An Investigation into the Genetic Variation of Chilling Requirement in Apple (*Malus* x *domestica* Borkh.) Progenies

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

Signature

Date: 12/02/02.

SUMMARY

Various experiments were undertaken over a period of five years to investigate the feasibility of initiating a large-scale programme of controlled apple breeding and selection for the improvement of climatic adaptation, using budbreak number (NB) as a practical criterion of selection. NB is preferred to time of budbreak as sole criterion on the grounds that early budbreak is associated with low NB under local conditions. Variation within and between adult and juvenile seedling families was investigated and the genetic control of the traits involved was assessed, as well as direct and correlated responses to selection. In initial experiments different rating criteria for NB as measure of chilling requirement were tested in association with vegetative and reproductive budbreak time and flowering duration, viz, a classification index based on number and distribution of budbreak (PDS grade), an index where shoot length with increased budbreak was included in the index calculation (PDS index) and budbreak number expressed as number per 100 cm of shoot length (NB index). Variance analysis (ANOVA and Variance component analyses) detected significant variation within seedling families for budbreak time and NB, but estimates of genetic components of variance between families were generally low. High genetic variance among seedlings within families is most likely due to the high level of heterozygosity in the parental cultivars as is characteristic of vegetatively propagated crops. Intra-class correlation coefficients for clones within and between families indicate moderate genetic determination for NB with broad sense heritabilities around 30 percent. Realized heritabilities calculated from response to two-way truncation selection were between 40 and 60 percent. For budbreak time (reproductive and vegetative), the broad sense heritability averaged around 75 and 69 percent, respectively, indicating a high degree of genetic determination. Significant response to selection for NB of one-year-old shoots of young seedlings and from seedlings grown into adult trees showed that pre-selection for increased budbreak successfully identified seedlings genetically inclined to more and better distribution of budbreak within a set time of 21 days after initial budbreak. Correlated responses indicated additional advantages of practical and horticultural value, viz, uniformity and position of budbreak, and the number and length of side shoots. In general, it is concluded from responses to two-way selection that utilizable genetic variance in NB is present within seedling families and thus that selection may successfully be applied as an early screening method for increased budbreak in adult trees.

The NB index of intact one-year-old shoots under prevailing sub-optimal winter conditions is therefore proposed as criterion of selection for improvement of climatic adaptation, and combined selection utilizing genetic variation between and within crosses as the selection method.

OPSOMMING

Verskeie proewe is oor 'n periode van vyf jaar uitgevoer om die toepaslikheid van 'n grootskaalse appelteel- en seleksieprogram vir die verbetering van klimaatsaanpasbaarheid te ondersoek met 'aantal knopbreke' (NB) as praktiese seleksiekriterium. NB word verkies bo tyd van knopbreek op grond daarvan dat vroeë knopbreek onder plaaslike toestande met lae NB gepaard gaan. Variasie binne en tussen volwasse en jong saailingfamilies en die genetiese beheer van die betrokke eienskappe is ondersoek, asook direkte en gekoreleerde seleksieresponsie. In die aanvangs-eksperimente is verskillende kriteria vir die kwantifisering van aantal knopbreke getoets as potensiële maatstawwe van die inherente kouebehoefte in appelsaailinge. Die tyd van vegetatiewe en reproduktiewe knopbreek en blomperiode is ook getoets. Die volgende indekse is gebruik: 'n klassifikasie-indeks om die aantal en verspreiding van knopbreke te beskryf (PDS graad), 'n indeks waar die lootlengte, met verhoogde aantal knopbreke, ingesluit is in die berekening van die indekswaarde (PDS indeks), en knopbreke uitgedruk as die aantal per 100 cm lootlengte (NB indeks). Variansie analise (ANOVA en variansie komponent analise) het betekenisvolle variasie binne saailingfamilies aangetoon vir tyd van, en aantal knopbreke. Ramings van genetiese komponente van variansie tussen families was relatief klein. Hoë genetiese variansie tussen saailinge binne families is waarskynlik te wyte aan die hoë vlak van heterosigositeit in die ouergenotipes, wat kenmerkend is van gewasse wat vegetatief voortgeplant word. Intraklas korrelasie koëffisiënte vir klone tussen en binne families het gedui op 'n middelmatige oorerflikheid in die breë sin (ongeveer 30 persent) vir aantal knopbreke. Verhaalde oorerflikhede wat bereken is vanaf responsie op twee-rigting afknottingsseleksie was tussen 40 en 60 persent. Vir tyd van knopbreek (vegetatief en reproduktief) was die breësin oorerflikhede ongeveer 75 en 69 persent, onderskeidelik, wat aanduidend is van 'n hoë graad van genetiese bepaling. Betekenisvolle responsie op seleksie vir NB van jong saailinge en saailingbome wat volwassenheid bereik het toon dat pre-seleksie vir knopbreke saailinge kan identifiseer wat geneties meer knopbreke en 'n beter verspreiding van knoppe binne 'n periode van 21 dae na die eerste knopbreek lewer. Gekorreleerde responsie op seleksie toon 'n addisionele voordeel van praktiese en tuinboukundige belang, naamlik, meer en langer sylote. In opsomming kan dit gestel word dat responsie op twee-rigting seleksie bruikbare genetiese variasie vir NB binne saailingfamilies ontgin het en dat seleksie vir verhoogde aantal knopbreke suksesvol toegepas

kan word. Die NB indeks op een-jaar-oue hout word dus voorgestel as seleksiekriterium vir verbetering van klimaatsaanpasbaarheid onder plaaslike sub-optimale wintertoestande, en gekombineerde seleksie "combined selection" wat genetiese variasie binne en tussen kruisings benut as seleksiemetode.

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1. INTRODUCTION

Apple is the most economically important deciduous fruit crop in the Western Cape Province of South Africa. During 2000/2001 17 million cartons were received for export, accounting for an estimated value of R178 million and approximately 38% of the total export value of deciduous fruits. Currently, cultivation covers some 21 500 hectares. There are four major production areas, viz, Elgin (34°S, 305 m, ca 850 Utah Chill Units (CU)), Koue Bokkeveld (33°S, 945 m, ca. 1300 CU), Langkloof (33°S, 722 m, ca 700 CU) and Vyeboom 34°S, 309 m, ca. 700 CU) which contribute respectively, 28%, 21%, 21% and 13% to the total orchard area. Other apple producing areas include Villiersdorp (9%), Montagu (2%), Piketberg (2%) and the Free State (4%).

Commercial cultivars planted develop abnormalities in growth behavior referred to as prolonged dormancy symptoms (PDS), because winters are not cold enough to satisfy winter chilling requirement (CR). Low winter chilling, fluctuations in temperature with exceptionally hot days and extended cold accumulation towards the end of winter and spring commonly occur. While temperatures in the Koue Bokkeveld are sufficiently cold for normal initial development of bud dormancy, the winter is too short for normal dormancy release. Producers in areas with low chilling conditions apply chemical agents in an attempt to induce more uniform budbreak and in order to achieve better fruit set and fruit quality. Dinitro-ortho-cresol mineral oil (DNOC) has been used but due to environmental and health concerns the product is to be phased out by 2005. Use of new alternative rest breaking products on the market will result in a loss of approximately R10 million income for the apple fruit industry annually.

For important horticultural traits, such as yield and fruit quality, it is unrealistic to expect levels of performance of cultivars to be the same in all environments. This is the main reason why some cultivars developed in other countries are not suitable for local production. Hence, there is an urgent need to develop cultivars better adapted to local low chilling conditions.

Knowledge about the genetic variance and inheritance of traits associated with CR is a prerequisite for the development of adapted cultivars by breeding and selection. The genetics of CR measured in terms of prolonged dormancy symptoms has not been adequately investigated and the genetic variability of budbreak number as selection criterion in planned breeding

programmes in fruit trees has not been assessed. The aim of this study was then to assess the potential of genetic manipulation and utilization of genetic variation in CR in order to provide guidelines for the design of such programmes.

In this study four main areas were covered to address the above problems and are presented in this dissertation as four chapters each written in the format of a single scientific article of which one chapter has already been accepted for publication (Chapter 4: HortScience, article HSMS 6655) and one chapter has been accepted contingent on an acceptable revision (Chapter 5: J. Amer. Soc, Hort. Sci., article JMS 9094). This has led inevitably to some repitition in the text across chapters on matters of introductory background, materials and literature references. The time spanned by the experiments has also meant some shifts in terminology, abbreviations and acronyms, for example, time of budbreak as TB (Chapters 1 and 4) and time of initial vegetative budbreak as IVB (Chapters 2 and 3). However, a full list of abbreviations and acronyms used precedes this introductory chapter.

Chapter 2 is a review of literature presented in two parts of which the first concerns dormancy and chilling requirement in a horticultural perspective and the second part concerns genetics and breeding for chilling requirement and climatic adaptation.

In Chapter 3 budbreak number is investigated as a measure of CR in young seedlings under controlled and uncontrolled conditions. The main aspects covered in these experiments are (1) the actual response to chilling measured by budbreak number, (2) variation between and within families of crosses and (3) response to selection.

In Chapter 4 variances of traits related to prolonged dormancy in adult apple seedlings planted in mild winter conditions are estimated, where in this experiment the specific objectives were to: (1) quantify the variation within and between adult apple seedlings families and (2) to test evaluation criteria for CR and prolonged dormancy.

In chapter 5 a quantitative genetic analysis of budbreak time and number is conducted in order to investigate chilling requirement in seedling families as indication of their adaptedness to local

growing conditions, where genetic parameters were estimated in an attempt to (1) explain the genetic control and variability of the criteria, (2) to assess their possible implication in breeding programmes, and (3) to explore the effectiveness of early screening for CR at a young seedling stage.

Chapter 6 describes experiments designed to examine the number of buds breaking as a criterion of selection against PDS in progeny groups obtained by crossing established cultivars. Specifically, the aims were (1) to evaluate within and between family variation for budbreak number in young seedlings, (2) to evaluate direct response to selection for high and low budbreak number within families, (3) to evaluate possible correlated responses in other related traits and (4) to examine residual genetic variance in the present material with a view to constructing an index for combined selection utilizing genetic variance between crosses as well as within crosses, for application in the continuation of the programme.

LIST OF ABBREVIATIONS AND ACRONYMS:

Cultivar names:

An	Anna
Au	Austin
Br	Braeburn
Fi	Fiesta
FR	Full Red
Fu	Fuji
GD	Golden Delicious
JR	Jona Red
Ki	Kirks
LW	Lady Williams
MD	Mollie's Delicious
Pr	Prima
Pc	Priscilla
RC	Red Chief
RG	Royal Gala
SE	Sharpe's Early
Sr	Summerred
St	Starking Delicious
Su	Summerking

Other names:

CU	Cold units
GDH	Growing degree hours
CR	Chilling requirements
PDS	Prolonged dormancy symptoms

Trait names:

TB	Budbreak time
NB	Budbreak number
IVB	Initial vegetative budbreak time
IRB	Initial reproductive budbreak time

2. LITERATURE REVIEW

2.1. INTRODUCTION

Many factors influence the adaptive potential of apple cultivars planted around the world. Some areas are more suitable than others for production due to temperature and soil conditions, light intensity and other environmental factors. This is the main reason why newly bred cultivars are not always suitable for all producing areas and why fruit tree breeding for improvement in economically important traits requires attention to traits related to environmental adaptedness as well.

Most temperate zone woody deciduous trees, including apple, require a certain degree of chilling to break dormancy before active shoot growth in the spring, a phenomenon generally referred to as the chilling requirement (CR). In apple cultivars adapted to a particular environment, budbreak occurs promptly and uniformly during spring and cultivars with timely budbreak during the early growing season are generally referred to as low CR genotypes.

Abnormal growth characteristics occur as a result of unmet CR during the rest period, or as a result of unfavourable temperatures during the period of normal budbreak. These abnormal growth characteristics observable under mild winter conditions have been referred to as symptoms of prolonged dormancy (PDS). If CR is not satisfied during the rest period, or when dormancy release is not successful, an absence, reduced, irregular or very long delay of lateral vegetative and reproductive budbreak within and among shoots occur.

Winter temperatures and the CR of genotypes affect the intensity of bud dormancy and, because buds on the shoot respond differently, the budbreak pattern and tree form can also be affected. A less uniform breaking of buds can lead to individual branches having increased autonomy while a more uniform budbreak leads to a more balanced tree structure.

Although plant adaptedness relates to the particular environmental factors prevailing, it also depends on the plant genetic composition. The prospects of breeding for adaptedness to specific environmental conditions is therefore of wide interest. For example, knowledge of the pattern of inheritance of CR and related traits would then be of importance for the development of effective breeding strategies. Previously, breeding for low CR has relied largely on subjective observation with little scientific basis. Genetic studies of fruit traits have usually been done retrospectively from breeding programme data and often came short in appropriate experimental design for estimation of variance components and heritability. The quantitative nature of the dormancy process, the environmental factors and their interactions involved have been complicating factors in these studies. The lack of an experimental approach has led to discrepancies in explanations of the genetic control of dormancy and CR. Some authors have suggested that major dominant genes are involved while others describe dormancy-related traits to vary in a quantitative manner. Generally, CR is now seen as a complex genetically determined trait, probably multigenic or, at least, partly controlled by multiple genes.

The complex nature of dormancy and CR in temperate fruit trees has led to various quantitative and semi-quantitative measures. Many authors have proposed climatic models and biological tests to predict development of dormancy and CR, but their use has rarely given satisfactory results. CR is usually estimated on the basis of vegetative bud development (time, number or distribution of budbreak) or by comparing new cultivars with the performance of cultivars with known or reputed CR.

To be able to breed apples adapted to specific climatic conditions, methods must be developed that will identify individual apple seedlings. Since apple seedlings have a long juvenile period, there is an urgent need to incorporate important traits such as CR early in a breeding program. Methods of pre-screening need to be simple because thousands of seedlings must be assessed each year. If significant response to early selection can be achieved, also for CR, this could have considerable benefits in tree breeding programmes. Published literature relating to the above introductory comments is reviewed in what follows below.

2.2. DORMANCY AND CHILLING REQUIREMENT IN DECIDUOUS FRUIT TREES

Dormancy is generally defined as the temporary suspension of visible growth of any plant structure containing a meristem, e.g. vegetative and reproductive buds (Lang, 1987) and may represent a continuous gradient of regulatory events or phases (Lang, 1985). Most temperate zone woody deciduous fruit trees, including apple, require a certain degree of chilling to be released from dormancy and to commence proper growth in the spring (Kester et al., 1977; Rodriguez and Sherman, 1985; Rowland et al., 1999; Sorensen, 1983). This phenomenon is generally referred to as the chilling requirement (CR) (Howe et.al., 1999; Martinez et al., 1999; Sorensen, 1983).

Dormancy is the outcome of a combination of many influences (Cook et al., 1998a) and the complex nature of dormancy and related processes in temperate fruit trees has led to various quantitative and semi-quantitative measures of dormancy release, and to a profusion of terms, often poorly defined and sometimes ambiguous. However, the following terminology has emerged as consistent. Control of dormancy by factors residing within the bud itself is referred to as *endodormancy* while control by factors in the plant outside of the bud as *paradormancy* and control by environmental factors as *ecodormancy* (Kahn, 1997; Lang et al., 1985). Endodormancy is also referred to as "winter dormancy" or "rest" (Fuchigami and Nee, 1987). The endodormant period ends when the CR is said to have been met. Total dormancy can be seen as the aggregate effect of all these factors.

For buds to break at spring they must first enter and then exit from dormancy (Cook and Jacobs, 2000). It is known that high temperatures gradually assume more control over bud development as trees approach completion of dormancy and that bud development progresses only if chilling is supplemented with temperatures favourable to growth (Brown, 1960; Kriebel and Wang, 1962; Worrall and Mergen, 1967). In warm climates it seems to be the amount, duration and interrelation of chilling and heat during the pre-meiotic stage of bud differentiation that determine whether or not this stage may proceed (Bailey and Hough, 1975). Bud exposure to warm temperatures is thus also necessary (Kester et al., 1977).

Buds have the ability to enter into and exit from dormancy even in warm temperatures (Mauget and Rageau, 1988), but cold temperatures are considered as the environmental trigger responsible for the initial induction (Cook, et al., 2000). Wilton (2000) describes dormancy in terms of two phases: the first phase commences at or close to leaf fall and requires a period of chilling to break down growth inhibitors present during this period, and the second phase is heat driven. Onset of the second phase depends on how warm spring temperatures are. In growing conditions with warm spring weather, development of buds to budbreak will be much shorter than in cool spring conditions. In warm climates the progression of dormancy may therefore differ from that in the cold climates. Cook and Jacobs, (2000) have shown that autumn conditions in warmer areas in South Africa are not inducive of normal entry into dormancy. In cold winter conditions entrance into and exit from dormancy are more rapid than in sub-optimal winter conditions.

Wide variation in CR exists among cultivars, wild species and hybrids (Hauagge and Cummins 1991a; Mahmood et al., 2000), but little is still known about the genetic basis of this variation. Cultivars described as low CR have a very shallow dormancy which is related to failure to develop a deep dormancy state (Hauagge and Cummins, 1991b), where deep and shallow dormancy are taken to refer to high and low bud CR, respectively. These cultivars, such as 'Anna', 'Dorsett Golden' and 'Ein Shemer', have been found to display total termination of dormancy in relation to the length of dormancy and no delayed foliation (Hauagge and Cummins, 1991b). Cultivars with a slow rate of winter development (prolonged rest period) and, therefore, presumably, high CR, are more resistant to fluctuating winter temperatures (Bailey and Hough, 1975).

2.2.1. PROLONGED DORMANCY SYMPTOMS

In conditions where winter chilling is effective, budbreak is prolific and occurs promptly and uniformly on terminal as well as lateral buds (Cook, et al., 1998a; Hauagge and Cummins, 1991c). An increase in the number of branches, flower size, pedicel length and fruit set, leaf fresh and dry weights and a reduced time of budbreak was found with increased chilling (Mahmood et al., 2000).

Abnormal growth characteristics occur at temperatures in the range between 4 and 9°C (Richardson et al., 1974) during the rest period, or as a result of unfavourable temperatures during the period of normal budbreak (Cook et al., 1998a; Hauagge and Cummins, 1991a; Mauget and Rageau, 1988). These abnormal growth characteristics observable under mild winter conditions have been referred to variously as symptoms of prolonged dormancy (PDS), delayed foliation or extended rest (Jacobs et al., 1981; Janick et al., 1996; Lesley, 1944). When dormancy release is not successful, an absence, reduced, irregular or very long delay of lateral vegetative and reproductive budbreak within and among shoots can occur (Cook, 1999a; Hauagge and Cummins, 1991c; Lammerts, 1945; Lesley, 1944; Mauget and Rageau, 1988). The opening of the terminal buds following insufficient chilling is slower (Crabbé, 1984) and the delay is further accentuated in the lateral buds, many of which remain dormant, resulting in the development of a reduced number of branches (Cook et al., 1999a). A less synchronized breaking of buds can lead to individual branches with increased autonomy. The length of the delayed budbreak is directly related to the lack of chilling and the less the chilling received the more uneven the pattern of budbreak.

The occurrence of paradormancy (correlative inhibition) largely accounts for the inhibition of budbreak of the upper lateral buds under sub-optimal conditions, resulting in poor sprouting and PDS of these buds (Jacobs et al., 1981). Regrowth of apple trees displaying PDS takes place mainly from terminal and lateral buds on the lower halves of one-year-old shoots with proximal lateral buds breaking more readily than the distal ones (Jacobs et al., 1981). This may lead to an increased basitonic breaking tendency (budbreak in lower, basal part of tree trunk), basal dominance and the vigor of individual, proximally situated branches relative to the development of the trunk. This leads to competition with the leader trunk and this problem intensifies with progressively less chilling (Cook et al., 1999a).

Other prolonged dormancy symptoms include deformed and nonviable flower parts and flower bud abscission, resulting in poor production, prolonged flowering duration, lower fruit set and uneven and small fruit size (Greybe, 1997; Jacobs et al., 1981; Janick et al., 1996; Lesley, 1944, Stushnoff and Quamme, 1983). High temperatures for an extended period of time during the rest

period stops flower bud differentiation resulting in bud drop (Bailey and Hough, 1975). The most prominent symptom is the absence or extended delay of lateral vegetative budbreak.

These symptoms commonly occur in orchards in the Western Cape region of South Africa since temperatures for normal dormancy release of most commercial cultivars are simply not met (Jacobs et al., 1981). Apple producers in these areas apply chemical agents in an attempt to induce more uniform budbreak and in order to achieve better fruit set and fruit quality. Up to this point, Dinitro-ortho-cresol mineral oil (DNOC) has been used, but due to environmental and health concerns, it is to be phased out by the year 2005.

2.2.2. ADAPTEDNESS TO SPECIFIC ENVIRONMENTAL CONDITIONS

Investigation of cultivar adaptedness is usually based on genotype and environmental interaction studies. CR is often referred to as an adaptive trait in trees and often associated with the terms adaptation and adaptedness, referring, in this context, to the way in which plants can survive and reproduce in specific environments (Hill et al., 1998), and reflected by the degree to which developmental events are synchronized with the particular climate (Dietrichson 1964). As such, adaptedness is a complex interaction between various environmental factors and the genetic composition of the plant.

The development of dormancy is an important adaptive strategy in woody perennials (Howe et al., 1999), and the adaptedness of many species and cultivars to a particular location may thus be determined by their CR (Kester et al., 1977). CR can prevent growth occurring during the transitory periods of warm temperatures through winter and can help synchronize plant growth with exposure to favorable environmental conditions (Rowland et al., 1999). CR can also determine to what degree fruit crops of temperate-zone origin will survive the cold winter and early spring without shoot and bud damage. Prolonged dormancy symptoms are thus indications of poor adaptedness to mild winter climates (Martinez et al., 1999; Mauget and Rageau, 1988). In apple genotypes adapted to the environment, budbreak occurs promptly and uniformly during spring (Hauagge and Cummins, 1991c) and it is normally accepted that apple cultivars with timely budbreak during the early growing season are low CR genotypes with good environmental

adaptedness (Bradshaw and Stettler, 1995; Chandler, 1960; Hauagge and Cummins, 1991a; Herter et al., 1988; Weinberger, 1944; Wilson et al., 1975).

2.2.3. BUDBREAK IN RELATION TO CHILLING REQUIREMENT IN FRUIT TREES

The pattern of budbreak is the consequence of numerous interactions between biological processes, not well understood, and climatic factors, for example, the inherent difference in depth of dormancy between terminal and lateral buds. During endodormancy, lateral buds appear less endodormant than terminal buds, but shifts take place during the dormancy period (Cook et al., 1998b; Hauagge and Cummins, 1991d). Cold accumulation appears to play a role in the normalization of bud growth potential and in the duration of budbreak (Cook et al., 1998a).

2.2.3.1. Positional effect of budbreak

Hormonal control over the correlative phenomena of budbreak determines tree architecture (Cook et al., 2000; Faust et al., 1997). Before budbreak in spring, a peak in endogenous cytokinins is observable that appears to act as a trigger for resumed growth and promotion of budbreak (Cutting et al., 1991; Faust et al., 1997; Shaltout and Unrath, 1983; Steffens and Stutte, 1989). The increase starts shortly before budbreak, increases rapidly with bud swelling and peaks at budbreak (Cutting et al., 1991; Faust et al., 1997). Numerous studies have been undertaken to describe the involvement of plant hormones in dormancy and dormancy release.

Differences in dormancy between the terminal and lateral buds due to different physiological states or correlative inhibition by hormonal control (Champagnat, 1978; Cook et al., 1998a; Jacobs et al., 1981; Mauget and Rageau, 1988), cause an increased developmental potential and a positional advantage of the terminal buds relative to the more proximal lateral buds. This results in dominance and acrotonic (distal) branching patterns (Cook et al., 1998a; Cook et al., 2000). Acrotony is a prerequisite for trunk formation and allows the leader shoot to dominate over the side shoots. Acrotony in apple shoots develops shortly before spring via this increased growth rate and the distribution of auxins and cytokinins in the shoots at budbreak may be involved in this development (Cook et al., 1998a). Furthermore, budbreak under forcing conditions remains

acrotonic. Even in intact apple shoots, the total inhibition of lateral buds that break in a more proximal position is less than that of more distal buds (Cook, et al., 1998c). Under complete chilling conditions a clearly defined acrotonic behavior in budbreak in apple shoots is evident. This has been described as a normal tendency during an extended period of chilling and results in what has come to be called apical dominance (Cook et al., 1999b).

The lack of an extended dormant period associated with the mild winters in the Western Cape of South Africa, impedes the development of acrotony (Cook et al., 1999a). This can be ascribed to low growth rate of buds (increased endodormancy) and less synchronization among buds at the time of spring budbreak, resulting in both delayed and more erratic budbreak. The most distal or apical buds are most commonly the buds that play a role in acrotony, as opposed to basitony, where long shoots originate from lateral buds of proximal origin (Cook et al., 1998a). Sprouting of upper lateral buds can be enhanced by terminal bud removal (Jacobs et al., 1981).

Basitony is the development of a lateral branching pattern in trees. In apple shoots basitonic behaviour is seldom expressed due to the inhibitions of the distal shoot parts on lower buds because of paradormancy (Cook et al., 1998a). For basitonic branching to occur, proximal buds need to be released from inhibition by distal shoot tissues (Cook et al., 2000). In sub-optimal growing conditions, associated with insufficient chilling and PDS, a basitonic growing tendency with increased breaking potential of lower lateral buds with poor terminal budbreak and growth potential (Cook, et al., 1998a; Cook et al., 1999a; Jacobs et al., 1981) has also been observed.

Basitony is expressed in apple shoots following certain treatments, such as girdling and the application of cytokinins and gibberellins during certain periods (Cook et al., 2000). Autumn application of commercial hormones to the proximal lateral buds increases the growth rate in spring to overcome distal inhibition associated with acrotony by distal tissues (Cook, et al., 2000; Cook et al., 2000; Faust et al., 1997; Steffens and Stutte, 1989). Application of hormones is commonly used commercially in South Africa to promote lateral branching (Cook et al., 2000).

Mesotonic branching involves an even distribution of branches on the main shoot (Crabbé, 1984). A greater ability of lateral budbreak in plum over the entire axis (mesotonic behavior) is an

indication of reduced dominance by the distal buds and may be a result of a shorter and less intense dormancy, i.e., a lower CR (Cook et al., 1998c). Normally with sufficient chilling more lateral buds gain the ability to overcome the dominance of distal buds (Cook et al., 1998c). With extended chilling a shift in growth habit from acrotony towards mesotony was observed in apple in cultivars 'Anna' and 'Northern Spy' (Faust et al., 1995).

2.2.3.2. Branching habit in relation to budbreak

Cultivars differ considerably in branching habit, from strongly acrotonic to mesotonic (Lespinasse and Delport, 1986). In non-manipulated apple trees, the pattern of budbreak normally determines tree architecture (Champagnat, 1978; Crabbé, 1984). Winter chilling and CR influences the intensity of bud dormancy and because buds on the shoot respond differently, the budbreak pattern and tree form also depend on CR and winter chilling and CR. Uniform budbreak provides for a more balanced tree structure.

Branching in spring occurs predominantly from the terminal and distal lateral buds (acrotony) via prolepsis following the period of dormancy (Cook et al., 1998c, Cook et al., 1999b). Occasionally, sylleptic branching on apple trees occurs on vigorously growing shoots, i.e., lateral shoots that develop concurrently with development of the main shoot, without a resting phase (Champagnat, 1978; Cook et al., 1998a). It appears that after their initiation, axillary meristems continue to grow, because of insufficient inhibition by the actively growing apical meristem to form sylleptic shoots (Hallé et al., 1978).

Sylleptic branching that occurs during the early developmental years of the tree life, is an indication of tree vigor and this capacity is used in the formation of feathered apple trees in the nursery (Cook et al., 1998a; Costes and Guédon, 1997). In the feathered tree, subordinate lateral branches develop in combination with a well-defined central shoot or future trunk (Cook et al., 1998a). It is also known that final tree form in orchards depends on the extent of sylleptic shoot formation and lateral branching in the nursery. Therefore, sylleptic branching is considered as an advantage for young tree establishment (Costes and Guédon, 1997) and sylleptic branching is of

practical and commercial importance for apple tree production in areas where chilling is inadequate (Cook et al., 2000).

Under conditions in the Western Cape of South Africa, final tree form in orchards depends largely on whether whips or feathered trees are planted. In the first instance the initial branching occurs via prolepsis and in the second instance via syllepsis (Cook et al., 1998a).

2.2.3.3. Mesotony versus basitonic autonomy

When trees are grown from non-headed, unbranched whips in South Africa, even in the Koue Bokkeveld area, budbreak is more erratic among buds on the same shoots, but also among trees on the same site (Cook et al., 1999a). The erratic budbreak pattern that occurs in association with shoot autonomy complicates the execution of treatments to establish successful apple plantings. It requires notching and scoring to promote lateral branching (Cook et al., 1998a), and the bending of shoots in growing seasons to manage the excessively vigorous lateral shoots (Cook et al., 1999a). Mild winters and the lack of an extended chilling period, and the accompanying ecodormant situation prevents the reinstatement of acrotony (Cook et al., 1998a). Under sufficient chilling conditions basitonic tendencies are poorly defined. During artificial chilling of nursery trees in cold rooms (4 °C) the normal apical control can be maintained and a more "normal" acrotonically branched tree can be developed (Cook et al., 1999a).

Certain cultivars have a higher tendency towards basal dominance or autonomous basal shoot formation (Lespinasse and Lauri, 1996). Extreme basal dominance in some nectarine cultivars, for example, occurs via sylleptic branching, and when these trees are trained from whips and not from feathered trees, the basal dominance can be eliminated (Cook et al., 1998a). Basal dominance of sylleptic shoots that develop during the growing season can be followed by acrotony on individual autonomous basal shoots. Acrotony that develops from unbranched whip trees will show a well-defined acrotony (Cook et al., 1998a). The acrotony form is more desirable from a tree-training perspective due to increased trunk-forming capacity

2.2.4. MEASUREMENT AND QUANTIFICATION OF DORMANCY AND CHILLING REQUIREMENT

Dormancy related traits are defined in the broad-sense as traits associated with any aspect of dormancy induction, maintenance or release (Mauget and Rageau, 1988; Howe et al., 1999). Numerous authors have used dormancy-related traits and phenological stages of tree development to predict the development and release of dormancy and CR as follows, leaving behind a picture of wide discordance in the interpretation of observations, approaches to the problem and terminology.

