A STUDY ON DORMANCY AND CHILLING REQUIREMENT OF PEACHES AND NECTARINES

by

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DECLARATION

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

SUMMARY

Most South African peach and nectarine production areas lack in adequate winter chilling. To address these issues, a conventional breeding programme was started, to develop new and improved stone fruit cultivars with special emphasis on climatic adaptation and pomological attributes. This study was conducted to test the accuracy of the scientific method called the phenological method of classification, currently used by the breeding programme to classify the selections in the second phase of evaluation according to chilling requirement.

During April 2000 and May 2001 and continuing until the next spring, 20 one-year-old shoots of 30 peach and nectarine selections were harvested fortnightly from an evaluation orchard on Bien Donné Experiment Farm, Simondium, Western Cape (34° S). All selections were previously categorised as high (>800 Utah chilling units [CU]), medium (400-800 CU) or low (<400 CU) chilling requirement based on phenologic observations. According to the preliminary classification of the selections included in this study, six selections were classified as high, three as medium and 21 as low chilling requirement. Two replicate bundles of shoots of each selection were prepared and forced at 25° C with continuous illumination until no further changes in bud burst occurred for a period of five days after which the shoots were then discarded. CU in the orchard were calculated according to the Utah and Infruitec models. The hours below 12° C and 7° C were also calculated. For each selection the number of days until 20% vegetative and reproductive bud break was plotted over day of year, Utah CU, Infruitec CU, hours below 12° C, and hours below 7° C, and expressed as a parabolic function. Similarly, the inverse of the number of days until 20% bud break or the rate of bud growth was also plotted against all the above variables. The area under these parabolas was statistically analysed using the CANDISC procedure of SAS Release 8.1. The groupings of the CANDISC procedure were more or less consistent with the preliminary groupings obtained with the phenoligical classification method.

On 16 May 2000 and 15 May 2001, 100 one-year-old shoots of the same peach and nectarine selections were harvested from the evaluation orchard on Bien Donné Experiment Farm, covered in wet paper towelling and black plastic bags and placed in a cold room kept at a temperature range between 4° C and 7° C. Two replicate bundles of 10 shoots of each selection were prepared fortnightly and forced at 25° C with continuous illumination until no further changes in bud burst occurred for a period of five days after which the shoots were then discarded. CU accumulated in the cold room at each transferral date was calculated according to the Utah model. For each selection the number of days until 20% vegetative bud break was plotted over Utah CU, and expressed as a parabolic function. Similarly, the inverse of the number of days until 20% bud break or the rate of bud growth was also plotted against the above variables. The area under these parabolas was statistically analysed using the CANDISC procedure of SAS Release 8.1. Once again, the groupings of the CANDISC procedure were more or less consistent with the preliminary groupings obtained with the phenoligical classification method.

Due to the nature of the scientific method used in this study, there is room for a certain margin of experimental error to occur, which could account for the misclassifications by the CANDISC procedure, when performed on the 2001 season's data. It can be concluded that the phenological method of classifying the selections, as currently used in the breeding programme, is consistent with the results of the scientific method described here. Therefore, it is recommended that the phenological method be used in future to classify the selections according to chilling requirement (CR), as this method is less time consuming and less costly to perform.

Finally the outcome of the analysis of one season's data was used as calibration data against which the other season's data was tested and the consistency of the results, using one set of discriminant functions, was tested. It can be concluded that a unique set of discriminant functions is necessary for each winter season to accurately classify selections according to CR with the CANDISC procedure.

OPSOMMING

'n Studie van dormansie en koue behoefte van perskes en nektariens

Gebrekkige winterkoue is 'n gegewe in die meeste Suid-Afrikaanse perske en nektarien-produksie-streke. Om die gevolge hiervan aan te spreek, is 'n konvensionele teelprogram tot stand gebring om verbeterde steenvrug cultivars te ontwikkel met voortreflike pomologiese eienskappe en wat aangepas is by die plaaslike klimaatstoestande. Hierdie studie is geloods om die akkuraatheid van die wetenskaplike metode, genoem die fenologiese klassifikasie metode, soos tans deur die teelprogram gebruik, waarvolgens seleksies volgens kouebehoefte geklassifiseer word, te toets.

Vanaf April 2000 en Mei 2001 tot en met die daaropvolgende lente, is 20 eenjaar-oue lote van 30 perske en nektatrien seleksies twee weekliks in 'n fase 2 evaluasie boord op Bien Donné Proefplaas, te Simondium in die Wes-Kaap (34° S) versamel. Al die seleksies was vooraf op grond van fenologiese waarnemings geklassifiseer in kategorieë van hoog (>800 Utah koue-eenhede [CU]), medium (400-800 CU) of laag (<400CU) ten op sigte van koue-behoefte. Hiervolgens val ses van die seleksies wat in die studie ingesluit is in die hoë-, drie in die medium- en 21 in die lae kategorie. Lote van elke seleksie is voorberei en in twee herhalings gebondel, waarna dit geforseer is teen 25° C met deurlopende beligting totdat geen verdere knopbreek vir 'n periode van vyf dae voorgekom het nie, waarna die lote verwyder is. CU in die boord is volgens die Utah en Infruitec modelle bereken. Die aantal uur onder 12° C en onder 7° C is ook bereken. Die aantal dae wat dit elke seleksie geneem het om 20% vegetatiewe en 20% reproduktiewe knopbreek te bereik is bereken en geplot teenoor die dag van die jaar, Utah CU, Infruitec CU, aantal uur onder 12° C en aantal uur onder 7° C en uitgedruk as 'n paraboliese funksie. Die inverse van die aantal dae tot 20% knopbreek, of die tempo van knopbreek, is op soortgelyke wyse geplot teenoor al bogenoemde veranderlikes. Die oppervlakte onder die parabole is statisties ontleed met behulp van die CANDISC prosedure van die SAS program (Vrystelling 8.1). Die groeperings wat met die CANDISC prosedure verkry is het grootliks

ooreengestem met die groeperings volgens die fenologiese klassifikasie metode.

Op 16 Mei 2000 en 15 Mei 2001, is 100 eenjaar-oue lote van dieselfde perske en nektarien seleksies in die evaluasie boord op Bien Donné versamel, toegedraai in klam handdoekpapier, in swart plastiek sakke geplaas en in 'n koelkamer geplaas waarvan die temperatuur konstant gehou is tussen die grense van 4° C en 7° C. Twee herhalings van 10 lote elk, van elke seleksie, is twee weekliks voorberei en geforseer by 25° C met deurlopende beligting totdat geen verdere knopbreek vir 'n periode van vyf dae plaasgevind het nie, waarna die lote verwyder is. Op elke oordragdatum is die aantal CU, wat in die koelkamer geakkumuleer het, volgens die Utah model bereken. Die aantal dae wat dit elke seleksie geneem het om 20% vegetatiewe knopbreek te bereik is bereken en geplot teenoor die Utah CU en uitgedruk as 'n paraboliese funksie. Die inverse van die aantal dae tot 20% knopbreek , dus die tempo van groei, is op soortgelyke wyse bererken en geplot teenoor bogenoemde veranderlikes. Die oppervlak onder die paraboliese funksies is statisties ontleed met behulp van die CANDISC procedure. Die groeperings van die CANDISC prosedure het weereens grootliks ooreengestem met die groeperings wat met die fenologiese metode van klassifikasie verkry is.

Weens die aard van die wetenskaplike metode wat tydens hierdie studie gebruik is, kom daar 'n sekere mate van eksperimentele fout voor, wat moontlik die misklassifikasies van die CANDISC prosedure kan verklaar, wanneer dit op die 2001 seisoen se data uitgevoer word. Die gevolgtrekking kan gemaak word dat die resultate van die fenologiese metode, soos dit tans deur die teelprogram gebruik word, ooreenstem met die resultate wat deur die wetenskaplike metode, wat hier beskryf word, verkry is. Dus word daar aanbeveel dat die fenologiese metode in die toekoms gebruik word om die seleksies te klassifiseer volgens koue behoefte (CR), aangesien hierdie metode minder tyd in beslag neem en goedkoper is om uit te voer.

Laastens is die resultaat van die analise van een seisoen se data gebruik as kalibrasiedata waarteen die data van die ander seisoen getoets is om so ooreenstemmendheid van die resultate te toets as slegs een stel diskriminant funksies gebruik word. Die slotsom was dat ‘n unieke stel diskriminantfunksies nodig is vir elke winter seisoen om die seleksies akkuraat met behulp van die CANDISC prosedure volgens koue-behoefte te klassifiseer.

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CONTENTS

1. LITERATURE REVIEW

Dormancy and chilling requirement of peaches and nectarines

1.1 Introduction

Winter dormancy of peach buds is terminated naturally by exposure to low, but not freezing temperatures (Guardian and Biggs, 1964). The duration of exposure to chilling temperatures required to terminate bud-rest has been observed to vary with cultivar. It has also been observed that the duration of exposure to chilling temperatures needed for normal resumption of bud-growth in the spring varies from year to year and from locality to locality with the same clonal material. A large part of this variation in the chilling requirement seems to be due to a partial reversal of the beneficial effects of chilling by rather warm temperatures immediately following exposure to cool temperatures (Erez et al., 1979a, Erez and Couvillon, 1987 and Erez et al., 1988, Erez et al., 1990). Thus, the amount of cool temperature needed for good bud-break increases when the buds are subjected to intermittent cool and prolonged warm periods.

The peach growing regions in South Africa have inadequate winter chilling to satisfy the dormancy-breaking threshold value of high chilling requirement cultivars (Linsley-Noakes, 1995). An accurate classification system of selections in the breeding programme will be an aid to identify suitable germplasm to develop medium and low chilling requirement cultivars suited to different specific micro climates.

1.2 Dormancy

1.2.1 Terminology

Dormancy in deciduous fruit trees is a phase of development that occurs annually and enables plants to survive cold winters (Saure, 1985). This statement holds true in seasonal climates where dormancy serves, amongst others, as an adaptive process against an unfavourable season. However, as Crabbé (1994) has stated, the primary role of dormancy is morphogenetic and consists of a temporal organisation of the plant's life. Borchert (1991) considered growth periodicity in trees to be primarily an inherent consequence of the developmental constraints of trees as large, long-lived plants pursuing a characteristic adaptive strategy. Dormancy in a general sense may be defined as "a state in which visible growth is temporarily suspended" (Samish, 1954 sited by Saure, 1985) i.e., "in which a tissue predisposed to elongate does not do so" (Doorenbos, 1953 sited by Saure, 1985). In the proposal of Lang et al. (1985), dormancy is defined as the temporary suspension of visible growth of any plant structure containing a meristem. This definition draws heavily on Romberger's work of 1963, which specifies three points of dormancy control, namely, 1) the environment, 2) apical control and 3) control within the affected organ. The focal point of dormancy and its regulation is the bud (Martin 1991). Lang et al. (1985) suggested the use of the Greek prefixes eco- (environment), para- (other than), and endo- (within) joined to the base word dormancy to differentiate among the points of control. The differentiation of the terms places emphasis on the condition or event that alters the dormancy state and where the condition or event is perceived. The environment has an overriding influence on all dormancies, but the first term, eco-dormancy, emphasises gross environmental conditions, such as temperature and water, that restricts growth. This is the dormancy of limiting conditions. Para-dormancy refers to signals that originate elsewhere in the plant and that are transported to the perceiving structure. In contrast, in endo-dormancy, the affected structure generates its own signal (Lang et al., 1987). Dormant buds in winter cannot grow until certain biochemical changes occur in that structure. These biochemical changes cannot occur autonomously in the structure. Winter chilling of an endo-dormant bud provides the environmental conditions that bring about the biochemical changes in the bud that allow growth. Chilling parts of the plant other than the bud will not result in the growth of the bud when warm conditions return. The bud itself must be chilled (Martin, 1991). More recent research necessitates further dividing the endodormant period into deep endo-dormancy (d-endodormancy), characterised by the inability to induce buds to grow under natural conditions, and a shallow endodormancy period (s-endodormancy), which is the stage where endodormancy can be overcome by artificial treatments (Faust et al., 1997).

1.2.2 Temperature effects on dormancy

It is well known that during endodormancy low temperatures may hasten and high temperatures may delay development, whereas the reverse is true in active plants. Dostál (1942, sited by Saure, 1985) has pointed out that many methods that are effective in forcing bud break early in dormancy, while the structure is still in an eco- or paradormant phase, tend to delay bud break later on, after the structure has entered endodormancy. Endo-dormancy, like dormancy in the general sense, has a fluctuating intensity. The stage of lower intensity towards the end of endodormancy as described by Samish (1954) and Vegis (1964), is now referred to as a transitional stage from endodormancy to ecodormancy (or s-endodormancy). Vegis (1964) observed that in this phase dormant organs are able to start their growth within the limits of certain external conditions, which are characteristic for each species or cultivar. He pointed out that bud break in this transitional stage may occur initially only in a narrow range of low temperature, which widens until growth activity has reached its maximum, and that temperatures exceeding this range may induce some kind of "secondary dormancy", now referred to as ecodormancy.

Bud break alone is not sufficient to determine the termination of endodormancy. Nor can the percentage of bud break within a certain period be taken as a valid indicator of the degree to which endodormancy is completed because many vegetative buds may fail to grow for reasons other than dormancy and because the influence of apical dominance may vary considerably among different cultivars and species. A more valid indicator should be the speed of bud break (Saure, 1985).

Couvillon and Hendershott (1974) observed that bud break may occur within 72-84 hr after raising the temperature in peaches that had completed endodormancy but had been prevented from growing by imposed dormancy (ecodormancy). Therefore, a rather short period of forcing appears sufficient to identify the completion of endodormancy definitely.

In cool regions the period of endodormancy is terminated rather soon. However, bud break is prevented for some time by a period of imposed dormancy due to adverse environmental conditions (ecodormancy), although considerable growth and development of the buds may occur during brief spells of favourable weather, permitting speedy growth after cessation of those limitations (Martin, 1991, Dennis, 1994, Crabbé, 1994).

1.2.2.1 Effective temperatures

It is generally accepted that deciduous fruit trees must be subjected to low temperatures for a certain period for dormancy release to occur and that this chilling requirement is genetically determined (Lesley, 1944, Fuchigami and Nee, 1987). Sufficient chilling in a cool-winter climate causes a rather quick decrease in the intensity of endodormancy; however, this decrease is masked under these conditions by a subsequent period of imposed dormancy. Insufficient chilling is the cause of the slower reduction of true dormancy (currently referred to as endodormancy) in warm-winter climates, where, therefor, endodormancy extends over a longer period. When there is no chilling, the intensity of true dormancy would not decrease at all, leading to an extreme case of prolonged dormancy (Saure, 1985, Crabbé, 1994).

Coville (1920) pointed out that low temperatures do not necessarily mean freezing; he found temperatures some degrees above freezing to be sufficient for breaking dormancy in blueberry. Chandler and Tufts (1934) noted that -1^{o} C to 0^{o} C was quite effective in promoting bud break of peach. However, Chandler et al. (1937) later reported that temperatures of 0.5^{o} C to 4.5^{o} C were as effective or even better than freezing temperatures, whereas temperatures of 9^{o} C were less effective. In more detailed experiments, Erez and Lavee (1971) obtained maximum rest-breaking efficiency in peach lateral leaf buds at 6^{o} C, and in terminal buds at 8^{o} C. In leaf buds 10^{o} C was only half as effective as 6^{o} C in breaking dormancy, and 18^{o} C was without any effect. Temperatures between 3^{o} and 6^{o} C had the same effect as 6^{o} C; in lateral leaf buds of weaker lateral shoots, 3^{o} C was even slightly better than 6^{o} C. Later, Erez and Couvillon (1982) reported that the maximum dormancy-breaking effect of continuous low temperature, for both vegetative and floral peach buds, occurred at 8° C; some small effect was observed at 0° C and 12° C, and no effect at 14° C. Richardson et al. (1974) considered 6° C to be the optimum dormancy-breaking temperature in some peach cultivars. They did not assume a chilling effect above 12.5° C and below 1.4° C. The Utah model assigns negative values to temperatures higher than 16° C, depending on temperature level. Gilreath and Buchanan (1981b) identified 8° C as the most effective temperature in peach cultivars having a low chilling requirement, with decreasing effect as observed at temperatures up to 14° C and down to 0° C.

