A study of apple fruiting branch development under conditions of insufficient winter chilling

By

Karen Maguylo

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Promoter: Prof. K.I. Theron
Dept. of Horticultural Science
Stellenbosch University
South Africa

Co-promoter: Dr. N.C. Cook
Deciduous Fruit Producers Trust
Research, Stellenbosch,
South Africa

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DECLARATION

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SUMMARY

A STUDY OF APPLE FRUITING BRANCH DEVELOPMENT UNDER CONDITIONS OF INSUFFICIENT WINTER CHILLING

Branch architecture is the position and length of lateral shoots along a main axis, and is dependant on competitions (dominance) among meristems and lateral shoots. In areas with inadequate winter chilling, branch architecture is altered, the dynamics of which are poorly understood. The aim of this work was to better understand the dynamics underlying plant architecture. In the first part of the study, the dynamics of apple branch architecture were characterized for two cultivars, Golden Delicious and Granny Smith, in areas with differing degrees of inadequate winter chilling (a warm area and a cool area). In an additional study, progeny of a mapped 'Telamon' (columnar habit) and 'Braeburn' (normal habit) population were used to quantify branch architecture in an effort to develop quantitative trait loci (QTLs) for branching habit. Although branch architecture could be quantified, it was difficult to relate these to known qualitative branching habits, as the columnar gene is dominant and limited the number of progeny that were not columnar.

With the exception of organogenesis in the season preceding growth, acrotonic tendencies (number of growing laterals, lateral length, fruit set) were not related to temporal (primigenic) dominance of the distally located buds or flowers within an axis. In the warm area, both relative time of budburst and flowering among buds within an axis did depict a loss of acrotony (positional dominance of the distally located buds and shoots within an axis). The first buds to burst and flower in the warm area had the greatest ability to grow out and set fruit, respectively, regardless of position within the shoot, implicating a role for primigenic dominance when chill unit accumulation was inadequate. Overall, temporal (primigenic) dominance in the warm area, and positional dominance (acrotony) in the cool area dictated lateral outgrowth and development.

OPSOMMING

‘N STUDIE VAN DIE ONTWIKKELING VAN APPELDRA-EENHEDE ONDER TOESTANDE VAN ONVOLDOENDE WINTERKOUE

Takargitektuur verwys na die posisie en lengte van laterale lote soos dit oor die hoofas versprei voorkom. Dit is afhanklik van kompetisie (dominansie) tussen meristeme en laterale lote. In areas met onvoldoende winterkoue word takargitektuur verander, maar die dinamika van hierdie veranderinge word nog nie goed verstaan nie. Die doel van hierdie navorsing was om die onderliggende dinamika wat plantargitektuur beïnvloed beter te verstaan. In die eerste deel van die studie is die dinamika van appeltakargitektuur van twee cultivars Golden Delicious en Granny Smith, in twee areas met verskillende mate van onvoldoende winterkoue bestudeer (’n warm en ’n koel area). In ’n verdere studie is die nageslag van ‘n ‘Telemon’ (kolomgroeiwyse) en ‘Braeburn’ (normale groeiwyse) kruising gebruik om takargitektuur te kwantifiseer. Dit is gedoen in ’n poging om kwantitatiewe eienskapslokusse vir vertakking te ontwikkel. Alhoewel takargitektuur kwantifiseer kon word, was dit moeilik om dit in verhouding te bring met kwalitatiewe vertakkingspatrone daar die kolomgroeiwyse-geen dominant is en die aantal indiwidue in die nageslag wat nie ’n kolomgroeiwyse gehad het nie beperk was.

Met die uitsondering van organogenese in die seisoen wat groei voorafgaan, is akrotoniese neigings (aantal laterale lote, laterale lootlengte, vrugset) nie beïnvloed deur tydelike (primigeniese) dominansie van distale knoppe of blomme binne ’n as nie. In die warm area het beide relatief tot knopbreek en blomtyd binne ’n assestelsel die verlies aan akrotonie beskryf (posisionele dominansie van distale knoppe en lote in assestelsel). Die eerste knoppe wat bot en blom in die warm area het die beste vermoë om te groei en vrugte te set, onafhanklik van hul posisie. Dit impliseer die rol van primigeniese dominansie wanneer ’n gebrek aan winterkoue ervaar word. Algemeen gesien was dit tydelike (primigeniese) dominansie in warm areas en posisionele dominansie (akrotonie) in die koeler area wat lateraal bot en ontwikkeling bepaal het.

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CONTENTS

This dissertation presents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters, therefore, has been unavoidable.

General Introduction

Branch architecture in apple (*Malus x domestica* Borkh.) is defined according to the location, type (vegetative or reproductive), and length of lateral shoots. This is related to both an inherent genetic basis for architecture and the response of the laterals buds and shoots to environmental constraints (Hallé *et al.*, 1978). Architecture depends on the competitions, whether positional or temporal, among lateral buds and shoots (Bell, 1991). One of the defining architectural characteristics in apple is acrotony or a dominance of the distally located proleptic buds and shoots. Acrotony exists on a number of levels (acropetal increases in organogenesis, budburst time and location, lateral outgrowth, and fruit-set) (Barthélémy & Caraglio, 2007; Cook *et al.*, 1998; Costes & Guédon, 2002; Lauri, 2007). In areas with inadequate winter chilling, one of the ways that branches respond to incompletion of endodormancy is to display symptoms of 'prolonged dormancy syndrome' which is characterized by erratic and prolonged budburst (Black, 1952; Saure, 1985). When this occurs, the acrotonic budburst tendency is also lost (Cook & Jacobs, 1999). The main ideas behind this study were to determine what is variant and what is invariant in terms of architecture with regards to inadequate winter chilling (Paper 1), to characterize dominance (positional and temporal) within apple shoots (Papers 1, 2 and 3), and to determine if architectural characteristics can be quantified and assessed early in a breeding program (Paper 4). As apple architectural characteristics are well-studied, it is easy to relate characteristics observed in this study to those observed in previous studies by other researchers.

Branch architecture is generally studied as a snap-shot in time (Hallé *et al.*, 1978). Characteristics are measured, and based on our knowledge of time of development of organs, related to competitive events occurring at a specific time. For example, in apple, differences observed in organogenesis of lateral buds within an axis are related to positional competition among those buds in the preceding season, i.e., within an axis there is an acropetal increase in spur leaf number and an increased ability to become reproductive (Lauri, 2007; Powell, 1995). Therefore, knowledge of organ production and lateral growth through time is crucial for understanding competitions among meristems when measured at a later time. Due to the fact that a thorough review has recently been written on architecture of trees (Barthélémy & Caraglio, 2007), in addition to the original work on architecture by (Hallé *et al.*, 1978), the literature review included in this dissertation will mostly cover a more basic aspect of plant architecture (i.e., the development of the meristem and factors influencing growth).

Reproductive buds are an ideal structure to analyze competitions among meristems within an annual shoot since spur leaf and flower number are evidence of competition in

autumn, and differences in fruit-set is evidence of competition during the following spring. In Paper 1, temporal and positional competitions among reproductive buds in 'Granny Smith' and 'Golden Delicious' two-year-old axes were characterized.

One of the theories of decreased budburst along one-year-old axes in inadequately chilled areas is that the terminal bud bursts before the laterals, establishing a primigenic dominance (Jacobs *et al.*, 1981; Saure, 1985). In the second paper, position (terminal or lateral) of the first bud to burst on one-year-old axes of 'Granny Smith' and 'Golden Delicious' was determined for each area, and, in 'Granny Smith', was related to lateral shoot characteristics. One of the main reasons for studying this in two areas was to determine whether lateral budburst and outgrowth was specifically related to environment (accumulated chill units) or more related to primigenic dominance of either the terminal or lateral buds.

Another part of this study (Paper 3) involved characterizing architecture of 'Granny Smith' and 'Golden Delicious' apple branches in two areas with different degrees of inadequate winter chilling. As acrotonic budburst is lost, the main objectives were to characterize the dynamics of budburst and relate this to final branch form (i.e., does length of a lateral have a relationship to position and/or time of development?). Branch architecture, by default, includes the location of latent lateral buds and aborted lateral shoots (Lauri, 2009). Lateral abortion was related to both position and relative time of budburst of a bud within an axis; bud latency was related to position within an axis. Lateral abortion (death of a lateral shoot) can either aid in the development of acrotony when buds are aborted more in the proximal section of the shoot or aid in the loss of acrotony when they are aborted in the distal sections of the shoot.

The final part of this study involved determining whether branch architectural characteristics could be quantified and related to known branching habits. For this, branching habits of progeny of a 'Telamon' x 'Braeburn' cross were quantified and clustered to form groups based on branching variables. These groups were then related to the known branching habits of apple. Along with the previous papers (determining what architectural characteristics are variant or invariant in relation to inadequate chilling), this would open the door to selecting for inheritable branch architecture characteristics early in a breeding program.

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Literature Review

Introduction

The overall architecture of the shoot system is derived from the activity of apical meristems. Apical meristems in the terminal position (terminal buds) provide the 'parent shoot' or main axis as well as give rise to leaves and axillary meristems which may or may not grow out in the year that they are formed (Barthélémy & Caraglio, 2007; Bell, 1991; Hallé *et al.*, 1978). As lateral proleptic buds, apical meristems provide the lateral shoots that define branch form. Branch architecture depends on the positional and temporal (i.e., primigenic dominance, or the dominance of a structure based on its time of development) competitions (dominance) among meristems, buds and lateral shoots along the main axis (Bell, 1991). These competitions influence resulting lateral organogenesis and size. Although meristems are plastic in their response to environmental cues, their characteristics are genetically determined (architecture) (Hallé *et al.*, 1978). Therefore, the dynamics among meristems not only define the mechanisms that underlie the development of the inherent architecture, but govern the architectural reaction to the environment as well which results in the final branch architecture.

One of the main defining developmental stages influencing branch dynamics in apple is the progression of dormancy, beginning with paradormancy, during which a bud's growth is inhibited by factors not internal to the bud. Therefore, branch architecture is altered in areas with inadequate chilling (warm areas). Apple branches grown in warm areas exhibit decreased and erratic budburst as well as an increased dominance of the terminal bud over lateral buds (Black, 1952; Cook & Bellstedt, 2001; Cook & Jacobs, 1999; Jacobs *et al.*, 1981; Saure, 1985; Strydom *et al.*, 1971). Competition among meristems differs with inadequate winter chilling (Cook & Bellstedt, 2001; Cook & Jacobs, 1999), and research in these areas will help distinguish between what is innate in branch architecture and what is influenced by the environment.

In order to accurately discuss the innate and environmental aspects of branch architecture, it is necessary to understand the mechanisms that underlie plant architecture, i.e., node number, internode length, activity of lateral and terminal meristems and relationships among these meristems (Bennett & Leyser, 2006; Rohde & Bhalerao, 2007). Because meristem competition is a continuous and dynamic process and architecture is observed at a point in time (static), it is important to understand when organogenesis occurs within the axes and how this can be determined using criteria measurable at a point in time (i.e., differences in spur leaf number reflect competitions occurring at shoot growth cessation in the year prior to

growth of the annual shoot) (Pratt, 1988; Pratt, 1990). In this review, I will define morphogenesis and growth of both the bud and apical meristem, specifically as these relate to competitions among meristems and branch form.

Meristems

Meristems Involved in Primary Growth- Apical meristems are areas of active cell division located distal to the youngest leaf primordia in a shoot or bud (Pratt, 1990). Maintenance of the apical meristem involves two processes: organ initiation and continuous renewal of itself (Scofield & Murray, 2006). The apical meristem is responsible for the creation of both the axillary meristems and primary vascular tissue (primary xylem and primary phloem). Organogenesis occurs on the flanks of the apical meristem and gives rise to the epidermis, cortex, and leaf primordia. The ontogenesis of vascular tissue is closely associated with that of leaf organogenesis (Rohde & Boerjan, 2001).

Axillary meristems are produced in the axils of the leaves (Bell, 1991; Bennett & Leyser, 2006; Garrison, 1955) and were originally continuous with the apical meristem that produced them (Pratt, 1967; Rohde & Boerjan, 2001). Unlike the apical meristem which is destined to be a shoot and modified to become a bud later in its development, an axillary meristem is destined to be a bud at its inception, or a shoot (via syllepsis), or to remain latent (Rohde & Boerjan, 2001). Outgrowth of axillary buds without an intervening period of rest is called syllepsis, which is in contrast to prolepsis in which there is a difference in time between organogenesis by the apical meristem and elongation of the shoots produced, via an intervening period of rest and bud formation (Bell, 1991; Hallé *et al.*, 1978). In prolepsis, budscales develop resulting in a distinct morphology: budscale scars separated by short internodes (Bell, 1991). The apical meristem indirectly inhibits syllepsis via a dominance phenomenon in the shoot called apical dominance (apically produced auxin indirectly suppresses lateral bud outgrowth), in which the outgrowth of an axillary meristem is inhibited by the apical meristem that produced it, resulting in a bud (Cline, 1991).

Tree and Branch Form

Tree Architecture- Architecture is essentially the genetically-blueprinted constructional organization of the tree (Hallé *et al.*, 1978) and is dependant on the activity of meristems since these are responsible for primary growth (Bell, 1991). Apple branch architecture (distribution of lateral types and sizes along a parent shoot) contains elements of both the invariant genetically-dictated architecture as well as the variant aspects that are in response to

environmental constraints. For a recent and very thorough review of architecture see Barthélémy and Caraglio (2007).