2.2.4.1. Cold Units

Various biological tests and climatic models have been reported to describe the optimum temperatures for dormancy release with varied results. According to Stushnoff and Quamme (1983), the optimum temperature for satisfying CR is between 0 and 7 °C, since temperatures below 0 °C inhibit enzymatic reactions. A temperature range of –1°C and 0°C was also found to be optimum (Chandler and Tufts 1933). Later reports of temperatures between 0.5 and 4.5 °C were shown to be more effective and temperatures of 9°C less effective. Erez and Couvillon (1987) reported that 6-8°C was the optimum temperature in their material.

Quantitative models of bud dormancy include the use of chill unit accumulation (CU) to describe specific phenological events and the determination of end points of dormancy (Allan et al., 1993; Fuchigami and Wisniewski, 1997; Linsley-Noakes et al., 1994; Richardson et al., 1974; Shaltout and Unrah, 1983, Weinberger, 1950; Wilson et al., 1975). The number of hours below 7.2 °C before budbreak occurs is frequently used as a measure of CU (Linsley-Noakes et al., 1994; Weinberger, 1950). For example, it has been estimated that 'Anna', a low chill requiring cultivar (Brooks and Olmo, 1972) needs approximately 200-300 hours below 7.2 °C to break bud dormancy, compared to 'Dorsett Golden' and 'Golden Delicious' which require 800-900 and 1050-1100 CU, respectively (Hauagge and Cummins, 1991c).

The Richardson- or Utah-model (Richardson et al., 1974) assigns specific values to specific temperature intervals (Table 1.). This model is based on a summation of the effective chilling hours in the winter season, where chilling contribution becomes less as the temperatures rise above or fall below these given mean hourly temperatures.

Table 1. Assignment of cold units according to the Utah-model (Richardson et al., 1974).

Mean hourly temperature (°C)	Assigned Richardson chilling unit (CU)
<1.4	0.0
1.5 – 2.4	0.5
2.5 – 9.1	1.0
9.2 – 12.4	0.5
12.5 – 15.9	0.0
16.0 – 18	-0.5
>18	-1.0

According to Richardson et al., (1974), CU accumulates only between 1.5 and 12.4 °C and not at lower temperatures. Because not all temperatures within this range are similarly effective in breaking, different values between 1.0 and –1.0 are assigned to different temperature intervals. The most effective temperatures for dormancy breaking lie between 2.5 and 9.1°C (Richardson et al., 1974). At temperatures higher than 15.9°C, a negative accumulation is supposed to occur.

The Utah-model has been widely used by the local industry, but tends to be less accurate in the warmer areas of South Africa. The use of the number of hours below 7.2°C as a measure of CR (Weinberger, 1950) applied in other fruit growing areas around the world might also not be appropriate in many warm temperate locations (Linsley-Noakes et al., 1994). The Utah-model transfers values from one month to the following and the effect of cold periods in warmer production areas could be masked (Greybe, 1997). Under Western Cape conditions this model should show negative CU accumulation and an adapted Utah-model was therefore developed. (Linsley-Noakes et al., 1994) in which minor changes to the model improve the accuracy by

deleting the carry-over effect of negating temperatures from one day to the next. The modified model assumes that after a certain period of cold, CU accumulation cannot be broken down by higher temperatures and this limits the negative influence of long periods of high temperatures on CU accumulation. Knowledge of the accumulation of CU can be useful in the forecasting of PDS and producers can use this information in deciding what and when to apply dormancy breaking chemicals and also which cultivars should be planted in a specific environment (Greybe, 1997).

In these biological tests, phenological stages of shoots after a specified time, or the time required for a specified phenological stage to develop, is normally used as the quantitative or semi-quantitative measurement. Criteria include length of the dormancy period (Hauagge and Cummins, 1991a; Lesley, 1944), bud activity, (Hauagge and Cummins, 1991d) bud growth capacity (expressed as the time required for budbreak) (Mauget and Rageau, 1988), rate of emergence of reproductive and vegetative buds (Cook et al., 2000; Halgryn et al., 2001), the percentage of vegetative buds breaking, time of bud set, time of budbreak (Wilson et al., 1975; Herter et al., 1988) and bud activity (Hauagge and Cummins, 1991a). Studies in budbreak dynamics are normally performed on shoots harvested at intervals in winter and just before spring after a given number of chilling hours have accumulated, and then to force them in a temperature controlled environment.

2.2.4.2. Budbreak time

Time of budbreak, also described as bud flush or budbreak, marks the initiation of shoot elongation and is used as an indicator of dormancy release and, hence, fulfilment of the CR. Time of bud set at the end of the growing season and budbreak after fulfilment of the CR is related to climatic cycles (Howe et al., 2000). CR has then been expressed as a time-temperature relationship (Kester et al., 1977) and it appears to be generally accepted that budbreak time is closely related to the bud CR, or that CR is a major determinant of time of budbreak (Bradshaw and Stettler, 1995; Chandler, 1960; Hauagge and Cummins, 1991a; Herter et al., 1988; Weinberger, 1944; Wilson et al., 1975). Rodriguez-A and Sherman, (1985), have found that time of bloom is mainly dependent on CR, rather than on heat accumulation.

Bud set and budbreak are also described as important adaptive traits in natural populations and that patterns of genetic variation of these traits have been molded by natural selection (Howe et al., 2000). Genotypes showing early budbreak during the growing season, such as the cultivar 'Anna', are thus considered to be low CR and therefore more widely adaptable (Chandler, 1960; Hauagge and Cummins, 1991c).

Under mild climatic conditions specifically, budbreak time has also been shown to be closely related to the CR (Hauagge and Cummins, 1991a; Oppenheimer and Slor, 1968). Selection for low CR genotypes in breeding programmes is then normally based on seedlings that break bud within a specified time period. Cultivars with low CR such as 'Anna', 'Ein Shemer' and 'Schlor' were indeed selected using early budbreak as selection criteria, i.e., selection of the earliest seedlings within families (Oppenheimer and Slor, 1968). In plum, budbreak in low CR cultivars have been found to exhibit an ability to be faster under forcing conditions (Cook et al., 1998b).

Various criteria for determining the time of dormancy release have been reported and may be summarized according to authors as follows:

Billington and Pelham (1991): used a scale of 1-9 to assess budbreak and leaf elongation to describe budbreak date (1 = 50% of lateral buds slightly swollen; 9 = 50 % of leaves fully elongated).

Erez and Lavee (1971): The time for 50% budbreak of a bundle of shoots after three weeks of forcing were used as measure of dormancy release.

Hauagge and Cummins (1991b): An index of bud activity, I50, where the I50 value is 50 if budbreak occurred in the first day of forcing at a temperature of 22°C and 16 h light, decreased by 1 for each subsequent day of delay.

Hauagge and Cummins (1991a): The length of the dormancy period expressed as the growing degree hours accumulated from leaf fall until budbreak.

Herter et al. (1988): The mean time of budbreak on cuttings with one bud was used under conditions where the effect of physiological correlations are nullified. Cuttings from the apical end of shoots were used and forced at 25°C, after which the time of budbreak for each bud was monitored in daily intervals.

Mauget and Rageau (1988): Dormancy has been measured by bud growth capacity, expressed as the time required for budbreak on single node cuttings isolated from the tree.

Mauget and Rageau (1988): The mean time of budbreak for the sampled buds was used as an indicator of dormancy – the higher the mean, the more dormant were the buds.

McEachern et al., (1978): Time of budbreak recorded in screening tests where cuttings from trees were forced under controlled conditions after receiving increments of chilling in the field.

Wilson et al. (1975): Time of budbreak recorded as the time at which a leaf tip was visible in 2-4 cm long cuttings taken from trees at monthly intervals. Ratings were given according to the month in wich budbreak occurred.

Growing degree hours has also been reported as an indirect measurement of chilling requirements, since growing degree hours accumulated between leaf fall and budbreak under alternating-temperature overwintering conditions is related to CR in subtropical conditions (Hauagge and Cummins, 1991a). Field based ratings on time of budbreak gave high correlation with a heat-requirement rating (Wilson et al., 1975).

2.2.4.3. Budbreak number

Normally, cultivar CR is determined by forcing dormant buds to budbreak at higher temperatures and assumes that the percentage budbreak increases with chilling (Linley-Noakes et al., 1994; Richardson et al., 1974; Shaltout and Unrath, 1983). It is proposed that budbreak should be more prolific, and will occur promptly and uniformly in low CR genotypes (Chandler, 1960; Hauagge and Cummins, 1991a).

Adaptability classes (referred to as degrees of adaptability) of seedlings have been expressed according to the number of lateral buds broken, uniformity of budbreak and internode elongation (Hauagge and Cummins, 1991c), or according to grades of adaptability related to lateral budbreak on a scale between 0 (least adapted) and 5 (no symptoms of prolonged dormancy observed) (Denardi et al., 1988). In maple, ratings of stages in budbreak have been applied by Kriebel and Wang, (1962). Halgryn et al., (2001), presented results on apple showing a simple relationship

between percentage budbreak and chilling varies considerably between cultivars, areas and years, and in some instances percentage budbreak decreased with chilling.

The number of buds that break on a shoot is dependent on an interaction between chilling and the cultivar branching habit (Halgryn et al., 2001). Chilling may affect the correlative phenomena between buds to enhance acrotonic (reduced percentage budbreak) branching patterns or enhance mesotonic (increased percentage budbreak) patterns of branching (Halgryn et al., 2001).

2.2.4.4. Budbreak rate

The rate of budbreak has also been used to quantify CR (Cook et al., 2000; Saure, 1985; Hauagge and Cummins, 199c, Halgryn et al., 2001). In cultivars where the % budbreak was negatively correlated with chilling, an increase in the rate of budbreak (days to 25% of bundle budbreak) was observed (Halgryn et al., 2001). In this work the rate of budbreak was found to be the most consistent criterium for describing the reaction of buds to chilling. The authors suggest that the rate of budbreak should be used as method to describe the reaction of buds to chilling and can be used for grouping cultivars according to chilling response.

Buds may fail to grow for reasons other than dormancy, e.g., apical dominance, which varies between cultivars (Hauagge and Cummins, 1991b). Bud growth rate (compared to days to budbreak) permits easy comparison of the relative development of buds in different positions (Cook et al., 1998a). The data was presented in the form: $(\text{days to 50\% budbreak})^{-1}$, which measures the bud developmental rate and indicates the growth potential of a bud. According to Champagnat (1978) and Faust et al. (1995) the rate of budbreak is determined by endodormant and paradormant (inhibition by distal shoot tissues) components.

2.2.4.5. Problems and discrepancies with quantification of chilling requirement

The wide variety of proposed climatic models and biological tests to predict development and termination of dormancy and CR was first pointed out by Mauget and Rageau (1988) and more recently reiterated by Halgryn et al., (2001) who note that chilling models to predict dormancy

completion were developed in climates cold enough to satisfy the CR of current cultivars and that these models have been used with limited success in areas with insufficiently cold winters. Another problem is the measurement of CR under a constant temperature regime that is sometimes higher than when measured under field conditions (Hauagge and Cummins, 1991a; Freeman and Martin, 1981). In this situation the continuous chilling treatment may not reflect the real situation under chilling deficient climates (Hauagge and Cummins, 1991b).

It has also been shown that field and controlled chilling differ in their effectiveness in the prediction and release of dormancy (Tehranifar et al., 1998; Mahmood et al., 2000), as expected since high temperatures frequently interrupt chilling temperatures in the field, and more so for warmer growing areas. Differences in CR determined under cold as opposed to subtropical winters have also been found in mature apple clones (Hauagge and Cummins, 1991a). In cherry, the response to chilling temperature of cut shoots differs from that of mature trees (Mahmood et al., 2000). In European plum, no correlation between cutting and fieldbased observations was found (Wilson et al., 1975).

Differences in budbreak between years can also cause discrepancies. In some years buds may not have completely entered dormancy before transfer to the cold room and thus delay the process of budbreak (Halgryn et al., 2001). In the cherry work referred to above, budbreak increased through successive chilling durations at 3.8°C up to a specific point (1000h chilling) and further chilling beyond this point reduced the percentage of budbreak.

In summary of the whole question of quantification and measurement of dormancy and chilling requirement, one can only conclude that this has for long been, and still is, a field of active and justifiable scientific exploration, from the point of view of important criteria of production efficiency in apple and other fruit tree crops. A review of workable criteria for breeding and selection for genetic improvement, implying large-scale screening of cross seedlings and clones, follows in which we are left with time to budbreak and budbreak number under field conditions as the best candidate traits at this point in time, with acknowledgement that we are then dealing with quantitative traits subject to possible multigenic inheritance and macro environmental

(between season and location) and micro environmental (between plots within orchards) variation.

2.3. GENETICS AND BREEDING FOR CHILLING REQUIREMENT IN DECIDUOUS FRUIT TREES

2.3.1. GENERAL STRATEGY IN FRUIT TREE BREEDING

Genetic analyses of fruit traits have usually been done retrospectively from breeding programme data and have often come short in appropriate experimental design for estimation of variance components and heritability of quantitative traits. Families are usually planted in single rows with no replication and randomization (Dicenta et al., 1993; Durel et al., 1998; Hansche et al., 1972; Hansche et al., 1965; Tancred et al., 1995). Due to the nature of the experimental material, measurements useful in estimating genetic parameters are then often confounded with variability due to yearly fluctuations in climate (Hansche, 1965). Yearly climatic differences are known to contribute to the variability of several traits in fruit crops (Hansche et al., 1966; Hansche et al., 1972; Tancred et al., 1995). Recorded examples include significant year X family interactions in a study concerning genetic variation in time of budbreak in Scottish birch (Billington and Pelham, 1991) and time of budbreak in peach (Lesley, 1944).

Breeding fruit and nut crops relies on knowledge of traits being selected for in terms of the genetic systems controlling their inheritance, and genetic and environmental factors that influence their expression (Chandrababu and Sharma, 1999). Strategies employed by breeders of various fruit crops generally include the establishment of high quality but genetically variable foundation stock. Genetic improvement of this stock is performed by selecting and mating genetically superior trees each generation and finally exploiting superior genotypes selected from these families for propagation as cultivars and cultivar replacements (Hansche et al., 1967). Fruit breeding is a long-term and costly process due to the long juvenility period and the large size of mature apple trees (Tancred et al., 1995).

2.3.1.1. Estimation of heritability

Progress in breeding programmes is conditioned by the magnitude and the nature of the genotypic and non-genotypic variation in the traits. Thus, knowledge of the pattern of inheritance

of CR is also important for development of effective breeding strategies (Hauagge and Cummins, 1991b). In perennial plant species, relationships among genetic traits affecting physiological processes can be investigated among families, within families or within individuals propagated vegetatively as clones (Kester et al., 1977). Heritability of quantitative traits is then based on a partitioning the variance among measurements of breeding stock phenotypes, σ_P^2, into genetic and environmental components (Wright, 1921). For example, one can partition σ_P^2 into a component attributable to genotypic differences among seedlings, σ_G^2, and a component attributable to variation in factors of the environment in which they were grown, σ_E^2, i.e., $\sigma_P^2 = \sigma_G^2 + \sigma_E^2$.

In fruit breeding populations these variance components are usually easily estimated. σ_P^2, is estimated as the phenotypic variance among individuals, σ_E^2 as the variance component within clones of a common genotype, and σ_G^2 as the variance component between clones or by subtraction. The ratio of the genetic variance to the phenotypic variance (σ_G^2/σ_P^2) provides a quantitative statement of the relative importance of genetic and environmental causes of variation. It also provides an estimate of the maximum possible value of heritability, referred to as "heritability in the broad sense". According to this definition of heritability, additive and non-additive components of genetic variance are inseparable. Estimation of the additive component of genetic variance (σ_A^2) requires an experimental design allowing for estimation of the covariance beween half-sibs or parent(s) and offspring, or realized response to selection with adequate control (Falconer and McKay, 1996) and leads to "heritability in the narrow sence", σ_A^2/σ_P^2, but high heritability estimates indicate that selection should be effective. The most important function of heritability is its predictive role in selection and expressing the reliability of the phenotypic value as breeding value.

2.3.1.2. Selection strategy

A long-term continuous process of fruit cultivar improvement depends on generation after generation improvement of breeding stocks (Hansche, 1983), i.e., on the continuous increase in

the frequency of alleles having favourable effects on the trait by recurrent selection in successive generations. When considerable additive genetic variance is present in a trait, simple selection procedures based on individual performance are likely to produce significant and predictable genetic gains (Hansche, 1965). Selected individuals are mated at random and the method is referred to as recurrent mass selection. Mass selection for fruit breeding is seen to be simple, and less expensive than more sophisticated methods that include progeny testing and inbreeding that would apply in the case of traits where genetic variance is predominantly non-additive. The time length of selection cycles is also minimized using mass selection.

Generally, in cross-fertilizing species, long-term recurrent selection in successive generations as described above implies the eventual fixation of all favourable alleles present in the initial foundation population and production of pure breeding homozygous lines for commercial exploitation. However, in fruit tree crops which can be propagated vegetatively by cloning, commercial exploitation of selected genetically superior genotypes as single seedlings or trees for subsequent cloning is possible at any stage and does not necessarily rely on homozygosity.

2.3.2. GENETIC VARIABILITY IN DORMANCY AND CHILLING REQUIREMENT

Many studies have revealed a genetic or partly genetic basis for variability in dormancy and other CR related traits and are reviewed in what follows, where a distiction is made between those using the heritability estimation approach and other approaches using the more classical analysis of segregation in progeny groups of crosses.

2.3.2.1 Estimates of heritability

Published estimates of heritability of dormancy traits in trees are summarized in Table 2. These and previous studies on apple have indicated relatively high narrow-sense heritabilities for the length of bud dormancy ranging from 0.66 to 0.69 (Hauagge and Cummins, 1991c). Narrow sense heritability for bud CR as determined by parent-offspring regression on time of bloom in peach was found to be 0.50 (Rodriguez-A and Sherman, 1985). Heritability estimates from parent-offspring regression for an index combining the number and distribution of budbreak

under subtropical winter conditions were found to be between 0.34 and 0.37 (Hauagge and Cummins, 1991c).

In general, it appears that the heritability of dormancy traits can be regarded as intermediate to high and these traits should respond positively to selection, but no indications in published literature have been found where this has actually been put to the test experimentally.

Table 2. Heritability estimates from studies performed on dormancy and related traits in trees.

Trait / Criteria	Fruit kind	Heritability estimate	Reference
Reproductive budbreak time	Almond	0.80	Kester et al., 1977
	Apricot	0.94	Couraujou et al., 1995
	Cherry	0.65	Hansche et al., 1966
	Peach	0.78	De Souza and Byrn, 1998
	Peach	0.93	Mowrey and Sherman, 1986
	Peach	0.39	Hansche et al., 1972
	Peach	0.67	Hansche, 1990
	Peach	0.50	Rodriguez-A and Sherman, 1985
	Apple	0.66 to 0.69	Hauagge and Cummins, 1991b
Vegetative budbreak time	Balsam poplar	0.21 to 0.47	Farmer, 1993
	Trembling Aspen	0.72	Thomas et al., 1997
	Hybrid poplars	0.80	Howe et al., 2000
	Scottish birch	0.65 to 0.63	Billington and Pelham, 1991
Length of dormancy	Douglas fir	0.44	Li and Adams, 1993
Degree of adaptability based on number of budbreak	Apples	0.34 and 0.37	Hauagge and Cummins, 1991b
Blind node propensity	Peach	0.23	De Souza and Byrne, 1998

In a study over successive years, Rodriguez et al. (1986) found that in peach CR genotypes accounted some 87% of the total variance and year effects for only 8%. High within-family genetic variation found for bud set and budbreak has been interpreted as evidence for adaptedness to specific environments (Howe et al., 2000).

2.3.2.2. Segregation analysis

In addition to heritability estimation studies, some further evidence on the nature of genetic variation in dormancy related traits has been reported from segregation observations and analyses of various kinds. In apple crosses where high CR parents were crossed with 'Anna', a low CR parent, it was indicated that variation in the length of bud dormancy, measured in terms of growing degree hours accumulated from leaf fall until budbreak, may be partly ascribable to major dominant genes, modulated by minor interactive genes (Hauagge and Cummins, 1991b). A normal distribution of length of dormancy was not found, implying that variation in the trait was not the result of the effects of additive genes alone. Highly heritable components of low CR, causing marked shifts in family distribution patterns were observed. The authors concluded that high narrow-sense estimates for the length of bud dormancy suggests that this trait is controlled largely by additive genetic variance (Hauagge and Cummins, 1991c).

Oppenheimer and Slor (1968) reported that variation in early budbreak might also be controlled by dominant genes. They postulated that early seedlings in their material could have derived from a recessive foreign parent and a heterozygous Israeli bred parent. A single recessive gene for non-dormancy was found in *Corylus avellana* L. (Thompson et al., 1985). Budsports reported in a range of temperate fruit crops are also evidence of the presence of genes with major effect on budbreak time (Topp and Sherman, 2000).

In contrast, other authors have described dormancy related traits to vary in a quantitative manner and CR is then seen as a complex genetically determined trait (Dennis, 1987; Howe et al., 1999; Howe et al., 2000). The quantitative nature of dormancy-related traits has also been demonstrated in controlled experiments on ripening date of apples (Tancred et al., 1995), cold hardiness in Douglas fir trees (Aitken and Adams, 1995), date of budbreak in Scottish birch (Billington and

Pelham, 1991) and the date of bud set and budbreak in *Picea abies* (L.) (Eriksson et al., 1978). Additive gene action of multiple factors in the determination of temperature response of bud set and budbreak was indicated in *Picea abies* (L.) (Eriksson et al., 1978). Dormancy related genes controlling other characteristics such as branching pattern may also play a role in the process of budbreak and dormancy release (Hauagge and Cummins, 1991c; Howe et al., 1999).

From the distribution classes for CR based on leafing response in peach seedlings, Lesley (1944) has suggested the presence of multiple genes with cumulative effects and absence of dominance. Distribution charts indicated normal distributions centering around the classes of the parental means. Slightly skewed distributions towards the low CR parent was evident, indicating one or a few genes with major effects (Lesley, 1944). Variability in the seven classes used was generally high. It was concluded that CR is based on the presence of multiple genes with cumulative effects. High heterozygosity was evident in these peach cultivars and selections.

Lammerts (1945) has also shown that low CR in peach based on leaf growth rate is due to the accumulation of the effects of multiple genes, some recessive and cumulative. Lesley (1944) and Lammerts (1945) have reported segregation in hybrid families of peach using grading systems. Later studies in peach CR based on leaf bud activity have also suggested multiple gene control with intermediate CR in the F_1 and a continuous distribution with recovery of parental types in the F_2 (Bowen, 1971). Kester (1977) suggested additive gene action in leafing and flowering time of almond.

CR is probably multigenic or, at least, partly controlled by multiple genes. The quantitative nature of the dormancy process and environmental factors and their interactions such as temperature, day length, drought and nutrient availability (Howe et al., 1999) complicates inheritance studies on CR. Soil moisture and nutrients also affect bud set (Howe et al., 2000).

2.3.3. BREEDING FOR CHILLING REQUIREMENT AND ENVIRONMENTAL ADAPTEDNESS

Breeding for low CR has largely relied on subjective observation and, in most cases, without a sound scientific basis (Roriguez-A and Sherman, 1985). However, observational and comparative information have appeared to serve plant breeders well in the development of new climatically adapted cultivars (Hesse, 1975). It apears that selection for lower CR often took place unintentionally and specific criteria for selection of low CR genotypes were not involved. The development of early-ripening peach (Bailey and Hough; 1958; Lammerts, 1945; Bowen, 1971; Lesley, 1944; Rodriguez and Sherman, 1985) and plum (Sherman et al., 1992) varieties, for example, have been important objectives of various breeding stations. More lately, the importance of studies on dormancy related traits at the population or family level, and the importance of genetic variation in these traits, have been emphasized (Howe et al., 1999).

Breeding fruit tree species for mild winter areas requires the use of germplasm with wide genetic variation in CR (Roriguez-A and Sherman, 1985). The pattern of genetic variability within available germplasm and the quality of genetic resources available substantially influences the choice of breeding material, and the success of the breeding programme (Kolliker et al., 1999). Understanding genetic variation in CR is important in designing breeding strategies and for evaluating the potential impact of global climatic changes (Howe et al., 1999). This aspect has not yet received adequate attention in the development of temperate fruits in areas where climatic conditions are sub-optimal for fruit production.

Two attributes important in breeding deciduous fruits for subtropical climates according to Stushnoff and Quamme (1983) are (1) cultivars should have a low winter CR and (2) a high heat requirement is needed to stimulate growth after dormancy. The most important requirement in breeding for low-chill adaptedness is to reduce CR as this allows the trees to break dormancy to a level that can be satisfied by the warmer winters (Topp and Sherman, 2000).

Three procedures have been proposed by Topp and Sherman (2000) to search for the low CR character: (1) selection for low CR within segregating families derived from crossing in

commercial high CR cultivars, (2) selection for low CR in subtropical locations and (3) use of different species as a possible source of low CR. Some Chinese peach varieties, for example, derive from wild species and have a short rest period (Lesley, 1944). The problem with the last method is poor fruit quality traits that are transmitted. Backcrossing to good quality parents is necessary, since in most cases low-chill parents have poor fruit quality (Hauagge and Cummins, 1991c). Once low-chill genes have been located, different selection strategies can be applied based on information on the inheritance of CR.

If emphasis is misplaced on traits of secondary importance, useful cultivars might not be produced in a breeding program (Bringhurst, 1983) unless secondary and primary traits are combined in an appropriate selection index for example, an index including fruit quality and CR traits. Selection within segregating families should preferably be performed under environmental conditions similar to those in which newly released cultivars will be tested and grown commercially.

2.3.4. BREEDING FOR LOW CHILL REQUIREMENT IN APPLE

Very few apple breeding programmes around the world are aimed specifically on low CR. Two institutions involved are The Volcani Institute of Agricultural Research in Israel (Oppenheimer and Slor, 1968) and the IAPAR Agronomical Institute in Curitiba, Brazil (Hauagge and Cummins, 1981). Also, only a few apple cultivars have been specifically developed for adaptedness to low chilling conditions (Hauagge and Cummins, 1991c). The cultivars developed by Oppenheimer and Slor (1968) are utilized by some breeders in programmes to produce cultivars with low CR and high quality fruit. These cultlivars were developed for subtropical regions and include 'Anna', 'Maayan', 'Michal' and 'Schlomit', developed with a CR of 200 to 300 hours below 7°C (Oppenheimer and Slor, 1968). These cultivars were selected from F_2 and backcross populations which had one adapted, unnamed, low CR seedling as the main gene source (Stushnoff and Quamme, 1983). Recent low CR cultivars that are becoming more widely available to apple producers include 'Adina', 'EarliDel', 'Goldina', 'Princessa', 'Primicia' and 'SummerDel' (Janick et al., 1996).

High CR genotypes are not subjected to selection pressures against PDS and these symptoms are likely to be more prevalent in families derived from high CR sources. Because the commercially available low CR cultivars normally have poor quality fruit, further development will require hybridization as reviewed by Hauagge and Cummins, 1991b. These authors concluded that rapid genetic progress toward the goal of high-quality, low CR cultivars can be expected from crosses between 'Anna' and high quality, high-CR cultivars. Combining high quality and disease resistance with low CR could revolutionize apple production in subtemperate and subtropical regions.

2.3.5. PRE-SELECTION FOR CHILLING REQUIREMENT

Since apple seedlings have a long juvenile period (4-10 years), there is an urgent need to incorporate important traits such as CR early in a breeding program (Hauagge and Cummins, 1991c). Methods of pre-screening need to be simple because thousands of seedlings must be assessed in each year of seedling development. If significant response to early selection can be achieved, it is considered as a benefit in tree breeding programmes because it offers a higher return on investment (Magnussen, 1988), mainly because the time between first leafing and first bloom may be several years. It is to the breeder's advantage to minimize the length of selection cycles, since, the shorter they are the greater will be the overall rate of response and the lower will be the cost of the response (Hansche, 1983). The gains from early selection depend on age-age correlations, developmental constraints and adverse genetic correlations (Rehfeldt, 1992).

Several examples of pre-selection in juvenile seedling trees have been reported. The use of secondary traits aiding in selection for time of bloom in peach (Rodriguez and Sherman, 1985), and increase in tree height in Balsam-poplars (Riemenschneider, 1992) and in Black cottonwood (Riemenschneider, 1994). In pre-selection for compact growth habit in apple, the number of side shoots and length-thickness ratio of the central shoot were good discriminating characteristics (Lapins, 1976). In *Prunus domestica* L. (European plum) prolonged winter dormancy with a more regular cropping performance could be achieved when selecting seedlings with high heat requirements (Wilson et al., 1975).

Criteria used previously to select spur type apples included the density of spurs on two-year old shoots, where spur types showed more than 20 spurs per meter, while standards had lower numbers (Blazek, 1992; Warrington et al., 1990). Low CR seedling peach trees were less influenced by short day treatments and lower growing temperatures than higher CR seedlings, suggesting pre-selection for mature tree CR (Lammerts, 1945). Bud set has also been used as an indirect selection criterium for improving frost hardiness and winter survival (Howe et al., 2000).

Inheritance values for the degree of adaptability, a classification index based on number of lateral buds broken, uniformity of budbreak and internode elongation, were higher at high CU accumulation compared to values obtained at low CU accumulation (Hauagge and Cummins, 1991c). According to these authors distinction between CR classes is less clear in colder winters. Oppenheimer and Slor (1968), also found that the continuous treatment of chilling may not reflect real situations occuring under chilling deficient climates.

2.3.5.1. Selection criteria for chilling requirement

Selection for low CR can be accomplished in field trials or by controlling the chilling regime under artificial conditions, i.e. test winters where winter temperatures are simulated under controlled conditions. The latter eliminates climatic variation and provides a systematic approach (Stushnoff and Quamme, 1983). With breeding experiments on complex traits such as CR, however, the work involved in testing numerous seedlings in this manner could be prohibitive and accurate determinations of CR are usually both cumbersome and time consuming (Hesse, 1975).

CR is usually estimated on the basis of vegetative bud development (time, number or distribution of budbreak) or by comparing new cultivars or seedlings with the performance of cultivars with known CR. By proper selection of controls with different CR, the unknown CR be assessed quite accurately (Hesse, 1975). In more accurate assessments for selections of unknown CR, scores obtained from the differences between given cultivars are used (Lammerts, 1941). Parents of control cultivars and seedlings (clones of seedlings) should be in the same growth stage, since juvenility factors may affect dormancy (Hauagge and Cummins, 1991c).