1.2.2.2 Negation of chilling

In warm regions, high temperatures during winter are well known to prolong dormancy. In studies conducted by Weinberger (1954, 1956, 1967a, b), he found that intermittent days with rather high temperatures (above 27°C and 32°C, respectively) delayed bud break more than did a moderate continuous temperature elevation (2° C or 3° C) throughout winter. The magnitude of this delay caused by warmth in November and still more in December (northern hemisphere) was positively correlated to the mean maximum but not to the minimum temperatures in these months. Weinberger (1967b) concluded that the delay in bud break was primarily the result of high temperatures in November and December and that, consequently, the dormancy-breaking effect of chilling must be at least partially reversible. However, in leaf buds the negative effect of a warm December could be completely overcome by subsequent cool weather.

Bennett (1949) observed that only a few hours of daily warm treatment at 22.8°C were sufficient to partially offset the effect of a daily 18-hr cold treatment. This was confirmed by Overcash and Campbell (1955), who found that the same total number of chilling hours at 4° C was less effective when chilling was interrupted by 8 hr above 21° C in a daily cycle. Erez et al. (1979b) found that 8hr at 15° C, a temperature that by itself could not remove dormancy, even increased the efficiency of chilling when used in a daily cycle with 6° C; however, no such promotion could be effected by 18° C, and higher temperatures increasingly negated the effect of chilling. However, even the highest temperature tested (24° C) lost its negating effect when the cycle length was extended, a low-chilling cultivar needing less extension than a high-chilling cultivar. This supports the thesis of Erez and Lavee (1971) that the reversal of the chilling effect by high temperatures may be prevented after some days by some kind of fixation process.

1.2.2.3 Influence of inadequate chilling on dormancy

In warm-winter regions, the period of endodormancy is extended; bud break occurs as soon as endodormancy is completed, or sometimes even during late dormancy, without an intervening period of imposed dormancy. If the winters become too mild and/or too short or, as in the tropics, are non-existent, endodormancy may be extended still further. Indeed, in deciduous fruit trees, dormancy becomes nearly irreversible in the tropics. Under these conditions, the symptoms of prolonged dormancy or delayed foliation may occur (Saure, 1985). These symptoms include the following:

- Delayed, protracted, and very weak leafing;
- Formation of bare, unbranched shoots that become increasingly shorter due to shorter internodes and increasingly swollen with each growth flush;
- Shortage of spurs capable of forming flower buds;
- Quickly declining vigour and, thus, early senescence of the trees; sometimes vigorous new growth from the base of the tree;
- Delayed and protracted flowering season;
- Poor fruit development and irregular ripening (Saure, 1985).

Black (1952) stated that during prolonged rest, flower buds in peach are frequently underdeveloped, the pistils in many cases being dwarfed. Consequently, the flower primordia often abort and the flower buds abscise in different stages of development. However as Brown (1958) pointed out after his observations, the abortion of flower primordia may occur early in autumn - and therefor cannot be attributed to insufficient chilling or prolonged dormancy. Zeller (1961) observed that abortion may occur under certain conditions and at certain periods even in a cool climate. Therefore, it is best to distinguish between symptoms of prolonged dormancy and symptoms of abnormal flower bud development, which both may occur simultaneously in warm-winter regions.

1.2.2.4 Chilling requirements

Couvillon and Erez (1985) concluded, from studies done on apple, cherry, peach and pear shoots, that fruit species do not have a specific heat requirement for bloom and bud break as proposed by Overcash (1965, sited in Couvillon and Erez, 1985) and Rom (1966, sited in Couvillon and Erez, 1985), but that budbreak and bloom dates are determined by the chilling requirement. Individual plants with a short chilling requirement growing under similar conditions with long-chilling individuals, will likely have their chilling requirement exceeded, resulting in a reduced fixed growing degree hours (GDH°) requirement for bud break and bloom, when compared with the long-chilling cultivar. During winters when excessive chilling temperatures occur, one would expect bloom on all species to occur with a reduced number of GDH°, in comparison with warm winters (Couvillon and Erez, 1985).

Studies conducted by Scalabrelli and Couvillon (1986) on 'Redhaven' peach show that peach bud type varies in response to chilling temperature as well as in length of the chilling period required for rest completion. Terminal vegetative buds have the shortest chilling requirement (600 hr) and are less affected by temperature level than lateral vegetative or floral buds which required 1340 hr and 2040 hr respectively, when 7.2°C was the chilling temperature, to reach the maximum bud break level. Their studies showed that there was little, if any, influence of correlative inhibition on lateral vegetative bud break, and one could conclude that the bud break differences were due primarily to the chilling influence. This reasoning is also supported by data that show an increasing lateral bud break response to increased chilling time, indicating that the lateral buds were inhibited by endodormancy and not correlative inhibition.

1.3 Phytohormones - role in the control of dormancy release

Many investigations have been conducted in order to relate the onset and release of endodormancy with changes in abscisic acid (Freeman and Martin,

1981) and gibberellin content. However, most of these experiments fail to properly distinguish between the possible states of the analysed buds. Adequate experimentation proves, for example, that the high initial content of abscicic acid declines in a few weeks as well in buds held at 22°C as at 4°C, the latter only permitting renewed growth. Thus, abscicic acid can be implied in the growth arrest but not in the maintenance of endodormancy (Saure, 1985, Martin, 1991).

Autumn abscicic acid levels could be involved in induction of dehydrins and in changes in permeability of membranes. This change deepens dormancy and the plant enters into the endodormant period. Dehydrins bind water. Dehydrin development and the increase in bound water in the buds leads to freeze protection (Arora and Wisniewski, 1996) but its concomitant effect is to deepen dormancy (Faust et al. 1997). The relatively viscous membranes change to become more fluid, thus allowing functioning under colder conditions. However in an article published in 1998, reporting on changes in water status in peach buds on induction, development and release from dormancy, Erez et al. (1998) proposes that the development of bound water in dormant buds is tightly coupled to cold hardiness, and not necessarily an indication of the depth of dormancy. They further state that because the phenomena of cold hardiness and dormancy are tightly interlinked, it is difficult to distinguish between them.

Throughout the chilling period, water slowly becomes freer, but the dehydrins do not disappear altogether yet. By an endogenous spontaneous mechanism, all these changes will take place regardless of the environmental conditions. With all these changes buds become ecodormant. During the last stages of endodormancy and during ecodormancy, buds are sensitive to cytokinins and other dormancy breaking chemicals (Martin, 1991).

The increase in gibberellin content often appears too late to be the factor removing domancy and is thus rather a consequence of dormancy release (Saure, 1985, Martin, 1991).

Saure (1985) suggested that cytokinins probably have some supplementary function in dormancy release, but is not the cause. There is an increase of cytokinins, which start shortly before budburst and increase rapidly with bud swelling, and peaks around budburst. This cytokynin peak in spring is believed to originate from the shoot and not the roots (Tromp & Ovaa, 1990; Cutting et al., 1991; Faust et al., 1997). Cytokinins trigger metabolic activities that are geared for growth, including DNA, RNA and protein synthesis, increase in the energy metabolism, and decrease in pathways important in resting tissues, for example energy metabolism shifts from the pentose pathway to the tricarboxylic acid pathway.

1.4 Founding clones of low-chill peach germplasm

The peach [*Prunus persica* (L.) Batsch] is adapted to temperate and subtropical zones. Most commercial production lies between latitudes 30° and 45° North and South (Hesse, 1975, Scorza and Sherman, 1996). Low mid-winter temperatures and spring frost limit peach production in the temperate zone. Insufficient chilling of reproductive and vegetative buds limits production in the tropics.

One of Vavilov's principles is that a wild species shows its greatest variability at or near its centre of origin (Scorza and Sherman, 1996). Frank N. Meyer travelled China early in the last century and found many apparently wild stands of peaches in the central provinces of China. The variability of the peach in China is quite apparent in the evidence given by Hederick (1917). Therefore it would be safe to deduct that the peach originated in central China (Scorza and Okie, 1990, Scorza and Sherman, 1996).

The Romans spread the peach throughout their realm. Although earlier migrations may have brought the peach to northern Africa and to Spain by the Moors, its spread through the European Mediterranean countries was mainly by the Romans. They undoubtedly brought the peach to present day France and may have carried it as far as England. The earliest described cultivars of France and England were often white- rather soft-flesh types, whereas those of Spain were described as firm and yellow-fleshed (Scorza and Sherman, 1996).

The era of exploration and colonisation in the sixteenth and seventeenth centuries was accompanied by distribution of plants from the home countries. The peach was introduced to continental America via the Spanish conquest of Mexico and into Florida as early as 1565 with the founding of St. Augustine. The Portuguese apparently introduced the peach to the east coast of South America at an early date (Scorza and Sherman, 1996).

Up to the American Revolution, North American peach culture relied almost solely on seedling stands. Between the Revolution and the Civil War, a number of cultivars came to the fore, usually seedlings of unknown origin (Scorza and Sherman, 1996). Some of the better were 'Early Crawford' and 'Late Crawford' and 'Oldmixon Cling'. 'Chinese Cling' was introduced to the United States by Charles Downing in 1850, from England. It had been discovered a few years previously in China by Charles Fortune, an English plant explorer. Downing sent trees of this new introduction to Henry Lyons in South Carolina, who first fruited the peach. Following the Civil War, Samuel H. Rumph of Marshallville, Georgia, fruited seedlings of 'Chinese Cling' – 'Belle of Georgia' ('Belle') and 'Elberta' came from this planting. 'Hiley' (a seedling of 'Belle') and 'J.H. Hale' (supposedly a seedling of 'Elberta') were found only a few years later. Among the most successful cultivars grown today in the United States, many, if not most, trace back to 'J. H. Hale', and hence through 'Elberta' or 'Belle' to 'Chinese Cling' (Scorza et al., 1985 sited in Scorza and Sherman, 1996). The most remarkable fact concerning this lineage is that most of the present-day cultivars in the United States are derived from a rather narrow base, and hence are genetically restricted. The peach's natural tolerance of inbreeding (Lesley, 1957) and the ease of recovery of quality fruit in segregating progenies of Chinese Cling offspring allowed U.S. breeders to repeatedly use this germplasm in the development of peach cultivars (Scorza and Okie, 1990).

Growers in Mexico have continued to utilise seedling trees since the introduction of peach seed by Spanish explorers in the 16th century. Moreover, after many generations, individual feral populations are now adapted to a wide range of environments from subtropics to cool highlands. In Mexico as in Spain, traditional peach fruit is a dual purpose non-melting type with yellow or orange flesh and no red overcolour. The Mexican cling (referred to as "criollo") populations exhibit a range of ripening time but due to the nature of seedling populations, these are mid-to-late season ripeners (Scorza and Sherman, 1996).

The Australian low-chill stonefruit industry is based on varieties from Wayne Sherman's Florida Breeding program. The introduction of low-chill cultivars in the 20 years period prior to 1995 resulted in a growth of $45 million in the Australian low-chill stonefruit industry. In 1995 plantings mainly consisted of Flordaprince and Flordagold peaches and Sundowner nectarines. However, new varieties are still being introduced from the University of Florida Breeding program. Up to 1995, 141 cultivars have been introduced, including peaches, nectarines, plums and apricots (Campbell et al.,1995).

The Florida breeding program is focussing on the introduction of non-melting flesh genes into a number of lines to promote the establishment of a firmer fleshed fruit that will travel well and have a longer shelf life (Campbell et al., 1995). However their objective is tree ripened fruit which retain firmness on the shelf, as a result the fruit will not necessarily have good cold storage ability, which is a prerequisite for South African produced stone fruit cultivars. The Australian low-chill stone fruit industry mainly supplies the local domestic market, thus long cold storage ability is not essential because of the closeness of the marketplace (Campbell et al., 1995). It can be deducted that as the Australian low-chill industry is dependent on an American breeding program that their genetic base is also restricted.

The South African canning peach industry is largely dependent on progeny of 'Chinese Cling' (Byrne and Bacon, 1999) which gave rise to the 'St. Helena peach', which was introduced by early settlers and which gave rise to the

Transvaal yellow cling types. 'Kakamas' was selected by A.D. Collins, a teacher from Kakamas, from seedlings obtained from the Transvaal cling types. The seedling was evaluated by prof. O.S.H. Reinecke, who released the cultivar 'Kakamas' in 1932 (Anon., 1971, Anon., 1973).

Dessert cultivars were introduced in the early years, for instance 'Babcock', 'Jubilee', 'Elberta' and 'Muir' from the United States of America, 'Duke of York', 'Early Rivers' and 'Peregrine', from the United Kingdom, and 'Goldmine', from New Zealand. However, only two of the cultivars mentioned, introduced from the U.S., have low chilling requirements (Sharpe, 1969, Anon., 1973).

Perez et al. (1993) studied the morphological and phenological polymorphisms in peach germplasm from Mexico and compared it to peaches from U.S., Brazil, Europe, and South Africa. This analysis clustered the Mexican and other central American criollos into one large group, indicating some cohesiveness but much variability. Other groups were formed by the South African peaches, the evergreen and related peaches, and the Brazillian with the low-chill U.S. cultivars.

1.5 Heredity of chilling requirement in peaches

Chilling requirement of peach cultivars is considered to be one of the characteristics that are obviously combinations of more or less inseparable components, in other words it is considered to be a complex trait (Lesley, 1944, Sharpe, 1961).

The genes for low chilling have been derived primarily from peaches of south China origin. The 'Honey' and 'Peen-tao' (also spelled pantao) types have been used as has the south China 'Hawiian' genotypes and 'Okinawa' (Scorza and Okie, 1990, Scorza and Sherman, 1996).

Sherman et al. (1988) determined the chill requirements of low chilling peaches growing in Florida. As noted by Weinberger (1950) (sited in Scorza and Sherman, 1996), and long recognised by those familiar with the response of peaches and nectarines to insufficient chilling, flower and vegetative buds may have different chilling requirements. When different, the vegetative buds nearly always have the greater requirement. For example, 'Elberta' flower buds require 850 chilling units, while the vegetative buds require 950 units.

Breeding for low chilling requirement has been largely empirical, but Lesley (1944), Lammerts (1945), and Sharpe (1961) have reported on segregation in hybrid populations. Lesley (1944) constructed distribution frequency charts, on the basis of his seven classes, following test winters. Most of the hybrid populations displayed normal distributions, centring around the class of the mean of the parents used. In some crosses evidence of slightly skewed distributions was obtained, indicating the possible presence of one or a few genes with major effects. In all classes variability was great, usually extending through most, if not all, of the seven classes. Both Lesley (1944) and later Sharpe (1961) concluded that the chilling requirement is based on multiple genes having a cumulative and similar effect on the phenotype, and the absence of dominance. Lesley concluded that all of the cultivars or selections used in the crosses were highly heterozygous for the multiple genes.

Lammerts (1945) evaluated several seedling populations for reaction to mild winters and recognised, following a "test" winter, a class that he designated "evergreen". These held much of their foliage throughout the winter. The number of segregants for this character suggested that it was controlled as a simple recessive character. It could be observed only following nearly frostless winters. Modifying factors were also involved, according to Lammerts (1945), so that low chilling requirement, as with Lesley (1944), was thought to depend on multigenic control. Later work with evergreen types has indicated that this character is incompletely dominant, with the heterozygotes exhibiting an intermediate phenotype. Environment, parental chilling requirement, and tree maturity affect the expression of the trait in heterozygotes, which can confuse classification (Rodriquez et al. 1994).

The results of these investigators appear to be similar to those described for other complex quantitative traits. Multigenic or polygenic control seems to predominate (Lang, 1994), but there is some evidence for the presence of one or a few genes having relatively major effects. Interaction with the season makes analysis difficult. The development of methods to more accurately characterise chilling requirement (Weinberger, 1961) would undoubtedly improve the efficiency of genetic characterisation for the trait.

1.6 Conclusion

To predict the suitability of specific cultivars to specific growing sites, knowledge of the bud break and bloom dates as well as their tendency to exhibit signs of delayed foliation under warm winter conditions is a prerequisite. These factors are all determined by the chilling requirement of the cultivar.