Fruiting Types- Independent of an architectural characterization, apple trees can be classified into four main branching habit groups based on growth (upright to weeping) and fruiting habit (Types I to IV) (Lespinasse, 1977), as well as position of the scaffold branches along the trunk (Lespinasse, 1992). Type I trees, representing one side of the spectrum, are spurred and mainly fruit on two-year-old wood and older, while Type IV trees have longer branches with a weeping habit, and fruit in distal positions and/or terminally on these brindle length shoots. Type II and III trees have intermediate fruiting and branching habits. The importance of the weeping habit of type IV trees is not only that the fruit are produced in the terminal positions of the axes but that the weeping habit may be partly due to the weight of the fruit on these longer shoots. In addition, due to the less or more autonomous nature of the laterals, Type I and Type IV are biennial and regular-bearing, respectively.

Fruiting type is a descriptive way to categorize degree of polyarchy and hierarchy among laterals, which is partially genetically determined. Hierarchy results in uneven competitions among laterals (i.e., Types I and II), while polyarchy results in laterals with equivalent competitive abilities (i.e., Type IV) (Lauri *et al.*, 1995). Polyarchy has also been referred to as basal dominance (Cook *et al.*, 1998). Even competition among laterals is related to autonomy of the laterals. Shorter laterals must rely on neighboring structures to sustain themselves, for example in fruit set of reproductive buds (Lauri *et al.*, 1996).

Shoot Types- Lateral vegetative shoots have varying degrees of preformation and neoformation. Preformation is suggested to be the architectural (positional, genetic) component of form, and neoformation to be the element of plasticity in response to environmental, exogenous, or even endogenous, constraints (Barthélémy & Caraglio, 2007). Of course, preformation may not always be an element of the final visible form if environmental factors prevent outgrowth of the preformed organs.

Shoots can be classified based on their length, the maximum of which is genetically regulated (Hu *et al.*, 2003), and their degree of preformation. Short shoots, or spurs, are entirely preformed and internodes do not elongate. Long shoots may either be only preformed with elongating internodes, or have a preformed growth followed by neoformed growth (Barthélémy & Caraglio, 2007; Bell, 1991; Costes *et al.*, 2006). Lateral shoots with only neoformed growth are considered sylleptic when outgrowth is simultaneous with that of

growth of the parent axis (Bell, 1991; Hallé *et al.*, 1978). When entirely neoformed shoots occur on older axes, outgrowth may be due to reiteration (duplication of the entire tree architecture; also called epicormic shoots or suckers) (Hallé *et al.*, 1978; Lauri *et al.*, 2009). The ability to reiterate is an important architectural feature that can be used to discriminate between apple genotypes (Lauri *et al.*, 2009).

Another way to classify branches is based on their relationship to the previous year's growth such as monopodial growth (i.e., terminal extension growth; e.g., in apple, when a vegetative lateral in one year is followed by vegetative growth in the following year), and sympodial growth (i.e. lateral extends via a bourse shoot or sub-terminal extension growth of vegetative lateral; e.g., in apple, when a reproductive lateral in one year is followed by a reproductive or vegetative lateral in the following year) (Bell, 1991; Hallé *et al.*, 1978).

Reproductive annual shoots have a short preformed shoot (bourse) terminating in an inflorescence and may have one or more relay axes (bourse shoots) (Pratt, 1988). The first few nodes of the bourse shoot can be preformed, although the bourse shoot is mainly due to neoformation (Costes *et al.*, 2006). The bourse shoot is partially preformed (it's meristem present in bud since its inception in the previous season) and therefore can be considered proleptic, even though there is a lack of budscale scars more commonly associated with syllepsis (Bell, 1991).

The Apple Fruiting Branch- In the first year of its growth, an apple annual shoot, hereafter referred to as shoot, produces several leaves and a meristem in the axil of each leaf (axillary meristems). In the following year, the buds on one-year-old axes (lateral buds) have five developmental fates in three stages: (1) Latent Stage (to remain dormant or latent); (2) Growing Stage (to grow as a (a) reproductive lateral with a fruit, (b) reproductive lateral without a fruit, or (c) vegetative lateral shoot); and (3) Ending Stage (to abort and produce a scar) (Lauri *et al.*, 1995). Both latent and ending stages represent non-growing meristems and are influential in defining branch architecture (Lauri, 2009). The relative amounts of growing and latent meristems differ according to genotype (Costes & Guédon, 2002; Lauri *et al.*, 2006) and may (Renton *et al.*, 2006) or may not (Lauri *et al.*, 2006) differ according to main axis length within a genotype (Lauri *et al.*, 2006).

In addition to relative quantities of bud types, lateral developmental sequences can be used to characterize branching pattern (Lauri *et al.*, 1995). Yearly developmental sequences can be identified for each lateral along the main axis. These specific sequences from one year to the next are both cultivar specific and related to fruiting type (Lespinasse (1977) Types I to

IV). Although all cultivars have the general movement from latent stage to growing stage to ending stage over a period of time related to the lifespan of the lateral, specific sequences can be used to discriminate between cultivars (Lauri *et al.*, 1995; Lauri *et al.*, 2006; Lauri *et al.*, 2009).

Two of the most common cultivar-discriminating sequences are bourse-over-bourse (the ability of a reproductive lateral in one year to be followed by another reproductive bud in the following year) and lateral abortion (the movement of a lateral from the growing stage to the ending stage). Both of these developmental sequences are important in regulation of bearing and are characteristic of not only individual genotypes but also of fruiting types (Lauri *et al.*, 2009).

Bourse-over-Bourse and Lateral Abortion- Bourse-over-bourse and lateral abortion may be functionally related (Lauri *et al.*, 1995; Lauri & Costes, 2004). Lateral abortion, which has a genetic or epigenetic basis (Lauri *et al.*, 2009; Lauri, 2009), is the ability to abort all potential growth from a lateral, usually due to non-production of a bourse shoot on, most commonly, an inflorescence that did not produce a fruit (Lauri *et al.*, 1995). In pear, it has been observed as abortion of vegetative lateral spurs or shoots in areas with inadequate winter chilling (du Plooy *et al.*, 2002). Although architecture is defined by growing laterals, lateral abortion plays a role in the development of architecture (Lauri *et al.*, 2009). Lauri and Lespinasse (1993) proposed that lateral abortion is linked to the functional autonomy of the remaining laterals (bourse-over-bourse) along an axis (Lauri *et al.*, 1995). While lateral abortion is known to have a basipetal increase along axes (i.e., via positional competitions) (Lauri, 2007), it is not currently known if lateral abortion is also related to time of budburst or development of a lateral shoot.

Bourse-over-bourse is associated with a minimum length of the bourse shoot. As there is an increased tendency from Type I to Type IV to have a longer annual growth period, bourse-over-bourse increases with fruiting type from 10% (Type I) to 65% (Type IV) (Lauri & Lespinasse, 1993). The functional autonomy of Type IV laterals may be due to the extended growth period resulting in a longer shoot subtending the bourse (Type IV cultivars bear on shoots approximately 15 cm in length) (Lauri & Lespinasse, 1993). This is in contrast to Type I cultivars which have shorter laterals, a disjunction between fruiting and vegetative structures, and therefore a lower propensity to become autonomous. Even though extinction and bourse-over-bourse are functionally related, it is uncertain whether this is because extinction triggers an increase in organogenesis and autonomy of the other meristems along

the axis, or if the bourse-over-bourse phenomenon restricts growth of adjacent meristems, resulting in a lateral aborting (Lauri, 2009).

Annual Cycle of Shoot Growth and Organogenesis

Shoot Growth Cessation, Initiation of the Terminal Bud and Autumn Syndrome- The first step towards dormancy is growth cessation of the shoot, or terminal bud set (Abbott, 1970; Heide & Prestrud, 2005; Olsen, 2006). The series of stages leading to dormancy includes: growth cessation, formation of budscales and winter buds, leaf senescence and abscission, and induction of endodormancy (Abbott, 1970; Heide & Prestrud, 2005; Olsen, 2006). Heide and Prestud (2005) refer to these stages as the "autumn syndrome". Leaf senescence and abscission occur acropetally along the shoot (Abbott, 1970; Heide & Prestrud, 2005) and are promoted by low temperatures (2 weeks exposure to 9ºC day/4ºC night temperatures (Lakso *et al.*, 1999); 1 to 2 weeks at 6, 9, or 12ºC after active growth at 21ºC (Heide & Prestrud, 2005)). In warm climates, leaf abscission is delayed.

Unlike most temperate tree species, growth cessation in apple is not induced by photoperiod; instead, growth cessation in apple is induced by low temperatures (<12ºC) (Heide & Prestrud, 2005). Growth cessation, leaf drop, and bud initiation occurred within 1 to 2 weeks of exposure to ≤12ºC and ≤9ºC temperatures in M9 and MM106, respectively. When MM106 was grown at 12ºC, growth cessation occurred only after 4 weeks and leaves were not shed entirely, but growth resumed immediately at 21ºC indicating that buds were not in endodormancy. At 12ºC, M9 ceased growing after 1 to 2 weeks, but could not resume growth at 21ºC even after 14 weeks.

Low night temperatures (21/9ºC; 10 hours) reduced growth rate but did not produce growth cessation in apple rootstock cultivars B9 and 'A2. However, 21/9ºC produced more shoot growth than those grown at a constant 15ºC, demonstrating that temperature fluctuations do not have simple responses (Heide & Prestrud, 2005).

Growth cessation generally involves both a decrease in internode length and a decrease in production of new leaves. Leaf production, and subsequent initiation of the terminal bud, is influenced by temperature (Heide & Prestrud, 2005). In contrast, internode length is, in part, influenced by photoperiod in apple rootstock cultivars M9 and MM106 (Heide & Prestrud, 2005). Since internode length is, in part, photoperiodically controlled, it indicates an involvement of both GAs and the phytochrome system which is involved in the perception of day-length (Howe et al 1996; Smith et al 1995). A decrease in internode length and bud

set, however, does not mean that dormancy will be induced. Photoperiod simply causes a decrease in shoot elongation (Olsen, 2003).

From Leaf Primordia to Budscales- The difference in budscale development between apical and axillary buds is that budscales are predetermined in axillary buds at their inception (Rohde & Boerjan, 2001), whereas in apical buds they are not predetermined but decided much later in their development (Fulford, 1966a). Both axillary bud initiation and development, and subsequently, release, are related to gradients among the buds along an axis, whereas initiation of apical buds is related to shoot growth cessation. The first sign of a change in the development of a leaf primordium to a budscale was found at, depending on the study, either the fifth (Fulford, 1966a) or eighth (Abbott, 1970) node below the apical meristem. Fulford (1966a) noticed that the change to budscale was characterized by the lateral growth of stipules and an increased development of the leaf base relative to the primordium as a whole while Abbott (1970) observed the first sign as a withering and abscission of the lamina and petiole, with the leaf base (and round scar at its apex) becoming the first, and outer, scale of the bud. The development of the budscale is a continuous process from leaf primordium to mature bud-scale so that within the bud at this point in time are the leaf primordia, immature budscales (all forms between leaf primordia and budscales including transition leaves), and budscales (Bell, 1991; Fulford, 1966a).

An active apical meristem is not free of inhibitions by other meristems. The rate of production of primordia by the apical meristem can be related to the extent at which it is inhibited by the primordia (leaves) adjacent to it and older leaf primordia inhibit, to a degree, the activity and development of the younger leaf primordia and other meristems in the apical region of the bud (Fulford, 1965). Budscales act to increase the separation of the foliage from the apical region of the bud in terms of nodes, and release the apical meristem from the effects of the foliage (allowing bracts to form) (Fulford, 1966a).

The rate of development of leaf primordia in the bud appeared to be progressively reduced as the season continued, so that the change to bud-scale development affected the primordium at an earlier stage of development (Fulford, 1966a).

Flower Development- Flower initiation begins after a critical node number is reached within the bud (Abbott, 1970; Fulford, 1966b; Huang, 1996; Luckwill, 1970). Flower initiation is related to plastochron (number of days between primordia production), which is not uniform; the plastochron is faster in earlier stages (~2 nodes/week) and then slows gradually. With a

long plastochron, the node number is not reached and the bud remains vegetative (Huang, 1996).

Time between shoot growth cessation of a shoot or spur and flower bud initiation is uniform for a cultivar and may last from two to five weeks (Huang, 1996). During the first signs of flower bud initiation, node formation in vegetative buds and reproductive buds differed (Huang, 1996). Normally, the number of nodes leveled off in vegetative buds while reproductive buds produced one to three more nodes before leveling off (bracts). However, in an area with a high temperature and humidity late in the summer, after the start of reproductive bud morphogenesis, reproductive and vegetative buds increase synchronously (increased nodes approximately three to four weeks more and then leveled off). The theory is that when vegetative growth is favored, vegetative buds continue to differentiate while reproductive buds stay at an initial stage for longer (Huang, 1996).

Bract Formation- Bract formation is related to the number of primordia differentiated within the bud, the degree of correlative inhibition of the buds, and possibly their vascular connections (Fulford, 1966b). Once the period of bract formation is over, flower morphogenesis proceeds rapidly (Bergh, 1985b; Costes, 2003; Foster *et al.*, 2003) so that only fully formed flowers are found in reproductive buds (Costes, 2003). In buds that have bracts formed, flowers will form as a consequence of the bracts, even if defoliation occurs on the tree (Fulford, 1966b).

The initial transition from a vegetative to a reproductive bud is characterized by a doming or rounding of the apical meristem (Bergh, 1985b; Foster *et al.*, 2003; Fulford, 1966b; Huang, 1996). Flower formation occurs only after bracts have been formed and never in their absence so bract formation can be used as an estimate of time of flower bud initiation (Fulford, 1966b).

Bract formation signifies a change in developmental fate although this change is reversible. Bracts form when they are one to two plastochrons old, indicating that a change in their development occurs shortly after their inception. There are no transitional structures between leaf primordia and bract. During bract formation, leaf primordia preceding the bracts were also modified to have flattened lamina and distinct stipular development; these primordia will become the characteristically small primary leaves of the flower cluster (Fulford, 1966b). If organogenesis ceases prior to completion of the entire bract formation period then an aberrant bud (reversion to a vegetative bud after earliest transition to

reproductive bud) will form that contains the bracts and these small primary leaves (Fulford, 1966b).

