovule micropyle, and (6) delivery of the male gametes to the mature embryo sac (Thompson, 1996; Wilhelmi and Preuss, 1997). The sequence is completed with double fertilisation and the subsequent growth and development of the embryo (Williams, 1970). Various factors such as environmental conditions (Nyéki and Buban, 1996; Sedgley and Griffin, 1989), stigma receptivity (Egea et al., 1991; Hedhly et al., 2003), ovule viability (Cerović and Ružic, 1992; Postweiler et al., 1985), pollen tube kinetics (Herrero, 1992) and self-incompatibility (Shivanna, 2003b; Tehrani and Brown, 1992) influence the success of these pollen-pistil interactions.

Pollen grains undergo some hydration in the stamen during the final stages of maturation (Lord and Russell, 2002). Pollen will generally not germinate until it has reached the stigma of a flower from the same species but some cases of *in situ* (germination inside anthers) has been reported in apple and almond which can adversely affect fruit set (Koul et al., 1985). When the pollen lands on the wet stigmatic surface of sweet cherry (Uwate and Lin, 1981), it adheres (Lord and Russell, 2002). Proteins and enzymes making up the pollen wall are rapidly released upon rehydration, and are important for inititial pollen-pistil recognition and pollen germination (Sedgley and Griffin, 1989). In *P. avium*, large increases in stigma secretions occur at or shortly after anthesis, as a result of degeneration and collapse of stigma papillae (Uwate and Lin, 1981). The thin-walled papillae cells have been shown to completely collapse 4 to 5 days after anthesis, but still support pollen germination for up to 10 days after anthesis (Stösser and Anvari, 1983).

After hydration, pollen grains germinate, forming a pollen tube which grows through the stigma secretion, entering the stigmatic tissue between the papilla cells (Heslop-Harrison, 1987; Wilhelmi and Preuss, 1997). Pollen tube growth through the style and ovary is confined to the nutrient-rich, mucilaginous intercellular secretions of the transmitting tissue (Knox et al., 1986; Shivanna et al., 1997). In *P. avium*, separation and partial collapse of the transmitting tissue cells had little or no effect on pollen tube growth (Stösser and Anvari, 1983). These extracellular secretions increase in the transmitting tissue following pollination (Uwate et al., 1982).

The nutrient content of pollen grains is unable to support and sustain pollen tube growth to the ovule (Shivanna et al., 1997). Pollen tube growth has been shown to be biphasic: a slow initial, autotrophic phase sustained by the pollen reserves and an accelerated phase involving a change to heterotrophic metabolism (Herrero, 1992; Mulcahy and Mulcahy, 1983). The carbohydrate content of the stylar extracellular matrix has been reported to decline with the passage of pollen tubes, while starch remains unused in unpollinated flowers (Cheung, 1996; Herrero and Arbeloa, 1989). Stösser and Anvari (1983) showed an abundance of starch reserves in sweet cherry at anthesis, which then disappeared 4 to 6 days later, as the pollen tubes grew down the style.

Directional cues for pollen tube guidance in the style are also believed to include pistil-specific glycoproteins (Hererro and Hormaza, 1996). One of these, the transmitting tissue-

specific (TTS) glycoprotein has been shown to attract pollen tubes, as well as adhering to the tube tip and being incorporated into the walls of the elongating pollen tubes. A nutritive role for the TTS protein was also suggested (Cheung, 1996). Polar growth of the pollen tube itself consists of a complex signalling network regulated by a tip-focused gradient of cytosolic intracellular [Ca^{2+}] and Rop GTPases (Camacho and Malho, 2003; Higashiyama et al., 2003; Iwano et al., 2004). Calcium concentrations are highest in the tip and rapidly decrease towards the distal regions of the pollen tube (Wilhelmi and Preuss, 1997).

After leaving the style, pollen tubes enter the ovary and grow along the surface of the placenta towards the ovule and micopylar opening. On arrival on the placenta, the pollen tubes no longer have a predetermined path of exudates and glycoproteins with which to navigate across the ovary wall, and ovules become the essential signal for pollen tube guidance (Shivanna et al., 1997; Wilhelmi and Preuss, 1997). The guidance of the pollen tube into the micropyle of the ovule is under the control of chemotrophic factors (Knox et al., 1986). Recent evidence suggests that secretions from the two synergid cells play a critical role in this final guidance. The synergids, which flank the egg cell at the micropylar end of the embryo sac, contain high concentrations of Ca^{2+}. Before or on arrival of the pollen tube, one of the synergids degenerates, causing a sudden increase in [Ca^{2+}] in the micropylar region (Cheung, 1996; Higashiyama et al., 2003). The pollen tube enters the degraded synergid and releases two sperm cells, one which fertilizes the egg cell and the second fertilizes the central cell (Wilhelmi and Preuss, 1997).

In peach, a placental protuberance, the obturator, has been shown to delay pollen tube growth (Herrero and Arbeloa, 1989). Pollen tube growth only resumed 4 to 5 days later once the starch of the obturator was fully hydrolyzed with a concomitant secretion of carbohydrates and proteins (Arbeloa and Hererro, 1987). Similar secretions in the exostome and micropylar canal are a prerequisite for pollen tube penetration into the micropyle in peach (Hererro, 2000). Pollen tubes have also been shown to ‘wander’ in the ovary cavity of peach and sour cherry without penetrating the ovule (Cerović, 1996; Hererro, 2000). In sour cherry this was frequently followed by fluorescence of the ovule, indicating a loss of viability.

1.2.3 Effective Pollination Period (EPP)

The Effective Pollination Period (EPP) is defined as the period during which the embryo sac remains viable and functional for fertilizing minus the time required for the pollen tube to

reach the embryo sac (Williams, 1966). The EPP and flower quality has been shown to play an important role in fruit set, and has a greater impact limiting production in some fruit crops, than lack of sufficient pollen transfer (Williams, 1970) or flower quantity (van Zyl and Strydom, 1982), although fruit set in almond was determined by both flower quantity and EPP (DeGrandi-Hoffman et al., 1989). Duration of EPP has been estimated under orchard conditions by hand pollinating flowers at varying time intervals after anthesis and recording final fruit set or by microscopic examination of the pistil, fixed at intervals after opening (Stösser and Anvari, 1982).

The three main parameters influencing the EPP in temperate fruit crops are: stigmatic receptivity, pollen tubes kinetics and ovule longevity. These in turn, are influenced by various physiological and environmental factors such as cultivar, flower quality, plant growth regulators, nutrition and temperature (Sanzol and Hererro, 2001). Temperature is probably the most critical of these factors as it has an influence on all of the different stages of the reproductive process (Hedhly et al., 2004). The influence of stigma receptivity and pollen tube growth will be discussed with special reference to ovule longevity.

1.2.3.1 Factors influencing EPP

a) Stigmatic receptivity

Stigma receptivity is defined as the ability of the stigma to support viable, compatible pollen (Heslop-Harrison, 2000). It has been implicated as a factor limiting the EPP and fruit set in almond (Yi et al., 2006), apricot (Burgos et al., 1991; Egea et al., 1991), kiwifruit (González et al., 1995), pear (Sanzol et al., 2003), sour cherry (Furukawa and Bukovac, 1989) and sweet cherry (Guerrero-Prieto et al., 1985). Receptivity has been shown to vary depending on temperature (Hedhly et al., 2003), stage of flower development (Egea et al., 1991), duration (Bubán, 1996), time of day (Orosz Kovács, 1996), and the presence or absence of stigmatic exudate (Knox et al., 1986).

Factors influencing stigma receptivity

Temperature

Temperature influences stigma receptivity in sweet cherry with high temperatures (20-30°C) reducing receptivity while low temperatures (10°C) increase it (Hedhly et al., 2003). Duration of receptivity varies among different fruit crops. In apple flowers, papillae collapse occurred one to two days after anthesis (Bubán, 1996) while in sweet cherry papillae lost turgidity 1 to

2 days after anthesis and collapsed completely after 4 to 5 days (Stösser and Anvari, 1983). Surprisingly, germination and pollen tube entry into the stigma still occurred nine to ten days after anthesis.

Pistil maturity

Stage of flower development has been shown to influence stigma receptivity with peach and sweet cherry stigmas being most receptive at anthesis (Sanzol and Hererro, 2001). In apricot, stigmas were most receptive, 2 to 4 days after anthesis (Egea et al., 1991) and in almond, when flowers were at the fully open stage with flattened petals (Yi et al., 2006). Although delayed maturation of the stigma limits EPP in some fruit crops, early degeneration has most frequently been shown to limit EPP in sweet cherry (Guerrero-Prieto et al., 1985) and apricot (Egea et al., 1991).

b) Pollen tube kinetics

The rate of pollen tube growth in *Prunus* pistils is known to be highly variable depending on pistilar genotype (Egea et al., 1991; Hedhly et al., 2005; Hormaza and Herrero, 1999), pollen genotype (Guerrero-Prieto et al., 1985; Hedhly et al., 2004), pollen competition (Hedhly et al., 2005; Hererro and Hormaza, 1996) or environmental factors (Cerović and Ružić, 1992; Hedhly et al., 2004; Jefferies et al., 1982; Keulemans, 1984; Keulemans and Van Laer, 1989).

The pollen tube growth rate through the pistil is not uniform and is influenced by the pistil. The tube accelerates on entering the stylar transmitting tissue and decelerates on entering the ovary cavity (Herrero, 1992). In peach, this has been associated with the pollen tube stopping at the obturator, and growth only resuming once cells produce a characteristic secretion (Arbeloa and Hererro, 1987). A slowing of pollen tube growth at the base of the style has also been noted in avocado (Sedgley, 1979) and almond (Pimienta and Polito, 1983), although evidence suggests this is due more to immaturity of the embryo sac than a physical restriction (Sedgley and Griffin, 1989).

Factors influencing pollen tube kinetics

Temperature and pistil genotype

Temperature is the most important environmental factor influencing pollen performance and plays a significant role in pollen germination (Egea et al., 1992), and the rate of pollen tube growth *in vivo*, with higher temperatures accelerating pollen tube growth while lower

temperatures retard its growth rate (Sanzol and Hererro, 2001). This has been reported in a number of crops such as apricot (Austin et al., 1998; Pirlak, 2002), apples (Williams, 1970), plum (Jefferies et al., 1982; Keulemans and Van Laer, 1989), sour cherry (Cerović and Ružić, 1992) and sweet cherry (Guerrero-Prieto et al., 1985; Hedhly et al., 2004). In sour cherry, the best rate of pollen tube growth was recorded at 15-20°C, while higher (25°C) and lower (5°C) temperatures resulted in fewer pollen tubes reaching the stylar base.

Prevailing ambient temperatures influence the pollen genotype in sweet cherry with high temperatures (30°C) favouring pollen tube growth of 'Cristobalina' (adapted to warmer conditions of SE Spain) while low temperatures (10°C) favoured 'Sunburst', which originated in Canada, indicating a the temperature adaptation of the pollen donor (Hedhly et al., 2004). 'Summit' sweet cherry, which is full compatible with both genotypes, was used as the female recipient.

In a trial of four self-incompatible sweet cherry cultivars, used as both pollen donor and recipient, Hormaza and Herrero (1999) showed clear differences between genotypes in their capacity to support pollen tube growth in the style. Pollen attrition down the style was similar for all crosses while the rate of pollen tube growth was highest in 'Summit' and 'Vignola' compared to 'Bing' and 'Burlat' styles. Hedhly et al. (2005) also noted differences in performance of a single pollen donor, 'Bing', in eight different pistil genotypes.

Pollen competition

The number of pollen grains deposited on the stigma of many plants species often greatly exceeds the number of ovules available for fertilization (Erbar, 2003; Hormaza and Hererro, 1994), particularly in ovule-limited species such as *Prunus* (Hormaza and Herrero, 1996). Plants have thus developed a means of natural selection among male gametophytes in pistilar genotypes which results in the dramatic attrition of pollen tubes as they grow towards the ovule, with only the fastest growing pollen tubes achieving fertilization (Erbar, 2003; Hedhly et al., 2005). This pollen competition has been shown to influence both pollen germination on the stigma, and pollen tube growth in the style (Hormaza and Herrero, 1996; Ter-Avanesian, 1978).

A positive correlation was found between number of pollen grains deposited on the stigma and the rate of pollen tube growth in the style of plum (Lee, 1980). Tonutti et al. (1991) found

that double pollination of 'Mora di Cazzano' sweet cherry extended the EPP from 2 to 3 days. This was attributed to faster pollen tube growth as a result of increased competition, due to a larger pollen load on the stigma.

Hormoza and Herrero (1996) found that under different pollen competition regimes, the number of pollen tubes at each stylar level was dependent on the size of the initial size of the viable pollen load, and the number of pollen tubes was reduced by the same proportion at each stylar level. The final number of tubes reaching the ovary cavity were very similar, and thus independent of initial pollen number.

In sweet cherry, a positive correlation was found between the number of pollen grains deposited on the stigma and germination percentage (Hormaza and Herrero, 1996). In *Petunia*, little or no germination occurred at pollen populations of less than 10 pollen grains while up to 75.2% germination was obtained at populations greater than 300 grains (Brewbaker and Majumder, 1961). Research on 'Bing' sweet cherry (Mayer et al., 1987) and 'Bluecrop' highbush blueberry (*Vaccinium corymbosum* L.) (Dogterom et al., 2000) have shown that they require approximately 100 and 125 pollen grains respectively to set a good fruit.

c) Ovule longevity

The viability of ovules and the embryo sac may be a limiting factor for fertilization and fruit set in fruit trees (Stösser and Anvari, 1982). Ovule viability plays an important role in the effective pollination period (EPP) with a number of crops showing poor fruit set due to a shortened EPP (Sanzol and Hererro, 2001). This is particularly evident in stone fruit such as cherries (Eaton, 1959; Eaton, 1962; Postweiler et al., 1985; Stösser and Anvari, 1982) and plums (Cerović et al., 2000) which contain two ovules in the ovary. The secondary ovule degenerates soon after pollination, resulting in only the primary ovule being available for fertilization (Pimienta and Polito, 1982; Postweiler et al., 1985). The duration of ovule viability in cherries varies considerably depending on the method of assessment, cultivar and temperature during anthesis (including pre- and post-anthesis) (Thompson, 1996).

Using the technique of fluorescent microscopy one is able to determine the viability of ovules at an early stage with aborted ovules developing an intense blue-green fluorescence while functional ovules exhibit low fluorescence or auto-fluorescence only (Anvari and Stösser,

1978a). Fluorescence is due to callose accumulation in nucellar and integument cells (Knox et al., 1986).

Factors influencing ovule longevity

Temperature

Temperature plays a significant role in the duration of ovule longevity with high temperatures shortening the period of ovule viability while lower temperatures increase its longevity (Cerović and Ružic, 1992; Postweiler et al., 1985). Ovule viability in sweet and sour cherries has been shown to vary from 1 to 2 days, at a constant temperature of 20°C and up to 5 days at 5°C (Postweiler et al., 1985). In sour cherries, viability varied from three to four days (25°C) up to nine days at 5°C (Cerović and Ružic, 1992). In a field trial conducted in western Oregon, 'Napoleon' sweet cherry ovules were shown to still be functional, 13 days after anthesis, at an average temperature of 10.6°C (Guerrero-Prieto et al., 1985). Temperatures up to 3 weeks after anthesis may influence the rate of embryo sac development and abortion in 'Italian' prune (Thompson and Lui, 1973). Beppu et al., (2001b) showed that the percentage of ovules with degenerated embryo sacs increased more rapidly at 15°C than at 25°C within 2 days of anthesis.

Cultivar

Differences in ovule viability and stage of development of the embryo sac at anthesis have also been noted between cultivars of the same species and between species. Ovule senescence in the plum cultivar 'Italian' was shorter than in 'Brooks', possibly as a result of a "stronger" flower genotype (larger flowers, earlier bloom) (Moreno et al., 1992). Cultivar differences were also shown to influence ovule viability in sweet cherry (Stösser and Anvari, 1982) with the functionality of the embryo sacs, at and shortly after anthesis, influencing their viability (Eaton, 1962).

In pears, viable embryo sacs in unpollinated flowers have been observed up to 15 days after full bloom (Herrero and Gascon, 1987) while in 'Brooks' plum, 80% of flowers still had viable ovules 20 days after anthesis (Moreno et al., 1992).

Defoliation

Ovule viability of 'Satohnishiki' sweet cherry has been shown to be influenced by autumn defoliation (Beppu et al., 2003). Defoliation reduced ovule longevity, possibly as a result of reduced carbohydrate reserves causing abnormal flower development.

Cross pollination

Cross pollination has been shown to influence ovule maturity and embryo sac development. Cross pollination in fruit crops induces various biochemical reactions in the pistilar tissues resulting in the prolonging of embryo sac viability (Herrero, 1992). Unpollinated apricot flowers showed more gradual and slower megagametophyte development than in pollinated flowers (Burgos and Egea, 1993), while a similar effect was noted in self- and cross-pollinated almond flowers (Pimienta and Polito, 1983). In pears, cross pollination did not affect embryo sac development but prolonged embryo sac viability by up to 10 days compared to the unpollinated flowers (Herrero and Gascon, 1987).

Winter chilling

Low productivity of some high chill apricot varieties grown in the Mediterranean region may be related to insufficient winter chilling (Legave, 1978b). Various floral anomalies such as short pistils and aborted or necrotic ovaries were observed in 'Reale d'Imola' apricot which possibly had a negative influence on ovule development (Guerriero et al., 1986). Winter chilling may influence the stage of ovule maturity at anthesis (and thus ovule longevity) with ovules from colder areas being slightly more mature, although it does not fully explain differences in fruit set (Egea and Burgos, 1998).

Nutrition

Improved nutritional status has been shown to improve ovule longevity and fruit set in fruit crops. Summer (soil) and post harvest (foliar) applied N fertilizer to apples (Williams, 1965) and pears (Khemira et al., 1998) respectively, resulted in significant increases in ovule longevity and fruit set.

Plant growth regulators

Various plant growth regulators and polyamines (putrescine, spermine, spermidine) when applied in autumn or at bloom, have succeeded in improving ovule longevity in fruit crops (Sanzol and Hererro, 2001). Applications of GA_3, at bloom, reduced ovule viability in cherry flowers (Beppu et al., 2005; Beppu et al., 2001b; Stösser and Anvari, 1982), while prolonging embryo sac viability in ‘Agua de Aranjuez’ pear (Herrero and Gascon, 1987). Endogenous GA_3, secreted during the period of rapid pollen tube growth, may be translocated to the ovule, increasing the longevity of the embryo sac (Herrero, 1992). Autumn applications of the GA-biosynthesis inhibitor, paclobutrazol, prolonged embryo sac viability in sweet cherry the

following spring (Beppu et al., 2005). Ethylene (ethephon or ethrel) applied at bloom reduced ovule viability in cherry flowers (Stösser and Anvari, 1982), while autumn applications to 'Italian' plum delayed ovule senescence, but had little effect on 'Brooks' flowers (Moreno et al., 1992).

Exogenous applications of polyamines have been shown to improve ovule longevity in apricot (Alburquerque et al., 2006) and pear (Crisosto et al., 1992) while higher endogenous polyamine contents have been associated with improved ovule viability in apricot (Alburquerque et al., 2006) and sour cherry (Schoonjans et al., 1989). Polyamines are involved in the stimulation of cell division and differentiation of floral organs (Evans and Malmberg, 1989) but their role in enhancing ovule longevity appears to be due to the improved nutritional status of the flower (Sanzol and Hererro, 2001).

1.3 DORMANCY, CHILLING & REST BREAKING AGENTS

Insufficient winter chilling is a major limiting factor to the production of deciduous fruit under warm climatic conditions (Allan, 1999; Couvillon, 1995). These low-chill conditions negatively impact on the yield potential of high-chill stone fruit such as sweet cherry. Most areas in South Africa are climatically marginal for the production of sweet cherries which have a chilling requirement exceeding 1100 Utah chill units (Mahmood et al., 2000). The sweet cherry cultivar 'Bing', which has been widely planted in new cherry plantings in the eastern Free State, Western Cape, Mpumalanga and KwaZulu-Natal regions, has been slow to come into production.

High day temperatures (>19°C) during winter, have been shown to cancel out the beneficial effect of night temperatures below 12°C which promote the accumulation of winter chilling (Erez, 1995). Since all deciduous fruit trees require a certain amount of chilling during winter to enable buds to break evenly, a lack of winter chilling can have an adverse effect on bud burst, resulting in delayed foliation, poor flowering and reduced fruit set (Couvillon, 1995; Martin, 1991). This prevention of normal bud burst is often associated with necrosis of floral buds. Stone fruit floral buds always die and abscise if they do not open in spring while apple buds can remain viable for up to a year (George and Erez, 2000).