As dormant organs are able to start their growth within the limits of certain external conditions, which are characteristic for each species or cultivar and which are determined by the genetic make-up of the cultivar, it is possible to group cultivars according to chilling requirement by observing the reaction of their shoots to forcing conditions after pre-exposure to different increments of effective chilling temperatures. If this procedure is started in the fall, the reaction of the shoots could give an indication of the progress of dormancy in each cultivar.

Bud break may occur within 72-84 hrs after raising the temperature in peaches that had completed endodormancy but had been prevented from growing by ecodormancy. Therefore, a rather short period of forcing appears sufficient to identify the completion of endodormancy definitely. An indicator of the termination of endodormancy should be the speed of bud break.

When cultivars are categorised into chilling requirement groups, it becomes possible to identify suitable parents to steer a breeding programme into a certain direction, in other words, to produce progeny with a chilling requirement falling within certain chosen parameters.

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2. PAPER 1: A STUDY ON DORMANCY AND CHILLING REQUIREMENT OF PEACHES AND NECTARINES

ABSTRACT

During April 2000 and May 2001 and continuing until the next spring, 20 1-year-old shoots of 30 peach and nectarine selections were harvested fortnightly from an evaluation orchard on Bien Donné Experiment Farm, Simondium, Western Cape (34° S). All selections were previously categorised as high (>800 Utah chilling units [CU]), medium (400-800 CU) or low (<400 CU) chilling requirement based on phenologic observations. According to the preliminary classification of the selections included in this study, six selections were classified as high, three as medium and 21 as low chilling requirement. Two replicate bundles of shoots of each selection were prepared and forced at 25° C with continuous illumination until no further changes in bud burst occurred for a period of five days after which the shoots were then discarded. CU in the orchard were calculated according to the Utah and Infruitec models. The hours below 12° C and 7° C were also calculated. For each selection the number of days until 20% vegetative and reproductive bud break was plotted over day of year, Utah CU, Infruitec CU, hours below 12° C, and hours below 7° C, and expressed as a parabolic function. Similarly, the inverse of the number of days until 20% bud break or the rate of bud growth, was also plotted against all the above variables. The area under these parabolas was statistically analysed using the CANDISC procedure of SAS Release 8.1. In most instances the number of hours below 12° C contributed most to the separation of selections into CR groupings. The groupings of the CANDISC procedure were more or less consistent with the preliminary groupings obtained with the phenoligical classification method. It is recommended that the phenological method be used in future to classify the selections according to CR.

Keywords: breeding, chilling requirement, dormancy, nectarine, peach

INTRODUCTION

Most South African peach and nectarine production areas lack in adequate winter chilling (Linsley-Noakes, 1995). Consequently, only medium (400 - 800 Richardson/Utah chilling units [CU]) to low (<400 CU) chilling requirement cultivars can be produced with any measure of success. To address these issues, a conventional breeding programme was started, to develop new and improved stone fruit cultivars with special emphasis on climatic adaptation and pomological attributes. To achieve this goal it is necessary to accurately determine the chilling requirement of these new selections.

This study was conducted to test the accuracy of the phenological method of classification, currently used by the breeding programme to classify the selections in the second phase of evaluation according to chilling requirement (CR).

MATERIALS AND METHODS

Plant material

Peach and nectarine selections in the second phase of evaluation of the ARC Infruitec-Nietvoorbij breeding programme were identified for inclusion in this study. The trees were planted in an evaluation orchard on Bien Donné Experiment Farm, Simondium, Western Cape (34° S). All selections were budded to Kakamas seedling rootstocks and planted 4.5 x 1.5 m. Six trees per selection, in their fourth to eighth leaf were used. Lesley (1944) stated that cultivars could be classified according to CR by recording certain phenological traits. All the selections were previously categorised as high (>800 CU), medium (400 – 800 CU) or low (<400 CU) CR based on the following phenologic observations during two seasons preceding this study: (1) date of 10% bud break, (2) date of 80% bud break, (3) duration of blossoming period (normally 8 to 10 days), (4) signs of delayed foliation (blossoming period longer than 10 days) and severity thereof and (5) recorded

CU (Infruitec model) at time of bud break at Bien Donné (warm), (6) Robertson (warm), and (7) in the Koue Bokkeveld (cold). The performance of these selections was also compared with that of control cultivars of known CR within the same orchards (Scorza and Sherman, 1996.). According to the preliminary classification of the selections included in this study based on phenologic observations, six selections were classified as high, three as medium, and 21 as low CR.

Methodology

Starting 20 April 2000 and 2 May 2001 (when shoot extension had ceased in all included selections), 20 1-year-old shoots per selection were collected fortnightly at random over the canopy (Scorza and Sherman, 1996). All shoots were trimmed at the basal end to a length of 30 cm. The shoots of each selection were bundled in two groups of ten shoots. These bundles were placed in plastic buckets with the base of the shoots in water containing 2.5 $mL.L^{-1}$ household bleach (5% sodium hypochlorite). All shoots were forced at a constant 25° C with continuous illumination (ca. 200 $\mu mol\ m^{-2}\ s^{-1}$ PAR). The solution in the buckets was replaced, and a fresh cut made at the base of each shoot, three times a week (Cook et al., 1998.). On Mondays, Wednesdays and Fridays bud burst of vegetative (green tip) and reproductive buds (pink tip), on the same shoot, was recorded until no further changes in bud burst occurred for a period of five days after which the shoots were then discarded.

Determination of CU

Meteorological data was collected from the weather station at Bien Donné from the beginning of April through to the end of August for both years. CU were calculated according to the Utah (Richardson *et al.*, 1974) and Infruitec models (Linsley-Noakes et al., 1994, Greybe, 1997). The hours below 12°C and 7°C were also calculated.

Data analysis

Occasionally bud burst occurred on fewer than five of the ten shoots per bundle. To avoid missing values, the time to 20% bud break was calculated as the time taken for bud burst to occur on two shoots per bundle, i.e. days to 20% bud burst. For each selection the number of days until 20% vegetative and reproductive bud break was plotted over day of year, Utah CU, Infruitec CU, hours below 12° C, and hours below 7° C, and expressed as a parabolic function. Similarly, the inverse of the number of days until 20% bud break or the rate of bud growth (Cannell, 1989), was also plotted against all the above variables. The area under these parabolas was statistically analysed using the CANDISC (Canonical Discriminant Analysis) procedure of SAS Release 8.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Days until 20% vegetative bud break 2000

The eigenvalues, as used in the CANDISC procedure, were λ_1 = 0.3944 and λ_2 = 0.0412. The first eigenvalue accounted for a high proportion of the total: $\lambda_1/(\lambda_1 + \lambda_2) = 0.9054$. The mean vectors were, thus, largely in one dimension and one discriminant function sufficed to describe 90.54% of the separation among the three groups. Therefore, the first pooled within-class standardised canonical coefficients (Can 1), represented in Table 1, were taken into consideration, to determine which variable contributed most to separating the groups. Thus hours below 12°C (h <12) contributed most to separating the groups (Can 1 = -11.57368761), while the second highest contribution was hours below 7°C (h <7) (Can 1 = 5.71885581). The linear discriminant function for chilling, as used in this analysis to classify the selections according to CR and which described the groups, is given in Table 2. The CANDISC procedure misclassified one high CR selection into the medium CR group. Six low CR selections were misclassified into the high category and three into the medium category. One medium CR selection was misclassified into the high grouping. Overall a 63.33% correct classification was achieved (Table 3). A visual presentation of the outcome of the CANDISC procedure is given in Figure 1. Included in this figure are the class means of the different groupings, showing their distribution in space relative to each other.

The six low CR selections (B, J, K, N, S and T), misclassified into the high CR grouping, can be largely attributed to the fact that these particular selections were all adversely affected by high temperatures experienced in the growth chamber during one weekend in May when the chamber malfunctioned, after which many of these shoots appeared shrivelled. This happened before 20% bud break and resulted in missing values. It is known that forcing shoots in this way has its limitations, with some shoots being adversely stressed despite the care taken. The misclassification of these low CR selections into the high CR grouping we therefore attribute to experimental error rather than to the inherent CR of the selections.

Furthermore, the boundaries given to the low, medium and high CR groupings artificially facilitate the classification of cultivars by CR. In reality, the CR of these selections forms a continuum from low to high CR. Therefore, some selections can be close to a boundary separating the groups, and displaying traits of both groups can be misclassified. During a marginal CU season, like 2000, the selection Ad fell in the upper quarter of the medium CR group but was misclassified with selections in the high CR group (Table 3). In 2000 a negative Utah CU accumulation was observed up to the fourth collection date. May 2000 was particularly warm and only 83 h <7 were recorded. By the fourth collection date only 47 Utah CU had accumulated making this model inaccurate.

Inverse of days until 20% vegetative bud break 2000

The eigenvalues were λ_1 = 0.2908 and λ_2 = 0.0333 and one discriminant function described 89.72% of the separation among the three groups. Here, as with the data based on days until 20% vegetative bud break, h <12 contributed most to separating the groups (Can 1 = -4.408866270), but the second highest contribution was collection date (Can 1 = 2.539793935), and not h <7 (Table 4). The linear discriminant function for chilling, as used by the CANDISC procedure to classify the selections by CR, is presented in Table 5.

One high CR selection (F) was misclassified into the medium CR group. Nine low CR selections (Ab, B, J, K, M, N, S, T and Z), including the same six that were misclassified using the days until 20% vegetative bud break, were misclassified into the high chilling category, and one (C) into the medium category. Two medium CR selections (Ad and Af) were misclassified into the low grouping. Overall only a 56.67% correct classification was achieved (Table 6; Figure 2). As this procedure was performed on the inverse of the days until 20% bud break, on the same plant material as in the previous section, the same inherent problems apply as discussed previously. When the CANDISC procedure was performed on the inverse of days until vegetative bud break (rate of growth to bud burst) it gave a less accurate classification (56.67% correct) of the selections compared to when it was performed on the days until 20% vegetative bud break (63.33% correct).

Days until 20% reproductive bud break 2000

The eigenvalues were λ_1 = 0.6390 and λ_2 = 0.0011 and one discriminant function described 99.82% of the separation among the groups. Therefore, the first pooled within-class standardised canonical coefficients (Can 1, Table 7), were considered to determine which variable contributed most to separating the groups. H <12 contributed most to separating the groups (Can 1 = 4.629872636) with collection date the second highest contribution (Can 1 = -2.211776789). The linear discriminant function for chilling, used to classify the selections according to CR and which describes the groups, is presented in Table 8. The CANDISC procedure misclassified four high CR selections (F, I, P and Q) into the medium CR group. Five low CR selections (B, J, K, N and T) were misclassified into the medium CR category. There was no misclassification from the medium CR grouping. Overall a 70.00% correct classification was achieved (Table 9; Figure 3).

Inverse of days until 20% reproductive bud break 2000

When looking at the response in terms of the inverse function the eigenvalues were λ_1 = 0.5391 and λ_2 = 0.1547 and one discriminant function described 77.70% of the separation among the three groups. Therefore, the first pooled within-class standardised canonical coefficients (Can 1, Table 10) were used to determine which variable contributed most to separating the groups. Once again, h <12 contributed most (Can 1 = -17.75852374) with h <7 second (Can

1 = 10.01445899). The linear discriminant function for chilling, as used to classify the selections according to CR and which describes the groups, is presented in Table 11. Eleven low CR selections (A, B, E, J, K, M, N, O, T, U and W), again including the same misclassified selections as above, were misclassified into the high chilling category and one (Ab) into the medium category. One medium CR selection (Af) was misclassified into the high grouping. Overall only a 56.67% correct classification was achieved (Table 12; Figure 4). As with the vegetative bud break data, the CANDISC procedure was less accurate when performed on the inverse of the days until 20% reproductive bud break (56.67% vs. 70.00%). Overall the difference between using vegetative vs. reproductive bud break was small.

Days until 20% vegetative bud break 2001

The eigenvalues were λ_1 = 1.6128 and λ_2 = 0.1350 and one discriminant function described 92.28% of the separation among groups. The collection date contributed most (Can 1 = -3.873591297) with h <12 second (Can 1 = 3.625219499) (Table 13). The linear discriminant function for chilling, as used to classify the selections according to CR and which describes the groups, is presented in Table 14. Only two high CR selections (L and X) are misclassified into the medium group, one low CR selection (J) was misclassified into the high group, and there was no misclassification within the medium CR group (Table 15; Figure 5). Overall a 90.32% correct classification was achieved, much higher than the 63.33% in 2000. This higher percentage could be due to the fact that fewer problems occurred during forcing and that 2001 winter was colder and therefore resulting in a generally better progression of winter dormancy.

Inverse of days until 20% vegetative bud break 2001

The eigenvalues were λ_1 = 0.6637 and λ_2 = 0.0734 and again one discriminant function described 90.05% of the group separation. Utah CU contributed most (Can 1 = -4.72476769) with h <12 second (Can 1 = 4.51986077) (Table 16). The linear discriminant function for chilling, used to classify the selections according to CR and which describes the groups, is presented in Table 17. Two high CR selections (F and X) were misclassified into the medium group. Selection X was also misclassified when the days until 20% vegetative bud break were used. Two low CR selections (D and J) were misclassified as high CR, and five (Ab, Ac, K, O and S) as medium. Selection J was also previously misclassified. Overall a 70.00% correct classification was achieved (Table 18; Figure 6). Once again the inverse of days until 20% vegetative bud break (rate of bud burst) gave a less accurate classification (70.00%) of the selections compared to the days until 20% vegetative bud break (90.32%), mainly due to the five misplaced low CR selections.

Days until 20% reproductive bud break 2001

The proportion of the eigenvalues ($\lambda_1 = 1.2141$ and $\lambda_2 = 0.1984$) indicates that one discriminant function described 85.96% of the group separation. According to the first pooled within-class standardised canonical coefficients (Can 1, Table 19) Infruitec CU contributed most (Can 1 = 3.300161662) with h <7 second (Can 1 = -2.817238409). The linear discriminant function for chilling, as used to classify the selections according to CR and which describes the groups, is presented in Table 20. Due to missing values only 25 selections could be used for the CANDISC procedure. No high CR selections were misclassified, three low CR selections (Ac, D and T) were misclassified as high CR, and one (J) as medium CR. One medium CR selection (Af) was classified as high CR. An overall 80.00% correct classification was achieved (Table 21; Figure 7).

Inverse of days until 20% reproductive bud break 2001

The eigenvalues were $\lambda_1 = 0.5381$ and $\lambda_2 = 0.0341$ and from their proportions it is clear that the mean vectors were largely in one dimension and one discriminant function described 94.04% of the separation among the three groups. Therefore, the first pooled within-class standardised canonical coefficients (Can 1, Table 22) indicate that h <12 contributed most to separating the groups (Can 1 = -11.94721944) with Infrutec CU second (Can 1 = 6.35562751). The linear discriminant function for chilling, as used to classify the selections according to CR and which describes the groups, is presented in Table 23. One high CR selection (F) was misclassified as medium CR. One low CR selection (O) was misclassified as high CR and three (M, N and S) as medium CR. An overall 80.00% correct classification was achieved (Table 24; Figure 8).

CONCLUSION

As mentioned earlier, some problems occurred during forcing in 2000 resulting in numerous misclassifications, in particular, of low CR selections. Furthermore, the boundaries given to the low, medium and high CR groupings are artificial aids to facilitate the classification of cultivars according to CR, but the CR of the selections included in this study forms a continuum from low to high CR without clear boundaries. During a marginal CU season, like the winter of 2000, a selection falling within the upper part of the medium CR grouping, could display traits similar to a selection falling within the lower part of the high CR grouping and could consequently be misclassified.

In most instances the number of hours below 12° C contributed most to the separation of the selections into CR groupings by the CANDISC procedure. This indicates that when criteria based on lower temperature were used, no clear distinction could be made between the selections according to CR.

The groupings of the CANDISC procedure obtained with the 2001 season's data are more or less consistent with the preliminary groupings obtained with the phenological classification method. Due to the nature of the scientific method used in this study, there is room for a certain margin of experimental error to occur, which could account for the misclassifications by the CANDISC procedure, when performed on the 2001 season's data. It can be concluded that the phenological method of classifying the selections, as currently used in the breeding programme, is consistent with the results of the scientific method described here. Therefore, it is recommended that the phenological method be used in future to classify the selections according to CR, as this method is less time consuming and less costly to perform.

