CROSS POLLINATION BIOLOGY OF APPLES WITH SPECIAL REFERENCE TO 'AFRICAN RED'.

BY

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DECLARATION

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

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KRUISBESTUIWINGSBIOLOGIE VAN APPELS MET SPESIALE VERWYSING NA 'AFRICAN RED'.

OPSOMMING

Oneffektiewe kruisbestuiwing in die boord kan toegeskryf word aan òf onverenigbaarheid tussen die hoof- en bestuiwingskultivar òf as gevolg van die blomtyd van die kruisbestuiwer wat nie genoegsaam oorvleuel met die van die hoofkultivar nie. Drie proewe is uitgevoer om 'n meer effektiewe proses daar te stel vir die toets van kultivarverenigbaarheid en om kultivars te probeer groepeer na gelang van hul reaksie op bepaalde hoeveelhede koue.

Meeste appelkultivars is selfonverenigbaar en benodig kruisbestuiwing vir genoegsame vrugset. As gevolg van verskille in die geneties gedefinieerde bevrugtingsverenigbaarheid tussen die stuifmeel van die manlike ouer (bestuiwer) en die eiersel van die vroulike ouer (hoofkultivar), verskil bestuiwers in hul vermoë om vrugte met sade te set. Vruggrootte en -massa is positief gekorreleerd met saadset alhoewel dit al gevind is dat die bestuiwer op sig self ook 'n invloed op vrugkwaliteit kan hê. 'African Red' appelbome op M7 onderstamme, in 'n evaluasie blok op 'n kommersiële plaas in die Koue Bokkeveld (32°55'N 19°27'E, Meditereense klimaat, ≈1060 Utah koue eenhede, en ≈530mm jaarlikse reënval; ligging 966 m), is gebruik om die invloed van 5 bestuiwers ('Granny Smith', 'Winter Banana', 'Cripps' Pink', 'Cripps' Red' and

'Simpson Crab') op vrugset, vrugmassa, -lengte en -deursnee oor twee seisoene te bepaal. Die mate waartoe 'African Red' self onverenigbaar is en die effek van blomposisie ("king" vs laterale blom) op vrugkwaliteit is ook bepaal. Geen een van die bestuiwers het vrugset beduidend beïnvloed nie. Ook is daar geen verskille gevind tussen die "king" en laterale blomposisies t.o.v. vrugset nie. Geen beduidende verskille is tussen bestuiwers gevind in die gemiddelde aantal of gewig van volsade geset nie. In albei jare was die vrugmassa beduidend gekorreleerd met saadset vir al vyf bestuiwerkultivars. In 1998 het 'Simpson Crab' vrugte geset wat beduidend langer was as vrugte wat geset het toe 'Cripps' Pink' as bestuiwer gebruik is. Daar is ook gevind dat 'African Red' hoogs selfonverenigbaar is.

Verenigbaarheidstoetse wat gebaseer is op die aantal vruggies wat ontwikkel nadat blomme van die hoofkultivar met die hand bestuif is, is 'n tydsame proses. Allele spesifieke PCR amplifikasie vir bekende S-allele van die onverenigbaarheids S-geen (S2, S3, S5 S7en S9) is suksesvol uitgevoer om die verenigbaarheid van genotipes vooraf te bepaal. Die resultate het goed vergelyk met wat in literatuur gevind is. Vir al die *Malus domestica* spesies wat getoets is, is ten minste een, en in sommige gevalle twee, van die S-allele gevind. Die blomappel 'Simpson' (*Malus baccata*) het egter nie een van die vyf S-allele opgelewer nie

Een-jaar-oue, 40 mm lang lote van verskeie appelkultivars, is in twee opeenvolgende jare (1998 en 1999) vanuit kommersiële boorde in beide die Elgin [34°S, 305 m, ca. 750 koue

eenhede (CU) (Richardson et al., 1974)] and Koue Bokkeveld (33°S, 945 m, ca. 1300 CU) areas van die Wes Kaap gsny. Die lote is geforseer om te bot by 'n konstante temperatuur van 25°C met deurlopende beligting, nadat elke groep lote aan 'n bepaalde hoeveelheid koue blootgestel is. Die effek van koue op bot van elke kultivar in beide areas is bepaal deur, 1) die totale persentasie knoppe wat gebot het, 2) die persentasie terminale knoppe wat gebot het, en 3) die tempo van bot [1/(dae tot 25% bot)] te meet. Daar is gevind dat bo-genoemde parameters beduidend tussen kultivars, en binne kultivars tussen areas, verskil. As 'n beskrywing van die reaksie van knoppe op koue het die tempo van bot die mees konstante resultate oor die twee opeenvolgende seisoene gelewer en kon hierdie parameter gebruik word om kultivars in groepe, na gelang van hul reaksie op koue, in te deel.

SUMMARY

Ineffective pollination of the main cultivar with the pollinator cultivar is due to either an incompatibility problem between the main and pollinator cultivar, or because the flowering times of the main and pollinator cultivars do not overlap adequately. Three trials were conducted to try and find a more effective way to determine cultivar compatibility and to group cultivars together according to their budburst reaction to chilling.

Most apple cultivars are self-incompatible and need cross-pollination for fruit set. Due to differences in the genetically defined fertilisation compatibility between the pollen from the male parent (pollinator) and the egg cell of the female parent, various apple pollinators differ in their ability to set fruit with viable seed. Fruit weight and size are positively correlated with seed set although it has been found that the pollinator can have a direct influence on fruit quality. 'African Red' apple trees on M7 rootstock in an evaluation block on a commercial farm in the Koue Bokkeveld region (32°55'N 19°27'E, Mediterranean climate, ≈1060 Utah chill units, and ≈530 mm rainfall annually; altitude 966 m) were used to assess the influence of 5 pollinators ('Granny Smith', 'Winter Banana', 'Cripps' Pink', 'Cripps' Red' and 'Simpson Crab') on fruit set, fruit weight and length and diameter. The degree to which 'African Red' is self-compatible was also assessed and the effect of flowering position ("king" vs. lateral) on fruit quality was determined. None of the pollinators showed a significantly higher fruit set. No

differences in fruit set were found between the "king" and lateral flowering positions. No significant differences were found in the average number or weight of well developed seeds between pollinators. In both years fruit weight was significantly correlated to seed number for all five pollinator cultivars. In 1998 'Simpson crab' gave fruit that were significantly more elongated than those of 'Cripps' Pink'. 'African Red' is highly self incompatible.

Compatibility assessments that are based on the number of fruit that develop after the flowers of the main cultivar had been hand pollinated in field trials are a time-consuming process. Allele-specific PCR amplification for some of the known S-alleles of the incompatibility S-gene (S2, S3, S5, S7 and S9) was carried out to successfully predict the compatibility of genotypes. The results compared well with that found in literature. For all the *Malus domestica* cultivars tested at least one, but in some instances both alleles of the S-gene were determined. 'Simpson crab' (*Malus baccata*) did, however, not possess any of the tested S-alleles.

One-year-old, ca. 40 mm long shoots of various apple cultivars were selected from commercial orchards in both the Elgin [34°S, 305 m, ca. 750 chill units (CU) (Richardson et al., 1974)] and Koue Bokkeveld (33°S, 945 m, ca. 1300 CU) regions of the Western Cape, South Africa in two consecutive years (1998 and 1999). Shoots were forced at a constant 25°C with continuous illumination after receiving their allocated chill units. The effect of chilling period on the budburst of each cultivar in both regions was estimated by determining, 1) the total proportion of budburst (%Bb), 2) the proportion of shoots with

terminal budburst (%TBb), and 3) the rate of budburst [1/(days to 25% budburst)]. It was found that these indices differed significantly between cultivars, and within cultivars between areas, as far as budburst patterns, in reaction to chilling, were concerned. The rate of budburst was the most consistent in describing the reaction of buds to different chilling periods and could be used to group cultivars together according to their budburst reaction to chilling.

Dedicated to those who had the ability and desire, but never

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CONTENTS

1. INTRODUCTION

With reports of wide variation in yield and fruit quality (size and shape) from one season to the next, it was decided to re-investigate orchard pollination requirements, especially for the new generation of apple cultivars, by looking at the different aspects influencing pollination.

The pollination problem is essentially two fold: incompatibility between the pollen from the male parent (pollinator cultivar) and the egg cell of the female parent (main cultivar) and a lack of synchronised flowering between the main and pollinator cultivar.

With the tendency to use fewer cultivars to serve as parents in deciduous fruit breeding programs throughout the world, the problem of cross incompatibility between closely related seedlings is increasing (Spiegel-Roy and Alston, 1982; Austin et al., 1998). Furthermore, most of the new cultivars introduced are bud sports (mutations). Modlibowska (1945) found that all the diploid bud mutations they evaluated were incompatible with the original cultivar, thus showing that the incompatibility genes were carried over to the progeny. The number of closely related cultivars that are planted in close proximity is increasing (Tustin and Cashmore, 1998; Labuschagne, pers. comm.).

Orchard design and planning has changed considerably over the last decade. In order to make management more efficient the range of apple cultivars planted in a single commercial orchard is restricted and the planting of solid blocks of a specific cultivar has

become common (Pheasant, 1985; Nel, pers. comm.). However, because of insufficient pollination, the planting of cross pollinator cultivars as rows instead of the scattered distribution of pollinators in the solid blocks in order to ensure adequate pollination pollen, is again increasing. Furthermore, in the past compatibility assessments were based on the number of fruit that developed after the flowers of the main cultivar was hand pollinated in field trials. This, however, is a time-consuming process and neither the climate nor the physiological condition of the test cultivar are taken into consideration. Allele-specific PCR amplification for some of the known S-alleles (S2, S3, S5, S7 and S9) was carried out to try and successfully predict the compatibility of genotypes in the laboratory

In most European apple producing countries cultivars flower within one or two weeks from one another due to extremely cold winters. With the marginal South African winters, prolonged flowering periods are common and cultivar full bloom dates differ considerably from one year to the next. The grouping of cultivars according to full bloom dates requires at least 10 years of field observations (Soltesz, 1997) and a more efficient way of determining time of bud burst has to be developed.

The purpose of the study was to improve our ability to make more effective pollination recommendations under South African conditions.

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THE POLLINATION BIOLOGY OF *MALUS DOMESTICA* BORKH.

2.1 Introduction.

In order for any organism not to become extinct, it needs to change and adapt to its environment continually. In the case of most flowering plants, including the apple, this is brought about by the diversification of genetic material (inheritance factors) (Wertheim, 1990). Natural selection is thus induced by cross pollination which will enable the new individual to be better adapted to its specific environment and more successful in competition e.g. being more resistant to disease or pests (Faergi and Van der Pijl, 1966).

Because of the above mentioned reason the apple has developed certain mechanisms, of which the self - incompatibility reaction is the most effective, to prevent inbreeding or self pollination and promote out breeding or cross pollination (Modlibowska, 1945; Faergi and Van der Pijl, 1966; Alston, 1996; Kao and McCubbin, 1996).

Although most apple cultivars are self incompatible (Janick and Moore, 1975) and the pollen of most other commercial cultivars is capable of inducing seed fertilisation, cross incompatibility can occur between cultivars that are related in terms of their progeny (Modlibowska, 1945; Owens, 1992). Also it has been found that triploid cultivars are usually incompatible with any other diploid cultivar due to the unbalanced gametes (different number of chromosomes) that are formed during meioses (Janick and Moore, 1975).

With the tendency to use fewer cultivars to serve as parents in deciduous fruit breeding programs through-out the world, the problem of cross incompatibility between closely related seedlings is increasing (Spiegel-Roy and Alston, 1982; Austin et al., 1998). Furthermore, it can be said that most of the new cultivars introduced are bud sports (mutations). Modlibowska (1945) found that all the diploid bud mutations they evaluated were incompatible with the original cultivar, thus showing that the incompatibility genes were carried over to the progeny. The fruit industry is therefore getting into a situation where a

number of closely related cultivars are planted in close proximity to one another (Tustin and Cashmore, 1998; Labuschagne, pers. comm.).

Orchard design and planning has changed considerably over the last decade. In order to make management more efficient, the range of apple cultivars planted in a single commercial orchard is restricted and the planting of solid blocks of a specific cultivar has become common (Pheasant, 1985; Nel, pers. comm.). Because of to little or incompatible pollination, reports of wide variations in orchard yield became evident (Williams, 1970; Jurich, pers. comm.). Some field consultants are now again recommending planting the cross pollinator cultivar as rows instead of the scattered distribution of pollinators in the solid blocks in order to ensure adequate pollination pollen.

For these and other reasons the re-investigation of orchard pollination requirements, especially for new apple cultivars, has become critical. The aim of this literature review is to look at the different factors affecting pollination excluding time of flowering.

2.2 Self-Incompatibility (SI)

In the case of the apple (*Malus domestica*), the male and female organs are present in the same flower. Because of this, and thus to prevent inbreeding as a consequence of continual self-pollination, flowering plants contain preventative mechanisms, e.g. differences in the length of the stamens and styles (Faegri and Van der Pijl, 1966; Wertheim, 1990). Of these mechanisms the most effective one is the so-called self-incompatibility (SI) mechanism (Broothaerts et al., 1996; Kao and McCubbin, 1996) which can best be described as a recognition system by which pistils can discriminate between pollen from different sources on a genetic level so as to allow only some to fertilise the ovules (Dodds et al., 1997).

Self-incompatibility can be expressed in three different ways. On the stigma where the germination of own pollen is inhibited, in the style where the pollen tube growth is resisted, and in the ovule where fertilisation is prevented. In the case of fruit trees the inhibition of

tube growth down the style is the most common expression of the SI system (Alston, 1996; Broothaert et al., 1996).

Gametophytic SI, which has been studied mainly in Solanaceous species such as *Petunia* spp. and *Solanum* spp., depends on and is controlled by a single gene, called the S-gene, which is situated near the surface of the stigma (Broothaerts, et al., 1995; Jansens, 1995; Alston, 1996; Broothaerts et al., 1996). Several different forms, or alleles, of the polymorphic S-gene has been identified (Broothaerts et al., 1995; Jansens et al., 1995). As soon as similar alleles in the pollen (tube) are recognised, the S-gene encodes for specific S-proteins, S-RNases, to be synthesised. These RNases are only present in the pistils of mature flowers and their function is to inhibit and stop further development of the pollen tube by breaking down the RNA of the developing pollen tube (Jansens et al., 1995; Alston, 1996; Broothaerts et al., 1996). This degradation thus either stops the pollen grain from germinating or stops the emerging pollen tube from growing further down the style (Kao and McCubbin, 1996; Dodds et al., 1997).

Many researchers (Herrero, 1992; Owens, 1992; Wilhelmi and Preuss, 1997) mentioned the deposition of callose and the formation of callose plugs as part of the incompatibility reaction. Suluman et al., 1997, however, found that in the case of *Brassica* the self-incompatibility system was unaffected by the lack of callose deposition in the stigma cells and that callose did not play a essential role in pollen rejection.

A second stage of the incompatible reaction, which permits fertilisation only to take place between gamets with different S-alleles, usually sets in if some of the pollen tubes managed to reach the ovary (Alston, 1996).

2.2.1 The reason for determining the S–allele.

In the past compatibility assessments were based on the number of seeded fruit which developed after the flowers of the main cultivar had been hand pollinated with the pollen of

the evaluated pollinator. This method is inadequate since factors other than compatibility, e.g. the condition of the test plant (physiological) and temperatures before and after anthesis (environmental), can have significant influences (Stott, 1972; Brown, 1991; Sakurai et al., 1997). Williams (1970) confirmed this by saying that if fruit set is used as a parameter of plant response to pollination it is necessary to include in the investigation a detailed study of all the factors involved from anthesis to fruit set.

Furthermore the early determination of the pollination requirements of new apple cultivars are very important, but the setting up of field trials is a time consuming process. By using fluorescence microscopy to evaluate pollen tube growth or to determine the different S-alleles, less time is needed and external factors can be eliminated, as tube growth in styles generally support field pollination results (Spiegel - Roy and Alston, 1982; Sakura et al., 1997). This information is essential in establishing the pollination requirements of new cultivars to ensure successful pollination in the orchard as well as in breeding programs for predicting the compatibility of S-genotypes for selecting parents that would avoid inbreeding (Kao and McCubbin, 1996; Sakurai et al., 1997). Sakurai et al. (1997) and Castano et al. (1997) also found that the S-allele genotyping was useful in confirming or challenging the pedigree of a cultivar.

2.2.2 Determination of the S-alleles

Got-1 is an isoenzyme that is closely linked to the S-locus (Battle et al., 1995). Alleles of Got-1 have been used as markers in recognition of the 11 S-alleles so far determined. Matsumoto et al. (1999) recently proposed a new S-allele, Sg, as part of the apple genome. After tissue analysis has been done, these markers, together with analysis of S-RNase activity, provide a means by which compatible pollinators can be recognised (Alston, 1996). Jansens et al. (1995) and Broothaerts et al. (1996), however developed an S-allele genotyping method using allele-specific polymerase chain reaction (PCR) amplification of genomic DNA followed by allele-specific restriction enzyme (endonuclease) break down. This method was used on certain apple cultivars with known S-phenotypes, and the S-allele

descriptions matched those suggested by the controlled pollination tests of Jansens et al. (1995). More recently Sassa et al. (1994) suggested that the S-genotypes could also be determined by establishing the profiles of the S-glycoproteins through two-dimensional gel electrophoresis (2D-PAGE). Glycoproteins are the products of S-alleles in the styles of apple. Sakurai et al. (1997) however, stated that the use of isoenzymes and S-glycoprotein profiles to analyse incompatibility groups is a time consuming process and they found that results were not always clear.

2.2.3 Degrees of incompatibility

When half the alleles of the pollentube of the pollinizer are recognised, the combination would be described as semi-compatible (Fig.1). When none of the alleles are recognised, the combination would in theory be fully compatible while if all the alleles are recognised the combination should be totally incompatible (Stahley, 1985; Alston, 1996; Kao and McCubbin, 1996). Spiegel-Roy and Alston (1982) further mentions four grades of pollen tube response: non-germinating pollen, short tubes bending upwards, tubes growing to one third of the length of the style, and pollen tubes growing the full length of the style.

Nevertheless, complete self- and cross incompatibility are rare in the case of apples (Modlibowska, 1945; Blazek, 1996). Torre-Grossa (1996) found that optimum pollination is not necessarily to get as high a set as possible. Because of economical implications in terms of a loss in fruit size and costly thinning programs, one would decide whether the cross-pollinator should be semi or fully compatible depending on the inherent set potential of the cultivar. Semi-compatible pollinators can thus be recommended to avoid overset and biennial bearing in the case of cultivars with high stylar receptivity combined with good pollen tube growth at low temperatures.

Although information on the S-allele status of a cultivar is a valuable tool in determining the best pollinator for a main cultivar, it is not the only feature that should be considered. Screening for high stylar activity and good pollen tube growth at low temperatures must be

a prerequisite to determine the best combination (Alston, 1996).

3. FACTORS EFFECTING COMPATIBILITY

Apart from the genotypic factor, compatibility also depends on temperature, pollen viability and stylar receptivity at the time of pollination (Alston, 1996).

2.3.1 Temperature

Self pollination followed by a period at temperatures 10°C and lower, results in inadequate tube growth, while at 15 - 25°C pollen tubes of own pollen has been found to reach the base of the style often (Modlibowska, 1945). Certain cultivars like 'Golden Delicious', when grown in the Netherlands is considered self incompatible, while in warmer growing areas it will be considered semi-compatible (Wertheim, 1976). Austin et al. (1998) also found with 'Sundrop' apricot that the temperature affected the rate at which self-incompatible pollen tubes were inhibited. Own pollen tube penetration was strongest at 10° to 15°C and weaker at higher or lower temperatures. This type of self-incompatibility which depends on environmental conditions, is referred to as pseudo-incompatibility (Wertheim, 1967; Wertheim, 1990).