2.3.5.1.1. Budbreak time

Lammerts (1945) has stated that delayed foliation in terms of 'leafing out dates', when leaf growth started on at least the terminal bud and most of the lateral buds, as rating criterion was more accurate than the use of arbitrary grade numbers. This method was the most easily measurable criterion in selecting peaches for mild climates. A significant correlation between peach and nectarine midparent flower bud CR and the resulting seedling families, based on time of bloom in the field, suggest that monitoring time of bloom of seedlings is effective in the prediction of flower bud CR (Roriguez-A and Sherman, 1985). Time required to flowering, measured after artificial chilling and forcing of budbreak at warmer temperatures, was used by Smith et al., (1992). It can probably be accepted that CR quantified in terms of time of budbreak has moderate to high heritability and that rapid genetic progress will be made in breeding through recurrent mass selection, according to Topp and Sherman (2000).

Seedlings classified for CR based on time of flower budbreak, and on cultivars with known CR, were also used by Roriguez-A and Sherman (1985). More than 75% open flower buds was considered as a reliable index of seedling CR. Parental flower bud CR was shown to be a good indicator of seedling progeny flower bud CR, but not of seed progeny CR. However, seed CR and flower bud CR were not as closely related as previously believed.

Oppenheimer and Slor (1968) describe a wide range in budreak time in seedling families of at least seven weeks. 'Anna', 'Ein Shemer' and 'Schlor', cultivars with low CR were selected using early leafing as selection criterium, i.e., seedlings that broke bud within three weeks of the earliest. Time of bloom was selected efficiently on the basis of leafing time in non-flowering juvenile apple seedlings (Oppenheimer and Slor, 1986). Topp and Sherman (2000) also claim that time of bloom can be used as an indicator of CR, the earliest blooming trees having the lowest CR.

Another technique that has been used is the placement and germination of seed under controlled chilling and a discard of those not germinating within a specified time period (Stushnoff and Quamme, 1983). Selection during seed stage for early germination has increased the efficiency of

the selection of peach genotypes for environments with less than 150 hours of chilling accumulation (Pérez Gonzales, 1990). Planting and screening on an intermediate site in terms of CU can accomplish further selection for low CR. There, seedlings should be differentiated between two extremes: some stop growing when daylengths shorten as autumn nights become colder and others should keep growing until frost kills new growth. The last group should be selected for sites with less chilling (Pérez Gonzales, 2000).

Significant positive correlations have been found between CR of seed and bud CR of the seed parents in apple, peach and peach X almond crosses, as reviewed by Roriguez-A and Sherman (1985). This relationships suggest that screening for CR can be performed at the seed stage by monitoring germination time to separate classes of high and low CR. However, low correlation was found between individual peach seed CR and the CR of the resultant seedling. Results suggest possible differences in the genetic bases of seed and seedling CR and that the technique is impractical when differences in CR are less than 300 CU.

Wilson et al. (1975) found a lack of correlation between time of budbreak in the field and dormancy ratings where pre-selection for prolonged winter dormancy was investigated in *Prunus domestica* L. In other words, selection for a long dormancy period did not result in seedlings with late budbreak. A positive relationship was found between time of budbreak and post dormant heat requirements. Thus, later budbreak and flowering could be achieved by selecting seedlings with a high heat requirement.

Flowering date is determined by the interaction of chilling hours received to break dormancy and heat units accumulated to commence flowering (Topp and Sherman, 2000; Billington and Pelham, 1991) and, hence, heat unit requirements should also be taken into consideration. Other secondary problems encountered when applying time of budbreak as selection criterion in warmer climates are bud failure, in which flower buds do not open in spring, reduced pollen viability associated with high bloom-time temperatures, reduced fruit setting associated with high minimum night temperatures (Topp and Sherman, 2000) and blind nodes with no functional vegetative or reproductive buds because of rapid growth during high temperatures (Sherman and Rodriguez, 1994). If budbreak is too early, the risk of frost damage is increased and an

excessively high bud CR will increase the probability that the CR will not be fully satisfied (Hauagge and Cummins, 1991c).

2.3.5.1.2. Number and distribution of budbreak

Chilling requirement (as adaptability values) of seedlings have previously been scored in classes based on a visual classification index according to number of lateral buds broken, uniformity of budbreak and internode elongation (Hauagge and Cummins, 1991d). Classification was also made according to a grade of adaptability from 1 to 10, where 1 indicated no lateral buds breaking and 10 indicated 100% buds breaking (Denardi et al., 1988). Lesley (1944) applied grading of cultivars and seedlings on the basis of leafing response in test winters. Grade 1 was assigned to a tree having the most advanced and even leafing, with no PDS, and required the least chilling. Grade 7 was assigned to one showing the most severe symptoms, resulting in total crop failure, and requiring the most chilling.

In summary it appears that the genetic variation in chilling requirement of seedling families obtained by crossing different cultivars has not been thoroughly explored and the genetics of chilling requirement measured in terms of prolonged dormancy symptoms and in relation to budbreak time has not been adequately investigated. It is also not known whether families and parental genotypes vary significantly in the occurrence of prolonged dormancy symptoms. Breeders have generally relied on selecting the best performing individuals from several years of test data.

It is believed that the genetic variability in the cold requirement character in the apple gene pool can be explored with greater success by establishing procedures which would maximize the discrimination of genetically superior parents and seedlings in segregating families, while minimizing the cost and time required for the selection process. Generally time of budbreak is seen as an indicator of CR, the earliest trees having the lowest CR. But the situation can be more complex because date of budbreak (budbreak time) is determined by the interaction of chilling hours received to break dormancy and other factors such as heat units accumulated to commence budbreak and factors determining inherent tree architectural traits which may influence the

position and number of budbreaks per se. One of the difficulties faced in working with woody plants is the multitude of genes that may be involved in controlling dormancy. CR is obviously an important variable involved in adaptedness of temperate species, including the apple and several traits and their interactions should be considered when optimizing a breeding programme.

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3. BUDBREAK NUMBER IN APPLE SEEDLINGS AS SELECTION CRITERION FOR IMPROVED ADAPTABILITY TO MILD WINTER CLIMATES

Abstract. The absence or long delay in budbreak is considered as the most prominent prolonged dormancy symptom (PDS) during incomplete dormancy release. Budbreak number (NB) was therefore evaluated as criterion to quantify seedling response to chilling and response to selection on excised and intact one-year-old apple (*Malus* x *domestica* Borkh.) seedling shoots under controlled and uncontrolled environmental conditions. Indexes based on (i) the number and distribution of budbreak (PDS grade), (ii) the number of budbreak, including shoot length where increased budbreak occurs in the calculation (PDS index) and (iii) budbreak number expressed as number per 100 cm length of shoot (NB index) were tested in association with budbreak time (TB). The indexes express the effects of cold treatments that induce earlier and higher numbers of budbreak. PDS index and NB index but not PDS grade, identified families with increased budbreak. Seedlings with high PDS grades and NB index values were also identified in families in which high chill requiring parents were used, indicating that TB as pre-selection criterion may fail to successfully identify seedlings with increased budbreak. Response to pre-selection for increased budbreak using PDS grades could be verified with the PDS and NB indexes in seedlings and clones. The NB index applied to intact one-year-old shoots under uncontrolled conditions is proposed as pre-selection criterion against PDS under prevailing sub-optimal winter conditions.

Additional index words. Malus x *domestica*, climatic adaptation, fruit breeding, selection response

Deciduous fruit trees require periods of low temperature for the termination (release) of winter dormancy, broadly referred to as the chilling requirement (CR). If dormancy release is unsuccessful, abnormal and undesirable growth characteristics such as reduced budbreak can occur. This is a common phenomenon on trees growing under mild winter climates such as prevail in the Western Cape of South Africa in areas where temperature requirements for normal dormancy release of current commercial cultivars are not met (Jacobs, et al., 1981; Cook and Jacobs, 2000).

CR is generally considered to be a complex genetically determined trait (Dennis, 1987; Howe et al., 1999) and quantitative or semi-quantitative measurements on a number of related traits have been proposed. Some authors have proposed biological tests and climatic models to predict development, release of dormancy and cultivar CR (Mauget and Rageau, 1988), but the measurement of CR is more usually based on bud development traits such as budbreak time, rate, number or percentage, or by comparing the performance of new cultivars or seedlings with that of cultivars with established CR. Predictions of the timing of various phenological events using models, e.g., budbreak at specified intervals on excised one-year-old shoots under forced conditions, have also been used (Cook and Jacobs, 2000; Halgryn, et al., 2001; Linsley-Noakes et al., 1994; Shaltout and Unrath, 1983; Wilson et al., 1975).

Quantitative models related to bud dormancy involve the use of chill unit (CU) accumulation to describe the phenological events and the determination of end points of dormancy (Allan et al., 1993; Fuchigami and Wisniewski, 1997; Linsley-Noakes et al., 1994; Richardson et al., 1974; Shaltout and Unrah, 1983, Weinberger, 1950; Wilson et al., 1975). According to the Utah-model the most effective temperatures for dormancy release lie between 2.5 and 9.1°C (Richardson et al. 1974). Because the Utah-model transfers values from one month to the following the effect of cold periods in warmer production areas can be masked at temperatures higher than 15.9°C (Greybe, 1997, Linsley-Noakes et al., 1994). An adapted Utah-model was therefore developed for the Western Cape (Linsley-Noakes et al., 1994) in which modifications of the model improve accuracy by deleting the carry-over effect of negating temperatures from one day to the next.

It appears to be generally accepted that budbreak time is closely related to bud CR (Bradshaw and Stettler, 1995; Chandler, 1960; Hauagge and Cummins, 1991a; Herter et al., 1988; Weinberger, 1944; Wilson et al., 1975). Under mild climatic conditions specifically, budbreak time has been shown to be closely related to bud CR (Hauagge and Cummins, 1991a; Oppenheimer and Slor, 1968). Genotypes showing early budbreak such as the cultivar 'Anna', are then regarded to be of low CR and therefore more widely adaptable (Chandler, 1960; Hauagge and Cummins, 1991a). Indeed, 'Anna' was selected using early leafing as selection criterion, i.e., from seedlings that broke bud within three weeks of the earliest budbreak

(Oppenheimer and Slor, 1968). Time of bloom has been selected for indirectly on the basis of leafing time in non-flowering juvenile apple seedlings (Oppenheimer and Slor, 1986). Topp and Sherman (2000) have also claimed that time of bloom can be used as an indicator of CR, the earliest blooming trees having the lowest CR. Lammerts,(1945) used the time when leaf growth starts on terminal and most lateral buds as an easily measurable criterion.

In warmer climates problems can be encountered when applying budbreak time as selection criterion since even when CR during dormancy is met, bud failure can occur because of temperatures not suitable for normal budbreak (Cook et al., 1998; Hauagge and Cummins, 1991a; Mauget and Rageau, 1988), leading to delayed and protracted budbreak. On the other hand, if budbreak is too early the risk of frost damage is increased in some growing areas (Hauagge and Cummins, 1991b). Hence, the interaction of chilling hours and heat to break dormancy can complicate the process of dormancy release (Topp and Sherman, 2000; Billington and Pelham, 1991) and selection for climatically adapted cultivars.

The rate of budbreak as an alternative criterion was found to be the most consistent in describing the reaction of buds to chilling and in the estimation of the CR of established cultivars (Halgryn et al., 2001; Cook et al., 2000; Saure, 1985). When the rate of budbreak is measured, large numbers of shoots from cultivars are harvested from different locations after completion of shoot extension. Bundles of shoots are then chilled and forced for different periods. Budbreak number in each bundle is recorded every two to three days until no further changes are observed. The time is recorded when budbreak is observed in the bundle of shoots on a specified number of shoots per bundle and data presented as the rate of bud development (Cook et al., 1999).

It is expected that budbreak should be more prolific (higher budbreak number), and will occur promptly and uniformly in low CR genotypes (Chandler, 1960; Hauagge and Cummins, 1991a). Adaptability grades (referred to as degrees of adaptability) of seedlings have then been defined according to the number of lateral buds broken, the uniformity of budbreak and internode elongation (Hauagge and Cummins, 1991c). Grades were defined on a scale between 0 (least adapted) and 5 (no symptoms of prolonged dormancy). Ratings of stages in budbreak have also been applied in maple by Kriebel and Wang, (1962). In another case classification in apple was

according to grades on a scale from 1 (no lateral buds breaking) to 10 (100% buds breaking) (Denardi et al., 1988). Lesley (1944) graded peach cultivars and seedlings on the basis of leafing response in test winters. A grade of 1 was assigned to trees having the most advanced and even leafing, with no PDS and requiring the least chilling. A grade of 7 was assigned to trees showing the most severe symptoms, resulting in total crop failure and requiring the most chilling.

Differences in dormancy between the terminal and lateral buds have been found to occur and are ascribed to different physiological states or correlative inhibition by hormonal control causing a positional advantage of terminal buds relative to the more proximal lateral buds (Champagnat, 1978; Cook et al., 1998; Jacobs et al., 1981; Mauget and Rageau, 1988). Buds may also fail to grow for reasons other than dormancy, e.g., correlative inhibition by terminal buds (Hauagge and Cummins, 1991b). Finally, the number of buds breaking has been found to depend on branching habit and interactions between chilling and branching habit (Halgryn et al., 2001).

Against this background of varying approaches to the problem of quantifying CR we have put together the results of five separate experiments carried out during the period 1995-2000 under controlled and uncontrolled conditions investigating in different ways and different materials some aspects of seedling budbreak number as a measure of CR and possible criterion of selection for improved adaptation to local conditions. The main aspects covered in these experiments are (1) the actual response to chilling measured by budbreak number, (2) variation between and within families of crosses and (3) responses to selection.

Variance analysis within and between families (2) and response to selection (3) relate to the detection and measurement of possible genetic variation in CR in families of seedlings obtained by crossing cultivars. Variance component analysis in fruit trees has been carried out by Bell and Janick, 1990; Curie, et al., 2000; De Souza, et al., 1998a; De Souza et al., 1998b; De Souza, et al., 2000; Durel, et al., 1998; Hansche, et al., 1972; Hansche, et al., 1975; Tancred, et al., 1995; Watkins and Spangelo, 1970. We have found no published work on planned experimental selection in fruit trees which was undertaken here to investigate the feasibility of initiating large-scale programmes of controlled breeding and selection for the improvement of adaptation in future, using budbreak number as a practical criterion of selection. We adopted the experimental

procedure of controlled two-way truncation selection for high and low budbreak number in the knowledge that this provides the best possible experimental control for the measurement of response to selection under conditions of limited resources available, i.e., better than maintaining a control population of equal size in which no selection is applied (Falconer and Mackay, 1996). Standard procedures for the experimental measurement of response and estimation of realized heritability then apply.

Materials and Methods

Controlled environmental conditions

Experiment 1. 100 three-year-old seedlings from each of four families ('Starking Delicious' X '2B-18-67', 'American Seedling' X 'Royal Gala', 'Starking Delicious' X '2A-16-15' and 'Braeburn' X '2B-26-59') were transferred to a cold room (4 ± 2°C) at the end of the growing season after leaf fall in June 1995. Seedlings received cold treatment for five periods corresponding to 800, 1000, 1200, 1400 and 1600 CU accumulation during which samples of 20 per family were transferred to a growth chamber (20 ± 2°C) to induce budbreak. Seedlings were randomly positioned in two replications. Twenty-one days after the initial budbreak, seedlings were graded on a scale from 0 to 5 for PDS as judged from the number and distribution of vegetative buds breaking illustrated in Fig.1. The number of days before terminal budbreak for each seedling and the number of days to 50% budbreak for each family were recorded, following the example of Hauagge and Cummins (1991a). Chilling units were calculated according to the method of Linsey-Noakes et al. (1995).

Experiment 2. One-year old excised shoots, approximately 30 cm long, were sampled from four families derived from crosses with the cultivar 'Golden Delicious' as common female parent and cultivars 'Prima', 'Summerking', 'Starking Delicious' and 'Braeburn' as male parents. Family seedlings were growing in randomized blocks and shoots were sampled from trees at two years and three years of age. Orchard management was typical of commercial practice except that no pruning or other tree growth manipulations such as rest breaking treatment were applied. Shoots were transferred to a cold room (4 ± 2°C) during the end of the growing season and then to a growth chamber (25 ± 2°C) to induce budbreak at six sampling dates corresponding to cold accumulation units 610, 800, 1020, 1200, 1400 and 1600 CU. 20 shoots from each family were

randomly positioned in 5 l plastic containers with 1 l water and 5 ml commercial bleach added. A NB index was calculated for each shoot as the number of budbreak per 100 cm length of shoot.

Fig. 1. An index based on the number of buds broken and uniformity of budbreak (PDS grade). This index grades seedlings according to the intensity of prolonged dormancy symptoms after budbreak. Grades range from 0 (no budbreak) to 5 (high number of budbreak). (This index is partly adopted from Hauagge and Cummins, 1991d).

Uncontrolled environmental conditions:

Experiment 3. Controlled hand crosses involving 10 apple cultivars made during 1993 resulted in eleven families with either 'Royal Gala' or 'Anna' as one of the parents. Seedlings were germinated and planted in 1 l plastic planting bags in 1994. The average family size was 278 seedlings. Data were recorded on two-year-old seedlings grown under low chill winter conditions of 737 CU. The TB of each seedling and the PDS grade was recorded 21 days after 50% of each family reached budbreak. Two-way mass selection for high (H selection) and low (L selection) number of budbreak according to PDS grade was performed late during the growing season (December 1995) on each family. After selection, prolonged dormancy symptoms were quantified according to a PDS index where shoot length with increased budbreak was considered

part of the calculation of the index value (examples shown in Fig. 2). NB index values were also calculated for each selected seedling (This same material was used in the subsequent investigation and measurement of response to selection by evaluation of clones of the selected trees described in Chapter 6.)

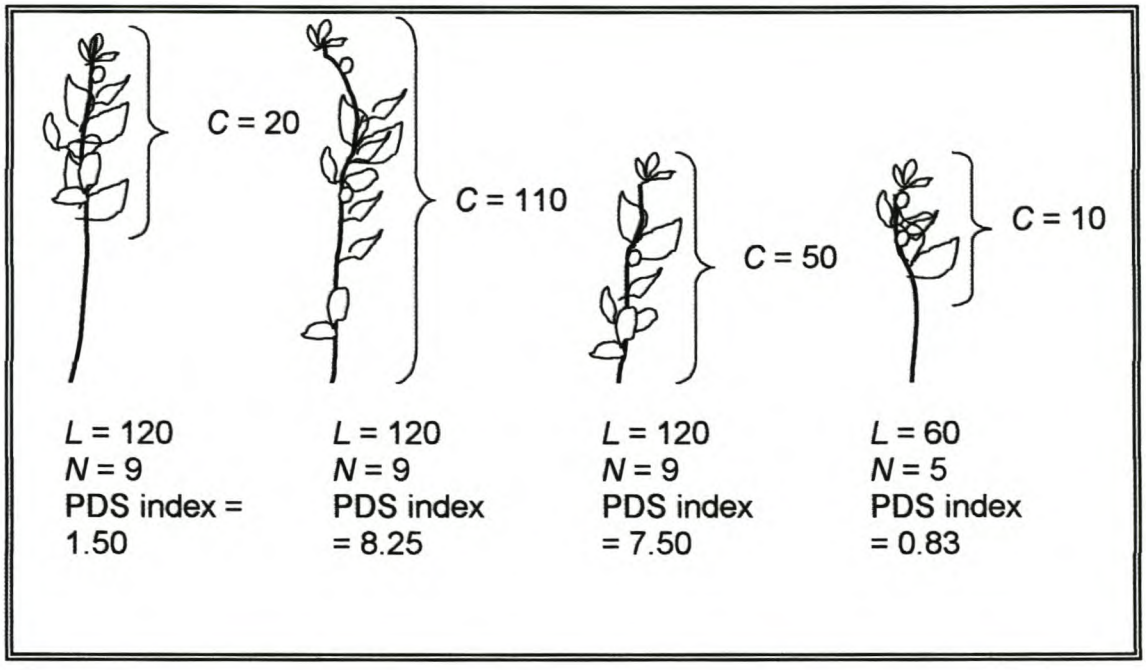

Fig. 2. Prolonged dormancy symptoms (PDS) measured according to a PDS index based on the number of buds broken per shoot, shoot length and distribution of budbreak. Index values were calculated by dividing the measured length of shoot where concentrated budbreak occurred (*C*), by the total length of shoot (*L*) and multiplied by the number of buds broken (*N*) for each seedling (*PDS index* = $C / L \times N$)

Experiment 4. Seedlings developed from ten open pollinated crosses were planted in 1 l plastic bags during the 1996 season. The average family size was 80 seedlings. The TB and NB index values were recorded on one-year-old intact shoots for each family over a period of three years (1997 to 1999). Seedling growth was revived each year to establish strong growing one-year-old shoots for data collection. Chilling accumulated during this period was 846, 878 and 708 CU, respectively. During two seasons (1998 and 1999), families were divided into two experimental

groups, one subjected to a cold treatment (+1400 CU) and the other to prevailing sub-optimal winter conditions.

Experiment 5. Seedlings of four families ('Braeburn' X 'Golden Delicious'; 'Golden Delicious' X 'Royal Gala'; 'Royal Gala' X 'Braeburn' and 'Mollies Delicious' open pollinated), were germinated and planted in pots in 1995. Families consisted of 400 seedlings divided into two experimental groups. During the first season the first group of 200 seedlings of each family received a cold treatment of 1400 CU accumulation and the second group was subjected to prevailing sub-optimal winter temperatures of 730 CU accumulation. Seedlings were planted in randomized blocks in a nursery. TB and NB index values were recorded on each seedling during 1996. Standard nursery practices were applied with no pruning and rest breaking treatments.

During the following season (1997), two-way selection for high (H) and low (L) budbreak number was applied based on the NB index values recorded during the 1996 season. Seedlings (50 in each selection group) with respectively the highest and lowest NB index values for each family - treatment combination were identified. After selection, half of each group (25 seedlings) received a cold treatment and the other half exposed to sub-optimal winter chilling conditions of 846 CU accumulation. TB and the NB index were recorded on each seedling.

As a follow-up of this experiment, 50 seedlings from each of the 1997 selection groups were cloned by budding during 1998. Two trees of each combination were budded on M25 rootstocks. One tree from each combination received winter chilling of 1600 CU accumulation and the other subjected to prevailing sub-optimal winter chilling of 708 CU accumulation. TB and NB index values were again recorded.

Data analysis. Linear regression analyses were performed using SAS Proc. Reg. procedure and analyses of variance (ANOVA) were carried out using SAS General Linear Model procedures (SAS Institute, Cary, N.C.) after testing for heterogeneity of variance by means of the Levene test (Snedecor and Cochran, 1991) and the Shapiro-Wilk test for normality (Shapiro and Wilk, 1965). Data sets were weighted for homogeneity of variance and transformed to reduce deviations from the normal distribution where necessary. Multiple comparisons were performed using Fisher's

and Student's t LSD tests. Correlation analyses were performed using the SAS Correlation procedure.

Results

Controlled environmental conditions:

Experiment 1. In this experiment where seedlings received cold treatment for five periods and forced at 20°C to induce budbreak, median values of the PDS grade showed that 'American Seedling' X 'Royal Gala' had increased budbreak at higher levels of chilling compared to other families. After transformation of the original PDS grades to location values, a generalized linear model technique was used to generate the maximum likelihood estimates of these values which were used in a regression analysis of PDS grade on CU. A separate regression line was fitted for each of the four families and the slopes of these lines were compared using Fisher's Least Significant Difference (LSD) test. The positive slopes of the lines represents an increasing tendency from lower classes to higher classes as the CU increases. Based on PDS grade medians, seedlings showed increased budbreak during cold accumulation with respect to the average regression (R^2 = 0.893) (Fig. 3A). Using the LSD test, it was found that the slopes of 'American Seedling X Royal Gala' (the family with the highest PDS grade increase) differed at 5% level of significance from 'Braeburn X 2B-26-59 (the family with the lowest PDS grade increase). Number of days at 20°C before terminal budbreak decreased during CU accumulation (R^2 = 0.866) (Fig. 3B). ANOVA of days at 20°C before terminal budbreak indicated non-significant differences between families. The number of days to 50% budbreak in all families decreased with cold accumulation (R^2 = 0.944) (Fig. 3C). The family 'Starking' X '2A-16-15' indicated a more delayed budbreak at higher chilling conditions compared to other families. ANOVA could not be performed on days to 50% budbreak because data were based on family mean response and not on individual seedlings. A negative association was found between PDS grade and days at 20°C before budbreak for all sampling dates (R^2 = 0.517).

Experiment 2. One-year old excised shoots were sampled from four families to test response to cold treatment on budbreak number. Regression analysis of NB index on CU for each family over two years are shown in Table 1, indicating significant association for year two only. Regression results indicate that only 2% (non-significant) of the variance in NB index is accountable to CU

in year one and 93% in year two. However a joint analysis of heterogeneity of regression by ANOCOVA (Covariance analysis) for NB index on CU over families showed no differences between families during the second year ($P = 0.1106$). A positive response of budbreak on CU during the second year indicates that experimental or sampling errors may have been involved during the first year's trial. Results during the second year do support NB index as a useful criterion of cold response because of its association with CU treatments.

Table 1. Regression analysis for NB index on CU for four families. Data were recorded on cut shoots forced in controlled conditions after exposure to chilling.

Family	Year 1			Year 2		
	F	*P*	R^2	*F*	*P*	R^2
GD X Pr	4.83	0.0302	0.0436	38.11	0.0001	0.2591
GD X St	2.89	0.0922	0.0268	41.55	0.0001	0.2604
GD X Su	3.04	0.0840	0.0282	37.40	0.0001	0.2407
GD X Br	0.02	0.9003	0.0001	41.56	0.0001	0.2671

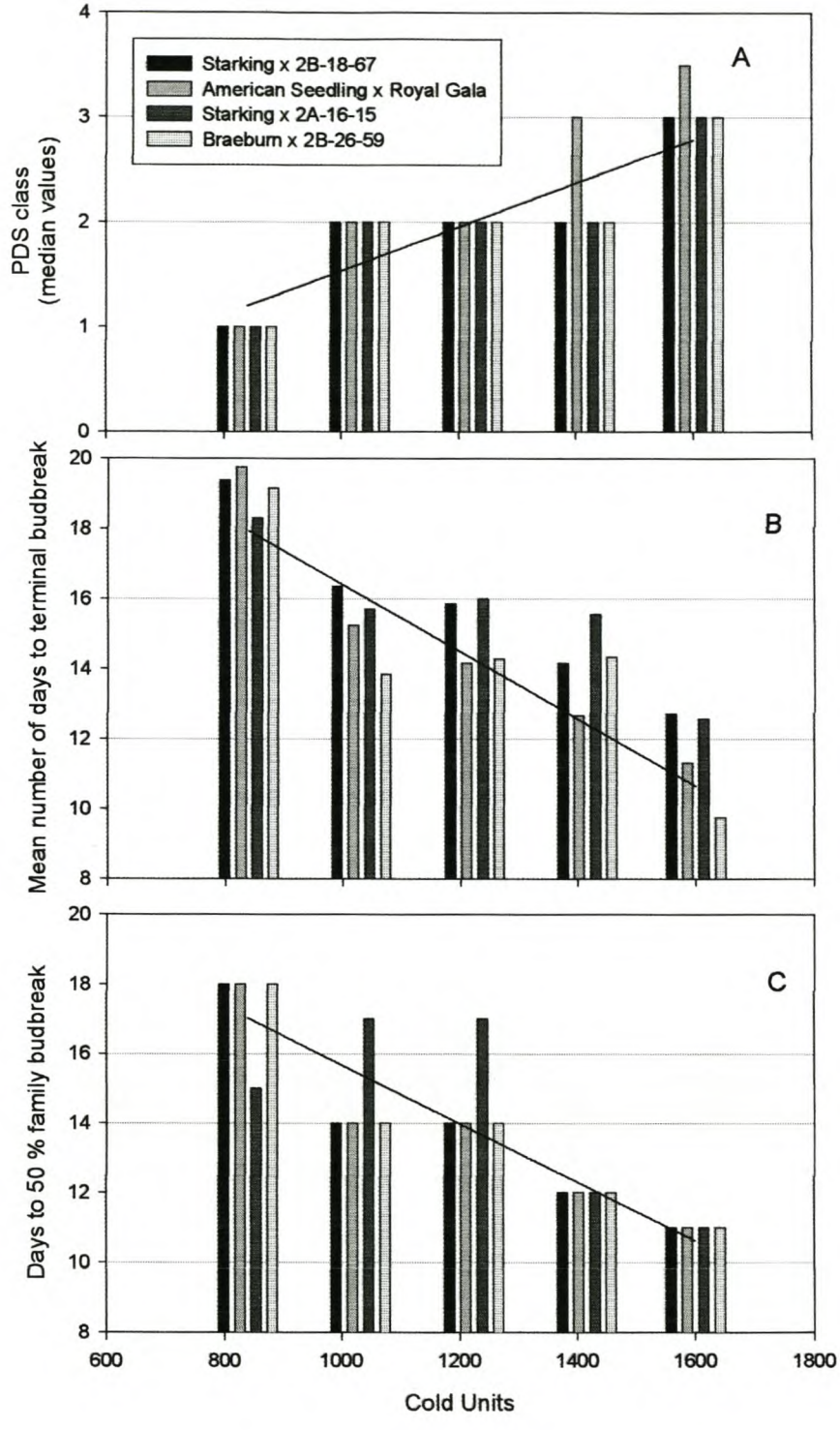

Fig. 3. Changes in (A) PDS grades, (B) number of days at 20 °C to terminal budbreak and (C) days to 50% family budbreak of three-year-old seedlings forced in a controlled environment (20 ± 2°C). Lines represents regression of criteria means on cold unit (CU) accumulation.