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TABLE 1: Pooled within-class standardised canonical coefficients based on the areas under the parabolas which describe the number of days until 20% vegetative bud break of the different variables of the year 2000.

Variable	Can1	Can2
Hr <12 °C	-11.5737	0.0234
Hr <7 °C	5.7189	1.8342
Infruitec chill units	1.5287	-0.0903
Richardson chill units	0.4284	0.7821
Day of collection	4.3699	-2.1361

TABLE 2: Linear discriminant function for chilling based on the areas under the parabolas which describe the number of days until 20% vegetative bud break of the different variables of the year 2000.

Variable	High	Medium	Low
Constant	-6.95755	-6.04935	-5.97126
Hr < 12 °C	-0.00246	-0.00295	-0.00450
Hr < 7 °C	0.01002	0.01026	0.01316
Infruitec chill units	-0.00111	-0.00098	-0.00061
Richardson chill units	0.00019	0.00014	0.00024
Day of collection	0.00201	0.00478	0.00792

TABLE 3: Number of observations and percent classified into chilling based on the areas under the parabolas which describe the number of days until 20% vegetative bud break of the different variables of the year 2000.

From Chilling	High	Medium	Low	Total
High	5	1[A]	0	6
%	83.33	16.67	0.00	100.00
Medium	1[B]	2	0	3
%	33.33	66.67	0.00	100.00
Low	6[C]	3[D]	12	21
%	28.57	14.29	57.14	100.00
Total	12	6	12	30
%	40.00	20.00	40.00	100.00
Priors	0.33333	0.33333	0.33333	

[A] Selection: X

[B] Selection: Ad

[C] Selections: B, J, K, N, S, T

[D] Selections: C, D, E

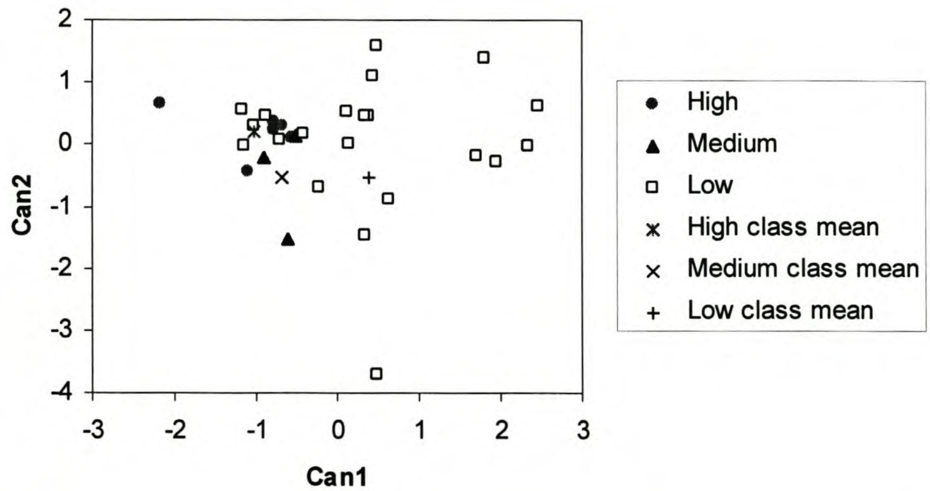

FIGURE 1: Discrimination between high, medium and low chilling requirement selections, using the area below the visual presentation of the polynomial equation, of the second degree, of the time until 20% vegetative bud break of the 2000 data in a canonical discriminant analysis.

TABLE 4: Pooled within-class standardised canonical coefficients based on the areas under the parabolas which describe the inverse of the number of days until 20% vegetative bud break of the different variables of the year 2000.

Variable	Can1	Can2
Hr < 12 °C	-4.4089	-0.2841
Hr < 7 °C	1.0516	-0.1248
Infruitec chill units	0.9213	0.5932
Richardson chill units	0.3756	-0.2750
Day of collection	2.5398	0.9184

TABLE 5: Linear discriminant function for chilling based on the areas under the parabolas which describe the inverse of the number of days until 20% vegetative bud break of the different variables of the year 2000.

Variable	High	Medium	Low
Constant	-4.90859	-7.21272	-5.70239
Hr < 12 °C	0.09075	-0.01028	0.21444
Hr < 7 °C	0.13134	0.18782	0.04162
Infruitec chill units	-0.17398	-0.12178	-0.20566
Richradson chill units	0.03486	0.03702	0.02081
Day of collection	0.57392	1.39267	-0.11530

TABLE 6: Number of observations and percent classified into chilling based on the areas under the parabolas which describe the inverse of the number of days until 20% vegetative bud break of the different variables of the year 2000.

From Chilling	High	Medium	Low	Total
High	5	1[A]	0	6
%	83.33	16.67	0.00	100.00
Medium	0	1	2[D]	3
%	0.00	33.33	66.67	100.00
Low	9[B]	1[C]	11	21
%	42.86	4.76	52.38	100.00
Total	14	3	13	30
%	46.67	10.00	43.33	100.00
Priors	0.33333	0.33333	0.33333	

[A] Selection: F

[B] Selections: Ad, Af

[C] Selections: Ab, B, J, K, M, N, S, T, Z

[D] Selection: C

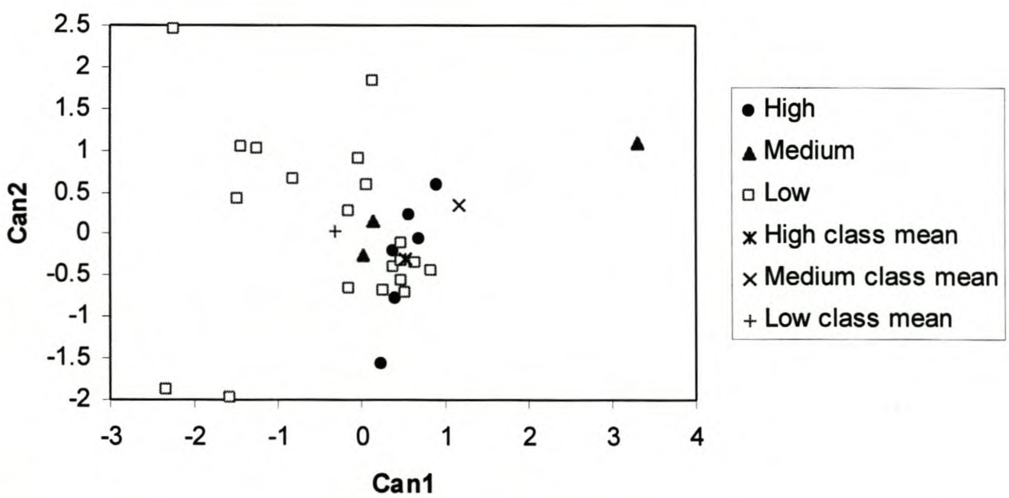

FIGURE 2: Discrimination between high, medium and low chilling requirement selections, using the area below the visual presentation of the polynomial equation, of the second degree, of the inverse of time until 20% vegetative bud break of the 2000 data in a canonical discriminant analysis.

TABLE 7: Pooled within-class standardised canonical coefficients based on the areas under the parabolas which describe the number of days until 20% reproductive bud break of the different variables of the year 2000.

Variable	Can1	Can2
Hr < 12 °C	4.6299	-2.9821
Hr < 7 °C	-1.0364	0.4350
Infruitec chill units	-0.3554	3.2173
Richardson chill units	-0.8509	-0.6524
Day of collection	-2.2118	0.2506

TABLE 8: Linear discriminant function for chilling based on the areas under the parabolas which describe the number of days until 20% reproductive bud break of the different variables of the year 2000.

Variable	High	Medium	Low
Constant	-6.27068	-5.43890	-3.28758
Hr < 12 °C	-0.00035	-0.00048	-0.00124
Hr < 7 °C	0.00032	0.00040	0.00083
Infruitec chill units	0.00056	0.00051	0.00068
Richardson chill units	-0.00015	-0.00011	6.82211E-6
Day of collection	0.00433	0.00510	0.00859

TABLE 9: Number of observations and percent classified into chilling based on the areas under the parabolas which describe the number of days until 20% reproductive bud break of the different variables of the year 2000.

From Chilling	High	Medium	Low	Total
High	2	4[A]	0	6
%	33.33	66.67	0.00	100.00
Medium	0	2	0	2
%	0.00	100.00	0.00	100.00
Low	0	5[B]	17	22
%	0.00	22.73	77.27	100.00
Total	2	11	17	30
%	6.67	36.67	56.67	100.00
Priors	0.33333	0.33333	0.33333	

[A] Selections: F, I, P, Q

[B] Selections: B, J, K, N, T

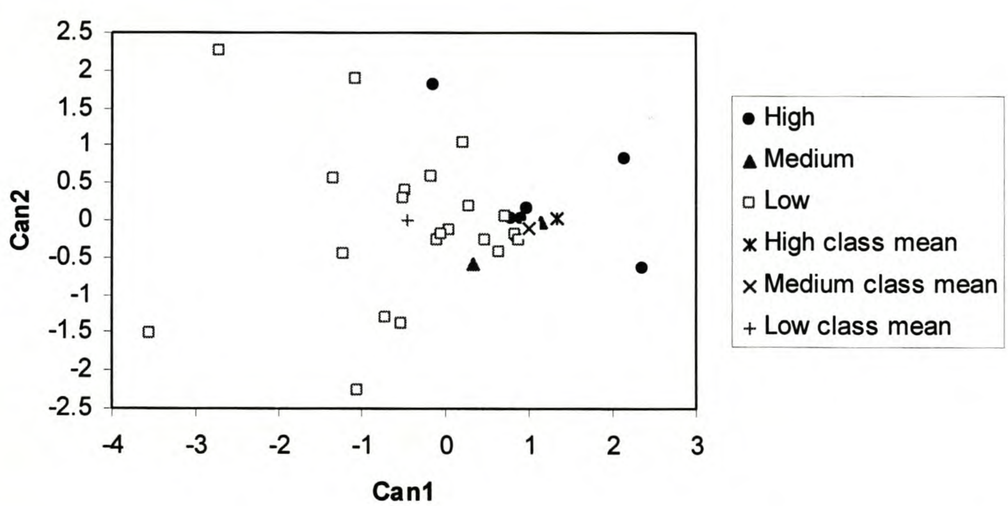

FIGURE 3: Discrimination between high, medium and low chilling requirement selections, using the area below the visual presentation of the polynomial equation, of the second degree, of the time until 20% reproductive bud break of the 2000 data in a canonical discriminant analysis.

TABLE 10: Pooled within-class standardised canonical coefficients based on the areas under the parabolas which describe the inverse of the number of days until 20% reproductive bud break of the different variables of the year 2000.

Variable	Can1	Can2
Hr < 12 °C	-17.7585	-3.6326
Hr < 7 °C	10.0145	-2.3172
Infruitec chill units	-0.6185	3.5192
Richardson chil units	0.6528	-0.4358
Day of collection	7.6568	3.5947

TABLE 11: Linear discriminant function for chilling based on the areas under the parabolas which describe the inverse of the number of days until 20% reproductive bud break of the different variables of the year 2000.

Variable	High	Medium	Low
Constant	-0.61371	-5.80220	-1.38698
Hr < 12 °C	-0.09039	-0.34489	-0.02348
Hr < 7 °C	0.10243	0.38655	-0.06309
Infruitec chill units	0.00196	0.04245	0.04301
Richardson chill units	0.00321	0.00886	-0.00385
Day of collection	0.71342	2.43882	0.47423

TABLE 12 : Number of observations and percent classified into chilling based on the areas under the parabolas which describe the inverse of the number of days until 20% reproductive bud break of the different variables of the year 2000.

From Chilling	High	Medium	Low	Total
High	6	0	0	6
%	100.00	0.00	0.00	100.00
Medium	1[A]	1	0	2
%	50.00	50.00	0.00	100.00
Low	11[B]	1[C]	10	22
%	50.00	4.55	45.45	100.00
Total	18	2	10	30
%	60.00	6.67	33.33	100.00
Priors	0.33333	0.33333	0.33333	

[A] Selection: Af

[B] Selections: A, B, E, J, K, M, N, O, T, U, W

[C] Selection: Ab

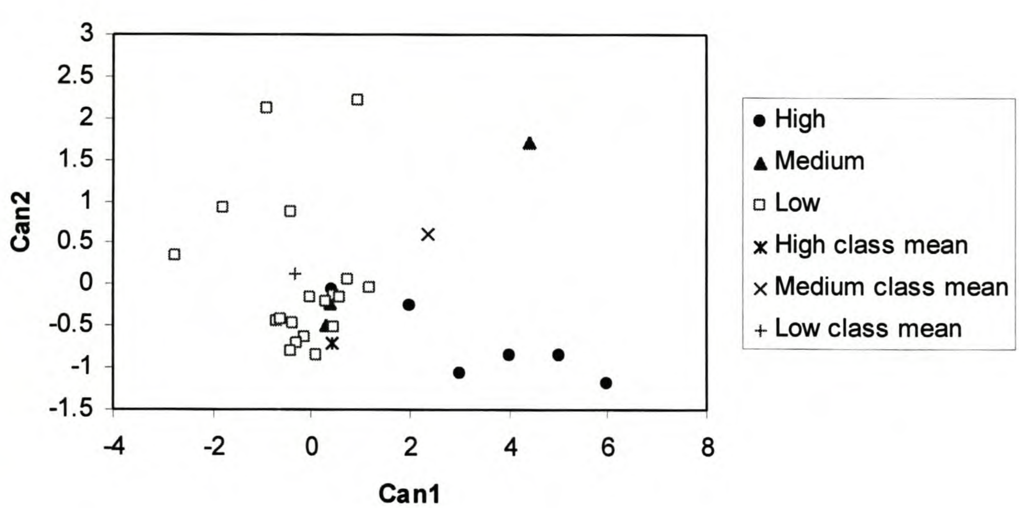

FIGURE 4: Discrimination between high, medium and low chilling requirement selections, using the area below the visual presentation of the polynomial equation, of the second degree, of the inverse of time until 20% reproductive bud break of the 2000 data in a canonical discriminant analysis.

TABLE 13: Pooled within-class standardised canonical coefficients based on the areas under the parabolas which describe the number of days until 20% vegetative bud break of the different variables of the year 2001.

Variable	Can1	Can2
Hr < 12 °C	3.6252	-0.3960
Hr < 7 °C	-1.2453	-2.5118
Infruitec chill units	2.3263	-2.1346
Richardson chill units	-0.3468	3.8714
Day of collection	-3.8736	1.2432

TABLE 14: Linear discriminant function for chilling based on the areas under the parabolas which describe the number of days until 20% vegetative bud break of the different variables of the year 2001.

Variable	High	Medium	Low
Constant	-22.23178	-19.84216	-9.83716
Hr < 12 °C	0.00435	0.00380	0.00201
Hr < 7 °C	-0.00582	-0.00805	-0.00375
Infruitec chill units	0.01955	0.01761	0.01611
Richardson chill units	-0.01618	-0.01304	-0.01479
Day of collection	-0.04966	-0.04121	-0.02381

TABLE 15: Number of observations and percent classified into chilling based on the areas under the parabolas which describe the number of days until 20% vegetative bud break of the different variables of the year 2001.