Prolonged dormancy in warm winter regions has become an obstacle to the economic production of stone fruit crops in these areas (Faust, 2000). Artificial means to compensate for

this lack of natural chilling has become an important factor in orchard management. A better understanding of dormancy release has allowed producers to use various cultural practices and rest breaking agents to manipulate the dormant period to increase budburst and obtain more even flowering in spring (Erez, 1987), enabling the expansion of temperate crops into marginal production areas.

1.3.1 Dormancy

Dormancy in deciduous fruit and nut trees of the temperate zones is a phase of development that occurs annually, resulting in a cessation of growth which allows them to survive unfavourable winter temperatures (Saure, 1985). Lang (1987) defined dormancy as "the temporary suspension of visible growth of any plant structure containing a meristem". Thus the focal point of dormancy and its regulation is the bud. In the dormant state, the tree is able to resist low temperatures but once buds start to grow in spring, resistance is lost (Martin, 1991).

The various stages of bud dormancy have been classified as follows (Lang et al., 1987):

- Paradormancy: buds are dormant as a result of correlative signals which originate elsewhere in the plant, e.g. apical dominance.
- Endodormancy: dormancy is regulated by factors within the bud itself. Winter chilling is necessary for endodormant buds to exit dormancy.
- Ecodormancy: cessation of bud growth due to unfavourable environmental factors, e.g. temperature, water, nutrients.

In temperate climates, buds of deciduous fruit trees enter a state of endo-dormancy in autumn as a result of environmental cues and will only exit this state once they have accumulated a certain amount of low temperature (Couvillon, 1995). Studies have indicated that low temperature is responsible for both the induction and release from endodormancy (Crabbé and Barnola, 1996). Heide and Prestrud (2005) demonstrated that low temperature (<12°C), and not photoperiod, is required for growth cessation and endodormancy induction in apples and pears while temperatures of 6 or 9°C are necessary for endodormancy release, and growth resumption in spring. Under mild winter conditions, buds may not receive their "correct" environmental cues to enter dormancy, with temperatures which normally promote chilling satisfaction enhancing dormancy (Cook and Jacobs, 2000).

The dynamics of dormancy induction, maintenance and release are still poorly understood in deciduous fruit trees grown in temperate climates, and particularly in regions with mild winter climates. Without an understanding of the temperatures influencing dormancy, it is difficult for researchers and producers to improve the management of dormancy release or to calculate the actual state of bud dormancy which is essential for proper timing of various cultural practices.

1.3.2 Chilling Requirement

Vegetative and floral buds of deciduous fruit trees must be exposed to low temperature for a certain minimum period to complete dormancy with the intensity of the chilling requirement being genetically determined (Martin, 1991; Saure, 1985). The effectiveness of chilling temperatures and the optimum temperature for chilling accumulation and the termination of endodormancy varies between species as well as between cultivars of the same species. Differences in chilling also exist between buds, with flower buds having a lower chilling requirement than vegetative buds (Martin, 1991).

Various chill unit models have been used to quantify chilling accumulation (Richardson et al., 1974; Shaltout and Unrath, 1983; Weinberger, 1950) but were found to be inaccurate under warm winter conditions (Erez et al., 1990; Linsley-Noakes and Allan, 1994). This led to the development of the Dynamic model (Fishman et al., 1987) and “Daily Positive chill unit model”/Infruitec model (Linsley-Noakes et al., 1994) which were improved models for peaches grown under these conditions (Erez et al., 1990).

Insufficient chilling is a common problem when growing temperate zone fruit crops in areas with warm winter climates (Couvillon, 1995). The chilling requirement of floral and vegetative buds is seldom fully satisfied under these low chill conditions leading to a condition termed delayed foliation (Jacobs et al., 2002; Saure, 1985). Typical symptoms include delayed vegetative and floral bud break, poor fruit set, sparse bud break levels and bud break spread over an extended period, resulting in fruit and flowers occurring on the same shoot. In severe cases, a total lack of bud break may occur (Erez, 1995).

Effective temperatures

Richardson, Seeley and Walker (1974) reported that temperatures between 2 and 9°C were the most beneficial in completing chilling in peach buds, with a maximum effect between 6 and

8°C (Erez and Couvillon, 1987). The upper limit of the chilling effect varies between 12.5°C (Richardson et al., 1974) and 16.5°C (Shaltout and Unrath, 1983), while temperatures above 18°C negate chilling depending on the duration and level of high temperatures (Erez, 1995). Chilling temperatures at or below -2°C and above 13°C had no effect and are not necessary to complete the chilling requirement and terminate endodormancy in peach (Erez and Couvillon, 1987). The optimum temperatures for chilling accumulation in sweet cherries was shown to vary between 3.2 and 3.7°C, with temperatures above 12°C being ineffective at breaking endodormancy (Mahmood et al., 2000).

Moderate temperatures are most effective in promoting bud break if applied in conjunction with chilling in a diurnal cycle (Erez, 1995). Temperatures of 13-15°C for 8 hours had a strong synergistic effect when alternated in diurnal cycles with low temperatures of 6°C for 16 hours (Erez and Couvillon, 1987). Chilling was most effective when these intermediate temperatures were applied during the final third of the chilling process. In sour cherries, a daily cycle of 5/15°C for 16/8 hours was found to be as effective as exposure to continuous 5°C (Felker and Robitaille, 1985).

The degree of chilling negation is dependent on the cycle length, level and duration of high temperatures during a cycle (Couvillon, 1995). Couvillon and Erez (1985) showed that high temperatures (19-23°C) applied for 6-8 hours in a diurnal cycle with 4°C resulted in nearly complete chilling negation in peach buds. The effect was greatest when applied during the early stages of the chilling period, with no negation recorded if three-quarters of the chilling requirement had accumulated.

1.3.3 Rest breaking agents (RBAs)

The interest in the artificial control of bud break of deciduous fruit trees is closely linked with the attempt to grow these species commercially in warm climates which have insufficient chilling (Erez, 1987). Most areas in South Africa are climatically marginal for the production of pome fruit, and high chill stone fruit such as sweet cherries which have a high chilling requirement. This led to chemical rest-breaking treatments being applied annually in the main apple producing areas to compensate for insufficient chilling (Terblanche and Strydom, 1973) and reduce symptoms of delayed foliation.

The response to the application of RBAs is often inconsistent as the efficacy of the active ingredient is dependent on the stage of bud development and rate of chemical applied (Fuchigami and Nee, 1987), method of application (George and Nissen, 1988), and prevailing environmental conditions (Erez, 1979). This is particularly important in *Prunus* species with separate floral and vegetative buds which are more sensitive to chemical toxicity than the mixed buds of pome fruit (Erez, 1987). Flower buds are more sensitive than vegetative buds, with flower bud toxicity and bud drop a common symptom.

Various RBAs have been used commercially worldwide including dinitro-o-cresol (DNOC) plus oil, thiourea, potassium nitrate, cyanamides and growth regulators with varying success (Erez, 1987; Erez, 1995). Research has shown that these chemicals can substitute for part of the chilling requirement, depending on the RBA, fruit type and cultivar grown (Erez, 2000).

1.3.3.1 DNOC plus oils

Simple plant and animal oils, and later mineral oils were used to compensate for a lack of chilling and break dormancy. Mineral oil was later combined with dinitro-o-cresol (DNOC) and has been the most widely used RBA in deciduous fruit orchards since the 1940's (Samish, 1945). It was recommended as a standard annual application in South African pome fruit orchards (Honeyborne, 1993) up until 2001 when it was withdrawn in South Africa as a result of its toxicity to humans and the environment (North, 2003).

The combination of oil and dinotrophenols acts as a powerful phosphorilation uncoupler (Samish, 1945) which causes temporary anaerobic conditions in the buds, leading to ethanol production and dormancy breaking. High day temperatures at, and the week following application, are vital to obtain a good rest-breaking effect (Erez, 1979).

1.3.3.2 Cyanamides

Calcium cyanamide was first used in Japan as a dormancy breaking agent in apples and grapevine buds (Kuroi et al., 1963). Its paste-like form and high concentrations, prevented its widespread commercial use (Erez, 1987). The discovery of hydrogen cyanamide, the supernatant of an aqueous suspension of $CaCN_2$, resulted in cyanamide becoming available as a commercial formulation of 50% H_2CN_2 (Shulman et al., 1986). The chemical, which was originally produced as a herbicide, has been shown to be effective in breaking dormancy in a number crops such as apple (North, 1992; Sagredo et al., 2005), apricot (Bartolini et al.,

1997), blueberry (Williamson et al., 2002), kiwifruit (McPherson et al., 2001), nectarine (George and Nissen, 1988), high-chill peach (Siller-Cepeda et al., 1992b), sweet cherry (Costa et al., 2004; Martínez et al., 1999; Snir and Erez, 1988) and table grapes (Lombard, 2003; Shulman et al., 1983).

a) Cyanamide metabolism and mode of action

Hydrogen cyanamide (HCN), when applied at sub-lethal doses, has been shown to inhibit or reduce catalase activity in grapevine (Shulman et al., 1986) and apricot buds (Bartolini et al., 1997), a response similar to chilled plant tissues (Patterson et al., 1984). Catalase plays an important role in plant metabolism by catalysing hydrogen peroxide to oxygen and water. The inhibition of catalase by HCN results in the plant detoxifying hydrogen peroxide via a chain of reactions which link with the pentose phosphate pathway, leading to an increased turnover of the pathway (Bichler, 1999).

Increased hydrogen peroxide levels, as a result of catalase inhibition, may increase levels of glutathione in the bud. Siller-Cepeda et al. (1992a) showed that HCN applied to peach buds, caused an initial decrease in glutathione content followed by a large increase, 24 hours after application. The concentration of glutathione was inversely proportional to the concentration of HCN applied. Exogenously applied glutathione had little or no influence on rest breaking of apple, pear or peach buds (Bennett et al., 1940).

b) Timing and rate of application

The correct timing and rate of application of HCN varies depending on the fruit genotype and cultivar (Fuchigami and Nee, 1987). HCN has been shown to promote early and more uniform bud break, and advance fruit maturity in a number of crops (Erez, 2000). It is particularly useful for advancing bloom and synchronising blossom among compatible cultivars (Erez, 1987). In sweet cherry, early applications advanced bloom of ‘Sam’ and ‘Rainier’ by up to 13 days while later ones resulted in a more compressed bloom period (Snir and Erez, 1988). Fruit maturity in ‘Sam’ was advanced by 2 weeks. A similar effect has been observed in apple (Bound and Jones, 2004) and peach (Siller-Cepeda et al., 1992b) buds with late applications delaying flowering whereas early ones advanced budburst and flowering.

The time of application has been shown to be more effective than concentration at advancing bloom and ripening time in ‘Burlat’ sweet cherry (Pasciano et al., 1997). Applications 53 days

before full bloom advanced flowering and ripening by 3 and 6 days, respectively, while no differences were noted between concentrations of 1, 2.5 and 4% HCN.

Fuchigami and Nee (1987) indicated that the influence of HCN on percentage bud break and phytotoxicity is dependent on the timing or physiological stage of the bud. Too early applications often being ineffective or late applications, close to natural bud burst, resulting in flower bud phytotoxicity (NeSmith, 2005). This effect is particularly evident in stone fruit crops (George and Nissen, 1988; Siller-Cepeda et al., 1992b), although sweet cherry buds appear to be far less sensitive, possibly due to better protection of the bud initials (Erez, 1995).

HCN has a marked effect on vegetative buds (Erez, 1987). Competition between vegetative and reproductive buds sometimes develops under conditions of advanced leaf development. This increased sink competition from excessive vegetative bud break negatively affects fruit set, and is typical of high concentrations of cyanamide applied to some stone fruit species (Erez, 2000). Trials on apricot (Bartolini et al., 1997), peach (Fernandez-Escobar and Martin, 1987; Siller-Cepeda et al., 1992b), kiwifruit (Richardson et al., 1994) and rabbiteye blueberry (Williamson et al., 2002; Williamson et al., 2001), showed that vegetative bud break was significantly enhanced by HCN with bud break increasing with HCN concentration, although flower bud phytotoxicity was most evident at the higher concentrations (1.5 to 2.0%) in the stone fruit and blueberries.

Leaf buds in stone fruit (Couvillon, 1995) and rabbiteye blueberries usually emerge after floral buds, as a result of their higher chilling requirement, leading to delayed canopy development. In years of low chilling and high fruit set, leaf emergence can be delayed for several weeks, resulting in very low leaf to fruit ratios, and ultimately low fruit set (Williamson et al., 2002; Williamson et al., 2001).

Monitoring of the stage of dormancy, using chilling models, is vital to prevent flower damage as resistance to HCN declines rapidly as buds are released from endo-dormancy (Erez, 1995). Cyanamides can only substitute for approximately 30% of the chilling requirement of a cultivar (Erez, 2000). Research conducted in California has shown HCN to be effective in breaking dormancy in ‘Bing’ sweet cherry when 49-57 Chilling portions (CP) (Fishman et al., 1987) had accumulated (Glozer, 2006). Erez (pers. comm.) reported that ‘Burlat’ and ‘Sam’

were responsive to HCN when approximately 42 and 70 CPs, respectively, had accumulated. In 'Tirinto' apricot, buds only became responsive to HCN once 500 Utah Chill units had accumulated (Bartolini et al., 1997).

c) Temperature effect

An interaction between HCN and temperature has been observed in stone fruit crops. Erez (1985) showed that HCN is temperature-dependent with the least damage to peach flower buds occurring at temperatures between 14 and 18°C with higher and lower temperatures enhancing the effect. In apples, the rest breaking response was associated with higher temperatures during and a few days after application (North, 1993).

1.3.3.3 Growth regulators

Cytokinins and gibberellic acid are known to break bud dormancy in some deciduous crops by substituting for part of the chilling requirement, but the high concentrations required invoked significant costs (Erez, 1987; Erez, 1995). A combination of the two, namely Promalin® (benzyladenine + GA_{4+7}) was effective in promoting flower and vegetative bud break in apricot and plum (Son and Kuden, 2005). Promalin® has also been shown to be highly effective at promoting bud burst, and inducing branching in young sweet cherry (Jacyna and Puchała, 2004; Miller, 1983) and apple trees (Erez, 1987).

In the mid 1980's, a cytokinin-like growth regulator, thidiazuron (*N*-phenyl-*N*'-1,2,3,-thidiazol-5-ylurea) (TDZ), was shown to improve bud burst in apples (Wang et al., 1986). Trials with peaches (Boozer and Pitts, 2002), pears, cherries and plums (Costa et al., 2004) showed similar effects, although Steffens and Stutte (1989) showed TDZ to be more effective in promoting bud burst when applied at the start of chilling period in apples.

The dormancy breaking effect of TDZ appears to be correlated with increases in RNA, DNA, S-adenosylmethionine (SAM) and protein with a corresponding acceleration in polyamine synthesis (Wang et al., 1986). Their studies indicated that TDZ has a pronounced influence on the release of lateral buds from dormancy in apple plants. It appears that TDZ is relatively immobile and stable in plant tissues and is not translocated in the apple plant as untreated buds on sprayed plants remained dormant after treatment (Steffens and Stutte, 1989).

1.3.3.4 Other chemicals (KNO_3, Thiourea, Armobreak®)

Potassium nitrate (KNO_3), alone or in combination with thiourea, has been shown to act as effective RBAs in apple (North, 1992), apricot (Küden and Son, 1997), nectarine (George and Nissen, 1988) and sweet cherry (Küden et al., 1997). KNO_3 is particularly effective in improving floral budbreak, especially in certain peach cultivars where abnormal flower development may occur (Erez, 1987). KNO_3 is a mild RBA when used alone, but in combination with Armobreak® (fatty amine polymer)(George et al., 2002; North, 1992) or Acer® (alkoxylated fatty amine)(North, 1995), has been shown to have considerably improved penetration and efficacy.

Thiourea has a strong phytotoxic effect on flower buds and was found to be effective mainly on leaf buds (Erez and Zur, 1981). It has subsequently been banned due to its mammalian phytotoxicity.

North (2003) has shown that various mixtures of inorganic (urea ammoniated nitrate and calcium ammonium nitrate) and organic nitrogen sources (choline chloride), combined with Acer®, have been effective at enhancing bud break in 'Golden Delicious' apples.

CONCLUSIONS

The quality of 'Bing' sweet cherry flowers begins with the initiation and differentiation of floral buds the previous summer. The buds then enter dormancy with all floral parts in a visible, immature stage (Diaz et al., 1981) with final ovule and pollen grain development taking place in spring before and at anthesis. A number of factors such as the environment (summer temperatures, winter chilling), plant growth regulators, and cultural practices influence these physiological processes. The time of floral initiation and development, under low chilling conditions in South Africa is unknown, and needs to be quantified in future research.

Pollination success in *Prunus* species is influenced by a number of factors ranging from weather conditions (pre-anthesis, during and post anthesis), flower quality, bloom synchrony, self-incompatibility, tree age and nutrition, and the complex interactions between these factors. Most sweet cherry varieties, including 'Bing' are gametophytically self incompatible

resulting in the inhibition of self- and cross-incompatible pollen tube growth in the upper third of the style (Tehrani and Brown, 1992). Cultivars require the efficient transfer of viable pollen from a compatible cross pollinizer/s, flowering synchronously with the main cultivar, to ensure adequate cross pollination and fertilization. Temperature and pollen-pistil interactions play an important role in the dynamics of pollen tube growth and ovule viability in sweet cherry and have a significant influence on fertilization and fruit set. No documented research has been conducted in South Africa on the most suitable pollinizers for 'Bing' as well as the climatic conditions affecting ovule longevity and pollen tube growth and their influence on the effective pollination period and final fruit set. Knowledge of the above factors will allow the correct advice on the most suitable pollinizer/s for 'Bing' grown under South African conditions.

Insufficient winter chilling is a major factor limiting the production of high-chill stone fruit such as sweet cherries grown under warm South African conditions. Typical symptoms include delayed vegetative and floral bud burst, floral abortion and bud drop resulting in poor flowering and fruit set. Artificial means to compensate for this lack of natural chilling has lead to the introduction of various cultural practices and RBAs to manipulate the dormant period and thus improve bud burst and flowering in the spring. Various RBAs such as HCN have been tested on sweet cherries worldwide but very little research has been conducted in South Africa. No data is available on the timing of HCN, alone or in combination with mineral oil, and thidiazuron plus mineral oil, on bud burst, flowering and fruit set in 'Bing' sweet cherry.

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2. PAPER 1: CROSS POLLINATION AND THE INFLUENCE OF TEMPERATURE ON POLLEN TUBE GROWTH AND OVULE LONGEVITY IN 'BING' SWEET CHERRY

Abstract

The cultivation of high-chill sweet cherry cultivars in areas receiving insufficient winter chilling often leads to poor fruit set. Among the possible explanations for this phenomenon are poor flowering, asynchronous flowering of pollinizers, low pollen production and flower abnormalities. Experiments using 2- to 3-year-old 'Bing' sweet cherry trees were conducted in the eastern Free State, South Africa, during the 2005 and 2006 seasons to determine the most suitable cross pollinizer/s for 'Bing' and to assess the influence of temperature and pollen-pistil interactions on pollen tube growth and ovule longevity. Significant differences in pollen germination occurred among pollinizers, although differences were noted in pollen performance on the stigma and style (*in vivo*) compared to the artificial media (*in vitro*), indicating a lack of correlation between *in vitro* germination and *in vivo* pollen-pistil interactions. Pollen tube growth, following cross pollination, was influenced by pollinizer genotype, temperature, and the number of pollen grains deposited on the stigma. The highest pollen tube growth rates in 'Bing' styles were recorded for the pollinizers 'Black Tartarian' (2006), 'Lapins' and 'Rainier' sweet cherries at temperatures of ~21°C. Temperature had the most significant influence on ovule longevity with the lower orchard temperatures extending ovule viability compared to the higher laboratory temperatures, although pollen tube growth rates were also reduced, thus shortening the effective pollination period. Cross pollination was also shown to extend ovule viability. The results indicate that 'Black Tartarian', 'Lapins' and 'Rainier' sweet cherries were the most suitable pollinizers for 'Bing'. Hand-pollination with pollen from these donors resulted in a several-fold increase in seed set over naturally-pollinated control flowers. It appears that the principle factors causing poor fruit set in 'Bing' sweet cherry are premature abortion of the ovule before fertilization and inadequate transfer of sufficient viable pollen under orchard conditions.

Key words: Cross pollination; ovule viability; pollen tube growth; *Prunus avium*; sweet cherry

Introduction

'Bing' is the main sweet cherry cultivar grown in South Africa and has a reputation for poor and irregular bearing, often resulting in very low yields. The cultivation of sweet cherries in warm climates which receive insufficient winter chilling often leads to low pollen production, pistil malformation (Oukabli and Mahhou, 2007), poor budburst and flowering (Iezzoni et al., 1990), while weather conditions during flowering impact on stigma receptivity, pollen tube dynamics and ovule viability, ultimately influencing fruit set (Sanzol and Hererro, 2001).