2.3.2 Ovule maturity

Another factor influencing pollen tube growth is the maturity of the ovule at the time of pollination. 'Cox's Orange Pippen' is able to pollinate itself if the temperature during the second half of the blossom period (ovule more mature) is higher than 15°C for four to five days (Williams et al., 1984). This of course would only be applicable if the ovule's life span is long enough (Jackson et al., 1983; Stösser and Anvari, 1983).

Although a cultivar might be receptable to certain pollen, including its own, it would not necessarily guarantee fertilisation. Here the difference between the time needed for the pollen tube to grow down to the ovule and the life span of the ovule would determine the time available for fertilisation to take place. This is referred to as the effective pollination period

(EPP) (Williams, 1966; Wertheim, 1976). The main factor influencing fertilisation is not the inhibition of the germination of the pollen on the stigma or the inability of the pollen tube to grow down because of the degradation of the style. It is the life span of the ovule that is the main cause for inadequate fertilisation (Stösser & Anvari, 1983).

2.3.3 Effect of mentor and pioneer pollen

2.3.3.1 Pioneer effect.

The pioneer effect was described as the effect of a double pollination with the compatible pollen of one or more pollinators (Visser, 1981). Visser and Verhaegh, 1980 and Visser et al., 1983 showed that seed set after pollination with compatible pollen was greatly improved by applying pollen twice with an interval of 1-2 days. The action of the second pollination is stimulated by the first, at its own expense, as the first pioneer pollen "prepares the way" for the second by removing the self incompatibility barriers in the style. (Visser and Verhaegh, 1980; Taylor and Hepler, 1997). Visser (1983) found that the double pollination resulted in a doubling of seed set.

It was found that when apple flowers were pollinated with compatible pollen (from a cross pollinator) followed by incompatible own pollen (pioneer effect) the fruit set did not differ significantly from the control which was pollinated once with compatible pollen (Visser et al., 1983). However, seed set were decreased. When self-pollination was followed by cross-pollination (the reverse) the fruit set was increased significantly without the seed set being affected. This indicates that own pollen served as pioneer pollen (Visser, 1983).

2.3.3.2 Mentor effect.

Visser (1983) described the mentor effect as the result of pollination with a mix (1:1 or 1:9) of the flowers own incompatible pollen and the compatible pollen of the pollinator. The competition between the pollen tubes was more severe in the mentor than in the pioneer situation. The mentor pollen had no effect on seed set, although both pioneer and mentor

pollination increased tube growth of own pollen in pear, and stimulated set in both apple and pear ("stimulative parthenocarpy") (Visser and Verhaegh, 1980). Herrero (1992) also reports on the synergistic effect of accumulated pollen grains on germination. He also reported that following mixed pollination, incompatible tubes could stimulate the stigma and style to allow the more rapid growth of the compatible tubes. It is evident that when one looks at overcoming self incompatibility, the pioneer approach, where self pollination is delayed by one or two days after the compatible pioneer pollination has taken place, is more effective than the mentor pollen approach where the compatible mentor pollen and the incompatible own pollen is mixed and applied simultaneously (Visser et al., 1983).

Therefore, although most apple cultivars would be considered self incompatible, the own pollen still plays a significant role in the set of fruit and this has been underestimated in the results of simple self pollination trials in the past (Visser, 1983). It may indirectly increase set by acting as pioneer pollen for the pollinator pollen or more directly in cooperation with compatible pollen serving as mentor or pioneer pollen, respectively. The weather during pollination time of course would affect this.

2.4. THE PHYSIOLOGY OF POLLINATION AND TUBE GROWTH.

The fertilisation process starts with a pollen grain coming to rest on the receptive surface (stigma) of a pistil. The function of the pollen grain and the tube emerging from it, is essentially to deliver two male gametes into the embryo sac, where double fertilisation will take place (Heslop - Harrison, 1987; Taylor and Hepler, 1997; Van Herpen, 1984). One gamete will fertilise the endosperm mother cell (2n) and the other one the egg cell (n).

Pollination can be divided into four characteristic stages: (1) pollen hydration and germination, (2) growth of the tube through the stigma, style and ovary, (3) pollen tube guidance to the ovule micropyle, and (4) delivery of the gametes to the embryo sac (Wilhelmi and Preuss, 1997).

During final maturation in the anther, the pollen grains undergo rapid dehydration before it is released as the anther bursts open (Wertheim, 1976; Taylor and Hepler, 1997). As the pollen grain lands on the wet apple stigma surface, it again becomes hydrated in order to re-establish the integrity of the membranes and to seal off the compartments which are leaky before sufficiently hydrated (Wilhelmi and Preuss, 1997; Grabe and Kristen, 1997). Papillae on the surface of the stigma secretes a nutrient-rich fluid which prevents it from drying out and induces germination (Wertheim, 1976).

The pollen grain contains two layers, viz., the inner (intine) and outer (extine) layer. Three germination pores exist in the extine, covered only by a thin layer. During germination the inner layer (intine) evaginates from the inside out and starts forming the pollen tube (Heslop - Harrison, 1987; Owens, 1992; Taylor and Hepler, 1997). Boron plays an important role in that it prevents the tube from bursting, due to the rapid uptake of water during germination (Wertheim, 1976; Vasil, 1963).

A number of researchers (Wertheim, 1990; Vasil, 1963; Stahley, 1985) mentioned the inhibitory effect of applied chemicals like herbicides, on the germination percentage of pollen grains on the stigma. Grabe and Kristen (1997) found that growing pollen tubes are able to synthesize their own essential amino acids and that glyphosate inhibits tube growth by blocking the aromatic amino acid pathway.

The tube will, after germination, invade or grow between the stigma cells and subsequently migrate into the style. In the style the tubes grow through an extracellular matrix, which is connected to the papillae on the stigma surface, and either forms a hollow channel or is secreted between style cells to make up a solid transmitting tract (Heslop-Harrison, 1987; Owens, 1992; Wilhelmi and Preuss, 1997). This complex matrix consists of lipids, proteins, carbohydrates and other molecules which is believed to provide the signals that are critical for directing and supporting pollen tube growth (Wilhelmi and Preuss, 1997). Stösser et al. (1996) found that although the transmitting tissue degrades (cells separate and collapse) after

3-5 days this has no influence on pollen tube growth in terms of rate of tube growth or the number of tubes penetrating the style. Pollination at intervals of up to 10-12 days after full bloom resulted in normal pollination and tube growth down to the ovule. Stösser and Anvari (1983) confirmed this by saying that fertilisation is not prevented by the senescence of the transmitting tissue or the stigmas, but that the limiting factor was in fact ovule longevity.

The growing tube is at first dependant on its own reserves for growth, but it later gradually gets more dependant on the style to supply it of nutrients (Herrero, 1992; Taylor and Hepler, 1997). It is suspected that the tube is at this stage guided by a concentration gradient of specific substances (molecules).

At the tip of the tube, the vegetative nucleus, which actively transcribes messages needed for growth, and the two male gametes are present. As the tube grows, the cellular content is transported to the tip and the volume of the tube remains relatively constant as plugs of callose are periodically deposited (Owens, 1990; Wilhelmi and Preuss, 1997). These plugs completely seal of the newer portion of the tube so that fertilisation can take place even if older sections of the tube dies (Heslop-Harrison, 1987).

Wertheim, 1976 stated the degree of callose formation is an indication of the degree of compatibility between the style and the pollen tube. High callose disposition indicating incompatibility reactions.

Generally, pollen tube entry into the micropyle is followed by intercellular growth through the nucellus to the embryo sac (Owens, 1992). After leaving the style , the tubes no longer have access to a predetermined path of exudate and have to navigate across the exposed surfaces of the ovary until it reaches an ovule. Recent studies show that essential signals most probably come from the ovules themselves (Taylor and Hepler, 1997; Wilhelmi and Preuss, 1997). Because only one pollen tube approaches each ovule, pollen tubes probably fend each other off. This meganism prevents polyspermy to take place and is analogous to

that seen in many animal systems (Heslop-Harrison, 1987; Herrero, 1992).

After the pollen tube arrives at the micropyle the tube ruptures at the tip and releases both the male gametes – one fertilises the egg cell and the other the endosperm mother cell (Wertheim, 1976).

Recent studies have indicated that gradients of calcium are essential in both pollen tube growth and guidance (Wilhelmi and Preuss, 1997). Calcium levels are highest in the tips of the pollen tubes and rapidly dissipate towards the distal regions (Heslop - Harrison, 1987; Taylor and Hepler, 1997). It was found that buffers that eliminate the gradients cause tube growth to cease (Wilhelmi and Preuss, 1997). Chemo-attraction also plays a very important role in the precise growth of the pollen tube into the micropyle and the fusion of the gametes cells with their targets (Heslop - Harrison, 1987; Wertheim, 1990).

2.4.1 Morphological changes following pollination.

In many flowers, pollination regulates a pollination syndrome that prepares the flower for fertilisation and embryogenesis, while shedding organs that have completed their function in pollen dispersal and reception (Porat et al., 1998). Herrero (1992) reported on the cytoplasmic and biochemical activities or changes in the pistil that precedes the pollen tube growth down the style. All these activities are devised to promote the development of the male gametophyte and to assist fertilisation. Visible changes associated with pollination include the growth and maturation of the ovary and ovules (Porat et al., 1998) and the stimulation of the transmitting tissue to release carbohydrates which are later used to support pollen tube growth (Taylor and Hepler, 1997). Pollination has also been shown to prolong embryo sac viability (Milutinovic, 1983).

Control of flower longevity will control the length of the pollination period that will influence fruit and seed set (Stead, 1992). The primary signal for the post-pollination syndrome to commence is a chemical signal coming from the pollen grain and is likely to be a

combination of IAA, ACC and ethylene (Herrero, 1992; Porat et al., 1998).

2.5. THE EFFECT OF FLOWER QUALITY AND EFFECTIVE POLLINATION PERIOD ON FRUIT SET.

The factors influencing set can be summarised by its effect on either the female gametophyte (ovules) and/or the male gametophyte (pollen).

Both the quality of the ovules and the pollen grains will in turn be determined by the flower quality and it is the short life span of the ovules of weak flowers that is the main cause for inadequate fertilisation (Stösser and Anvari, 1983)

2.5.1 Factors influencing flower quality.

The quality of flower buds is expressed by bud size, ovule size, ovule longevity, and spur leaf area which will determine the ability of the flower to set high quality fruit (Rom, 1985; Dennis, 1986).

Flower induction already starts in the year preceding bloom. The internal stimulus that triggers induction is not yet known, but probably is a combined effect of both the internal physiological condition of the tree and external environmental effects (Rom, 1985). The first morphological changes (initiation process) starts to take place in the first week of January (in South Africa) and from March to August the flower components (pistil, petals, sepals etc.) start developing until just prior to bloom when budburst will occur if the chilling and heat requirements have been satisfied.

The process and sequence of development can be interrupted or stopped at any stage if conditions become unfavorable, when flower inhibitors are utilised or vegetative growth is increased. This may result in spurs or buds with only one or a few poorly develped flowers (Rom, 1985).

2.5.1.1 Light interception

Spurs at the top of the canopy are larger in diameter, have larger spur leaf area and an increased leaf efficiency (specific leaf weight) than buds in the middle and bottom of the tree (Rom, 1985). This confirms the importance of light and proper tree training for high quality bud development.

2.5.1.2 Temperature

High temperatures during the summer will reduce reproductive bud initiation by changing the rates of shoot growth. High temperatures in the weeks before bud break is also correlated with poor fruit set as rapid development of the bud in spring results in poor flower quality and insufficient fertilisation (Rom, 1995; Tromp and Borsboom, 1996).

2.5.1.3 Endogenous plant hormones

Plant growth regulators play a very significant role in flower initiation. Gibberellins are produced in seeds of developing fruit and inhibit flower initiation. Thus a big crop may inhibit the initiation of buds for the following year and so reduce cropping the following year (Rom, 1985; Wertheim, 1976). This explains the biennual bearing phenomena and the importance of early fruit thinning. Cultivars with a tendency to biennial bearing has longer effective pollination periods in the years of heavy cropping than in the alternating years (Williams, 1970).

Other hormones, like ethylene, may increase flower initiation. Auxins also have been reported to indirectly increase flowering because of fruit thinning or the production of internal ethylene (Rom, 1985; Dennis, 1986).

2.5.2 Factors influencing the effective pollination period (EPP).

With an extensive EPP, the rapid transfer of pollen by the pollination agent is not that critical. But if the EPP is short, yield will be limited if pollen transfer does not take place shortly after anthesis when differentiation of the egg cell commences (Milutinovic et al., 1996).

Wertheim (1976) noted that the egg apparatus starts degenerating as soon as cell division stops. Shortly after anthesis cell division is still active, but then decreases rapidly. In the case of "normal strength" flowers, cell division lasts for up to five days. In the case of strong flowers, cell division can take place for up to 10 days after anthesis.

The EPP can be determined by either hand pollinating a limited number of flowers at varying intervals after flower opening and recording the subsequent presence or absence of seeded fruitlets, or by examining the egg apparatus in flowers preserved in sequence after opening (Williams, 1965). Under field conditions the EPP is in the region of 3 or 5 days in diploid and triploid apple cultivars, respectively. This period can differ from 2-10 days depending on the year and cultivar (Williams, 1970; Paprstein annd Blazek, 1996).

Therefore every factor affecting the EPP will inevitably affect fruit set, directly or indirectly.

2.5.2.1 Nutrition

Optimal nutrition is critical for optimal tree performance. When nitrogen was applied in the summer after harvest, once shoot growth has ceased, the effective pollination period was lengthened (Williams, 1966; Williams, 1970). The nitrogen affected the EPP by lengthening the life span of the egg apparatus and by increasing the rate of pollen tube growth (Wertheim. 1990; Williams, 1965). This is due to the formation "strong" flowers following the autumn nitrogen application (Tukey, 1985). Rom (1985) found that a summer or early post-harvest application of nitrogen had little effect and that it is an autumn application that will increase ovule viability, extending the EPP the following spring. Autumn applied nitrogen may also improve leaf size, but excessive amounts will stimulate shoot growth and inhibit flower quality (Rom, 1985).

Zinc is one of the micronutrients that is essential for high quality flowers. Zinc is necessary for chlorophyll production or stability. Chlorophyll is responsible for photosynthesis as well as cell expansion and continued growth. A zinc shortage may therefore limit growth and

development through carbohydrate limitations and reduce bud development (Tukey, 1985)

Cytokinins are essential for cell division and growth and a lack of this hormone can lead to retarded pollen tube growth and subsequent poor fertilisation. Plants with a low concentration of cytokinins usually also have depressed levels of boron. Boron is essential for the proper development of apical meristems and pear blast is a case of severe boron deficiencies where the whole bud collapses due to tissue instability (Tukey, 1985; Rom, 1985).

2.5.2.2 Flower quality

It was also found that the stigmas of the flowers of trees with optimal nitrogen levels were receptive for longer periods than the flowers of below optimum trees (Wertheim. 1990; Williams, 1965). This is probably because of the fact that stronger flowers were developed at optimum N-levels and climatic conditions as most flower(bud) formation takes place during the summer preceding bloom. Stronger flower buds will inevitably prolong the EPP as the ovule longevity of stronger flowers will be longer than that of weaker or normal flowers. Stösser et al. (1996) and Wertheim (1976) further classifies the king flower as stronger than the laterals in the cluster.

2.5.2.3 Rootstocks

The fact that the embryo sac stays in tact as long as cell division takes place is probably because of the constant influx of hormones together with different nutrients needed for cell division (Wertheim, 1976). One would therefore be able to hypothesise that factors affecting the flow of nutrients to the embryo sac would also influence the ovule longevity, and therefore the EPP. This could explain the differences in set induced by different rootstocks. Wertheim (1990) for instance noted the reason why Quince Adams (a clone of Quince C) was able to set more flowers than Quince A was because nutrients were able to reach the flowers more readily.

2.5.2.4 Temperature

Van Herpen (1984) found that in the case of *Petunia hybrida* the temperature during pollen development in the case of incompatible pollen and stylar development in the case of the compatible pollen, plays a significant role in the length of the pollen tubes. In the case of incompatible pollen, the tube is dependant on its own reserves for growth as additional pollen enzyme systems that prepares the way towards the ovary through the intercellular substance, is not switched on or activated. Pollen tube length is therefore dependent on the pollen reserves which in turn depends on the temperature at which pollen development took place. The higher the temperature regime (25.5°C vs. 19.5°C) the more reserves accumulated in the pollen and the longer the pollen tubes and the higher the germination percentages, were.

In the case of the compatible pollen, the length of the tube is dependant on the mass of intercellular substance which is dependant on the temperature during stylar development. The higher the temperature the more free sugars get converted to starch leading to a increase in the intercellular substance, higher pollen germination and longer pollen tube growth (Van Herpen, 1984).

Pollen tube growth not only depends on the temperature during the programic phase (the period between the landing of the pollen on the stigma and the double fertilisation), but also on the temperature regime during the preceding flower and plant development (Jackson et al., 1983; Van Herpen, 1984; Owens, 1992).

Tromp & Borsboom (1996) noted that it is the temperature after anthesis that has the greatest influence on fruit set and that the temperature during full bloom plays a less significant role if temperature during bloom allows for proper pollination to take place shortly after anthesis. They found that with 'Golden Delicious' that the temperature, three weeks after pollination, did not have any significant effect on set, but that between three and six weeks higher post-bloom temperatures had a negative effect on set as significant fruit drop occurred. It is important to keep in mind that they measured set 6 weeks after pollination while the linear relationship between temperature and fertilisation that Williams (1970) found was measured

as fertilisation only up to 10 days after pollination with detached flowers. Fruit drop was therefore not taken into consideration. The shorter EPP at 17°C than at 13°C found by Tromp & Borsboom (1994, 1996) does not fit in the original EPP concept which assumes that the EPP is shortened at the lower temperature (Stösser et al., 1996). This could probably be explained by the fact that the increase in the rate of pollen tube growth was exceeded by the more rapid decrease in ovule viability.

This is however contradictory to what Williams (1969) found, viz., a linear relationship exist between temperature and fertilisation between 7°C and 15°C . At higher temperatures (15°C-25°C) the simple correlation between temperature and tube growth was no longer found. High temperatures accelerated ovule senescence. Williams (1970) further proposed the modification of the EPP equation to the longevity of the ovule minus 1 day for each 1°C that the mean temperature is below 17°C.

Jackson et al. (1983) found that with 'Cox's Orange Pippin', set was increased when low temperatures (5-10°C) were applied 2-3 months before anthesis. The results further suggested that female flower fertility is involved, because pollen from a common source was used. Low pre-bloom temperatures can therefore lead to increased fruit set even when post pollination conditions are adverse.

Other climatic effects that may influence set and EPP would include hot dry winds that might dry out the stigmas making them less receptive to the pollen grains. Also high humidity may prevent or reduce the ability of the anther to open and release the pollen. Rain also slows these processes down (Stahley, 1985).

To summarise, pollen tube growth and senescence is temperature dependant. Both ovule senescence and tube growth rate is accelerated by increasing temperature, but below a certain minimum temperature pollen tube growth will cease and no fertilisation will take place. This minimum temperature is cultivar dependent.

2.5.2.5 Cultivar differences

As previously noted, Williams found that for each rise of 1°C, one day less is required to effect fertilisation leading to a requirement of only 2 days at 15°C. Variation between cultivars was however found, as considerable differences in style length exists between different cultivars. The reaction of pollen tube growth rate to temperature can be significantly affected by the cultivar to further cause variation (Modlibowska, 1945; Stott, 1972;Williams, 1970). Williams (1970) found that 'Sweet Alford' apple gave poor results when grown in cooler areas due to slow pollen tube growth.

Milutinovic (1983) and Dennis (1986) found that there were definite differences between cultivars with regards to the functional capabilities and longevity of the embryo sac and pollen. The latter also depended on type of bearing unit and the position of the flower in the cluster (Lee et al., 1994).