Uncontrolled environment

Experiment 3. In this experiment data were recorded on two-year-old seedlings from controlled crosses grown under low chill winter conditions. Median values for PDS grade did not differ between families. The percentage of seedlings per family with high PDS grades, ranged from 6.6% ('Braeburn' X 'Anna') to 20.7% ('Royal Gala' X 'Fuji') (Table 2). Seedlings with high PDS grades (4 and 5) from high CR parents comprised 12.8% of all seedlings and 10.3% from low CR parents and all seedlings with grade 4 and 5, showed budbreak within 21 days of 50% family budbreak. In families where Anna was used as a parent, early budbreak occurred after accumulation of 737 CU. The date on which the first family, viz, 'Royal Gala' X 'Anna', reached 50% budbreak (the "initial budbreak" used as reference point for other families) was mid August 1995. The last families reached 50% budbreak 35 days after the initial budbreak date (Table 2). The time range between the first and the last seedlings for budbreak was 59 days. PDS index values after selection is shown in Table 3. Significant differences of PDS index values were detected between families for both H and L groups ($P < 0.01$). Mean PDS index values for the H group (7.12) differed significantly from group L (0.26). Average correlation over families for PDS index and NB index based on covariance analysis was significant for H ($r = 0.728$) and L ($r = 0.612$) groups (Table 3).

Table 2. Mean family budbreak time and percentage of seedlings within high PDS classes before two-way selection was applied according to the PDS class index.

Family	Number of Seedlings	Days after budbreak	% Seedlings in PDS class 4, 5[x]
RG X An[y]	233	5.610	14.02
Br X An[y]	167	0.00[z]	6.69
Br X RG	174	17.03	6.66
RG X Pc	327	25.49	12.00
RG X Br	325	24.56	6.57
RG X FR	311	31.69	13.97
RG X RC	288	30.38	14.81
RG X Fu	140	19.64	20.70
RG X Fi	230	28.88	19.51
RG X LW	333	35.45	13.37
RG X JR	167	31.00	9.70

[x] PDS classes 4 and 5 refer to seedlings identified visually with high numbers and even distribution of budbreak

[y] 'Anna' has one of the lowest chilling requirements found in *Malus* x *domestica* (Brooks and Olmo, 1972)

[z] Initial budbreak of 'Braeburn' X 'Anna' used as reference for budbreak time in other families

Table 3. Mean PDS index and NB index values calculated for seedling families after selection based on PDS grade. Correlation coefficients indicate association between PDS index and NB index values based on covariance analysis.

Family	Number selected		Mean PDS Index		Mean NB Index		*r*	
	H	L	H	L	H	L	H	L
RG X An[z]	41	43	10.86	0.55	17.47	4.95	0.75	0.78
Br X An[z]	30	31	5.59	0.42	22.56	10.14	0.62	0.63
Br X RG	31	32	8.53	0.12	19.78	4.22	0.38	0.57
RG X Pc	59	59	6.81	0.03	18.82	3.16	0.84	0.58
RG X Br	61	61	8.33	0.59	15.99	3.74	0.89	0.63
RG X FR	56	59	6.09	0.01	18.80	3.16	0.60	0.16
RG X RC	54	53	7.38	0.13	17.09	2.60	0.83	0.59
RG X Fu	27	28	4.10	0.80	15.75	5.10	0.70	0.63
RG X Fi	42	42	6.02	0.29	13.30	3.06	0.79	0.84
RG X LW	63	63	5.34	0.09	13.78	2.97	0.74	0.67
RG X JR	31	30	5.57	0.01	14.32	2.60	0.56	0.87

H and L refer to selection for high and low number of budbreak, respectively

[z] 'Anna' has one of the lowest chilling requirements found in *Malus* x *domestica* (Brooks and Olmo, 1972)

Experiment 4. Differences among families developed from open pollinated seed, indicate significant genetic variance for TB and NB index values on exposure to the cold treatment ($P < 0.01$) and to sub-optimal temperatures ($P < 0.01$) (Table 4). Open pollinated families with lower NB exposed to chilling also showed low mean NB values under sub-optimal temperatures, i.e., 'Golden Delicious', 'Granny Smith' and 'Michal'. Families with high mean NB values when exposed to chilling showed high values under unfavourable conditions, i.e., 'Austin, 'Early O'henimuri' and 'Vista Bella'. All families with early budbreak did not show high NB index values. TB was earlier when cold treatment was applied to seedlings (average difference of 16 days) and cold treatment induced budbreak in seedlings (average increase of 6 buds per 100 cm

shoot length). ANOVA and ANOCOVA on family means indicate significant genetic variance ($P < 0.01$) and negative genetic covariance ($P < 0.01$) between TB and NB index.

Table 4. Mean TB and NB index values on one-year-old seedling shoots after exposure to chilling conditions and sub-optimal winter conditions (control). Data was collected over a two-year period for chill treatment (+ 1400 CU) and over three years for the control (sub-optimal growing conditions).

Family	TB		NB Index	
(open pollinated)	Chill[z]	Control[z]	Chill	Control[z]
Golden Delicious	279 ab	301 ab	16.30 d	10.14 b
Granny Smith	270 bcd	292 b	16.20 d	10.25 b
Michal	267 cd	261 c	15.43 d	10.35 b
Sweet Cornelly	232 f	260 c	17.67 cd	10.78 b
Dorsett Golden	287 a	305 a	15.54 d	11.71 b
Braeburn	275 bc	294 b	21.38 b	13.29 ab
Anna	258 e	236 d	22.99 ab	16.16 a
Austin	270 bcd	267 c	22.28 ab	16.82 a
Early O'Henimuri	266 de	263 c	20.43 bc	16.95 a
Vista Bella	278 b	294 b	24.86 a	17.63 a

[z] Year X Family interaction used as error term in ANOVA

ANOVA performed for each treatment separately

Experiment 5. Cold treatment in this experiment involving four seedling families, initially of 200 seedlings each per treatment in 1996, induced significantly higher budbreak (37%) than the untreated controls in the first test period and there was significant heterogeneity among families (Table 5a). The higher budbreak and heterogeneity among families carried over to the second period of testing after selection in 1997 (67%) and to the cloned seedlings in 1999 (33%). Fig. 4 illustrates the overall effect of chilling on NB and the response to selection averaged over all families for the various combinations of chill and control treatments. Clearly, the initial chill

treatment of seedlings was most effective in stimulating NB and resulted in significantly higher response to selection in both seedlings and clones. Cold treatment induced earlier TB (10 to 15 days) compared to the untreated controls. Heterogeneity among families was significant in most cases according to ANOVA (Table 5b). Table 6 illustrates the overall effect of chilling on TB and the correlated response to selection for NB averaged over all families for the combinations of chill and control treatments. On average the H group was only three days earlier in TB compared to the L group.

Table 5a. Family response to cold treatment measured as NB index values. Chilling of 1400 CU was applied on one experimental group and the second group was subjected to prevailing sub-optimal winter temperatures (control).

Family	1996 Seedlings before selection		1997 Seedlings after selection		1999 Clones after selection		
	Chill	Control	Chill	Control	Chill	Control	Mean
Br X GD	33.03 a	22.11 b	22.34 a	13.02 ab	9.77 a	5.53 a	17.63
GD X RG	26.94 c	18.01 c	16.52 b	8.89 c	8.61 b	4.39 b	13.98
MD[z]	32.05 a	28.82 a	26.90 a	15.78 a	8.42 b	5.24 a	19.54
RG X Br	29.19 b	19.62 b	18.21 b	10.57 bc	6.93 b	5.01 a	14.92
Mean	30.30	22.14	20.99	12.07	8.43	5.04	

Different letters indicate significant difference at $P \leq 0.05$.

[z]Open pollinated family

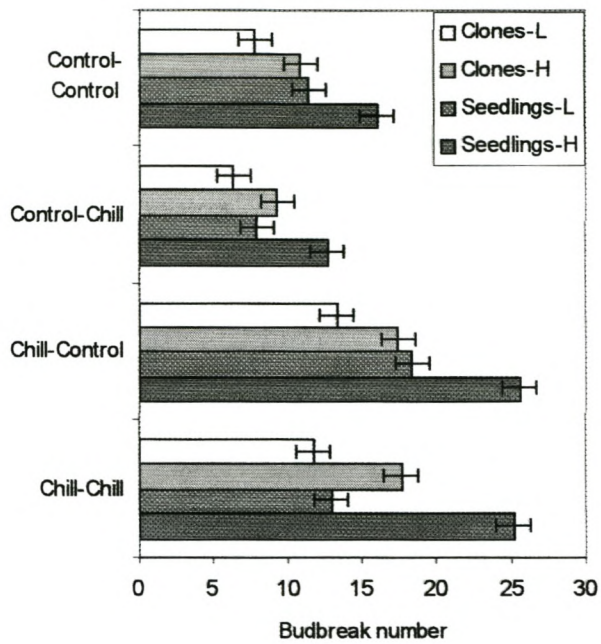

Fig. 4. Budbreak number averaged over families for high (H) and low (L) selections and for different induced chill and control treatment combinations (see text, Exp. 5). Standard Error = 1.14 calculated from ANOVA.

Table 5b. Family response to cold treatment measured as time of budbreak. Chilling of 1400 CU was applied on one experimental group and the second group was subjected to prevailing sub-optimal winter temperatures (control).

Family	1996 Seedlings before selection		1997 Seedlings after selection		1999 Clones after selection		
	Chill	Control	Chill	Control	Chill	Control	Mean
Br X GD	273.45 d	284.35 c	261.35 b	272.01 a	311.83 b	324.79 d	291.52
GD X RG	275.37 a	293.86 a	266.38 a	276.84 a	312.96ab	332.07 a	296.16
MD[z]	274.92 b	285.12 b	263.78ab	272.36 a	313.66 a	329.97 b	293.47
R G X Br	274.51 c	284.06 d	264.00ab	276.93 a	312.39 b	327.23 c	291.95
Mean	274.56	286.85	263.88	274.56	312.78	328.51	

Different letters indicate significant difference at $P \leq 0.05$.

[z]Open pollinated cross

Table 6. Budbreak time averaged over families for high (H) and low (L) selections and for different induced chill and control treatment combinations (see text, Exp. 5).

Treatment	Budbreak number			
	Seedlings		Clones	
	H	L	H	L
Chill – Chill	261.51	264.22	311.58	312.47
Chill – Control	274.35	281.27	325.44	330.89
Control – Chill	263.27	266.53	313.00	313.83
Control – Control	270.18	272.56	326.52	331.12

Discussion

Since apple seedlings have a long juvenile period (4-10 years), there is an urgent need to incorporate important traits such as chilling requirement into breeding programmes at an early stage (Hauagge and Cummins, 1991b). Pre-selection criteria need to be simple and effective because clonal testing is not possible when working with very large numbers of young seedlings. Seedling selection for adaptability to local chilling conditions such as prevail in the Western Cape will then require selection criteria that identify the seedling responses involved as accurately as possible.

Normally, horticultural studies based on phenological events of budbreak are performed on shoots cut from trees during winter and just before spring after a given number of chilling hours have accumulated, and then to force the cuttings in a temperature controlled atmosphere. The chilling regime can be controlled under artificial conditions, i.e., test winters, where winter temperatures are simulated. The latter can eliminate climatic variations and provide a more systematic approach to the problem (Stushnoff and Quamme, 1983).

In breeding experiments on complex traits such as chilling requirement, however, the work involved in testing numerous seedlings in controlled environments has been found to be prohibitive (Hesse, 1975). Furthermore, controlled chilling can also differ from field chilling in the effectiveness of prediction of release of dormancy and chilling requirement (Tehranifar et al., 1998; Mahmood et al., 2000) since high temperatures frequently interrupt periods of chilling in the field. Differences in CR determined under cold vs. sub-optimal winter conditions have also been found in mature apple clones (Hauagge and Cummins, 1991a). It may be more difficult to determine the deepest dormancy, i.e., when the buds are fully dormant, when cold accumulates at a quicker rate as is the case under controlled conditions (Hauagge and Cummins, 1991b). The use of cut shoots has also shown that response to chilling can differ from whole trees in terms of their chilling requirement (Mahmood, et al., 2000; Wilson et al., 1975), and in some years buds may not have completely entered dormancy before transfer to the cold room, thus delaying the process of budbreak (Halgryn et al., 2001). Hauagge and Cummins (1991a) have found that excised

shoots in which the terminal buds have been removed are not reliable for measuring bud dormancy in apple.

Cold temperatures are considered as the environmental trigger responsible for the reduction of CR during winter (Cook, et al, 2000; Cook et al., 1998). Cold has a normalizing effect on the duration of budbreak and the pattern of budbreak seems to be more uniform with increased chilling (Cook et al., 1998). Budbreak number has also been found to increase with increased chilling (Linley-Noakes et al., 1994; Richardson et al., 1974; Shaltout and Unrath, 1983). In apple genotypes adapted to the environment, budbreak occurs promptly and uniformly during spring (Chandler, 1960; Hauagge and Cummins, 1991d). In our experiments where cold treatments were applied (Exp. 1, 2, 4 and 5), earlier and more uniform budbreak was also found and increased cold induced budbreak excepting the first year in the excised shoot assay (Exp. 2). The indexes used in this study quantified the increased budbreak during cold accumulation implying that indexes may also be applied to identify seedlings better adapted to the environment.

Family differences in the controlled environment where chilling could be systematically increased are evident for PDS grade and the number of days to 50% family budbreak, but not for number of days at 20°C (Exp.1). Family differences in the NB index were also evident in excised shoots in the second year of evaluation (Exp. 2). Under uncontrolled conditions, family variation for budbreak time was evident but family differences in PDS grade could not be shown (Exp. 3). Differences in PDS index and NB index successfully identified families with increased budbreak (Exp. 3, 4 and 5) and selection groups (Exp. 3 and 5), and family differences for these indexes were repeatable over years. In Exp. 5, ANOVA identified variation in NB values between seedling families over a period of three years in the young seedlings and seedling clones, and families with increased budbreak showed fairly consistent values over the different treatments.

A negative genetic association between TB and NB index was indicated (Exp. 4). In Exp. 3 and 4, however, seedlings with high PDS grades and NB index values were also found in families where high CR genotypes with later budbreak time were used as parents, indicating that early TB as pre-selection criterion may exclude seedlings with increased budbreak. The rate of budbreak

has also been proposed as a way of quantifying CR of cultivars, but for breeding purposes this approach seems not to be of much use, since clonal replication of large numbers of seedlings consisting of one shoot is a long-term process, laborious and expensive.

Selection at high CU has been found to be less practical to apply than overwintering in sub-optimal winter conditions (Hauagge and Cummins, 1991b; Oppenheimer and Slor, 1968). Under controlled conditions budbreak can increase through successive chilling durations up to a specific point but further chilling can reduce budbreak (Halgryn et al., 2001; Mahmood et al., 2000). Another problem is that the measurement of CR under a constant temperature regime can be different to that measured under field conditions (Hauagge and Cummins, 1991a; Freeman and Martin, 1981). Together with other authors (Hauagge and Cummins, 1991b; Oppenheimer and Slor, 1968), we are also inclined not to encourage the use of controlled chilling for pre-selection since the treatment is unlikely to be the same as the prevailing climatic conditions. Past attempts to adapt the Utah-model for use under local conditions reflect a similar field approach to the problem of evaluating chilling requirement. Our experiment with cut shoots finds that results vary from one year to the next. One possible reason for this is the difficulty in determining the optimum time to collect shoots as dictated by the progression and state of dormancy and dormancy release. The use of the cut shoot assay for pre-selection purposes should be re-evaluated because of contradictions found in this experiment.

The high correlation between PDS index and NB index values (Exp. 3) showed that both indexes can be used to distinguish between seedlings for budbreak number but, because the PDS index (Exp. 1 and 3) is more laborious and time consuming to apply, the use of the NB index (Exp. 2, 3, 4 and 5) is recommended. The increase and uniformity of budbreak in response to cold treatment is an indication of the usefulness of indexes quantifying budbreak number. Although the number of budbreak on intact shoots does not distinguish between correlative factors on budbreak within and outside the seedlings, such as hormonal control, results from these experiments indicate that budbreak number after a specified period does quantify the phenotypic reaction of individual seedlings, as a response to chilling.

Variation between families in our material was clearly detectable using the PDS and NB indexes. Response to selection based on PDS grade indicates that selection on a visual basis can be applied as a pre-selection for increased budbreak, but the ordinal nature of the measurement scale of this index creates limitations for quantitative genetic analyses. It is important to note that seedling age can have some effects on length of bud dormancy (Hauagge and Cummins, 1991c) and possibly on budbreak number. The gains of early selection depend on age-age correlations (Rehfeldt, 1992) and, therefore, it remains to test whether the seedling response to selection for increased budbreak is carried over to an adult phase in order to improve adaptation to local winter chilling conditions. The NB index applied on intact one-year-old shoots under uncontrolled conditions is proposed as pre-selection criterion for selection to local adaptability.

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4. GENOTYPIC VARIATION IN PROLONGED DORMANCY SYMPTOMS IN APPLE PROGENIES

Abstract. Variability of characteristics associated with prolonged dormancy in apple *(Malus* x *domestica* Borkh.) progenies planted in the Western Cape region of South Africa was recorded over a three-year period. The time of initial vegetative and reproductive budbreak, the number of vegetative and reproductive budbreak and the flowering duration were used as criteria. Data were collected on bearing trees of apple seedling families where the cultivars 'Anna' and 'Golden Delicious' were used as a common parent. Analysis of variance detected significant variation among seedling families for time of budbreak, number of budbreak and flowering duration in 'Golden Delicious' families. 'Braeburn' X 'Golden Delicious' consistently produced seedlings with higher numbers of budbreak and 'Golden Delicious' X 'Prima' showed significantly lower numbers than other 'Golden Delicious' families. In 'Anna' families significant differences were found for time of budbreak. 'Anna' families showed higher variability within families than 'Golden Delicious' families. Comparisons of progenies of 'Anna' and 'Golden Delicious' showed large differences in variation for the time of budbreak and for duration of flowering. Mean budbreak of 'Anna' progenies did not differ from 'Golden Delicious' progenies. Associations were found between initial time of budbreak and number of budbreak as well as time and flowering duration. Results reveal high genetic variance in prolonged dormancy symptoms among seedlings within apple families which can be directly ascribed to high levels of heterozygosity in the cultivars used, and should be explored further for the purposes of breeding and selection.

Additional index words. Malus x *domestica*, chilling requirement, climatic adaptation

Apple production areas of South Africa experience fluctuations in temperature during and between winters that harmfully affect the ultimate fruit set and fruit quality. The average winter chilling periods in two of the most important apple producing areas, Elgin (34°S) and Bokkeveld (33°S), are 850 and 1300 chill units (CU), respectively. Apple producers in these areas apply chemical agents to induce more uniform budbreak and thereby achieve better fruit set and fruit quality. Dinitrortho-cresol mineral oil (DNOC) is widely used, but is costly and because of

environmental and health concerns, use of this chemical is to be phased out by 2005. Hence, there is an urgent need to develop apple cultivars better adapted to these low chill conditions. This will reduce production costs by eliminating the need for application of DNOC and other dormancy-breaking chemicals and may contribute to extending the overall production area for apples as well.

The complex nature of dormancy and related processes in temperate fruit trees has led to various quantitative and semi-quantitative measures of dormancy release and to a profusion of poorly defined terms, that are sometimes ambiguous. However, the following terminology has emerged as consistent and useful, and has been adopted in the present study. Control of dormancy by factors residing within the bud itself is referred to as *endodormancy* while control by factors in the plant but outside of the bud as *paradormancy* and control by environmental factors as *ecodormancy* (Kahn, 1997; Lang, et al., 1985). Endodormancy includes the requirement for exposure to low temperatures before active shoot growth in the spring, generally referred to as the *chilling requirement* (Sorensen, 1983). The time of budbreak, as measured from an appropriate origin date, is related to endodormancy (Bradshaw and Stettler, 1995). The endodormant period ends when the chilling requirement has been met. The chilling requirement is a major determinant of time of budbreak. Abnormal growth characteristics observable under mild winter conditions, i.e., temperatures not low enough to meet the chilling requirement, have been variously referred to as symptoms of prolonged dormancy, delayed foliation or extended rest (Jacobs et al., 1981; Janick et al., 1996). Prolonged dormancy symptoms occur as a result of an unmet chilling requirement at temperatures in the range between 4 and 9°C (Richardson et al., 1974) during the rest period, or as a result of unfavorable temperatures during the period of normal budbreak (Cook, et al., 1998; Hauagge and Cummins, 1991a; Mauget and Rageau, 1988). Symptoms include reduced break of vegetative and reproductive buds, prolonged flowering duration, lower fruit set and uneven fruit size. The most prominent symptom is the absence or delay of lateral vegetative budbreak. Prolonged dormancy symptoms are an indication of poor adaptation to mild winter climates (Martinez et al., 1999). These symptoms commonly occur in orchards in the Western Cape region of South Africa since the chilling requirement for normal dormancy release of most commercial cultivars is simply not met.

Most studies of chilling requirements have been based on clones of single cultivars and rootstocks (Cook et al., 1998; Mauget and Rageau, 1988; Powell, 1986) resulting in some understanding of the mechanism of dormancy. However, it appears that the genetic variation in chilling requirement of seedling families obtained by crossing different cultivars has not been thoroughly explored. We believe that the assessment of genetic variation in seedling families from crosses of parents from diverse genetic backgrounds will be an aid in understanding the basic factors controlling winter chilling requirement and dormancy release. Since apple breeding is a costly and long term process due to the long juvenile period of trees, results of such studies demonstrating genetic variability in families of seedlings should be useful to breeders in providing guide-lines for the design of efficient breeding and selection programs.

The present study was undertaken to obtain estimates of the variances of characteristics related to prolonged dormancy in adult apple seedlings planted in mild winter conditions. The specific objectives were: (1) to quantify the variation within and between adult apple seedlings families for further exploration in a breeding program for improved climatic adaptation and (2) to develop evaluation criteria for winter chilling requirement and prolonged dormancy.

Materials and Methods

Plant material. Apple seedlings from controlled crosses were randomly selected from eight families planted in orchards for fruit quality breeding. The crosses had been made for the purpose of evaluation of fruit characteristics with no attention to any specific mating design. Four families with the high chilling 'Golden Delicious' as one parent and four with the low chilling 'Anna' as one parent were used. 'Anna' has one of the lowest chilling requirements found in *Malus* x *domestica* (Brooks and Olmo, 1972), ± 300 CU, compared to ± 1500 CU for 'Golden Delicious' (Hauagge and Cummins, 1991b). 'Golden Delicious' was used as a common female parent with 'Prima', 'Summerking', 'Starking Delicious' and 'Braeburn' as male parents. 'Anna' was used as a common male parent in crosses with 'Austin', 'Sharpe's Early', 'Kirks' and 'Summerred'. No records are available on chilling requirements of the other parents, other than for 'Braeburn' (± 1100 CU), 'Prima' (± 1100 CU) and 'Summerred' (± 999 CU) (Hauagge and Cummins, 1991b; R. Hauagge, personal communication). Seedlings from each family were randomly selected and

the same seedlings were used for measurements during the growing seasons of 1996, 1997 and 1998. The aim was 60 seedlings from each family where possible, but numbers were ultimately between 50 and 60. There was no selection performed at the seedling stage and there was no chance of inadvertent selection for a trait with possible linkage to chilling requirement before the trees were put into the field. The first set of data was recorded during 1996 in the seventh growing year of the 'Golden Delicious' families and the fifth growing year of the 'Anna' families.

Planting design. Seedlings were planted in an orchard in the Western Cape region of South Africa where inadequate winter chilling for standard apple cultivars is normally experienced. The original plantings were not planned to meet the specific aims of the present study. Sibling seedlings within families were planted adjacently in family rows and all four families with a common parent were growing together. However, the areas in which seedlings were planted were frequently inspected and appeared to be adequately uniform and free of any sources of serious bias which might influence the results. Subsequent measurements on clonally propagated selections of this material in properly designed trials provided some support for the assumption of negligible or no bias in the results. Genetic parameters have previously been estimated in trials of this nature in several fruit tree crops (Dicenta et al., 1993; Hansche et al., 1972; Tancred et al., 1995). The four 'Anna' families were planted approximately 100 m from the 'Golden Delicious' families and here it seemed advisable to judge any differences between these major groups with caution. Orchard management was typical of standard commercial practice except that no pruning, chemical thinning or rest-breaking treatments were performed.

Data collection and measurements. Preliminary observations identified three major criteria quantifying seedling reaction to winter chilling and prolonged dormancy under sub-optimal climatic growing conditions: (1) the date of initial vegetative budbreak (IVB), scored as the time of the first sign of green leaves emerging from any vegetative bud, (2) the total number of reproductive and vegetative budbreak and (3) the flowering duration. The number of reproductive budbreak was scored as the number of reproductive buds that had burst at the date of last reproductive budbreak (LRB), i.e., the time when the last flower buds on the tree had reached the balloon stage. The number of vegetative budbreak was scored as the number of buds that had

burst 21 days after the IVB, a period regarded as adequate for apple trees to express ability to overcome the state of dormancy (Faust et al., 1995; Hauagge and Cummins, 1991b). The flowering duration was calculated as the difference between the date of initial reproductive budbreak (IRB), scored at the first sign of flowers in the tight cluster stage and LRB.

Three intact branches per seedling tree at even height and growing direction were randomly selected to record the number of buds and shoots on three years of growth. Normally, one-year-old shoots are used as analogous units to represent adult tree behavior (Cook et al., 1999; Costes and Guédon, 1997). In this study, branches with three season's growth were therefore regarded as suitable and representative. The total length of the three-year-old selected branches was recorded and the number of side shoots on these branches was recorded in the following classes: 0.5 - 5 cm, 5-15 cm, 15-30 cm as well as shoots exceeding 30 cm. Budbreak was then expressed as the number of buds per 100 cm length of shoot. During the second and third year of data collection the number of vegetative budbreak was recorded at 21 days and 42 days after IVB in order to assess the increase of budbreak over time as an indication of the period of vegetative budbreak. Chill units were calculated according to a modified Utah model found to be more suitable for local chilling conditions where negative CU values are not carried from one day to the next (Linsley-Noakes et al., 1994).

Variance structure. The variance structure in the seedling families may be broken down as follows, where the primary interest lies in estimating the underlying causal components of variance from the observations, applying standard quantitative genetic principles (Falconer and Mackay, 1996):

(i) variance of seedling trees within families of the same cross

$$\sigma_W^2 = \sigma_g^2 + \sigma_e^2$$

where σ_g^2 is a genetic component (generated by crossing in this case) and σ_e^2 a component ascribable to environmental variables within the trial orchard

(ii) variance between families

$\sigma_B^2 = \sigma_G^2 + \sigma_W^2$

where σ_G^2 is the genetic variance between families for a given common parent ('Golden Delicious' or 'Anna').

If we had have had repeated measurements on clones of a given genotype (say clones of size *n* per seedling) in just one season, the analysis of variance (ANOVA) and expected mean squares (EMS) for the estimation of components would then read:

Between families (N trees per family)	$\sigma_e^2 + n\sigma_g^2 + Nn\sigma_G^2$
Between seedlings within families	$\sigma_e^2 + n\sigma_g^2$ (A)
Within clones of one seedling	σ_e^2 (B)

The intra-class correlation coefficient relevant to selection between seedling within crosses is

$t = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$ estimated by $\frac{A-B}{A+(n-1)B}$ with standard error of the estimate

$$\mathrm{SE}(t) = \sqrt{\frac{2[1+(n-1)t]^2(1-t)^2}{n(n-1)(N-1)}}$$

The equivalent intra-class correlation coefficient relevant to selection between families is

$t = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_W^2}$ (used here for comparative purposes only). In the present experiments the repetition of measurement was in fact on the same tree in different seasons, involving possible genotype-environment interactions at two levels, *viz*,

(i) year X family interaction, σ_{GE}^2 and

(ii) year X seedling interaction within families, σ_{gE}^2

Conceptionally, ANOVA and EMS can then be done in two parts (Kempthorne, 1961), assuming y years of measurement and N trees per family:

(i)	Years (Y)	not relevant	
	Families (F)	$\sigma^2 + N\sigma^2_{GE} + Ny\sigma^2_G$	
	Y X F interaction	$\sigma^2 + N\sigma^2_{GE}$	
	Residual	σ^2	
(ii)	Seedlings within families	$(\sigma^2_e + N\sigma^2_{gE}) + y\sigma^2_g$	(A)
	Y X trees within families	$(\sigma^2_e + \sigma^2_{gE})$	(B)

Note that in the latter analysis, the environmental variance (within orchard) and genotype-environment interaction cannot be estimated separately, since only one observation is made on each tree each year. Estimation of the intra-class correlation follows the same steps as above but the actual value is reduced if genotype-environment interaction is present.

ANOVA was carried out on measurements for separate groups ('Anna' and 'Golden Delicious' families). Separate analyses were carried out for each year, and a joint analysis over the three years to test for Year and Year X Family (Y X F) interaction effects and to estimate variance components and intra-class correlations. The seedling-within-families mean square was used to compare between families if Y X F interaction was not significant, i.e. differences between family means were compared with differences between seedlings within families. In cases where significant Y X F interaction was found, the mean square for Y X F was used as error term in the ANOVA. The analyses were performed using SAS General Linear Model procedures (SAS Institute, Cary, N.C.) after testing for heterogeneity of variance by means of the Levene test (Snedecor and Cochran, 1991) and the Shapiro-Wilk test for normality (Shapiro and Wilk, 1965). Data sets were weighted for homogeneity of variance and transformed to achieve normal distributions if the tests showed skewed distributions. Multiple comparisons were performed using Student's t LSD test. Variance components and intra-class correlation coefficients were calculated using the SAS Variance Component Estimation procedure.

Results

Winter chilling conditions recorded up to the end of August for the three years of observation were 621 CU for 1996, 676 CU for 1997 and 748 CU for 1998. Unusually cold temperatures for this area were experienced during the first year (1996) when the total CU accumulation toward the end of November was 921.

Between - family variation. ANOVA detected significant levels of variation for IVB among 'Anna' families (Table 1), and significant variation among 'Golden Delicious' families for IVB and number of budbreak (Table 1). Family means over three years of measurement are shown in Figure 1. 'Anna' X 'Austin' was consistently earlier than the other families. The 'Golden Delicious' X 'Braeburn' family showed budbreak earlier than the other 'Golden Delicious' families and had a higher number of budbreak. 'Golden Delicious' X 'Prima' showed a lower number of budbreak. In 'Golden Delicious' families the between – family variance for IVB, expressed as a percentage of the total variance was 19.6% compared to 4.8% for 'Anna' families (Table 2). Corresponding values for the number of budbreak were 8.7% and 0.5%, respectively. Differences among families for flowering duration were not significant for either set (Table 1) and intra-class correlation coefficients (Table 2) also indicate relatively low between family variation in this variable.