Adequate fruit set in sweet cherries is dependent on the successful completion of a sequence of reproductive events that take place during the progamic phase (Hedhly et al., 2004). This starts with the availability of an adequate source of viable, compatible pollen, the effective transfer of the pollen to a receptive stigma and pollen tube growth down the style and into the viable ovule. The sequence is completed with fertilisation and the subsequent growth and development of the embryo and fruit (Williams, 1970).

Most sweet cherry cultivars, including 'Bing', are gametophytically self-incompatible (GSI) and cross-incompatible causing the arrest of pollen tube growth within the top third of the stylar tissue (Tehrani and Brown, 1992). Sweet cherries have been classified into 22 compatibility groups with cultivars having the same two S-alleles being assigned to the same group (Iezzoni et al., 2005). GSI has resulted in modern orchards being inter-planted with compatible cultivars or pollinizers whose bloom times overlap and flower synchronously with the main cultivar (Nyéki et al., 2003; Thompson, 1996). Newly planted 'Bing' orchards in South Africa are frequently planted with four or more pollinizers due mainly to a lack of knowledge of suitable cross compatible cultivars and the variability in their time of bloom in relation to 'Bing' (personal observation).

The short period of flower receptivity and pollen-pistil interactions often limits fruit tree productivity and fruit set leading to the concept of the effective pollination period (EPP) (Sanzol and Hererro, 2001). EPP is defined as the period during which the embryo sac remains viable and functional minus the time required for the pollen tube to reach and fertlize the egg cell (Williams, 1970). The viability of the embryo sac and ovules plays a significant role in fruit set with a number of crops showing poor fruit set due to a shortened EPP. This is particularly evident in stone fruit such as cherries (Beppu et al., 2001; Eaton, 1959; Postweiler et al., 1985; Stösser and Anvari, 1982) and plums (*Prunus domestica* L.) (Cerović et al.,

2000) which contain only two ovules in the ovary. The secondary ovule degenerates soon after pollination, leaving only the primary ovule available for fertilization (Pimienta and Polito, 1982; Postweiler et al., 1985).

Temperature plays an important role in the duration of the EPP in *Prunus* by influencing stigma receptivity (Egea et al., 1991; Hedhly et al., 2003), ovule longevity (Cerović and Ružic, 1992; Cerović et al., 2000; Guerrero-Prieto et al., 1985; Postweiler et al., 1985) and pollen tube growth (Cerović and Ružić, 1992; Guerrero-Prieto et al., 1985; Hedhly et al., 2004). High temperatures have been shown to shorten the period of ovule viability while lower temperatures increased its longevity (Cerović and Ružic, 1992; Postweiler et al., 1985). Beppu et al. (2001) showed a rapid degeneration of the embryo sac and ovule viability in sweet cherry flowers kept at 25°C, resulting in markedly reduced fruit set.

The influence of temperature on the rate of pollen tube growth has been reported in apricot (Austin et al., 1998), plum (Keulemans and Van Laer, 1989), sour cherry (Cerović and Ružić, 1992) and sweet cherry (Hedhly et al., 2004). High temperatures (>20°C) accelerate pollen tube growth while lower temperatures decrease it. Thus high temperatures during bloom accelerate pollen tube growth rates, but shorten the EPP by decreasing ovule longevity (Sanzol and Hererro, 2001).

The number of pollen grains deposited on the stigma of *Prunus* species usually greatly exceeds the number of ovules available for fertilization (Hormaza and Herrero, 1996). Competition between pollen grains and pollen-pistil interactions have been shown to influence both pollen germination on the stigma and pollen tube dynamics in the style (Hormaza and Hererro, 1994; Ter-Avanesian, 1978). A positive correlation was found between number of pollen grains deposited on the stigma and percentage germination in sweet cherry (Hormaza and Herrero, 1996) while a similar positive correlation was found between pollen number and the rate of pollen tube growth in the style of plum (Lee, 1980).

The objectives of this study were, firstly, to determine the most suitable cross pollinizer/s for ‘Bing’ sweet cherry by means of controlled laboratory and orchard cross pollination trials. Secondly, to determine the influence of temperature on ovule longevity and pollen tube growth and their effect on fertilization in the laboratory trials, and fertilization and fruit set in the orchard trial, using fluorescence microscopy.

Materials and methods

Experiments were conducted during the 2005 and 2006 seasons at Ash Creek farm, Clarens (28°28'S; 28°19'E, 1860 m.), eastern Free State, South Africa. In 2005, the experiment was performed in a laboratory on 2- to 3-year-old 'Bing' sweet cherry branches. In 2006, the experiments were performed in both a laboratory and in a commercial orchard, on 2- to 3-year-old 'Bing' branches on 5-year-old (6^{th} leaf) 'Bing' sweet cherry trees on 'Gisela® 5' rootstock. Orchard trees were spaced 4.5 x 2.0 m and were trained to a modified central leader. Pollinizer trees were spaced every fifth tree within the row. Temperature and relative humidity data were measured in the laboratory, and in the orchard at a height of 1.5 m, using Tinytag Plus sensors (Gemini Data Loggers, UK). In the 2006 orchard trial, 6 honey bee hives per hectare were introduced into the orchard at 5-10% bloom and a further 4 hives per hectare were placed in the orchard at 50-60% bloom to determine the effects of natural pollination. An indication of bee activity was done by counting the number of bees visiting a branch per 10 minutes. Counts (n=6) of approximately 10 to 12 bees per branch per 10 minutes were obtained with 80% being nectar feeders and 20% pollen gatherers.

Pollen collection and storage

Flowers were collected at the balloon or first white stage (Stage 6) (Ballard et al., 1982) from healthy 4- and 5-year-old pollinizer trees in a commercial orchard at Ash Creek farm, Clarens. 'Stella' trees were forced to flower early by means of the earlier application of 1% hydrogen cyanamide and by individually covering the trees with polyethylene covers. The anthers from each pollinizer cultivar were carefully separated from the flowers immediately after collection, and placed in separately labelled open petri-dishes. Anthers were air-dried in a warm (20 to 22 °C), wind free place for 24 to 48 hours until anther dehiscence. Anthers and pollen were then placed into labelled, loosely stoppered glass vials. These were in turn placed in a small glass dessicator, held over a dessicant, and kept in the refrigerator at ~4 °C until used 1 to 2 days later. The collected pollen was used for *in vitro* pollen germination tests and for hand pollination in the laboratory and orchard.

2005 Season

Pollen germination

Pollen samples were left at room temperature (~75-85 % RH) for 6 hours to rehydrate before determining viability. Agar plate preparation was adapted from the procedure described by Cerović and Ružić (1992). Pollen collected from 'Rainier', 'Van', 'Sweet Anne', 'Lapins' and

'Stella' was applied to labelled petri-dishes containing a 1 % agar medium, 12 % sucrose and 15 ppm boric acid, using a sterilized toothpick. Petri dishes were sealed with Parafilm® and incubated at room temperature (mean temperature = 21.7 ± 2.6 °C) and 95-100 % RH for 24 hours. Pollen viability was recorded as the percentage pollen grains germinated with pollen tubes greater than the diameter of the pollen grain. Evaluation was done with a stereo microscope (100X magnification) in three different fields of vision. Six replicates consisting of 400 to 500 pollen grains per treatment were assessed.

Pollen tube growth and ovule viability

Uniform, 2- to 3-year-old branches, with flowers at the first white stage, were cut from 4-year-old (5th leaf) 'Bing' sweet cherry trees on 'Gisela® 5' rootstocks. The basal 2 to 3 cm was cut under water in the laboratory and branches were forced overnight in a 5% sucrose solution. The following day the branches were placed in buckets containing tap water which was changed every 2 days throughout the experiment. When flowers reached the first bloom stage (Stage 7) (Ballard et al., 1982), they were emasculated and hand pollinated, when the control flowers reached anthesis, with pollen collected from 'Rainier', 'Van', 'Sweet Anne', 'Lapins' and 'Stella' (Table 2.1). Anthesis was considered as "the stage of external flower development after the stigma and anthers were exposed by the expansion of the petals but before the flowers were fully open" (Eaton, 1962). Approximately 25 flowers per branch, on each of 6 branches per treatment, were hand pollinated. Six un-pollinated branches were kept as controls. The most advanced flowers (fully open) at the time of emasculation and those least developed were removed to improve uniformity. To determine the number of pollen tubes in the different pistilar sections and ovule viability, three flowers per branch (18 pistils used for analysis) were collected daily, for 5 days after hand pollination. Calyx and stamens were removed and the pistils were fixed in FAA (1 part 40 % formalin: 8 parts 80 % alcohol: 1 part acetic acid) for 24 hours (Preil, 1970). Sampling was terminated after five days due to wilting and abscission of stylar tissue. After fixing in FAA, pistils were placed in 80 % ethanol and stored in a refrigerator at ~4 °C until analysed.

2006 Season

Pollen germination

Pollen samples were left at room temperature (~75-85 % RH) for 6 to 8 hours to rehydrate before determining viability. The "hanging drop" preparation was adapted from the procedures described by Parfitt and Ganeshan (1989), and Keulemans (1987). Preparations

were made by suspending the pollen in 2 ml Eppendorf® tubes containing a solution of 20 % sucrose (w/v) and 30 ppm boric acid. Tubes were then placed in an incubator at 21 °C for 24 hours. After the required time, 5 µl was transferred from the test tube to a standard cavity slide with a cover slip. Pollen viability was recorded as the percentage of pollen grains germinated with pollen tubes greater than the diameter of the pollen grain. Evaluation was done with a light microscope (100X magnification) in three different fields of vision. Eight replicates consisting of 400 to 500 pollen grains per treatment were assessed.

Pollen tube growth and ovule viability - Laboratory

Uniform, 2- to 3-year-old branches, with flowers at the first white stage, were cut from 5-year-old (6th leaf) 'Bing' sweet cherry trees on 'Gisela® 5' rootstocks. The basal 2 to 3 cm was cut under water in the laboratory and branches were forced overnight in a 5% sucrose solution. The following day the branches were placed in basins containing tap water which was changed every 2 days throughout the experiment. When flowers reached the first bloom stage, they were emasculated and hand pollinated, when the control flowers reached anthesis, with pollen collected from 'Rainier', 'Sweetheart', 'Stella', 'Black Tartarian' and 'Lapins' (Table 2.1). Approximately 20 flowers per branch, on each of eight branches per treatment (eight replicates), were hand pollinated. Eight un-pollinated branches were kept as controls. The most advanced flowers (fully open) at the time of emasculation and those least developed were removed to improve uniformity.

To determine the number of pollen tubes in the different pistilar sections and ovule viability, 2 to 3 flowers per branch (20 pistils used for analysis) were collected on days 1, 2, 3, 5 and 7 after hand pollination. Calyx and stamens were removed and the pistils were fixed in FAA for 24 hours. After fixing in FAA, pistils were place in 80 % ethanol and stored in a refrigerator at ~4 °C until analysed.

Pollen tube growth and ovule viability - Orchard

The influence of temperature and cross pollination on compatibility, pollen tube growth and ovule longevity were investigated. The experiment was set up as a randomized complete block design, with eight 5-year-old (6th leaf) 'Bing' sweet cherry trees on 'Gisela® 5' rootstock, grown under 20% black hail netting, from a commercial orchard at Ash Creek farm, Clarens. Treatments were then randomly allocated to each of seven 2- to 3-year-old branches per tree (Table 2.1). All branches, except the 'open pollinated control', were isolated

at the first white stage using cheese-cloth bags with a gauze diameter less than 0.25 mm. Flowers from all experiments, except the 'open pollinated control', were emasculated at the first bloom stage, and stigmas were hand pollinated with a glass rod when the 'open pollinated control' flowers had reached anthesis. Branches were re-covered with cheese-cloth bags immediately after hand pollination and bags were left on until ~21 days after hand pollination. The glass rod was sterilised with 96 % ethanol solution following each treatment application.

To determine the number of pollen tubes in the different pistilar sections and ovule viability, two to three randomly selected flowers per branch were collected on days 1, 2, 3, 5 and 7 after hand pollination. At least 20 flowers per branch were left for the determination of initial and final fruit set. Calyx and stamens were removed and the pistils were fixed in FAA for 24 hours. After fixing in FAA, pistils were place in 80 % ethanol and stored in a refrigerator at ~4 °C until analysed.

Pistil preparation and microscope examination

Pollen tube growth and ovule viability were monitored on squash preparations of pistils by means of fluorescence microscopy using the technique of Martin (1959) with the following adaptations: after softening pistils in 8N NaOH, they were rinsed in distilled water for 1 hour and then placed in a 6 % hydrogen peroxide solution in a refrigerator for 3-4 hours to clear the tissue. Cleared pistils were then rinsed twice, for 10 minutes, in distilled water and then stained with aniline blue dye (0.1% solution in 0.1 N K_2HPO_4) for 8-12 hours. For observation under the fluorescent microscope, pistils were mounted on slides with a drop of aniline blue stain and glycerine, and gently flattened with a cover slip. Mounted samples were kept in a refrigerator at ~5 °C until observed under the microscope. Preparations were observed using a Zeiss light microscope equipped with a UV filter system consisting of a 330-385 nm exciter filter and an LP528 barrier filter. Data collection included: number of germinated pollen grains on the stigma surface; number of pollen tubes in the top third section of the style, at the top of the ovary and penetrating the ovule; and ovule viability.

Statistical analyses were performed using Genstat 9.1 (Rothamsted Experimental Station, 2006). Percentage data for pollen germination, "pollen tube penetration" and fruit set were arcsine square-root transformed and then subjected to analysis of variance (ANOVA) with differences between means determined using the least significant differences test at the 0.05

significance level. Data for number of pollen tubes reaching the top of the ovary, percentage of ovules penetrated by pollen tubes and percentage of viable ovules were analysed as response variables using ANCOVA with pollen treatment as a main factor and days after hand-pollination (log-transformed) as a covariate. Regression models were also fitted to the relationship between days after hand-pollination and the various response variables outlined above. Logarithmic regression models were selected as these accounted for the least variance (highest R^2 value). Percentage data for ovules penetrated by pollen tubes and viable ovules were arcsine square-root transformed before linear regression analysis.

Results

Climate

Mean daily maximum and average air temperatures were higher during the 2005 laboratory experiment (T_{max} 25.9 $\pm$ 2.9 °C; T_{avg} 20.9 $\pm$ 2.9 °C) than both the 2006 laboratory (T_{max} 22.2 $\pm$ 2.2 °C; T_{avg} 19.1 $\pm$ 2.3 °C) and orchard (T_{max} 23.4 $\pm$ 5.9 °C; T_{avg} 15.7 $\pm$ 5.9 °C) experiments. Mean daily minimum orchard air temperature was substantially lower (6.9 $\pm$ 5.9 °C) than the 2005 laboratory (16.8 $\pm$ 2.9 °C) and 2006 laboratory (15.4 $\pm$ 2.2 °C) experiments (Table 2.2; Fig. 2.1). The minimum temperature recorded in the orchard after HP was 5.9 °C although a severe frost on 31 August 2006 recorded temperatures of -1.4 to -2.7 °C. Relative humidity varied between 17.1 and 100% in the orchard while the ranges for the indoor experiments were smaller, with lower maximum and minimum RH being recorded during the 2005 laboratory experiment compared to the 2006 laboratory experiment (Table 2.2).

Bloom synchrony

Time of bloom of the pollinizers was assessed over both seasons. ‘Lapins’ and ‘Black Tartarian’ were classified as early-blooming cultivars reaching full bloom approximately 2 to 4 days before ‘Bing’. ‘Rainier’, ‘Van’, ‘Sweet Anne’ and ‘Sweetheart’ flowered synchronously with ‘Bing’ in 2005, while ‘Van’ flowered 4 to 5 days after ‘Bing’ in 2006. ‘Stella’ was classified as a late blooming cultivar flowering ~7 to 9 days later than ‘Bing’ in both seasons (Fig. 2.2).

Pollen germination (in vitro) and “pollen tube penetration” (in vivo)

Pollen viability was assessed by means of *in vitro* germination tests during 2005 and 2006 using the ‘agar-plate’ and the ‘hanging drop’ techniques, respectively. Thus differences in viability could not be compared between years. Percentage germination was low in both

years, but significant differences were noted between pollinizers (Table 2.3). During 2005, ‘Stella’, ‘Van’ and ‘Lapins’ had significantly higher percentage germination than ‘Rainier’ and ‘Sweet Anne’. During 2006, ‘Black Tartarian’ and ‘Lapins’ had the highest percentage germination followed by ‘Stella’ and ‘Rainier’. Very low pollen germination was observed for ‘Sweetheart’.

Due to pollen grains being washed off the stigma during lab preparation for microscopy work, “pollen tube penetration” *in vivo* was determined as the average number of pollen tubes in the upper third of the style as a percentage of the total number of germinated pollen grains on the stigma, one day after hand pollination. This gave an indication of pollen performance on the stigma and the upper section of the style. “Pollen tube penetration” for each cultivar was higher *in vivo* (Table 2.4) than pollen germination *in vitro* (Table 2.3). During the 2005 laboratory experiment, ‘Van’ showed the second highest percentage germination *in vitro* (Table 2.3) but the lowest “pollen tube penetration" *in vivo* (Table 2.4). ‘Lapins’ generally had the highest germination percentage *in vivo* across all three experiments, while ‘Black Tartarian’ showed good germination *in vitro* and “pollen tube penetration” *in vivo* during 2006.

The effect of number of pollen grains deposited on the stigma and pollen tube dynamics was noted in the experiments. The number of germinated pollen grains on the stigma, one day after hand pollination, was proportional to percentage “pollen tube penetration” in the 2006 orchard experiment (Table 2.4). The relationship was not as apparent in the two laboratory experiments.

Number of pollen tubes reaching the top of the ovary

Pollen tube attrition was noted down the length of the style with a large reduction in the number of pollen tubes reaching the top third of the style and entering the ovary. The number of pollen tubes reaching the top of the ovary varied between the experiments ranging from 2.5 to 4.1 in the laboratory 2005 (Fig 2.3), 1.3 to 4.3 in the laboratory 2006 (Fig. 2.4) and 0 to 3.4 in the orchard 2006 (Fig. 2.5), 3 days after hand pollination. Pollen tubes appeared to reach a maximum on this day.

Regression lines were fitted to determine the relationship between days after hand pollination and the average number of pollen tubes reaching the top of the ovary. These regression

curves, based on a logarithmic model, explained a high percentage of the variance in the average number of pollen tubes reaching the top of the ovary (R^2 values ranged between 0.71 to 0.89). In the laboratory in 2005, 'Rainier', 'Stella' and 'Lapins' pollen tubes reached the top of the ovary 1 day after hand pollination while 'Van' and 'Sweet Anne' tubes took 2 days to reach the ovary. The number of pollen tubes reaching the top of the ovary for each day after hand pollination differed significantly among pollen sources, with 'Rainier', 'Stella' & 'Lapins' showing the highest number of pollen tubes and 'Van' and 'Sweet Anne' the lowest (Fig. 2.3). In both the 2006 laboratory (Fig. 2.4) and orchard experiments (Fig. 2.5), the highest number of pollen tubes reaching the top of the ovary occurred with 'Black Tartarian', followed by 'Lapins' and 'Rainier'. 'Stella', 'Sweetheart', and the 'open pollinated control' in the orchard 2006, showed the lowest number of pollen tubes in the top of the ovary. It is worth noting that these three treatments also showed very low numbers of germinated pollen grains on the stigma (Table 2.4).

Temperature had an influence on pollen tube growth rates, although the differences were not significant. The rate of pollen tube growth, particularly day 1 and 2 after hand pollination, was highest in the laboratory 2005 (Fig. 2.3) and lowest in the orchard 2006 (Fig. 2.5) indicating the influence of ambient temperature on pollen tube growth. When comparing the 2006 results, slower pollen tube growth occurred in the orchard with no pollen tubes having reached the top of the ovary by day 1 after hand pollination (Fig. 2.4; Fig. 2.5).

Unusual pollen tube growth was noted in the top of the ovary cavity where tubes showed signs of 'branching' (Fig. 2.6a) and curling into a ball (Fig. 2.6b). This was particularly evident in cases where both ovules had aborted (bright fluorescence) or where the primary ovule was showing some sign of fluorescence.

Pollen tubes penetrating the ovule

An effect of pollinizer (pollen donor) and temperature were noted across the trials with pollen tube penetration being more rapid at the higher temperatures recorded in the laboratory in both years compared to the orchard. In the laboratory 2005, 'Stella', 'Rainier' and 'Lapins' pollen tubes entered the ovary cavity and penetrated the ovule within 2 days of hand pollination, while 'Sweet Anne' and 'Van' pollen tubes first penetrated the ovule, 4 days after hand pollination (Fig. 2.7).