2.5.2.6 Wood and tree age

The age of the wood on which the flower clusters are born seem to have a great influence on the flowers quality and set potential and flowers on one-year-old wood often set poorly (Lee et al., 1994). Substantial morphological differences exist between flowers on wood of different ages. Clusters on young one-year-old wood typically have smaller leaf areas, lower flower weight and fewer flowers per cluster than those on older wood resulting in a reduction in ovule longevity and therefore the EPP (Robbie & Atkinson, 1994). Lee et al. (1994) found that spur terminal flowers flowered for a longer period of time and produced more and "stronger" flowers than lateral buds did. This resulted in fruit containing 16.5% more seeds due to a more viable and stronger egg apparatus that caused the spur flowers to have a longer EPP.

2.6. INFLUENCE OF POLLINATOR ON SEED NUMBER AND FRUIT CHARACTERISTICS.

Stösser et al. (1996) stated that, except for pear, no parthenocarpy is known in pome or stone fruit and that fertilisation and the beginning of seed formation is necessary for fruit set. Tromp and Borsboom (1994), however, found seedless fruit in pollination studies with 'Golden Delicious'.

Fruit shape and weight are important in determining fruit prices in the market place. Tromp (1995) noted that fruit with higher seed counts had a more regular shape than those with few or no seeds while Brault and De Oliveira. (1995) found a positive relationship between the number of well-formed seeds and fruit symmetry. It is therefore important for a pollinator to not only be compatible with the main cultivar, but to also ensure seed formation. Keulemans et al. (1994) found a difference in seed set and weight between a number of pollinators tested. De Witt et al. (1996) and Keulemans et al. (1996) found that the higher the seed count in fruit the higher the fruit weight. Fruit weight is of course not only influenced by the number of seeds as other factors like crop load, bud quality and vegetative growth also plays a role. These factors together with seed count all relate to each other and together they influence the sink - source relationship (Tukey, 1985).

The metaxenia effects refers to the influence of the pollinator on the fruit characteristics outside the seed, while the xenia effect refers to the effect of the pollen on the seed qualities. Church and Williams (1983) quoted Nebel, 1936 describing metaxenia as 'any phenomenon that may be caused by the action of the zygote upon the surrounding maternal tissue and indicated that the pollinator might actually change fruit characteristics because of the different genetic content of the embryo.

It has been found that the influence of pollinizers on fruit weight is not only dependant on the number of seeds but also on the pollinator itself (Putter et al., 1996). Keulemans et al. (1996) found that certain pollinators simply induced a systematic higher fruit weight than others

irrespective of the fruit set or seed number. Also Rejman (1983) found that the pollinator can influence the set percentage, seed count per fruit, colour and weight of fruit. He found that some characteristics of the fruit like weight and skin colour might not only depend on the number of seeds developed from the different pollinators, but also on the genetic make-up of the pollen used and its influence on maternal tissues. Church and Williams (1983) did not find any metaxenia effect when different crab apples were tested as pollinators for 'Cox's Orange Pippin'.

It can thus be concluded that in order to improve fruit quality in terms of weight and shape, optimal pollination in the orchard is of high importance. It would also seem that as some pollinators show a systematic positive or negative effect on fruit size, compatibility tests alone in the future might not be adequate for producers to make the right pollination choices.

7. CONCLUSION

Pollination can be affected by a wide range of factors from weather to flower quality and tree condition and the possibility of complex interactions between these factors makes it particularly hard to formulate pollination policies for unique situations. However, research has looked at most of these factors individually and certain sensible deductions were made which certainly helped in managing the pollination process.

As far as the potential of the female gametophyte is concerned it was found that for high quality flowers with a long effective pollination period, a relatively low level of pollen transfer may be adequate but that the same level would be inadequate, if the blossom is weak with a short effective pollination period.

Without knowledge of the fertilisation potential of the ovule it would be difficult to give proper pollination recommendations and research in this area has to go on especially with the new cultivars being planted.

Effective pollination would be hard to achieve if climatic conditions are not conducive to high female fertility and pollen tube growth rates. For this reason Williams (1970) suggested that the process of effective pollen transfer should be addressed to stabilise yields in the orchard once the correct pollinator has been identified. The choice of pollinator is probably the most important factor which determines pollination success or failure.

Furthermore, at least two different pollinators should be used in order to ensure that both the early and late flowers are pollinated especially in marginal areas with prolonged bloom periods (Pheasant, 1985).

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PAPER 1: DETERMINING THE S-ALLELES OF DIFFERENT APPLE (*MALUS DOMESTICA* BORKH.) POLLINATORS.

Abstract

Compatibility assessments that are based on the number of fruit that develop after the flowers of the main cultivar had been hand pollinated in field trials are a time-consuming process. Allele-specific PCR amplification for some of the known S-alleles (S2, S3, S5, S7 and S9) was carried out to successfully predict the compatibility of genotypes. The results compared well with that found in literature. For all the *Malus domestica* cultivars tested at least one, but in some instances both alleles of the S-gene were determined. 'Simpson crab' (*Malus baccata*) did, however, not possess any of the S-alleles tested for. Further work needs to be done on the possibility of other crab apples presently used as pollinators in the apple industry (e.g. *Malus grandiflora*) also not displaying any known S-alleles and the subsequent effect on fertilisation, fruit set and fruit quality.

Introduction

In the case of the apple (*Malus domestica* Borkh.) the male and female organs are present in the same flower. Therefore, to prevent inbreeding as a consequence of continual self-pollination, the apple uses the self-incompatibility (SI) mechanism (Broothaerts et al., 1996). In the case of fruit trees the inhibition of tube growth down the style is the most common expression of the SI system (Alston, 1996; Broothaert et al., 1996).

Compatibility assessments are normally based on the number of fruit that develop after the flowers of the main cultivar were hand pollinated with the pollen of the evaluated

pollinator in field trials. This method is time-consuming (Spiegel - Roy & Alston, 1982) and physiological and environmental factors that significantly influences fruit set are not taken into account (Sakurai et al., 1997). Therefore, it has become imperative to find a different method to determine compatibility. Gametophytic SI depends on and is controlled by a single gene, called the S-gene, which is situated near the surface of the stigma (Broothaerts, 1995; Jansens et al., 1995; Broothaerts et al., 1996; Alston, 1996). Several different forms, or alleles, of the polymorphic S-gene have been identified (Broothaerts, 1995; Jansens et al, 1995). As soon as similar alleles in the pollen (tube) are recognised by the stylar tissue, the S-gene encodes for specific S-proteins called S-RNases to be synthesised. These RNases stop further development of the pollen tube by breaking down its RNA (Jansens et al., 1995; Alston, 1996; Broothaerts et al., 1996).

Jansens et al. (1995) and Broothaerts et al. (1996), developed an S-allele genotyping method using allele-specific polymerase chain reaction (PCR) amplification of genomic DNA, followed by allele-specific restriction enzyme (endonuclease) digestion. This method was used on certain apple varieties with known S phenotypes, and the S-allele descriptions matched those suggested by the controlled pollination tests of Jansens et al. (1995).

Determining the S–alleles should enable the researcher/breeder to successfully predict the compatibility of genotypes for selecting pollinators for newly developed cultivars so as to optimise fruit quality and yield (Sakurai et al., 1997). Castano et al. (1997) also found that S-allele genotyping is useful in confirming or challenging the pedigree

of a cultivar.

In this paper we will report on the determination of the S-alleles of the newly released apple variety 'African Red', and five possible pollinators for this cultivar.

Material and Methods

Plant material. Immature (2 to 3-week old) leaves of the different pollinator cultivars ('Simpson Crab', 'Granny Smith', 'Cripps' Red', 'Winter Banana' and 'Cripps' Pink') and of the new cultivar ('African Red') were collected from 5-year-old trees planted on M793 rootstocks on a commercial farm (Paardekloof) in the Witzenberg Valley, Koue Bokkeveld region (32°55'N 19°27'E; Mediterranean climate; ≈1060 Utah chill units, and ≈530 mm rainfall annually, altitude 966 m). Leaves were stored at -80°C until used.

Isolation of genomic DNA. DNA was isolated from leaf material using the method of Lavi et al. (1991). Procedures used were briefly as follows: 1 to 2 g of leaves were ground in a mortar and pestle, together with 10 ml extraction buffer (5% (w/v) CTAB, 1.4 M NaCl, 0.1% (v/v) 2-mercaptoethanol, 100 mM Tris-HCl (pH 8.0) and 20 mM EDTA). This mixture was incubated for 1 h at 60°C with occasional gentle shaking. After being cooled to room temperature, the mixture was extracted twice with chloroform-isoamyl alcohol [24:1 (v/v)]. The DNA was then precipitated overnight at room temperature by adding 1% CTAB precipitation buffer (1% (w/v) CTAB, 0.1% (v/v) 2-mercaptoethanol, 50 mM Tris-HCl (pH 8.0), 10 mM EDTA). After centrifugation, the pelleted DNA was washed with 1M CsCl and precipitated with

100% ethanol for 30 min at -20°C. The pellet was then washed with 70% ethanol and dissolved in deionised water.

Allele-specific PCR amplification. Allele-specific PCR amplification for the S-alleles, S2, S3, S7 and S9 was carried out with 50 ng of genomic template DNA and allele-specific primers in a total reaction volume of 35 μl. The reaction mixture consisted of 20 mM $(NH)_2SO_4$, 75 mM Tris-HCl (pH 9.0), 0.01% Tween (w/v), 3 mM $MgCl_2$, 0.2 mM of each dNTP, 1 μM of each primer, 17 mM 2-mercaptoethanol and 0.75U *Taq* polymerase (Advanced Biotechnology). PCR conditions were as follows: 1 cycle of 94°C for 2 min, 30 cycles of 94°C for 1 min, annealing temperature at an optimal temperature for each primer combination for 1 min, 72°C for 1min, and a final cycle of 72°C for 2 min. Annealing temperatures for each primer pair and cultivars used as positive controls are listed in Table 1. Reaction conditions for allele S5 were as described for the Expand™ Long Template PCR System (Boehringer Mannheim), using 1x buffer 3, 0.5 mM of each dNTP, 300nM of each primer, 3.25 mM $MgCl_2$ and 2.625U *Taq*. Restriction digestion of PCR fragments were performed as described in Jansens et al. (1995).

Results and Discussion

The results compare well with those found in literature (Jansens et al., 1995). S-genes identified for the positive controls (Table 1) were identical to those found by Broothaerts et al. (1996).

PCR fragments obtained for alleles S2 and S7 are shown in Fig. 1. The fragment lengths for S2 are 449bp and for S7 440bp as previously described by Broothaerts et al.

(1996). 'Cripps' Red', 'Cripps' Pink', 'Winter Banana' and 'Golden Delicious' (positive control) showed fragments for S2. 'Cripps' Red' and 'Jonathan' (positive control) showed fragments for S7. These PCR fragments were digested with restriction enzymes to confirm their identical base pair sequences. The PCR products for S2 and S7 were cut into two fragments: 349bp & 100bp for S2 and 228bp & 212bp for S7 as previously described by Broothaerts et al. (1996). The fragments are shown in Fig. 2. Analyses for S3, S5 and S9 were conducted in the same way and are shown in Fig. 3-7. The PCR product for S5 was not restriction digested as this S-allele was not present in any of the tested cultivars. The S-alleles obtained for each of the six cultivars are listed in Table 2. Allele-specific primers are not yet available for all known S-alleles. We can therefore not be certain if 'Granny Smith' and 'Cripps' Pink' are homozygous for S3 or S2, respectively. It is also unknown whether 'Simpson crab' (*Malus baccata*) differs from other commercially grown apple (*Malus domestica*) cultivars at DNA level. Only when all possible alleles can be amplified, will it be possible to know if 'Simpson crab' contains any S-alleles. The lack of results with 'Simpson crab' never the less necessitates a critical assessment of other crab apples as they are widely used in the apple industry as pollinators e.g. *Malus grandiflora, Malus floribunda and Malus scheideckeri.* Schotsmans (1997) for instance found that when 'Hillierri crab' (*Malus scheideckeri*) was compared to *Malus domestica* cultivars as a pollinator for 'Royal Gala' it resulted in a decrease in fruit size due to a decrease in seed set, which might be an indication of reduced compatibility.

Broothaerts et al. (1999) only recently analysed two crab apple cultivars, *Malus simcoe* and *Malus floribunda* 821. *M simcoe* has the S-alleles S3 and S27, while in the case of

Malus floribunda, S26 was present (I. Nerum, pers. commun.). This indicates that these crab apples do have S-alleles. Matsumoto et al. (1999) recently proposed a new S-allele, S*g*, in the apple genome. Unfortunately the primer sequence for these new alleles are not available yet in South Africa.

Conclusion

Allele-specific PCR analyses proved to be a useful and rapid technique of determining the pollination group (S-alleles) to which a cultivar belongs but can at this stage not be seen as an end in itself and more research needs to be done on the reliability of this tool/technique. Knowledge of the S-alleles in apple cultivars will aid breeders in the selection of parents for hybridization and provide important information concerning pollination requirements in the future.

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TABLE 1. Primer pairs (Jansens et al., 1995), annealing temperatures and positive controls used in PCR amplification reactions.

Allele	Primer pair	Annealing temperature	Positive control
S2	OWB122 and OWB123	64°C	Golden Delicious
S3	OWB134 and OWB145	56°C	Granny Smith
S5	OWB139 and OWB138	62°C	Fiesta
S7	OWB126 and OWB127	62°C	Jonathan
S9	OWB154 and OWB155	62°C	Jonathan

TABLE 2. S-allele typing of the five pollinators cultivars and the new cultivar, 'African Red'.

Cultivar	S-genotype
'African Red'	*S3S9*
'Granny Smith'	S3 -
'Cripps' Red'	S2S7
'Cripps' Pink'	S2 -
'Winter Banana'	S2S3
'Simpson Crab'	-
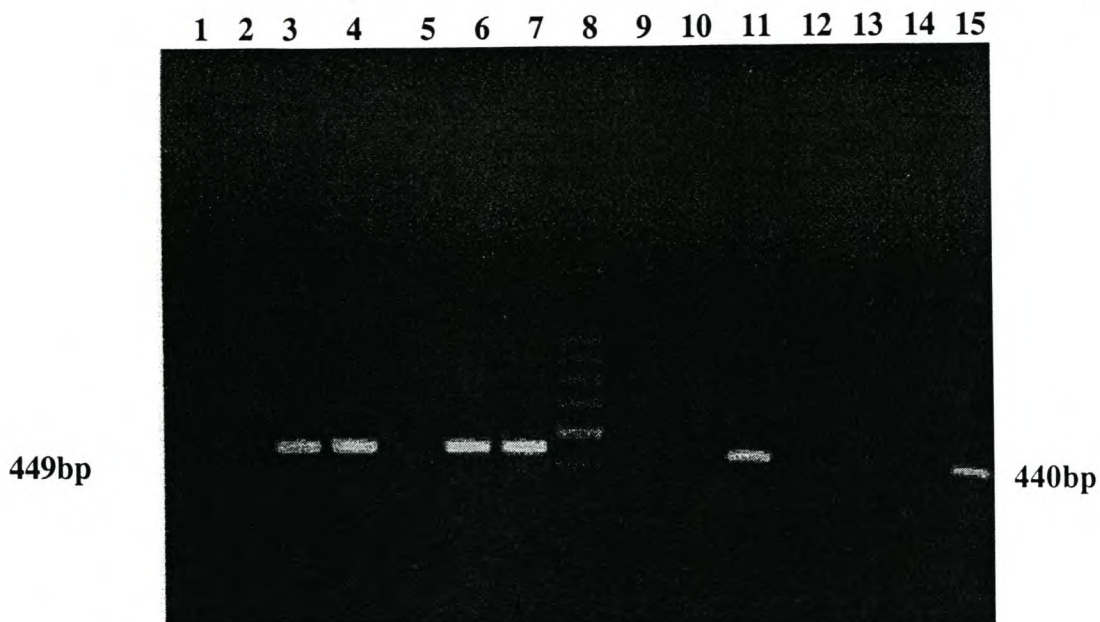
Fig 1. H2+7PCR: S-genotype analysis for the S2-allele (lanes 1-7) and S7-allele (lanes 8-15). Lane 1 and 9 – 'Simpson' (*Malus baccata*), lane 2 and 10 – 'Granny Smith', lane 3 and 11 - 'Cripps' Red', lane 4 and 12 - 'Cripps' Pink', lane 5 and 13 - 'African Red', lane 6 and 14 – 'Winter Banana', lane 7 – 'Golden Delicious', lane 15 – 'Jonathan' and lane 8 - DNA size standard (Promega).
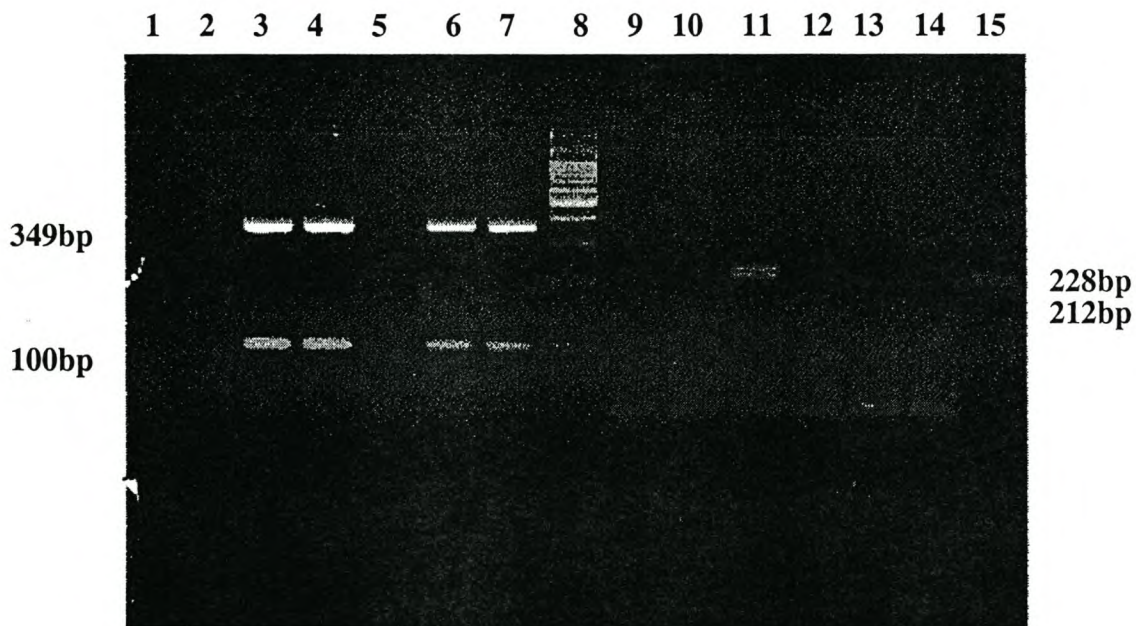
Fig 2. H2+7RE: PCR products shown in figure (H2+7PCR) after restriction enzyme digestion with *Eco*RV for S2-products and *Acc*I for S7-products.
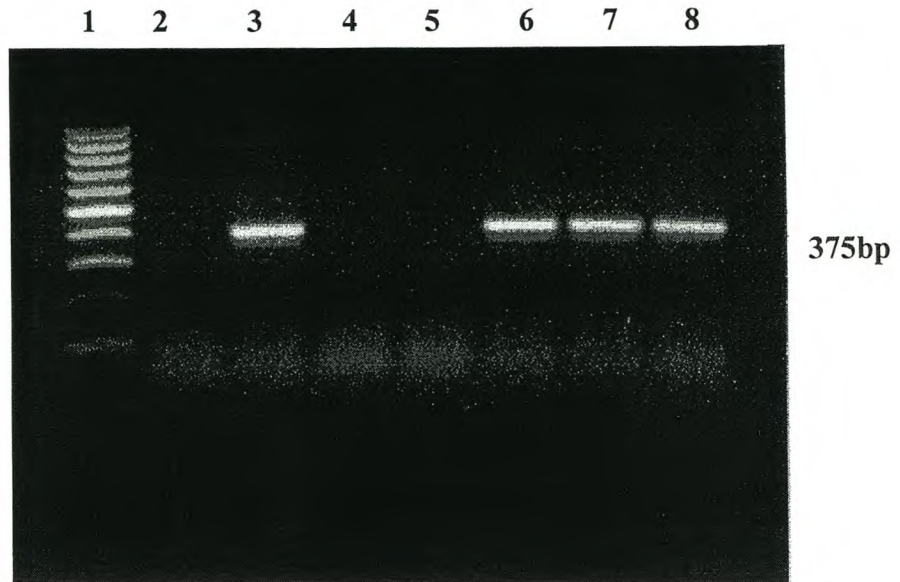
Fig 3. H3PCR: S-genotype analysis for the S3-allele. Lane 1 - DNA marker (Promega), lane 2 – 'Simpson' (*Malus baccata*), lane 3 – 'Granny Smith', lane 4 - 'Cripps' Red', lane 5 - 'Cripps' Pink', lane 6 - 'African Red', lane 7 – 'Winter Banana' and lane 8 – 'Granny Smith'.