Floral Morphogenesis- A minimum number of bracts determines when the flower is formed and once the period of bract formation is over, floral commitment is irreversible (Fulford, 1966b). The timing of flower initiation is not related to the subsequent time of flower or fruit maturity, however it does differ between cultivars (Hoover *et al.*, 2004). The rate of primordia production within the bud is related to how much it is inhibited by the leaves so that sepals have an increased production rate due to the presence of bracts (Fulford, 1966b).

When subtending growth (in the apical meristem) or inhibition by the apical meristem ceases (in axillary buds, when a certain number of nodes exist between the apical and axillary meristem) rounding, doming, and flattening of the apex have all been reported to occur (Bergh, 1985b; Costes, 2003; Foster *et al.*, 2003; Huang, 1996). Lateral meristems within the bud initiate bracts before the terminal, each subtending a lateral floral meristem (Foster *et al.*, 2003; Huang, 1996). Lateral meristem bracts within the bud develop acropetally and their size is dependant on their position with the middle one being the largest followed by the distal, and the proximal being the smallest in size (Huang, 1996). After lateral bracts are developed, the bract subtending the terminal meristem is formed (Foster *et al.*, 2003).

After bract formation, the time at which dominance of the terminal meristem over lateral meristems occurs within the bud has been observed to begin at different stages. What is not disputed by any of the authors is that the terminal meristem is dominant during flower initiation, and that the development of floral structures occurs more quickly in the terminal meristem than a lateral meristem despite the fact that initiation of lateral floral meristems within the bud preceded that of the terminal (Bergh, 1985b; Foster *et al.*, 2003; Huang, 1996). On a global view, the factors that influence floral organogenesis do not affect the reproductive bud as a whole but act locally within a meristem (Fulford, 1966b).

Among the lateral floral meristems within the bud, floral organogenesis proceeds acropetally so that the most proximal forms first and the most distal (closest to the terminal) forms last (Foster *et al.*, 2003; Huang, 1996).

Organ development of each floral meristem (lateral and terminal) develops in the following centripetal order: sepals, petals, stamens and carpels (Bergh, 1985b; Dennis, Jr., 1986; Foster *et al.*, 2003). Rate of appendage formation differs according to cultivar (Dennis, Jr., 1986). By autumn, all floral organs are microscopically visible except for the ovules (Dennis, Jr., 1986), and buds enter endodormancy with a preformed number of primordia

(Costes, 2003). Abbott (1970) reports this as "nine budscales, three transition leaves, six true leaves and three bracts. This axis is terminated by a flower primordium (the 'King' flower) and the lateral flower primordia are formed in the axils of three bracts and the three distal leaves." However, preformation is also influenced by position within the axis. Terminal buds on long shoots have more organs than spurs, which in turn have more organs than axillary buds. Organogenesis of the preformed bud is not due to growth cessation of the subtending shoot since terminal buds grow longer than spurs. Organogenesis is more related to the morphogenetic gradient along the axis (i.e., competition among buds) (Costes, 2003).

Organogenesis in buds may (Costes, 2003) or may not (Bergh, 1985b; Gordon *et al.*, 2006) stop at endodormancy. Although the preformation part of the bud should occur prior to dormancy, in areas with inadequate winter chilling, there may be a blur between preformation and neoformation. In apple grown in South Africa, floral organs (carpels, sepals, petals, and pollen sacs) develop slowly during winter (Bergh, 1985b). In peach grown in California, organogenesis of vegetative buds was found to occur during dormancy so that from leaf fall to budburst, the number of primordia in the bud doubled (although transition to reproductive did not occur) (Gordon *et al.*, 2006). Therefore, bud fate (reproductive or vegetative) is determined in the preceding season but organ number may increase through dormancy in inadequately chilled areas.

Dormancy

Bud development and/or maintenance can be due to either environmental (e.g. photoperiod, cold temperatures or water shortage) or internal (e.g. correlative inhibition or apical dominance) factors, and indicates the establishment of dormancy (Crabbé & Barnola, 1996). Dormancy is a temporary suspension of visible growth of any plant structure containing a meristem (Lang *et al.*, 1987; Olsen, 2006). Dormancy is also a major determinant of branch architecture (Champagnat & Côme, 1986). Many reviews have recently (Anderson *et al.*, 2001; Arora *et al.*, 2003; Chao, 2002; Chao *et al.*, 2007; Horvath *et al.*, 2003; Olsen, 2003; Olsen, 2006; Rohde *et al.*, 2007; Rohde & Bhalerao, 2007; Shimizu-Sato & Mori, 2001) and more historically (Bachelard, 1980; Champagnat, 1983; Champagnat, 1989; Champagnat & Côme, 1986; Saure, 1985; Vegis, 1964) been written about dormancy, so it will only be defined here and discussed as it relates to architecture, specifically in terms of competition among meristems.

Dormancy can be subdivided into three types (paradormancy, endodormancy, and ecodormancy) as defined by (Lang *et al.*, 1987). Endodormancy is defined as a dormancy

imposed from within the bud (Lang *et al.*, 1987), and can be divided into three stages: induction, maintenance, and release (Olsen, 2006). Basic decisions about dormancy are involved in dormancy induction and dormancy release (Erez, 2000). Paradormancy is defined as a dormancy controlled by signals originating outside the affected meristem (Lang *et al.*, 1987), and includes such controls as apical dominance (Cline, 1997) and correlative inhibition by sections of the axis (Champagnat, 1983; Cook & Bellstedt, 2001) over lateral bud outgrowth. Release from paradormancy can occur at any time prior to endodormancy by removing the inhibiting structure (e.g. removing leaves during foliar inhibition) (Champagnat, 1983). Paradormancy can be thought of as a series of correlative inhibitions gradually approaching closer to the bud during summer and autumn until endodormancy, dormancy occurring only within the affected structure itself, is finally reached (Champagnat & Côme, 1986).

Chilling Requirement and Dormancy Progression- Entrance into endodormancy in apple is synonymous with dormancy induction (Olsen, 2003). Endodormancy can be reached as early as midwinter and release from endodormancy requires a period of chilling, known as the chilling requirement (Olsen, 2006). The chilling requirement ensures maintenance of dormancy since outgrowth of the buds will not occur before the chilling requirement is reached. In areas characterized by inadequate winter chilling, budburst may still occur when temperatures are adequately warm in the spring. However, this will only occur after a period of time related to the depth to which the bud is dormant. Depth of dormancy is then determined using this delayed budburst phenomenon, and depth of dormancy has been shown to differ among locations and cultivars (Cook & Jacobs, 2000), as well as among bud types and position within the axis (Cook & Bellstedt, 2001; Jacobs *et al.*, 1981; Mauget & Rageau, 1988). If the buds are released from endodormancy (i.e., the chilling requirement has been met) and the temperatures are not conducive to growth, the bud will remain in an ecodormant phase until environmental conditions are favorable for growth. Typically, the dormancy progression for terminal buds is an entrance into endodormancy via paradormancy and then ecodormancy (Olsen, 2003). In lateral bud dormancy progression, paradormancy is followed by endodormancy, and then paradormancy (Faust *et al.*, 1995; Mauget & Rageau, 1988) and/or ecodormancy (Williams *et al.*, 1979) until budburst. Faust *et al.* (1995) determined that the higher chilling cultivars have both a longer endodormant period and stronger correlative inhibition by the terminal (apical dominance, and later acrotony) during both paradormant phases.

Delay in budburst is a way to measure depth of dormancy and dormancy progression through winter (Champagnat, 1989). A decreasing budburst potential, or increasing number of days to budburst, (entrance into dormancy) is followed by an increasing budburst potential, or decreasing number of days to budburst (exit from dormancy). The maximum depth of dormancy occurs between these two phases and is the point at which the greatest number of days to budburst at warm temperatures occurs (Champagnat, 1983). Dormancy progression of an individual bud includes both the entrance into and exit from dormancy. Each bud along an axis has both a different time of entrance to dormancy and exit from dormancy, and therefore dormancy progression, and this is related to both endodormancy of the bud as well as correlative inhibitions acting on the bud (Champagnat, 1983).

Although dormancy can be subdivided into the three phases (Lang *et al.*, 1987), it is a continuous process and therefore has a high degree of overlap (Faust *et al.*, 1995; Olsen, 2003). Over-wintering buds are in a dormant state that is due to both endodormancy and paradormancy (Faust *et al.*, 1995), in the form of correlative inhibition, and therefore depth of dormancy differs according to position of the bud within the axis (Champagnat, 1983), and differs between both terminal and lateral buds (Mauget & Rageau, 1988). Buds along an axis differ in both depth of and rate of release from endodormancy. Removal of inhibiting organs changes both the dormancy progression and chilling requirement of a bud (Champagnat, 1983; Cook & Bellstedt, 2001; Crabbé & Barnola, 1996; Cronjé *et al.*, 2004).

Bud endodormancy is triggered by cold (Crabbé & Barnola, 1996; Heide & Prestrud, 2005) or freezing (Cook *et al.*, 2005; Cook & Jacobs, 2000) temperatures. This temperature-related signal for endodormancy induction is not perceived in the leaves, and is most likely perceived in the bud itself (Cook *et al.*, 2005). A freezing treatment prior to chilling accumulation enhances bud dormancy (Cook *et al.*, 2005). Cold temperatures also fulfill the chilling requirement, and are therefore responsible for both maintaining dormancy and subsequently releasing buds from dormancy (Crabbé & Barnola, 1996). In areas with adequate winter chilling, such as Belgium, buds enter endodormancy in autumn and begin to exit dormancy in late winter (Cook *et al.*, 1998). Prior to budburst, the buds exit dormancy rapidly. Once the chilling requirement has been met, the buds wait in an ecodormant phase until spring temperatures are warm enough for the buds to burst. In areas with inadequate chilling, endodormancy is prolonged and 'delayed foliation' occurs (Jacobs *et al.*, 1981; Saure, 1985; Strydom *et al.*, 1971).

Buds can enter and exit dormancy even at warm temperatures (Mauget & Rageau, 1988), although at a slower rate, and even before leaf abscission has occurred on the shoots

(Cook *et al.*, 2005). In warm areas that experience a freezing event, maximum bud dormancy can be reached before chilling hours have accumulated since chilling unit accumulation begins with endodormancy induction (i.e., freezing temperature) (Cook & Jacobs, 2000). Bud dormancy, when induced by freezing temperature in a warm area, mimics the dormancy progression of terminal buds in an area with adequate winter chilling such as Belgium (Cook *et al.*, 1998). In contrast, if a freezing event does not occur, there is a late entrance into endodormancy and depth of endodormancy will only increase with chilling accumulation (Cook & Jacobs, 2000). As temperatures rise in the spring, the chilling requirement may not have been met and the buds have a prolonged endodormancy and resulting 'delayed foliation'. Cultivars considered as low chill requiring have a shallower dormancy than those with higher chill requirements (Hauagge & Cummins, 1991).

Budburst

The beginning of growth in the spring has two phases which are regulated differently: (1) bud burst (release from dormancy) and (2) stem elongation (Borchert, 2000; Erez, 2000) or the ability to pursue growth (Costes & Guédon, 2002). A sharp increase of cytokinins in the xylem sap is associated with budburst in apple (Cook *et al.*, 2001). Budburst without stem elongation can be observed in areas with inadequate winter chilling where a vegetative bud can burst and form a rosette structure, specifically with the use of rest-breaking agents, and is an indication of incomplete dormancy release (Erez, 2000). Budburst occurs in the spring either after the bud has completed endodormancy and is waiting in the state of ecodormancy for spring temperatures to rise, or, in areas of inadequate winter chilling, after a period of time corresponding to the depth of dormancy of the individual bud (Jacobs *et al.*, 1981). Both bud type and position influence the ability of a bud to break dormancy and start growing (Cook *et al.*, 1998; Cook & Jacobs, 1999). When the chilling requirement has been satisfied, the breaking of dormancy generally occurs in synchrony, and can be called a flush (Cline & Harrington, 2007). A budburst flush may also occur during the season as re-growth after a growth arrest (multiple flushes per year). Specifically in areas with inadequate winter chilling, terminal buds are hypothesized to burst well before lateral buds and establish a primigenic dominance; reproductive buds, which are considered to have a lower chilling requirement (Naor *et al.*, 2003), burst before vegetative buds. This less uniform and delayed budburst is called 'delayed foliation', or 'prolonged dormancy syndrome' and commonly occurs when the chilling requirement during endodormancy has not been reached (Black, 1952; Saure, 1985; Strydom *et al.*, 1971; Tromp, 2005).

Budburst and stem elongation are regulated by either the independent action of or interaction between several hormones: auxin, cytokinins, abscissic acid (ABA), gibberellins, and a new carotenoid-derived hormone (Bennett & Leyser, 2006; Beveridge, 2006; Leyser, 2003; McSteen & Leyser, 2005; Napoli *et al.*, 1999; Ongaro & Leyser, 2008; Schmitz & Theres, 2005; Shimizu-Sato & Mori, 2001). Auxin indirectly inhibits lateral bud outgrowth (apically derived auxin doesn't enter the inhibited bud (Booker *et al.*, 2003)). In fact, auxin increases in a bud once its meristem begins active growth (McSteen & Leyser, 2005; Ongaro & Leyser, 2008). Cytokinins, which are produced in both root and shoot tissues, independently promote budburst (Cline, 1991; Sachs & Thimann, 1967; Shimizu-Sato & Mori, 2001). In addition, cytokinin levels increase when buds are activated to grow (Emery *et al.*, 1998). Research suggests that not only do cytokinins promote budburst, but are, in fact, necessary for budburst to occur (Bangerth, 1994; Turnbull *et al.*, 1997). Interaction between auxin and cytokinin results in auxin rapidly suppressing cytokinin synthesis (Nordström *et al.*, 2004). ABA is not influential in budburst but is negatively correlated with rate of elongation after budburst, and therefore influential in the size of the lateral (Emery *et al.*, 1998). During active shoot growth, gibberellins are responsible for internode elongation and, eventually, bud set (Olsen, 2006). In addition to the previously known hormones involved in bud outgrowth and branching, recent research shows that the new carotenoid-derived hormones, strigolactones, are long-distance signals that move acropetally in the axis to inhibit bud outgrowth (Gomez-Roldan *et al.*, 2008; Ongaro & Leyser, 2008; Schmitz & Theres, 2005; Umehara *et al.*, 2008).