Flowers pollinated with ‘Black Tartarian’ and ‘Rainier’ had the highest percentage of pistils with tubes penetrating the primary ovule, 3 days after hand pollination in the laboratory 2006 while ‘Sweetheart’ pollen penetrated the ovule 7 days after hand pollination with no tube penetration recorded for ‘Stella’ (Fig. 2.8). The percentage of pistils with pollen tubes penetrating the ovule in the orchard 2006, showed a similar trend with ‘Black Tartarian’, ‘Rainier’ and ‘Lapins’ showing significantly faster and higher ($P<0.001$) pollen tube penetration than ‘Stella’, ‘Sweetheart’ and the ‘open pollinated control’ which all recorded no pollen tube penetration of the ovule (Fig. 2.9).

A number of cases were noted of pollen tubes entering fluorescing ovules with some pollen tube tips appearing to form a ball in the micropyle and nucellar regions (Fig. 2.6c). Some pistils contained pollen tubes penetrating both the viable primary ovule and the secondary fluorescing ovule (Fig. 2.6d).

Ovule viability

Viability of the ovule was determined by fluorescence microscopy (Anvari and Stösser, 1978a) with intense fluorescence of the entire ovule indicating a non-viable ovule (Fig. 2.10a) while no fluorescence indicated a viable ovule (Fig. 2.10b). Fluorescence commenced at the chalazal end (Fig. 2.10c), with central fluorescence of the nucellar region (Fig. 2.10d) being noted in approximately 17% of orchard 2006 ovules by day 5 and 7 after HP. Ovule viability decreased rapidly in the laboratory 2005 with only 16.7% of pistils containing viable primary ovules 3 days after anthesis, and no viable ovules 5 days after anthesis. The laboratory 2006 experiment also showed a rapid decline in ovule viability with 18.8% of pistils containing viable primary ovules 5 days after anthesis, and no viable ovules by day 7 after anthesis. Ovule viability in the orchard 2006 was significantly higher than both laboratory trials with 34.8% of pistils containing viable ovules 7 days after anthesis (Fig. 2.11).

Ovule degeneration in cross pollinated flowers, in 2005 in the laboratory, occurred a lot slower than in the control flowers, with approximately 44.5% of pistils still containing viable ovules, 5 days after anthesis (Fig. 2.12). The differences were not significant for the 2006 experiments (Fig. 2.13; Fig. 2.14).

Temperature appeared to have played an important role in the viability of the primary ovule in the ‘Bing’ pistils. A definite trend was noted between the mean temperatures recorded in the

three experiments (Fig. 2.1) and the viability of the ovules in the un-pollinated controls (Fig. 2.11). The highest mean temperature was recorded in the laboratory 2005 experiment, corresponding with the most rapid decrease in ovule viability while the lowest mean temperature, recorded in the orchard 2006, resulted in the highest ovule viability.

Fruit set

Fruit set was calculated as the number of fruit set as a percentage of the total number of hand pollinated flowers. Significant differences in percentage fruit set were recorded between pollinizers for the orchard 2006 experiment although initial and final fruit set were low across all treatments. 'Black Tartarian', 'Lapins' and 'Rainier' showed significantly higher ($P<0.001$) final fruit set compared to 'Sweetheart', 'Stella' and the 'open pollinated control' (Fig. 2.15). No fruit set was recorded in the bagged controls indicating that 'Bing' is allogamous and that the cheese-cloth bags were effective in preventing cross pollination. There was a strong positive relationship between average fruit set in 'Bing' flowers and the average number of germinated pollen grains recorded for each donor on 'Bing' stigmas ($R^2 = 0.93$, $df = 4$)

Discussion

The synchronous flowering of compatible pollinizers with 'Bing' sweet cherry is vital to ensure that adequate pollen is available for cross pollination by pollinators, in this case, honey bees. Flowering dates of the cross pollinizers tested showed that all had a 30 to 50% overlap of bloom date with 'Bing', except 'Stella' which had less than 5% overlap with 'Bing'. Nyéki et al. (2003) concluded that self-incompatible stone fruit cultivars require at least a 50% overlap in flowering date with cross pollinizers while sweet cherries may need at least a 70% overlap with compatible pollinizers. The poor bloom synchrony between 'Stella' and 'Bing' precludes 'Stella' as a suitable cross pollinizer for 'Bing'.

Percentage germination *in vitro* was relatively low in both years, although significant differences were noted among pollinizers (Table 2.3). The germination percentages compare favourably to research by Choi and Andersen (2005) on sweet cherries, where flowers collected at the balloon stage, equivalent to the collection stage of the current experiments, gave pollen germination of 19.3%. This compared to 3.4% for pollen collected from flowers at the delayed dormant stage (~35 days prior to natural full bloom day) and forced to flower, and 44.0% germination from flowers collected at full bloom which had opened naturally.

These differences were probably due to the different stages of maturation of the pollen as some pollen degeneration was observed in the forced flowers. The high germination percentage of 'Stella' is quite surprising as the trees were forced to flower early by means of the early application of hydrogen cyanamide and covering of trees with polyethylene. This resulted in some early bloom but flower quality was very poor and flowers had to be collected at a very early, first white stage in order to obtain un-dehisced anthers.

The behaviour of pollen tubes *in vitro* did not always reflect their behaviour *in vivo* with 'Van' having the second highest rate of germination *in vitro*, but the lowest rate of "pollen tube penetration" *in vivo* in 2005 (Table 2.3; Table 2.4). This latter result probably reflects differences in the female parent genotype's ability to support pollen tube germination on the stigma and initial tube growth in the style by modulating pollen performance from various pollen donors (Hedhly et al., 2005). Thus pollen-pistil interactions probably explain the lack of correlation observed between the pistil and *in vitro* germination (Hormaza and Hererro, 1994). The higher *in vivo* "pollen tube penetration" versus *in vitro* pollen germination, observed across all pollinizers, compares favourably with work on various sweet cherry cultivars, including 'Bing' (Hormaza and Herrero, 1999). They also showed that the fastest germinating pollen grains on the stigma did not always reflect higher pollen tube growth rates further down the style, indicating that fertilization success is highly dependent on pollen-pistil interactions throughout the entire pistil.

The number of germinated pollen grains on the stigma appeared to affect pollen tube growth, especially during 2006 (Table 2.4; Fig. 2.3; Fig. 2.4). Pollen competition on the stigma has been shown to influence pollen germination and pollen tube growth in the styles of plum (Lee, 1980), sweet cherry (Hormaza and Herrero, 1996) and pome fruit (Visser et al., 1988). Hormaza and Herrero (1996) showed in sweet cherry that the number of pollen tubes reaching each stylar level was dependent on the initial pollen load. The difficulty of ensuring uniform deposition of pollen on all stigmas may have influenced the rates of pollen tube growth with significant differences in the number of germinated pollen grains on the stigma. Poor pollen dehiscence from 'Stella' and 'Sweetheart' anthers probably contributed to this effect, although 'Stella' did show the highest "pollen tube penetration" in both laboratory experiments even though it recorded the lowest number of germinated pollen grains on the stigma.

The results of the experiments indicate that temperature, pollen competition and genotype played a role in the dynamics of pollen tube growth in 'Bing'. The time taken for pollen tubes to reach the top of the ovary varied between seasons and between the laboratory and outdoor treatments indicating that temperature played a role in the rate of tube growth. Relatively small differences were noted though, possibly as a result of the fairly narrow range of average temperatures recorded of between 15.7 and 20.9°C. Research on various *Prunus* species has shown that pollen tube growth rate is related to temperature (Hedhly et al., 2005; Hedhly et al., 2004; Jefferies et al., 1982; Keulemans, 1984) with optimum constant temperatures for apricot, sweet and sour cherry ranging between 15-20°C (Cerović and Ružić, 1992; Pirlak, 2002).

The type of pollinizer had a significant influence on pollen tube growth, although it should be noted that the number of germinated pollen grains on the stigma may have modified the effect. 'Rainier' and 'Lapins' pollen showed consistently higher numbers of pollen tubes reaching the top of the ovary during both seasons with 'Black Tartarian' showing the highest number of pollen tubes in the ovary during the 2006 season (Fig 2.10). The results are supported by work on sweet cherry (Guerrero-Prieto et al., 1985; Hedhly et al., 2005; Hormaza and Herrero, 1999), almond (Kodad and Socias i Company, 2006), avocado (Robbertse et al., 1998) and sour cherry (Cerović et al., 1998; Cerović and Ružić, 1992) who showed variations in pollen tube growth between pollen donor genotypes, often influenced by temperature-genotype interactions. Research on 'Sunburst' and 'Cristobalina' sweet cherry showed differences in their adaptation to various climates with 'Cristobalina' better adapted to warmer climates, reflecting the temperature adaptation of the pollen donor (Hedhly et al., 2005). This possibly explains the results of 'Black Tartarian' and 'Lapins' which have a more moderate chilling requirement and were thus better adapted to the prevailing climatic conditions in the trials, although it does not fully explain the effect of 'Rainier' pollen. Cruzan (1993) suggested that differences in the growth of pollen tubes in the style among pollen donors may be due to inhibition of pollen tube growth rather than differences in pollen vigour.

The contrasting performance of 'Stella' between the two seasons is possibly due to the low number of pollen grains deposited on the stigma in 2006 (Table 2.4). A "population effect" has been observed in some species where a minimum number of pollen grains are required for pollen germination to occur (Hormaza and Hererro, 1994). Research on 'Bing' sweet cherry has shown that stigmas need approximately 100 pollen grains to set a good fruit (Delaplane

and Mayer, 2000; Mayer et al., 1987). In *Petunia*, little or no germination occurred at pollen populations of less than 10 pollen grains while up to 75.2% germination was recorded at populations greater than 300 grains (Brewbaker and Majumder, 1961).

Pollen tubes penetrated viable, non-fluorescing primary ovules although some cases were noted where the tubes entered fluorescing ovules. Temperature appeared to play a role in the time taken for pollen tubes to enter the ovule. In the laboratory in 2005, the first pollen tubes entered the primary ovule 1 to 2 days after hand pollination (Fig. 2.7). In 2006, it took 3 days for the first tubes to penetrate the ovule in the laboratory (Fig. 2.8), and between 4 and 5 days in the orchard (Fig. 2.9), reflecting the effect of different temperatures on pollen tube penetration. The effect of temperature on the time pollen tubes take to reach the ovule has been recorded in a number of species. In their experiments, Stösser and Anvari (1983) found that pollen tubes in sweet cherries required 6 to 8 days to reach the ovule. In 'Napoleon' sweet cherry, pollen tubes reached the ovule by the 4th day after pollination at 13.7°C and by the 6th day at 10.6°C (Guerrero-Prieto et al., 1985). In sour cherry, Cerović and Ružić (1992) showed that pollen tubes penetrated the micropyle 2 to 3 days after pollination at optimum temperatures of 15-20°C while temperatures <10°C resulted in pollen tubes only penetrating the micropyle, 4 to 6 days after pollination. A similar result was obtained with 'Sundrop' apricot pollen tubes which reached the ovary after 24 hours and penetrated the micropyle after 48 hours, when flowers were kept at 25°C, while ovule penetration in field trials typically took 12-14 days (mean temperature 10.2°C) (Austin et al., 1998). The mean temperature recorded in this experiment shows that the temperature was favourable for pollen tube growth and entry into the ovule although the optimum temperature for fertilization is unknown. Higher temperatures accelerate pollen tube growth and pollen tube penetration of the ovule but also shorten the viability of the ovule and embryo sac, leading to shortened EPP (Postweiler et al., 1985; Sanzol and Hererro, 2001).

The influence of pollen donor was observed in both years with 'Stella', 'Rainier' and 'Lapins' pollen penetrating the ovule, 1 to 2 days after hand pollination compared to 4 days for 'Van' and 'Sweet Anne'. In the orchard 2006, 'Black Tartarian', 'Rainier' and 'Lapins' pollen tubes penetrated the ovules within 4 to 5 days of hand pollination. The lack of ovule penetration in 'Stella', 'Sweetheart' and the 'open pollinated control' reflects the very low initial number of germinated pollen grains on the stigma. This is also reflected in the low final fruit set obtained in these treatments. The low number of germinated pollen grains on the stigma, pollen tube

penetration and fruit set recorded in the 'open pollinated control's indicates poor pollen transfer by pollinators. This is quite surprising when one considers that the orchard was supplied with 10 hives per hectare. The recommended number of hives per hectare varies from country to country but 3 to 5 strong hives per hectare is generally recommended for sweet cherries (Benedek, 2003; Thompson, 1996). Counts of approximately 10 to 12 bees per branch per 10 minutes indicated a relatively high degree of bee activity in the orchard. This figure is similar to the 25-35 bees per minute per mature cherry tree recommended by Thompson (1996).

The unusual behaviour of pollen tubes in the top of the ovary and in the ovule indicates a possible loss of pollen tube guidance. This phenomenon has been observed in sour cherry (Cerović, 1996; Cerović and Ružic, 1992) where the authors presumed that the embryo sac and its constituents were involved in the mechanism of regulating pollen tube growth. A number of authors have shown that the synergids are the origin of the chemotropic response (Cheung, 1996; Higashiyama et al., 2003; Wilhelmi and Preuss, 1997) thus any degeneration of the embryo sac and egg apparatus can result in a loss of tube guidance. Fluorescence of the ovule, possibly as a result of early degeneration of the embryo sac and egg cell, may have been the reason for the low percentage of pollen tubes penetrating 'Bing' ovules. The observation of pollen tubes entering fluorescing, non-viable ovules agrees with the work of Cerović and Ružic (1992) but is in contrast to research by Anvari and Stösser (1978a) who reported that pollen tubes never penetrated fluorescing ovules.

The observation of ovule fluorescence is an easy and accurate method of determining ovule viability in 'Bing' sweet cherry pistils. The results indicate that senescence of the primary ovule is mainly influenced by temperature, with the higher mean temperatures under laboratory conditions causing a rapid decline in ovule viability compared to the lower orchard temperatures. This is confirmed by various researchers who have shown that temperature influences the duration of ovule longevity in *Prunus* species (Cerović and Ružic, 1992; Cerović et al., 2000; Postweiler et al., 1985). Ovule viability in sweet and sour cherries has been shown to vary from 1 to 2 days, at a constant temperature of 20°C and up to 5 days at 5°C (Postweiler et al., 1985). In sour cherries, viability varied from 3 to 4 days at a constant 25°C and up to 9 days at 5°C (Cerović and Ružic, 1992). In a field experiment conducted in western Oregon, 'Napoleon' sweet cherry ovules were shown to still be functional, 13 days after anthesis, at an average temperature of 10.6°C (Guerrero-Prieto et al., 1985). The

relatively large proportion of ovules with central fluorescence noted during the orchard 2006 trial may indicate early embryo sac and nucellar degeneration. Work on 'Satohnishiki' sweet cherry showed that trees kept at temperatures of 20 to 25°C from budburst until petal fall showed a rapid degeneration of the embryo sac and nucellus with 90% of ovules degenerated, within 4 days of anthesis, markedly reducing fruit set (Beppu et al., 2005; Beppu et al., 1997; Beppu et al., 2001).

Morphological examinations of the embryo sacs were not conducted, thus differences may exist between the aging of the ovule, indicated by fluorescence, and embryo sac viability (Tonutti et al., 1991). Work in 'Nonpareil' almond showed that early fluorescence in the chalazal region of the nucellus, where the funicular trace enters the ovule, was due to callose deposition which may result in the disruption of metabolite transport into the nucellus during the final stages of development (Pimienta and Polito, 1982). This has been shown to occur in other *Prunus* species (Sanzol and Hererro, 2001).

Cross pollination influenced ovule longevity by delaying ovule degeneration and thus increasing the percentage of pistils containing viable ovules. This was especially evident during the 2005 laboratory experiment (Fig. 2.12), which showed rapid degeneration of ovules in the control pistils, while the effect was not significant in the 2006 experiments (Fig. 2.13; Fig. 2.14). Contradictory results have been reported in the literature. Herrero (1992) reported that cross pollination in fruit crops induces various biochemical reactions in the pistilar tissues resulting in the delayed degeneration of the embryo sac. Cross-pollinated apricot (Burgos and Egea, 1993) and almond (Pimienta and Polito, 1983) flowers showed faster embryo sac development and reduced ovule longevity compared to unpollinated flowers while prolonged embryo sac viability and longer ovule viability was shown to occur in cross pollinated pears (Herrero and Gascon, 1987) and sour cherry (Cerović and Mićić, 1999). The prolongation of embryo sac viability in sour cherry, which was promoted by compatible pollen tubes, may enhance the chances of fertilization success and increase fruit set. It should be noted that many apricot cultivars grown under Mediterranean climates show an important delay in the maturation of the embryo sac at anthesis (Alburquerque et al., 2004; Egea and Burgos, 1994; Egea and Burgos, 1998). Other factors such as insufficient winter chilling (Egea and Burgos, 1998; Guerriero et al., 1986; Legave, 1978), pre-bloom temperatures (plant growth regulators (Beppu et al., 2005; Herrero, 1992; Herrero and

Gascon, 1987) and tree nutrition (Khemira et al., 1998) may have also influenced ovule and embryo sac longevity in these experiments.

Conclusions

One year of orchard data makes it difficult to confirm all the results obtained in the experiments as environmental conditions preceding, during and post bloom change from year to year, and the synchrony of flowering of pollinizers and 'Bing' varies between years. Historical data shows this is particularly evident in low chill accumulation years. All pollinizers showed some synchrony of bloom with 'Bing' except 'Van' in 2006, and 'Stella' in both years which flowered approximately 7-9 days later than 'Bing', precluding it as a suitable cross pollinizer. All the pollinizers tested showed relatively low *in vitro* pollen germination percentages but good "pollen tube penetration" of the stigma and upper section of the style indicating the importance of pollen-pistil interactions where the female genotype and temperature are able to modulate pollen performance from various pollen donors (Hedhly et al., 2005). No pollen incompatibility was noted in any of the pollen-pistil combinations indicating that all pollinizers were cross compatible with 'Bing'. Stigma receptivity was not assessed in the trials but has been shown to limit pollen germination and penetration of the transmitting tissues (Hedhly et al., 2003).

The average temperatures recorded during the trials seemed to favour pollen tube growth, especially 'Black Tartarian', 'Lapins' and 'Rainier' pollen, indicating that pollen tube growth rates did not appear to be a factor limiting fertilization and fruit set in 'Bing'. The 2006 laboratory pollen tube growth rate and fertilization results, although conducted at higher temperatures and showing faster growth rates, were probably a relatively good indicator of pollen tube growth and fertilization in orchard pistils (Jefferies et al., 1982). The converse results obtained with 'Stella' in the two seasons indicates a possible pollen "population effect" (Hormaza and Hererro, 1994) during the 2006 season. A similar effect appears to have influenced the rate of pollen tube growth and ovule penetration in 'Sweetheart' in the laboratory and orchard, and the 'open pollinated control' in the orchard during 2006.

Temperatures during and after anthesis played a significant role in the viability of the primary ovule in 'Bing' pistils. The higher and relatively constant temperatures in the laboratory accelerated ovule senescence resulting in a rapid decline in ovule viability, while the lower temperatures in the orchard resulted in more gradual senescence. Although the lower

temperatures in the orchard increased ovule longevity, they also slowed the rate of pollen tube growth and penetration of the ovule, resulting in an effective pollination period of only 2 to 3 days. It is crucial that pollination is not delayed and that pollen from a suitable pollinizer such as ‘Black Tartarian’, ‘Lapins’ and ‘Rainier’ is available. Ovule degeneration is closely linked to environmental conditions and will thus vary from year to year. Cross pollination increased ovule longevity thus any measures which increase pollen transfer can possibly improve ovule viability.

The low fruit set recorded in the trial is typical of ‘Bing’ production under conditions of insufficient winter chilling and the temperature extremes experienced during bloom in South Africa. The principle factors causing low and unreliable fruit set in ‘Bing’ sweet cherry appear to be premature abortion of the ovule, possibly as a result of early degeneration of the embryo sac resulting in a shortened EPP, and inadequate transfer of sufficient viable pollen under orchard conditions. The latter was unexpected, given the high rate of visits to flowers by bees, and deserves further investigation. Thompson (1996) indicated that if fruit set on hand pollinated branches was significantly higher than that on open pollinated branches, insufficient transfer of pollen is contributing to reduced orchard fruit set.

The influence of temperatures during late summer and autumn and the lack of winter chilling on embryo sac and egg apparatus degeneration also need to be further investigated. Cytological observations need to be conducted in conjunction with fluorescent microscopy to elucidate the relationship between the two.

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Table 2.1 Pollen donors (pollinizers) used in laboratory and orchard cross pollination treatments during the 2005 and 2006 seasons.

2005	2006	
Laboratory	Laboratory	Orchard
	Treatments	
Control – no cross pollination	Control – no cross pollination	Control – no cross pollination
‘Rainier’	‘Rainier’	Control – open pollination
‘Van’	‘Black Tartarian’	‘Rainier’
‘Sweet Anne’	‘Sweetheart’	‘Black Tartarian’
‘Lapins’	‘Lapins’	‘Sweetheart’
‘Stella’	‘Stella’	‘Lapins’
		‘Stella’

Table 2.2 Air temperature and relative humidity means as measured during the laboratory (2005), and the laboratory and orchard (2006) trials.