From Chilling	High	Medium	Low	Total
High	4	2[A]	0	6
%	66.67	33.33	0.00	100.00
Medium	0	3	0	3
%	0.00	100.00	0.00	100.00
Low	1[B]	0	21	22
%	4.55	0.00	95.45	100.00
Total	5	5	21	31
%	16.13	16.13	67.74	100.00
Priors	0.33333	0.33333	0.33333	

[A] Selections: L, X

[B] Selection: J

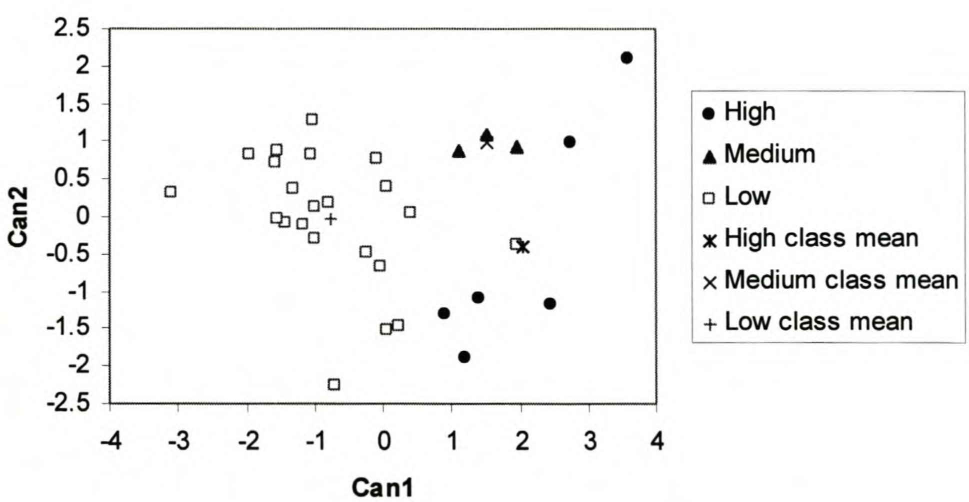

FIGURE 5: Discrimination between high, medium and low chilling requirement selections, using the area below the visual presentation of the polynomial equation, of the second degree, of the time until 20% vegetative bud break of the 2001 data in a canonical discriminant analysis.

TABLE 16: Pooled within-class standardised canonical coefficients based on the areas under the parabolas which describe the inverse of the number of days until 20% vegetative bud break of the different variables of the year 2001.

Variable	Can1	Can2
Hr < 12 °C	4.5199	1.9063
Hr < 7 °C	1.1184	0.2833
Infruitec chill units	0.9888	-24.4879
Richardson chill units	-4.7248	20.9423
Day of collection	-1.0843	1.8684

TABLE 17: Linear discriminant function for chilling based on the areas under the parabolas which describe the inverse of the number of days until 20% vegetative bud break of the different variables of the year 2001.

Variable	High	Medium	Low
Constant	-2.32642	-2.80501	-7.16000
Hr < 12 °C	0.17560	0.16317	0.36317
Hr < 7 °C	0.53439	0.54218	0.77232
Infruitec chill units	-1.00788	0.05911	-0.59686
Richardson chill units	0.49771	-0.53392	-0.20277
Day of collection	0.94973	0.19280	0.07035

TABLE 18: Number of observations and percent classified into chilling based on the areas under the parabolas which describe the inverse of the number of days until 20% vegetative bud break of the different variables of the year 2001.

From Chilling	High	Medium	Low	Total
High	3	2[A]	0	5
%	60.00	40.00	0.00	100.00
Medium	0	3	0	3
%	0.00	100.00	0.00	100.00
Low	2[B]	5[C]	15	22
%	9.09	22.73	68.18	100.00
Total	5	10	15	30
%	16.67	33.33	50.00	100.00
Priors	0.33333	0.33333	0.33333	

[A] Selections: F, X

[B] Selections: D, J

[C] Selections: Ab, Ac, K, O, S

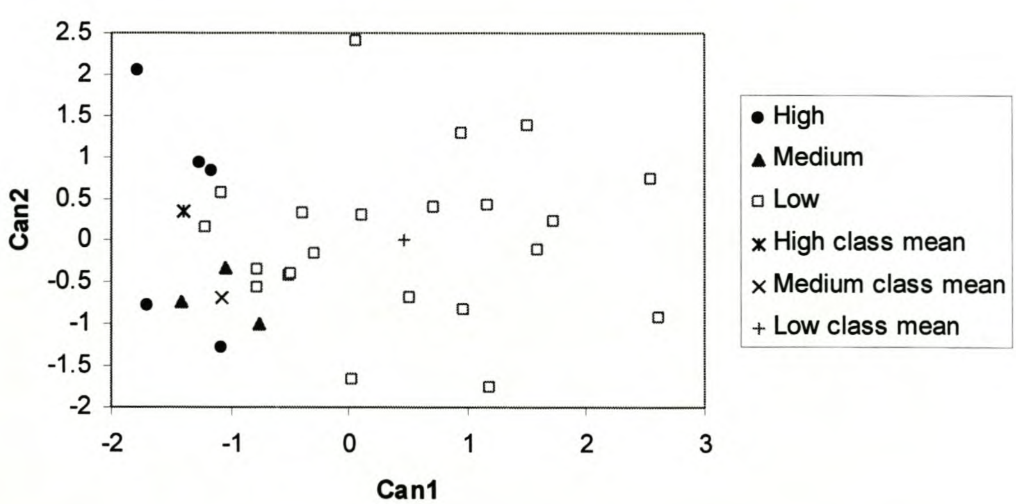

FIGURE 6: Discrimination between high, medium and low chilling requirement selections, using the area below the visual presentation of the polynomial equation, of the second degree, of the inverse of time until 20% vegetative bud break of the 2001 data in a canonical discriminant analysis.

TABLE 19: Pooled within-class standardised canonical coefficients based on the areas under the parabolas which describe the number of days until 20% reproductive bud break of the different variables of the year 2001.

Variable	Can1	Can2
Hr < 12 °C	0.0197	-1.9158
Hr < 7 °C	-2.8172	-3.2229
Infruitec chill units	3.3002	3.6389
Richardson chill units	-0.0242	0.1328
Day of collection	-0.2969	0.2292

TABLE 20: Linear discriminant function for chilling based on the areas under the parabolas which describe the number of days until 20% reproductive bud break of the different variables of the year 2001 .

Variable	High	Medium	Low
Constant	-24.66553	-33.88750	-13.85829
Hr < 12 °C	0.00190	0.00102	0.00117
Hr < 7 °C	0.00018	-0.00596	-0.00052
Infruitec chill units	-0.00119	0.00436	-0.00062
Richardson chill units	0.00034	0.00037	0.00036
Day of collection	0.00354	0.00263	0.00531

TABLE 21: Number of observations and percent classified into chilling based on the areas under the parabolas which describe the number of days until 20% reproductive bud break of the different variables of the year 2001.

From Chilling	High	Medium	Low	Total
High	2	0	0	2
%	100.00	0.00	0.00	100.00
Medium	1[A]	1	0	2
%	50.00	50.00	0.00	100.00
Low	3[B]	1[C]	17	21
%	14.29	4.76	80.95	100.00
Total	6	2	17	25
%	24.0	8.00	68.00	100.00
Priors	0.33333	0.33333	0.33333	

[A] Selection: Af

[B] Selections: Ac, D, T

[C] Selection: J

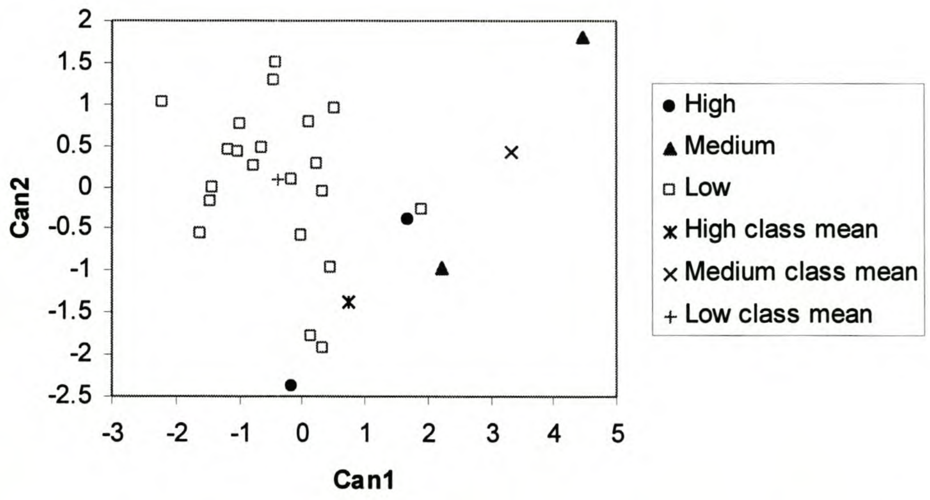

FIGURE 7: Discrimination between high, medium and low chilling requirement selections, using the area below the visual presentation of the polynomial equation, of the second degree, of the time until 20% reproductive bud break of the 2001 data in a canonical discriminant analysis.

TABLE 22: Pooled within-class standardised canonical coefficients based on the areas under the parabolas which describe the inverse of the number of days until 20% reproductive bud break of the different variables of the year 2001.

Variable	Can1	Can2
Hr < 12 °C	-11.9472	-8.1819
Hr < 7 °C	1.0779	1.8671
Infruitec chill units	6.3556	5.0284
Richardson chill units	0.7189	0.1062
Day of collection	4.0801	2.5567

TABLE 23: Linear discriminant function for chilling based on the areas under the parabolas which describe the inverse of the number of days until 20% reproductive bud break of the different variables of the year 2001 .

Variable	High	Medium	Low
Constant	-2.07164	-0.74413	-3.32712
Hr < 12 °C	-0.11215	0.03492	0.44515
Hr < 7 °C	0.54601	0.36903	0.23226
Infruitec chill units	0.13205	-0.03713	-0.43182
Richardson chill units	-0.03984	-0.04291	-0.09591
Day of collection	-0.09054	-0.57228	-2.06932

TABLE 24: Number of observations and percent classified into chilling based on the areas under the parabolas which describe the inverse of the number of days until 20% reproductive bud break of the different variables of the year 2001.

From Chilling	High	Medium	Low	Total
High	1	1[A]	0	2
%	50.00	50.00	0.00	100.00
Medium	0	2	0	2
%	0.00	100.00	0.00	100.00
Low	1[B]	3[C]	17	21
%	4.76	14.29	80.95	100.00
Total	2	6	17	25
%	8.00	24.00	68.00	100.00
Priors	0.33333	0.33333	0.33333	

[A] Selection: F

[B] Selection: O

[C] Selections: M, N, S

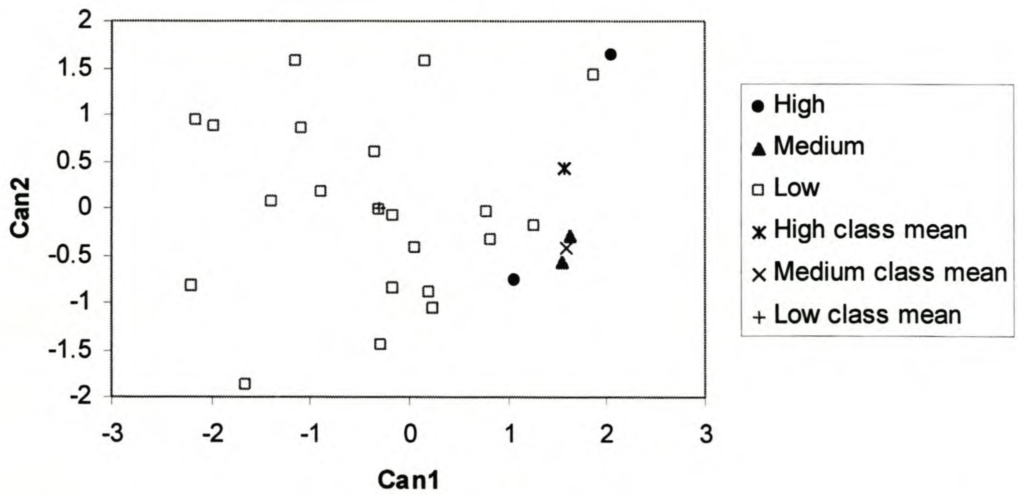

FIGURE 8: Discrimination between high, medium and low chilling requirement selections, using the area below the visual presentation of the polynomial equation, of the second degree, of the inverse of time until 20% reproductive bud break of the 2001 data in a canonical discriminant analysis.

3. PAPER 2: A STUDY ON DORMANCY AND CHILLING REQUIREMENT OF PEACH AND NECTARINE SHOOTS GIVEN ARTIFICIAL CHILLING IN A COLD ROOM

ABSTRACT

On 16 May 2000 and 15 May 2001, 100 one-year-old shoots of 30 peach and nectarine selections were harvested from an evaluation orchard on Bien Donné Experiment Farm, Simondium, Western Cape (34° S), covered in wet paper towelling and black plastic bags and placed in a cold room kept at a temperature range between 4° C and 7° C. All selections were previously categorised as high (>800 Utah chilling units [CU]), medium (400-800 CU) or low (<400 CU) chilling requirement based on phenologic observations. According to the preliminary classification of the selections included in this study, six selections were classified as high, three as medium and 21 as low chilling requirement. Two replicate bundles of 10 shoots of each selection were prepared fortnightly and forced at 25° C with continuous illumination until no further changes in bud burst occurred for a period of five days after which the shoots were then discarded. CU accumulated in the cold room at each transferral date, was calculated according to the Utah model. For each selection the number of days until 20% vegetative bud break was plotted over Utah CU, and expressed as a parabolic function. Similarly, the inverse of the number of days until 20% bud break or the rate of bud growth, was also plotted against the above variables. The area under these parabolas was statistically analysed using the CANDISC procedure of SAS Release 8.1. The groupings of the CANDISC procedure were more or less consistent with the preliminary groupings obtained with the phenoligical classification method. The boundaries given to the low, medium and high CR groupings artificially facilitate the classification of cultivars by CR. In reality some selections can be close to a boundary separating the groups, and displaying traits of both groups can be misclassified. It can be concluded that the inherent CR of the selections, as determined by their genetic make-up, is still expressed even under conditions of maximum CU accumulation, as it was still possible to group the selections according to CR under these conditions.

Keywords: breeding, chilling requirement, dormancy, nectarine, peach

INTRODUCTION

Most South African peach and nectarine production areas lack of adequate winter chilling (Linsley-Noakes, 1995). Consequently, only medium (400 - 800 Richardson/Utah chilling units [CU]) to low (<400 CU) chilling requirement cultivars can be produced with any measure of success. To address these issues, a conventional breeding programme was started, to develop new and improved stone fruit cultivars with special emphasis on climatic adaptation and pomological attributes. To achieve this goal it is necessary to accurately determine the chilling requirement of these new selections.

This study was conducted to test the reaction of selections, classified according to phenological observations, into high (>800 chilling units CU), medium (400 to 800 CU) and low (<400 CU) chilling requirement (CR) to being chilled artificially, at known increments, until a maximum of CU is accumulated.

MATERIALS AND METHODS

Plant material

The same as set out in Paper 1.

Methodology

Except for the following aspects, the methods used were the same as set out in Paper 1. Starting on 16 May 2000 and on 15 May 2001 (when shoot extension had ceased in all included selections) 100 shoots of each selection were cut, covered in wet paper towelling and black plastic bags and placed in a cold room kept at a temperature range between 4 and 7° C. Twenty shoots of each selection were taken out of the cold room two weeks later and fortnightly thereafter, until all shoots were transferred to forcing conditions. As the cold room was kept at a temperature range between 4 and 7° C, only the Richardson model (Richardson et al., 1974) was used to calculate CU as the Infruitec model would have given the same outcome under these circumstances (Linsley-Noakes et al., 1994).

Data analysis

Occasionally bud burst occurred on fewer than five of the ten shoots per bundle. To avoid missing values, the time to 20% bud break was calculated as the time taken for bud burst to occur on two shoots per bundle, i.e. days to 20% bud burst. For each selection the number of days until 20% vegetative bud break was plotted over Utah CU, and expressed as a parabolic function. The inverse of the number of days until 20% bud break, expressing the relationship between bud growth following different periods of chilling (Cannell, 1989), was also plotted against Richardson CU. The area under the parabolas was statistically analysed using the CANDISC (Canonical Discriminant Analysis) procedure of SAS Release 8.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Days until 20% vegetative bud break 2000

The eigenvalues, as used in the CANDISC procedure, were λ_1 = 0.3821 and λ_2 = 0.0837. The first eigenvalue accounted for a high proportion of the total: $\lambda_1 / (\lambda_1 + \lambda_2) = 0.8203$. The mean vectors were, thus, largely in one dimension and one discriminant function sufficed to describe 82.03% of the separation among the three groups. Therefore, the first pooled within-class standardised canonical coefficients (Can 1), presented in Table 1, were taken into consideration, to determine which variable contributed most to separating the groups. Because only one model was used to express CU, the quadratic regression parameters were used as variables throughout: Variable A = the intercept, variable B = the coefficient of the linear term and variable C = the coefficient of the quadratic term. Thus variable B contributed most to separating the groups (Can 1 = 17.3475, while the second highest contribution was made by variable C: Can 1 = 12.5490). The linear discriminant function for chilling, as used in this analysis to classify the selections according to CR and which describes the groups, is presented in Table 2. The CANDISC procedure misclassified three high CR selections (F, I and P) into the medium CR group, and one (selection Q) into the low CR group. Five low CR selections (B, D, E, J and K) were misclassified into the high CR category and 2 (Ac and G) into the medium category. One medium CR selection (Ag) was misclassified into the high grouping. Overall a 62.5% correct classification was achieved (Table 3). Figure 1 presents the grouping of the selections into classes, according to CR, and the class means are indicated.