Fig 4. H3RE: PCR products shown in figure (H3PCR) after restriction enzyme digestion with *Pst*I.

Fig 5. H5PCR: S-genotype analysis for the S5-allele. Lane 1 – 1kb DNA marker (Promega), lane 2 – 'Simpson' (*Malus baccata*), lane 3 – 'Granny Smith', lane 4 - 'Cripps' Red', lane 5 - 'Cripps' Pink', lane 6 - 'African Red', lane 7 – 'Winter Banana' and lane 8 – 'Fiesta'.

Fig 6. H9PCR: S-genotype analysis for the S9-allele. Lane 1 - DNA marker (Promega), lane 2 – 'Simpson' (*Malus baccata*), lane 3 – 'Granny Smith', lane 4 - 'Cripps' Red', lane 5 - 'Cripps' Pink', lane 6 - 'African Red', lane 7 – 'Winter Banana' and lane 8 – 'Jonathan'.

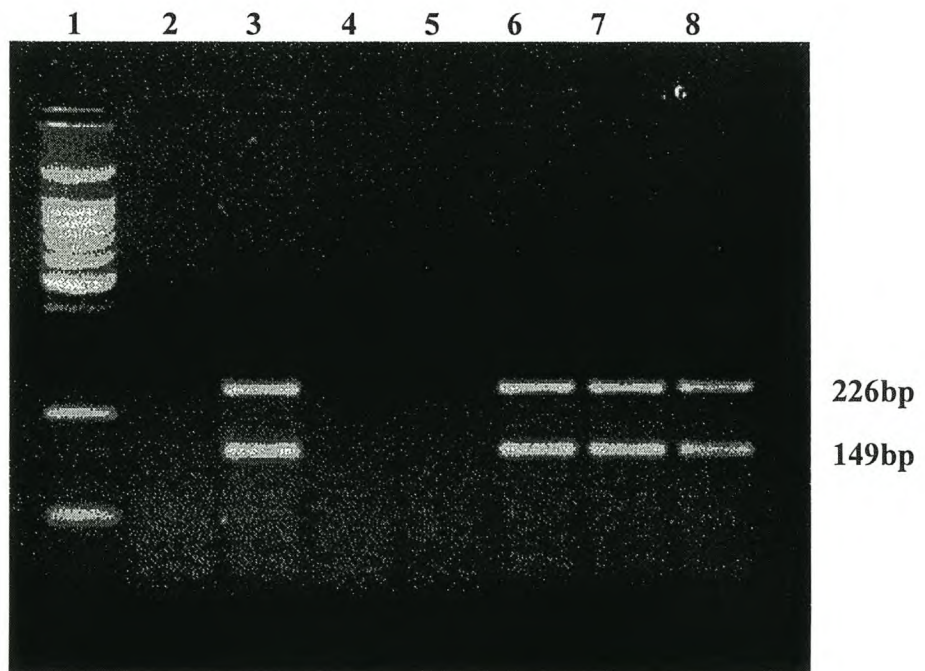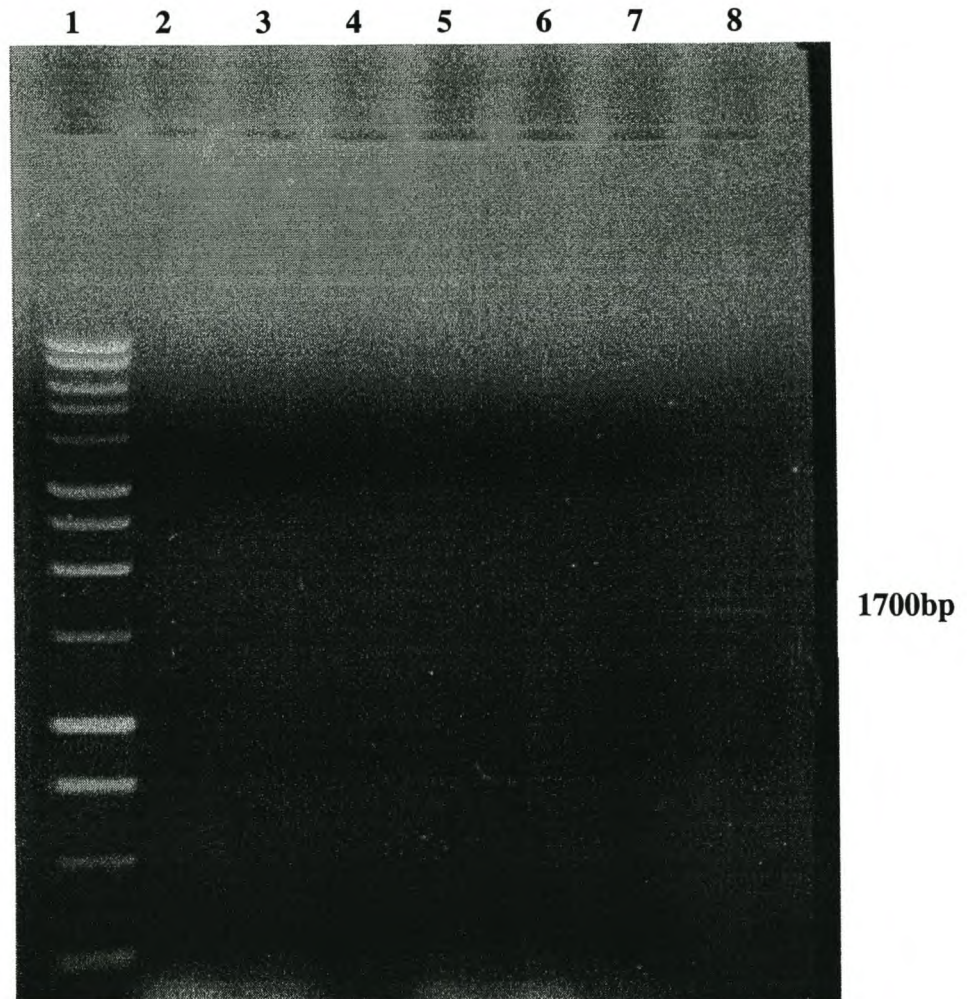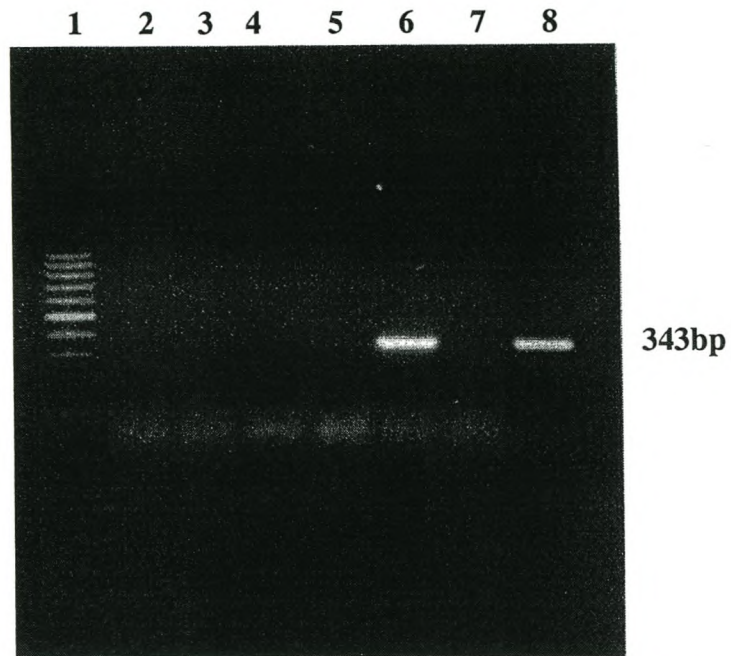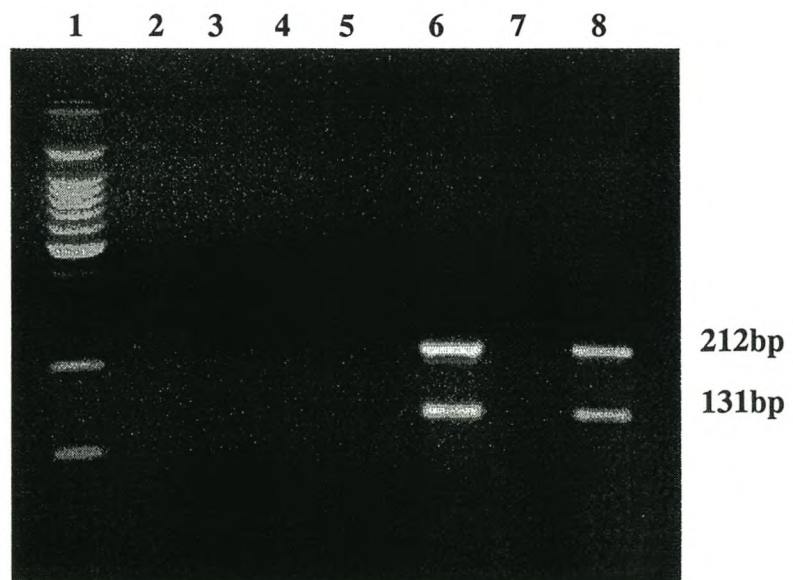Fig 7. H9RE: PCR products shown in figure (H9PCR) after restriction enzyme digestion with *Eco*RI.

PAPER 2: POLLINATION BIOLOGY: THE INFLUENCE OF DIFFERENT POLLINATORS ON FRUIT SET AND QUALITY.

Abstract

Most apple cultivars are self-incompatible and need cross-pollination to induce fruit set. Due to differences in the genetically defined fertilisation compatibility between the pollen from the male parent (pollinator) and the egg cell of the female parent, various apple pollinators differ in their ability to set fruit with viable seed. Fruit weight and size are positively correlated to seed set although it has been found that the pollinator may have a direct influence on fruit quality. 'African Red' apple trees on M7 rootstock in an evaluation block on a commercial farm in the Koue Bokkeveld region (32°55'N 19°27'E, Mediterranean climate, ≈1060 Utah chill units, and ≈530 mm rainfall annually; altitude 966 m.) were used to assess the influence of 5 pollinators ('Granny Smith', 'Winter Banana', 'Cripps' Pink', 'Cripps' Red' and 'Simpson Crab') on fruit set, fruit weight and length and diameter. The degree to which 'African Red' is self-compatible was also assessed and the effect of flowering position ("king" vs. lateral) on fruit quality was determined. None of the pollinators showed a significantly higher fruit set over the two seasons that the trial was conducted. No differences were found between the "king" and lateral flowering positions in fruit set. No significant differences were found between pollinators in the average number or weight of well developed seeds. In both years fruit weight was significantly correlated to seed number for all five pollinator cultivars. In 1998 using 'Simpson crab' resulted in fruit that were significantly more elongated than those of 'Cripps' Pink'. 'African Red' is highly self incompatible. The EPP for 'African Red' varies between 4 and 6 days depending on the pollinator cultivar.

Introduction

Fruit weight and size are economically important characteristics in fruit production. Most apple cultivars are self-incompatible and need cross-pollination to induce fruit set (Dennis, 1986). Various apple pollinators differ in their ability to set fruit with viable seed. This can be ascribed to a difference in the genetically defined fertilisation compatibility between the pollen from the male parent (pollinator) and the egg cell of the female parent in order to produce seed (Goldway et al., 1999). In the case where the pollinator has the same S-alleles as the main cultivar no pollen should be compatible. Where half of the pollinator's S-alleles are the same as that of the main cultivar, half of the pollen should be compatible. When none of the alleles are recognised, the combination would in theory be fully compatible (Stahley, 1985; Alston, 1996).

The higher the seed count in fruit the higher the fruit weight and the more regular the fruit shape (De Witt et al., 1996; Keulemans et al., 1994). It has further been found that the influence of pollinators on fruit weight is not only dependent on the number of seeds, but that the pollinator itself can also have an influence (Putter et al, 1996; Keulemans et al., 1996). This is known as the metaxenia effect (Wertheim, 1976). Rejman (1983) found that the pollinator can influence the set percentage, seed number per fruit, colour and weight of fruit. Church and Williams (1983), however, did not find any difference in fruit characteristics when 'Cox's Orange Pippin' was pollinated with different crab apples.

Therefore, in order to improve fruit quality in terms of weight and shape, optimal pollination in the orchard is of great importance. Because of strong evidence that some

pollinators show a systematic positive or negative effect on fruit size, compatibility tests alone in the future might not be sufficient to choose the right pollinator.

The effective pollination period (EPP) is defined as the longevity of the egg apparatus minus the time necessary for pollen tube growth (Williams, 1966; Williams, 1970). The EPP is determined by hand pollinating a limited number of flowers at varying intervals after flower opening and recording the subsequent presence or absence of seeded fruitlets (Williams, 1965). Should the period be extensive, the rapid transfer of pollen from the pollination agent would not be that critical. But if the period is restricted, yield might be limited if pollen transfer does not take place shortly after anthesis when differentiation of the egg cell commences (Milutinovic et al., 1996).

The research reported on in this paper was done in order to (a) determine the effect of different pollinators on fruit set and whether it corresponded with the S-alleles identified in Paper 1, (b) to determine whether pollinators had an influence on the number and weight of seeds and if this had an influence on fruit weight, (c) to determine if the EPP of 'African Red' was pollinator dependent, (d) how the pollen tube growth rate between pollinators differed and if this was connected to fruit set and EPP, and (e) to what degree 'African Red' is self-compatible.

Materials and Methods

Plant material and site. Trials were conducted over two seasons, 1997/1998 and 1998/1999. 'African Red' apple trees on M7 rootstock planted in 1993 were used. Trees

were spaced 1m between rows and 0.5 m between the trees in the row. The site is an evaluation block on a commercial farm, Wakkerstroom, in the Witzenberg Valley, Koue Bokkeveld region (32°55'N 19°27'E, Mediterranean climate, ≈1060 Utah chill units, and ≈530 mm rainfall annually; altitude 966 m).

Pollen collection and pollenation procedure. Flowers in the balloon stage of flower development were collected from healthy trees in commercial orchards in the Witzenberg Valley. The anthers of each cultivar were removed and placed in separately labelled open glass, petri-dishes that were placed in a desiccator. The anthers were allowed to dry for approximately 36 h at 22°C. At the dehiscence of the anthers the pollen from each cultivar was placed in labelled vials that were sealed with cork plugs. These in turn were placed in a smaller desiccator and kept in a refrigerator at ≈4°C until they were used 2 days later in the trials. The viability of the pollen was tested on hanging drops of 10% sucrose at 25°C as described by Schotsmans (1997). In trials 1, 2 and 3 the flower clusters were covered by fastening a grease proof bag (size 1) with a clothes peg over the cluster at balloon stage 16 h before the trials commenced. Flowers were hand pollinated using a glass rod. Pollen was applied to the stigma surface after flower petals were removed. Clusters were again closed after pollination. Following each treatment, the rods were sterilised by dipping them in a 95% ethanol solution.

Trial 1: This trial was conducted to determine the rate of pollen tube growth down the flower styles. Clusters were thinned to two lateral flowers per cluster, and these were pollinated as described above. Five pollinators ('Granny Smith', 'Winter Banana',

'Cripps' Pink', 'Cripps' Red' and 'Simpson Crab') were used on eight consecutive days in a factorial design and repeated 10 times.

Trial 2: The effect of different pollinators on fruit set and fruit quality was compared in this trial. Clusters were thinned to two lateral flowers per cluster, and these were pollinated as described above. Five pollinators, 'Granny Smith', 'Winter Banana', 'Cripps' Pink', 'Cripps' Red' and 'Simpson Crab' were used on eight consecutive days in a factorial design and repeated 20 times.

Trial 3: This trial was conducted to compare the fruit set and fruit quality of different positions within the cluster i.e. "king" vs. lateral flower. Clusters were thinned leaving either the "king" or one lateral flower per cluster, and these were pollinated as described before. A factorial design was used with two positions pollinated on eight consecutive days using 'Simpson Crab' as pollinator. At 2, 4, 8, 24, 28 and 48 hours, flowers were harvested and fixed by putting them into a FAA solution [ethanol (90%) + formaldehyde (5%) + acidic acid (5%)].

Trial 4: This trial was conducted to determine to what degree 'African Red' is self-compatible and the influence of "mentor" pollen. Clusters were thinned to two lateral flowers per cluster, and these were pollinated as described above. A factorial design was used with three treatments being repeated 20 times using 'Simpson Crab' as pollinator In the first treatment 'Simpson Crab' pollen was applied followed by an application of own pollen (mentor effect). In the second treatment own pollen was applied first followed by

'Simpson Crab' pollen and in the third treatment only own pollen was applied.

Microscopic examination of pollen tube growth used in trial 1. In the first year the pollinated pistils were prepared and examined based on a method similar to that of Kho and Bar (1968) and Martin (1958). In the next year the method was modified according to the method of Dumas and Knox (1983) which is more suited to apple style examinations. The pollinated flowers were fixed in FAA. The FAA was replaced by 70% ethanol and the material stored in a refrigerator (0.5°C). It was then softened and cleared by heating at 65°C for 1 h in an 8 N KOH solution. Flowers were rinsed twice for 5 min in distilled water to get rid of the excess KOH. Material was then put into a 3% sodiumhipochloride solution (Jik) for 30 seconds. It was again rinsed three times for a total of 10 min in distilled water. The material was then put in a phosphate buffer (0.1N K_3PO_4, pH 12.4) for 10 min, and then stained with aniline blue dye (0.1% solution in 0.1N K_3PO_4, pH 12.4). The material was kept in the darkroom overnight before examining under UV light. For observation the styles were mounted in a few drops of fresh aniline blue. Preparations were kept in a refrigerator (0.5°C). The stained specimens were studied under fluorescent light using a Zeiss HBO 50W super-pressure mercury lamp in a Zeiss microscope Model Axioscope H at 10x magnification.

To evaluate pollen tube growth, the styles of each pistil was divided into 4 sections (0-0.5, 0.5-1, 1-1.5 and 1.5-2) from the top (0) to the bottom (2) part of the style. Tube growth and the percentage germination was recorded over a period of 48 hours.

Data collected at harvest. At the 8th and 10th of February in 1998 and 1999 respectively, the fruit were harvested and the following data were recorded: (1) total fruit set, (2) fruit weight, (3) fruit length and diameter, (4) number and position of full and aborted seeds per fruit and (5) weight of full seeds per fruit.

Statistical analysis. The General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) was used to analyse the data (SAS Institute Inc., 1990). Single degree of freedom polynomial contrasts were fitted where applicable.

Results and Discussion

Pollen tube growth and germination studies.

Germination potential. The hanging drop method used to obtain an indication of pollen viability, indicated large differences in the germination potential of the five pollinators used (Table 1). In the first season (1998) all pollinators gave a relatively low germination percentage. In 1999, the percentage germination for 'Cripps' Pink' and 'Simpson' was quite high. However, the microscopic evaluation of the actual germination of the pollen on the stigmas of the 'African Red' flowers gave contradicting results where 'Cripps' Pink' and 'Simpson' gave the lowest average germination potentials. It must be said that the pollen's interaction with the stigmatic surface can not be imitated in the laboratory and will in a significant way influence pollen germination and could explain the contradiction between what was found in the field and in the laboratory. No significant differences were found in the final or average germination percentages of pollen, on the stigmas of the

'African Red' flowers, in both years. (Table 2). The percentage of pollen grains germinating in 1999 was on average 10% more than in 1998 possibly due to higher temperatures (≈ 6.35°C warmer) and less rain during the 1999 flowering period.