	Temperature (°C)			Relative humidity (%)	
	T_{max}	T_{min}	T_{avg}	RH_{Max}	RH_{Min}
Laboratory 2005	25.9	16.8	20.9	60.0	9.1
Laboratory 2006	22.2	15.4	19.1	64.7	22.2
Orchard 2006	23.4	6.9	15.7	100	17.1

Table 2.3 Percentage germination of pollen grains, as a measure of pollen viability, using the 'Agar-plate' (2005) and 'hanging drop' (2006) techniques.

Pollenizer	Pollen germination (%)	
	Agar-plate (2005 season)	Hanging drop (2006 season)
'Stella'	23.2 a*	31.6 b
'Lapins'	18.4 a	37.5 a
'Rainier'	11.8 b	30.6 b
'Van'	20.6 a	-
'Sweet Anne'	11.2 b	-
'Black Tartarian'	-	38.7 a
'Sweetheart'	-	18.4 c
LSD (5%)	5.42	3.90
Pr > F	0.004	<0.001

* Means followed by the same letter within the same column do not differ significantly at P=0.05.

Table 2.4 Number of germinated pollen grains on the stigma and "pollen tube penetration" *in vivo*, 1 day after hand pollination, in 'Bing' sweet cherry pistils during the laboratory (2005), and the laboratory and orchard (2006) trials.

Polleniser	2005		2006			
	Laboratory		Laboratory		Orchard	
	No. of germinated pollen grains	Pollen tube penetration[#] (%)	No. of germinated pollen grains	Pollen tube penetration (%)	No. of germinated pollen grains	Pollen tube penetration (%)
'Stella'	35.4 b	63.4 b*	5.2 c	72.2 a	3.1 cd	32.5 b
'Lapins'	73.4 a	79.7 a	11.9 bc	72.0 ab	60.4 b	68.5 a
'Rainier'	84.7 a	53.0 c	26.1 b	43.4 c	35.0 bc	67.6 a
'Van'	42.3 b	33.7 d	-	-	-	-
'Sweet Anne'	41.8 b	47.6 c	-	-	-	-
'Black Tartarian'	-	-	124.5 a	63.4 ab	110.2 a	65.5 a
'Sweetheart'	-	-	6.8 c	57.5 bc	1.1 d	29.8 b
Control - open	-	-	-	-	2.6 cd	18.3 b
LSD (5%)	13.77	6.14	18.73	14.54	33.47	20.45
Pr > F	<0.001	<0.001	<0.001	0.002	<0.001	<0.001

[#] "Pollen tube penetration" *in vivo* was determined as the average number of pollen tubes in the upper third of the style as a percentage of the total number of germinated pollen grains on the stigma, one day after hand pollination.

[*] Means followed by the same letter within the same column do not differ significantly at P=0.05.

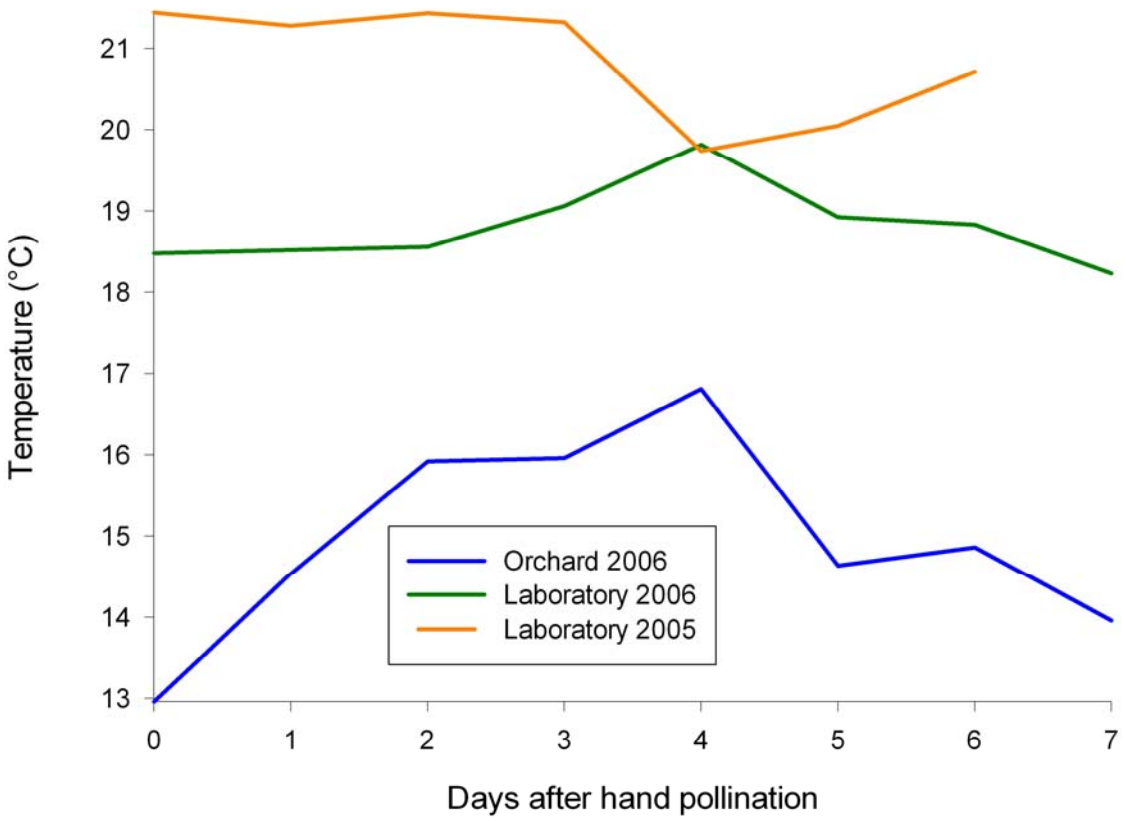

Figure 2.1 Mean ambient air temperatures recorded during and after hand pollination of 'Bing' sweet cherry in the laboratory (2005), and in the laboratory and orchard (2006) trials.

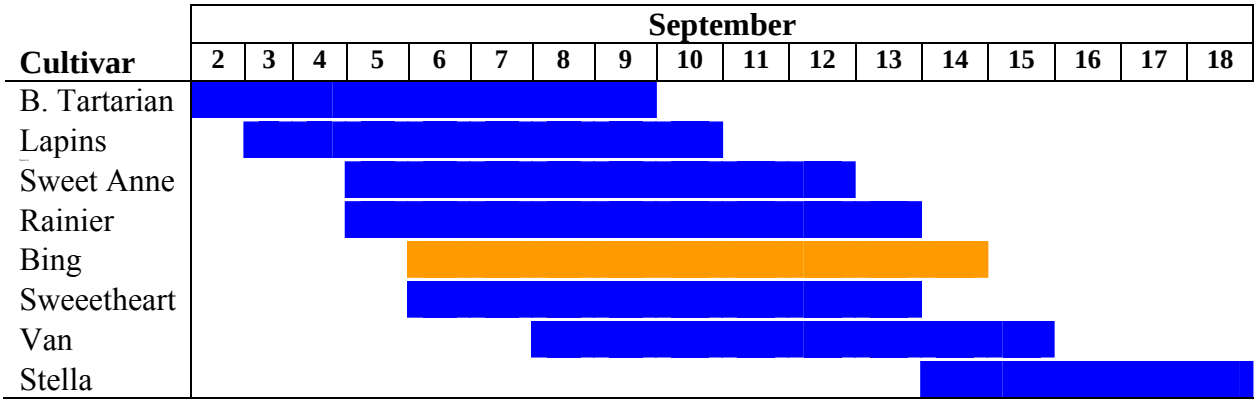

Figure 2.2 Bloom overlap of the various cross pollinizers with 'Bing' sweet cherry, tested during the 2005 and 2006 seasons, determined by visual observation.

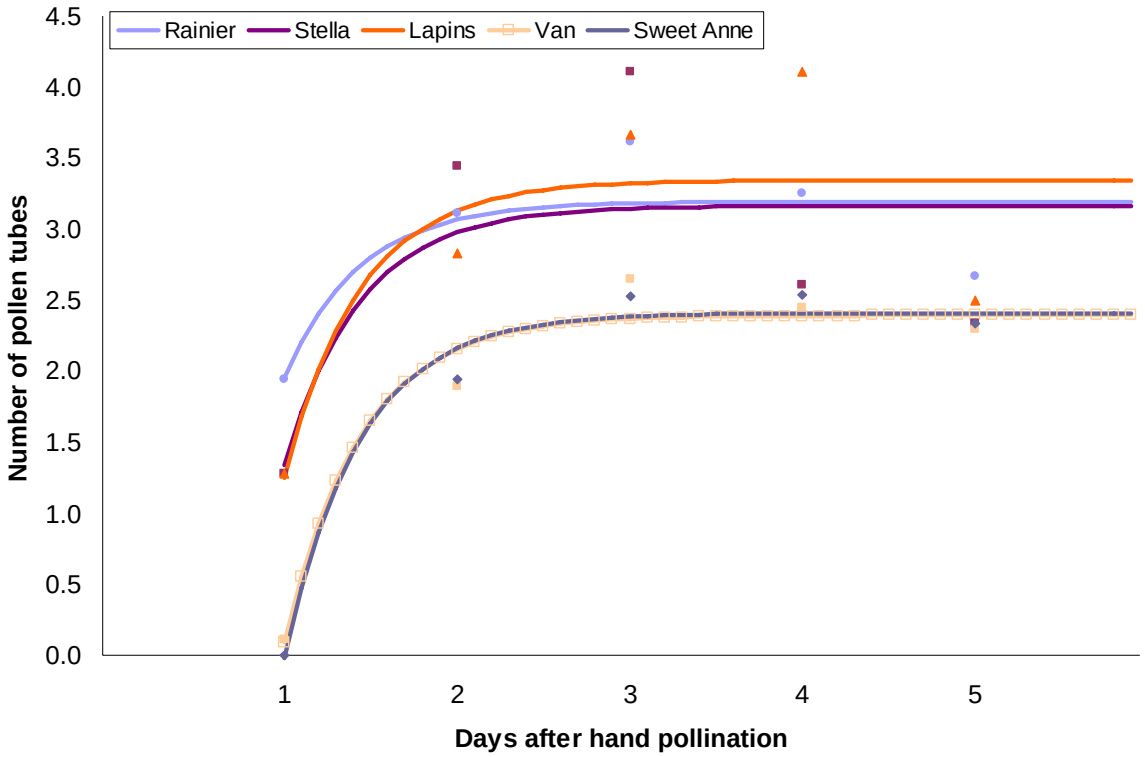

Figure 2.3 Relationship between number of pollen tubes reaching the top of the ovary and days after hand pollination of 'Bing' sweet cherry flowers in the laboratory during 2005.

Source of Variation	d.f.	Mean square	*F* value	Pr > *F*
Days[z]	2	7.46	23.87	<0.001
Polliniser	4	1.45	4.63	0.014
Days x Polliniser	4	0.17	0.54	0.709
Residual	14	0.31		

$R^2 = 0.709$

[z] Days after hand pollination

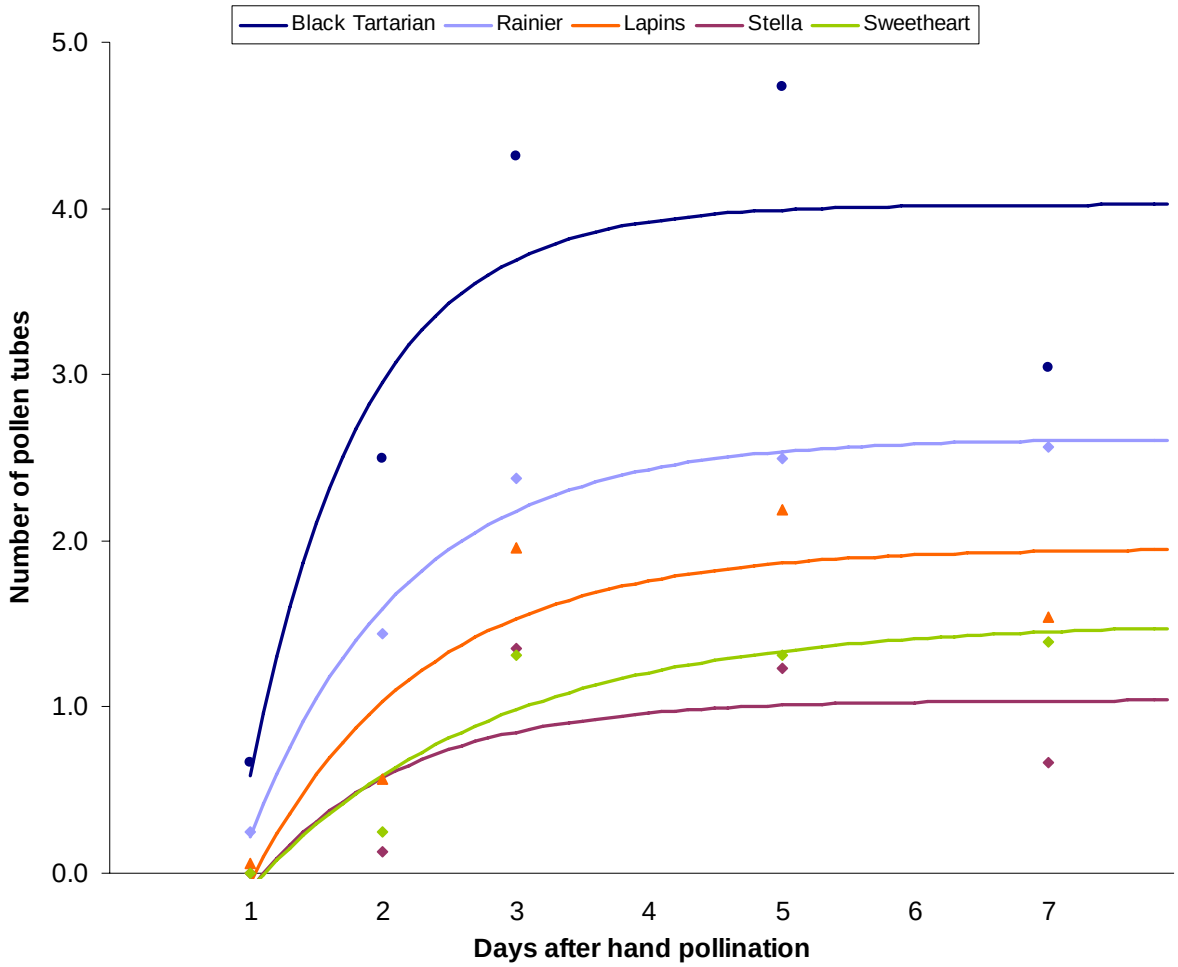

Figure 2.4 Relationship between number of pollen tubes reaching the top of the ovary and days after hand pollination of 'Bing' sweet cherry flowers in the laboratory during 2006.

Source of Variation	d.f.	Mean square	*F* value	Pr > *F*
Days[z]	2	7.42	19.87	<0.001
Pollinisor	4	4.57	12.25	<0.001
Days x Polliniser	4	0.56	1.50	0.275
Residual	24			

$R^2 = 0.771$

[z] Days after hand pollination

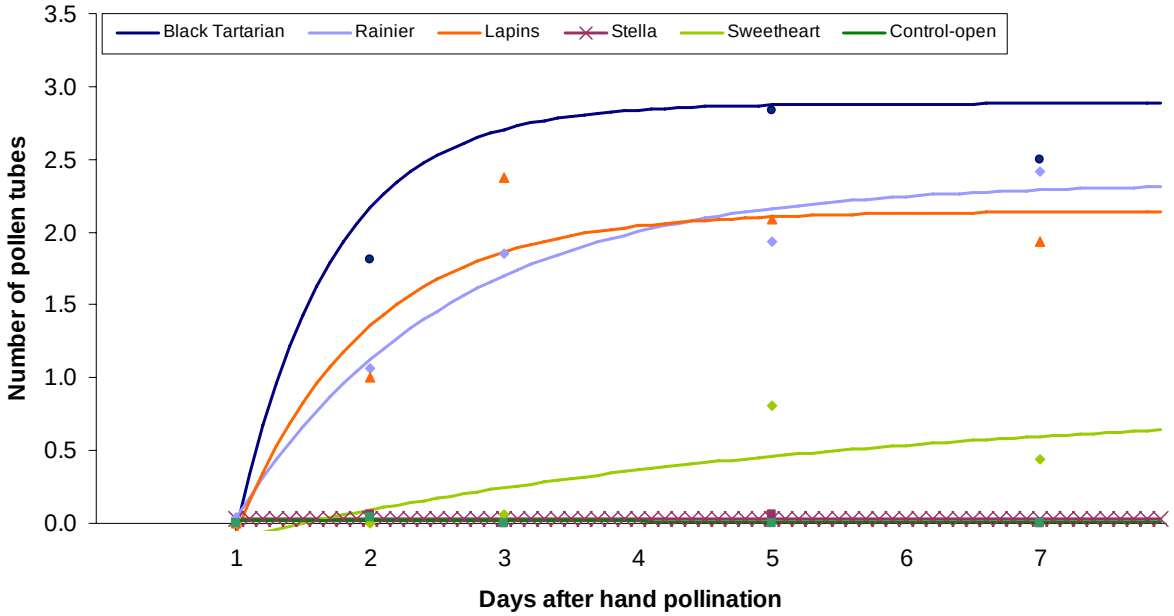

Figure 2.5 Relationship between number of pollen tubes reaching the top of the ovary and days after hand pollination of 'Bing' sweet cherry flowers in the orchard during 2006.

Source of Variation	d.f.	Mean square	*F* value	Pr > *F*
Days[z]	2	3.58	27.93	<0.001
Polliniser	5	4.09	31.89	<0.001
Days x Polliniser	5	1.13	8.86	0.001
Residual	12			

$R^2 = 0.894$

[z] Days after hand pollination

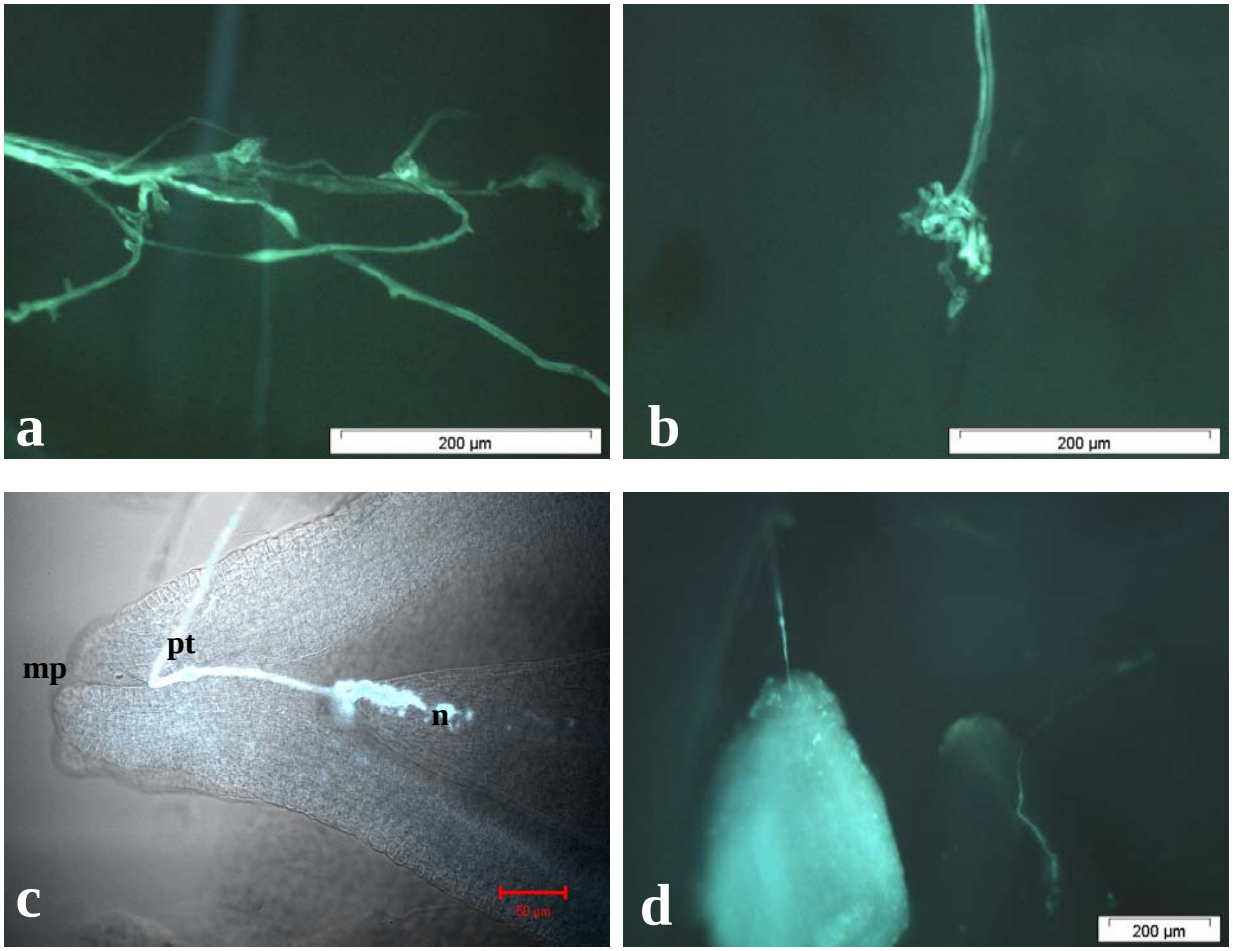

Figure 2.6 Pollen tube growth in 'Bing' sweet cherry pistils; (a) pollen tube 'branching'; (b) pollen tube curling into a ball in the ovary cavity; (c) pollen tube penetration of the primary ovule; (d) and pollen tubes penetrating of both the viable primary ovule (right) and aborted secondary ovule (left). Abbreviations: pt = pollen tube; mp = micropyle; n = nucellus.