Reproductive Bud Development

In spring, just prior to budburst, the final part of floral organogenesis (pollen sacs, carpels, and ovule primordia) occurs. As the bud swells, carpels elongate, pollen sacs form in the anthers, and the filaments of stamens elongate (Bergh, 1985b). Cell number increase in the cortex of developing flowers is slow during autumn and winter, but from late winter until full bloom increases rapidly (Bergh, 1985a). In research on the apple cultivar Starking Delicious, a maximum number of divisions (25 or 26) were shown to occur in the developing apple fruit primordia between formation of the flower primordia and 35 to 50 days post anthesis; 21 of the divisions occur prior to anthesis. Since the number of divisions is stable, differences in fruit cell numbers may be due to the difference in the number of cells involved in formation of the flower primordia and exemplify competitive correlative influences occurring during this time (Bergh, 1985a).

Inflorescences have a determinate axis (bourse) that supports, in basipetal order, a terminal (king) flower, lateral flowers and spur leaves (Buban & Faust, 1982; Pratt, 1988). One or more bourse shoots, depending on cultivar, are formed on the bourse, and the first 2-5 nodes of these bourse shoots are preformed in the preceding year (Pratt, 1988). After budburst, the bourse elongates and has been shown to stop when the king flower reaches the balloon stage. Bourse length differs among cultivars, location in the canopy and time of initiation (Kudo & Kyotani, 2000). Bourse volume is positively related to return bloom suggesting that the location of stored carbohydrates close to the terminal bud of the bourse shoot is beneficial and necessary for inflorescence development, fruit set and fruit development in the following year (Lespinasse & Delort, 1993). Younger buds on an annual shoot, initiated later in the preceding season, have longer and more slender bourses while older buds, initiated earlier, have shorter and more compact bourses (Abbott, 1970; Kudo & Kyotani, 2000). Abbott (1970) observed further differences between young and old reproductive buds of apple. When older buds burst, the flowers are immediately visible with no extension of transition leaves and only a few small preformed primary leaves while flowers are numerous, sessile (attached at the base and not raised on the peduncle) and set well. In contrast, the slender, elongated young buds have extended scales and prominent transition leaves. The leaves are large and grow out well before the flowers. At full bloom, young buds have a long bourse, a lower number of flowers with long stalks and a bourse bud destined to become a vegetative shoot. Young buds are usually associated with decreased fruit set (Abbott, 1970). Since relative time of initiation results in different inflorescence morphology, individual inflorescence morphology can be used to determine competitions among inflorescences based on time of development. The leaves at the base of the bud are dominant over more apical, as well as more basal leaves and flowers in young buds (Abbott, 1970; Fulford, 1966a). Abbott (1970) proposed that the difference between earlier and later initiated reproductive buds was based on a "zone of dominance" that moves up from the base of the bud, and that this change in dominance may be related to the initiation of endodormancy.

Spur leaves are the first to emerge from dormant buds and comprise most of canopy until after bloom (Fulford, 1966b). More photosynthate for early growth comes from spur leaves than from reserves (Hansen, 1967b). Since they are the only leaves present during fruit set and cell division, they effect both fruit size and shape, as well as calcium level (Rom & Ferree, 1984a). Spur leaves are essential for fruit set; they influence fruit size up to 60 days after full bloom (DAFB) (Rom & Ferree, 1984b).

Spur size (dry weight) has been shown to vary within an axis (Hansen, 1967a); spur leaf area, number and average leaf size vary within a tree and between genotypes (cultivars) (Rom & Ferree, 1984a). However, spur size (dry weight), inferring vascular connection, is only one of several factors related to leaf development (Rom & Ferree, 1984a). Spurs with fewer leaves are less likely to flower. This may be due to correlative inhibition by other organs, which causes budscales to form sooner which reduces the number of leaves that can expand, as well as affecting the development of the bud itself (Fulford, 1966b).

The size of the bourse shoot is positively related to the number of leaves in the inflorescence (Lauri & Trottier, 2004), and is influenced by rootstock (Chun *et al.*, 2002). In the second week after anthesis, the bourse shoot is a strong sink and competes for carbohydrates with the developing fruit during the time when cell numbers in the fruit are still increasing. Cell division decreases during this time, however, once the additional leaves produced by the bourse shoot are exporting carbohydrates, the rate of cell division in the developing fruit increases, and, ultimately, spurs with bourse shoots have been shown to produce larger fruit than those without bourse shoots. If no fruit are on the spur, the bourse shoot will export carbon to a neighboring fruiting spur (Abbott, 1960; Bergh, 1985a).

Active Shoot Growth

A plant's primary growth is the result of many processes that can be grouped into two separate events: organogenesis and extension (Barthélémy & Caraglio, 2007). During neoformed growth, the axis elongates with progressive development of primordia. Leaves are the most visible structures produced. Once a leaf reaches a certain number of nodes below the apical meristem, it's axillary bud forms (Rohde & Boerjan, 2001). Axillary meristems develop continuously while the terminal meristem grows and may even grow out sylleptically. The axillary buds, then, have a developmental gradient in which the oldest axillary buds (proximally located along the axis) have the highest number of primordia (budscales and foliage leaves) while the youngest may have only generated budscales (Rohde & Boerjan, 2001), even though final differentiation in the over-wintering bud may be completely different to this.

Growth Rhythms- Shoot growth in apple is rhythmic. Growth rhythm can be defined according to either (1) internode elongation ("units of extension") or (2) mitotic activity and organogenesis ("units of morphogenesis") (Hallé *et al.*, 1978). A unit of extension is an uninterrupted period of extension and begins with expansion of preformed scales leaves (in

the case of proleptic shoots) or production and expansion of leaves (sylleptic shoots) and ends with the short internodes associated with foliage leaves. A unit of extension is synonymous with growth unit or "flush" of growth in apple and one or more units of extension within a growing season comprise an annual shoot (axis). In contrast, a unit of morphogenesis is based on organogenesis and so a single unit of morphogenesis is a continuous production of primordia, regardless of whether these primordia expand or not. The onset of mitotic activity signals the onset of a unit of morphogenesis. Proleptic shoots of apple begin in the middle of a unit of morphogenesis (although the beginning of a unit of extension) since some leaves are initiated in the previous season. Annual shoots and growth units in apple, then, are defined according to units of extension (Hallé *et al.*, 1978). A growth arrest, or limit between two growth units along an annual shoot, is distinguished by an area with short internodes and bud scale scars (Barthélémy & Caraglio, 2007).

Growth Units vs. Annual Shoots- During the season, growth-limiting conditions may cause organogenesis to slow or stop. When conditions are again sufficient for growth, shoot growth will resume. Growth arrests may be reactions to such things as environmental- or water-limiting conditions, or competitions among meristems along the axis or within the plant, and are dependant on plant age (ontogeny) (Rohde & Boerjan, 2001). With an increase in the time of the growth arrest, the developing bud may transition to reproductive and proceed with floral morphogenesis. The resulting flowers are weak when growth resumes. This phenomenon has been referred to as 'extended bourse' by (Seleznyova *et al.*, 2008), and is common in areas with inadequate winter chilling (personal observation).

A growth unit is the part of the axis that was formed during the "non-interrupted phase of lengthening" (Costes *et al.*, 2006). This growth may either begin with syllepsis or a type of prolepsis. Prolepsis can occur as a result of either bud release from endodormancy (beginning of an annual growth) or bud release from para- or eco-dormancy (beginning of an intra-annual growth unit). In the case of intra-annual growth units, the second of the two growth units is the result of prolepsis from para- or eco-dormancy. Each growth unit is technically the same annual "age"; however, each behaves as a separate annual shoot in some ways (Lauri, 2007; Lauri & Terouanne, 1998). Since organogenesis is known to progress basipetally along the axis in *Picea* spp. (Powell, 1995) and growth units have acrotonic bud organogenesis in apple (Lauri, 2007), it can be deduced that organogenesis develops basipetally once the terminal bud of the growth unit becomes paradormant. An annual shoot is the amount of growth, comprised of one or more growth units, that occurs during a growing season (Costes *et al.*,

2006), and physiologically speaking, since annual growth is a consequence of temperate climates, we can say ends just prior to the budscales of a bud that undergoes endodormancy. In addition, due to the induction of endodormancy, discontinuities in the secondary xylem cause an annual ring to form since no secondary xylem is produced during endodormancy of the cambium. The annual ring is the boundary between early and late wood produced during the annual growth. Between growth units of the same annual shoot, false rings may be produced but they lack the outer boundary of the "true ring" (Hallé *et al.*, 1978).

Dominance in the Annual Shoot and the Development of Acrotony

Dominance in axes can be observed along axes in ways such as the differential length, size, and organogenesis of laterals (Champagnat, 1978; Lauri, 2007), the differential acquisition of ability to become active (budburst or flowering) (in reference to acrotony, Champagnat, 1978; Cook *et al.*, 1998; Hallé *et al.*, 1978), and therefore the resulting differential activity, or primigenic dominance, of lateral buds and shoots (relative time of bursting and flowering) (Bangerth, 1989; Bangerth & Ho, 1984; Cook *et al.*, 1998), and the differential ability to maintain and/or re-establish growth relative to surrounding laterals on a growth unit or axis (such as the ability to retain fruit after fruit-set (Racskó *et al.*, 2008; Paper 3)) .

Acrotony is a type of dominance that describes morphological relationships among proleptic buds and lateral shoots within a growth unit or axis (Champagnat, 1978). Typically it is defined as "the increase in vigor (length, diameter, number of leaves) of the vegetative proleptic branches (from dormant buds) from the bottom to the top position of the parent growth unit" and has more recently included in its definition architectural features and bud organogenesis (Lauri, 2007). However, acrotony can be, and has been, defined on different levels.

Some researchers (Champagnat, 1978; Hallé *et al.*, 1978) define acrotony as a greater ability of lateral shoots to occur in the distal position of the axis implying an increase in budburst in the this position irrespective of the lengths of the resultant lateral shoots. A recent review of plant architecture (Barthélémy & Caraglio, 2007) supports this idea that acrotony is the increase in number of growing laterals moving distally along the axis (further indicating that the increase in length of the lateral shoots should be referred to as acrotonic branching (Barthélémy & Caraglio, 2007) as budburst and outgrowth are regulated differently (Borchert, 2000; Costes & Guédon, 2002; Erez, 2000)). In fact, to many researchers, this greater ability to burst in the distal section of the axis is, if not the only, at least a part of the idea of acrotony (e.g., as an increase in percentage of growing laterals in the distal position of an axis (Lauri,

2007) or as an increase in the budburst potential of the terminal bud and/or lateral buds in the distal position (Cook *et al.*, 1998)).

Whether defined as acrotony or acrotonic budburst, etc., there is further evidence of positional dominance of the distal part of the axis. Powell (1995) observed that organogenesis occurs in a basipetal direction along an axis in *Picea* spp. so that there is an increase in organ number in the buds moving distally along an axis, or an acrotonic organogenic activity. This is also observed in apple as an increase in preformed spur leaf number in the distal part of one-year-old axes (Lauri, 2007).

Acrotonic development, as well as loss of acrotony (Cook & Jacobs, 1999), also occur via morphogenetic gradients that develop during endodormancy (Crabbé & Barnola, 1996). Within an axis, there is an initial basitonic bursting tendency that becomes acrotonic as dormancy progresses (Champagnat, 1983; Cook *et al.*, 1998; Crabbé & Barnola, 1996; Jacobs *et al.*, 1981). More proximally-located buds have higher growth rate and are less dormant than more distally-located buds from autumn to mid-winter (Champagnat, 1983; Cook *et al.*, 1998; Crabbé, 1981). This basitonic bursting tendency, specifically in areas where endodormancy is not completed, may result in primigenic dominance of the first bud to be active. In studies of tomato fruits, however, primigenic dominance determines relative size along the truss even though position determines maximum potential fruit size (Bangerth & Ho, 1984).

The phase of acrotony that occurs after budburst ("acrotonic branching" to Barthélémy and Caraglio (2007); Cline and Harrington (2007)) exemplifies the positional dominance that distally located laterals have over more proximally located laterals and/or correlative dominance among laterals within the same axis (Cline & Harrington, 2007). This results in the acrotonic habit that is typical of branches on trees such as apple (Lauri, 2007). While a positional dominance plays a role in the increase in length and size of laterals moving distally along the axis as indicated when all buds burst at the same time (in reference to acrotony, Cline & Harrington, 2007; in reference to apical control, Wilson, 2000), it is also hypothesized that primigenic dominance plays a role in the development, and therefore loss, of acrotony when budburst is differential along an axis (Cook *et al.*, 1998; Lauri, 2007).

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<u>PAPER 1:</u> Primigenic and positional dominance among reproductive buds in branches of 'Golden Delicious' and 'Granny Smith' apple grown in areas with inadequate winter chilling

Abstract

Temporal (relative time of budburst and flowering) and positional (relative position within the shoot) influences on reproductive buds were recorded on two-year-old shoots of 'Granny Smith' and 'Golden Delicious' apple (*Malus x domestica* (Borkh.)) trees grown at two locations in South Africa, a cool area, Koue Bokkeveld and a warm area, Warm Bokkeveld, with adequate and inadequate winter chilling, respectively. Inflorescence size (leaf number, leaf area, and flower number) did not differ temporally or with position. For both cultivars, fruit set in the cool area was acrotonic and independent of relative flowering time, while temporal (primigenic) dominance was more important describing relationships among reproductive buds in the warm area. Inflorescence size and fruit set indicate a separation of environmental (inadequate winter chilling) and innate factors in competition among reproductive buds along a shoot.