Rate of pollen tube growth. Another factor, apart from germination percentage that will influence the effectiveness of a pollinator is the rate of pollen tube growth. From Table 3 it is clear that no differences were found in 1998 in the final pollen tube length after 48 hours or the rate of tube growth as expressed by the average tube length. In 1999, small differences occurred with 'Granny Smith' pollen tubes being significantly shorter and slower than those of 'Simpson crab'. No other significant differences were found.

Fixatives that contain aldehydes like FAA should in the future be avoided as it complicated the microscopic examination of the styles with fluorescent light. Carnoy's fixative (6 parts ethanol, 3 parts chloroform, and 1 part acetic acid) should be used, as it would probably render greater visibility of the pollen tubes (Prof. H. Robbertse, Univ. of Pretoria, personal commun.). Although not always very bright, the germinating pollen tubes and tube growth were easily observed.

Effective pollination period (EPP).

In the 1998 season, all the pollinators evaluated for 'African Red' induced a high average set percentage, 57.8 to 47.9%, except for 'Simpson crab' where the set percentage was only 28.7% (Table 4). As is to be expected the set was reduced the longer pollination was delayed after full bloom (Fig. 1a). When fitting regression lines to the set percentages

obtained over time, the fit was quite good (P=0.0071 to 0.1191). The gradients of these lines indicate the rate at which set decreased over time and can be used to estimate the EPP. From the coefficients it is clear that this rate did not differ significantly for the five pollinators evaluated. However, the rate for 'Cripps' Red' was the lowest while 'Winter Banana' decreased the fastest. The line for 'Simpson crab' was below those of the other four pollinators due to a lower set potential from the start. The reason why 'Simpson crab' (*M baccata*) had the shortest EPP and gave a considerably lower set percentage in 1998 than the rest of the *M domestica* pollinators (Table 4) can at this stage possibly be contributed to the fact that it differs from 'African Red' at species level. The EPP of 'African Red' for a set percentage of 30% varied between 4 and 6 days depending on the pollinator cultivar.

In the 1999 season the best set percentages achieved (set at the optimum day) were much lower than in the previous season (Table 4). Fitting regression lines also proved more difficult as the fit for 'Cripps' Pink', 'Granny Smith' and 'Winter Banana' was non-significant (Fig. 1b). No consistent results were found in fruit set between the pollinators over the two years (Table 4). In 1998, although not significant, 'Cripps' Red' gave a higher average set percentage than the rest of the pollinators (Table 4). In Paper 1 we reported that both S-alleles of 'Cripps' Red' (S_2S_7) differs from that of 'African Red' (S_3S_9) and this might explain 'Cripps' Red' giving the higher average set percentage. However, in 1999, 'Cripps' Pink', of which the S-alleles also differs from that of 'African Red', (S_2 -) had set significantly more fruit than 'Cripps' Red' (Table 4). The only consistency found between the two years was that 'Simpson crab' gave the lowest average

fruit set in both years.

No consistent results were found in fruit set between the "king" and lateral flowering positions over the two years (Table 5).

From Table 6 it is clear that no significant differences existed between treatments 1 (mentor pollen followed by own pollen) and treatment 2 (own pollen followed by mentor pollen) in fruit set. Treatment 3 did not set any fruit and is an indication of the strong/significant self-incompatibility system in 'African Red'.

Seed set and weight.

Effect of pollinator (Trial 2). In 1998, no significant differences were found between pollinators in the number or average weight of well developed seeds (Table 7a). 'Simpson crab' as pollinator resulted in the least number of full seeds developing. 'Winter Banana' resulted in setting fruit with significantly more aborted seeds than 'Cripps' Red' and 'Simpson crab'.

In 1999, again no significant differences were found between pollinators in the average number or weight of well developed seeds (Table 7b). 'Winter Banana' and 'Cripps' Red' as pollinators, however, resulted in the most well developed seeds. 'Simpson crab' again, together with 'Granny Smith', had the least number of well developed seeds. 'Simpson crab' had the most aborted seeds, followed by 'Cripps' Pink'. Seed abortion is an indication that double fertilisation did not take place. In both years seed number and seed

weight were very well correlated (Tables 8 and 9).

In 1998, an increase in the number of well developed seeds was found until two days after full bloom, after which it decreased (Fig. 2a). The slight increase towards the end of the pollination period cannot be explained. The initial increase could be due to the fact that 'African Red' flowers were not fully matured at full bloom and the stigmas or egg apparatus unreceptive (Wertheim, 1976). The seed weight followed a similar trend (Fig. 2b). The number of aborted seeds were low during the initial pollination period, but increased steeply towards the end of the pollination period (Fig. 2c).

In 1999, the resultant number of well developed seeds and their weight remained relatively constant during the whole pollination period (linear trend) (Fig. 2a,b). The number of aborted seeds showed the opposite trend to the previous year (Fig. 2c). This cannot be explained, but one must bear in mind that the fruit set and EPP data were also very inconsistent (Fig. 1b, Table 4). In general, the seed set and weight were higher in 1999 ($\approx$55%) compared to 1998 ($\approx$46%). As mentioned previously, the germination percentage was also higher in 1999, but why this did not result in higher fruit set is unknown. Temperatures during the post bloom period were higher in 1999 (data not presented) which could have stimulated competing vegetative growth, resulting in more severe November drop.

Effect of flowering position (Trial 3). There were no significant differences between the "king" and lateral positions in the cluster for either seed set or seed weight in both years

(Table 10). The fruit developing from the "king" flower, however, did give the higher seed set and weight. In 1998 the lateral flowering position resulted in fruit with more aborted seeds, but not significantly more than the "king" flower position. In 1999 the opposite was true (Table 10). No significant differences were observed in the number or weight of well developed seeds or in the number of aborted seeds that developed when "king" or lateral flowers were pollinated on consecutive days after full bloom (data not presented).

Effect of mentor and own pollen (Trial 4). From Table 11 it is clear that there were no significant differences between treatments 1 (mentor pollen followed by own pollen), and treatment 2 (own pollen followed by mentor pollen) as far as well developed seed number, seed weight and aborted seed number were concerned. Treatment 3 (only own pollen) did not set any fruit, which indicates that 'African Red' is fully self-incompatible.

Fruit dimensions and weight.

Effect of pollinator (Trial 2). In 1998 pollination with 'Simpson crab' resulted in fruit that were significantly more elongated than those pollinated with 'Cripps' Pink' (Table 12). This could possibly be explained by the fact that 'Simpson crab' also set the least number of seeds in 1998 (Table 7a) which possibly caused the more elongated fruit shape as was also found by Brault (1995). During the 1999 season, there were no significant differences between the pollinators used. Generally the pollinators that had set the longest fruit also set the widest fruit. This was especially true in 1999 (Table 12). As pollination was delayed after full bloom a slight downward trend could be observed in fruit length in both years (Fig. 3a). In 1998, a downward trend was observed for fruit diameter (Fig. 3b) while

a slight upward trend was observed in 1999 with cultivar interactions. (Fig. 3c). From the covariate analyses it is clear that part of the change in fruit dimensions can be attributed to seed content (Fig. 3a-c). This effect of the seed number on fruit dimensions is verified by the correlation coefficients in most cases (Table 8 and 9). In 1998, a good correlation between seed content and fruit length and diameter were found for all pollinators. However, in 1999 the correlation coefficient for 'Cripps' Red' and 'Cripps' Pink' between seed content and fruit diameter and length were negative and not significant. Also for 'Simpson crab' the correlation was non-significant (Table 8 and 9). In both years fruit weight was also significantly correlated to seed number for all five pollinator cultivars. No significant differences were found between pollinators for fruit weight (Table 12).

Effect of flowering position (Trial 3). In 1998, no significant differences in fruit length were found between the "king" and lateral flowering positions in the cluster although fruit from the "king" flower were on average longer and significantly larger in diameter, than those from the lateral flower (Table 13). In 1999 no significant differences were found between the "king" and lateral flowering positions. When different flowers were pollinated on consecutive days after full bloom, no significant differences were found in fruit length and diameter (data not presented).

Effect of mentor and own pollen (Trial 4). From Table 14 it is clear that there were no significant differences between treatment 1 (mentor pollen followed by own pollen) and treatment 2 (own pollen followed by mentor pollen) as far as fruit length and diameter were concerned.

CONCLUSION

It was difficult to confirm test results from the one year to the next through field pollination trials because of fluctuating experimental and environmental conditions, as was found by Sakurai et al. (1997) and Jansens et al. (1995). Pollinators did not differ significantly from one another in germination potential or pollen tube length. No consistent significant differences were found between pollinator cultivars as far as fruit set was concerned. 'Simpson crab' gave the lowest set in both years. Differences in set percentage between *Malus domestica* ('African Red') and *Malus baccata* ('Simpson crab') species might be ascribed to an incompatibility reaction between the pistil of the main cultivar and pollen tubes of the pollinator, whereby foreign pollen from other species is discriminated against and fertilisation prevented (Dodds et al., 1997). The fact that 'Cripps' Red' gave a higher set percentage than the rest of the pollinators in 1998 corresponds well with the S-alleles as reported in Paper 1 where 'Cripps' Red' was S2 and S7 and 'African Red' S3 and S9. This was, however, not confirmed during the following season. 'Cripps' Pink' and 'Cripps' Red' are of common ancestry ('Golden Delicious' x 'Lady Williams'), but were in two distinct pollination groups (both S-alleles differed) and both of these cultivars gave a relatively high set percentages on the optimum day despite the fact that 'Golden Delicious' was also used as a parent for 'African Red' ('Golden Delicious' x 'Dietrich Starking'). Apples that are in distinct pollination groups can therefore be intercrossed for the production of new hybrids. Allele-specific PCR analyses is a useful and rapid technique of determining the pollination group (S-alleles) to which a cultivar belongs and, to a certain extent, supported the field pollination trials but can at this stage not be seen as an end in itself and more research needs to be done on the reliability of

this tool/technique.

No significant differences were found between pollinators in effective pollination period (EPP) in both years but varied between 4 and 6 days depending on the pollinator cultivar for a percentage set of 30%. No consistent results were found in fruit set between the "king" and lateral flowering positions over the two years. Fruit size was well correlated with seed number for all 5 pollinator cultivars but no pollinator gave a significantly better fruit size. 'Simpson crab' gave fruit that were significantly more elongated than those of 'Cripps' Pink' in 1998. 'Simpson crab' also set the least number of seeds in 1998 which possibly caused the more elongated fruit shape. During the 1999 season, there were no significant differences between the pollinators used as far as fruit length and diameter were concerned. Generally the pollinators that had set the longest fruit also set the widest fruit. 'African Red' did not set any fruit when pollinated by its own pollen and suggests the presence of a strong incompatibility system in this cultivar.

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Table 1. Percentage germination of pollen grains using the hanging drop-method in the laboratory as an indication of pollen viability.

	1998	1999
Cripps' Pink	40	88
Simpson	30	95
Winter Banana	35	46
Cripps' Red	35	45
Granny Smith	55	46

Table 2. The average (of the 5 times that the percentage of pollen grains that germinated were counted) and final (at 48 hours after pollination) pollen germination percentage on the stigmatic surface for the 5 different pollinators used to pollinate 'African Red'.

	1998		1999	
Pollinator	**Final germination %**	**Average germination %**	**Final germination %**	**Average germination %**
Winter Banana	88.40 a	80.38 a	84.07 a	78.22 a
Cripps' Red	83.00 a	73.67 a	92.30 a	81.87 a
Simpson	96.00 a	57.32 a	80.16 a	64.96 a
Cripps' Pink	93.02 a	64.68 a	71.05 a	68.13 a
Granny Smith	85.90 a	65.73 a	65.60 a	71.25 a
LSD (5%)	59.20	24.12	47.74	26.82

Table 3. The average (of the 5 times that the length of the pollen tubes growing down the styles were measured) and final (at 48 hours after pollination) pollen tube length of the 5 different pollinators used to pollinate 'African Red' as measured by the section of the style reached by the pollen tubes

Pollinator	1998		1999	
	Final pollen tube length	**Average pollen tube length**	**Final pollen tube length**	**Average pollen tube length**
Winter Banana	1.50 a	0.96 a	1.11 ab	1.05 ab
Cripps' Red	0.70 a	0.74 a	1.13 ab	1.06 ab
Simpson	1.73 a	0.57 a	1.15 a	1.12 a
Cripps' Pink	0.73 a	0.70 a	1.05 ab	1.07 ab
Granny Smith	0.55 a	0.78 a	1.02 b	1.02 b
LSD (5%)	1.231	0.502	0.127	0.073

Table 4. Fruit set of 'African Red' as influenced by different pollinators. % Set (optimum) is the highest percentage set recorded for the pollinator over the 8 days.

Pollinator	% Set (optimum).	% Set (average).
1998		
Granny Smith	72.5 a	47.92 a
Simpson	55.0 a	28.71 b
Cripps' Pink	82.5 a	51.07 a
Cripps' Red	72.5 a	57.78 a
Winter banana	77.5 a	51.00 a
	*	*
1999		
Granny Smith	42.5 a	34.19 ab
Simpson	60.0 a	23.12 b
Cripps' Pink	32.5 a	35.85 a
Cripps' Red	40.0 a	26.56 b
Winter banana	37.5 a	28.28 ab
LSD (5%)	*	8.16*

*=The Chi square (χ^2) test for difference between 2 binomial proportions were used.

A χ^2 value greater than 3.84 is significant at the 5% significance level.

Table 5. Fruit set of 'African Red' as influenced by different flowering positions.

Position in cluster	% Set (average).	% Set (optimum).
1998		
King flower	22.8 a	40 a
Lateral flower	17.0 a	40 a
	*	*
1999		
King flower	30.0 b	50.0 b
Lateral flower	43.75 a	75.0 a
LSD (5%)	*2.09*	*

*=The Chi square (χ^2) test for difference between 2 binomial proportions were used.

A χ^2 value greater than 3.84 is significant at the 5% significance level.

Table 6. Fruit set of 'African Red' when pollinated by mentor pollen (Simpson crab) followed by own pollen (treatment 1), when pollinated by own pollen followed by the mentor pollen (treatment 2), or only pollinated by own pollen (treatment 3) in 1999.

Treatment	Average % set.
1	40 a
2	45 a
3	*0*
LSD (5%)	*39.36*

Table 7a. Seed set and weight of 'African Red' as influenced by different pollinators in

1998.

Pollinator	Average number of well developed seeds.	Average weight of well developed seeds (g).	Average number of aborted seeds.
Cripps' Pink	4.67 a	0.23 a	1.60 ab
Cripps' Red	4.47 a	0.22 a	0.71 bc
Winter Banana	4.82 a	0.24 a	2.26 a
Granny Smith	4.71 a	0.24 a	1.58 ab
Simpson	4.43 a	0.23 a	0.13 c
LSD (5%)	*4.64*	*0.02*	*0.88*

Table 7b. Seed set and weight of 'African Red' as influenced by different pollinators in

1999.

Pollinator	Average number of well developed seeds.	Average weight of well developed seeds (g).	Average number of aborted seeds.
Cripps' Pink	5.43 a	0.234 a	0.19 ab
Cripps' Red	5.40 a	0.239 a	0.08 b
Winter Banana	5.40 a	0.246 a	0.12 b
Granny Smith	5.03 a	0.240 a	0.11 b
Simpson	5.07 a	0.220 a	0.25 a
LSD (5%)	*5.90*	*0.027*	*0.12*

Table 8. The correlation between seed number and fruit weight, diameter, length and seed weight for 'African Red' 1998.

	Pollinator cultivar				
	Granny Smith	**Winter Banana**	**Cripps' Red**	**Cripps' Pink**	**Simpson**
Fruit weight:					
Pearson correlation coefficient	0.4461	0.3046	0.3024	0.4334	0.3411
Significance level	0.0001	0.0002	0.0001	0.0001	0.0026
Fruit diameter					
Pearson correlation coefficient	0.4769	0.1947	0.3528	0.3931	0.4233
Significance level	0.0001	0.0185	0.0001	0.0001	0.0001
Fruit length					
Pearson correlation coefficient	0.2967	0.1525	0.2353	0.3555	0.2672
Significance level	0.0004	0.0661	0.0034	0.0001	0.0196
Seed weight					
Pearson correlation coefficient	0.9464	0.9502	0.4880	0.9706	0.9376
Significance level	0.0001	0.0001	0.0001	0.0001	0.0001

Table 9. The correlation between seed number and fruit weight, diameter, length and seed weight for 'African Red' 1999.

	Pollinator cultivar				
	Granny Smith	**Winter Banana**	**Cripps' Red**	**Cripps' Pink**	**Simpson**
Fruit weight:					
Pearson correlation coefficient	0.4931	0.5229	0.2864	0.2446	0.4887
Significance level	0.0001	0.0001	0.0072	0.0085	0.0001
Fruit diameter					
Pearson correlation coefficient	0.3607	0.2781	-0.0367	-0.0290	0.0574
Significance level	0.0004	0.0095	0.7386	0.7581	0.6270
Fruit length					
Pearson correlation coefficient	0.3429	0.1762	-0.0619	-0.0878	-0.0724
Significance level	0.0008	0.1047	0.5737	0.3510	0.5401
Seed weight					
Pearson correlation coefficient	0.6646	0.9569	0.7901	0.8261	0.8587
Significance level	0.0001	0.0001	0.0001	0.0001	0.0001

Table 10. Seed set and weight of 'African Red' as influenced by different flowering positions.

Treatment	Average well developed seed count	Average well developed seed weight (g)	Average aborted seed count
1998:			
King flower	4.76 a	0.239 a	0.18 a
Lateral flower	3.78 a	0.189 a	0.33 a
LSD (5%)	*1.13*	*0.06*	*0.33*
1999:			
King	5.36 a	0.280 a	0.33 a
Lateral	5.06 a	0.260 a	0.29 a
LSD (5%)	*0.73*	*0.05*	*0.31*

Table 11. Seed set and weight of well developed and aborted seeds of 'African Red' when pollinated by mentor pollen (Simpson crab) followed by own pollen (treatment 1) when pollinated by own pollen followed by the mentor pollen (treatment 2), or only pollinated by own pollen (treatment 3) in 1999.

Treatment	Average well developed seed count.	Average well developed seed weight (g).	Average aborted seed count.
1	3.9 a	0.162 a	0.5 a
2	3.5 a	0.161 a	0.2 a
3	_[2]	-	-
LSD (5%)	*2.8*	*0.116*	*0.58*

[2] No fruit set in treatments.

Table 12. Fruit dimensions (fruit length and diameter) of 'African Red' as influenced by different pollinators.

Pollinator	Average fruit length (mm)	Average fruit diameter (mm)	Average fruit weight (g)
1998:			
Winter Banana	55.65 ab	65.71 a	115.16 a
Simpson	56.21 a	65.58 a	111.23 a
Granny Smith	55.7 ab	65.20 ab	116.38 a
Cripps' Red	55.14 ab	64.87 ab	117.06 a
Cripps' Pink	54.66 b	64.20 b	113.80 a
LSD (5%)	*1.10*	*1.27*	*6.24*
1999:			
Winter Banana	49.90 a	56.28 a	96.19 a
Simpson	48.98 a	56.09 a	92.28 a
Granny Smith	49.66 a	56.04 a	96.87 a
Cripps' Red	50.20 a	56.58 a	97.05 a
Cripps' Pink	50.17 a	55.30 a	96.67 a
LSD (5%)	*2.03*	*2.14*	*4.99*

Table 13. Seed set and weight of 'African Red' as influenced by different flowering positions.

Position in cluster	Average fruit length (mm)	Average fruit diameter (mm)
1998		
King flower	58.28 a	67.47 a
Lateral flower	54.79 a	62.40 b
LSD (5%)	*2.71*	*3.11*
1999		
King flower	51.80 a	58.65 a
Lateral flower	52.59 a	58.77 a
LSD (5%)	*1.89*	*1.65*

Table 14. Fruit length and diameter of 'African Red' when pollinated by mentor pollen (Simpson crab) followed by own pollen (treatment 1) and when pollinated by own pollen followed by the mentor pollen (treatment 2) in 1999.