Year X Family interaction. Significant Y X F interaction is apparent for 'Golden Delicious' crosses for all measurements, but only for flowering duration in 'Anna' crosses (Table 1). Expressed as a percentage of the total variance (Table 2), Y X F interaction was small compared to other components contributing to the total variance.

Table 1. Analysis of variance for three criteria associated with prolonged dormancy symptoms in 'Anna' and 'Golden Delicious' families. Data were recorded on adult trees in sub-optimal winter chilling conditions during three years (1996, 1997 and 1998).

	Anna Families				Golden Delicious Families			
Criteria and Source of variation	*Df*	*MS*	*F*	*P*	*Df*	*MS*	*F*	*P*
Budbreak time (IVB)								
Year	2	9.559	9.76	0.0001	2	68.272	24.84	0.0001
Family	3	11.029	11.26	0.0001	3	76.689	27.91	0.0001
Y X F interaction	6	271.590	0.08	0.9978	6	2.748	2.75	0.0120
Residual	699	0.979			686	1.000		
Budbreak number								
Year	2	12.227	12.23	0.0001	2	183.087	65.39	0.0001
Family	3	2.605	2.60	0.0509	3	33.165	11.84	0.0062
Y X F interaction	6	0.741	0.74	0.6166	6	2.800	2.80	0.0107
Residual	699	1.000			688	1.000		
Flowering duration								
Year	2	198.070	21.45	0.0018	2	101.150	22.82	0.0016
Family	3	9.096	0.99	0.4603	3	10.723	2.42	0.1645
Y X F interaction	6	9.233	4.50	0.0002	6	4.433	4.15	0.0004
Residual	672	2.051			642	1.069		

Table 2. Variance components and intra-class correlation coefficients for criteria associated with prolonged dormancy symptoms in four 'Anna' and four 'Golden Delicious' families.

		Source of variation					Intra-class correlation	
Criteria	Fam.	Year (Y)	Family (F)	Y X F	Within F	Residual	t_1	t_2
IVB	An	26.87	37.87	0.00 (± 0.00)	659.07 (± 63.04)	58.65	0.05	0.92 (± 0.06)
	GD	21.06	26.99	4.15 (± 2.85)	53.04 (± 6.08)	32.79	0.20	0.62 (± 0.19)
Total NB	An	1.36	0.19	0.05 (± 0.22)	16.24 (± 2.16)	19.53	0.01	0.45 (± 0.20)
	GD	15.09	3.89	0.91 (± 1.23)	10.81 (± 1.40)	14.12	0.09	0.34 (± 0.21)
Flower	An	0.83	0.01	0.14 (± 0.09)	0.67 (± 0.12)	1.39	0.00	0.32 (± 0.19)
duration	GD	0.44	0.04	0.07 (± 0.05)	0.17 (± 0.05)	0.90	0.02	0.12 (± 0.10)

Table 3. Within-family variation for initial time of budbreak, number of budbreak and flowering duration. Values are averaged over three years.

	TB				Total NB				Flowering duration			
Family	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
An X Au	192	283	232.75	27.29	2.77	20.39	10.75	4.60	17.00	73.30	42.17	12.65
An X SE	196	288	242.82	29.56	3.03	24.65	11.68	4.53	13.00	78.60	39.71	14.76
An X Ki	194	288	240.85	23.97	3.15	22.90	12.37	4.49	6.50	78.00	43.86	14.35
An X Su	203	285	250.00	26.17	4.27	23.99	11.59	4.10	47.50	66.00	37.36	11.83
LSD (0.05)			12.26				7.07				1.88	
GD X Pr	267	304	282.98	8.89	2.59	20.79	10.63	3.52	8.00	57.30	29.41	8.31
GD X St	263	298	278.66	8.04	4.32	25.34	12.67	4.16	9.50	43.30	25.54	6.78
GD X Su	267	299	283.62	8.36	2.60	27.50	12.95	4.38	10.00	40.00	23.40	7.75
GD X Br	260	290	271.53	6.17	8.49	24.38	15.87	3.82	12.30	43.60	28.18	5.65
LSD (0.05)			9.16				6.01				1.52	

Within family variation. Significant variation between seedlings for all traits measured reflects that the genotypic variation present within families was much higher than that between families (Table 2). A high degree of variation was present within 'Anna' families for IVB and for flowering duration as is evident from values and variances in Table 3 and Fig. 2. The variance component between seedlings for IVB was 84% and 38% of the total variance for 'Anna' and 'Golden Delicious' crosses, respectively (Table 2). Corresponding values for the number of budbreak were 43% for 'Anna' and 24% for 'Golden Delicious' crosses. Relatively high intra-class correlation coefficients within crosses are evident for IVB (0.92 for 'Anna' and 0.62 for 'Golden Delicious') compared to correlation coefficients for number of budbreak (0.45 for 'Anna' and 0.34 for 'Golden Delicious') (Table 2). Intra-class correlations for flowering duration were lower in both cases (0.32 for 'Anna' and 0.12 for 'Golden Delicious'). A low number of budbreak (less than 10 buds per 100 cm length of shoot) was found in many 'Anna' and 'Golden

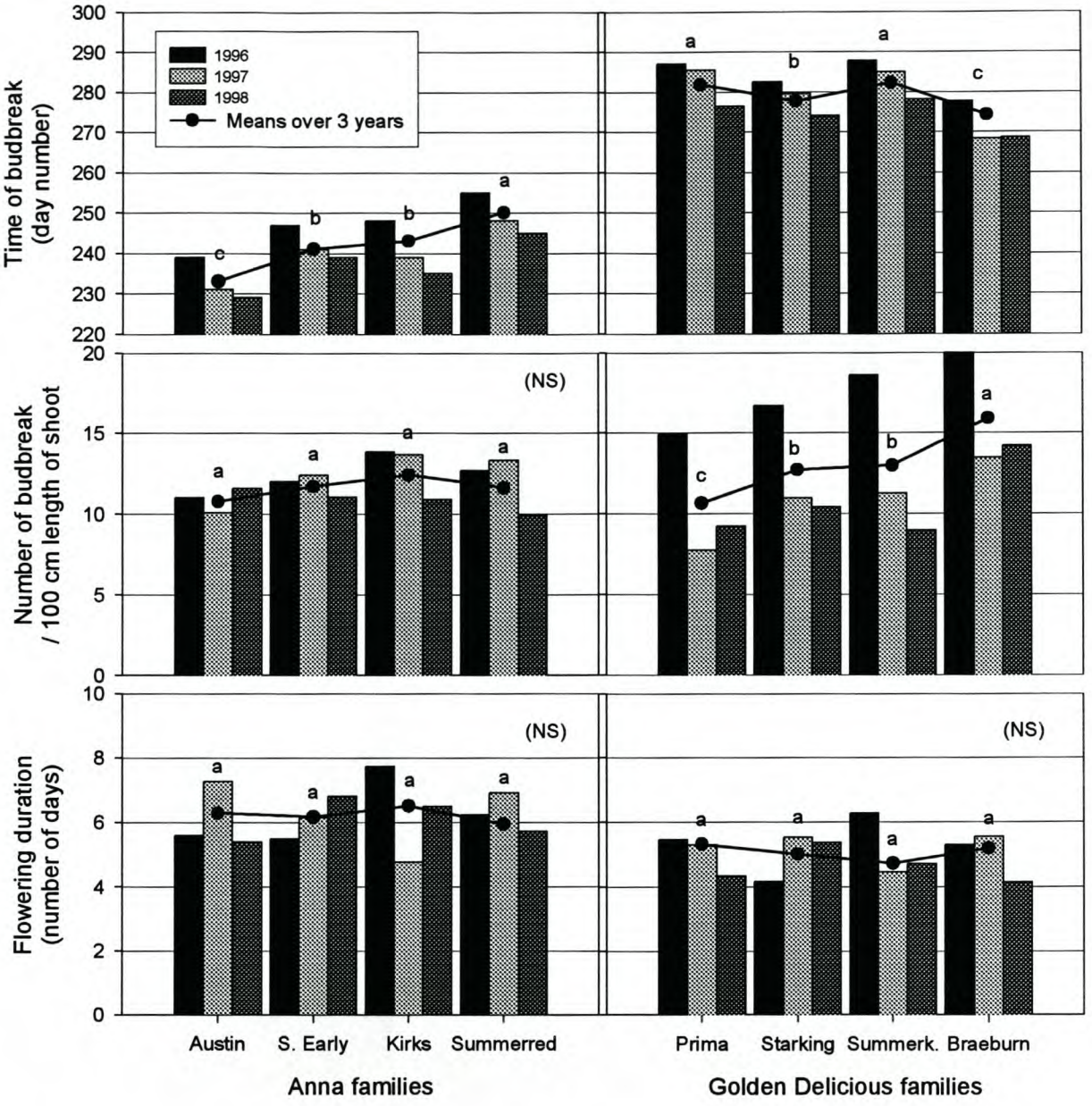

Fig. 1. Between-family variation for mean time (IVB) and total number of budbreak and for mean flowering duration over three years in 'Anna' and 'Golden Delicious' progenies. Families include 'Anna' as a common parent with 'Austin', 'Sharpe's Early', 'Kirks' and 'Summerred' and 'Golden Delicious' as a common parent with 'Prima', 'Starking Delicious', 'Summerking' and 'Braeburn'. ANOVA was performed separately for 'Anna' and 'Golden Delicious' progenies. Student's *t* LSD test was used to separate means. Letters indicate significant differences between means at $P \leq 0.05$ (Values for flowering duration were log transformed before analysis).

Delicious' seedlings (40.7 and 23.9%, respectively) (Fig. 2B). In general, 'Anna' families showed greater variation than 'Golden Delicious' families for all traits.

Variability between the 'Anna' and 'Golden Delicious' progenies. As pointed out in the introduction, the original plantings were not specifically planned to compare 'Golden Delicious' and 'Anna' progenies and any such comparison should therefore be made with care. However, some marked differences unlikely to be attributable to within season environmental factors at the experimental site became apparent and should perhaps not be ignored in future testing and selection decisions. These are illustrated in Fig. 2 and may be summarized as follows.

Seedlings of 'Anna' differed markedly from those of 'Golden Delicious' in the mean and distribution of IVB (Fig. 2A) showing initial budbreak at around 180 days compared to 250 days in 'Golden Delicious'. For each year 65%, 55% and 60% respectively, of 'Anna' seedlings exhibited budbreak earlier than the earliest seedlings of 'Golden Delicious'. A much wider time range from first to last budbreak was also apparent in the seedlings of 'Anna' (93 days), compared to the 'Golden Delicious' seedlings (36 days). This is also evident from the coefficients of variation: 11.7% in 'Anna' crosses and 3.1% in 'Golden Delicious'.

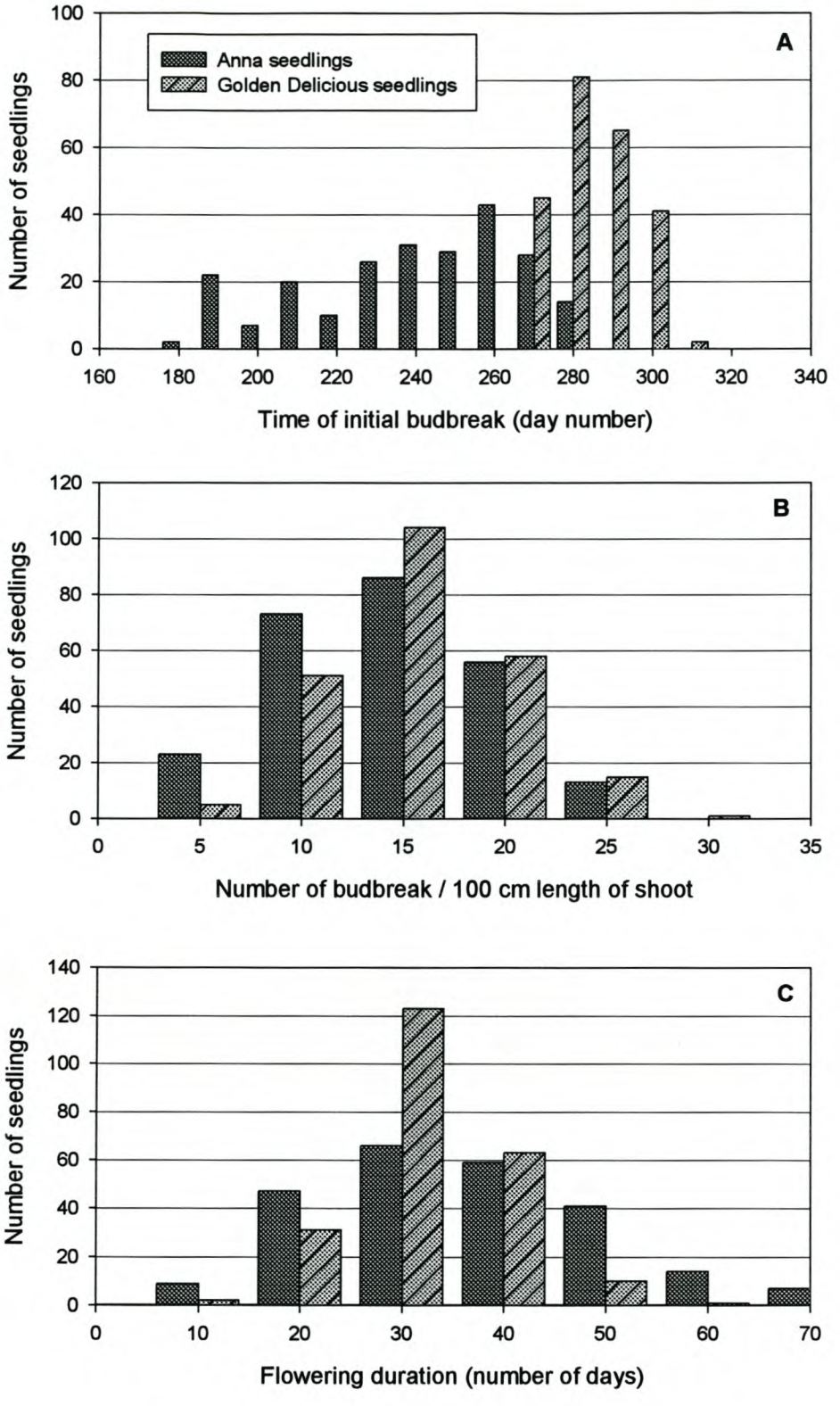

Fig. 2. Seedling frequency distribution of means over three years for (A) time of initial budbreak (B), total number of budbreak and (C) flowering duration in eight apple families.

Although more seedlings in 'Anna' crosses appear in the lower distribution classes for total number of budbreak (Fig. 2B), the mean numbers do not appear to differ significantly. 'Golden Delicious' seedlings exhibited higher budbreak (17.48) in the first season compared to 12.32 for 'Anna' seedlings (Fig. 1) and the percentage of seedlings with budbreak numbers in excess of 15 per 100 cm shoot length over all three seasons was 28% as against 31% in 'Golden Delicious' based on the data summarized in Fig. 2B. 'Anna' seedlings exhibited a wider range in flowering duration by some 13 days (Fig. 2C).

Year to year performance. The ANOVA indicates significant differences between years in all measurements in both family groups and might have been predicted from the different weather patterns recorded during these years in the region. However, yearly fluctuations in 'Golden Delicious' families were considerably higher than in 'Anna' families. Variance component analysis showed 15% attributable to year effects for IVB and 34% for number of budbreak in 'Golden Delicious' compared to 3.4% and 3.6%, respectively, in 'Anna' (Table 2). Yearly fluctuations for flowering duration were greater, being approximately 27% for both family groups.

Correlation analyses. A positive association between IVB and number of budbreak was found in 'Anna' families, but in 'Golden Delicious' families the association was negative (Table 4). As indicated, more than half of the 'Anna' seedlings sprouted earlier than seedlings from 'Golden Delicious'. Of these, 39% showed low numbers of budbreak (below 10 buds per 100 cm length of shoot) (Fig. 3A). During the second and third years, budbreak in 'Golden Delicious' seedlings occurred after final winter chilling accumulation and therefore no correlation analysis could be performed between data collected on CU accumulation and data on number of budbreak and flowering period.

Table 4. Correlation analyses for number of budbreak and flowering duration with time of budbreak and chill units (CU) accumulated in 'Anna' and 'Golden Delicious' families over a period of three years.

		Time of budbreak		CU	
Criteria	Year	An	GD	An	GD
Total number of budbreak	1996	0.66	-0.31	0.67	-0.29
	1997	0.32	-0.63	0.39	[z]
	1998	0.12	-0.36	0.23	[z]
Flowering duration	1996	-0.33	-0.39	-0.33	-0.40
	1997	-0.66	-0.22	-0.59	[z]
	1998	-0.54	-0.23	-0.48	[z]

All values were significant at $P = 0.001$, except for total number of budbreak and time of budbreak of 'Anna' families in 1998 ($P = 0.07$)

N values approximately equal (N = 216 - 236)

[z] Budbreak occurred after total CU accumulation, therefore, no correlation analysis was possible for data on chill units, number of budbreak and flowering duration on 'Golden Delicious' families.

Low correlations between the number of budbreak 21 days after IVB and the difference between number of buds sprouted 42 and 21 days after IVB were found in both years for both 'Anna' crosses ($r = -0.385$ and $r = -0.175$, respectively) and 'Golden Delicious' crosses ($r = 0.113$ and $r = -0.168$, respectively), implying little or no budbreak after 21 days. Although the overall correlation coefficients was low, more seedlings in 'Anna' families with early budbreak showed a prolonged period of vegetative budbreak when compared with 'Golden Delicious' families (Fig. 3B). Higher correlations for IVB and flowering duration and for CU accumulation and flowering duration were found in 'Anna' families than in 'Golden Delicious' families (Table 4 and Fig. 3C).

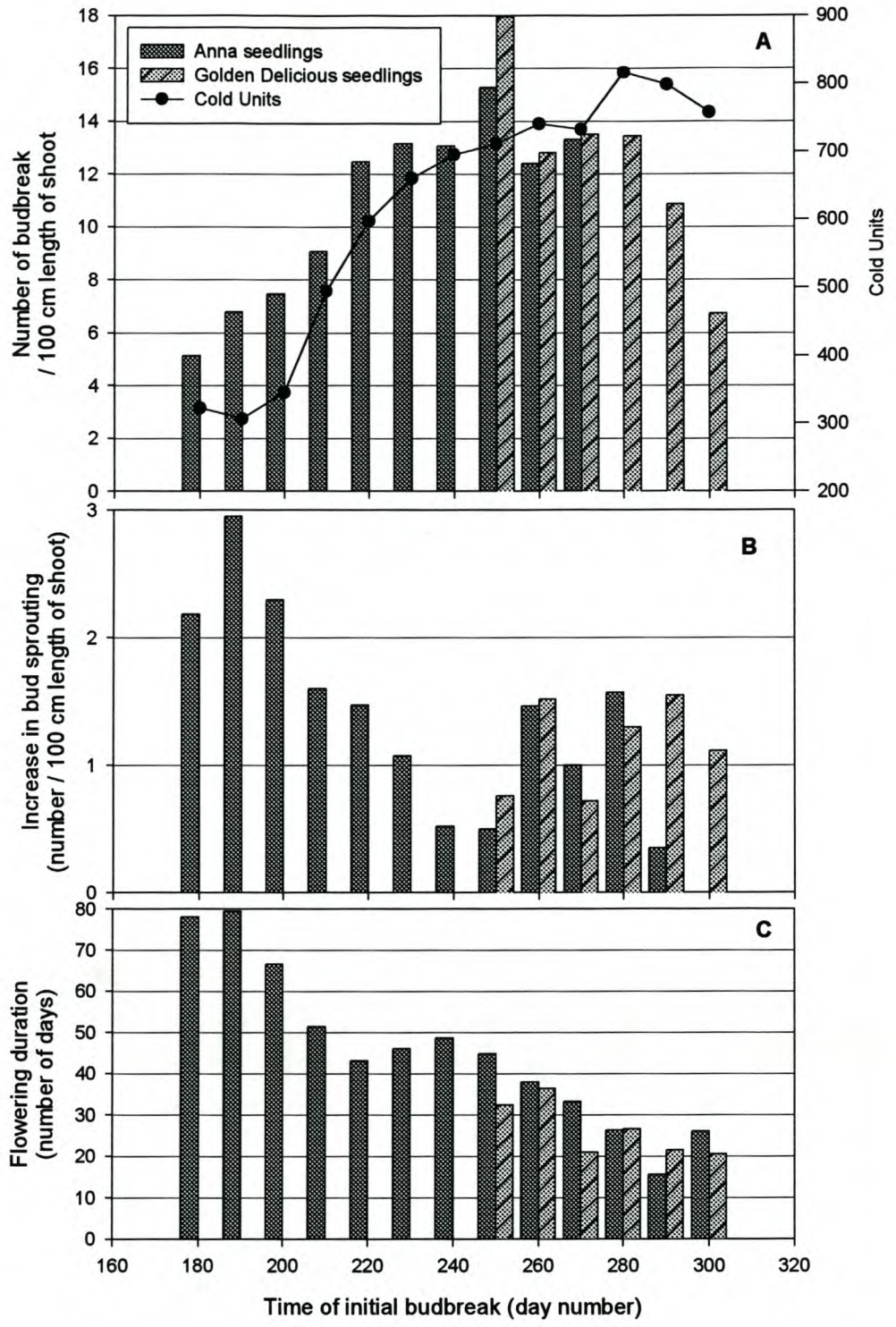

Fig. 3. Distribution curves for (A) mean number of budbreak over three years according to time of budbreak (IVB) and accumulation of cold units (B) for increased vegetative budbreak

21 days to 42 days after IVB and (C) mean flowering duration over three years in eight apple progenies.

Discussion

Low winter chilling and fluctuations in temperature with exceptionally hot days and extended cold accumulation towards the end of winter and spring were prevalent during this investigation. Prolonged dormancy is common under these climatic conditions. A substantial number of seedlings exhibited symptoms that included bare shoots without budbreak, delay of vegetative and reproductive budbreak and prolonged flowering periods. These symptoms were observed in seedlings of the high chill parent, 'Golden Delicious', and also in seedlings of the low-chill 'Anna'. Some seedlings of 'Anna' showed lower numbers of budbreak than seedlings of 'Golden Delicious'.

Measurable variation between and within apple seedlings for characteristics associated with prolonged dormancy was found in all families and was generally higher for 'Anna'. 'Anna' X 'Austin' within the 'Anna' families and and 'Braeburn' X 'Golden Delicious' within the 'Golden Delicious' families, gave many seedlings with early budbreak. The chilling requirements of 'Braeburn' and 'Prima' were estimated to be similar, (1100 CU) when percentage terminal budbreak was used as a rating criterion on excised shoots (R. Hauagge, personal communication). In the present study, the 'Braeburn' X 'Golden Delicious' cross showed a higher mean number of budbreak and the 'Golden Delicious' X 'Prima' cross showed a significantly lower mean number of budbreak compared to the other 'Golden Delicious' families. Quantification of the genetic variance in these families will provide a better basis for future choices of parents with regard to these traits.

Within-family seedling variance was generally higher than that for between families for all measurements. It is common knowledge that the apple cultivars are highly heterozygous and this is reflected in the variation in seedling families (Brown, 1960). Variation for time of budbreak within 'Anna' families was more than ten-fold higher than for 'Golden Delicious' families. Variation in flowering duration and the period of vegetative budbreak was also higher in 'Anna'

than in 'Golden Delicious' families. High numbers of seedlings in the middle classes suggest additive gene action, as has been reported previously for other fruit tree traits (Bell and Janick, 1990; Hansche et al., 1966 and 1972). However, dominance for the length of dormancy period in 'Anna' crosses has also been reported (Hauagge and Cummins, 1991c).

Yearly climatic differences are known to contribute to the variability of several traits in fruit crops (Hansche et al., 1966; Hansche et al., 1972; Tancred et al., 1995). We assessed consistency in family performance by estimating Year and Year X Family interaction variance components. Yearly differences contributed more to total variation than between family differences. High yearly fluctuations in family means for time and number of budbreak was found, particularly in 'Golden Delicious' seedlings. During the first season, the 'Golden Delicious' families showed a higher mean number of budbreak than 'Anna' families and this might be attributable to the higher CU accumulation. This may reflect greater responsiveness of 'Golden Delicious' seedlings to seasonal changes, as was also found in the variance components for year effects. Year to year variation in flowering duration was relatively large and can probably be explained by alternate bearing.

Year X Family interaction for all criteria was low as a percentage of total variation. The interaction that occurred suggests consistent or small variation in crossover performance (switching of ranks) of individual families from year to year. Year X Family interaction occurring in 'Golden Delicious' crosses for time and number of budbreak appeared not to be of the cross-over type interaction, implying that a family superior in one year performed well in other years as well. However, Year X Family interactions for flowering duration do appear to be of a cross-over type, implying that a family may have superior flowering relative to others during one particular season. This may also be an explanation for lower between-family differences for flowering duration than for other traits. Certain cultivars are more prone to bear heavy crops in alternative years, and this tendency can be transmitted to their seedlings.

Absence of significant Year X Family interaction for number of budbreak in the 'Anna' families, suggests that a single year of testing might be adequate for selection and would imply much saving in cost and time. On the other hand, significant interaction as found in 'Golden Delicious'

families point to a program of multi-year testing and selection based on mean performance averaged over years. The alternative strategy often suggested in breeding annual crops is to judiciously select a range of test locations likely to simulate the range of climatic and other environmental factors that normally occur from year to year.

Correlation between time and number of budbreak and flowering duration indicate that selection for early budbreak will not automatically select seedlings without prolonged dormancy symptoms and conversely that early budbreak is probably not useful for selecting for local conditions. Time of budbreak reflects chilling requirement under favorable climatic conditions (Hauagge and Cummins, 1991a; Weinberger, 1944) but early budbreak under unfavorable, fluctuating temperatures seemed to result in abnormal behavior. Budbreak in seedlings of 'Golden Delicious' was later during a period of constant and higher temperatures, i.e., conditions that are more suitable for dormancy release. Absence of sufficient winter chilling seemed to result in prolonged dormancy symptoms in these seedlings. Distribution of seedlings according to time and number of budbreak identifies between day 230 (mid August) and day 290 (mid October) as optimal (Fig. 3), and these points can be viewed as truncation points for the selection of adapted seedlings in local low-chill conditions. Other cross combinations should be tested to verify this observation.

Prediction of the timing of various events using phenological models, e.g., budbreak at specified intervals on excised one-year-old shoots under forced conditions, is frequently used in horticultural studies. These models have been used in the investigation of chilling requirements of cultivars and in studies of dormancy release (Cook and Jacobs, 2000; Linsley-Noakes et al., 1994; Shaltout and Unrath, 1983; Wilson et al., 1975). The percentage of shoots with budbreak after a specified time, or the time of budbreak after a specified percentage of sprouting has occurred, have been used as criteria in these investigations. This approach for pre-screening and breeding purposes would seem not to be of much use, since no replication is possible when working with the very large numbers of young seedlings that would be necessary in the type of breeding program we propose. The occurrence of prolonged dormancy symptoms in seedlings where early budbreak occurred underlines the complex nature of environmental adaptation and suggests that the inherent chilling requirement of seedlings should not be the only consideration. Although the number of budbreak on intact shoots does not distinguish between paradormancy

and ecodormancy factors as a response to chilling, it does quantify the phenotypic reaction of individual seedlings to climatic stimuli after a sufficient period following commencement of budbreak.

This study suggests that the variation in prolonged dormancy symptoms is measurable and can be further explored for breeding purposes. High genetic variation within crosses is directly related to variation generated by crossing heterozygous parents. Since the families involved different cultivars in each case and all seedlings were grown in the same environment this must lead to the conclusion that the variation is at least partially genetic and therefore amenable to change by selection. Although the experimental design was not appropriate for estimating heritability, we nevertheless feel confident that a one-shot mass selection among seedlings within crosses based on our measurements of prolonged dormancy symptoms should result in genetic response and improved adaptation to local conditions. It remains to devise some procedure for early selection, perhaps by defining independent culling levels or an appropriate selection index using time and number of budbreak as predictive selection tool.

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5. GENETIC VARIATION IN CHILLING REQUIREMENT IN APPLE PROGENIES

Abstract. Genetic variation in chilling requirement was investigated over three growth periods using clonal progenies of six apple (*Malus* x *domestica* Borkh.) families derived from crosses of high and low temperature requiring cultivars. Two quantitative measurements related to chilling requirement, viz, the time of initial budbreak (vegetative and reproductive) and the number of breaking buds over a specified time interval, were used as evaluation criteria. Genetic and environmental variances of the traits are presented as intra-class correlation coefficients for clones within and between families. For budbreak time, reproductive and vegetative, the broad sense heritability averaged around 75 and 69 percent respectively, indicating a high degree of genetic determination in this material. For budbreak number, moderate to low genetic determination was found with broad sense heritabilities around 30 percent. Estimates of genetic components of variance between families were generally very low in comparison to the variance within families and predict potentially favourable responses to truncation selection on the traits within these progeny groups. Analysis of the data show that the distribution of budbreak time is typical of quantitative traits with means distributed closely around midparent values. Skewed distributions towards low budbreak number were obtained in varying degrees in all families.

Additional index words. Malus x *domestica*, fruit breeding, heritability, climatic adaptation, selection

Adaptedness refers to the way in which plants can survive and reproduce in specific environments (Hill, et al., 1998) and is reflected by the degree to which developmental events are synchronized with the climate (Dietrichson, 1964). Many factors influence the adaptive potential of apple (*Malus* x *domestica* Borkh.) cultivars planted around the world and for important horticultural traits, such as yield and fruit quality, it is unrealistic to expect the same level of performance in all environments. This is the main reason why cultivars bred in other countries are not always suitable for local production and why fruit tree breeding for improvement in economically important traits requires attention to traits related to adaptedness as well. Adaptedness is a complex interaction between various environmental factors and the plant. Breeding for climatic adaptation is of increasing interest among plant breeders.