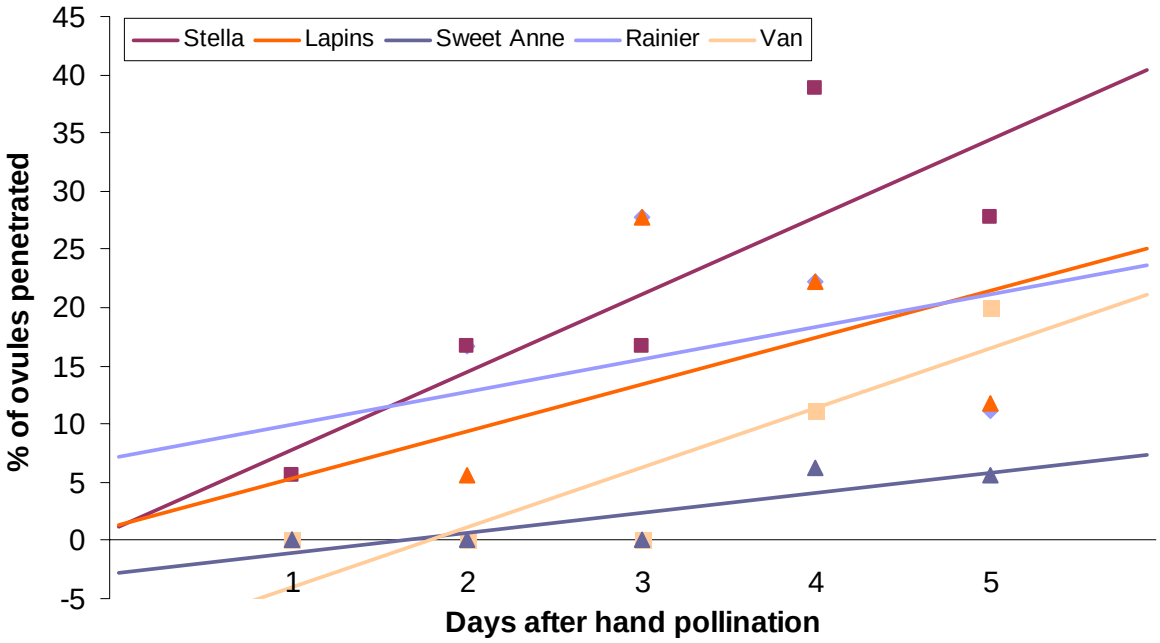

Figure 2.7 Relationship between percentage of pistils with the primary ovule penetrated by pollen tubes and days after hand pollination of 'Bing' sweet cherry flowers in the laboratory during 2005.

Source of Variation	d.f.	Mean square	*F* value	Pr > *F*
Days[z]	1	1380.87	19.45	<0.001
Polliniser	4	365.36	5.15	0.008
Days x Polliniser	4	15.44	0.22	0.925
Residual	15	165.37		

$R^2 = 0.571$

[z] Days after hand pollination

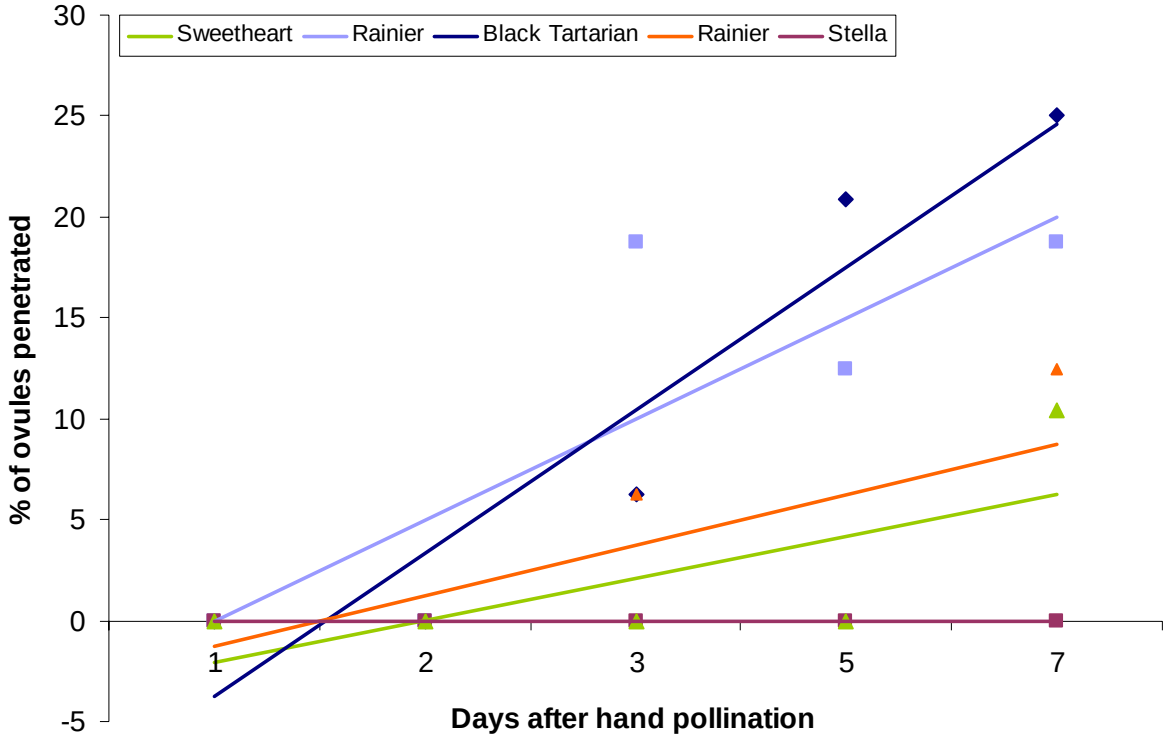

Figure 2.8 Relationship between percentage of pistils with the primary ovule penetrated by pollen tubes and days after hand pollination of 'Bing' sweet cherry flowers in the laboratory during 2006.

Source of Variation	d.f.	Mean square	*F* value	Pr > *F*
Days[z]	1	1091.19	29.41	<0.001
Polliniser	4	189.49	5.11	0.008
Days x Polliniser	4	105.61	2.85	0.061
Residual	15	117.84		

$R^2 = 0.685$

[z] Days after hand pollination

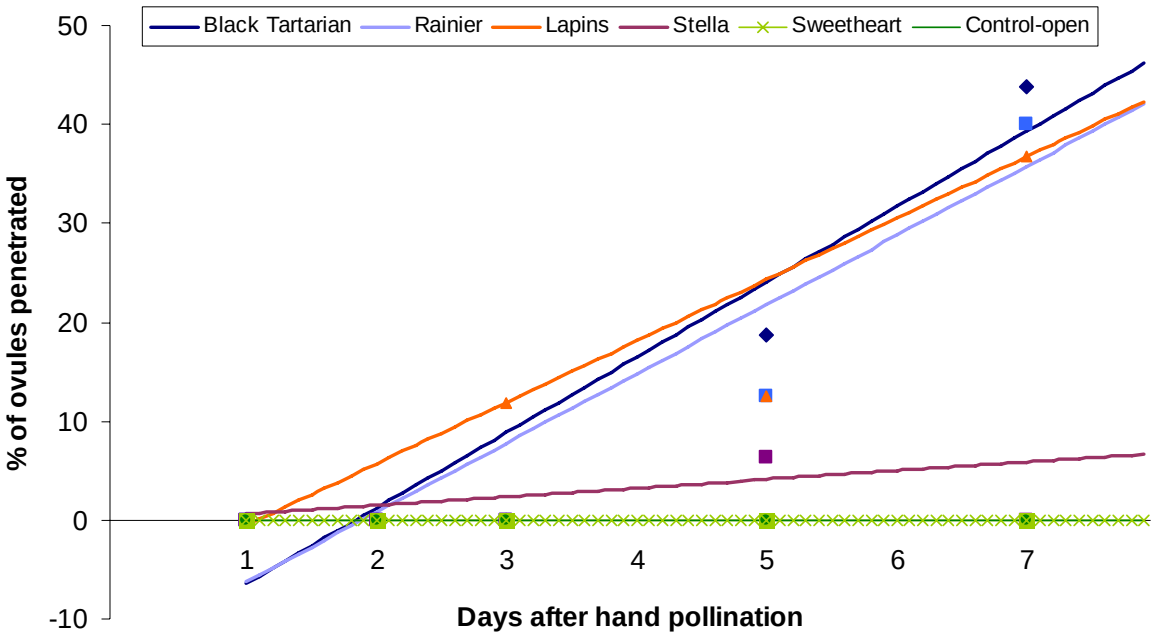

Figure 2.9 Relationship between percentage of pistils with the primary ovule penetrated by pollen tubes and days after hand pollination of 'Bing' sweet cherry flowers in the orchard during 2006.

Source of Variation	d.f.	Mean square	*F* value	Pr > *F*
Days[z]	1	1820.27	65.14	<0.001
Polliniser	5	254.67	9.11	<0.001
Days x Polliniser	5	314.77	11.26	<0.001
Residual	18			

$R^2 = 0.843$

[z] Days after hand pollination

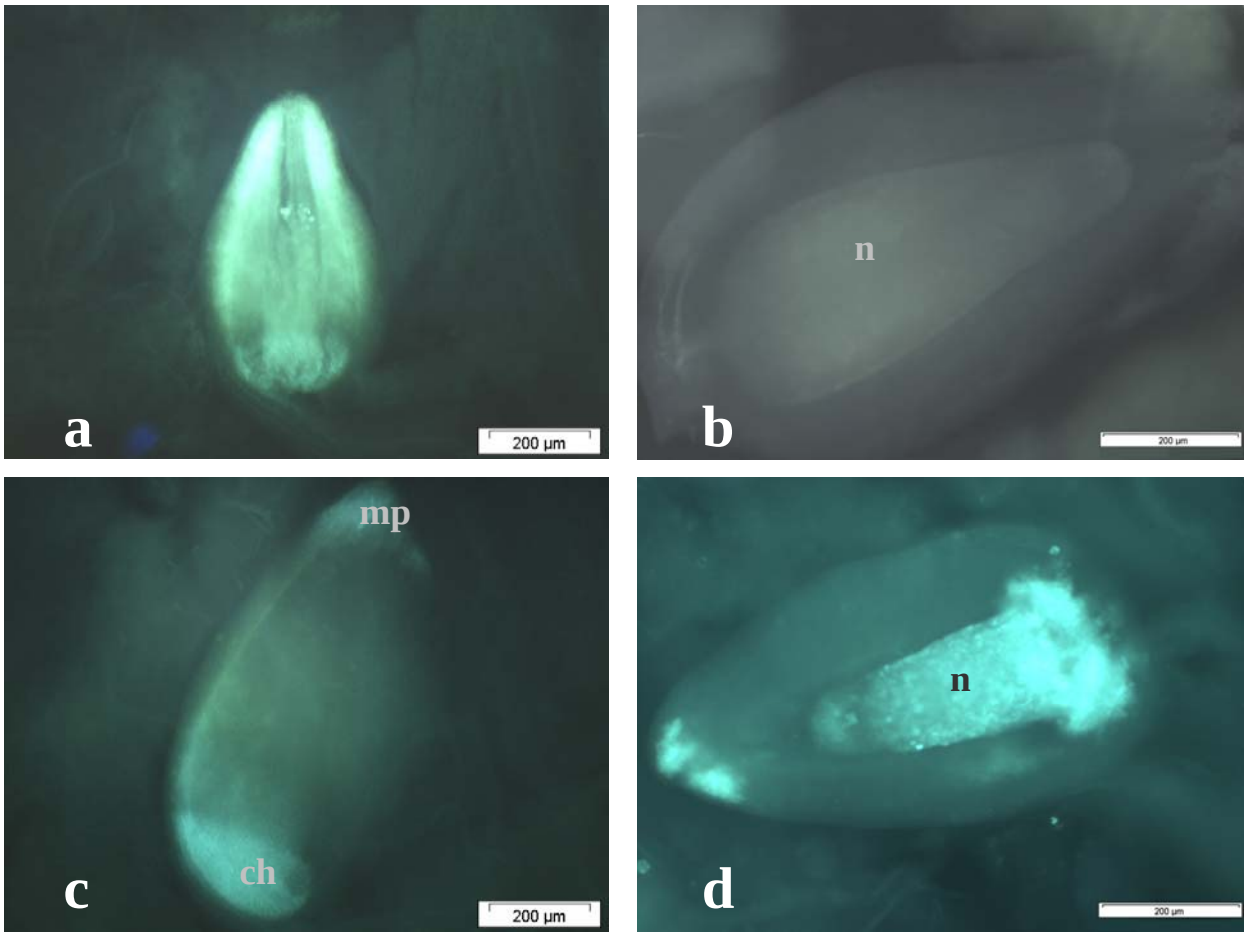

Figure 2.10 Different stages of ovule viability in 'Bing' sweet cherry; (a) intensely fluorescing non-viable ovule; (b) viable ovule; (c) slight fluorescence in the chalazal and micropylar regions; (d) central fluorescence of the nucellus. Abbreviations: ch = chalaza; mp = micropyle; n = nucellus.

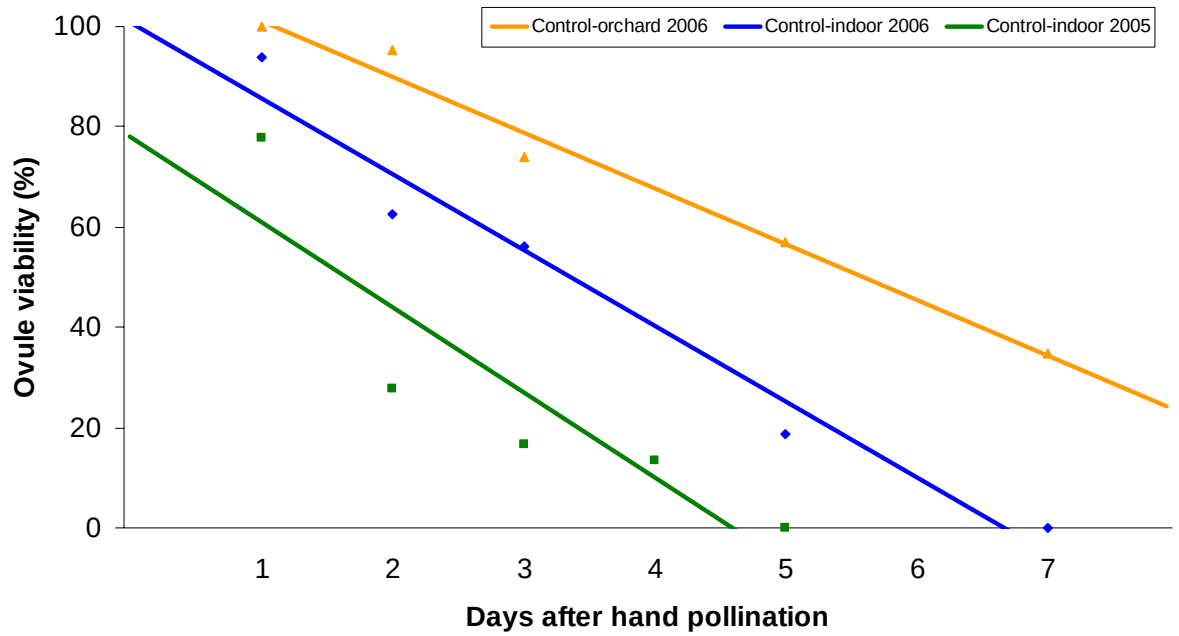

Figure 2.11 Relationship between percentage of pistils with viable primary ovules in 'Bing' sweet cherry flowers and days after hand pollination for all unpollinated controls across both seasons.

Source of Variation	d.f.	Mean square	*F* value	Pr > *F*
Days[z]	1	54.83.1	127.68	<0.001
Polliniser	2	2076.3	48.35	<0.001
Days x Polliniser	2	96.5	2.25	0.162
Residual	9	42.9		

$R^2 = 0.941$

[z] Days after hand pollination

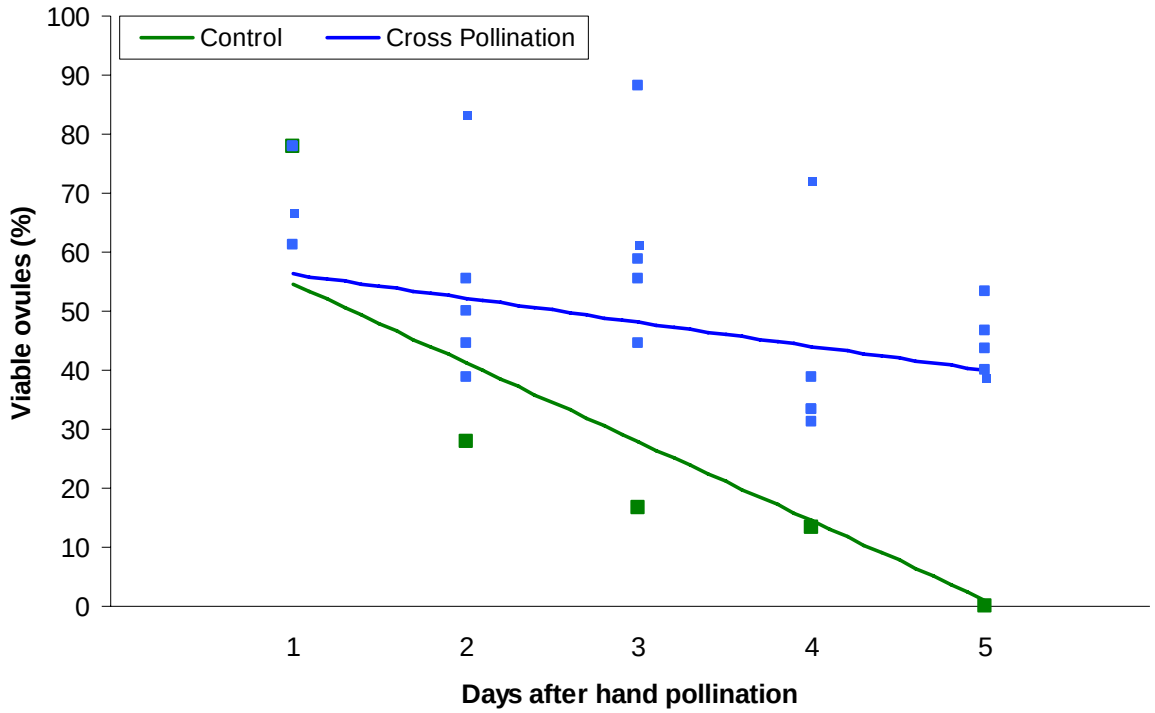

Figure 2.12 Relationship between percentage of pistils with viable primary ovules in unpollinated control versus cross pollinated 'Bing' sweet cherry flowers and days after hand pollination in the laboratory during 2005.