Treatment	Average fruit length (mm)	Average fruit diameter (mm)
1	38.44 a	46.72 a
2	40.23 a	42.40 a
LSD (5%)	*29.23*	*31.01*

Fig. 1a. The effective pollination period of 'African Red' as influenced by different pollinators Granny Smith (GS), Cripps' Pink (CP), Simpson (Simp), Cripps' Red (CR) and Winter Banana (WB) for the 1998 season.

Fig. 1b. The effective pollination period of 'African Red' as influenced by different pollinators Granny Smith (GS), Cripps' Pink (CP), Simpson (Simp), Cripps' Red (CR) and Winter Banana (WB) for the 1999 season.

Fig. 2a. The number of well developed seeds in 'African Red' fruit following hand pollination on consecutive days after full bloom. Data pooled for pollinator (Trial 2).

Fig. 2b. The weight of well developed seeds in 'African Red' fruit following hand pollination on consecutive days after full bloom. Data pooled for pollinator (Trial 2).

Fig. 2c. The number of aborted seeds in 'African Red' fruit following hand pollination on consecutive days after full bloom. Data pooled for pollinator (Trial 2).

Fig. 3a. The change in fruit length of 'African Red' over time for trial 2. The unadjusted means for length were used.

Fig. 3b. The change in fruit diameter of 'African Red' over time for the 1998 season for trial 2.

Fig. 3c. The interaction between pollinators (GS = Granny Smith, CP=Cripps' Pink, Simp=Simpson Crab, CR=Cripps' Red, WB=Winter Banana) and the influence of time on the fruit diameter of 'African Red' for the 1999 season. The unadjusted means for diameter were used.

Fig. 1a
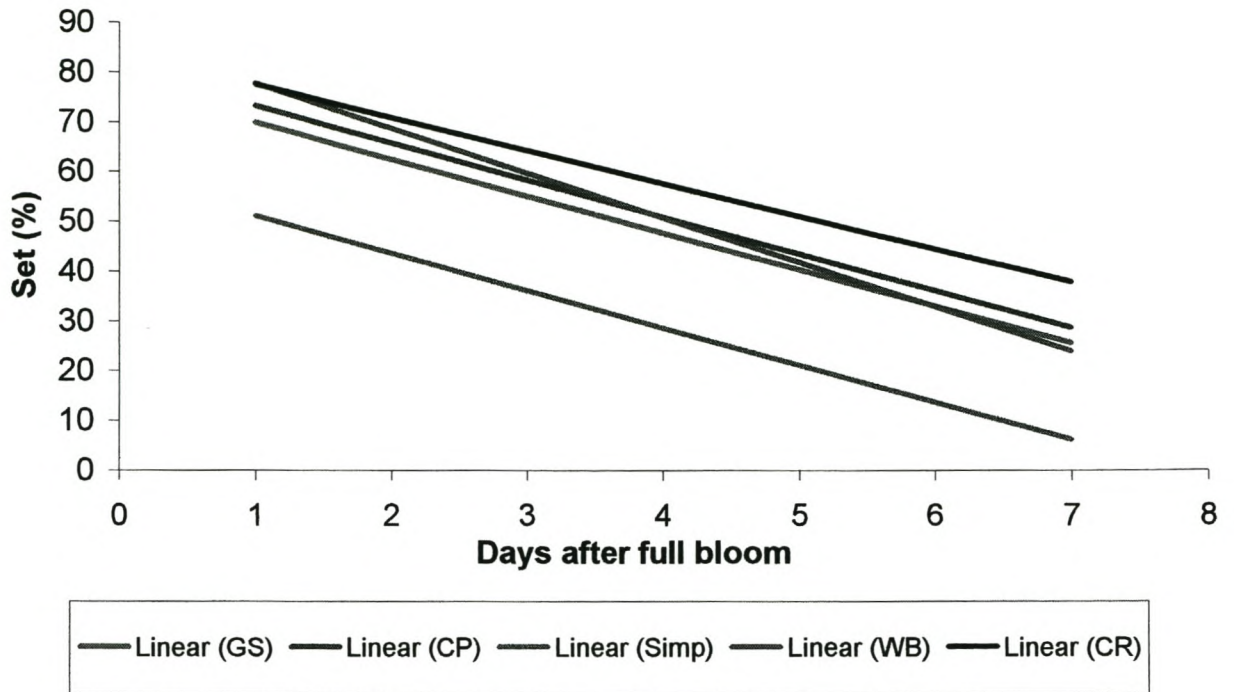
Pollinator		Coefficients	Standard Error	P-value
Cripps' Red	a	84.0714	15.1388	0.0026
	b	-6.5714	3.3851	0.1099
Cripps' Pink	a	80.6429	17.6003	0.0059
	b	-7.3929	3.9355	0.1191
Granny Smith	a	77.3571	14.6172	0.0032
	b	-7.3571	3.2685	0.0742
Winter Banana	a	86.7143	9.0858	0.0002
	b	-8.9286	2.0316	0.0071
Simpson	a	58.6429	14.6844	0.0104
	b	-7.4821	3.2835	0.0717

Fig. 1b
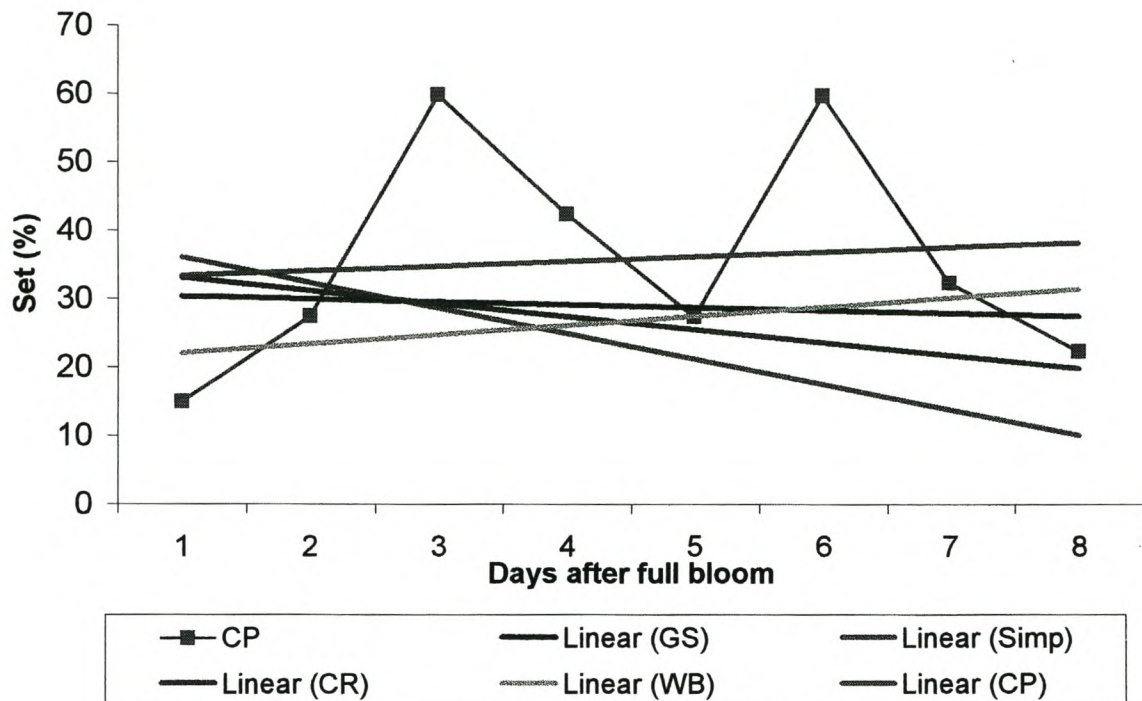
Pollinator		Coefficients	Standard Error	P-value
Cripps' Red	a	35.0000	4.0745	0.0001
	b	-1.8750	0.8069	0.0591
Cripps' Pink	a	32.5893	14.0506	0.0595
	b	0.7440	2.7824	0.7981
Granny Smith	a	30.8036	7.2395	0.0054
	b	-0.3869	1.4336	0.7963
Winter Banana	a	20.7143	6.9741	0.0250
	b	1.3690	1.3811	0.3598
Simpson	a	39.7321	3.3718	0.0000
	b	-3.6905	0.6677	0.0015

Fig. 2a

Number of well developed seeds/fruit vs Days after full bloom — Trial 2. 1998, Trial 2. 1999
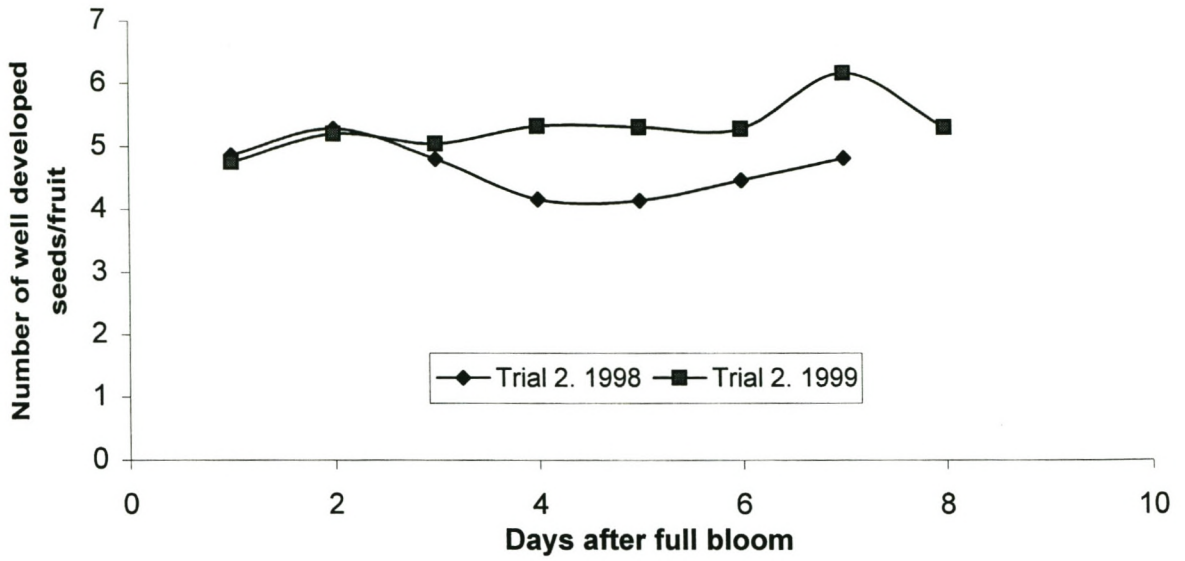
Source '98	DF	Pr>F
Pollinator	4	0.4607
Time	6	0.0001
Poll.x Time	23	0.6373

Source '99	DF		Pr>F
Block	19		0.2523
Pollinator	4		0.5683
Time	7		0.0228
Linear		1	0.0069
Quad		1	0.4776
Poll x Time	28		0.3502

Fig. 2b
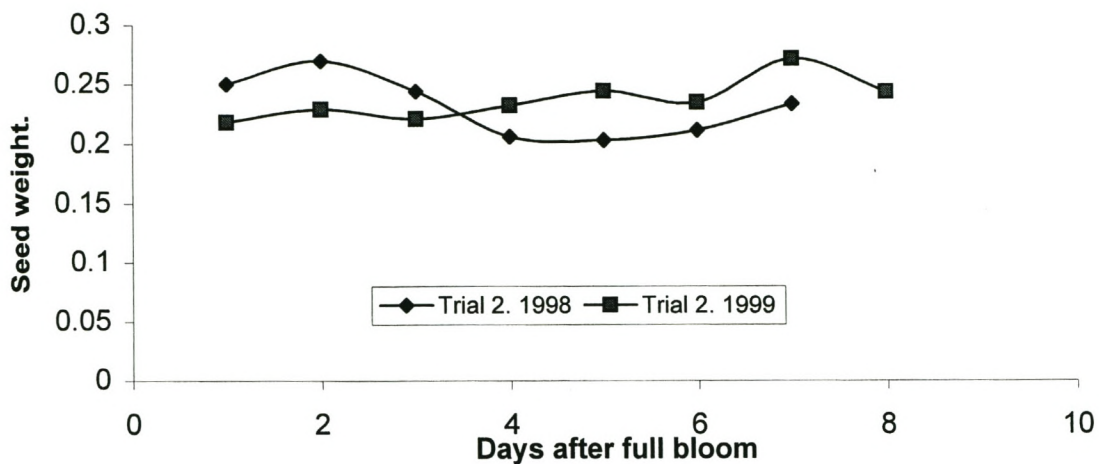
Source '98	DF	Pr>F
Pollinator	4	0.1803
Time	6	0.0001
Poll.x Time	23	0.4758

Source'99	DF		Pr>F
Block	19		0.0002
Pollinator	4		0.7539
Time	7		0.0105
Linear		1	0.0031
Quad		1	0.7262
Poll x Time	28		0.2026

Fig. 2c
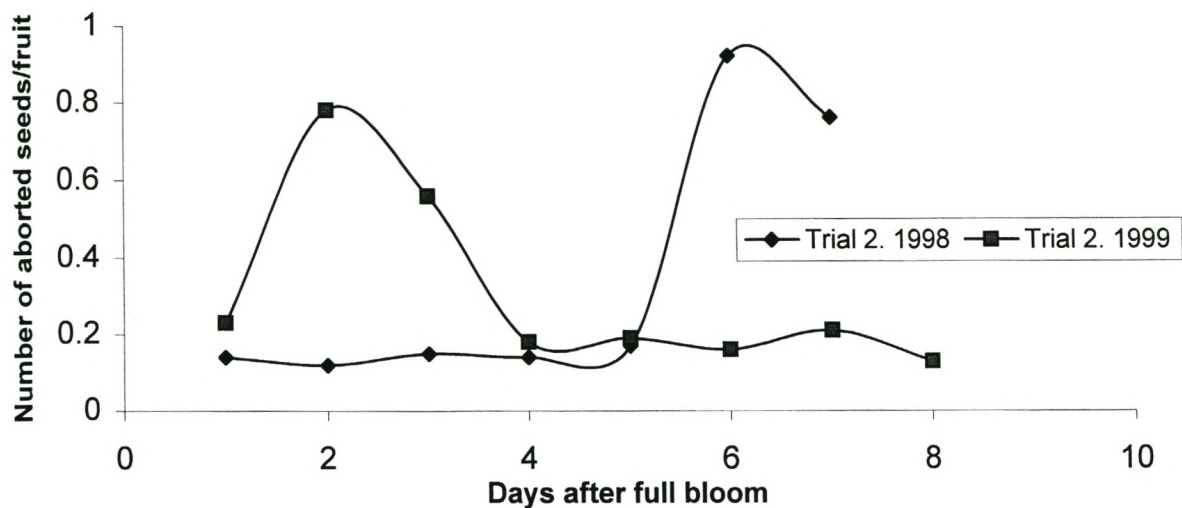
Source '98	DF	Pr>F
Pollinator	4	0.0001
Time	6	0.5829
Poll.x Time	23	0.8485

Source '99	DF	Pr>F
Block	19	0.2759
Pollinator	4	0.0141
Time	7	0.1336
Poll.x Time	28	0.0937

Fig. 3a
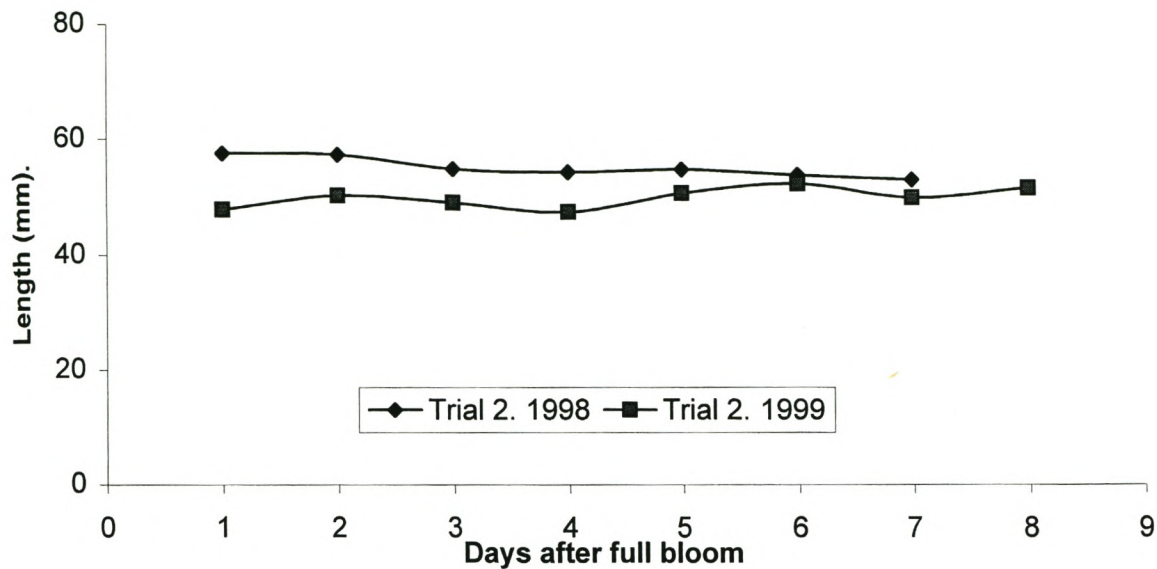
Source '98	DF	Pr>F
Pollinator	4	0.0786
Time	6	0.0001
Poll.x Time	23	0.0100

Source'99	DF	Pr >F	
Seed weight	1		0.0018
Block	19	0.0001	0.0001
Pollinator	4	0.2044	0.2296
Time	7	0.0097	0.0197
Linear	1	0.0507	
Quad	1	0.8457	
Poll x Time	28	0.0866	0.0948

Fig. 3b
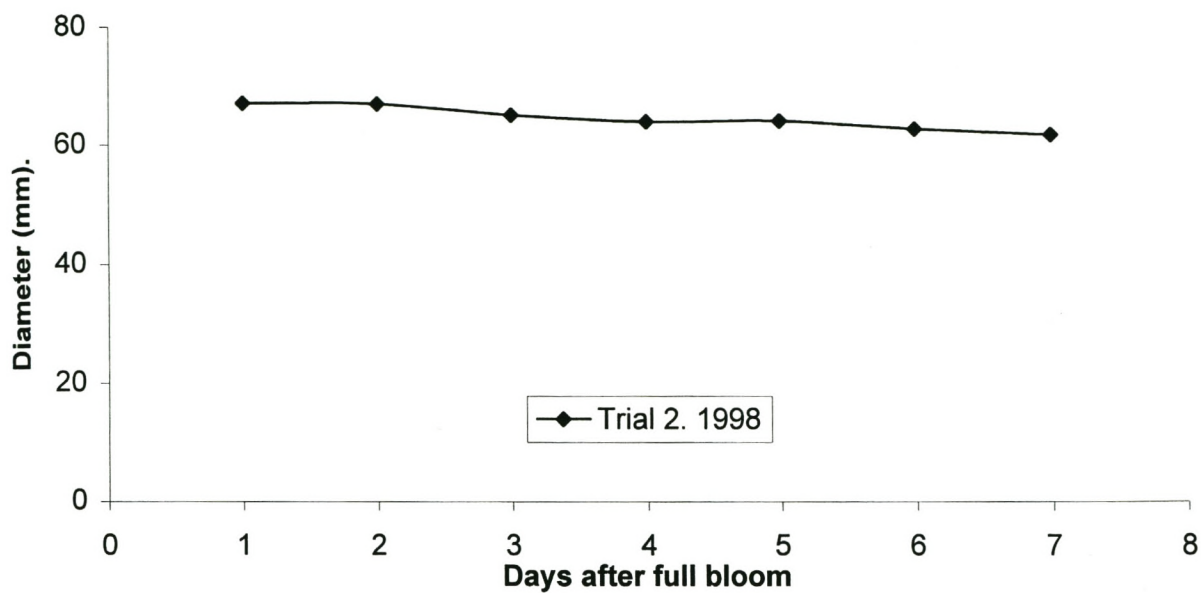
Source '98	DF	Pr>F
Pollinator	4	0.0999
Time	6	0.0001
Poll.x Time	23	0.0132
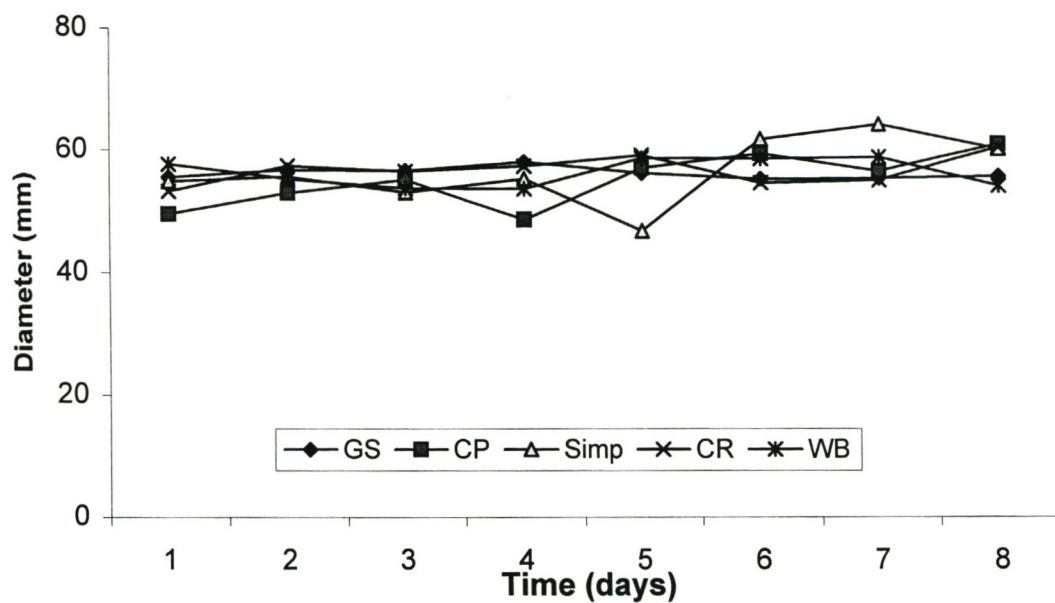
Source'99	DF		Pr >F	
Seed Weight	1			0.0001
Block	19	0.0001		0.0001
Pollinator	4	0.8816		0.9410
Time	7	0.0111		0.0565
Linear		1	0.0050	
Quad		1	0.7518	
Poll x Time	28	0.0001		0.0772

PAPER 3: GENOTYPIC RESPONSE OF APPLE (*MALUS DOMESTICA* BORKH.) BUDS TO CHILLING PERIOD.