Most temperate zone woody deciduous trees, including apple require a certain degree of chilling to break endodormancy before active shoot growth in the Spring, (Rodriguez & Sherman, 1985; Sorensen, 1983), a phenomenon generally referred to as the chilling requirement (CR) (Martinez et al., 1999; Sorensen, 1983). Wide variation in CR exists among cultivars, wild species and hybrids (Hauagge and Cummins 1991a). The number of hours below 7.2 °C before budbreak occurs, is frequently used as measure of CR and expressed as cold unit (CU) accumulation (Linsley-Noakes et al., 1994; Weinberger, 1950). For example it has been estimated that 'Anna', a low CR cultivar (Brooks and Olmo, 1972) needs approximately 200-300 hours below 7.2 °C to break bud dormancy, compared to 'Dorsett Golden' and 'Golden Delicious' which require 800-900 and 1050-1100 CU, respectively (Hauagge and Cummins, 1991a).

Generally, CR is seen as a complex genetically determined trait, probably multigenic or, at least, partly controlled by multiple genes (Dennis, 1987; Howe et al., 1999). Inheritance studies on CR are complicated by the quantitative nature of the dormancy process and environmental factors such as temperature, daylength, drought and nutrient availability (Howe et al., 1999). The quantitative nature of dormancy-related traits has been demonstrated in controlled experiments on cold hardiness in Douglas fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) trees (Aitken and Adams, 1995), date of budbreak in Scottish birch (*Betula pubescens* Ehrh.) (Billington and Pelham, 1991) and the date of bud set and budbreak in *Picea abies* (L.) (Eriksson et al., 1978). Dormancy related genes controlling other characteristics such as branching pattern may also play a role in the process of budbreak and dormancy release (Hauagge and Cummins, 1991b; Howe et al., 1999).

Timing of bud set at the end of the growing season and budbreak after fulfilment of the CR is tied to climatic cycles (Howe et al., 2000) and these traits are frequently used in studies relating to dormancy and dormancy release. Time of budbreak, also described as bud flush or bud burst, marks the initiation of shoot elongation as an indicator of dormancy release and fulfilment of the CR. Selection for low CR genotypes in breeding programmes is normally based on seedlings that break bud within a specified time period. Cultivars with low CR such as 'Anna', 'Ein Shemer' and 'Schlor' were selected using early budbreak as selection criterium, i.e., selection of the

earliest seedlings in the seedling families (Oppenheimer and Slor, 1968). Classification and grading systems for adaptedness using number and distribution of budbreak were also previously applied in apple by Hauagge and Cummins (1991c) and by Denardi et al., (1988). Ratings of stages in budbreak was applied in maple by Kriebel and Wang, (1962).

According to local observations and previous studies in the Western Cape of South Africa, the most prominent symptom of incomplete dormancy release is the absence of budbreak or a long delay in budbreak (Cook and Jacobs; 2000; Jacobs, et al., 1981) resulting in low numbers of buds and uneven distribution of buds on shoots. These symptoms are collectively referred to as prolonged dormancy and are found in trees in areas where the temperature requirements for normal dormancy release of prevailing commercial cultivars are not met (Cook and Jacobs, 2000; Jacobs, et al., 1981).

From the above it is evident that CR can be measured and expressed in several different ways depending on the criteria used and the traits measured. The genetics of CR measured in terms of prolonged dormancy symptoms and in relation to budbreak time has not been adequately investigated. We also do not know whether families and parental genotypes vary significantly in the occurrence of these symptoms. To better assess the potential for genetic manipulation, the extent of genetic variation in above symptoms must be better understood.

In this study we have conducted a quantitative genetic analysis of budbreak time and budbreak number in order to investigate CR in seedling families as indication of their adaptedness to local growing conditions. Genetic parameters were estimated in an attempt to explain the genetic control and variability of the criteria, to assess their possible implication in breeding programmes, and to explore the effectiveness of early screening for CR at a young seedling stage.

Materials and Methods

Plant material. The progenies evaluated were derived from two sets of crosses, viz, four families involving the high CR cultivar 'Golden Delicious' as one parent and two families involving the low CR cultivar 'Anna' as one parent. 'Golden Delicious' was the common female parent in

crosses with cultivars 'Prima', 'Summerking', 'Starking Delicious' and 'Braeburn' as male parents. 'Anna' was the common male parent in crosses with 'Austin' and 'Sharpe's Early'. 'Anna' is generally regarded as one of the lowest CR cultivars found in *Malus* x *domestica* and originates from an Israeli cultivar, Red Hadassiya X 'Golden Delicious' (Brooks and Olmo, 1972). 'Golden Delicious' is a popular commercial cultivar, originated as a chance seedling with 'Golden Reinette' and 'Grim's Golden' as reputed parents. No records are available on chilling requirement of parents we used other than for 'Anna' (± 300 CU), 'Golden Delicious' (± 1500 CU), 'Braeburn' (± 1100 CU), 'Prima' (± 1100 CU) and 'Summerred' (± 999 CU) (Hauagge and Cummins, 1991a; R. Hauagge, personal communication). Seedlings (60 seedlings in case of 'Golden Delicious' families and 100 seedlings in case of 'Anna') for these trials were selected at random from adult trees originally planted in the field for fruit quality evaluation and then clonally replicated by budding on M793 rootstocks.

Planting design. Clonal replicates of seedlings were planted in an orchard in the Western Cape region of South Africa where temperate climatic conditions characterised by low winter chilling are normally experienced (Elgin, 34°S; ± 300 m). An orchard of 0.70 ha, established in the Springs of 1997 and 1998 was used as planting site. Tree spacing was 1 m within rows and 3 m between rows. Parents and progenies from the two sets of crosses were planted adjacently in two trials each consisting of seven replications in randomized blocks. Sibling seedlings within cross families were planted adjacently in progeny rows within blocks and parents used in crosses were planted at random within their progeny rows. Orchard management was typical of commercial practice except that no pruning or other tree growth manipulations such as winter oil treatment for rest breaking, were applied.

Data recorded. The initial time of vegetative and reproductive budbreak of each seedling and the number of vegetative and reproductive buds breaking were recorded as criteria of winter CR. In the first two seasons, all buds were scored on the whole tree and recorded as the number of buds per 100 cm length of shoot. In the third year, four one-year-old shoots were selected at random on each seedling and budbreak expressed as the number of buds per 100 cm length of shoot.

The initial reproductive budbreak (IRB) was recorded at the first sign of flowers in the tight cluster stage, and the date of initial vegetative budbreak (IVB) at the time when leaves started to emerge from a vegetative bud. IRB and IVB were recorded once a week. The number of days for IVB and IRB was recorded from January 1 onwards. During the first two seasons the number of buds breaking was recorded 21 days after IVB, a period regarded as adequate for apple trees to express ability to overcome the state of dormancy (Faust et al., 1995; Hauagge and Cummins, 1991a). During the third season, the number of buds breaking was counted 21 days after the last seedling reached IVB. The shoots that developed on each seedling were assigned to the following three classes: 0.5-30 cm, 30-60 cm and 60 – 120 cm. The number of shoots in each class was recorded for each tree. The total length of the main shoot was also recorded. Chill units were calculated according to the modified Utah equation (Richardson et al., 1974), that was regarded more suitable for local chilling conditions where negative CU values are not carried from one day to the next (Linsley-Noakes et al., 1994). The growing degree hours (GDH) (Anderson, et al., 1986) were calculated daily as the difference between the mean and assumed base temperatures (10 °C).

Data were collected on seedlings when they were two, three and four years of age during 1997 - 2000. The first records was for the second (one year after budding) and following growing seasons in the case of 'Golden Delicious' seedlings during 1997 and during 1998 for 'Anna' seedlings. Data were collected on all traits for a full three year period, except IRB in 'Golden Delicious' families, where the number of seedlings flowering during the first two seasons was insufficient so data from only one year were available.

Data analysis. Analysis of variance (ANOVA) was performed on all measurements for each of the 'Anna' and 'Golden Delicious' families. Separate analyses were performed for each year and a joint analysis for the three years in order to test for Year X Family interaction effects. The mean square for seedlings within families was used for the comparison between families. Where significant Year X Family interaction was found in the joint analysis, the mean square for Year X Family was used as error. The analyses were performed using SAS General Linear Model Procedures (SAS Institute, Cary, N.C.) after testing for heterogeneity of variance using the Levene test (Snedecor and Cochran, 1989) and the Shapiro-Wilk test for normality (Shapiro and

Wilk, 1965). Weighting appeared to be advisable and the data transformed to logs where necessary in some cases. Variance components and intra-class correlation coefficients were calculated using the SAS Variance Component Estimation Procedure.

Variance structure. The variance structure has been discussed in terms of standard quantitative genetic principles (Falconer and Mackay, 1996) and is briefly qualified for completeness. In the present experiments, the repetition of measurements was different clones of single seedlings, where

(i) variance of seedling trees within families of the same cross

$$\sigma_W^2 = \sigma_g^2 + \sigma_e^2$$

where σ_g^2 is a genetic component, generated by crossing in this case, and σ_e^2 a component ascribable to variable environment within the trial orchard

(ii) variance between families

$$\sigma_B^2 = \sigma_G^2 + \sigma_W^2$$

where σ_G^2 is the genetic variance among families for a given common parent, i.e., 'Golden Delicious' or 'Anna'.

Repeated measurements on clones of trees (n = 7 per tree) performed per season, result in the ANOVA and Expected Mean Squares (EMS) for estimation of components as follows:

Between families (N trees per family)	$\sigma_e^2 + n\sigma_g^2 + Nn\sigma_G^2$	
Between trees within families	$\sigma_e^2 + n\sigma_g^2$	(A)
Within clones	σ_e^2	(B)

The intra-class correlation coefficient relevant to selection between trees within families is then

$$t = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2} \text{ estimated by } \frac{A - B}{A + (n-1)B} \text{ with standard error}$$

$$SE(t) = \sqrt{\frac{2[1+(n-1)t]^2(1-t)^2}{n(n-1)(N-1)}}$$

The equivalent intra-class correlation coefficient relevant to selection between families is

$$t = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_W^2}.$$

Results

Winter chilling conditions. Normal winter chilling conditions for the region were experienced during this investigation. Relatively low CU accumulation for apple production occurred during the four years, being respectively 763, 897, 622 and 724 measured from the beginning of May until the end of August each year. Figure 1 shows the weekly accumulation of CU and GDH calculated from data collected over a period of four years (1997 – 2000).

Budbreak time, reproductive buds. Budbreak in 'Braeburn' seedlings was significantly earlier than in the others (Table 1). 'Golden Delicious' was intermediate for IRB and 'Prima', 'Starking Delicious' and 'Summerking' were the later flowering parents. During all years of the experiment, 'Anna' was the earliest flowering parent, 'Sharpe's Early' was the latest and 'Austin' was intermediate ($P = 0.0012$). A genetic basis for these differences is evident in the progenies where 'Anna' X 'Austin' seedlings showed significant early flowering compared to 'Anna' X 'Sharpe's Early' seedlings in the joint analysis over three years ($P = 0.0050$). Very low numbers of 'Golden Delicious' seedlings flowered during the second and third years after cloning. In 'Anna' families, 85.2% of seedlings flowered in the second year in comparison to 42.1% seedlings of 'Golden Delicious' crosses. Records on reproductive budbreak time in 'Golden Delicious' families were therefore analyzed for the third season only.

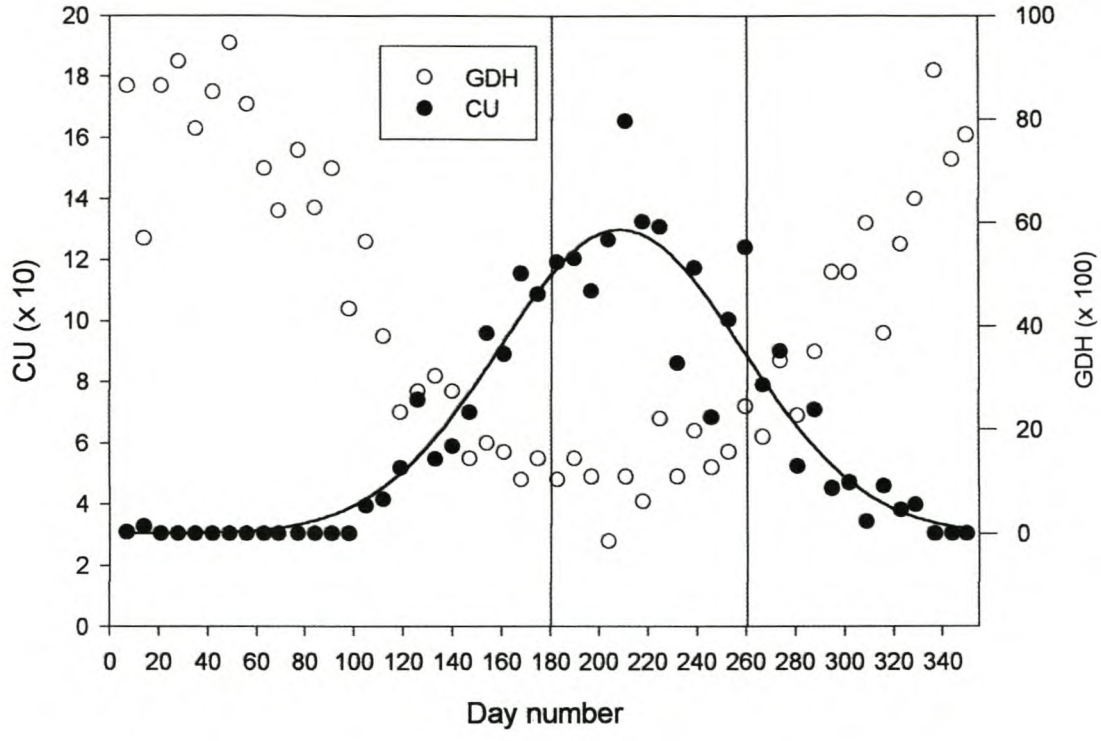

Fig. 1. Distribution of mean chill unit (CU) and growing degree hours (GDH) during four seasons in the Elgin area in South Africa (34 °S; 305 m) plotted on a weekly basis (Day 1 represents January 1). Vertical lines represent the mean start of reproductive and vegetative budbreak dates in 'Anna' progenies at ± 300 CU accumulation (end June) and ± 875 CU (mid September) in 'Golden Delicious' progenies.

Budbreak time, vegetative buds. 'Golden Delicious' X 'Starking Delicious' and 'Golden Delicious' X 'Braeburn' showed earlier vegetative budbreak compared to the other two 'Golden Delicious' families ($P = 0.0007$) (Table 2). Parents did not differ for IVB, largely due to large genotype X year interactions. The cross 'Anna' X 'Sharpe's Early' was significantly later compared to 'Anna' X 'Austin' ($P = 0.0239$). This is also evident in the parental means where 'Austin' sprouted earlier than 'Sharpe's Early', but later than 'Anna' ($P = 0.0004$). The succession in IVB in 'Anna' parents and families over three years of data recording was again consistent. The estimated phenotypic correlation between IRB and IVB was strong for the 'Anna' progeny ($r = 0.739$; $P = 0.0001$; $N = 1700$) and for 'Golden Delicious' progeny ($r = 0.864$; $P = 0.0001$; $N = 4100$).

Table 1. Means for reproductive budbreak time in six apple families and parents. ANOVA were performed separately for parents and progenies for the 'Golden Delicious' and 'Anna' groups. Multiple comparisons were performed using Student's t LSD test. Standard deviations (STD) for progeny data are included.

Parents and Families	Year 1[y]	Year 2[y]	Year 3	Joint analysis[z]
Golden Delicious	-	-	298.782 b	-
Prima	-	-	305.278 a	-
Starking	-	-	305.167 a	-
Summerking	-	-	304.750 a	-
Braeburn	-	-	289.586 c	-
GD X Pr*	-	-	297.253 b	-
GD X St	-	-	291.796 c	-
GD X Su	-	-	299.046 a	-
GD X Br	-	-	285.818 d	-
STD	-	-	12.65	-
An	219.736 c	202.765 c	188.635 c	203.712 b
Au	257.525 b	257.205 b	252.395 b	255.708 a
SE	285.375 a	278.298 a	274.723 a	279.465 a
An X Au	258.562 b	243.242 b	233.278 a	245.027 b
An X SE	267.757 a	253.537 a	248.169 a	256.487 a
STD	24.92	26.73	32.26	30.31

[y]ANOVA on 'Golden Delicious' seedlings could not be performed because of low reproductive budbreak number during the first two years of data recording.

[z] In cases of significant Year X Family interaction the mean square for Year X Family was used as error term in the ANOVA.

*See page 4 for list of abbreviated cultivar names

Table 2. Means for vegetative budbreak time in six apple families and parents. ANOVA were performed separately for parents and progenies for the 'Golden Delicious' and 'Anna' groups. Multiple comparisons were performed using Student's t LSD test. Standard deviations (STD) for progeny data are included.

Parents and Families	Year 1	Year 2	Year 3	Joint analysis[z]
GD	292.783 b	310.132 a	297.620 c	300.178 a
Pr	296.650 ab	283.136 c	305.211 a	294.999 a
St	294.033 b	297.625 b	301.467 ab	297.708 a
Su	299.793 a	306.688 a	298.370 bc	301.617 a
Br	296.136 ab	298.750 b	292.424 d	295.770 a
GD X Pr	292.080 b	297.793 a	298.991 a	296.288 a
GD X St	289.005 c	290.614 c	291.961 c	290.527 b
GD X Su	294.588 a	293.943 b	296.658 b	295.063 a
GD X Br	288.139 c	283.924 d	286.582 d	286.215 c
STD	16.39	13.49	11.25	13.95
An	233.755 c	213.686 c	213.288 c	220.243 c
Au	262.826 b	250.025 b	253.342 b	255.397 b
SE	286.020 a	263.102 a	279.851 a	276.324 a
An X Au	253.829 b	243.411 b	238.345 b	245.195 b
An X SE	262.016 a	251.941 a	251.576 a	255.178 a
STD	21.85	27.89	29.02	25.56

[z] In cases of significant Year X Family interaction the mean square for Year X Family was used as error term in the ANOVA.

Total budbreak number. Variation among individual seedlings for the number and uniformity of budbreak, and, thus, for the prevalence of prolonged dormancy, was of such an order that it was possible to identify trees with low and high budbreak numbers visually (Fig. 2). Low numbers of buds breaking (less than 10 buds per 100 cm length of shoot) were found in many cross derived 'Anna' and 'Golden Delicious' seedlings (72 and 47%, respectively). The mean budbreak number in the 'Golden Delicious' X 'Prima' family was significantly lower than the other 'Golden Delicious' families (P = 0.0176), indicating a genetic basis for this trait (Table 3). Although 'Golden Delicious' X 'Starking Delicious' seedlings showed high mean budbreak numbers, the joint ANOVA did not detect significant differences between 'Golden Delicious' families other than for the cross 'Golden Delicious' X 'Prima'. Parental means for budbreak number indicate that 'Prima', 'Anna', 'Austin' and 'Sharpe's Early' are generally low, 'Golden Delicious' and 'Braeburn' intermediate, and 'Summerking' and 'Starking Delicious' were high (Table 3). ANOVA on parents showed that 'Prima' was significantly lower and 'Starking Delicious' significantly higher in budbreak number (P = 0.0051) which is consistent with the above mentioned family means. 'Anna' families did not differ significantly and 'Anna' as parent showed lower numbers compared to 'Austin' and 'Sharpe's Early' (P = 0.0001) during the first year. From Fig. 3 it is clear that mean budbreak number increases during the season in the 'Anna' progeny, which can be associated with CU and GDH accumulation. Correlation analyses for IVB and total budbreak number in the 'Anna' crosses was significant in year one for parents (r = 0.537; P = 0.0001; N = 142) and for families (r = 0.316; P = 0.0001; N = 1371); and in the second year for parents (r = 0.226; P = 0.0072; N = 140) and for families (r = 0.311; P = 0.0001; N = 1344). No association was found in 'Golden Delicious' crosses between budbreak time and budbreak number.

Fig. 2. 'Anna' cross seedlings during the first year of the experiment after budding with high budbreak numbers (A) and with low budbreak numbers (B). 'Anna' seedling during second year after budding with high budbreak numbers (C) and with low budbreak numbers (D). 'Golden Delicious' seedling with high (E) and low (F) budbreak numbers during second year after budding.

Table 3. Means for total budbreak number (vegetative and reproductive) in six apple families and parents used in crosses. ANOVA were performed separately for parents and progenies for the 'Golden Delicious' and 'Anna' groups. Multiple comparisons were performed using Student's t LSD test. Standard deviations (STD) for progeny data are included.

Parents and Families	Year 1	Year 2	Year 3[y]	Joint analysis[z]
GD	12.726 a	16.936 c	3.415 b	11.026 b
Pr	5.836 c	12.774 d	3.719 b	7.443 c
St	15.258 a	26.015 a	9.362 a	16.878 a
Su	15.140 a	20.679 b	3.850 b	13.223 ab
Br	9.967 b	16.485 c	4.185 b	10.212 b
GD X Pr	12.412 b	14.502 b	4.043 c	10.319 b
GD X St	14.169 a	18.189 a	6.088 a	12.815 a
GD X Su	12.780 b	18.222 a	4.845 b	11.949 a
GD X Br	13.974 a	18.039 a	4.870 b	12.285 a
STD	7.63	8.02	5.00	8.51
An	8.589 b	8.380 b	1.956 a	6.308 a
Au	14.868 a	10.156 a	1.780 a	8.935 a
SE	14.585 a	8.900 ab	1.368 a	8.284 a
An X Au	8.374 a	10.819 a	2.272 a	7.155 a
An X SE	8.394 a	10.466 a	2.258 a	7.032 a
STD	6.43	6.04	2.22	6.32

[y] Four one-year-old shoots were selected at random on each seedling tree for bud counting.

[z] In cases of significant Year X Family interaction the mean square for Year X Family was used as error term in the ANOVA.

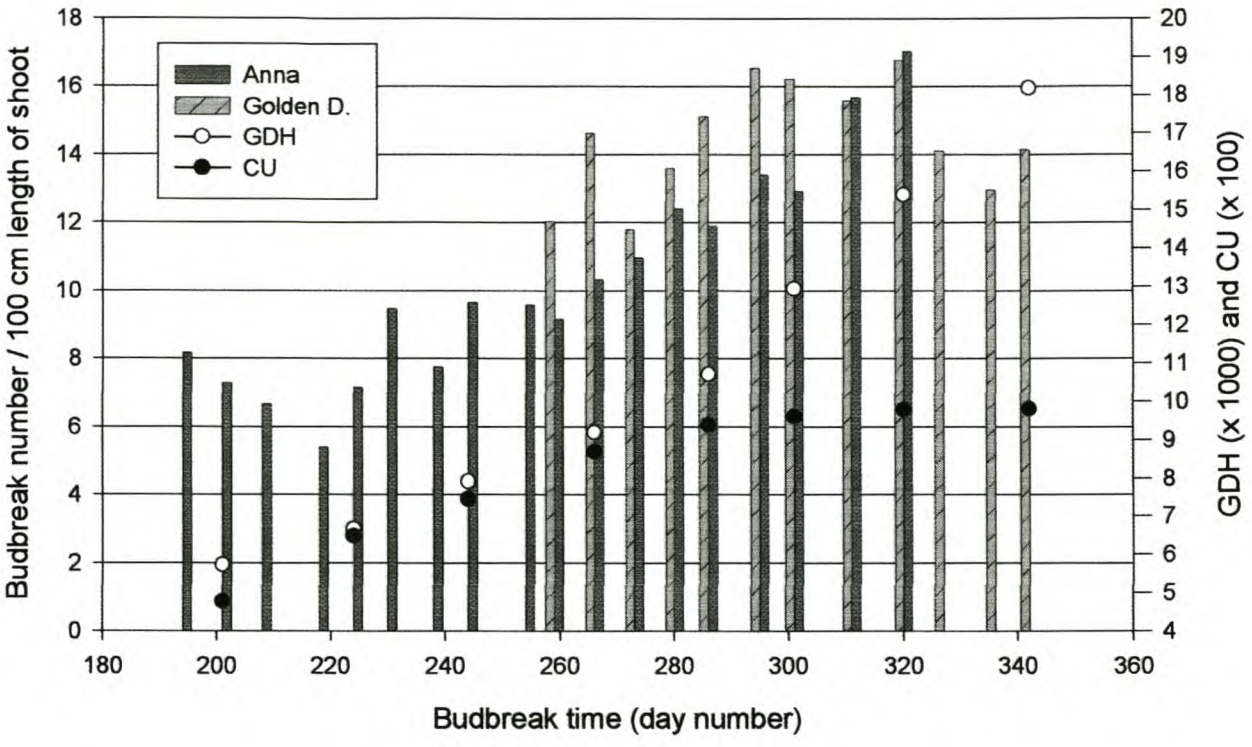

Fig. 3. Distribution curve for mean budbreak number of 'Anna' progenies and 'Golden Delicious' progenies recorded over two years on clones of young seedling trees according to budbreak time (IVB) and accumulation of CU and GDH.

Distribution curves. Patterns of distribution for budbreak time and number were uniform over the three-year period. All families showed continuous distributions for IRB, (Fig. 4A) IVB (Fig. 4B) and budbreak number (Fig. 4C). Only two distribution curves are shown for each trait to illustrate the tendency in 'Golden Delicious' and 'Anna' families, respectively. Within the two groups, the distribution patterns were quite consistent. Accentuated decreases in frequency of extremes and progeny means around midparents indicate possible additive gene effects for IRB and IVB. There was no evidence of segregation due to single genes of major effect for IRB and IVB. On the other hand, skewed distributions towards lower budbreak number were evident in all families, especially in the 'Anna' cross families (Fig. 4C), and might be indicative of major gene effects in this trait. The ranges for IRB in 'Anna' families (161 days) were wider than in 'Golden Delicious' families (72 days). In contrast to the IVB in 'Anna' families (142 days), 'Golden Delicious' families also showed a narrower range (96 days). Large numbers of 'Anna' seedlings

showed earlier reproductive budbreak time (65% of seedlings) and vegetative budbreak time (60% of seedlings) in comparison to 'Golden Delicious'.

Genetic and environmental variance components. Variance component analyses summarized in Table 4 were performed on the data for each year separately. Broad sense heritabilities and standard errors were calculated from the estimates of genetic and residual variance components. The IRB and IVB heritabilities calculated over a three year period were high (in the order of 70%), while that for budbreak number was moderate to low (around 30%). Estimated heritabilities were similar in the three years of data recording for budbreak time, but for budbreak number some difference is apparent (h^2 = 0.47 in year two and h^2 = 0.17 in year three). By comparison, genetic differences between families appear to be consistently small (ranging from 0% to 18%) probably attributable to the fact that families within the two groups shared one common parent. These relatively small differences between families were nevertheless statistically significant in the analyses of variance for IRB and IVB in both population groups for budbreak number in 'Golden Delicious' families. In calculations of family means as deviations from mid-parent values, significance was found for time of vegetative budbreak in 'Anna' crosses only. Mean gene effects, for which parents contributed by different alleles, indicate a degree of dominance towards the later parents, 'Austin' and ' Sharpe's Early.

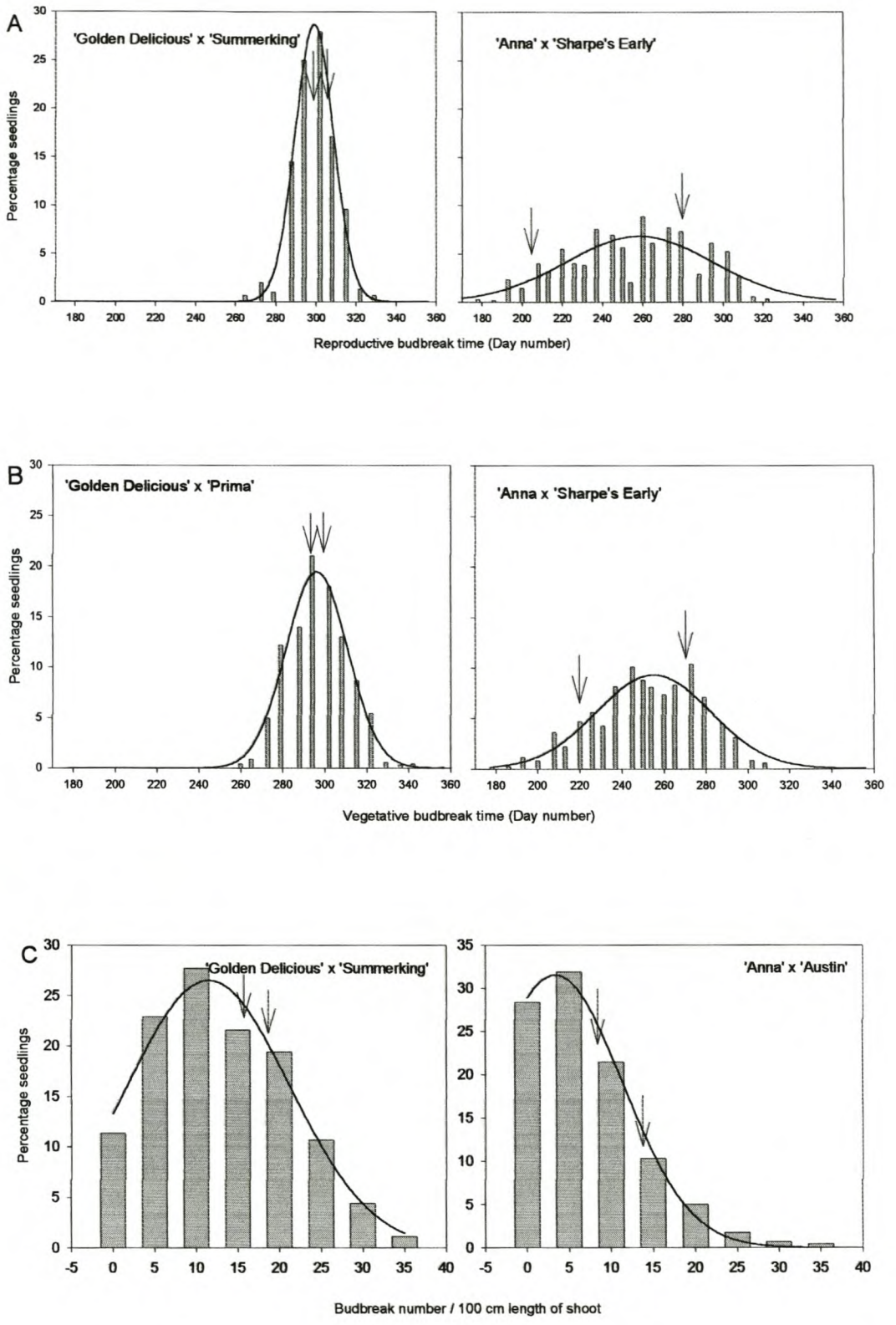

Fig. 4. Frequency distributions of (A) reproductive budbreak time (B) vegetative budbreak time and (C) budbreak number based on means of seedling clones in apple families. Means of

parents are indicated with arrows. The data presented were collected over a three-year period and illustrate the tendency which was consistent in all populations.