Inverse of days until 20% vegetative bud break 2000

The eigenvalues were λ_1 = 0.4217 and λ_2 = 0.0204 and one discriminant function described 95.39% of the separation among the three groups. Therefore, the first pooled within-class standardised canonical coefficients (Can 1), presented in Table 4, indicates that variable A contributed most to separating the groups (Can 1 = -4.2965, while the second highest contribution was made by variable B: Can 1 = -3.7285). The linear discriminant function for chilling, as used in this analysis to classify the selections according to CR and which describes the groups, is presented in Table 5. Three high CR selections (selections F, I and P) were misclassified into the medium CR group, and one (selection Q) into the low CR group. Five low CR selections (selections B, D, G, H and K), including three of the five selections that were misclassified using the days until 20% vegetative bud break, were misclassified into the high CR category and three (selections Ac, E and J) into the medium category. One medium CR selection (selection Ag) was misclassified into the high grouping. Overall a 59.38% correct classification was achieved (Table 6; Figure2). When the CANDISC procedure was performed on the inverse of days until 20% vegetative bud break (rate of growth to bud burst) it gave a less accurate classification (only 59.38%

correct) of the selections compared to when it was performed on the days until 20% vegetative bud break (62.50% correct).

Days until 20% vegetative bud break 2001

The proportion of the eigenvalues (λ_1 = 0.4576 and λ_2 = 0.0745; $\lambda_1 / [\lambda_1 + \lambda_2]$ = 0.8600) indicates that one discriminant function described 86.00% of the separation among the three groups. Therefore, the first pooled within-class standardised canonical coefficients (Can 1), presented in Table 7, indicates that variable B contributed most to separating the groups (Can 1 = 4.1867, with the second highest contribution made by variable A: Can 1 = 3.8120). The linear discriminant function for chilling, used to classify the selections according to CR and which describes the groups, is presented in Table 8. The CANDISC procedure misclassified two high CR selections (L and X) into the medium CR group, and two (F and Q) into the low CR group. Two low CR selections (D and J) were misclassified into the high CR category and three (Ac, B and K) into the medium category. One medium CR selection (Ag) was misclassified into the high grouping. Overall a 65.63% correct classification was achieved (Table 9; Figure 3).

Inverse of days until 20% vegetative bud break 2001

Here the eigenvalues were λ_1 = 0.4788 and λ_2 = 0.0889 and one discriminant function described 84.34% of the separation among the groups. Therefore, the first pooled within-class standardised canonical coefficients (Can 1), presented in Table 10, indicates that variable B contributed most to separating the groups (Can 1 = 1.6598, with the second highest contribution made by variable A: Can 1 = 1.5260). The linear discriminant function for chilling, as used in this analysis to classify the selections according to CR and which describes the groups, is presented in Table 11. Two high CR selections (Q and X) were misclassified into the medium CR group, and one (F), which was also misclassified using the days until 20% vegetative bud break, was misclassified into the low CR group by the CANDISC procedure. Seven low CR selections (Ac, B, D, G, H, J and T), including the two that were misclassified using the days until 20% vegetative bud break, were misclassified into the high CR category and one (K) into the medium category.

No medium CR selection was misclassified. Overall a 64.52% correct classification was achieved (Table 12; Figure 4).

General discussion

The two weekly intervals between transferral of shoots from the cold room to forcing conditions were too long, with resultant large CU increments accumulating between transferral dates. On the first transferral date in 2000, for example, 360 CU have already accumulated, on the second date 696 CU and on the third, 1032 CU. This masked the different responses of the different genotypes to chilling. For instance on the first transferral date, enough CU have accumulated to satisfy the CR of selections in the low CR - as well as some selections in the medium CR groupings and on the third date of transferral, the CR of all the selections were met irrespective of CR grouping. This resulted in a larger number of misclassifications by the CANDISC procedure. Weekly intervals between transferrals would have given more satisfactory smaller increments of CU accumulation.

It is known that forcing shoots in this way has its limitations, with some shoots being adversely stressed despite the care taken. The misclassification of the low CR selections into the high CR grouping we therefore attribute to procedure problems rather than to the inherent CR of the selections. When looking at the numbers per se, it seems as if a large number of selections in the low CR category were misclassified, however when these numbers are expressed as a percentage of the total number of selections in the low CR category, it is quite low compared to the percentages of misclassification realised in some instances in the other CR categories.

The boundaries given to the low, medium and high CR groupings artificially facilitate the classification of cultivars by CR. In reality, the CR of these selections forms a continuum from low to high CR. Therefore, some selections can be close to a boundary separating the groups, and displaying traits of both groups can be misclassified.

Furthermore, using only two replicates increased the experimental error, which in turn led to a larger percentage of misclassification.

CONCLUSION

As an overall correct classification of 63.01% was achieved, under these conditions with the CANDISC procedure, it can be concluded that the shoots reacted similarly to artificial chilling as to temperature conditions within the range of temperatures known to cause the accumulation of CU, occurring naturally in the orchard where these shoots were collected and a relatively good classification was achieved. It can further be concluded that the inherent CR of the selections, as determined by their genetic make-up, is expressed even under conditions of maximum CU accumulation, as it was still possible to group the selections according to CR under these conditions.

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TABLE 1: Pooled within-class standardised canonical coefficients based on the areas under the parabolas which describe the number of days until 20% vegetative bud break of the different variables of the year 2000.

Variable*	Can1	Can2
A	5.4318	2.9539
B	17.3475	-0.8600
C	12.5490	-3.7106
Area	0.00000000	0.00000000

* Quadratic regression paramaters: A = intercept; B = coefficient of linear term; C = coefficient of quadratic term.

TABLE 2: Linear discriminant function for chilling based on the areas under the parabolas which describe the number of days until 20% vegetative bud break of the different variables of the year 2000.

Variable*	High	Medium	Low
Constant	-11.39266	-7.54033	-8.66046
A	1.02334	0.50140	1.62251
B	-2.47707	0.97799	-9.10966
C	-4011	-2136	-5945
Area	0.02546	0.03075	-0.00227

* Quadratic regression paramaters: A = intercept; B = coefficient of linear term; C = coefficient of quadratic term.

TABLE 3: Number of observations and percent classified into chilling based on the areas under the parabolas which describe the number of days until 20% vegetative bud break of the different variables of the year 2000.

From Chlling	High	Medium	Low	Total
High	2	3[A]	1[B]	6
%	33.33	50.00	16.67	100.00
Medium	1[C]	3	0	4
%	25.00	75.00	0.00	100.00
Low	5[D]	2[E]	15	22
%	22.73	9.09	68.18	100.00
Total	8	8	16	32
	25.00	25.00	50.00	100.00
Priors	0.33333	0.33333	0.33333	

[A]Selections: F, I, P

[B]Selection: Q

[C]Selection: Ag

[D]Selections: B, D, E, J, K

[E]Selections: Ac, G

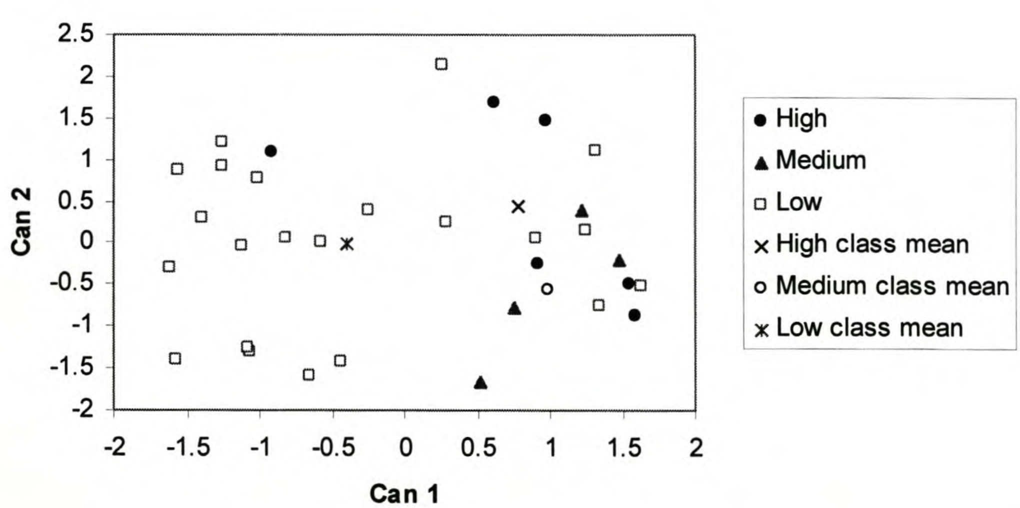

FIGURE 1: Discrimination between high, medium and low chilling requirement selections, using the area below the visual presentation of the polynomial equation, of the second degree, of the time until 20% vegetative bud break of the 2000 data in a canonical discriminant analysis.

TABLE 4: Pooled within-class standardised canonical coefficients based on the areas under the parabolas which describe the inverse of the number of days until 20% vegetative bud break of the different variables of the year 2000.

Variable*	Can1	Can2
A	-4.2965	8.4125
B	-3.7285	14.8889
C	1.2265	6.5965
Area	0.00000000	0.00000000

* Quadratic regression paramaters: A = intercept; B = coefficient of linear term; C = coefficient of quadratic term.

TABLE 5: Linear discriminant function for chilling based on the areas under the parabolas which describe the inverse of the number of days until 20% vegetative bud break of the different variables of the year 2000.

Variable*	High	Medium	Low
Constant	-5.24831	-6.99625	-5.69213
A	36.32337	41.30186	27.01860
B	216.14680	254.36196	169.73109
C	-55415	-63851	-40934
Area	1.12052	1.30321	1.11166

* Quadratic regression paramaters: A = intercept; B = coefficient of linear term; C = coefficient of quadratic term.

TABLE 6: Number of observations and percent classified into chilling based on the areas under the parabolas which describe the inverse of the number of days until 20% vegetative bud break of the different variables of the year 2000.

From Chlling	High	Medium	Low	Total
High	2	3[A]	1[B]	6
%	33.33	50.00	16.67	100.00
Medium	1[C]	3	0	4
%	25.00	75.00	0.00	100.00
Low	5[D]	3[E]	14	22
%	22.73	13.64	63.64	100.00
Total	8	9	15	32
	25.00	28.13	46.88	100.00
Priors	0.33333	0.33333	0.33333	

[A]Selections: F, I, P

[B]Selection: Q

[C]Selection: Ag

[D]Selections: B, D, G, H, K

[E]Selections: Ac, E, J

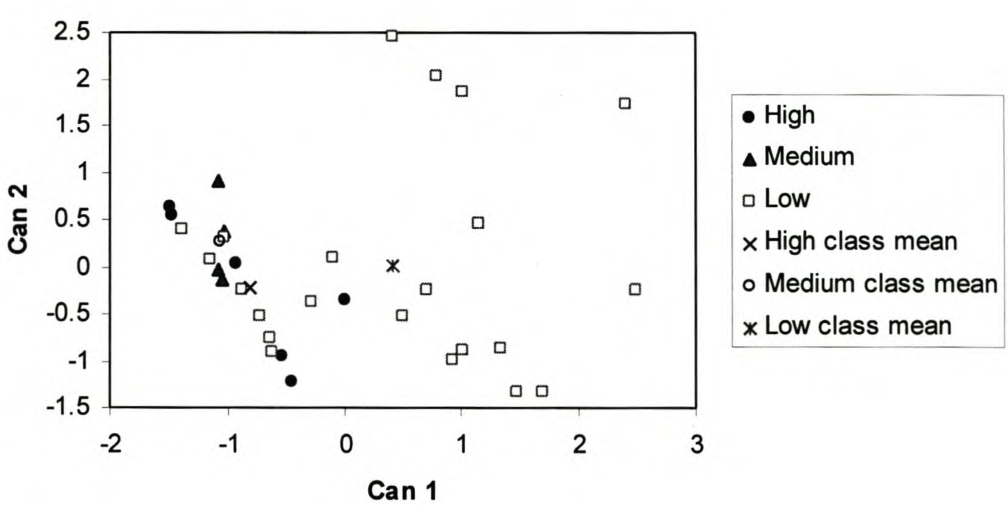

FIGURE 2: Discrimination between high, medium and low CR selections, using the area below the visual presentation of the polynomial equation, of the second degree, of the inverse of time until 20% vegetative bud break of the 2000 data in a canonical discriminant analysis.

TABLE 7: Pooled within-class standardised canonical coefficients based on the areas under the parabolas which describe the number of days until 20% vegetative bud break of the different variables of the year 2001.

Variable*	Can1	Can2
A	3.8120	3.7622
B	4.1867	6.3035
C	1.2196	2.0468
Area	0.000000000	0.000000000

* Quadratic regression paramaters: A = intercept; B = coefficient of linear term; C = coefficient of quadratic term.

TABLE 8: Linear discriminant function for chilling based on the areas under the parabolas which describe the number of days until 20% vegetative bud break of the different variables of the year 2001.

Variable*	High	Medium	Low
Constant	-8.00689	-10.10030	-3.98357
A	0.19412	0.25699	0.08397
B	1.52193	-0.36077	1.18374
C	-676.26870	-600.19495	-275.14123
Area	0.03430	0.03016	0.02457

* Quadratic regression paramaters: A = intercept; B = coefficient of linear term; C = coefficient of quadratic term.

TABLE 9: Number of observations and percent classified into chilling based on the areas under the parabolas which describe the number of days until 20% vegetative bud break of the different variables of the year 2001.

From Chlling	High	Medium	Low	Total
High	2	2[A]	2[B]	6
%	33.33	33.33	33.33	100.00
Medium	1[C]	2	0	3
%	33.33	66.67	0.00	100.00
Low	2[D]	3[E]	17	22
%	9.09	13.64	77.27	100.00
Total	5	7	19	31
	16.13	22.58	61.29	100.00
Priors	0.33333	0.33333	0.33333	

[A]Selections: L, X

[B]Selection: F, Q

[C]Selection: Ag

[D]Selections: D, J

[E]Selections: Ac, B, K

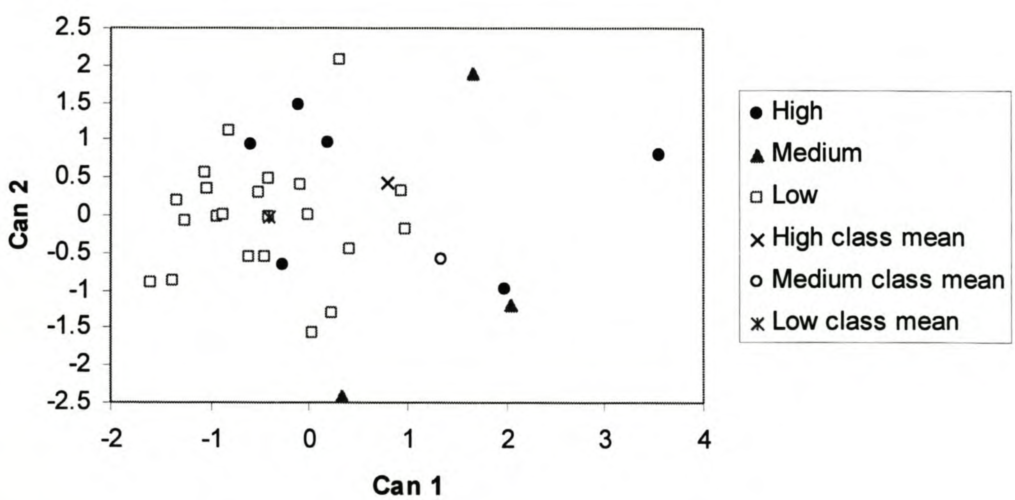

FIGURE 3: Discrimination between high, medium and low chilling requirement selections, using the area below the visual presentation of the polynomial equation, of the second degree, of the time until 20% vegetative bud break of the 2001 data in a canonical discriminant analysis.