Introduction

Fruit set is considered to be the most important factor affecting yield (Dennis, Jr., 1981). In apple (*Malus x domestica*), fruit set is the result of both the ability of a flower to reinstate growth of the ovary after anthesis (i.e., initial fruit set) and to survive competition from neighboring flowers and/or neighboring inflorescences (i.e., final fruit set) (Goldwin, 1992). Because of this competition among flowers and inflorescences, fruit set is a measure of correlative phenomena (dominance and competition) among reproductive laterals within an annual shoot, or among flowers within an inflorescence. The competition can either be temporal (primigenic dominance when the first bud to either burst or flower is dominant) (Racskó *et al.*, 2008), or positional (topophysis) (Bredmose *et al.*, 1999; Bredmose *et al.*, 2001). Positional competition includes acrotony or basitony in the case of competition among inflorescences; and apical dominance or basal dominance in the case of competition among flowers within an inflorescence.

Reproductive buds of apple are mixed (contain leaves and flowers) and found in terminal positions of short (spur) and long shoots, and/or in distal lateral positions along one-year-old shoots. Inflorescences have a determinate axis (bourse) that supports, in basipetal order, a terminal (king) flower, lateral flowers and spur leaves (Buban & Faust, 1982; Pratt, 1988). Number and area of spur leaves are positively related to fruit set of flowers within the inflorescence (Ferree *et al.*, 2001; Lauri *et al.*, 1996). Since these leaves are the primary source of photosynthate during cell division of the developing fruit they have an impact on fruit size (Ferree & Palmer, 1982; Lauri & Kelner, 2001; Petri & Leite, 2004), yield and yield efficiency (Rom and Ferree, 1984).

Correlative phenomena among flowers within an inflorescence are well documented (Bangerth, 2000; Lloyd *et al.*, 1980; Miranda *et al.*, 2005). Generally, primigenic dominance predicts fruit set ability of a particular flower within an inflorescence (Bangerth & Ho, 1984), while the ability of an inflorescence to set at least one fruit depends on competition between inflorescences (Lauri *et al.*, 1996). For example, primigenic dominance is evident when one flower is pollinated before another in a tomato truss (i.e., first pollinated becomes largest fruit), even though position influences the maximum potential sink strength and fruit size (Bangerth & Ho, 1984).

Both positional and temporal competitions dictate relationships between inflorescences within the same shoot. Positional competition among all laterals is typically acrotonic in apple, with the increase in lateral length moving from proximal to distal along the

shoot, or acrotony (Bell, 1991; Brunel *et al.*, 2002; Cook *et al.*, 1998a; Hallé *et al.*, 1978; Napoli *et al.*, 1999; Puntieri *et al.*, 2007; Wilson, 2000). Acrotony can be defined as "the increase in vigor (length, diameter, number of leaves) of the vegetative proleptic branches (from dormant buds) from the bottom to the top position of the parent growth unit" (Lauri, 2007). Acrotony, although evident when the distally located buds burst first (Lauri, 2007), may not necessarily be dependant on sequence of budburst but on position within the shoot and the development of a positional dominance of the distally located buds that occurs with adequate chilling (Cook *et al.*, 1998b; Cook *et al.*, 2001; Cook & Bellstedt, 2001). Bud organogenesis, budburst percentage (Brunel *et al.*, 2002; Lauri, 2007) and reproductive growth (ie. flower and leaf number per spur and fruit set) have been shown to adhere to a similar acrotonic tendency as vegetative laterals (Lauri, 2007; Powell, 1995).

Temporal dominance can be experimentally induced. In apple, the largest inflorescences (leaf numbers) have the highest fruit set (Lauri & Terouanne, 1999) and are located in apical positions along the shoot (Lauri, 2007). However, when the largest inflorescences are removed, fruit set in the remaining inflorescences increases, indicating that a type of primigenic dominance occurs where large inflorescences are autonomous and smaller inflorescences are reliant on import of carbohydrates (Lauri *et al.*, 1996; Lauri & Terouanne, 1999).

Naturally, buds along a shoot do not retain the same potential to grow (Bell, 1991) due to two main dormancy-related characteristics: (1) buds differentially enter and exit endodormancy based on their position due to correlative inhibition by the terminal bud (i.e., paradormancy) (Cook *et al.*, 1998b; Cook & Bellstedt, 2001), and (2) reproductive buds require less chilling than vegetative buds (Naor *et al.*, 2003). The development of acrotony is due to the dominance that the terminal and distal lateral buds have over more proximally located buds (Cook *et al.*, 1998b; Cook & Bellstedt, 2001). During dormancy the acrotonic growth tendency is lost (Cook *et al.*, 1998b). Buds in more distally located positions initially have a deeper dormancy, and therefore less potential to grow, than more proximally located buds during the progression of dormancy (Champagnat, 1983). As the chilling requirement is fulfilled, the distally located buds have a higher potential to grow (Champagnat, 1983; Cook *et al.*, 1998b). Budburst potential becomes basitonic before developing the acrotonic tendency once again (Cook *et al.*, 1998b; Cook & Jacobs, 1999).

Under ideal conditions (i.e., when chilling is adequate), primigenic dominance is not as readily evident within an annual shoot since budburst time is more uniform than when dormancy is not completed. With increasing winter temperatures there is a decrease in the

number of buds that burst (Naor *et al.*, 2003; Paper 1) and the period over which budburst occurs is prolonged (i.e., delayed foliation) (Cook *et al.*, 1998b; Cook & Jacobs, 1999; Paper 1; Erez & Lavee, 1974; Jacobs *et al.*, 1981). The terminal bud has been shown to burst first and establish dominance (Jacobs *et al.*, 1981; Naor *et al.*, 2003) followed by budburst of the proximal buds. The distal shoot tissues are probably still inhibited by the terminal at this point (when the terminal has primigenic dominance and still inhibits lateral budburst) (Jacobs *et al.*, 1981) since pruning the terminal bud before chilling reinstates the original acrotonic budburst potential (Cronjé *et al.*, 2004).

The aim of our study was to determine what happens among reproductive buds within an apple shoot when winter chilling is inadequate. We used fruit set and inflorescence size to characterize the positional (acrotonic) and/or temporal (sequential budburst/flowering) influences on competition between reproductive structures within the annual shoot. To do this, we used two apple cultivars grown in two areas with differing amounts of winter chilling in order to have different budburst patterns. We used the two-year-old section of the shoot (thereby using terminal inflorescences on spurs) since this growth maintains the acrotonic growth tendency (Lauri, 2007) and had a higher density of inflorescences than one-year-old section of the shoot in our trial, thereby allotting more inflorescences across position and time.

Materials and Methods

Plant Material and Sites – The trial was carried out on two apple cultivars with differing growth habits and architectural features: Golden Delicious on M793, and Granny Smith on seedling rootstock, in the 2006-2007 season. These cultivars have different shoot architectures, types III and IV, respectively, according to the typology of Lespinasse and Delort (1986).

The cultivars were selected in commercial orchards located in areas with both a lower (Warm Bokkeveld; 33º20’ S, 19º19’ E, 496m, 1119 PCU) and higher (Koue Bokkeveld; 33º12’ S, 19º19’ E, 1045m, 1698 PCU) chilling unit accumulation historically. In each area, one commercial farm was used. Hereafter the Koue Bokkeveld and Warm Bokkeveld will be referred to as the cool area and warm area, respectively. Meteorological data was collected from the orchard used for the trial or the local area of the orchard. The net chilling units were calculated according to the daily positive chill unit model (PCU; Linsley-Noakes *et al.*, 1994).

In the cool area, ‘Golden Delicious’ trees were planted in 1997 at a spacing of 4.5 x 2.5 m; and ‘Granny Smith’ trees were planted in 1933 at a spacing of 5.6 x 5.6 m. In the warm

area, the ‘Golden Delicious’ trees were planted in 1995 and the ‘Granny Smith’ trees were planted in 1995 at a spacing of 5.0 x 2.5 m. All trees were in full production in 2007.

All trees received standard irrigation and fertilization. No trees received any chemical restbreaking. The flowers and fruit were neither hand nor chemically-thinned. Flowers were open pollinated by honey bees and there were no pollination problems during the time of this trial. In addition, there were no adverse environmental conditions that would prevent pollination or fruit set.

For all cultivar/area combinations, 20 branches were randomly selected in June 2006 at shoulder height from 10-20 trees within one row. Selected branches started growing in September (spring) 2004, and therefore were comprised of a two-year-old shoot section, one-year-old shoot section and a terminal bud. Since acrotony is evident within a single growth unit (Lauri, 2007), the two-year-old section of these branches had only one growth unit. The branches were approximately horizontal when selected, but never below horizontal.

Branch Measurements - Nodes were numbered along the two-year-old shoot section (hereafter referred to as shoot) of the selected branches from the proximal to distal end. The shoot was partitioned into four quadrants based on node number, with quadrant 1 being the most proximal and quadrant 4 being the most distal.

Starting in early September, the shoots were monitored every day. Budburst and flowering dates of the reproductive buds were recorded for each node along the shoot. The objective was to characterize the differences between the first, second, third and fourth buds to burst or flower along a shoot. Since the date of the first reproductive bud within a shoot to burst or flower was not the same across all shoots, the reproductive buds were considered sequentially. The first bud to burst along a single shoot was first in the sequence (sequence 1), the second bud to burst was sequence 2, and so forth. If two buds along the same shoot burst on the same day, then they were both considered as the same sequence number.

All reproductive nodes along the shoot were considered. For each reproductive node, the numbers of leaves and flowers were counted at anthesis. The king flower was then removed from every cluster in order to count the number of cells in the receptacle at relative time of anthesis (data not shown). The removal of the king flower has no effect on whether the entire inflorescence sets a fruit (Medrano *et al.*, 2000; Miranda *et al.*, 2005; Vaughton, 1993). Apple inflorescences that have all of their flowers have the same ability to set fruit as those with up to two (including the king) flowers removed (Miranda *et al.*, 2005).

Length and maximum width of the inflorescence leaves were measured non-destructively to the nearest centimeter (cm). Length and maximum width of inflorescence leaves from shoots not used in this study were also measured. These leaves were then harvested and actual area was determined using the LI-3100C (Li-Cor, Inc.). A linear regression equation was computed using leaf length x width and leaf area (Table 1). Areas of all leaves in the inflorescence were added together for total leaf area per inflorescence. The length of each bourse was also measured.

Fruit set was considered as the ability of an inflorescence to set at least one fruit, and was calculated as the number of inflorescences with at least one fruit divided by the total number of inflorescences (Lauri & Terouanne, 1999). Initial fruit set, or ovary stimulation that results in a fruit after anthesis (Goldwin, 1992), was considered as the presence or absence of a fruit in the inflorescence in October (ie. after anthesis). Final fruit set, or the ability of the fruits to survive competition (Goldwin, 1992), was considered in December [i.e., after final ("June") drop].

Data Analysis **-** In order to determine the influence of position and relative flowering time on the ability of an inflorescence to set fruit, fruit set was analyzed per quadrant number and per sequence number. Shoots were pooled to increase the minimum number of reproductive nodes per quadrant and/or sequence number to a minimum of five. Only relative frequencies with a minimum value of 5 were considered in these analyses. Fruit set was then analyzed as non-parametric data using the β approximation of the Kruskal-Wallis H test. Multiple means comparison test was used to test differences between either quadrants or sequence of budburst (Kruskal and Wallis 1952).

Two variables were considered as factors influencing the inflorescence size/ leaf number: position within the shoot (1-4) and sequential budburst time (1-4). The influence of position and sequential budburst time on leaf number, leaf area, and total leaf area per inflorescence was studied for each cultivar/site combination individually. Each reproductive node was considered as one case. Inflorescence variables were considered normally distributed and the one way ANOVA (Fisher F test) followed by the Fisher LSD was used to analyze them. Although the interaction between position and sequential budburst time would have been interesting in this study, a factorial could not be done due to missing factor combinations (Milliken & Johnson, 1984), specifically in relation to reproductive nodes not being present in all positions.

All statistical analyses were done using StatSoft (2008) with the exception of the p-values of the β approximation for the non-parametric data, which was calculated by hand using the tables in Kruskal-Wallis (1952).

Results

Temporal and Positional Aspects of Reproductive Buds and Reproductive Budburst – The mean number of reproductive budburst sequences for 'Golden Delicious' was 3.7 in the cool area and 3.6 in the warm area (Table 2). The mean number of reproductive budburst sequences for 'Granny Smith' was 2.1 in the cool area and 3.1 in the warm area.

Few, if any, reproductive buds occurred in the most proximal position along the annual shoot. For 'Golden Delicious', the mean number of reproductive buds per shoot was 8.7 in the cool area and 7.3 in the warm area (Table 3). The mean number of reproductive buds per shoot for 'Granny Smith' was 6.4 in the cool area and 5.6 in the warm area (Table 3). Budburst of reproductive buds was linearly related to flowering date (data not shown).

For 'Golden Delicious', the majority of the first reproductive buds to burst were in quadrant 4 in the cool area (0.47) and quadrant 3 in the warm area (0.44) (Table 4). In the cool area, sequences 2 through 4 broke predominantly in quadrant 3 (0.43, 0.47, and 0.75, respectively). This was also the case in the warm area (0.39, 0.36, and 0.38, respectively), although not as convincingly since the percentage of sequences to burst in quadrants 2-4 was more similar (Table 4).

For 'Granny Smith', the majority of buds to burst first were in quadrant 4 for both the cool (0.44) and warm (0.53) areas (Table 4). Further sequences (2-3 for the cool area and 2-4 for the warm area) were more likely to burst in quadrant 3 (Table 4).