Table 4. Intra-class correlation coefficients for reproductive and vegetative budbreak time and total budbreak number in 'Anna' and 'Golden Delicious' families.

	'Golden Delicious' families			'Anna' families		
Criteria / source of variation	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
Vegetative budbreak time						
t_1	0.001	0.003	0.183	0.002	0.003	0.001
t_2	0.615	0.574	0.655	0.686	0.824	0.827
SE (t_2)	0.051	0.054	0.048	0.036	0.023	0.022
Reproductive budbreak time						
t_1	-[y]	-	0.189	0.058	0.060	0.001
t_2	-	-	0.652	0.721	0.821	0.813
SE (t_2)	-	-	0.048	0.032	0.023	0.024
Total budbreak number						
t_1	0.001	0.000	0.000[z]	0.000	0.000	0.000[z]
t_2	0.397	0.290	0.300[z]	0.232	0.465	0.169[z]
SE (t_2)	0.058	0.056	0.056[z]	0.040	0.044	0.036[z]

Table 4 continued (footnotes)

[y] Variance component analyses could not be performed because of low reproductive budbreak number in 'Golden Delicious' families.

[z] Four one-year-old shoots were selected at random on each tree for bud counting.

Discussion

Genetic analyses of fruit traits have usually been done retrospectively from breeding programme data and often came short in appropriate experimental design estimation of variance components and heritability. Families are usually distributed in single rows with no replication and randomization (Dicenta et al., 1993; Durel et al., 1998; Hansche et al., 1972; Tancred et al., 1995). In this study the design was in accordance of what is expected for analyses of continuous traits. The trials were performed on parents and offspring grafted onto the same rootstock which gives the trees similar physiological status, combined with an experimental design allowing the evaluation of genetic parameters in the six apple families. The design of the experiment allows for a partitioning of the total variance of the measurements into components between families (genetic), between seedlings within families (genetic) and within clones (environmental), and for the calculation of corresponding intra-class correlation coefficients, according to standard quantitative genetic principles (Falconer and MacKay, 1996). The intra-class correlation within clones of seedlings is then an estimate of heritability (broad sense in this case) to be used as indicator of the prospects of achieving genetic improvement by means of truncation selection between seedling clones within families. Measurements were performed on the basis of continuous quantitative assessments and not by subjective judgement on a nominal or ordinal scale.

Normal winter conditions for the Western Cape was experienced during this investigation. Winter chilling was frequently interrupted by moderate to warm days and low CU accumulation that resulted from temperature fluctuations. Winters also differed from season to season in terms of chill and heat unit accumulation. It is clear that many of the 'Anna' seedlings needed a shorter

chilling period compared to 'Golden Delicious' seedlings for dormancy release to occur. The intermediate flowering time of the parent 'Golden Delicious' in combination with three later flowering parents, 'Prima', 'Starking Delicious' and 'Summerking' resulted in non-significant differences between these families. The parent 'Braeburn', however, shifted the progeny mean towards earlier reproductive budbreak. The parental influence was also clear in 'Anna' progenies, with the earlier flowering seedlings from 'Austin' X 'Anna' showing more early flowering seedlings compared to the 'Sharpe's Early' X 'Anna' family. The succession in flowering time in 'Anna' parents and families was constant over the three years and the range was much wider than in 'Golden Delicious' families, indicating a wide genetic variation to choose from in a selection programme. 'Anna' seedlings were observed to be highly reproductive, flowering at a very young stage, indicating precocity in their adaptive response to local conditions, which is a benefit in apple cultivar development.

Means for IVB among parents of 'Golden Delicious' crosses did not differ significantly. Among the 'Golden Delicious' progenies, however, significant differences in IVB were observed where 'Starking Delicious' and 'Braeburn' induced early budbreak. Although the 'Braeburn' progeny showed early reproductive and vegetative budbreak, the period of ripening was largely extended towards the end of the cropping season compared to the other three families (unpublished data). 'Golden Delicious' families showed a much later and narrower range of IVB compared to the 'Anna' families. It is possible that seedlings may differ in their rapidity of response to favourable conditions after their chilling requirement was satisfied (Worrall and Mergen, 1967), since it is known that a genetic variability exist for heat requirement after CR is satisfied during the rest period (Hauagge and Cummins, 1991d). The response of 'Golden Delicious' seedlings may be partly a function of accumulated GDH, since the first budbreak started at a time when GDH accumulation increased during mid September. 'Austin' was significantly earlier in IVB compared to 'Sharpe's Early' with a marked genetic influence on progeny means. The stability of family ranking for IVB indicates that families can effectively be assessed for budbreak time and that parents can be identified accordingly.

Broad sense heritabilities calculated in this study indicate that IRB (h^2 = 0.75) and IVB (h^2 = 0.69) are highly heritable and that the variation between seedlings can be primarily ascribed to genetic factors. Previous studies on apple have indicated relatively high narrow-sense heritability for the length of bud dormancy (Hauagge and Cummins, 1991b). Moderate genetic control of budbreak time have been reported for Douglas-fir (h^2= 0.44) (Li and Adams, 1993) and for Balsam poplar (h^2 = 0.21 to 0.47) (Farmer, 1993), and strong genetic control for Trembling aspen (0.72) (Thomas, et al., 1997) and hybrid poplars (h^2 = 0.80) (Howe, et al., 2000). High heritabilities for budbreak time suggest that response to selection will be successful and that genetic advance is expected to be relatively rapid. Two limitations are acknowledged concerning the heritability estimates. First, though collected over multiple years, the data are from a single site and thus the heritability estimates are biased upward to the extent that genotype X location interactions would exist for the traits investigated. The multiple years of data are taken from the same trees and thus two observations on a single tree may not be independent. For these traits it seems especially important if there is the potential for cumulative effects of budbreak differences over several years. Second, the heritability estimates come from a very limited sample of apple germplasm and given the small number of crosses, the reference population for these estimates are probably limited only to these crosses and could probably not be generalized to all 'Anna' or all 'Golden Delicious' crosses.

Previous studies on apple where high chilling parents were crossed with 'Anna' suggest that length of bud dormancy measured in terms of GDH accumulated from leaf fall until budbreak is ascribable to major dominant genes, modulated by minor interactive genes (Hauagge and Cummins, 1991b). Oppenheimer and Slor (1968) also found evidence that early budbreak appears to be controlled by dominant genes. From the distribution classes for CR based on leafing response in peach seedlings, Lesley (1944) suggested the presence of multiple genes with cumulative effects and absence of dominance. Lammerts (1945) has shown that the low CR in peach based on leaf growth rate is due to the accumulation of the effects of multiple genes, some recessive and cumulative. Studies in peach CR based on leaf bud activity have also suggested multiple gene control (Bowen, 1971). Continuous distributions and midparent values in the crosses in our trials indicate that additive effects of genes are probably more important than non-additive effects in the total genetic variance of these traits.

Prolonged dormancy symptoms were common and easily observable under climatic conditions experienced during this investigation. A substantial number of seedlings exhibited severe symptoms that included bare shoots without budbreak and delay of vegetative and reproductive budbreak. These symptoms were observed in seedlings from high chill parents of the 'Golden Delicious' progenies, and also in seedlings of the low-chill 'Anna' progenies. Some seedlings of 'Anna' showed lower numbers of budbreak than seedlings of 'Golden Delicious' in contrast to previous reports where 'Anna' demonstrated total termination of dormancy and no delayed foliation (Hauagge and Cummins, 1991b). Parental means indicated that some cultivars are more inclined to lower budbreak numbers than others. The three parents used in the 'Anna' crosses, viz, 'Anna', 'Austin' and 'Sharpe's Early' generally had low budbreak number. The parent 'Prima' and its progeny were also low, similar to results previously obtained from adult trees (Chapter 4). 'Golden Delicious' and 'Braeburn' families were intermediate while 'Summerking' and 'Starking Delicious' families were high. 'Anna' families did not differ significantly as was observed previously in adult trees (Chapter 4).

Offspring of low X low crosses as well as offspring of low X intermediate crosses tend towards low budbreak number. Offspring of intermediate X intermediate, and intermediate X high crosses are intermediate. Asymmetry in the distributions towards low budbreak number were noticed. In contrast to budbreak time, broad sense heritability of budbreak number was moderate to low (h^2 = 0.30). Heritability estimates from parent-offspring regression for an index combining the number and distribution of budbreak under subtropical winter conditions were found to be between 0.34 and 0.37 by Hauagge and Cummins (1991b). The relatively low heritablity indicates low expected response to selection for this trait and suggests the need for selection based on clonal progeny means, i.e., family selection.

It is generally accepted that vegetative budbreak time is a reflection of the accumulated chilling and heat requirement under favorable climatic conditions (Hauagge and Cummins, 1991d; Weinberger, 1944) and that genotypes showing early budbreak during the growing season, such as the cultivar 'Anna', are low chill requiring and, therefore, more widely adaptable. It is also expected that budbreak should be more prolific, and will occur promptly and uniformly (Hauagge

and Cummins, 1991a). Thus, in mild climates budbreak time is related to bud CR (Hauagge and Cummins, 1991d; Oppenheimer & Slor, 1968). For the given level of chilling, the response of the 'Anna' seedlings was more rapid than that of 'Golden Delicious seedlings. Significant association between budbreak time and budbreak number was found for 'Anna' parents and progeny, but not for 'Golden Delicious' parents and progeny. Similar results have been reported previously on adult trees where a positive association between budbreak time and number was found in 'Anna' families and a negative association in 'Golden Delicious' families (Chapter 4). The high percentage of seedlings in 'Anna' families with low budbreak numbers compared to 'Golden Delicious', indicates that selection for early budbreak will not automatically identify seedlings lacking prolonged dormancy symptoms. Early budbreak is probably not a useful trait to select in climates with unfavorable fluctuating temperatures and low winter chilling. Distribution of seedlings according to budbreak time and number identifies a truncation point (around day 260) at maximum CU accumulation and onset of GDH accumulation as optimal for adaptation for seedlings in the low-chill conditions of our experiment.

The association found between budbreak time and number, and the high number of seedlings in the 'Anna' progeny with prolonged dormancy symptoms, has important practical implications for breeding programmes. The patterns of budbreak observed is a result of interaction between genetic and controlling environmental stimuli (CU and GDH accumulation) and not solely the direct result of inherent variation in chilling requirement as generally measured in terms of budbreak time. It is known that high temperatures gradually assume more control over bud development as trees approach completion of rest and that bud development progresses only if chilling is supplemented with temperatures favourable to growth (Brown, 1960; Kriebel and Wang, 1962; Worrall and Mergen, 1967). Although the interrelationships among the traits are not understood, the correlated response indicates that selection for early budbreak should result in negative effects on budbreak number and the occurrence of prolonged dormancy symptoms. Selection for number of buds breaking should be more difficult than selecting for time of budbreak. Previously, heritability values of 30 - 40 percent for quantitatively inherited traits have been described as favorable and should guarantee adequate efficiency of mass selection in the field (Durel et al., 1998).

The success of any crop improvement program depends, among other factors, on the amount of genetic variability for the trait under selection (De Souza, 1998). The substantial genetic variation within families found in the present study can most probably be explained by the fact that apples are cross-fertilizing, that within family variation is directly related to variation within cultivars and that new genetic variation is generated by crossing and segregation. Genetic variation between families appears to be consistently small and may be explained by the fact that families within the two groups all shared one of two common parents.

In this study we used budbreak time and number on a continuous scale to investigate the variation in chilling requirement in apple families and to determine the genetic and environmental components of variation. Trees were grown under experimental conditions where fluctuations in environmental effects could be controlled to some extent by replication and randomization. In general, the results have exposed significantly high levels of genetic variation within families, of an order indicating that the traits are amendable to genetic improvement by selection. The best strategy for producing families segregating for early budbreak will be to cross two cultivars resulting in a high midparent value and where the simplest method to select superior individuals is based on their own performance. Clonal testing should not be necessary for selection for budbreak time but would be advisable for the budbreak number.

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6. SELECTION FOR INCREASED BUDBREAK IN APPLE

Abstract. Significant response to selection for budbreak number (NB) based on data recorded on one-year-old shoots of young apple (*Malus* x *domestica* Borkh.) seedlings (Experiment I) and branches from adult seedling trees (Experiment II) has been demonstrated in clonally propagated seedling trees. Between family variation for NB was low and masked by year X family interaction effects. Correlated response in uniformity and position of budbreak, and in the number and length of side shoots, was found. Realized heritability for NB was estimated as 40-60%. Correlated responses in the time of budbreak (TB) within 'Golden Delicious' and 'Anna' selection groups showed some similarities but differences between the two groups were found with respect to family means and midparent values. The highest realized response was observed in 'Anna' crosses. Significant variation between families and parents were found for NB in Experiment II. Association between TB and NB, according to midparent and cross groupings, and according to the parental means, indicate a positive genetic correlation between these traits. Significant response to two-way selection confirms the presence of utilizable genetic variance within seedling families for NB and the results indicate that this procedure may be successfully applied as an early screening method for increased budbreak in adult trees. Combined selection utilizing genetic variance between crosses as well as within crosses is proposed as the best procedure to increase the frequency of seedlings with increased budbreak and to improve adaptation to high winter chilling conditions.

Additional index words: Malus domestica, prolonged dormancy symptoms, chilling requirement, climatic adaptability, fruit tree breeding, two-way selection, selection response, realized heritability, combined selection

Dormancy is generally defined as the temporary suspension of visible growth of any plant structure containing a meristem, e.g. vegetative and reproductive buds (Lang, 1987) and may represent a continuous gradient of regulatory events or phases (Lang, 1985). Chilling Requirement (CR) relates to the need for exposure to low temperatures for temperate fruit trees to be released from dormancy and to commence proper growth (Howe et.al., 1999). Generally, CR

is seen as a complex genetically determined trait, probably multigenic or partly controlled by multiple genes (Dennis, 1987; Howe et al., 1999).

If CR temperatures do not occur during the rest period or when dormancy release is not successful, an absence or long delay of lateral vegetative and reproductive budbreak occurs (Cook, et al., 1998; Hauagge and Cummins, 1991a; Mauget and Rageau, 1988). The length of the delay is directly related to the lack of chilling and the less the chilling received the more uneven the pattern of budbreak (Mauget and Rageau, 1988). These abnormalities are described as prolonged dormancy symptoms (PDS) (Jacobs, et al., 1981; Janick et al., 1996). Most apple cultivars planted in the Western Cape region of South Africa (34°S) exhibit PDS because winters are not cold enough to meet their chilling requirements. Chemical treatments are commonly used to induce uniform bud break and to obtain better fruit set and fruit quality but the practise has come under threat and will be banned in the near future.

Since vegetative budbreak under normal chilling is prolific and occurs uniformly in a relatively short period of time we have examined response to selection based on the number of buds breaking as a criterion of selection against PDS. Specifically, the aims of this study were (1) to evaluate direct response to selection for high and low budbreak within families, (2) to evaluate possible correlated responses in other related traits and (3) to evaluate residual genetic variance for budbreak in young seedlings. The experimental procedure of two-way truncation selection, high and low, was adopted since this provides the best possible experimental control for the measurement of response under conditions of limited resources available, i.e., in this case better than maintaining control populations of equal size in which no selection is applied (Falconer and MacKay, 1996, p195).

Materials and Methods

Plant material and selection procedures.

Experiment I. Families derived from cultivars 'Anna' and 'Royal Gala'.

The initial material consisted of a very large population of two year-old seedlings, approximately 3 400, planted in one-liter plastic planting bags during 1994, comprising 11 families and average family size 280. The cultivar 'Anna' was a common male parent in two families and 'Royal Gala'

the common parent in 9 families. A pre-selection based on visual evaluation of NB was applied in one year-old shoots towards the middle of the growing season in December 1995. Two groups were selected, one for high NB (H selection) and one for low NB (L selection). Approximately 45 H and 45 L seedlings were selected within each family. By visual evaluation we could screen the entire population relatively quickly and obtain the highest possible selection intensity for the limited facilities available for the programme later. Alternatively, we could have reduced numbers by random sampling followed by more accurate selection based on actual counts but at a lower selection intensity. As we had no prior information on which to determine optimal sample size we opted for visual evaluation.

After the visual pre-selection, the actual number of buds breaking was counted and expressed as the number of buds per 100 cm length of shoot (NB). During the following three years (1996, 1997, 1998) both budbreak time (TB) and NB were recorded on each individual seedling. NB was scored 21 days after initial budbreak at the time when leaves started to emerge from a vegetative bud. Seedling growth was revived each year to establish strong growing one-year-old shoots.

Further selections within the H and L groups were performed based on NB scores averaged over the period of three consecutive years after the initial visual pre-selection. Twenty seedlings of each of the H and L groups were selected and cloned by budding on M793 rootstock in a nursery. The clones of selected seedlings were transplanted to plastic bags during the following season and grouped in a randomized blocks experiment to evaluate selection response as the difference between the H and L group means. Trees were allowed to develop without pruning or any other growth manipulation.

Experiment II. Selections from 'Anna' and 'Golden Delicious' families.

In a second trial, seedlings were selected for high and low NB based on the numbers of vegetative and reproductive buds breaking on adult trees collected over the period of three years (1996, 1997 and 1998). These trees originated from families of two different sets of crosses, viz, four families with the high chill 'Golden Delicious' as parent and four families with the low chill 'Anna' as parent. Trees from 'Golden Delicious' and 'Anna' planted in orchards for fruit

evaluation purposes were, respectively, seven and five years of age. 'Golden Delicious' was used as a common female parent in crosses with 'Prima', 'Summerking', 'Starking Delicious' and 'Braeburn' as male parents. 'Anna' was used as a common male parent in crosses with 'Austin', 'Sharpe's Early', 'Kirks' and 'Summerred'. 'Anna' has one of the lowest chilling requirements found in *Malus* x *domestica* (Brooks and Olmo, 1972), ± 300 CU, compared to ± 1500 CU for 'Golden Delicious' (Hauagge and Cummins, 1991a). Of the other parents CR records were available only for 'Braeburn' (± 1100 CU), 'Prima' (± 1100 CU) and 'Summerred' (± 999 CU) (Hauagge and Cummins, 1991a; Hauagge, personal communication).

Thirteen to fifteen seedlings were selected for each of the H and L groups out of a total of 60 seedlings scored per family. Selected seedlings were clonally propagated by budding on M793 rootstock in a nursery. The clonal trees were transplanted to plastic bags during the following season and arranged in a randomized blocks experiment to evaluate selection response, again as the difference between H and L groups and to evaluate residual variation within H and L groups. The trial consisted of 5 blocks with single trees from the two selection groups within each block. Parental cultivars used in the crosses were included in the trial as well for the purpose of comparison of midparent values with H and L groups for the various crosses. Trees were allowed to develop without pruning or any other tree growth manipulation. The first data were collected during the 2000 growing season.

Measurements recorded in clonal trials. The following measurements were recorded on young trees after clonal propagation: NB 21 days after initial vegetative budbreak, TB, the number and length of side shoots, the total number of non-sprouted buds (Experiment I), shoot circumference (Experiment I) and the total shoot lengths on each seedling (I and II). The number of shoots was recorded in the following classes: <5 cm, 6-15 cm, 16-30 cm and >30 cm. NB was again expressed as the number of budbreak per 100 cm length of shoot. Data were recorded on four equal sections (section 1 lower to section 4 upper) over the length of the tree. The number of side shoots (sylleptic branching during the first season) of each tree was recorded. Cold units (CU) were calculated according a modified Utah-model (Linsley-Noakes et al., 1994).

Analysis of selection data. The analysis uses the standard prediction formula for response to truncation selection, viz, $R = HS$ where S is the selection differential, i.e., the difference between means of high and low selection groups in the case of two-way selection, and H is the ratio of genetic variance (V_G) to phenotypic variance (V_P) for the trait in the population under selection. When R and S are calculated from actual data as in the present experiments the estimate of H is R/S, known as the realized heritability (Falconer & Mackay, 1996, p197). The usage requires some qualification as follows (JH Louw, personal communication): (i) The population under selection is the family of a cross between cultivars of which the genomes are likely to be highly heterozygous, including loci for the trait of interest. Genetic variance of the trait therefore refers to *total* genetic variance including additive, dominance and epistatic components. H is then referred to as ***broad sence*** heritability (Falconer and Mackay, 1996, p123). (ii) Selection response, R, refers to follow-up measurements repeated on selected individuals (seedlings), and not to progenies of selected individuals as is the usual usage. Follow-up measurements in these experiments were made on the same seedlings in seasons subsequent to selection, or on vegetative clones of selected seedlings. (iii) The usage is in keeping with the prediction of response in socalled one-step selection in plant breeding, i.e., selection in populations consisting of fixed (and reproducable) genotypes such as inbred lines and line crosses (Wricke and Weber, 1986, p179). In apples and other tree crops, reproduction of genotypes is accomplished by vegetative cloning. (iv) Since estimation relies on *observed* selection differential and response, no assumption of normality in the distribution of the trait is required, i.e., $R/S = V_G/V_P$ is the regression of genotype on phenotype in the conventional sence regardless of the underlying distribution. (v) Families of seedlings such as those for common parents Royal Gala, Anna and Golden Delicious in crosses with other cultivars are sets of full-sib families and the two-way selection was within families. Any ongoing programme of crossing and selection would presumably aim to utilize genetic variance between families as well, by selection based on individual seedling performance (or the mean seedling clone performance) P_w, combined with the family mean, P_f. If P_w is expressed as a deviation from the family mean and P_f as a deviation from the population mean, the standard index for combined selection is applicable, viz,

$$I = k_w P_w + k_f P_f \text{ where } k_w = \frac{1-r}{1-t} \text{ and } k_f = \frac{1+(n-1)r}{1+(n-1)t}.$$

n is the family size, *r* the family relationship (= ½ for full-sibs) and *t* the intraclass correlation for the trait within families (Falconer and Mackay, 1996). The residual variance of NB in the three sets of crosses may then be analysed in these terms with the aim of formulating the next step of a programme of selection for this material and a future programme of crossing involving other genetic resources.

General data analysis. Analysis of variance (ANOVA) was carried out for all measurements for each year and for all years to test for Year X Family interaction effects where applicable. The Year X Family interaction mean square was used as error term in the ANOVA to test significant differences between families in Experiment I. ANOVA to investigate differences between selection groups for individual years was also based on Selection X Family interaction terms. The analyses were performed using SAS General Linear Model procedures (SAS Institute, Cary, N.C.) after testing for heterogeneity of variance by means of the Levene test (Snedecor and Cochran, 1991) and the Shapiro-Wilk test for normality (Shapiro and Wilk, 1965). Data sets were weighted for homogeneity of variance and transformed to to obtain closer approximations to the normal distribution where necessary. Multiple comparisons were performed using Student's t LSD test. Correlation analyses were performed using the SAS Correlation procedure.

Results

Response to selection for budbreak number within families.

Experiment 1. Significant differences were detected between the H and L selection groups for NB, indicating significant selection response over the set of 11 crosses (Fig. 1). Some families showed greater response, e.g, 'Anna X 'Braeburn', and in others a lower response to selection was found, eg, 'Royal Gala' X 'Fiesta'. The response was positive and significant over the full period of three years after the initial visual selection (Table 1a). The response (*R*) calculated as the difference between H and L overall means translates to a realized heritability of around 44%, where the selection differential (*S*) was calculated from mean NB counts after the initial visual selection.

Response to the second phase of selection was verified in the clonal trial over the two following years containing 20 selections in each group. Significant differences between the selection groups were again clearly evident (Fig. 2, Table 1b). A distinct difference in growth habit between the selection groups during the first year was also evident as illustrated in Fig. 3. NB on the main shoots of the H group (sections 1 to 4) was more uniform compared to the L group and the L group showed higher NB in the upper parts (sections 3 and 4) of the main shoots (Table 2). During the second year of this trial, higher NB was evident in the lower parts of trees in the H group compared to the L group and may be explained by the greater extent of side shoot development in this group. NB in the L group remained higher in sections 3 and 4 (Table 2). The observed selection differentials and responses in this phase of selection translates to a realized heritability of around 53%.

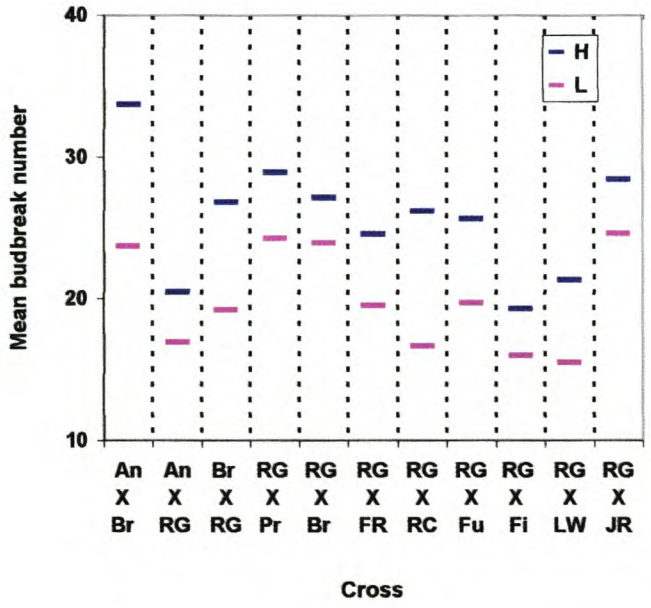

Fig. 1. Response to initial selection for high (H) and low (L) budbreak number within individual families (crosses) averaged over three seasons (1996-1998). Selection was based on visual evaluation in 1995.

Table 1a. Mean response to selection for high (H) and low (L) budbreak number averaged over all families (crosses) for initial visual selection within families (crosses)

	Mean budbreak number					
	Selection	Response				
	1995	1996	1997	1998	Mean (1996/98)	Realized heritability
H	16.52[z]	38.28 ± 1.90	21.84 ± 1.11	16.88 ± 1.43	25.67 ± 1.87	
L	3.86[z]	31.14 ± 1.90	15.52 ± 1.11	13.32 ± 1.43	19.99 ± 0.87	
Mean		34.71	18.68	15.10		0.44

[z] Scored after initial visual selection

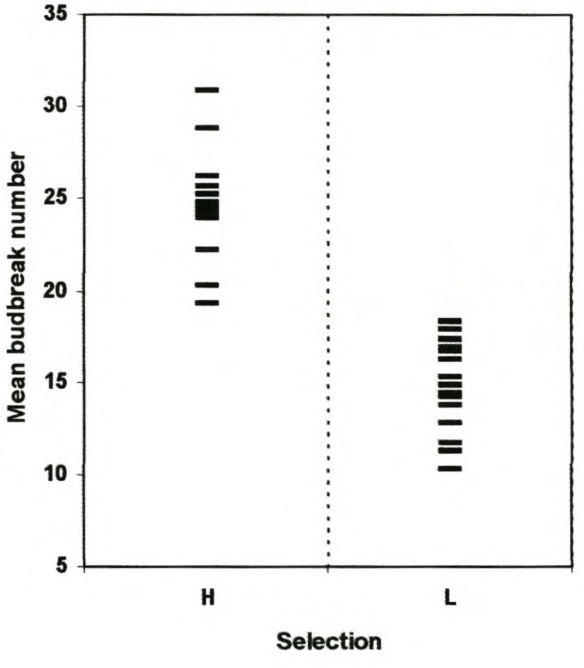

Fig. 2. Response to second phase selection for high (H) and low (L) budbreak number averaged over two seasons (1999-2000). Selection of the 20 highest and 20 lowest was based on three-year means (1996-1998) after pooling all families (crosses).

Table 1b. Mean response to selection for high (H) and low (L) budbreak number averaged over all families (crosses). Selection on three-year means (1996-1998), all families (crosses) pooled.

	Mean budbreak number				
	Selection	Response			
	1996/98	1999	2000	Mean (1999/00)	Realized heritability
H	37.98	34.27 ± 1.11	15.49 ± 0.53	24.88 ± 0.62	
L	19.06	20.50 ± 1.11	9.25 ± 0.53	14.87 ± 0.62	
Mean		27.39	12.37	19.87	0.53

Table 2. Variation in tree characteristics of seedling clones selected for high and low NB. *P*-values indicate differences between the H and L selection groups.

Trait	Year 1 (1999)			Year 2 (2000)		
	H	L	*P*	H	L	*P*
NB [yz]						
Total tree length	34.27	20.50	0.0001	15.49	9.25	0.0001
Section 1	8.79	3.97	0.0001	4.69	2.36	0.0001
2	8.53	5.16	0.0001	4.28	2.30	0.0001
3	8.24	6.30	0.0001	3.87	2.63	0.0001
4	8.49	7.13	0.0001	3.28	2.81	0.0392
Number of side shoots [z]						
Total tree length	13.32	7.90	0.0001	39.53	24.53	0.0001
Section 1	7.94	4.76	0.0001	11.44	5.82	0.0001
2	7.68	5.38	0.0001	13.11	9.20	0.0001
3	-	-	-	12.44	9.05	0.0001
4	-	-	-	6.29	4.68	0.0002
Length of side shoots [z]						
Total tree length	117.09	54.911	0.0001	394.88	282.79	0.0001
Section 1	86.36	44.731	0.0001	156.48	94.31	0.0001
2	46.24	20.769	0.0001	110.99	95.47	0.0180
3	-	-	-	103.77	87.10	0.0042
4	-	-	-	59.25	58.51	0.8178
TB	294.12	300.64	0.0001	277.34	286.49	0.001
Shoot length	165.04	169.47	0.0598	218.12	219.46	0.5955
Shoot circumference	11.91	12.13	0.2710	16.11	15.97	0.5351
Internode length	2.28	2.26	0.2425	2.17	2.11	0.0059
Total buds	72.32	75.39	0.0024	100.15	105.22	0.0017
% Budbreak	94.37	51.13	0.0001	51.36	28.18	0.0001

Table 2 continued (footnotes)

[y] ANOVA performed on Log transformed data.

[z] Individual seedlings with no budbreak and shoot formation were excluded in the analysis to normalize the distribution for ANOVA.

Sections refer to position of tree from lower parts (1st Section) to top of trees (4th Section) on which data were recorded.