Abstract

One-year-old, ca. 40 mm long shoots of various apple cultivars were, selected from commercial orchards in both the Elgin [34°S, 305 m, ca. 750 chill units (CU; Richardson et al., 1974)] and Koue Bokkeveld (33°S, 945 m, ca. 1300 CU) regions of the Western Cape, South Africa. Shoots were forced at a constant 25°C with continuous illumination after receiving their allocated number of chill units. The effect of chilling period on the budburst of each cultivar in both regions was estimated in two consecutive years (1998 and 1999) by determining: 1) the total proportion of budburst (%Bb), 2) the proportion of shoots with terminal budburst (%TBb), and 3) the rate of budburst [1/(days to 25% budburst)]. It was found that these indices differed significantly between cultivars, and within cultivars between areas, as far as budburst patterns, in reaction to chilling, were concerned. The rate of budburst was the most consistent in describing the reaction of buds to different chilling periods and could be used to group cultivars together according to their budburst response to chilling.

Introduction

Apple (*Malus domestica* Borkh.) flowers generally require cross-pollination to be able to set fruit (Dennis, 1986). To ensure effective pollination it is important that the time of bloom of the pollinator and main variety overlap (Kronenberg, 1985). Grouping cultivars for syncroniced bloom requires at least 10 years of field observations (Soltesz, 1997, Göndör-Pinter et al., 1994). For the developing South African fruit industry where older

varieties are regularly replaced with new and improved ones, and where full bloom dates vary considerably between seasons due to marginal winter chilling, this method of determining cross pollination combinations is ineffective. A more effective method is required.

The deciduous fruit tree originated in a temperate zone with cold winters and warm summers (Wertheim, 1990). The trees developed a dormant phase during winter season to survive cold temperatures (Faust et al, 1997; Saure, 1985). Dormancy is a physiological state of rest that can only be overcome by the quantitative accumulation of a certain amount of cold (chilling) (Faust et al, 1997; Powell, 1986). Only part of this chilling requirement can be overcome by other means. One of the most important factors determining the time of bud burst and therefore full bloom is temperature (Hauagge and Cummins, 1991b; Young and Werner, 1985). The relationship between temperature and the release from dormancy is poorly understood and is probably a complex interaction of different factors e.g. autumn conditions, chilling, thermal time and photoperiod (Cannell, 1989).

A wide variation between cultivars for the length of bud dormancy has been reported (Hauagge and Cummins, 1991a; Anderson and Seeley, 1993) and researchers have attempted to determine the chilling requirements of different cultivars. The results have shown many discrepancies, particularly in the way researchers have attempted to calculate the chilling requirement of a variety (Saure, 1985). Measuring the proportion of buds bursting after exposure to a certain amount of chilling and subsequent forcing in a growth

chamber, is commonly used. Saure, (1985) stated that the determination of the percentage budburst alone is not sufficient to determine the end of true dormancy. Reasons being that buds may fail to grow for reasons other than dormancy (Hauagge and Cummins, 1991a; De Real-Laborde, 1987) and that the influence of factors that also inhibit budburst, i.e., apical dominance, may vary considerably between different varieties and species (Vegis, 1964; Saure, 1985; Wertheim, 1990) due to differences in growth habit and genetic make-up (Hauagge and Cummins, 1991c; Brown, 1991). Hauagge and Cummins (1991d) suggest the rate of budburst and the capacity for shoot growth after the exposure of buds to an amount of chilling as a more valid indicator.

In this study an attempt was made to quantify the reaction of different cultivars from different areas (origins) to chilling using different methods of determining the end of dormancy, in order to group them according to budburst reactions. This may enable more effective pollinator recommendations in the future.

Material and Methods

Plant material. Trials were conducted over two seasons and at two sites. In 1998 one-year-old shoots from 'Royal Gala', 'Fuji' (Akifu strain), 'Golden Delicious', 'Granny Smith', 'Topred', 'Braeburn', 'Cripps Pink', 'Cripps Red' and 'African Red' apple trees were harvested on the 20th and 21st of May from a commercial farm in the Koue Bokkeveld region in the Western Cape, South Africa [33°S, 945 m, ca. 1300 chill units (CU)] and a commercial farm in the Elgin region in the Western Cape, South Africa (34°S, 305 m, ca. 750 CU) respectively. One hundred (100) shoots of each cultivar were cut from each

location. The .shoots were brought to the laboratory and cut to a length of 40cm, discarding the basal portion.

In 1999 one-year-old shoots from 'Royal Gala', 'Fuji' (Akifu strain), 'Golden Delicious', 'Granny Smith', 'Topred', 'Braeburn', 'Cripps Pink', 'Cripps Red' and 'African Red' trees were harvested on the 12th and 11th of May in the Ceres and Elgin regions respectively. Two hundred (200) shoots of each cultivar were cut at two different locations within the same region. The shoots were brought to the laboratory and cut to a length of 40cm, discarding the basal portion.

Treatments and experimental design. In 1998, 20 shoots for each cultivar area combination were exposed to four chilling periods at 4°C: 600 h, 900 h, 1200 h, and 1500 h.

In 1999, the shoots were exposed to five chilling periods at 4°C: 600 h, 800 h, 1000 h, 1200 h, 1400 h. Each cultivar was represented by two groups of 20 shoots from two locations within the same area for each of the five chilling periods. After the shoots received their allocated amount of chilling, they were placed in 5L white plastic buckets (three to four bundles per bucket), with their bases in ≈1L of water containing 5ml/L household bleach (5% sodium hypochlorite). The buckets with shoots were placed in a growth chamber that maintained a constant 25°C with continuous illumination (≈215 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR). The water was replaced every second day. The number of shoots in each bundle, with burst buds (green tip), was recorded every second day until no further change was observed. Total budburst (measuring both the terminal and lateral buds) and

percentage terminal budburst were recorded for each cultivar. Rate of budburst was calculated as [1/(days to 25% terminal budburst)].

At the time of harvest, the shoots in the warmer Elgin area had already accumulated 86 positive chill units (PCU) in 1998 and 57 PCU in 1999 as calculated according to the Utah model (Richardson et al., 1974) without a negative CU value carried from one day to the next, i.e. chilling reversal (Lindsley-Nokes et al., 1994). In Ceres, 380 PCU had accumulated at the time of harvest in 1998 and 315 PCU in 1999.

Statistical analysis. In 1998 a factorial analyses of variance was performed on the data from 9 varieties in 2 areas using 4 chilling treatments and one replication. Within each cultivar, area x chilling interaction was tested for. Chilling effects were expressed in terms of single degree polynomials. Third order interactions were used as error degrees of freedom after being tested for normality. Those showing significant (P<0.05) cultivar x area interactions were presented separately. For those not showing any interaction, a combined regression for the two areas was fitted per cultivar.

In 1999 a factorial analyses of variance were performed on the data from 8 cultivars in 2 areas using 5 chilling treatments and two replications. Within each cultivar, area x chilling period interactions were tested for. Chilling effects were expressed in terms of single degree polynomials. Those showing significant (P< 0.05) cultivar x area interactions were presented separately. For those not showing any interaction, a combined regression for the two areas were fitted per cultivar.

Results

Proportion of total budburst (%Bb). In 1998 two distinct groups could be identified: one group in which the %Bb increased with chilling and another group where the proportion total budburst decreased with chilling (Fig. 1, Table 1). No cultivar x area interactions were observed in 1998. In 1999 a general upward trend was observed with chilling, except for 'Granny Smith' and 'Braeburn' from Elgin. Cultivar x area interactions were found for 'Golden Delicious' and 'Braeburn'.

Proportion of shoots with terminal budburst (%TBb). Except for 'Cripps' Red' and 'Cripps' Pink' from Ceres and 'Braeburn' from both areas that showed a downward trend, all of the cultivars tested in 1998 either showed an increase in %TBb or remained fairly stable with chilling (Fig. 2, Table 2). Some cultivars ('Cripps' Pink', 'Cripps' Red', 'Royal Gala', 'Topred') again showed interaction with area.

In 1999 all of the cultivars tested, except for 'Granny Smith' and 'Braeburn', either showed a general increase in %TBb or remained fairly stable with chilling. 'Golden Delicious', 'Topred' and 'Royal Gala' showed area interactions. Area significantly affected the reaction of 'Cripps' Red' and 'Cripps' Pink' to chilling in 1998 (cultivar x area interaction), but not in 1999.

Rate of budburst. In most cultivars the rate of budburst increased with chilling in both years (Fig. 3, Table 3). In 1998, 'Cripps' Pink', 'Royal Gala', 'Cripps' Red' showed an area interaction. The varieties that did not show area interaction exhibited an increase in

the rate of budburst with chilling. All of the varieties of which total budburst was negatively correlated with chilling, showed an increase in rate of budburst as chilling increased.

Discussion

In temperate climates, in order for the buds on a shoot to start growing in the spring (budburst), it has to, after entering into a state of winter rest (dormancy), again exit out of dormancy (Cook & Jacobs, 2000). Temperature plays a major role in dormancy release (Cannel, 1989). The amount of chilling (chill units) and thermal time (heat units) required for budburst are interrelated and the relationship between dormancy release and temperature is a poorly understood and complex process (Cannel, 1989). Chilling models, that were developed in countries with cold winters under conditions of "complete chilling", have been used with limited success in South Africa, with its "insufficiently cold" winters. These models determine cultivar chilling requirement by forcing buds (that are naturally not inclined to rapid development) to budburst at higher temperatures and it is assumed that percentage budburst in general increases with chilling (Shaltout & Unrath, 1983; Lindsley-Nokes et al., 1994; Richardson et al., 1974). Our results have shown this not to be the case with locally grown shoots, even following artificial chilling, and that the simple relationship between percentage budburst and chilling is cultivar and area dependent. Under sub-optimal winter chilling, as found under South African conditions, budburst was slower and more erratic than under conditions of "complete chilling" (Cook et al., 1998; Cook and Jacobs, 1999). The entrance to bud dormancy does not procede normally under local conditions (Cook and Jacobs, 2000). It is possible that shoots used in this study were

not properly dormant before transfer to chilling conditions.

Forcing shoots to budburst is a crude way of trying to determine chilling requirement and difficulties are encountered when attempting to measure the number of effective chill units required to satisfy chilling requirement by forcing (Cannel, 1989). A model by which budburst, as a response to chilling, can be measured is therefore needed to enable the grouping of varieties according to their reaction to varied chilling. This model has to be consistent under local conditions and not change significantly with cultivar and area.

By determining the %Bb, the effect of chilling on the branching, and thus growth habit, of the cultivar is what is being measured. Since cultivars differ in growth habit, use of the Utah model to determine chilling requirement based on the number of buds that burst as chilling increases, may be less effective. Furthermore, because of correlative inhibition of the lateral buds by the terminal bud (Martin, 1987; Cook et al., 1998; Cook and Jacobs, 1999), the proportion of total budburst will be influenced by the reaction of the terminal buds to chilling which again will depend on the year and cultivar. It was found that all of the cultivars of which total budburst were negatively correlated with chilling, showed an increase in growth potential as chilling increased in 1998. This shows that these varieties ('Braeburn', 'Cripps' Pink', 'African Red', 'Fuji') possibly had more of a tendency towards the acrotonic growth habit versus the more mesotonic growth habit of those cultivars that showed an increase in the total number of buds (laterals and terminals) that burst. The inconsistency from year to year and between cultivars within the same year, can be seen from Fig. 1. De Real-Laborde (1987), noted that a possible reason for the

inconsistencies might be due to the fact that similar temperatures may cause a different response on budburst depending on the time during the rest process at which chilling was applied. This could be true also in terms of a differential response of the terminal and lateral buds to chilling temperatures.

The %TBb indicates the ability of the terminal buds to sprout. The terminal bud is essentially independent of distal inhibitions as dormancy is primarily comprised of factors within the terminal bud. It would be expected that temperature would play a major role in terminal bud burst (Cook et al., 1998). With some cultivars in 1998, the terminal bud lost its ability to sprout as chilling increased (Fig. 2). In 1999 certain cultivars increased in their ability to sprout, but then again decreased after 1000 CU (Fig. 2).

From the above mentioned results it can be concluded that the parameters currently used to determine chilling requirement [proportion of total budburst (%Bb) and proportion of shoots with terminal budburst (%TBb)] are inconsistent from year to year and between cultivars within the same year (Fig. 1 and 2).

In this study it was found that the rate of budburst more consistently corresponded to chill accumulation, except in a few instances. 'Royal Gala' in 1998 from the Ceres and Elgin areas showed poor growth (Fig. 3), and exhibited extremely low vigour (deep dormancy) even after 1200 CU. In 1999 'Royal Gala' entered the deepest dormancy, but also exited dormancy rapidly. The growth potential of 'Cripps' Pink' in 1998 also increased with chilling, but then again decreased after 1000 CU.

The main question to be answered, therefore, is what cultivars can be grouped together when exposed to chilling and would it be possible to use the rate of budburst model as a consistent tool in accomplishing this? Most local production areas accumulate less than 800 CU. If the rate of budburst or growth potential is used as an indication of chilling requirement (Fig 3), 'Topred', 'Golden Delicious' and 'Royal Gala' should have the lowest growth potential (highest chilling requirement) at 800 CU whilst 'Granny Smith', 'Braeburn' and 'Fuji' should have the highest growth potential (lowest chilling requirement) at 800 CU (Fig. 3). This corresponds well with phenological full bloom observations recorded over a number of years in South Africa as 'Granny Smith' and 'Braeburn' are classified as being part of the group of earlier flowering cultivars (highest growth potential after being exposed to favourable growing conditions in spring) while 'Topred' and 'Golden Delicious' are considered to be late flowering cultivars. Saunders and Halgryn (1997) (unpublished data) tried to quantify cultivar chilling requirement by collecting one-year-old shoots and forcing them to budburst at 20°C in a glasshouse. The number of Utah chill units required to achieve 60% total budburst was then determined as the chilling requirement of the cultivar evaluated. Cultivars were classified according to their chilling requirement as "high", "medium" or "low". 'Fuji' and 'Braeburn' were classified together with 'Royal Gala' and 'Topred' as having approximately the same "high" chilling requirement. 'Golden Delicious' was considered as having a "medium" chilling requirement. This does not correspond to the above mentioned deductions made from the growth rate model and from the phenological full bloom observations. A possible reason might be that, as previously mentioned, the effect of chilling on the growth habit of the cultivars is what was being determined and not necessarily the chilling requirement.

Similar amounts of chilling may cause different responses to budburst depending on the branching habit of the cultivar tested.

Hauagge and Cummins (1991a) state that under mild winter conditions with wide temperature fluctuations during the dormancy season, important interactions among cultivars and environmental factors, other than chill unit accumulation, are responsible for terminating bud dormancy. This study confirms the complexity of dormancy biology under the mild South African winters and it is apparent that there are significant differences between cultivars, and within cultivars between areas, as far as budburst, and therefore also flowering patterns, are concerned.

In order to make effective cross pollination recommendations in the future, the aim should be to find a way to group cultivars according to their reaction on different amounts of chilling and not necessarily to quantify cultivar chilling requirement. A model that is fairly consistent and that is as far as possible representative of the field situation, is proposed. As also proposed by Saure (1985), Cannel (1998) and Hauagge and Cummins (1991d), the use of the "rate of budburst" model is recommended as being the most consistent. A better understanding of the phenological and physiological laws that govern budbreak, will greatly increase the chances to accurately predict time of budbreak and enable more effective pollination recommendations to be made to producers in the future.

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Table 1 Polynomial R^2 and significance level for the change in the proportion of total budburst with chilling period.

Cultivar	Polynomial function	R^2	P>F
1998 - No cultivar x area interaction			
Braeburn	y = 18.740-0.0020x	0.03	0.6909
Fuji	y = 12.644-0.004x	0.10	0.4344
Golden Delicious	y = -7.544+0.0136x	0.47	0.0603
Granny Smith	y = 1.829+0.0170x	0.69	0.0103
African Red	y = 16.61-0.0044x	0.36	0.3996
Topred	y = 2.225+0.0081x	0.21	0.2603
Cripps' Pink	y = 20.184-0.0054x	0.13	0.3817
Cripps' Red	y = -4.821+0.0178x	0.62	0.0202
Royal Gala	y = -0.748+0.0089x	0.21	0.2506
1999 - No cultivar x area interaction			
Cripps' Red	y = 3.0+0.0095x	0.72	0.0001
Topred	y = 2.0+0.0098x	0.79	0.0001
Granny Smith	y = 17.3-0.0014x	0.23	0.0774
Cripps' Pink	y = 3.4+0.0093x	0.58	0.0001
Fuji	y = 6.3+0.0065x	0.29	0.0143
Royal Gala	$y = 8.7+0.0048x+0.0001x^2$	0.91	0.0001
1999 – Cultivar x area interaction			
Golden Delicious - Ceres	$y = -11.4+0.039x-0.00001x^2$	0.43	0.1825
Golden Delicious - Elgin	$y = 34.7-0.059x+0.00003x^2$	0.71	0.0139
Braeburn - Ceres	y = 10.3+0.0055x	0.30	0.1120
Braeburn - Elgin	y = 23.2-0.0054x	0.12	0.3309

Table 2 Polynomial R^2 and significance level for the change in the proportion of shoots with terminal budburst with chilling period.