Source of Variation	d.f.	Mean square	*F* value	Pr > *F*
Days[z]	1	1897.94	25.27	<0.001
Polliniser	1	1715.31	22.84	<0.001
Days x Polliniser	1	728.27	9.70	0.004
Residual	26	75.10		

$R^2 = 0.654$

[z] Days after hand pollination

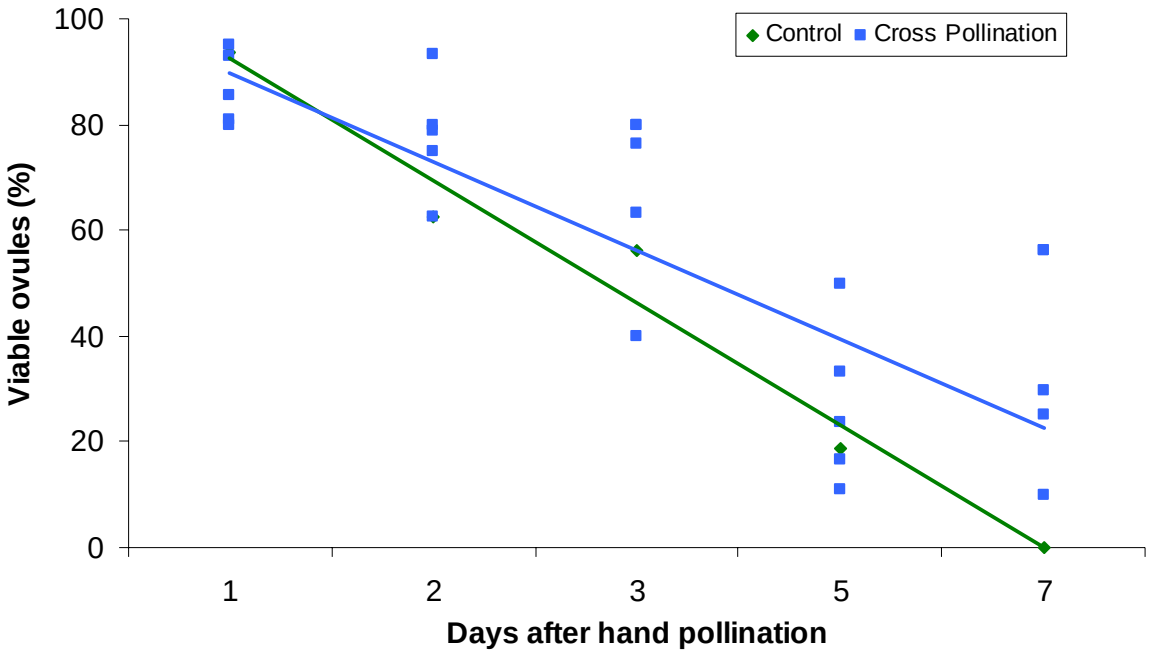

Figure 2.13 Relationship between percentage of pistils with viable primary ovules in unpollinated versus cross pollinated 'Bing' sweet cherry flowers and days after hand pollination in the laboratory during 2006.

Source of Variation	d.f.	Mean square	*F* value	Pr > *F*
Days[z]	1	9361.5	57.10	<0.001
Polliniser	1	261.0	1.59	0.218
Days x Polliniser	1	355.6	2.17	0.153
Residual	26	163.9		

$R^2 = 0.665$

[z] Days after hand pollination

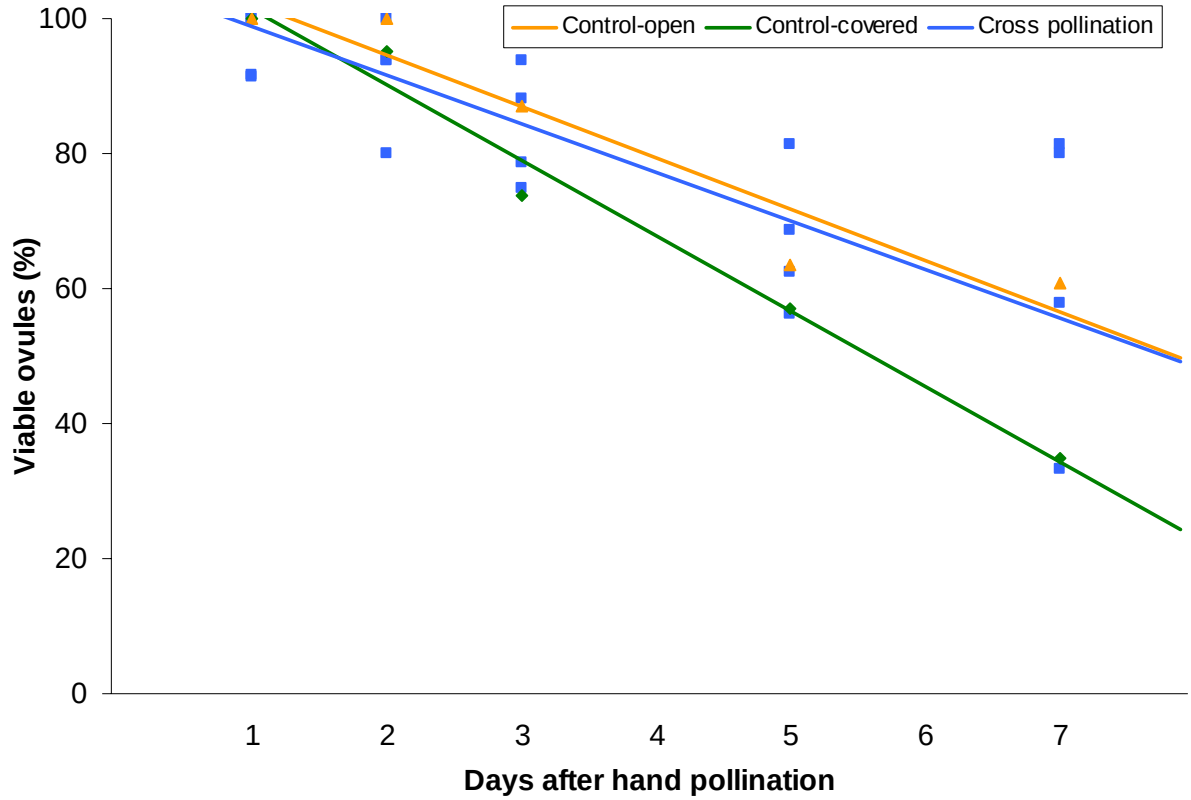

Figure 2.14 Relationship between percentage of pistils with viable primary ovules in unpollinated versus cross pollinated 'Bing' sweet cherry flowers and days after hand pollination in the orchard during 2006.

Source of Variation	d.f.	Mean square	*F* value	Pr > *F*
Days[z]	1	6765.5	64.88	<0.001
Polliniser	2	89.6	0.86	0.434
Days x Polliniser	2	91.1	0.87	0.428
Residual	29			

$R^2 = 0.651$

[z] Days after hand pollination

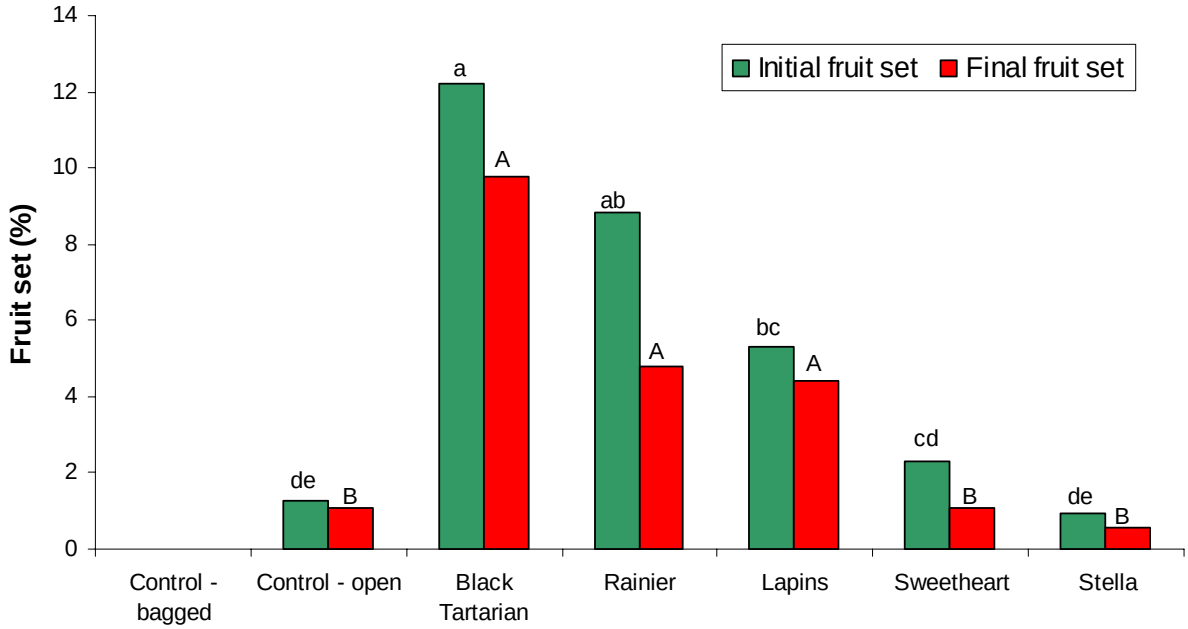

Figure 2.15 Initial and final fruit set of hand cross-pollinated 'Bing' sweet cherry flowers in the orchard during 2006. Means followed by the same letter within the same fruit set parameter do not differ significantly at $P = 0.05$.
Initial fruit set: LSD (5%) = 5.90, P<0.001. Final fruit set: LSD (5%) = 6.06, P<0.001.

3. PAPER 2: INFLUENCE OF TIMING AND CONCENTRATION OF DIFFERENT REST BREAKING AGENTS ON DORMANCY BREAKING IN 'BING' SWEET CHERRY UNDER SOUTH AFRICAN CONDITIONS

Abstract

Inadequate winter chilling is a major limiting factor to the successful production of sweet cherries in South Africa with low chill conditions negatively impacting its cropping potential. Trials aimed at assessing the impact of various rest breaking agents (RBAs) and timing of application on vegetative and floral bud break, and yield were conducted on 4-year-old 'Bing' sweet cherry trees on 'Gisela® 5' rootstock. Two experiments were conducted near Clarens (28°28'S; 28°19'E, 1860 m) and Reitz (28°0'S; 28°28'E; 1717 m) in the eastern Free State, during 2005 and 2006 respectively. The 2005 experiment evaluated five treatments, viz; 1% and 2% Dormex® (hydrogen cyanamide, HCN); 1% Dormex® + 3% mineral oil; and 3% Lift® (thidiazuron and mineral oil) sprayed on three dates (29 July 2005, 5 August 2005 and 12 August 2005) preceding expected "green-tip" stage of flower development, plus an unsprayed control. In 2006 four treatments were evaluated, viz; 1% Dormex®; 1% Dormex® + 3% mineral oil; 3% Lift® applied on three dates (26 July 2006, 7 August 2006 and 12 August 2006) and an unsprayed control. No interaction was observed between time of application and type of RBA. RBAs were effective at improving budburst and yield during both seasons with the time of application of RBAs having the most significant influence on budburst and production efficiency in 'Bing' sweet cherry trees. RBAs were most effective at improving vegetative budburst when applied 9 to 16 days before "green-tip" stage of flower development. Floral budburst and yield were increased by 1% Dormex® + 3% mineral oil and 3% Lift®, but results varied between seasons indicating that time of RBA application should be based on chilling accumulation and bud development stage and not based on calendar date.

Keywords: hydrogen cyanamide; insufficient chilling; rest breaking agents; sweet cherry; thidiazuron; timing

Introduction

Insufficient winter chilling is a limiting factor to the production of deciduous fruit under warm climatic conditions (Couvillon, 1995). Most areas in South Africa are climatically marginal for the production of high-chill stone fruit such as sweet cherries (*Prunus avium* L.) which have a chilling requirement exceeding 1 100 Utah chill units (UCU) (Mahmood et al.,

2000). Pheno-climatography modelling showed that 'Bing' sweet cherry grown at Prosser, Washington State University, USA, required a minimum of 880 UCU to complete endo-dormancy (Ashcroft et al., 1977; Richardson et al., 1986). The chilling requirement of floral and vegetative buds is seldom fully satisfied leading to uneven floral bud burst, delayed foliation and poor fruit set (Erez, 1995; Saure, 1985). Stone fruit floral buds are particularly susceptible, with prevention of normal bud break often leading to bud necrosis (George and Erez, 2000). Artificial means to compensate for this lack of chilling, by the annual application of rest breaking agents (RBAs), has become standard practice in the sweet cherry industry in South Africa.

The discovery of hydrogen cyanamide (HCN) in the 1980's, an aqueous suspension of calcium cyanamide, resulted in cyanamide becoming commercially feasible as a rest breaking spray (Shulman et al., 1986). The chemical has been shown to be effective in the breaking of dormancy in a number crops such as apples (*Malus domestica* Borkh.)(North, 1992; Sagredo et al., 2005), apricot (*Prunus armeniaca* L.) (Bartolini et al., 1997), highbush blueberry (*Vaccinium corymbosum* L.) (Williamson et al., 2002), nectarines (*P. persicae* L. Batsch) (George and Nissen, 1988), high chill peaches (*P. persicae* L. Batsch) (Siller-Cepeda et al., 1992), grapes (Lombard, 2003; Shulman et al., 1983), and sweet cherries (Costa et al., 2004; Snir and Erez, 1988).

HCN promotes early and more uniform budbreak, and is particularly useful for enhancing bloom and synchronising blossoming among sweet cherry and almond cultivars which require maximum blossom levels for cross pollination (Erez, 1987; Erez, 2000). In 'Sam' and 'Rainier' sweet cherry, early applications tended to advance bloom by up to 13 days, while later applications resulted in a more compressed bloom period (Snir and Erez, 1988). Fruit maturity in 'Sam' was advanced by 2 weeks. The time of application of HCN has been shown to be more effective than concentration at advancing bloom in 'Burlat sweet cherry (Pasciano et al., 1997).

Fuchigami and Nee (1987) indicated that the influence of HCN on percentage bud burst and phytotoxicity is dependent on the timing or physiological stage of dormancy of the bud. Late applications, close to natural bud burst, often result in flower bud phytotoxicity (NeSmith, 2005), while too early applications are often ineffective as the buds are still endodormant (Erez, 2000). Stone fruit crops have simple buds which are particularly susceptible to

chemical burn, although sweet cherry buds appear to be less sensitive (Erez, 1995). HCN can only substitute for approximately 30% of a cultivar's chilling requirement (Erez, 1995; Erez, 2000). Research conducted in California has shown that 'Bing' sweet cherry trees were responsive to HCN when 49-57 Chilling Portions (Fishman et al., 1987) had accumulated (Glozer, 2006).

Leaf buds in stone fruit usually emerge after floral buds as a result of their higher chilling requirement, leading to delayed foliage development (Couvillon, 1995). In blueberries, leaf emergence during years of low chilling and high fruit set, can be delayed for several weeks, resulting in low leaf-to-fruit ratios and low final fruit set (Williamson et al., 2002; Williamson et al., 2001). HCN has been shown to have a marked effect on vegetative buds (Erez, 1987), significantly enhancing vegetative bud burst in apricot, peach and rabbiteye blueberries in areas with insufficient winter chilling.

Thidiazuron (*N*-phenyl-*N*'-1,2,3,-thidiazol-5-ylurea; TDZ), a cytokinin-like growth regulator, has been shown to improve bud burst in apples (Wang et al., 1986), pears, cherries and plums (Costa et al., 2004), although work on apples by Steffens and Stutte (1989) showed TDZ to be more effective in promoting bud burst when applied at the initiation of chilling. The dormancy breaking effect of TDZ appears to be correlated with increases in RNA, DNA, S-adenosylmethionine (SAM), protein and accelerated polyamine synthesis (Wang et al., 1986).

The objectives of this study were, firstly, to determine the effectiveness of low concentrations of Dormex®, alone and in combination with mineral oil, and Lift®, and secondly, to determine the optimum timing of applications, to enhance budburst, fruit set and yield of 'Bing' sweet cherry in a climatically marginal region with inadequate winter chilling.

Materials and methods

Experiments were conducted over two seasons on 4-year-old (5th leaf) 'Bing' sweet cherry trees on 'Gisela® 5' (Gi 5) rootstock grown in commercial orchards near Clarens (28°28'S; 28°19'E, 1860 m) and Reitz (28°0'S; 28°28'E; 1717 m), eastern Free State, South Africa. Trees were spaced 4.5 x 2.0 m and were trained to a modified central leader (Solaxe) system. Hourly air temperature was measured using Tinytag Plus data loggers (Gemini Data Loggers, UK) and used to calculate Daily Positive Utah Chill units (PCUs) (Linsley-Noakes *et. al*., 1994) and Chilling Portions (CPs) according to the Dynamic model (Fishman *et. al*., 1987).

Rest breaking agents used in the experiments were: Dormex® (hydrogen cyanamide 520 g/L, HCN; SKW Trostberg AG, Germany), Lift® (thidiazuron 3 g/L in mineral oil; Almond Agro Chemicals, South Africa) and Budbreak® (mineral oil 869.3 g/L; BASF, South Africa).

2005 season

During the 2005 season in the Clarens area, the treatments consisted of Dormex® (1% and 2% v/v), Dormex® 1% (v/v) plus Budbreak® oil 3% (v/v) and Lift® 3% (v/v) applied at three dates (29 July 2005, 5 August 2005 and 12 August 2005) prior to expected "green-tip" stage of flower development. Chilling accumulation was calculated from 1 May 2005 until each of the three dates of application. Trees were sprayed to runoff using a knapsack sprayer at the three dates above, corresponding to 23, 16 and 9 days prior to predicted "green-tip" [Stage 3, (Ballard et al., 1982)].

Data were collected from two 2-year-old branches, situated on opposite sides of the tree, and five 1-year-old shoots which were randomly selected per tree. Branch diameters were measured at the base of the 2-year-old branch, and branch cross-sectional area (BCSA) calculated. Percentage vegetative budburst data on 1-year-old shoots, and floral and vegetative budburst of spurs on 2-year-old branches were collected on 17 September 2006 (post bloom). Floral buds were considered open at stage 3 to 4, i.e. buds at "green-tip" to "tight cluster", based on the Ballard et al. (1982) rating chart while vegetative buds were considered open when the tips of the first two leaves were clearly visible. Yield efficiency (g. cm^{-2} BCSA) and branch fruit density (number of fruit/cm^2 BCSA) were calculated from all fruit, harvested at commercial maturity on the 2-year-old branches.

The experiment was set out as a randomised complete block design with four single-tree replicates and two factors (treatment and time). Each replicate consisted of three trees with the centre tree used for data collection. One of the border trees consisted of a pollenizer (either 'Stella', 'Sweet Anne', 'Van' or 'Rainier'). Analysis of variance was conducted using the PROC GLM procedures of SAS, release 9.1 (SAS Institute, 2002) initially as a one-way classification (with control) and secondly as a two-way factorial analysis (without control) with RBAs and time as the main effects.

2006 season

During the second (2006) season in the Reitz area, the treatments consisted of Dormex® 1% (v/v), Dormex® 1% (v/v) plus Budbreak® oil 3% (v/v) and Lift® 3% (v/v) applied at three dates (26 July 2006, 7 August 2006 and 12 August 2006) prior to expected budburst. Chilling accumulation was calculated from 1 May 2006 until the three dates of application. Trees were sprayed to runoff using a knapsack sprayer at the three dates above, corresponding to 29, 17, 12 days prior to predicted "green-tip".

Data were collected from three 2-year-old branches and five 1-year-old shoots were randomly selected per tree and used for data collection. Branch diameters were measured at the base of the 2-year-old branch, and BCSA calculated. Percentage vegetative budburst data on 1-year-old shoots, and floral and vegetative budburst of spurs on 2-year-old branches were collected on 16 September 2006 (full bloom). Floral buds were considered open at stage 3 to 4, i.e. buds at "green-tip" to tight cluster, based on the Ballard et al. (1982) rating chart while vegetative buds were considered open when the tips of the first two leaves were clearly visible. Yield efficiency ($g.cm^{-2}$ BCSA) and branch fruit density (number of $fruit/cm^2$ BCSA) were calculated from all fruit, harvested at commercial maturity, on the 2-year-old branches.

The experiment was set out as a randomised complete block design with five single-tree replicates and two factors (treatment and time). Each replicate consisted of three trees with the centre tree used for data collection. One of the border trees consisted of a pollenizer (either 'Stella', 'Black Tartarian', 'Van' 'Giant Heidelfinger' or 'Rainier'). Analysis of variance was conducted using the PROC GLM procedures of SAS, release 9.1 (SAS Institute, 2002) initially as a one-way classification (with control) and secondly as a two-way factorial analysis (without control) with RBAs and time as the main effects.

Results

Daily Positive Utah chill (Infruitec) unit accumulation was higher, during the 2006 versus the 2005 season, for the second and third spray dates, but slightly lower for the first spray date (Fig. 3.1; Table 3.1). Cumulative chilling portions for the three application dates were substantially higher during the 2006 season compared to the 2005 season. Based on PCUs, buds had received less than 70% of their chilling accumulation (Erez, 2000) by the third spray date in both years. The correlation between CP:PCU under mild winter climates has been shown to be slightly less than 1:20 (Allan et al., 1999). Thus in terms of CPs, none of the buds

appear to have received 70% of their chilling requirement by the third spray date in 2005, but all buds had received ~ 70% of their chilling requirement by the first spray date in 2006.