TABLE 10: Pooled within-class standardised canonical coefficients based on the areas under the parabolas which describe the inverse of the number of days until 20% vegetative bud break of the different variables of the year 2001.

Variable*	Can1	Can2
A	1.5260	-0.2344
B	1.6598	1.9873
C	0.4122	1.3667
Area	0.000000000	0.000000000

* Quadratic regression paramaters: A = intercept; B = coefficient of linear term; C = coefficient of quadratic term.

TABLE 11: Linear discriminant function for chilling based on the areas under the parabolas which describe the inverse of the number of days until 20% vegetative bud break of the different variables of the year 2001.

Variable*	High	Medium	Low
Constant	-4.68605	-4.50684	-7.75511
A	9.73185	-38.42409	20.30030
B	196.75582	167.34885	311.40604
C	337.81956	12613	-6281
Area	0.86991	0.84494	1.07446

* Quadratic regression paramaters: A = intercept; B = coefficient of linear term; C = coefficient of quadratic term.

TABLE 12: Number of observations and percent classified into chilling based on the areas under the parabolas which describe the inverse of the number of days until 20% vegetative bud break of the different variables of the year 2001.

From Chlling	High	Medium	Low	Total
High	3	2[A]	1[B]	6
%	50.00	33.33	16.67	100.00
Medium	0	3	0	3
%	0.00	100.00	0.00	100.00
Low	7[C]	1[D]	14	22
%	31.82	4.55	63.64	100.00
Total	10	6	15	31
	32.26	19.35	48.39	100.00
Priors	0.33333	0.33333	0.33333	

[A]Selections: Q, X [C]Selections: Ac, B, D, G, H, J, T

[B]Selection: F [D]Selections: K

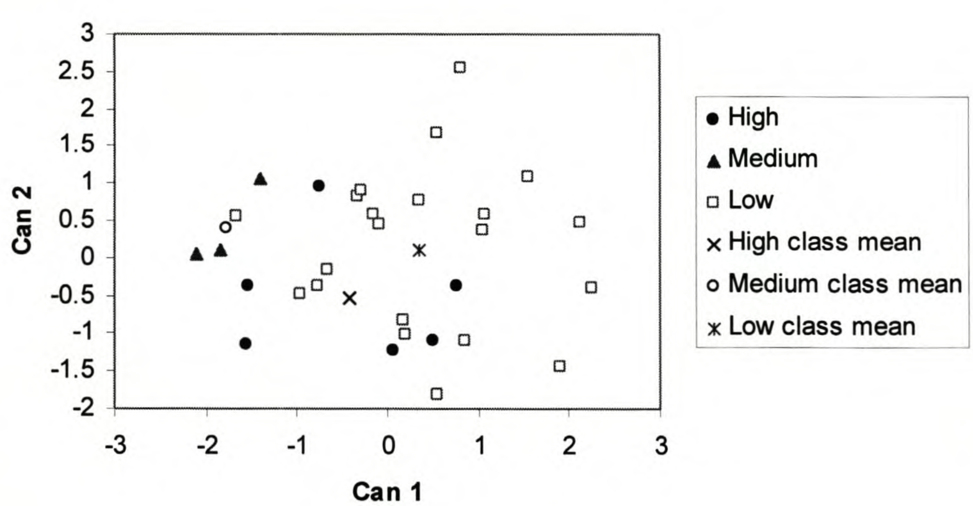

FIGURE 4: Discrimination between high, medium and low CR selections, using the area below the visual presentation of the polynomial equation, of the second degree, of the inverse of time until 20% vegetative bud break of the 2001 data in a canonical discriminant analysis.

4. PAPER 3: A STUDY ON DORMANCY AND CHILLING REQUIREMENT OF PEACHES AND NECTARINES USING ONE YEAR'S DATA AS CALIBRATION DATA

ABSTRACT

The same plant material and methods were used as set out in Paper 1. The area under the parabolas was statistically analysed using the CANDISC procedure of SAS Release 8.1. The outcome of the analysis of one season's data was used as calibration data against which the other season's data was tested and the consistency of the results, using one set of discriminant functions was tested. It can be concluded that a unique set of discriminant functions is necessary for each winter season to accurately classify selections according to CR with the CANDISC procedure. This explains the poor reclassification achieved when the discriminant functions of the CANDISC procedure of one season are used as calibration data to test the data of the other season against.

Keywords: breeding, chilling requirement, dormancy, nectarine, peach

INTRODUCTION

Most South African peach and nectarine production areas lack of adequate winter chilling (Linsley-Noakes, 1995). Consequently, only medium (400 - 800 Richardson/Utah chilling units [CU]) to low (<400 CU) chilling requirement cultivars can be produced with any measure of success. To address these issues, a conventional breeding programme was started, to develop new and improved stone fruit cultivars with special emphasis on climatic adaptation and pomological attributes. To achieve this goal it is necessary to accurately determine the chilling requirement of these new selections.

This study was conducted to test whether the discriminant functions used by the CANDISC procedure (Canonical Discriminant Analysis) to discriminate between high (>800 chilling units [CU]), medium (400 to 800 CU) and low (<400 CU) CR selections, bred by the breeding programme, could be used unaltered from season to season, with similar results.

MATERIALS AND METHODS

Plant material

As set out in Paper 1.

Method

As set out in Paper 1.

Data analysis

Occasionally bud burst occurred on fewer than five of the ten shoots per bundle. To avoid missing values, the time to 20% bud break was calculated as the time taken for bud burst to occur on two shoots per bundle, i.e. days to 20% bud burst. For each selection the number of days until 20% vegetative and reproductive bud break was plotted over day of year, Utah CU, Infruitec CU, hours below 12^{o} C, and hours below 7^{o} C, and expressed as a parabolic function. Similarly, the inverse of the number of days until 20% bud break or the rate of bud growth (Cannell, 1989), was also plotted against all the above variables. The area under these parabolas was statistically analysed using the CANDISC (Canonical Discriminant Analysis) procedure of SAS Release 8.1 (SAS Institute Inc., Cary, NC, USA).

The outcome of the analysis of one season's data was used as calibration data against which the other season's data was tested. This was done by classifying the data of the second season according to the discriminant functions generated from the data obtained in the first season. The consistency of the results, using one set of discriminant functions is reported on in this paper.

RESULTS AND DISCUSSION

Days until 20% vegetative bud break

From the comparative table of pooled within-class standardised canonical coefficients (Table 1), it is clear that in the two different seasons reported on different variables (the quadratic regression parameters of each chilling model were used as variables throughout: Variable A = the intercept, variable B = the coefficient of the linear term and variable C = the coefficient of the quadratic term) contributed most to discriminating between selections. For instance when the 2001 season's data was used as calibration data, based on the number of hours below 7^{o} C, variable B contributed most in separating the selections whereas if the 2000 season's data was used as calibration data, variable A made the biggest contribution. Also, different variables made the biggest contributions in the two different seasons when the data based on Infruitec and Richardson CU is compared between seasons. This is an indication that different discriminant functions are used in the two different seasons to discriminate between selections.

When the CANDISC procedure was performed on the 2000 season's data, with the discriminant functions of the CANDISC procedure of the 2001 season, in other words tested against the outcome of the CANDISC procedure of the 2001 season, as calibration data, only a 27.59% correct classification, overall was achieved (Table 2). When the CANDISC procedure was performed on the data of the 2001 season as a test against the outcome of the CANDISC procedure of the 2000 season as calibration data, a 60.00% correct classification, overall was achieved (Table 3).

Inverse of days until 20% vegetative bud break

When the comparative table of pooled within-class standardised canonical coefficients (Table 4) is studied, it is clear again that in the two different seasons reported on, different variables contributed most to discriminating between selections. For instance when the 2001 season's data was used as calibration data, based on the number of hours below 7^{o} C, variable A contributed most in separating the selections whereas when the 2000 season's data was used as calibration data, variable B made the biggest contribution. Also, different variables made the biggest contribution in the two different seasons when the data based on Collection day and Richardson CU is compared between seasons. Again, this is an indication that different discriminant functions are used in the two different seasons to discriminate between selections.

When the outcome of the CANDISC procedure of the 2001 season was tested against the outcome of the CANDISC procedure of the 2000 season, as calibration data, only a 55.17% correct classification, overall was achieved (Table 5). When the outcome of the CANDISC procedure of the 2000 season was tested against the outcome of the CANDISC procedure of the 2001 season, as calibration data, only a 42.82% correct classification, overall was achieved (Table 6).

Days until 20% reproductive bud break

Once again it is clear from the comparative table of pooled within-class standardised canonical coefficients (Table 7), that in the two different seasons reported on, different variables contributed most to discriminating between selections. For instance when the 2001 season's data was used as calibration data, based on the number of Infruitec CU, variable B contributed most in separating the selections whereas if the 2000 season's data was used as calibration data, variable A made the biggest contribution. Also, different variables made the biggest contributions in the two different seasons when the data based on Richardson CU is compared between seasons. Thus once again, different discriminant functions are used in the two different seasons to discriminate between selections.

When the CANDISC procedure was performed on the 2001 season's data, with the discriminant functions of the CANDISC procedure of the 2000 season, in other words tested against the outcome of the CANDISC procedure of the 2000 season, as calibration data, only a 12.00% correct classification, overall was achieved (Table 8). When the CANDISC procedure was performed on the data of the 2000 season as a test against the outcome of the CANDISC procedure of the 2001 season as calibration data, only a 26.67% correct classification, overall was achieved (Table 9).

Inverse of days until 20% reproductive bud break

When the comparative table of pooled within-class standardised canonical coefficients (Table 10) is studied, it is clear that different variables contributed most to discriminating between selections, in the two different seasons reported on. When the 2001 season's data was used as calibration data, based on the number of Infruitec CU, variable A contributed most in separating the selections whereas when the 2000 season's data was used as calibration data, variable C made the biggest contribution. Different variables made the biggest contributions in the two different seasons when the data based on Richardson CU, Collection day and Hours below 12^{o} C is compared between seasons. It indicates that different discriminant functions are used in the two different seasons to discriminate between selections.

When the CANDISC procedure was performed on the 2001 season's data, with the discriminant functions of the CANDISC procedure of the 2000 season, as calibration data, only a 4.00% correct classification, overall was achieved (Table 11). When the CANDISC procedure was performed on the data of the 2000 season as a test against the outcome of the CANDISC procedure of the 2001 season as calibration data, a 9.68% correct classification, overall was achieved (Table 12).

CONCLUSION

It can be concluded that a unique set of discriminant functions is necessary for each winter season to accurately classify selections according to CR with the CANDISC procedure, as different variables are used in each season to discriminate between selections, as can be seen in the tables of pooled within-class standardised canonical coefficients. This can surely be attributed to the unique set of climatic conditions prevailing in each winter season, which cause a slightly different reaction in the shoots of the different selections.

This explains the poor reclassification achieved when the discriminant functions of the CANDISC procedure of one season are used as calibration data to test the data of the other season against.

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LINSLEY-NOAKES, G.C., 1995. Temperature as a factor in nectarine production in the Western Cape. Ph.D. dissertation, University of Natal.

TABLE 1: Pooled within-class standardised canonical coefficients of days until 20% vegetative bud break.

	Can 1		Can 2	
Variable*	Calibration 00	Calibration 01	Calibration 00	Calibration 01
A col.day	437.3110	1009.4202	-366.3341	99.9994
B col. day	939.6449	1642.5031	-619.8455	186.9576
C col. day	502.9116	638.5891	-255.3551	74.3496
Area col. day	0.0000	0.0000	0.0000	0.0000
A Hr < 7	-67.0879	34.1046	-92.8428	-23.5941
B Hr < 7	-47.4554	98.8253	-149.0634	-40.6079
C Hr < 7	8.7624	71.1364	-64.1478	-23.0608
Area Hr < 7	0.0000	-0.6453	0.0000	0.3886
A Hr < 12	-57.2259	-163.0275	132.1440	4.8116
B Hr < 12	-206.6887	-128.3572	183.6999	10.5318
C Hr < 12	-147.8748	14.3619	57.3346	33.6176
Area Hr < 12	0.0000	-0.8814	0.0000	-0.0008
A Infr CU	40.9312	35.6459	27.6842	-34.5362
B Infr CU	74.7389	-20.1046	64.0795	-56.1191
C Infr CU	39.3781	-49.3377	41.3799	-46.5684
Area Infr CU	0.0000	0.5506	0.0000	-0.3501
A Rich CU	10.6128	-10.1815	-1.9662	30.5582
B Rich CU	-3.1025	-15.2371	-0.1500	34.3940
C Rich CU	12.1325	-6.5738	-1.0811	21.5342
Area Rich CU	0.0000	-0.4566	0.0000	-0.4534

*Quadratic regression paramaters: A = intercept; B = coefficient of linear term; C = coefficient of quadratic term, col. day = collection day, Hr < 7 = Hours below 7^{o} C, Hr < 12 = Hours below 12^{o} C, Infr CU = Infruitec chilling units, Rich CU = Utah chilling units.

TABLE 2: Number of observations and percent classified into chilling when the discriminant functions of the 2001 season were used as calibration functions (days until 20% vegetative bud break).

From Chilling	High	Medium	Low	Total
Calibration				
High 01	6	0	0	6
%	100.00	0.00	0.00	100.00
Test				
High 00	6	0	0	6
%	100.00	0.00	0.00	100.00
Calibration				
Medium 01	0	3	0	3
%	0.00	100.00	0.00	100.00
Test				
Medium 00	2	0	0	2
%	100.00	0.00	0.00	100.00
Calibration				
Low 01	1	1	19	21
%	4.76	4.76	90.48	100.00
Test				
Low 00	9	10	2	21
%	42.86	47.62	9.52	100.00
Calibration				
Total 01	7	4	19	30
%	23.33	13.33	63.33	100.00
Test				
Total 00	17	10	2	29
%	58.62	34.48	6.90	100.00
Priors	0.33333	0.33333	0.33333	

TABLE 3: Number of observations and percent classified into chilling when the discriminant functions of the 2000 season were used as calibration functions (days until 20% vegetative bud break).

From Chilling	High	Medium	Low	Total
Calibration				
High 00	5	1	0	6
%	83.33	16.67	0.00	100.00
Test				
High 01	3	1	2	6
%	50.00	16.67	33.33	100.00
Calibration				
Medium 00	0	2	0	2
%	0.00	100.00	0.00	100.00
Test				
Medium 01	0	0	3	3
%	0.00	0.00	100.00	100.00
Calibration				
Low 00	0	3	18	21
%	0.00	14.29	85.71	100.00
Test				
Low 01	5	1	15	21
%	23.81	4.76	71.43	100.00
Calibration				
Total 00	5	6	18	29
%	17.24	20.69	62.07	100.00
Test				
Total 01	8	2	20	30
%	26.67	6.67	66.67	100.00
Priors	0.33333	0.33333	0.33333	

TABLE 4: Pooled within-class standardised canonical coefficients of the inverse of days until 20% vegetative bud break).