Positional effects on fruit set - Few, if any, reproductive buds occurred in the most proximal position (quadrant 1) along the shoot (Table 4) so fruit set was not calculated for this area. Fruit set in quadrant 1was not calculated since their were either no or less than five reproductive buds in this quadrant.

In 'Golden Delicious' grown in the cool area, initial fruit set in quadrant 4 (96%) was greater than in both quadrants 2 (71%) and 3 (83%), and quadrant 3 was greater than in quadrant 2 ($p<0.01$); final fruit set was equal in quadrants 3 (77%) and 4 (86%), both of which had greater set than in quadrant 2 (59%) ($p<0.01$) (Table 5 and Fig 1A). Initial fruit set of 'Golden Delicious' grown in the warm area did not differ by quadrant, while final fruit set was greater in quadrant 4 (45%) followed by quadrant 3 (33%) and then quadrant 2 (24%)

($p<0.05$) (Table 5 and Fig 1 A). Very few inflorescences lost their ability to retain fruit (final fruit set) in the cool area while in the warm area almost half of the inflorescences that originally set fruit (initial fruit set) in quadrants 2 and 3 lost their ability to retain fruit, as did one-third of the inflorescences in quadrant 4 (Fig 1 A).

Initial fruit set of 'Granny Smith' grown in the cool area was greater in quadrant 4 (77%) than quadrants 2 (47%) and 3 (40%), while final fruit set was greatest in quadrant 4 (48%) followed by quadrant 2 (40%) and then quadrant 3 (27%) ($p<0.05$ in both cases) (Fig 1 B). This was not due to the influence of growth unit (Lauri, 2007) in our trial since each shoot only had one growth unit. Fruit set according to position did not differ in 'Granny Smith' in the warm area (Table 5 and Fig 1 B). In the cool area most of the inflorescences losing their ability to retain fruit were in quadrant 4, and there were only a few inflorescences that lost all of their fruit in quadrant 2 (Table 5 and Fig 1 B).

Effects of flowering sequence on fruit set **-** In both 'Golden Delicious' and 'Granny Smith' in the warm area, the first reproductive bud to flower along the shoot has the greatest initial fruit set (0.82) and final fruit set (0.70) (Fig 1 C and D). Fruit set decreased with increasing sequence number ($p<0.001$ in both cases), with the exception of 'Golden Delicious' initial fruit set sequence 1 (0.82) and 2 (0.96) which were not significantly different at $\alpha=0.05$, and final fruit set of 'Granny Smith' in which sequence 2 (0.38) was greater than sequence 4 (0.20), while sequence 3 (0.28) was not significantly different from either of sequences 2 or 4 (Table 6 and Fig 1 C). There were relatively few inflorescences to drop fruit in sequence 1 of 'Golden Delicious', while all 'Granny Smith' sequences lost similar amounts of fruit.

In the cool area, 'Golden Delicious' initial fruit set and final fruit set was lower in sequence 1 (0.60 and 0.52, respectively) than the other sequences (Fig 1 C). Drop was similar between all sequences (Fig 1 C). Initial fruit set of 'Granny Smith' in the cool area did not was greatest in sequence 3 (Fig 1 D). Final fruit set sequence 2 (0.47) was less than sequence 1 (0.65) and 3 (0.65), while sequence 4 (0.53) did not differ from any other sequence (Fig 1 D). Very little drop occurred in sequence 1 (Fig 1 D).

Influence of position and budburst sequence on inflorescence characteristics **-** Position had no influence on the inflorescence characteristics of leaf number (Fig 2 A), leaf area per leaf (Fig 2 C), and bourse length (Fig 3 A), with the exception of 'Golden Delicious' in the cool area which had a lower leaf area per leaf in quadrant 2 (Fig 2 C). In addition, there was no influence of budburst sequence on any of these characteristics for the cultivars when grown in

the cool area with the exception of a greater bourse length in sequence 1 of 'Golden Delicious' grown in the warm area (other sequences being equal) (Figs 2 B and D, and Fig 3 B). 'Granny Smith' also had a greater bourse length in sequence 1 as compared to sequences 2 through 4, which were all equal (Fig 3 B). In the warm area, leaf number per inflorescence (Fig 2 B) and bourse length (Fig 3 B) was significantly greater in sequence 1 than in sequence 4, although sequence 1 did not differ from sequences 2 and 3, while only 'Granny Smith' exhibited a reduced leaf area per leaf from in sequence 4 as compared to sequence 1 (sequences 2 and 3 being equal to both 1 and 4) (Fig 2 B). Leaf area per leaf was not affected by sequence number in 'Golden Delicious' in the warm area. Therefore, no acrotony was observed.

Discussion

Using fruit set as a measure of competition in apple annual shoots, our results show that competition between reproductive laterals is either the result of position in the shoot (as in the cool area which had adequate chilling) (Fig 1 A and B) or time of activity (as in budburst sequence in the warm area with inadequate chilling) (Fig 1 C and D). These competitions are irrespective of inflorescence size (spur leaf number, flower number, spur leaf area per leaf) (Fig 2 A and C) with the exception of a possible link to bourse length in the warm area (Fig 3 A). In other studies, one-year-old shoots show differences in fruit set between inflorescences of an annual growth related to differences in inflorescence size (leaf and flower number) (Lauri *et al.*, 1996; Lauri, 2007) , as well as bourse shoot length (Lespinasse & Delort, 1993).

The relationship between inflorescence size and fruit set in one-year-old shoots may reflect an increased competition among inflorescences that does not exist in two-year-old shoots since two-year-old shoots contain terminal inflorescences on spurs and shoots, while one-year-old shoots have lateral flowers. Inflorescence size is relatively equal along two-year-old shoots (Fig 2 A and B) as the differences between positions equalize with age and therefore fruit-set is less dependant on inflorescence size and more dependant on other factors.

The differences between one- and two-year old shoots in inflorescence size and fruit set may also be due to the different types of competition that occur within the shoot. Competition among reproductive buds can occur at three periods of time: (1) during floral initiation and organogenesis of the inflorescence which occurs in the summer and autumn preceding bud growth (Fulford, 1966a; Fulford, 1966b), (2) during the winter when the

acrotonic budburst potential is lost and regained during the progression of dormancy (Champagnat, 1983; Cook *et al.*, 1998b), and (3) during flowering (Goldwin, 1992). In one-year-old shoots, competition may be higher during initiation and organogenesis (between lateral inflorescences along the shoot), while two-year-old shoots (with apical inflorescences along the shoot that are more autonomous) may have higher competition during flowering.

Influence of position in the shoot and time of activity on reproductive buds - Positional and temporal dynamics of reproductive buds can be observed at three different times during their development: (1) during initiation and organogenesis, (2) as correlative influences during dormancy, and (3) as competition during fruit set. Acrotonic trends and the loss of this acrotony are implicated in each of these processes.

Initiation and organogenesis - Buds within the two-year-old shoot are initiated in the preceding summer, when positional advantage (leaf number, size, etc) is still apparent in the subtending laterals as these still have leaves present on them. Reproductive buds (leaf primordia and apex size and shape) in *Picea* spp. are known to develop basipetally so that the first buds to initiate and differentiate are in the more distal positions in one-year-old shoots (Powell, 1995). This gives an initial reproductive bud size (leaf and flower primordia number) advantage according to acrotonic position. Reproductive bud size (leaf number) also differs along apple shoots in an acrotonic fashion in one-year-old shoots (Lauri, 2007). This was initially attributed to acrotony but may be an indication of the basipetal development of buds along the shoot that would have continued with an increased growing season. In a previous study on apple by (Hauagge & Cummins, 1991), the number of buds becoming reproductive increased when defoliation was delayed. If the growing season is long during organogenesis, as in both locations in our study, then all the buds have ample time to develop (basipetally) along the shoot, and the limit to the number of leaves/flowers per spur should be the genetically-dictated maximum. As in *Picea*, all buds along the shoot can accumulate the maximum inflorescence primordia numbers given a long enough period of time before entrance into dormancy.

Correlative phenomena during dormancy – In our study, the different budburst patterns (time and position of budburst) between areas with adequate (cool area) and inadequate (warm area) winter chilling are depictive of the basitonic to acrotonic gradient of budburst potential that occurs with chilling (Champagnat, 1983; Cook *et al.*, 1998b). The re-establishment of acrotony is a slow process. This retention of the basitonic-acrotonic gradient shows that the gradient of precedence is maintained for reproductive buds. Since

reproductive buds have a lower chilling requirement than vegetative buds (Naor *et al.*, 2003), the gradient of budburst potential that occurs during dormancy is less reliant on chilling hours of an individual bud type, but on position within the shoot. In addition, this progression of dormancy is not related to bud size in terms of flower and leaf numbers since all reproductive buds exiting dormancy were of equal size in both cultivars.

Competition during fruit set –The acrotonic (positional) fruit set tendency was evident in both cultivars in the cool area. In the warm area, primigenic dominance was observed, and again in both cultivars. Environment, in this case most likely chilling accumulation, has a greater impact on fruit set within the shoot than did either of the cultivars in our study. However, the acrotonic fruit set tendency was only observed in the competition among inflorescences that occurred at initial fruit set and which was maintained through final fruit set (Fig 1 A and B) since inflorescence size was equal (Fig 2 A and C).

In the cool area, acrotonic fruit set trends were not the result of relative time of anthesis along the shoot. Although time of activity (primigenic dominance) influences have been implicated in being the cause of acrotony (Bangerth, 1989), this has, to our knowledge, not been verified. In our study, acrotonic competition between reproductive buds after winter chilling was not dependant on time of activity. However, relative flowering time, and subsequent budburst, may depict the loss of acrotony in that the first reproductive buds to burst were had the highest fruit set in the warm areas (Fig 1 C and D) while position was not a strong factor in the initial fruit set (Fig 1 A and B). The first buds to flower in the warm area had the greatest ability to set fruit regardless of position in the shoot or cultivar, implicating a primigenic dominance effect with limited chilling (i.e., limited reserves). The first buds to flower on the shoot were clearly the most competitive (Fig 1 C and D) even though this was not related to inflorescence size (Fig 2 B and D). In the cool area, there was adequate chilling accumulation (allowing for an acrotonic budburst potential (Cook *et al.*, 1998b)) in addition to an adequately warm autumn (ample time to acquire the maximum number of flower and leaf primordia in each overwintering bud) and still an acrotonic fruit set trend. This implicates another, or an innate, acrotonic influence that occurs within the shoot (i.e., hormones, carbohydrates), and one that is independent of a bud's time of activity. With more chilling, buds have the ability to re-establish acrotony (see Paper 1), and this is also likely on the two-year-old axis as evidenced by these fruit set positional competitions.

Since there are sufficient buds and reserves with adequate chilling, one possibility is that the shoot can preferentially select the ideal position for reproductive bud growth. The distal position is advantageous (increased light interception in the exterior canopy and

potential for highest photosynthesis (Campbell *et al.*, 1992); buds are initiated first and therefore greatest bourse leaf numbers and organogenic potential (Lauri, 2007)). In warmer areas, with a potential for having an inadequate amount of reproductive buds or viable reproductive buds bursting, the benefit is for the first bud to set regardless of whether it has the positional advantage. This 'first come first serve' approach of primigenic dominance in inadequately chilled areas secures that at least one reproductive bud has the opportunity to set fruit.

Based on this research and previous studies, we propose the following: (1) maximum spur leaf and flower number per bud are innate, although development of reproductive buds most likely occurs basipetally in the two-year-old shoot (as it is known to do in the one-year-old shoot) and not all buds along the shoot may reach their full potential; (2) the loss of acrotony occurs during the winter preceding budburst and is observed temporally (ie. differential flowering, and therefore budburst, potential along the shoot); (3) with no obvious difference in bud size nor time of activity along a shoot there is an innate acrotonic fruit set tendency; and (4) in warm winter areas, primigenic dominance is more important in determining which inflorescences set fruit than in cold winter areas.

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Table 1. Linear regression equations (and R^2 for the equation) computed using leaf length x width (LW) and area (LA) of leaves on two-year-old annual shoots of Golden Delicious and Granny Smith apple cultivars grown at two sites, a warm area (Warm Bokkeveld) and a cool area (Koue Bokkeveld). P-value was less than 0.0001 in all cases.

Cultivar	Site	Equation	R^2
'Golden Delicious'	Cool Area	LA = 0.6717 (LW) + 0.2245	0.9049
	Warm Area	LA = 0.6302 (LW) + 0.2437	0.9150
'Granny Smith'	Cool Area	LA = 0.7386 (LW) - 0.1857	0.9666
	Warm Area	LA = 0.7296 (LW) - 0.1022	0.9750

Table 2. Means (± standard errors) for the total number of budburst sequences of reproductive buds observed on two-year-old (2YO) axes of 'Golden Delicious' and 'Granny Smith' apple branches grown in two sites, Koue Bokkeveld (cool area) and Warm Bokkeveld (warm area).

Cultivar	Site	Number of budburst sequences for reproductive buds
'Golden Delicious'	Cool Area	3.7 ± 1.4
	Warm Area	3.6 ± 1.9
'Granny Smith'	Cool Area	2.1 ± 0.8
	Warm Area	3.1 ± 1.1

Table 3. Number of reproductive buds (RB) per two-year-old axes of 'Golden Delicious' and 'Granny Smith' apple cultivars grown at two sites, a warm area (Warm Bokkeveld) and a cool area (Koue Bokkeveld). Data are mean ± standard error for 20 shoots.

Cultivar	Site	Number of reproductive buds per shoot
'Golden Delicious'	Cool Area	8.7 ± 5.4
	Warm Area	7.3 ± 2.4
'Granny Smith'	Cool Area	6.4 ± 3.3
	Warm Area	5.6 ± 3.5

Table 4. Percentage of buds from each budburst sequence (1-4) that occurred in a particular position (quadrants 1-4) along the two-year-old shoots for Golden Delicious and Granny Smith apple cultivars grown at two sites, a warm area (Warm Bokkeveld) and a cool area (Koue Bokkeveld).