Cultivar	Polynomial function	R^2	P>F
1998 - No cultivar x area interaction			
Braeburn	$y = 98.250-0.0233x$	0.21	0.2596
Fuji	$y = 53.750+0.0041x$	0.01	0.8508
Golden Delicious	$y = -34.25+0.0808x$	0.89	0.0004
Granny Smith	$y = -4.50+0.0733x$	0.66	0.0139
African Red	$y = 45.00+0.025x$	0.45	0.3258
1998 - Cultivar x area interaction			
Cripps' Red - Ceres	$y = 73.0-0.0100x$	0.01	0.8901
Cripps' Red - Elgin	$y = -10.0+0.0833x$	0.60	0.2266
Royal Gala - Ceres	$y = -61.5+0.0966x$	0.90	0.0491
Royal Gala - Elgin	$y = 30.5+0.0066x$	0.01	0.8953
Cripps' Pink - Ceres	$y = 98.5-0.0200x$	0.14	0.6314
Cripps' Pink - Elgin	$y = 38.5+0.0466x$	0.69	0.1717
Topred - Ceres	$y = 10.5+0.0650x$	0.90	0.0526
Topred - Elgin	$y = 34.0+0.0033x$	0.00	0.9445
1999 - No cultivar x area interaction			
Braeburn	$y = 30.4+0.149-0.00008x^2$	0.35	0.0315
Cripps' Red	$y = 84.3+0.0112x$	0.37	0.0043
Granny Smith	$y = 42.8+0.109+0.00005x^2$	0.14	0.2851
Cripps' Pink	$y = 68.0+0.025x$	0.58	0.0001
Fuji	$y = 83.2+0.029+0.00001x^2$	0.15	0.2429
1999 - Cultivar x area interaction			
Golden Delicious - Ceres	$y = -55.4+0.263x-0.0001x^2$	0.75	0.0155
Golden Delicious - Elgin	$y = 124.9-0.086x+0.00004x^2$	0.17	0.5236
Topred - Ceres	$y = 34.1+0.0503x$	0.50	0.0329
Topred - Elgin	$y = 94.6+0.0030x$	0.05	0.5462
Royal Gala - Ceres	$y = 43.4+0.0755x-0.00002x^2$	0.83	0.0119
Royal Gala - Elgin	$y = 47.5+0.082x-0.00003x^2$	1.00	-

Table 3 Polynomial R^2 and significance level for the change in the rate of budburst with chilling period. No curves were fitted for Royal Gala in 1998 due to zero values obtained when less than 25% of the shoots burst buds.

Cultivar	Polynomial function	R^2	P>F
1998 - No cultivar x area interaction			
Braeburn	$y = 0.043+0.00003x$	0.65	0.0156
Fuji	$y = 0.012+0.00005x$	0.00	0.8127
Golden Delicious	$y = -0.019+0.00006x$	0.76	0.0048
Granny Smith	$y = -0.009+0.00007x$	0.86	0.0009
African Red	$y = 0.037+0.00002x$	0.64	0.2000
Topred	$y = 0025+0.00005x$	0.33	0.1358
1998 - Cultivar x area interaction			
Cripps' Red - Ceres	$y = 0.059+0.00003x$	0.00	0.9509
Cripps' Red - Elgin	$y = -0.054+0.00001x$	0.86	0.0712
Royal Gala - Ceres	-	-	-
Royal Gala - Elgin	-	-	-
Cripps' Pink - Ceres	$y = 0.006+0.0006x$	0.87	0.0673
Cripps' Pink - Elgin	$y = -0.141-0.0005x+0.0000003x^2$	0.82	0.0026
1999 - No cultivar x area interaction			
Cripps' Red	$y = 0.352+0.0006x+0.000003x^2$	0.54	0.0014
Topred	$y = 0.180-0.0003x+0.000001x^2$	0.73	0.0001
Braeburn	$y = 0.083+0.0001x$	0.00	0.9479
Golden Delicious	$y = -0.003+0.00006x$	0.50	0.0007
Granny Smith	$y = 0.217-0.0003x+0.000001x^2$	0.51	0.0023
Cripps' Pink	$y = 0.216-0.0003x+0.000002x^2$	0.63	0.0002
Fuji	$y = 0.241-0.0003x+0.000001x^2$	0.25	0.0842
Royal Gala	$y = 0.369-0.0007x+0.000003x^2$	0.55	0.0120

Fig. 1.

The change in the proportion of total budburst for Bra=Braeburn, Fuj=Fuji, GD=Golden Delicious, GS=Granny Smith, AR=African Red, TR=Top Red, CP=Cripps' Pink, RG=Royal Gala, CR=Cripps' Red with chilling (chill units).

Fig. 2.

The change in the proportion of shoots with terminal budburst for Bra=Braeburn, Fuj=Fuji, GD=Golden Delicious, GS=Granny Smith, AR=African Red, TR=Top Red, CP='Cripps' Pink', RG=Royal Gala, CR='Cripps' Red' with chilling (chill units).

Fig. 3.

The change in the rate of budburst of shoots for Bra=Braeburn, Fuj=Fuji, GD=Golden Delicious, GS=Granny Smith, AR=African Red, TR=Top Red, CP=Cripps' Pink, RG=Royal Gala, CR=Cripps' Red with chilling (chill units).
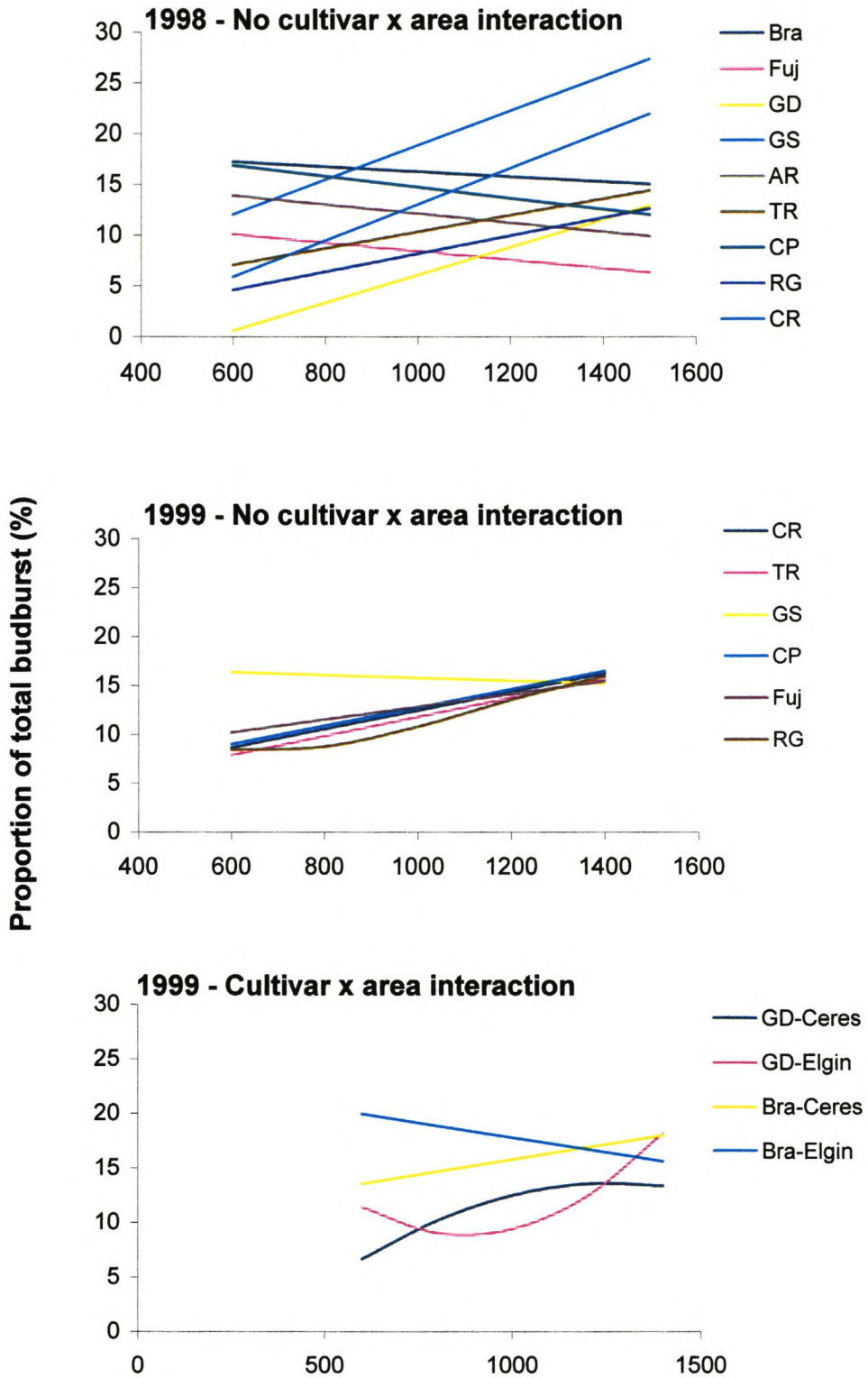
Fig. 1

1998 - No cultivar x area interaction

Legend: Bra, Fuj, GD, GS, AR, TR, CP, RG, CR

1999 - No cultivar x area interaction

Legend: CR, TR, GS, CP, Fuj, RG

Proportion of total budburst (%)

1999 - Cultivar x area interaction

Legend: GD-Ceres, GD-Elgin, Bra-Ceres, Bra-Elgin

Fig. 2   100

Fig. 3   101

1998 - No cultivar x area interaction

1998 - Cultivar x area interaction.

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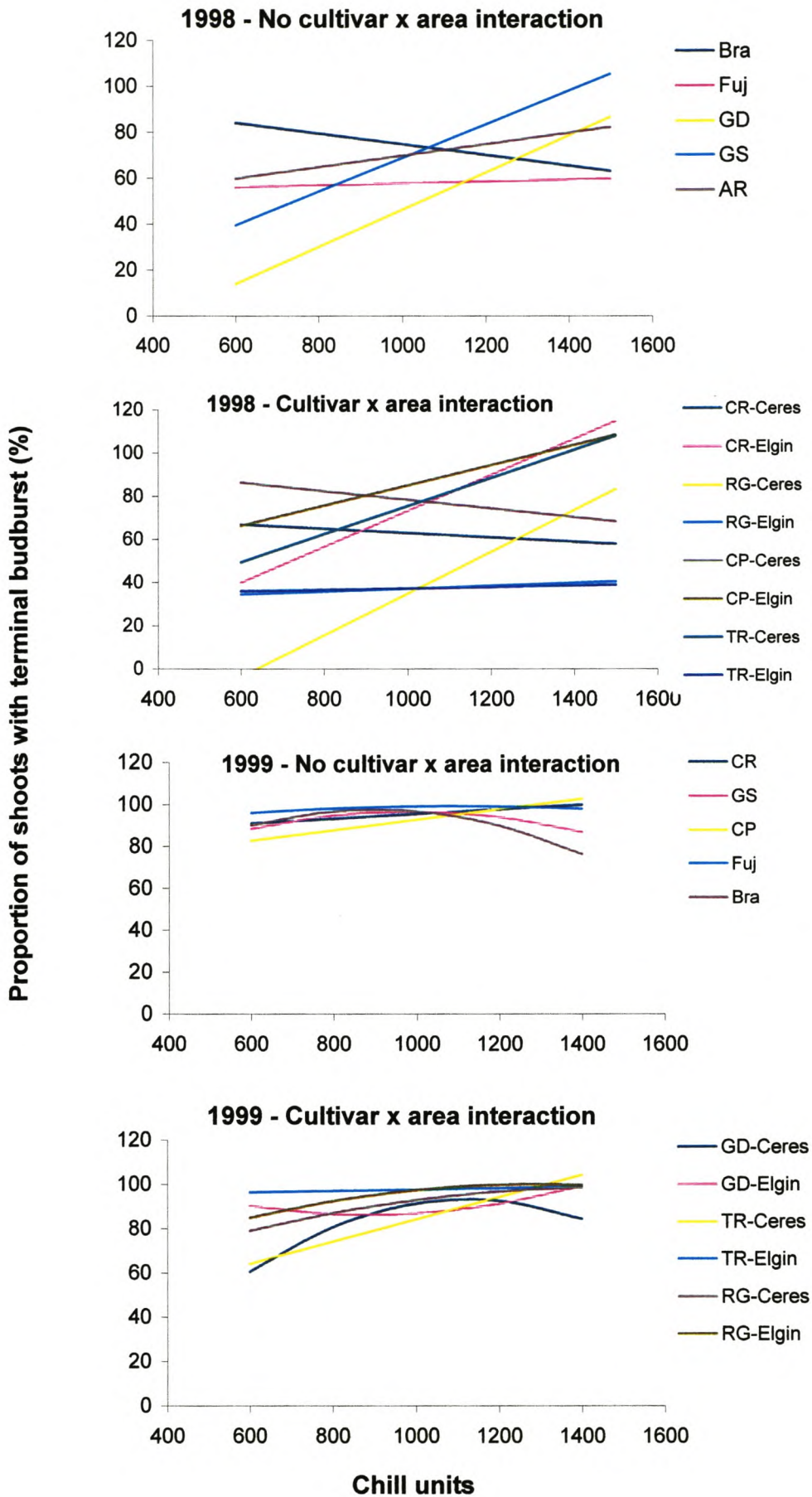
Growth rate [1/(Days to 25% budburst)]

Chill units
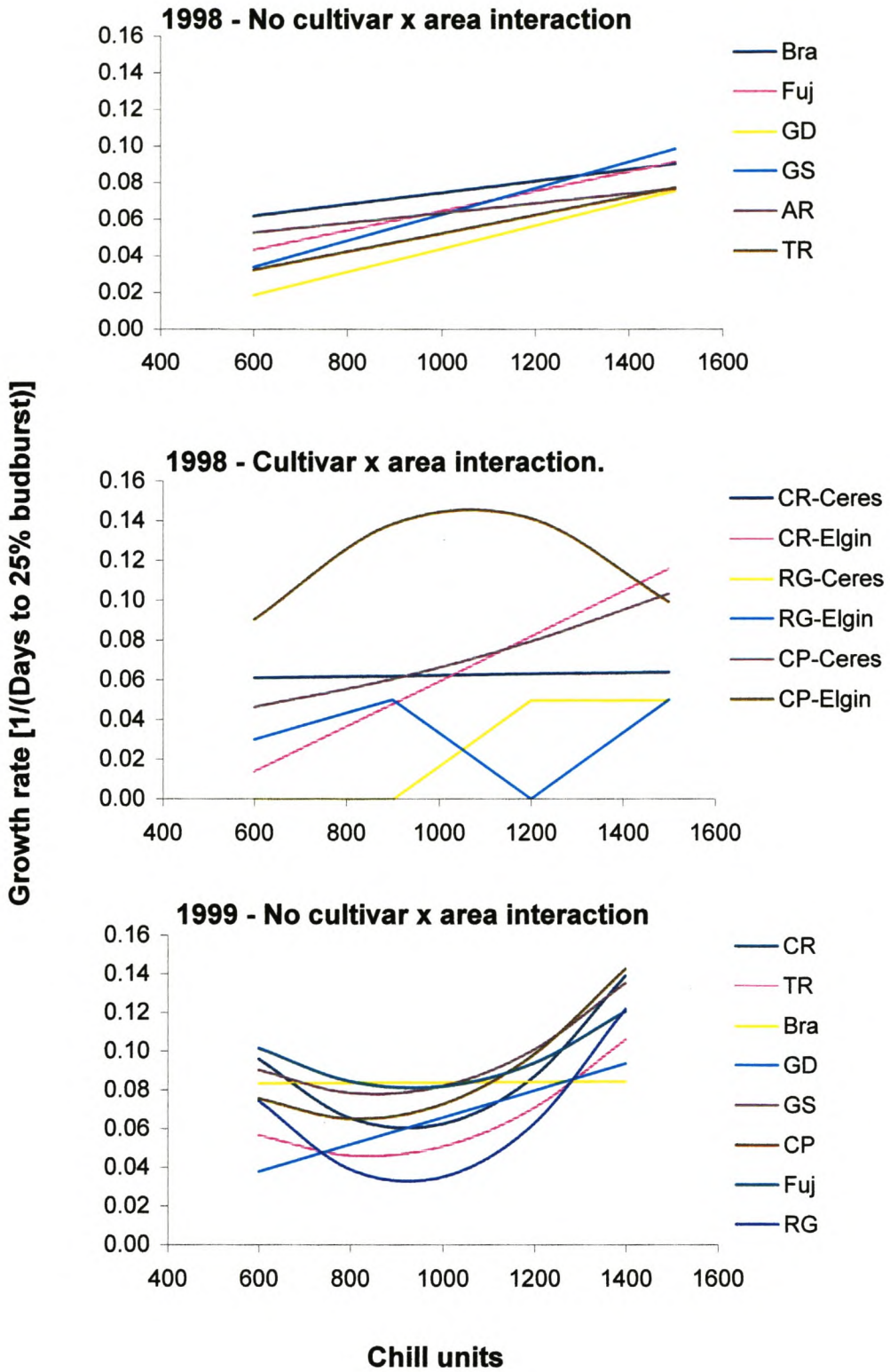
CONCLUSION

Allele-specific PCR amplification for some of the alleles of the S-gene, that regulates incompatibility, were successfully carried out. For all the *Malus domestica* cultivars tested at least one, but in some instances both alleles of the S-gene were determined. 'Simpson crab' (*Malus baccata*), however, did not possess any of the S-alleles tested for. Further work needs to be done on the possibility of other crab apples presently used as pollinators in the apple industry (e.g. *Malus grandiflora*) also not displaying any known S-alleles and the subsequent effect on fertilisation, fruit set and fruit quality. Allele-specific PCR analyses proved to be a useful and rapid technique of determining the pollination group (S-alleles) to which a cultivar belongs and, to a certain extend, supported the field pollination trials but can at this stage not be seen as an end in itself and more research needs to be done on the reliability of this tool/technique. Knowledge of the S-alleles in apple cultivars will aid breeders in the selection of parents for hybridization and provide important information concerning pollination requirements in the future. No consistent differences were found between the pollinators tested ('Simpson crab', 'Cripps' Pink', 'Cripps' Red', 'Winter Banana', 'Granny Smith') in their ability to induce fruit set or influence fruit weight for 'African Red'. The crab apple 'Simpson' (*Malus baccata*) did however give the lowest set in both years possibly due to the fact that it differs from 'African Red' (*Malus domestica*) at species level. 'African Red' did not set any fruit when pollinated by its own pollen and suggests the presence of a strong incompatibility system in this cultivar. No significant differences were found between pollinators for effective pollination period (EPP) in both years but the EPP of

'African Red' varied between 4 and 6 days depending on the pollinator cultivar for a percentage set of 30%. From the above it is obvious that their are a number of other, possibly more important factors other than the pollinator cultivar, that is influencing fruit quality and set potential. However, based on information on the S-alleles involved and the average set percentages recorded, it is recommended that, except for 'Simpson crab', any one of the pollinators tested would be suitable to successfully pollinate 'African Red'.

This study further confirms the complexity of dormancy biology under the mild South African winters and it is apparent that there are significant differences between cultivars, and within cultivars between areas, as far as budburst, and therefore also flowering patterns, are concerned. The aim in the future should be to find a way to group cultivars according to their reaction on different amounts of chilling and not necessarily to quantify cultivar chilling requirement. A model that is fairly consistent and that is as far as possible representative of the field situation, is proposed. As also proposed by Saure (1985); Cannel (1998) and Hauagge and Cummins (1991d), it is recommended to use the "rate of budburst" model as being the most consistent. According to this model, the time of budbreak for 'African Red' should coincide with that of 'Royal Gala' and 'Golden Delicious'. Limited phenological full bloom observations of 'African Red' confirms this. A better understanding of the phenological and physiological laws that govern budbreak, will greatly increase the chances to accurately predict time of budbreak and enable more effective pollination

recommendations to be made to producers in the future. Further research in this area is encouraged.

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STATISTICS

Statistics is available from Mr. F. Calitz at the ARC-Infruitec/Nietvoorbij research institute. Data is stored on the ARC computer network.