	Can 1		Can 2	
Variable*	Calibration 00	Calibration 01	Calibration 00	Calibration 01
A col.day	-763.29006	641.3566	94.0878	749.9111
B col. day	-1513.5666	1153.7361	100.4153	1468.3108
C col. day	-771.3235	507.9105	12.2028	704.5974
Area col. day	0.0000	0.0000	0.0000	0.0000
A Hr < 7	-72.0664	-3.7380	27.1753	-19.7159
B Hr < 7	-78.8169	-1.4293	46.8198	-2.7292
C Hr < 7	-19.9918	0.2782	25.9530	7.9244
Area Hr < 7	0.0000	0.0000	0.0000	0.0000
A Hr < 12	234.4698	6.7231	-27.9453	-28.9883
B Hr < 12	409.2688	45.2213	16.0340	-42.3240
C Hr < 12	211.3250	47.4556	28.7360	31.4539
Area Hr < 12	0.0000	0.0000	0.0000	0.0000
A Infr CU	-53.4729	-52.4730	-23.0994	-51.3613
B Infr CU	-143.2998	-111.3013	-90.1477	-153.1058
C Infr CU	-98.5500	-56.6273	-67.2752	-134.5274
Area Infr CU	0.0000	0.0000	0.0000	0.0000
A Rich CU	4.7524	11.9087	7.6365	31.2758
B Rich CU	3.1333	22.9026	2.0784	53.4653
C Rich CU	1.0873	14.0596	4.4257	45.9013
Area Rich CU	0.0000	0.0000	0.0000	0.0000

*Quadratic regression paramaters: A = intercept; B = coefficient of linear term; C = coefficient of quadratic term, col. day = collection day, Hr < 7 = Hours below 7° C, Hr < 12 = Hours below 12° C, Infr CU = Infruitec chilling units, Rich CU = Utah chilling units.

TABLE 5: Number of observations and percent classified into chilling when the discriminant functions of the 2000 season were used as calibration functions (inverse of days until 20% vegetative bud break).

From Chilling	High	Medium	Low	Total
Calibration				
High 00	5	0	0	5
%	100.00	0.00	0.00	100.00
Test				
High 01	0	0	5	5
%	0.00	0.00	100.00	100.00
Calibration				
Medium 00	0	2	0	2
%	0.00	100.00	0.00	100.00
Test				
Medium 01	0	0	3	3
%	0.00	0.00	100.00	100.00
Calibration				
Low 00	1	0	20	21
%	4.76	0.00	95.24	100.00
Test				
Low 01	0	5	16	21
%	0.00	23.81	76.19	100.00
Calibration				
Total 00	6	2	20	28
%	21.43	7.14	71.43	100.00
Test				
Total 01	0	5	24	29
%	0.00	17.24	82.76	100.00
Priors	0.33333	0.33333	0.33333	

TABLE 6: Number of observations and percent classified into chilling when the discriminant functions of the 2001 season were used as calibration functions (inverse of days until 20% vegetative bud break).

From Chilling	High	Medium	Low	Total
Calibration				
High 01	4	1	0	5
%	80.00	20.00	0.00	100.00
Test				
High 00	0	2	3	5
%	0.00	40.00	60.00	100.00
Calibration				
Medium 01	0	3	0	3
%	0.00	100.00	0.00	100.00
Test				
Medium 00	0	1	1	2
%	0.00	50.00	50.00	100.00
Calibration				
Low 01	0	2	19	21
%	0.00	9.52	90.48	100.00
Test				
Low 00	2	8	11	21
%	9.52	38.10	52.38	100.00
Calibration				
Total 01	4	6	19	29
%	13.79	20.69	65.52	100.00
Test				
Total 00	2	11	15	28
%	7.14	39.29	53.57	100.00
Priors	0.33333	0.33333	0.33333	

TABLE 7: Pooled within-class standardised canonical coefficients of days until 20% reproductive bud break.

	Can 1		Can 2	
Variable*	Calibration 00	Calibration 01	Calibration 00	Calibration 01
A col.day	135.4674	-272.8326	-494.6921	-935.4194
B col. day	214.8945	-378.9829	-940.9418	-1528.0990
C col. day	84.6311	-99.0485	-453.0493	-600.0552
Area col. day	0.0000	0.0000	0.0000	0.0000
A Hr < 7	-133.8119	-11.4224	51.2303	6.0883
B Hr < 7	-192.3859	-24.3864	75.4012	28.8185
C Hr < 7	-74.1096	-16.0392	28.8901	39.3973
Area Hr < 7	0.0000	0.0000	0.0000	0.0000
A Hr < 12	30.7731	82.3235	4.0754	156.5657
B Hr < 12	75.0816	113.3668	13.5761	138.2821
C Hr < 12	40.2620	2.0626	15.4870	-1.3178
Area Hr < 12	0.0000	0.0000	0.0000	0.0000
A Infr CU	59.9337	-20.2253	-12.9346	-62.5564
B Infr CU	58.9196	-64.0733	-15.6216	-41.1075
C Infr CU	15.2718	-1.0917	-9.1161	16.4976
Area Infr CU	0.0000	0.0000	0.0000	0.0000
A Rich CU	6.6925	-1.4080	2.8001	2.0777
B Rich CU	-0.3454	26.7384	-2.1969	-14.4370
C Rich CU	5.9081	12.0515	3.6627	-38.2046
Area Rich CU	0.0000	0.0000	0.0000	0.0000

*Quadratic regression paramaters: A = intercept; B = coefficient of linear term; C = coefficient of quadratic term, col. day = collection day, Hr < 7 = Hours below 7° C, Hr < 12 = Hours below 12° C, Infr CU = Infruitec chilling units, Rich CU = Utah chilling units.

TABLE 8: Number of observations and percent classified into chilling when the discriminant functions of the 2000 season were used as calibration functions (days until 20% reproductive bud break).

From Chilling	High	Medium	Low	Total
Calibration				
High 00	6	0	0	6
%	100.00	0.00	0.00	100.00
Test				
High 01	0	3	0	3
%	0.00	100.00	0.00	100.00
Calibration				
Medium 00	0	2	0	2
%	0.00	100.00	0.00	100.00
Test				
Medium 01	0	2	0	2
%	0.00	100.00	0.00	100.00
Calibration				
Low 00	1	0	21	22
%	4.55	0.00	95.45	100.00
Test				
Low 01	1	18	1	20
%	5.00	90.00	5.00	100.00
Calibration				
Total 00	7	2	21	30
%	23.33	6.67	70.00	100.00
Test				
Total 01	1	23	1	25
%	4.00	92.00	4.00	100.00
Priors	0.33333	0.33333	0.33333	

TABLE 9: Number of observations and percent classified into chilling when the discriminant functions of the 2001 season were used as calibration functions (days until 20% reproductive bud break).

From Chilling	High	Medium	Low	Total
Calibration				
High 01	3	0	0	3
%	100.00	0.00	0.00	100.00
Test				
High 00	3	0	3	6
%	50.00	0.00	50.00	100.00
Calibration				
Medium 01	0	2	0	2
%	0.00	100.00	0.00	100.00
Test				
Medium 00	2	0	0	2
%	100.00	0.00	0.00	100.00
Calibration				
Low 01	0	0	20	20
%	0.00	0.00	100.00	100.00
Test				
Low 00	10	7	5	22
%	45.45	31.82	22.73	100.00
Calibration				
Total 01	3	2	20	25
%	12.00	8.00	80.00	100.00
Test				
Total 00	15	7	8	30
%	50.00	23.33	26.67	100.00
Priors	0.33333	0.33333	0.33333	

TABLE 10: Pooled within-class standardised canonical coefficients of the inverse of days until 20% reproductive bud break.

	Can 1		Can 2	
Variable*	Calibration 00	Calibration 01	Calibration 00	Calibration 01
A col.day	-709.7202	28250.9799	-177.7356	-1000.9811
B col. day	-1715.8552	4847.1294	-333.5407	-1886.6916
C col. day	-1029.5804	2120.7255	-154.2334	-903.2509
Area col. day	0.0000	0.0000	0.0000	0.0000
A Hr < 7	-45.8238	-97.8537	-47.2267	71.7429
B Hr < 7	-85.8922	-277.1623	-75.4748	163.6836
C Hr < 7	-46.9010	-202.4437	-33.0071	97.3322
Area Hr < 7	0.0000	0.0000	0.0000	0.0000
A Hr < 12	57.4415	38.7378	32.1309	28.3195
B Hr < 12	246.2597	-18.1203	21.5835	171.6623
C Hr < 12	235.1029	-87.6828	-20.9194	120.9665
Area Hr < 12	0.0000	0.0000	0.0000	0.0000
A Infr CU	20.1081	-403.1191	19.5875	73.1849
B Infr CU	-41.7700	-330.6819	44.8955	-44.1239
C Infr CU	-83.5510	-11.2050	29.3756	-57.9122
Area Infr CU	0.0000	0.0000	0.0000	0.0000
A Rich CU	14.0104	135.6361	8.8002	-28.4248
B Rich CU	-1.5417	144.0595	1.8933	-17.4620
C Rich CU	15.5565	76.6969	8.6229	-18.9859
Area Rich CU	0.0000	0.0000	0.0000	0.0000

*Quadratic regression paramaters: A = intercept; B = coefficient of linear term; C = coefficient of quadratic term, col. day = collection day, Hr < 7 = Hours below 7° C, Hr < 12 = Hours below 12° C, Infr CU = Infruitec chilling units, Rich CU = Utah chilling units.

TABLE 11: Number of observations and percent classified into chilling when the discriminant functions of the 2000 season were used as calibration functions (inverse of 20% reproductive bud break).

From Chilling	High	Medium	Low	Total
Calibration				
High 00	6	0	0	6
%	100.00	0.00	0.00	100.00
Test				
High 01	0	3	0	3
%	0.00	100.00	0.00	100.00
Calibration				
Medium 00	0	2	1	3
%	0.00	66.67	33.33	100.00
Test				
Medium 01	1	0	1	2
%	50.00	0.00	50.00	100.00
Calibration				
Low 00	1	0	21	22
%	4.55	0.00	95.45	100.00
Test				
Low 01	2	17	1	20
%	10.00	85.00	5.00	100.00
Calibration				
Total 00	7	2	22	31
%	22.58	6.45	70.97	100.00
Test				
Total 01	3	20	2	25
%	12.00	80.00	8.00	100.00
Priors	0.33333	0.33333	0.33333	

TABLE 12: Number of observations and percent classified into chilling when the discriminant functions of the 2001 season were used as calibration functions (inverse of 20% reproductive bud break).

From Chilling	High	Medium	Low	Total
Calibration				
High 01	3	0	0	3
%	100.00	0.00	0.00	100.00
Test				
High 00	1	3	2	6
%	16.67	50.00	33.33	100.00
Calibration				
Medium 01	0	2	0	2
%	0.00	100.00	0.00	100.00
Test				
Medium 00	0	1	2	3
%	0.00	33.33	66.67	100.00
Calibration				
Low 01	0	1	19	20
%	0.00	5.00	95.00	100.00
Test				
Low 00	1	20	1	22
%	4.55	90.91	4.55	100.00
Calibration				
Total 01	3	3	19	25
%	12.00	12.00	76.00	100.00
Test				
Total 00	2	24	5	31
%	6.45	77.42	16.13	100.00
Priors	0.33333	0.33333	0.33333	

5. GENERAL DISCUSSION AND CONCLUSION

Winter dormancy of peach buds is terminated naturally by exposure to low, but not freezing temperatures (Guardian and Biggs, 1964). The duration of exposure to chilling temperatures required to terminate bud-rest has been observed to vary with cultivar. It has also been observed that the duration of exposure to chilling temperatures needed for normal resumption of bud-growth in the spring varies from year to year and from locality to locality with the same clonal material. A large part of this variation in the chilling requirement seem to be due to a partial reversal of the beneficial effects of chilling by rather warm temperatures immediately following exposure to cool temperatures (Erez et al., 1979a, Erez and Couvillon, 1987 and Erez et al, 1988, Erez et al., 1990). Thus, the amount of cool temperature needed for good bud-break increases when the buds are subjected to intermittent cool and prolonged warm periods.

To predict the suitability of specific cultivars to specific growing sites, knowledge of the bud break and bloom dates as well as their tendency to exhibit signs of delayed foliation under warm winter conditions is a prerequisite. These factors are all determined by the chilling requirement of the cultivar (Couvillon and Erez, 1985).

Most South African peach and nectarine production areas lack of adequate winter chilling (Linsley-Noakes, 1995). Consequently, only medium (400 - 800 Richardson/Utah chilling units [CU]) to low (<400 CU) chilling requirement (CR) cultivars can be produced with any measure of success.

A conventional breeding programme was started, to develop new and improved stone fruit cultivars with special emphasis on climatic adaptation and pomological attributes. When cultivars are categorised into CR groups, it becomes possible to identify suitable parents to steer a breeding programme into a certain direction, in other words, to produce progeny with a CR falling within certain chosen parameters. Currently the breeding programme uses a phenological method of classification to classify the selections in the second phase of evaluation according to CR.

In this study the data obtained from shoots under forcing conditions was statistically analysed using the CANDISC (Canonical Discriminant Analysis) procedure of SAS Release 8.1 (SAS Institute Inc., Cary, NC, USA) and the outcome compared with the outcome of the preliminary groupings obtained with the phenological classification method.

Some problems occurred during forcing in 2000 resulting in numerous misclassifications, in particular, of low CR selections. Furthermore, the boundaries given to the low, medium and high CR groupings are artificial aids to facilitate the classification of cultivars according to CR, but the CR of the selections included in this study forms a continuum from low to high CR without clear boundaries. During a marginal CU season, like the winter of 2000, a selection falling within the upper part of the medium CR grouping, could display traits similar to a selection falling within the lower part of the high CR grouping and could consequently be misclassified.

In most instances the number of hours below 12° C contributed most to the separation of the selections into CR groupings by the CANDISC procedure. This indicates that when criteria based on lower temperature were used, no clear distinction could be made between the selections according to CR.

The groupings of the CANDISC procedure obtained with the 2001 season's data are more or less consistent with the preliminary groupings obtained with the phenological classification method. Due to the nature of the scientific method used in this study, there is room for a certain margin of experimental error to occur, which could account for the misclassifications by the CANDISC procedure, when performed on the 2001 season's data. It can be concluded that the phenological method of classifying the selections, as currently used in the breeding programme, is consistent with the results of the scientific method described here. Therefore, it is recommended that the phenological method be used in future to classify the selections according to CR, as this method is less time consuming and less costly to perform.

When shoots were artificially cooled in a cold room kept at a constant temperature range between 4° C and 7° C before being transferred to forcing conditions an overall correct classification of 63.01% was achieved with the CANDISC procedure. It can be concluded that the shoots reacted similarly to artificial chilling as to temperature conditions within the range of temperatures known to cause the accumulation of CU, occurring naturally in the orchard where these shoots were collected and a relatively good classification was achieved. It can further be concluded that the inherent CR of the selections, as determined by their genetic make-up, is expressed even under conditions of maximum CU accumulation, as it was still possible to group the selections according to CR under these conditions.

A unique set of discriminant functions is necessary for each winter season to accurately classify selections according to CR with the CANDISC procedure, as different variables are used in each season to discriminate between selections, as can be seen in the tables of pooled within-class standardised canonical coefficients (Paper 3). This can surely be attributed to the unique set of climatic conditions prevailing in each winter season, which cause a slightly different reaction in the shoots of the different selections. This explains the poor reclassification achieved when the discriminant functions of the CANDISC procedure of one season are used as calibration data to test the data of the other season against.

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6. ADDENDUM A

TABLE 1: List of peach and nectarine selections included in this study, with their CR indicated according to the phenological method of classification.

Selection	Code	CR	Cross Combination
M 1	A	Low	1
17C-38-39	B	Low	2
17C-40-11	C	Low	2
17C-39-5	D	Low	2
17C-39-21	E	Low	2
M 2	F	High	3
17C-8-25	G	Low	4
5B-10-9	H	Low	26
M 3	I	High	5
S4B-3-19	J	Low	6
5C-15-28	K	Low	7
M 4	L	High	8
PE93-26	M	Low	9
S4D-7-20	N	Low	10
S4B-22-2	O	Low	11
PE94-38	P	High	12
PE94-52	Q	High	13
20D-13-13	S	Low	14
5B-21-7	T	Low	15
M 5	U	Low	16
M 6	V	Low	17
5B-6-8	W	Low	18
M 7	X	High	19
5B-5-28	Y	Low	18
5D-3-4	Z	Low	20
M 8	Aa	Low	21

Continued on next page

TABLE 1 (continued): List of peach and nectarine selections included in this study, with their CR indicated according to the phenological method of classification.

Selection	Code	CR	Cross Combination
5B-25-3	Ab	Low	22
NE94-16	Ac	Low	23
NE94-50	Ad	Low	24
NE94-52	Ae	Medium	24
NE94-56	Af	Medium	24
NE94-68	Ag	Medium	25