Cultivar	Site	Budburst Sequence	Position 1 (proximal)	2	3	4 (distal)
'Golden Delicious'	Cool Area	**1**	0%	26%	26%	47%
		2	0%	29%	43%	29%
		3	6%	24%	47%	24%
		4	0%	25%	75%	0%
	Warm Area	**1**	0%	28%	44%	28%
		2	6%	28%	39%	28%
		3	7%	29%	36%	29%
		4	13%	25%	38%	25%
'Granny Smith'	Cool Area	**1**	6%	17%	33%	44%
		2	0%	8%	50%	42%
	Warm Area	**1**	5%	16%	26%	53%
		2	9%	26%	52%	13%
		3	0%	23%	54%	23%

Table 5. Fruit set significance parameters of the β-approximation of the Kruskal-Wallis H-test. H test value and p-values for initial fruit set (Initial) and final fruit set (Final) according to position (quadrants) within the two-year-old shoots of 'Golden Delicious' and 'Granny Smith' apple cultivars grown at two sites, a warm area (Warm Bokkeveld) and a cool area (Koue Bokkeveld).

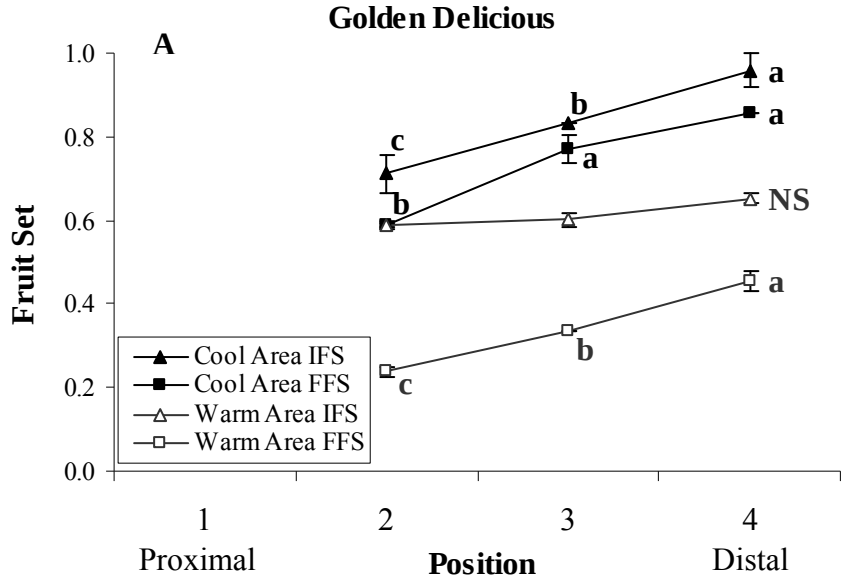
Cultivar	Site	Fruit Set	H	*P*
'Golden Delicious'	Cool Area	Initial	8.62	**
		Final	8.97	**
	Warm Area	Initial	5.51	NS
		Final	10.62	*
'Granny Smith'	Cool Area	Initial	4.87	*
		Final	2.51	*
	Warm Area	Initial	6.93	NS
		Final	8.37	NS

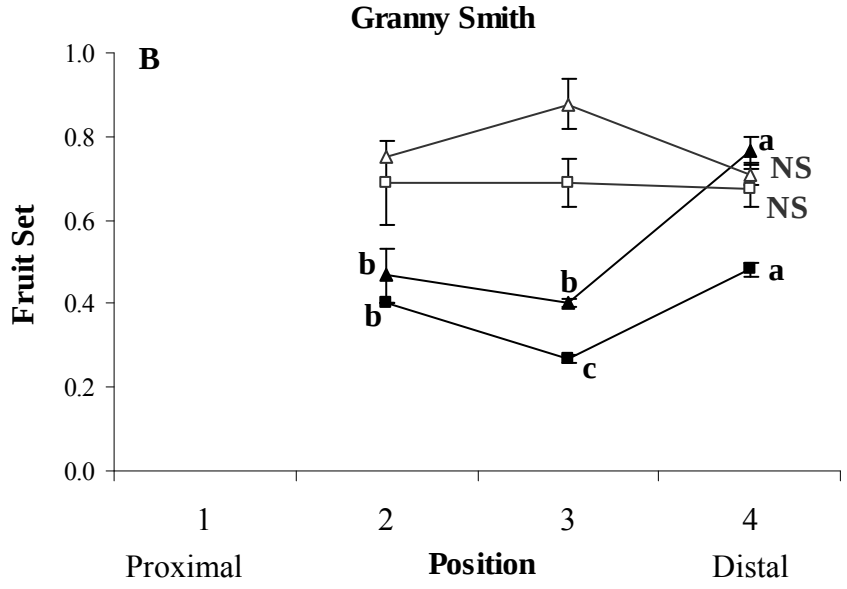
*, **, ***: significant differences between means at the 0.05, 0.01, and 0.001 levels, respectively

Table 6. Fruit set significance parameters of the β-approximation of the Kruskal-Wallis H-test. H test value and p-values for initial fruit set (Initial) and final fruit set (Final) according to sequential flowering for Golden Delicious and Granny Smith apple cultivars grown at two sites, a warm area (Warm Bokkeveld) and a cool area (Koue Bokkeveld).

Cultivar	Site	Fruit Set	H	*P*
'Golden Delicious'	Cool Area	Initial	9.30	**
		Final	11.04	***
	Warm Area	Initial	13.73	***
		Final	13.43	***
'Granny Smith'	Cool Area	Initial	7.86	*
		Final	9.10	**
	Warm Area	Initial	14.24	***
		Final	13.54	***

*, **, ***, NS: significant differences between means at the 0.05, 0.01, and 0.001 levels, and non-significant, respectively


Golden Delicious
A
Fruit Set
1.0
0.8
0.6
0.4
0.2
0.0
Cool Area IFS
Cool Area FFS
Warm Area IFS
Warm Area FFS
a
a
NS
a
b
a
b
c
b
c
1
2
3
4
Proximal
Position
Distal


Granny Smith
B
Fruit Set
1.0
0.8
0.6
0.4
0.2
0.0
a
NS
NS
b
b
a
b
c
1
2
3
4
Proximal
Position
Distal

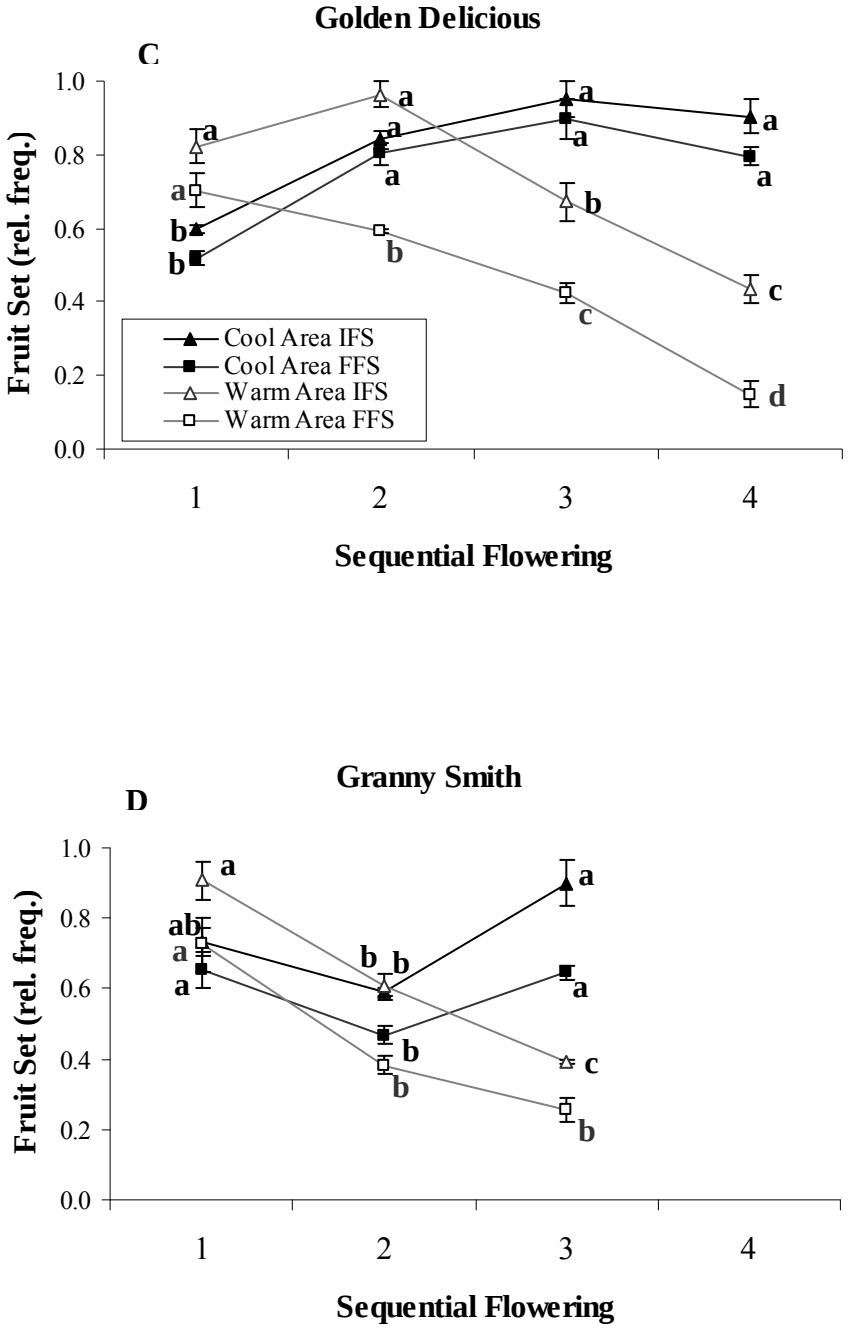
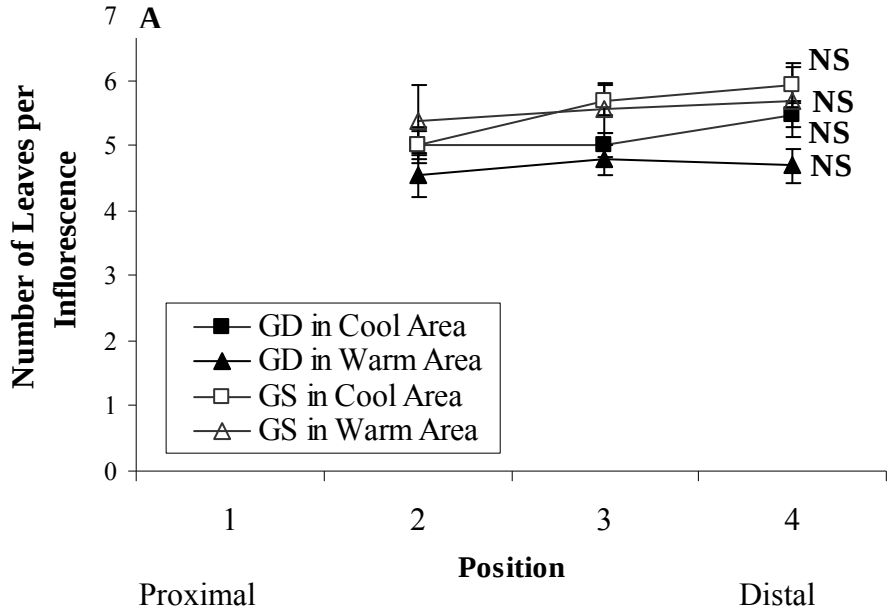
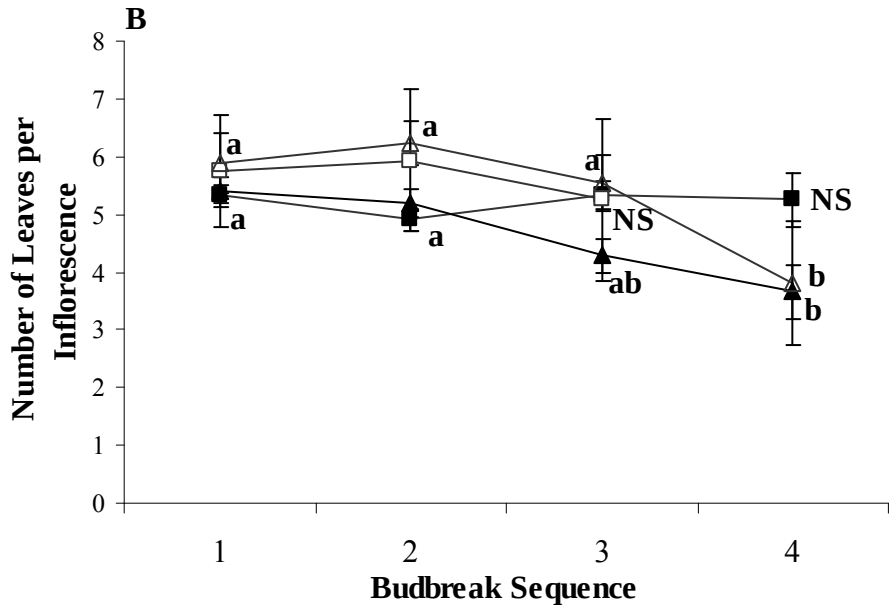


Figure 1. Relationship between fruit set and both (A and B) relative position (quadrant) along the shoot (1=proximal and 4= distal) according to node number and (C and D) flowering sequence (1=first reproductive bud to flower along a shoot and 2= second to flower within the shoot, etc.) within a two-year-old shoot section. Fruit set is percentage of reproductive nodes within a relative position or sequence of flowering that set at least one fruit. Data were collected from both a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld) sites for both 'Golden Delicious' (GD) (A and C) and 'Granny Smith' (GD) (B and D) apple cultivars. Means and standard errors were calculated using pooled shoot data. Letters signify significant differences α=0.05 using mean separation of the Kruskal-Wallis test (K-W) between either relative position or sequential flowering within a single cultivar-site combination. NS = nonsignificant.