2005 season

All treatments, with the exception of 1% Dormex® + 3% oil which was applied on 29 July, improved vegetative budburst on 1-year-old shoots and spurs, and floral budburst on spurs, compared to the unsprayed control (Table 3.2). Although all RBA treatments improved budburst, all trees showed symptoms of delayed foliation due to insufficient winter chilling at the time of application as a result of the unseasonably warm winter. All treatments increased fruit density and production efficiency, compared to the unsprayed control (Table 3.2). No symptoms of phytotoxicity were noted for any treatments.

No significant interactions between RBA treatments and date of application were observed. RBA treatments affected only vegetative budburst on 1-year-old shoots significantly with budburst increasing with Dormex® concentration from 1 to 2% (Table 2). No significant differences were noted between 1% and 2% Dormex® for budburst on spurs, fruit density and production efficiency.

The time of application of the various RBA treatments significantly influenced budburst, fruit density and production efficiency. Time of spray application had the most beneficial effect on budburst and yield when sprayed on 12 Aug. corresponding to 9 days before “green tip” and ~34 days before full bloom (Table 3.2). The flower buds on 12 Aug. were at the “side-pink” stage of development (Fig. 3.2), between stages 1 (first swelling) and 2 (side green) of the Ballard et al. (1982) rating chart. Budburst increased with date of application with the exception of floral budburst on 5 Aug. Vegetative budburst on 1-year-old shoots and on spurs was higher ($P<0.001$) for the 5 Aug. and 12 Aug. compared to 29 July application. Floral budburst was 21.4% and 22.9% higher ($P=0.016$) for 12 Aug. application compared to 29 July and 5 Aug. respectively. The 29 July spray date increased the earliness of vegetative budburst and time of flowering by 3 to 5 days (data not shown) but had no influence on time to harvest. Fruit density and production efficiency increased with time of application with 12 Aug. resulting in significantly higher fruit number and yield per cm^2 BCSA than the first two spray dates (Table 3.2).

2006 Season

Severe frosts on 30 (-2.3°C) and 31 (-3.9°C) August may have negatively influenced the results as approximately 10-20% flower bud damage on unsprayed trees was noted throughout the orchard block.

Similarly to the 2005 trial, no interaction was detected between RBA treatments and date of application. All treatments, with the exception of 1% Dormex® which was applied on 12 Aug., improved vegetative budburst on 1-year-old shoots and spurs, and floral budburst on spurs, compared to the unsprayed control (Table 3.3). Compared to the 2005 season, flower buds showed a distinct reduction in percentage budburst for the third (12 Aug.) application (16 days prior to actual "green-tip"), especially the two Dormex® treatments. Lift® showed a slight decrease in floral budburst for the 12 Aug. application, with no significant differences between dates of application. Fruit density and production efficiency was highest for the 26 July spray but generally lower for the third spray on 12 Aug., compared to the unsprayed control.

Time of application of the various RBA treatments showed significant differences between spray dates for floral budburst, fruit density and production efficiency (Table 3.3). Floral budburst was significantly higher for the 26 July and 7 Aug. sprays compared to the 12 Aug application. The buds on 7 Aug. were at the "side-pink" stage of development, between stages 1 (first swelling) and 2 (side green) of the Ballard et al. (1982) rating chart. Vegetative buds on spurs showed the converse effect to the flower buds with 7 Aug. and 12 Aug. spray dates resulting in increased ($P=0.002$) budburst compared to 26 July spray. Fruit density and production efficiency showed the same effect as flower buds with fruit number and yield per cm^2 BCSA being significantly reduced between the 26 July and 12 Aug. applications. The effect of Lift® on vegetative budburst on 1-year-old shoots and floral and vegetative budburst on spurs was very similar among the three spray dates.

Discussion

This study has demonstrated that the time of application of various RBAs could be a more important factor than the type of RBA used in breaking dormancy and promoting budburst and yield in 'Bing' sweet cherry grown under insufficient chilling conditions. No interactions were noted between time of application and RBA treatment indicating that the various RBAs showed a similar response in terms of vegetative budburst and yield at each spray date, particularly at the first spray date in both years.

The stage of bud development is influenced by the satisfaction of chilling, to overcome endodormancy, and the subsequent accumulation of heat units during eco-dormancy (Richardson et al., 1986). PCU accumulation during both seasons, and CPs during 2005, were less than 70% of the chilling requirement for 'Bing' indicating that the Dormex® treatments may not have been able to fully substitute for the chilling requirement of the cultivar (Erez, 1995; Erez, 2000). Glozer (2006) showed that 'Bing' sweet cherry buds were responsive to HCN when 49-57 CPs have accumulated. This was confirmed during the 2006 season, when 48 CPs had accumulated by the 26 July spray date. The lack of delayed foliation on treated trees during the 2006 season indicates that at least 70% of chilling was probably satisfied at the time of application.

The application of Dormex® and Lift® showed an improvement in budburst and yield compared to the unsprayed control in both years. The results agree with the work of Costa et al. (2004) who showed that both chemicals are effective RBAs at improving vegetative and floral budburst in 'Bing' sweet cherry. No significant differences in vegetative and floral budburst on spurs, and yield were noted between 1% and 2% Dormex® during the 2005 season, indicating that 1% Dormex® can be used effectively to promote flowering and fruit set in 'Bing' sweet cherry. This agrees with previous research in South Africa, and Mexico, which showed 1% Dormex® to be effective at improving vegetative and floral budburst across a range of sweet cherry cultivars, including 'Bing' (Costa et al., 2004; Martínez et al., 1999). The addition of 3% mineral oil to 1% Dormex® did not significantly enhance efficacy but did increase production efficiency in both seasons.

Improved vegetative budburst on 1-year-old shoots was observed during 2006 indicating a possible increase in number of spurs and flower clusters the following season. Concentrations of HCN less than 2% have been shown to significantly enhance vegetative budburst in peach (Fernandez-Escobar and Martin, 1987; Siller-Cepeda et al., 1992), sweet cherry (Costa et al., 2004) and rabbiteye blueberries (Williamson et al., 2001). Poor vegetative budburst on 1-year-old shoots and delayed foliage development on spurs was noted on the control trees during 2005. This poor leaf emergence, lead to low leaf:fruit ratios and was the likely cause of the low fruit density and production efficiency on these trees. Delayed canopy development and reduced fruit set, enhanced by marginal winter chilling, has been observed in rabbiteye blueberries (Williamson et al., 2002).

The time of the application of the different RBAs had the most beneficial effect on budburst and yield when sprayed on 12 Aug. in 2005 (~35 days before full bloom) and 26 July (~51 days before full bloom) in 2006. The higher floral budburst and production efficiency recorded for the 12 Aug. spray (Table 2) in 2005 possibly indicates that buds may not have fully exited endo-dormancy by the time of application, allowing RBAs to more effectively promote budburst closer to "green-tip" and full bloom. Siller-Cepeda et al. (1992) showed that budburst in 'Redhaven' peach buds was highest with treatments of 0.5% and 1% HCN applied during the late stages of endo-dormancy.

The poor floral budburst and production efficiency observed for the 12 Aug. spray in 2006 indicates that late applications, once buds have been released from endo-dormancy can result in flower bud damage occurring, as a result of phytotoxicity (Erez, 2000). The response to the time of application of HCN has been shown to be inconsistent as its efficacy is dependent on the intensity of bud endo-dormancy and tissue sensitivity (Erez, 1987; Fuchigami and Nee, 1987). Siller-Cepeda et al. (1992) reported 80-90% damage to 'Redhaven' peach buds when sprayed with 2% HCN or higher eco-dormancy. Late applications of HCN have also been shown to cause substantial flower bud mortality in nectarines (George and Nissen, 1988), rabbiteye blueberries (Williamson et al., 2001) and strong inhibition of flower bud development in 'Springcrest' peach (Fernandez-Escobar and Martin, 1987). The floral buds were thus at a more advanced stage of physiological development on 12 Aug. 2006 and therefore more sensitive to phytotoxic concentrations of HCN. Weather conditions during applications were slightly cooler in 2006 than 2005 with minimum temperatures being 5.5°C colder. The low minimum temperatures during and after spraying, and the severe frost damage on 30/31 August 2006 may have influenced the degree of phytotoxicity of the late third application as reflected in Table 3 where a declining trend is visible. Erez (1987) reported that cool conditions may enhance floral bud damage in peaches and plum sprayed with HCN although the interaction is still unclear.

The effect of Lift® on the time of floral and vegetative budburst on spurs in both seasons appears to be independent of the time of application as all three spray dates showed similar budburst percentages. This may indicate that bud tissues are receptive to Lift® over a wider range of physiological bud stages, therefore increasing the "window" for application. Work on 'Anna' and 'Delicious' apples showed that TDZ was most effective at promoting bud burst

on 2-year-old wood before and after chilling accumulation (Steffens and Stutte, 1989), i.e. during para- and eco-dormancy.

Conclusions

Rest breaking agents were effective at improving budburst and yield over both seasons with the application time of RBAs having the most significant influence on budburst and production efficiency in 'Bing' sweet cherry trees. No interaction was observed between time of application and type of RBA. Most of the year-to-year variability in budburst and phytotoxicity can probably be attributed to the degree of chilling accumulation prior to applying treatments and the physiological state of the bud at the time of application (Siller-Cepeda et al., 1992).

Rest breaking agents were most effective at improving vegetative budburst when applied ~ 9 to 16 days before actual "green-tip" across both seasons. Dormex® 1% + 3% oil and 3% Lift® were the most effective at breaking floral bud dormancy and improving yield efficiency although the time of application varied between seasons. Treatments applied 9 days and 33 days before "green-tip" were most effective during 2005 and 2006, respectively. An interaction of phytotoxicity and late winter frosts may have been the reason for the decreased floral budburst, fruit density and production efficiency noted with 12 Aug. spray in 2006. The timing of RBAs could thus be based on a combination of chill unit accumulation and visual bud development stage and not only on calendar date. In low chill years (<700 PCU's and <40 CPs by end of July) applications could be based on physiological stage of bud development ("side-pink") while in high chill years (>850 PCUs and >50 CPs), applications could be based on both chilling accumulation and the visual appearance of the bud. Lift® showed promising results with a wider "window" of application than Dormex®.

Further studies are required to identify the best rate of Lift® and optimize the timing of Dormex® and Lift® to improve floral budburst and yield in 'Bing', as the results were inconclusive due to the possible interaction of the late frost and the time of RBA application. This research suggests that 'Bing' is climatically poorly suited to the current production areas of the eastern Free State which experience insufficient winter chilling, and research is required to identify new lower chill cultivars with improved climatic adaptation.

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Table 3.1 "Green-tip" and chill unit accumulation for the 2005 and 2006 seasons.

Season	Application date	Predicted "green-tip"	Actual "green-tip"	Chill units	
				PCU	CP
2005	29 July	22 Aug	21 Aug	659	39
	5 August			704	41
	12 August			740	43
2006	26 July	24 Aug	28 Aug	637	48
	7 August			736	54
	12 August			770	57

Table 3.2 Effect of RBA treatments and time of application on budburst, fruit density and production efficiency of 'Bing' sweet cherry in the Clarens area during 2005.

Treatment	Vegetative budburst (%)		Floral budburst	Fruit density (No. of fruit cm^{-2} BCSA[#])	Production efficiency (g cm^{-2} BCSA[#])
	1-yr-old shoot	Spurs	Spurs		
Analysis including control (One-way classification)					
Dormex (1%)					
29 July	23.2 fg[*]	86.8 abc	73.0 abc	2.3	23.0
5 Aug	26.3 defg	88.2 abc	70.0 abc	15.4	59.9
12 Aug	35.6 bcdef	94.1 ab	100.0 a	29.4	135.2
Dormex (2%)					
29 July	30.5 cdefg	87.4 abc	79.2 abc	13.4	102.1
5 Aug	51.8 a	93.3 abc	54.1 c	2.7	26.9
12 Aug	43.3 abc	97.9 ab	100.0 a	45.1	263.4
Dormex (1%) + oil (3%)					
29 July	21.0 g	75.6 dc	51.4 c	5.6	49.6
5 Aug	39.0 abcd	97.0 ab	65.3 bc	17.5	119.5
12 Aug	46.5 ab	100 a	87.7 ab	20.8	135.4
Lift (3%)					
29 July	22.2 g	81.5 bcd	90.0 ab	12.1	76.2
5 Aug	25.4 efg	96.3 ab	98.2 a	22.5	159.7
12 Aug	37.2 bcde	98.7 ab	91.3 ab	18.6	144.5
Control	21.6 g	67.9 d	51.7 c	0.4	3.2
Pr > F	0.0001	0.0224	0.0189	0.1327	0.0626
Factorial analysis without control					
Rest breaking agent					
Dormex (1%)	28.3 b	89.7	81.0	15.7	72.7
Dormex (2%)	41.9 a	92.9	77.8	20.4	130.8
Dormex (1%) + oil (3%)	35.5 ab	90.9	68.1	14.7	101.5
Lift (3%)	28.3 b	92.2	93.2	17.7	126.8
Time					
29 Jul	24.2 b	82.9 b	73.4 b	8.4 b	62.7 b
5 Aug	35.6 a	93.7 a	71.9 b	14.5 ab	91.52 b
12 Aug	40.6 a	97.7 a	94.8 a	28.5 a	169.6 a
Pr > F					
RBA	0.0024	0.8412	0.0919	0.8993	0.5020
Time	<0.0001	0.0004	0.0162	0.0257	0.0209
Time x RBA	0.1404	0.4083	0.3146	0.3770	0.3373

[#]Branch cross-sectional area

[*]Means followed by the same letter within the same column are not significantly different at P = 0.05.

Table 3.3 Effect of RBA treatments and time of application on budburst, fruit density and production efficiency of 'Bing' sweet cherry in the Reitz area during 2006.

Treatment	Vegetative budburst (%)		Floral budburst	Fruit density (No. of fruit cm^{-2} BCSA[#])	Production efficiency (g cm^{-2} BCSA[#])
	1-yr-old shoot	Spurs	Spurs		
Analysis including control (One-way classification)					
Dormex (1%)					
26 Jul	42.5 b[*]	69.6 bc	76.0 ab	20.2	151.2
7 Aug	40.1 b	82.6 ab	72.4 abc	7.8	68.6
12 Aug	59.3 ab	91.5 a	50.2 c	7.1	56.3
Dormex (1%) + oil (3%)					
26 Jul	47.3 b	66.8 bc	92.9 a	16.1	121.9
7 Aug	80.9 a	94.5 a	93.7 a	17.2	116.5
12 Aug	69.2 ab	93.6 a	51.2 c	12.7	87.8
Lift (3%)					
26 Jul	52.5 ab	90.2 a	83.0 ab	20.1	158.2
7 Aug	63.8 ab	90.3 a	70.7 abc	7.2	64.8
12 Aug	58.9 ab	94.5 a	64.5 bc	7.4	60.8
Control	9.08 c	58.6 c	50.5 c	9.5	74.4
Pr > F	0.0045	0.0001	0.0013	0.2701	0.2292
Factorial analysis without control					
Rest breaking agent					
Dormex (1%)	47.3	81.3	66.2	11.7	92.0
Dormex (1%) + oil (3%)	65.8	85.0	79.3	15.3	108.8
Lift (3%)	58.4	91.7	72.7	11.6	94.6
Time					
26 Jul	47.4	75.5 b	83.9 a	18.8 a	143.8 a
7 Aug	61.6	89.4 a	78.9 a	10.7 ab	83.3 b
12 Aug	62.5	93.2 a	55.3 b	9.1 b	68.3 b
Pr > F					
RBA	0.1436	0.1049	0.1789	0.5706	0.8008
Time	0.1986	0.0020	0.0004	0.0442	0.0198
Time x RBA	0.4957	0.1694	0.3207	0.6034	0.6848

[#]Branch cross-sectional area

[*]Means followed by the same letter within the same column are not significantly different at P = 0.05.

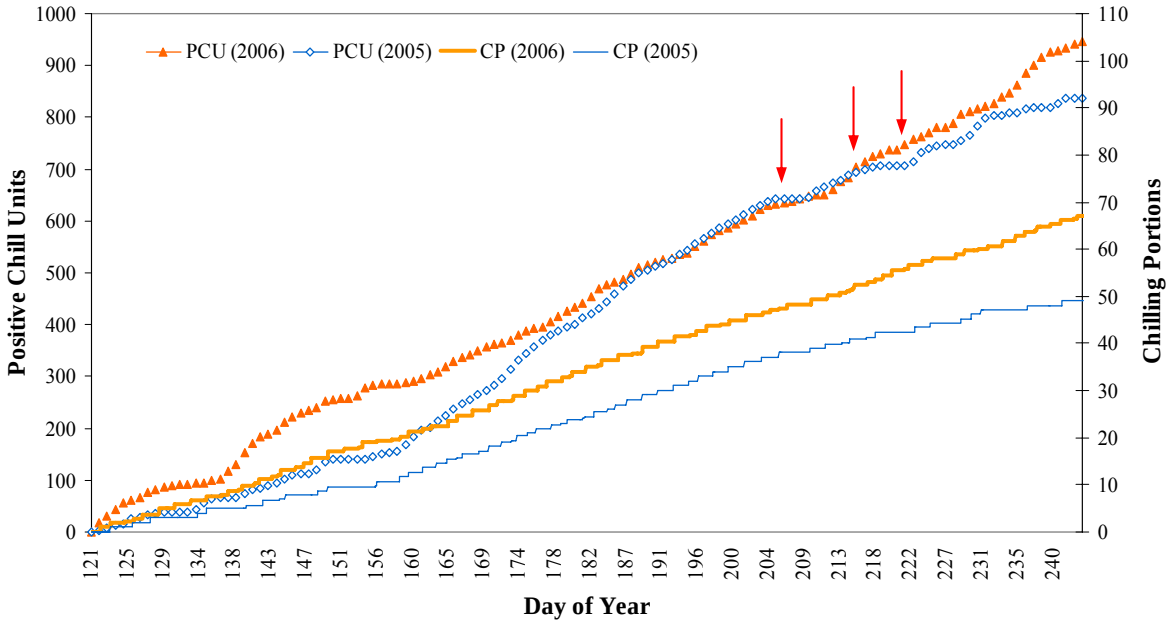

Figure 3.1 Chilling accumulation during the 2005 and 2006 seasons. Arrows indicate the approximate timing of RBA spray applications.

Figure 3.2 'Bing' sweet cherry spur showing flower buds at "side pink" stage of development.

CONCLUSIONS

The current research has helped answer a number of questions concerning 'Bing' sweet cherry production in the South African. All the pollinizers tested in the trials were cross compatible and showed some synchrony of bloom with 'Bing', except 'Stella' which flowered too late and precluded it as a suitable pollinizer. 'Black Tartarian', 'Rainier' and 'Lapins' showed the best pollen performance and fruit set in the orchard experiment, indicating that they are the most suitable cross pollinizers for 'Bing'. Pollen performance appeared to be mediated by both temperature and pollen-pistil interactions. Poor transfer of pollen in the orchard for the 'open pollinated control', and "pollen competition" on the stigma appear to have played a role in the low fruit set recorded for some pollen donors.

The viability of the primary ovule was influenced by the prevailing temperature conditions with the higher laboratory temperatures significantly reducing ovule viability compared to the lower orchard temperatures. Although the lower temperatures in the orchard increased ovule longevity, they slowed tube penetration of the ovule, resulting in an effective pollination period of only 1 to 2 days. Cross pollination improved ovule longevity thus the maximization of pollen transfer within 2 to 3 days of anthesis could improve ovule viability and final fruit set. The loss of pollen tube direction in the ovary, and central fluorescence of the ovule may indicate early degeneration of the embryo sac in the orchard trial.

The principle factors causing low fruit set in 'Bing' sweet cherry appear to be premature abortion of the ovule and inadequate transfer of sufficient viable pollen under field conditions. Further research is required on the influence of late summer and winter temperatures on embryo sac degeneration and optimization of the number of bee hives during bloom.

The two rest breaking agents, Dormex®, alone or in combination with mineral oil, and Lift® were effective at improving budburst and yield over both seasons with the application time of RBAs having the most significant influence on budburst and production efficiency in 'Bing' sweet cherry trees. Applications of RBAs approximately 9 to 16 days before actual "green tip" had the most significant effect on vegetative bud burst with variable results obtained for floral budburst. Most of the year-to-year variation in floral bud burst and phytotoxicity can probably be attributed to the degree of chilling accumulation prior to the application of treatments and the physiological state of the bud at the time of application. The timing of

RBAs should thus be based on a combination of chill unit accumulation and visual bud development stage and not solely on calendar date.

The current research suggests that ‘Bing’ sweet cherry is poorly suited climatically to the current production areas of the eastern Free State and short-term research needs to identify methods of improving chilling and fruit set by means of evaporative cooling and fruit set-enhancing plant growth regulators. Longer term work requires the identification of new, lower chill cultivars with improved climatic adaptation to South African conditions.