Fig. 3. Clones of seedlings for (A) high and (B) low budbreak number, one year after budding on M793 rootstocks (second growing season). One photo represents three clones of one seedling selection.

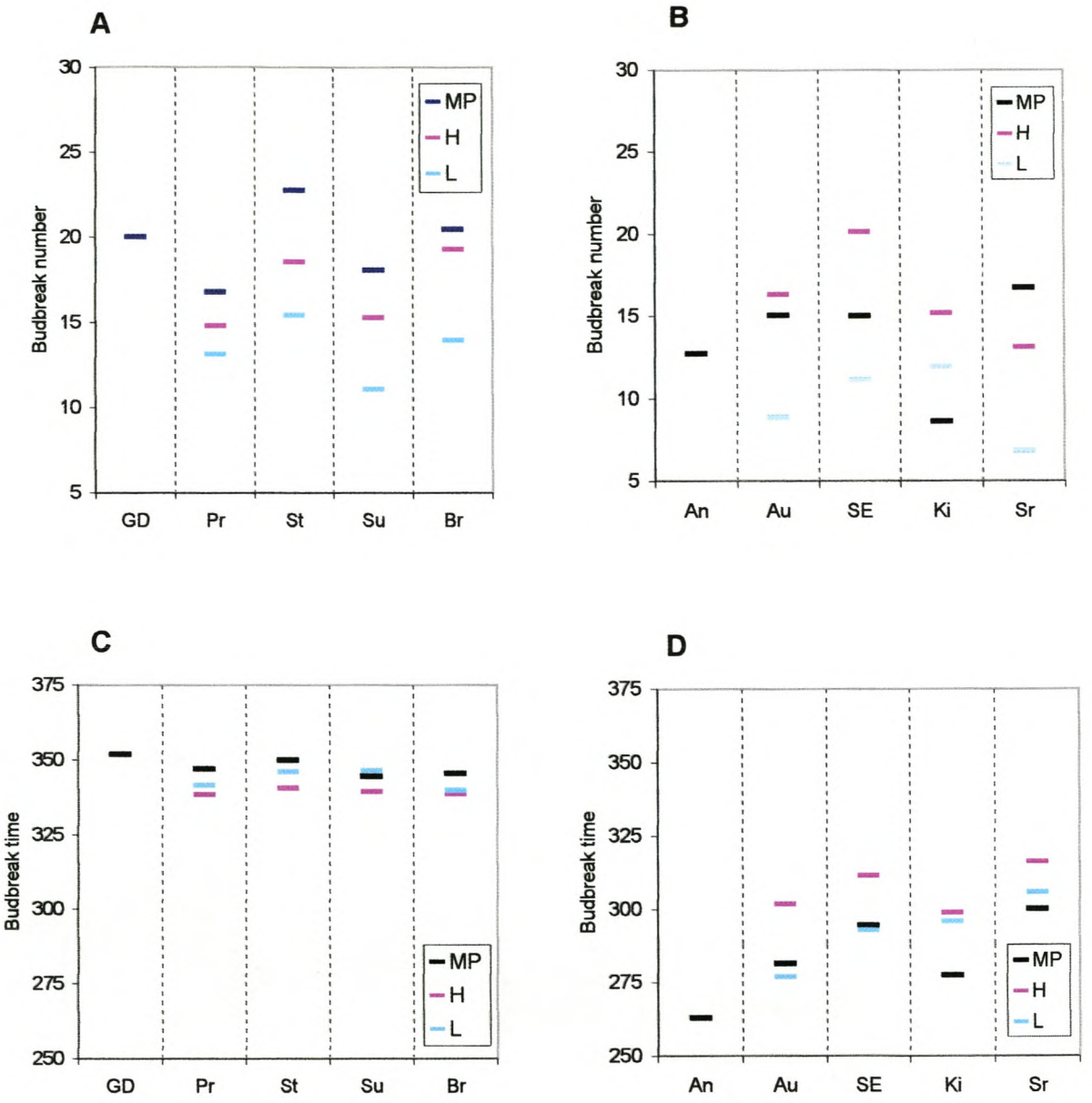

Fig. 4. Correlated response for budbreak number for common parent (A) GD or (B) An, midparent value (MP) and high (H) and low (L) selections in crosses. Correlated response in budbreak time for (C) An and (D) GD crosses.

Experiment II. Significant differences for NB between the H and L groups were evident in 'Anna' (P <0.01) and 'Golden Delicious' selections (P = <0.01) (Fig. 4A and B). On average, 'Anna' selections had a lower mean (12.94) than 'Golden Delicious' (15.21) (P <0.01), in agreement with the observed difference between these two common parental cultivars grown in the same trial. Realized heritability calculated from the selection differential and response to selection was around 63% in 'Anna' families and 40% in 'Golden Delicious' families (Table 3). Both H and L groups were consistently lower than the midparent value in all 'Golden Delicious' crosses and in one 'Anna' cross (with 'Summerred'). These are unexpected results in terms of quantitative genetics unless heterosis (in these cases negative) is associated with these crosses and will have to be explored further.

Table 3. Mean response to selection for high (H) and low (L) budbreak number averaged over all crosses with mean mid-parent value (MP) and realized heritability.

	Mean budbreak number		
	Selection (1996 / 98)	Response / MP (2000)	Realized heritability
(a) An crosses			
MP		14.35 ± 1.23	
H	17.33	16.19 ± 1.80	
L	7.11	9.69 ± 1.80	
Mean H / L		12.94	0.63
(b) GD crosses			
MP		19.51 ± 1.23	
H	17.97	16.97 ± 1.15	
L	9.09	13.45 ± 1.15	
Mean H / L		15.21	0.40

Correlated response in budbreak time

Experiment I. Significant differences between 'Anna' and 'Royal Gala' families were evident for TB. 'Anna' selections were recorded as 28 days earlier in the H group and 46 days earlier in the L group. Within 'Anna' families, the H group was 14 days later than the L group, and in 'Royal Gala' families the H group was four days earlier than the L group.

Experiment II. In the correlated response of TB, large differences between 'Anna' and 'Golden Delicious' selections were found. On average, 'Anna' selections were 42 days earlier than 'Golden Delicious' (P <0.01) (Fig. 4, C and D), and budbreak occurred over a more prolonged period in 'Anna' selections. Budbreak in H groups occurred significantly later than in L groups of 'Anna' selections, and earlier in the H groups of 'Golden Delicious' selections. Midparent values for TB in 'Golden Delicious' selections were generally higher than H and L group means and lower than the H groups in 'Anna', excepting 'Austin' and 'Sharpe's Early' in the L groups.

The genetic association between TB and NB may be analysed in various ways in this material, in terms of means of midparent and cross groupings (Fig 5A), where cross performance is estimated using the overall means of H and L groups, and in terms of parental means taken on their own (Fig 5B). Taking parents alone (Fig. 5B), a positive genetic association (r = 0.679) is evident. Another approach is the regression of cross means for one trait (TB) on midparent values for the second trait (NB) and vice versa, as is done in parent-offspring investigations of genetic correlation between traits (Falconer and Mackay, 1996, p316). This is illustrated in Fig. 6 and again confirms a positive genetic association between TB and NB at this level, i.e., 'Anna' and 'Golden Delicious' parental and cross groups.

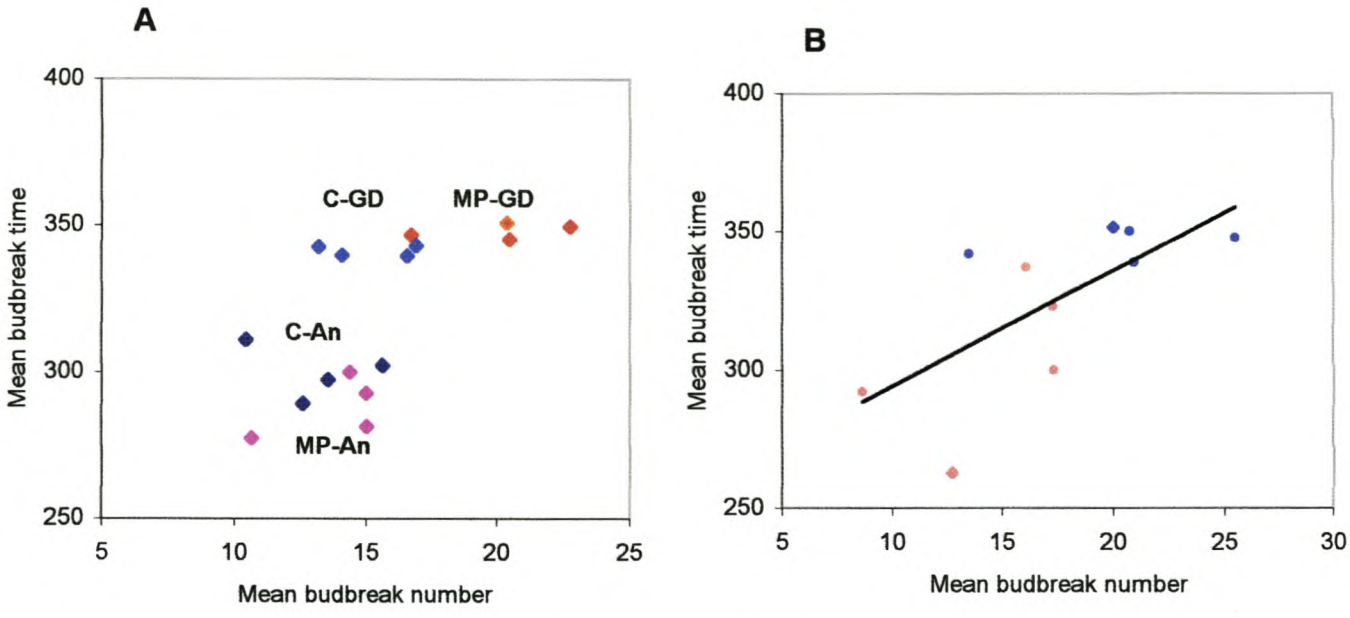

Fig. 5. Genetic associations between budbreak time and budbreak number in groupings according to (A) midparent value and crosses and (B) according to parents alone. MP-GD: midparent values for GD crosses; MP-An: midparent values for An crosses; C-GD cross means for GD crosses; C-An: cross means for An crosses. C-GD and C-An obtained from the means of high and low selection groups.

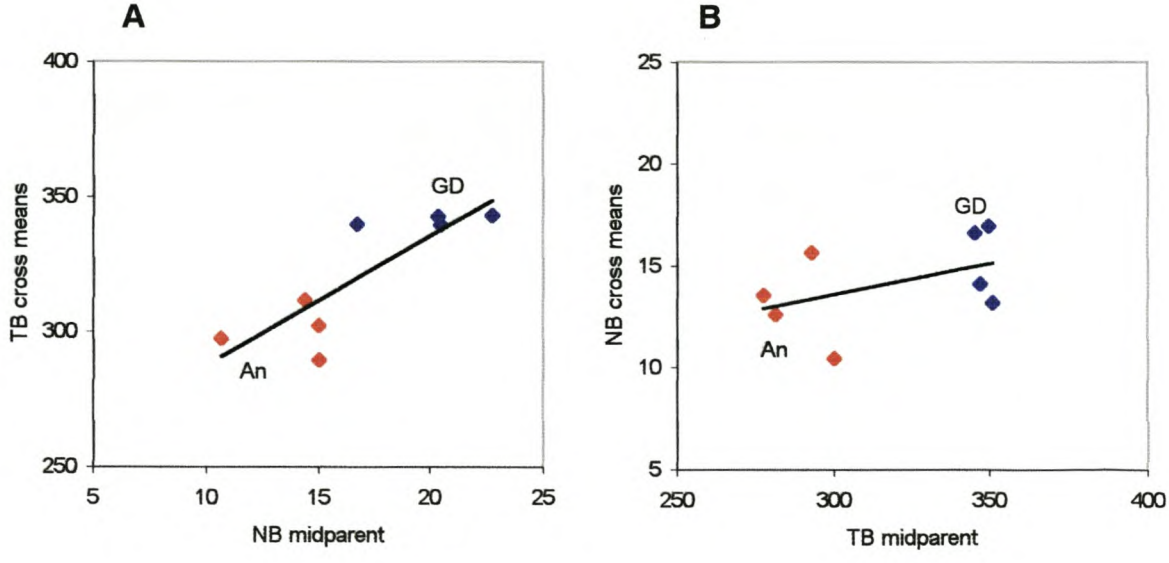

Fig. 6. Regression of cross means for (A) TB on midparent values of NB and (B) regression of cross means for NB on midparent values of TB.

Correlated response in other traits

Experiment I. Following the second selection cycle, budbreak expressed as percentage of total buds was higher in the H group than in the L group. Significant differences between H and L groups for uniformity and position of budbreak, and the number and lengths of side shoots in the cloned seedlings were evident (Table 2). Side shoots were longer in the H group compared to L in both years. Strong growing shoots (shoots exceeding the mean length of all long shoots) in the lower part of trees was 57% in the H group and 50% in L. Means for main shoot length, shoot circumference and internode length did not differ significantly between the selection groups. The L group showed slightly more total buds on main shoots compared to H. More seedlings in the L group had no shoots forming and no buds breaking during both years (data not shown).

Experiment II. 'Anna' had significantly higher reproductive budbreak than 'Golden Delicious' (P <0.01). More reproductive buds occurred on trees in the L group than in the H group. This was also observed for the number of side shoots where the H group produced more shoots. 'Austin' and 'Summerred' had the highest number of reproductive budbreak and 'Kirks' and 'Anna' produced the highest numbers of side shoots.

Between family variance

Experiment I. After the initial visual two-way mass selection, significant differences between families were found in both the H and L groups (P <0.01). During the three years following the initial selection, yearly variation masked family differences. Multiple comparisons suggest low genetic variation for NB between families when Year X Family interaction was applied as error term during this period.

Experiment II. Means for NB suggest that 'Austin' and 'Summerred' selections have low NB, whereas 'Prima', 'Kirks' and 'Summerking' have intermediate NB, and 'Starking Delicious', 'Braeburn' and 'Sharpe's Early' high NB (Table 4). Parental means for 'Kirks', 'Prima' and 'Anna', are low (Table 5, Fig. 5B), 'Sharpe's Early', 'Austin' and 'Summerred' intermediate and 'Summerking', 'Golden Delicious' and 'Braeburn' high. 'Starking Delicious' was consistently

superior. Within the 'Anna' crosses, 'Anna' X 'Austin' was early in budbreak and 'Anna' X 'Summerred' late. Differences between 'Golden Delicious' crosses for TB were not statistically significant. Parental means differed significantly for TB (Table 5), with 'Anna' the earliest and 'Golden Delicious' the latest. 'Austin' and 'Kirks' can be regarded as early cultivars and 'Summerking', 'Starking Delicious' and 'Prima', as late cultivars according to these results.

Table 4. Means for traits in apple seedlings selected for high and low NB (vegetative plus reproductive buds) based on data collected on adult trees.

Families	TB		NB [xy]		Number of reproductive buds [y]		Number of side shoots [yz]	
	H	L	H	L	H	L	H	L
GD X Pr	338.34	341.38	14.77	13.42	0.28	0.45	3.81	3.70
GD X St	340.48	345.90	18.55	15.39	0.16	0.14	2.31	2.20
GD X Su	339.21	346.24	15.27	11.05	0.33	0.25	1.16	1.75
GD X Br	339.65	339.73	19.29	13.93	0.78	1.31	3.31	2.05
Mean	339.41 c	343.12 d	17.04 c	13.95 b	2.62 ab[z]	4.67 abc[z]	7.30 a[z]	6.98 a[z]
An X Au	301.80	276.83	16.32	8.87	2.49	7.76	7.79	5.63
An X SE	311.47	292.95	20.15	11.17	2.30	2.50	5.37	3.83
An X Ki	298.83	295.82	15.19	11.93	3.34	5.00	13.75	9.00
An X Sr	316.13	305.66	13.08	7.80	3.09	2.40	13.70	9.30
Mean	307.02 b	292.40 a	16.32 c	10.10 a	6.75 bcd[z]	8.61 cd[z]	12.67 c[z]	9.09 b[z]

[x] Weighted analyses performed

[y] ANOVA performed on Log transformed data.

[z] Individual seedlings with no budbreak and shoot formation were excluded in the analyses to normalize the distrbution for ANOVA.

Means separated with General Linear models procedure

Table 5. Means for parents used in the development of apple families from which seedlings were selected for high and low number of budbreak.

Parents	TB	NB [xy]	Number of reproductive budbreak	Number of sylleptic shoots
GD	351.67 a	20.01 abcd	0.33 de	1.67 c
Su	350.29 a	20.74 abc	1.57 cd	0.21 de
St	347.73 ab	25.49 a	0.00 e	0.13 de
Pr	341.93 bc	13.49 de	2.07 cd	0.00 e
Br	338.93 c	20.93 abc	5.60 c	2.80 c
Sr	337.07 c	16.09 cde	16.40 a	1.20 cd
SE	322.69 d	17.32 bcde	8.15 ab	1.77 c
Au	299.87 e	17.35 bcde	13.47 ab	1.53 c
Ki	292.00 f	8.62 f	1.60 cde	6.07 b
An	262.93 g	12.70 de	8.50 b	10.43 a

[x] Weighted analyses performed

[y] ANOVA performed on Log transformed data.

Letters indicate significant differences at $P < 0.05$

Analysis of selection response. Results of the analysis of residual variance in the three sets of crosses having one parent in common in the two experiments are further summarized in Table 6, giving the intraclass correlation coefficients, t, and the derived weights k_w for P_w and k_f for P_f, the latter calculated for family sizes of 5, 10 and 20 which should more or less cover the range of family sizes possible in follow-up testing and selection in this material. Note that family size relates to the number of clones per family in this material which also had replicated measurements (trees) within clones. The weight assigned to the family mean P_f increases with

increase in n and declines with increase in t as predictable, but in material of this nature the magnitude of t can be manipulated by the choice of the clone size, t increasing with increase in clone size. This raises the interesting question of the optimum utilization of limited available resources, i.e., the optimum balance between number of seedlings per cross and clone size when the available space for any one cross is limited.

Table 6. Analysis of residual variance for NB in H and L groups in terms of the intraclass correlation coeffisient t within crosses and weighting factors k_w and k_f for combined selection as defined in the text, and for different numbers of clones per cross, n. Data for An and GD refer to Experiment 1 and RG to Experiment 2.

Common parent		H vs L	Crosses	Clones within crosses	t	k_w	k_f		
							$n = 5$	$n = 10$	$n = 20$
An									
	df	1	3	48					
	MS	807.9	134.3	18.3	0.327	0.74	1.30	1.39	1.45
GD									
	df	1	3	48					
	MS	228.8	104.1	28.9	0.167	0.60	1.80	2.20	2.52
RG									
	df	1	9	18					
	MS	412.3	64.4	19.7	0.435	0.89	1.09	1.12	1.13

Age-age correlations: Correlation analyses were performed to test the association between measurements on two-year-old seedling clones and measurements on the original adult parental trees on which the selection in Experiment II was based (Table 7). The first year's set of data on adult trees was recorded during 1996 in the seventh growing year of the 'Golden Delicious' families and the fifth growing year of the 'Anna' families. Correlation analysis based on covariance indicates a positive association between adult and juvenile seedlings for TB in the L group ($r = 0.363$) and in the H group ($r = 0.493$), and for NB in the L group ($r = 0.313$) and for the H group ($r = 0.127$).

Table. 7. Correlation over selections for TB and NB based on covariance of data recorded on adult and juvenile seedling clones.

		TB			NB		
Family	N	Mean Adult	Mean Juvenile	*r*	Mean Adult	Mean Juvenile	*r*
H							
GD X Pr	14	278.86	338.34	0.194	15.22	14.77	-0.427
GD X St	13	272.92	340.48	0.179	18.29	18.55	0.039
GD X Su	14	281.50	339.21	0.215	17.74	15.27	0.228
GD X Br	14	268.21	339.65	0.250	20.41	19.29	-0.185
An X Au	14	249.50	301.80	0.765	17.19	16.32	0.609
An X SE	13	257.76	311.47	0.849	17.87	20.15	0.261
An X Ki	13	249.77	298.83	0.789	17.79	15.19	0.172
An X Sr	14	255.69	316.13	0.725	16.45	13.08	0.331
L							
GD X Pr	14	288.57	341.38	0.028	7.29	13.42	-0.176
GD X St	14	280.86	345.90	-0.465	8.22	15.39	0.072
GD X Su	13	290.62	346.24	0.318	7.30	11.05	0.564
GD X Br	15	270.80	339.73	0.485	13.55	13.93	0.364
An X Au	15	215.20	276.83	0.491	5.25	8.87	0.598
An X SE	13	223.15	292.95	0.732	5.54	11.17	0.242
An X Ki	13	229.69	295.82	0.572	7.84	11.93	0.476
An X Sr	13	247.53	305.66	0.793	8.03	7.80	0.367

Discussion

A significant response to selection in opposite directions for high and low NB was confirmed in clonally propagated trees based on records of one-year-old shoots of young seedlings and branches from adult trees. In Experiment I, NB counts during three seasons following pre-selection clearly confirm a genetic basis for the observed variation. In Experiment II, where data on adult trees were used as a measure of selection response and tested on young clonal trees, significant response and genetic variation was also found. The highest realized response for NB occurred in 'Austin', 'Sharpe's Early' and 'Summerred' selections derived from 'Anna' crosses. Correlated selection response for TB was generally higher in 'Anna' crosses than in 'Golden Delicious' crosses.

Correlated responses between families showed some similarities within the 'Golden Delicious' and 'Anna' groups, but differences between the two groups were clearly evident with regard to family means and midparent values. Midparent values for NB and TB in 'Golden Delicious' were generally higher than for H and L selection means. Discrepancies in midparent values between 'Golden Delicious' and 'Anna' can perhaps only be explained by the relationships between parents that were used in crosses, i.e., 'Anna' was selected from a backcross population which had one, low chill requiring Israeli seedling as the main gene source (Stushnoff and Quamme, 1983).

Realized heritabilities for NB were estimated between 40 and 60%. In Experiment II the realized heritability for 'Anna' families was around 65% and 40% for 'Golden Delicious' families. In previous experiments performed locally, estimates of genetic components of variance between and within families in the absence of selection gave estimates of broad sense heritability for NB around 30% (Chapter 4). Heritability estimates from studies performed on dormancy and related traits in trees include reproductive budbreak time in almond: 80% (Kester et al., 1977), in apricot: 94% (Couraujou, et al., 1995), in cherry: 65% (Hansche et al., 1966), in peach: 39% (Hansche et al., 1972), and in apple: 67% (Hauagge and Cummins, 1991b). For degree of adaptability based on budbreak number in apple a heritability estimate of 36% (Hauagge and Cummins, 1991b) and for blind node propensity in peach, 23% (De Souza and Byrne, 1998). In general, it appears that

the heritability of dormancy traits related to budbreak time can be regarded as intermediate to high and these traits should respond positively to selection.

Correlated responses to selection for NB were found in the uniformity and position of budbreak, and in the number and lengths of side shoots. After the second selection cycle in Experiment I, differences between selection groups H and L were clearly evident and characterized by a more active growth pattern in the H group with higher budbreak numbers and side shoot formation compared to the L group. These shoots are known as sylleptic side shoots that develop on vigorously growing shoots concurrent with development of the main shoot, without a resting phase (Champagnat, 1978; Cook, et al., 1998). Final tree form in orchards depends on the extent of sylleptic shoot formation and lateral branching in the nursery. Sylleptic branching is considered as an advantage for young tree establishment (Cook, et al., 2000; Costes and Guédon, 1997). In Experiment II, 'Anna' selections exhibited significantly higher reproductive budbreak and number of side shoots than 'Golden Delicious' selections, in agreement with observations in previous trials under local conditions where 'Anna' families were found to have precocious behaviour (ability to flower at juvenile stage) compared to 'Golden Delicious' families (Chapter 4).

Variance between families was masked by yearly variation in Experiment I. Parental means for NB in Experiment II suggest that 'Kirks', 'Prima' and 'Anna' have low NB and 'Starking' the highest. 'Prima' has repeatedly been found to be low in NB in other experiments (Chapters 3, 4 and 5). TB in 'Anna' crosses differed significantly, but not so in the case of 'Golden Delicious' crosses. Differences in TB was also the case among the parents. Means between families for the two selection groups showed significant variation for NB. Significant variation in parental means for NB indicates that 'Braeburn' and 'Starking Delicious' can produce higher NB in breeding families. In previous experiments these cultivars have also shown increased budbreak compared to others, viz, 'Braeburn' in Chapter 4 and 'Starking Delicious' in Chapter 5.

Association between TB and NB according to midparent values and cross groupings, and according to parental means, imply a positive genetic association between these traits. Based on data collected from adult trees, 'Anna' selections showed earlier TB that occurred over a longer

period when compared to 'Golden Delicious' selections. This extended period of budbreak has also been found in the evaluation of adult families (Chapter 4) and in young clonal families (Chapter 5). The H selections of 'Anna' were later in TB than the L selections and the H selections of 'Golden Delicious' were earlier than the L selections.

Significant response to early selection is considered as a benefit in tree breeding programmes because it offers a higher return on the investment (Magnussen, 1988), mainly because the time between first leafing and first bloom may be several years. Many examples of pre-selection in juvenile seedlings have been described, for example, pre-selection and secondary traits aiding in selection for time of bloom in apple (Rodriguez and Sherman, 1985), increase in tree height in Balsam-poplars (Riemenschneider, 1992) and in Black cottonwood (Riemenschneider, 1994). In pre-selection for compact growth habit in apple, the number of side shoots and length-thickness ratio of the central shoot have been found to be good discriminating characteristics (Lapins, 1976). In *Prunus domestica* L. prolonged winter dormancy with a more regular cropping performance was achieved when selecting seedlings with high heat requirements (Wilson, et al., 1975). Criteria used previously to select spur type apples included the density of spurs on two-year old shoots, where spur types showed more than 20 spurs per meter, while standards had lower numbers (Blazek, 1992; Warrington, et al., 1990).

In our experiments pre-selection was based on an easy recognizable character, NB on shoots of two-year old intact seedlings planted in plastic bags. The initial assessment was not time-consuming and did not depend on a series of measurements. Response to two-way selection confirms the presence of utilizable genetic variance in seedling families for NB and results indicate that this procedure may be successfully applied as an early screening method for increased budbreak in adult trees. Correlated responses have both adverse and favorable implications for the breeding programme. On the one hand, selection for early TB will not be effective in identification of seedlings with increased NB. Selection for NB, on the other hand, may lead to increased shoot formation that may support better tree shapes and easier horticultural manipulation.

From a practical perspective, selection using NB has resulted in superior seedlings with good prospects for improving adaptation to local areas with low chill winter conditions. The positive correlation found between original seedling performance and clonal selection means, further supports the use of NB as one of the most important symptoms of prolonged dormancy. The utilization of genetic variance between crossses (families) should be included in any future breeding programme by means of the index combining individual seedling or clone performance within families and the family mean. This will further increase the potential of breeding stock which is of major importance in the improvement of vegetatively propagated cultivars. The rate at which selection increases the frequency of seedlings with increased budbreak depends on the number of seedlings per cross and on clone size for which the optimum balance under limited facilities available needs to be further explored.

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7. CONCLUDING REMARKS

- Visual selection using the PDS grade (Chapters 3, 6) and NB index (Chapter 6) successfully identified individual seedlings with increased budbreak. NB index on intact shoots could successfully identify the phenotypic reaction of individual seedlings to climatic stimuli after a period of 21 days following commencement of budbreak.
- An increase in budbreak number and uniformity in response to cold treatment was found using PDS grade, PDS index and NB index as rating criteria.
- Budbreak number is preferred to time of budbreak as sole criterion on the grounds that early budbreak is associated with low budbreak number under local conditions.
- This study resulted in the detection of significant genetic variation within and between apple seedling families for traits associated with chilling requirement, viz, budbreak time (variously referred to as TB, IVB, or IRB), flowering period and number of budbreak (variously referred to as PDS grade, PDS index, or NB index).
- In some experiments family differences for budbreak number were found to be repeatable over years and in others the variance between families was masked to some extent by yearly variation. Overall, low genetic variance between families could be be explained by the fact that families within the groups studied shared one common parent.
- Within family variance was generally higher than for between families for all traits. It can be concluded that high genetic variation within families is directly related to variation generated by segregation of heterozygous combinations of alleles in the parents used in crossing.
- Continuous distributions in crosses indicate that additive effects of genes are probably more important than non-additive effects in the total genetic variance of traits investigated (Chapters 4 and 5). Asymmetry in the distributions towards low budbreak number in one experiment was, however, noted (Chapter 5).
- Broad sense heritability for budbreak time was 69 % and for budbreak number 30 % calculated from genetic components of variance (Chapters 4 and 5). Realized heritability calculated from response to two-way truncation selection was between 40 and 60 % for budbreak number (Chapter 6). We therefore we feel confident in reporting that the heritability of this trait is intermediate to high in the segregating families tested.

- Response to selection based on PDS grade indicates that selection on a visual basis at high intensity can be applied as pre-selection criterion for increased budbreak, but the ordinal nature of the measurement scale creates limitations for quantitative genetic analyses.
- Correlated responses to selection for budbreak number was shown to include improved uniformity and position of budbreak and the number and length of side shoots.
- Differences in growth patterns between selections for high and low budbreak number were evident and were characterized by a more active growth pattern in the H selections with higher budbreak number and side shoot formation. This is regarded as an advantage for young tree establishment.
- The distribution of seedlings according to budbreak time and number identifies a truncation point for budbreak time as optimal for adaptation of seedlings under prevailing local conditions (Chapter 4). A culling level for budbreak time at this point and selection on budbreak number within the selected group will hopefully prevent a genetic shift to later budbreak in breeding families.
- Repeated backcrossing with selection using idependent culling levels for budbreak time and number is recommended as a feasable breeding programme for improvement in adaptation of apple to local conditions. Significant Year X Family interaction in budbreak number in some experiments point to a program of multi-year testing and selection based on mean performance averaged over years. Clonal testing should not be necessary for selection for budbreak time but would be advisable for budbreak number as this improves the accuracy of selection. Combined (index) selection utilizing genetic variance between crosses as well as within crosses is also proposed as a procedure to increase selection response.
- The positive correlation between original seedling values (adult and juvenile) and clonal selection means for budbreak number raises prospects for successful early selection against the symptoms of prolonged dormancy. It remains to be established whether the seedling response to selection for increased budbreak is carried over to adult trees.