A
Number of Leaves per Inflorescence
7
6
5
4
3
2
1
0
NS
NS
NS
NS
GD in Cool Area
GD in Warm Area
GS in Cool Area
GS in Warm Area
1
2
3
4
Position
Proximal
Distal


B
Number of Leaves per Inflorescence
8
7
6
5
4
3
2
1
0
a
a
a
a
a
NS
NS
ab
b
b
1
2
3
4
Budbreak Sequence

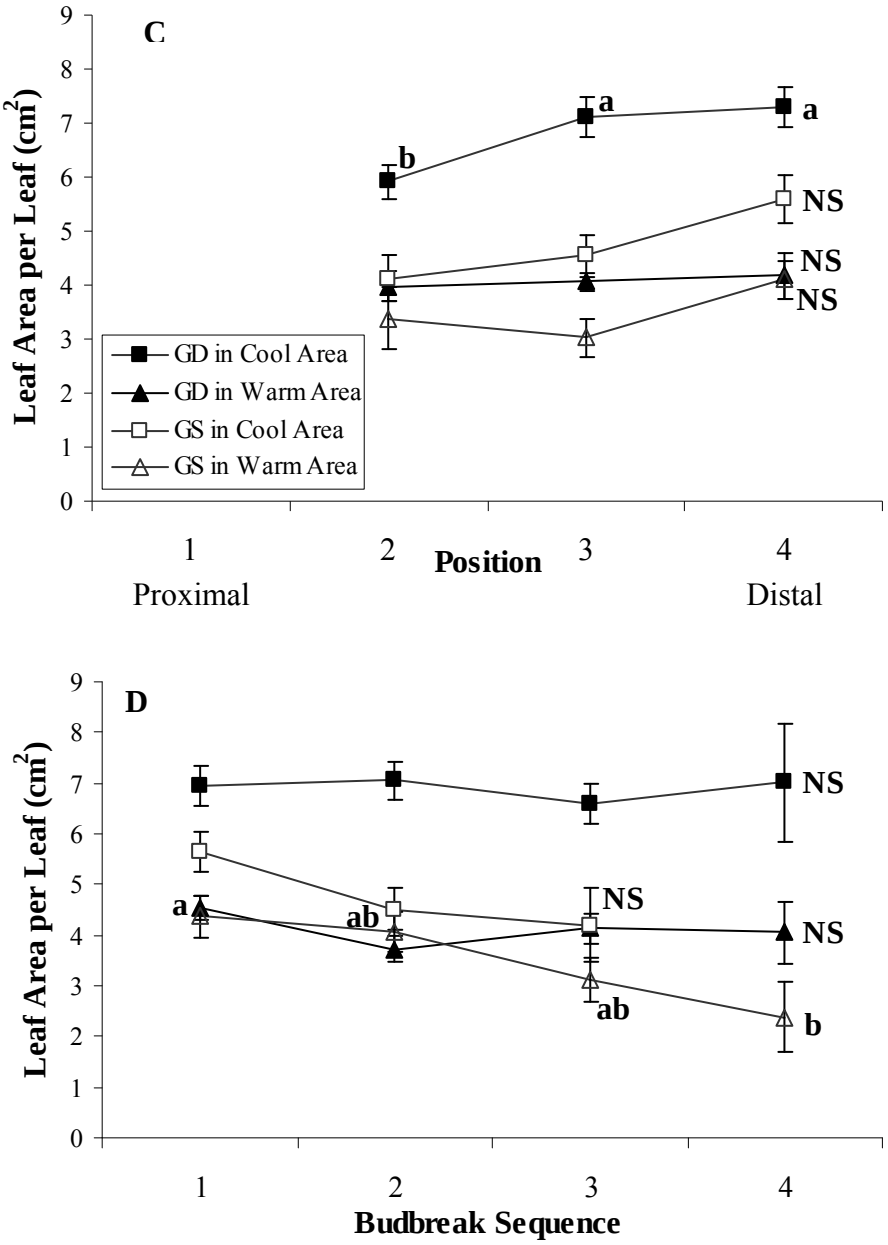


Figure 2. Relationship between inflorescence size characteristics and both (A, C, and E) position (quadrant) within the two-year-old shoot section (1=proximal and 4=distal) and (B and D) budburst sequence (1=first reproductive bud to reach greentip along a shoot and 2= second to reach greentip along the shoot, etc.). Inflorescence characteristics considered were: (A and B) number of leaves per inflorescence, and (C and D) mean leaf area per leaf within an inflorescence at the onset of anthesis. Data was collected from two apple cultivars, 'Golden Delicious' (GD) and 'Granny Smith' (GS), and two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld). Means and standard errors were calculated for both position and sequential budburst within a cultivar-site combination. Letters signify significant differences $\alpha=0.05$ using Newman-Keuls multiple range test between either relative position or sequential flowering within a single cultivar-site combination. NS = nonsignificant.

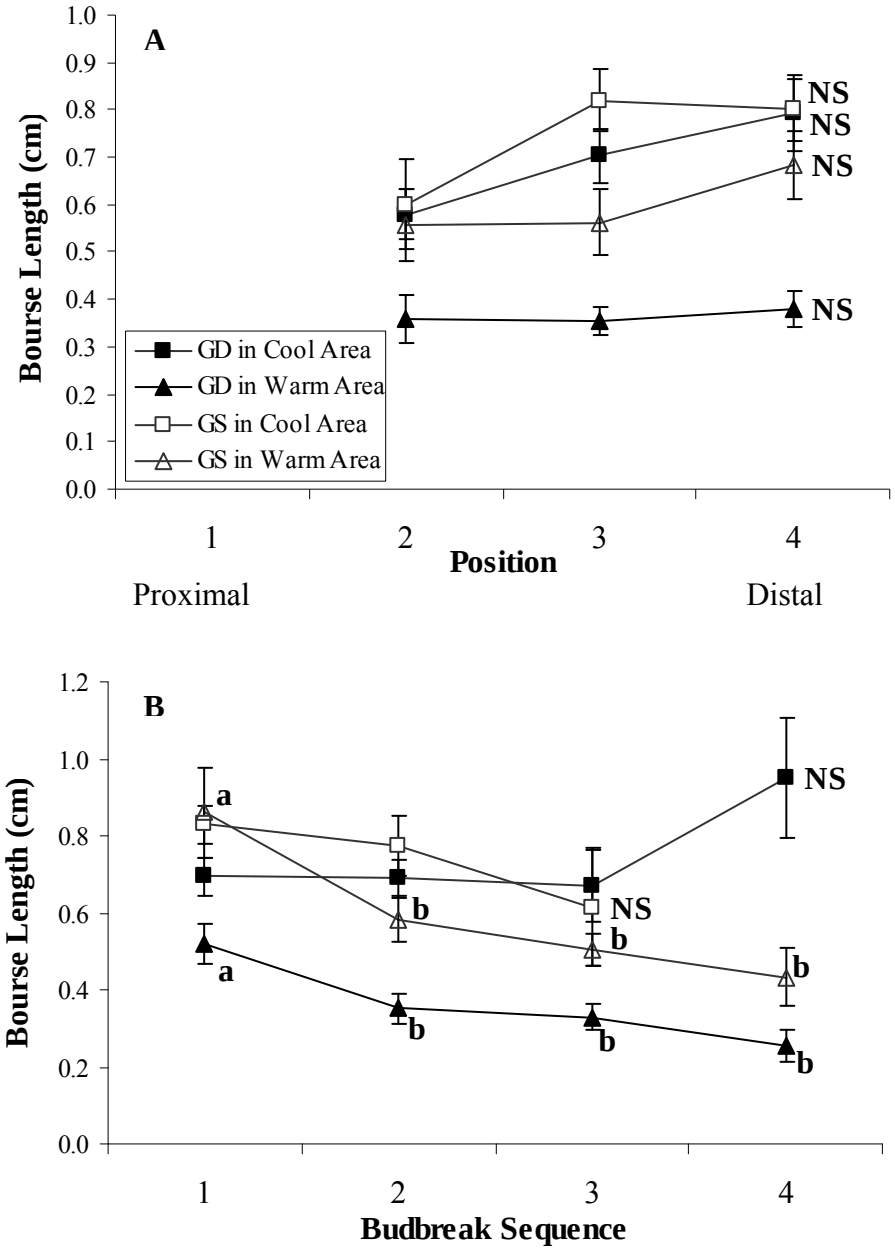


Figure 3. Relationship between bourse length and both (A) position (quadrant) within the two-year-old shoot section (1=proximal and 4=distal) and (B) relative budburst sequence (1=first reproductive bud to flower along a shoot and 2= second to flower within the shoot, etc.). Data was collected from two apple cultivars, 'Golden Delicious' (GD) and 'Granny Smith' (GS), and two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld). Means and standard errors were calculated for both position and sequential budburst within a cultivar-site combination. Letters signify significant differences $\alpha=0.05$ using Newman-Keuls multiple range test between either relative position or sequential flowering within a single cultivar-site combination. NS = nonsignificant.

PAPER 2. Environment and position of first bud to burst on apple (*Malus x domestica* Borkh.) shoots affects lateral outgrowth

Abstract

A study was conducted to determine which bud (terminal or lateral) breaks first, and therefore has primigenic dominance, on 'Granny Smith' and 'Golden Delicious', one-year-old apple (*Malus x domestica* Borkh.) shoots grown in two locations in Western Cape, South Africa, with differing degrees of inadequate winter chilling. Lateral budbreak and growth was influenced by the position of the first bud to break on the shoot, but did not differ between locations. On 'Granny Smith' shoots with primigenic dominance of the terminal, lateral budbreak and growth was suppressed, in accordance with the typical 'delayed foliation' commonly observed in warm winter climates. However, when at least one lateral broke before the terminal, lateral budbreak and growth were similar to what has been observed in cold winter areas. In addition, primigenic dominance of laterals was more common in a warm area than a cool area, and more common in 'Granny Smith' than 'Golden Delicious'.

Introduction

Branching in apple is essentially dependant on the bursting and subsequent outgrowth of proleptic shoots. Proleptic shoots are characterized by their formation in one season, progression through dormancy which requires a period of chilling, and subsequent growth in the spring (Bell, 1991; Hallé *et al.*, 1978). In warm winter climates, trees receive inadequate winter chilling to break endodormancy, and consequently suffer from what has been termed 'prolonged dormancy syndrome' or 'delayed foliation'. One main symptom of prolonged dormancy syndrome is a prolonged, delayed and often absent vegetative budburst (Black, 1952; Saure, 1985; Strydom *et al.*, 1971). Not only does this result in a direct loss of future bearing sites (Black, 1952; Strydom *et al.*, 1971), but it also impacts the acrotonic habit of the branch (Cook & Jacobs, 1999).

Terminal and lateral buds differ in their progression through dormancy (Cook *et al.*, 1998; Cook & Jacobs, 1999; Mauget & Rageau, 1988; Williams *et al.*, 1979). The dormancy progression of terminal buds is characterized by a rapid entrance into dormancy in autumn (decreasing bud growth potential) until a maximum depth has been reached which is followed by an exit from dormancy (increasing bud growth potential) that is initially slow, becoming rapid just before budburst in spring (Cook *et al.*, 1998). In an area with inadequate chilling, the dormancy progression of terminal buds is altered. Buds enter dormancy at a slower and more gradual rate and maximum depth only occurs much later in the winter (Cook & Jacobs, 2000). Buds then burst at a time related to their dormancy depth, or remaining chilling requirement, resulting in a delayed foliation.

In contrast, lateral bud dormancy begins with paradormancy in the preceding season. Paradormancy is imposed outside the bud, but within the plant and can take the form of, for example, such things as either a subtending leaf or apical dominance (Lang *et al.*, 1985). In lateral bud dormancy progression, paradormancy is followed by endodormancy, and then again paradormancy (Faust *et al.*, 1995; Mauget & Rageau, 1988) and/or ecodormancy (Williams *et al.*, 1979) until budbreak. Faust et al. (1995) determined that the higher chilling cultivars have both a longer endodormant period and stronger correlative inhibition by the terminal (apical dominance, and later acrotony) during both paradormant phases.

In the spring, lateral growth has two phases that are differentially regulated: (1) budbreak, or release from dormancy, and (2) elongation (Borchert, 2000; Erez, 2000). Lateral budbreak (number of growing laterals) on an annual shoot is related to genotype, and may (Renton *et al.*, 2006) or may not (Costes & Guédon, 2002; Lauri *et al.*, 2006) be respective of the length of the annual shoot. Budbreak is known to be influenced by winter chilling

accumulation, decreasing with a decrease in chilling (Black, 1952; Hauagge & Cummins, 1991a; Jacobs *et al.*, 1981; Petri & Leite, 2004; Saure, 1985; Strydom *et al.*, 1971). For example, in a study by Costes and Guédon (2002) with adequate chilling, 'Gala' and 'Fuji' had 43% and 38% percent growing laterals (budbreak percent), respectively. In an area with 1000 chill unit (CU) accumulation (North Carolina (NC) model), 'Gala' and 'Fuji' had from 2-14% and 1.7% lateral budbreak, respectively (Petri & Leite, 2004).

In warm winter climates, the terminal is the first to break (Jacobs *et al.*, 1981). The decrease in or absence of lateral budbreak has been attributed to the primigenic dominance (dominance related to the sequence of budbreak (Bangerth, 1989)) of the terminal over the laterals (Saure, 1985). Researchers have attributed the absent or decreased budbreak when chilling was not adequate to the correlative inhibition by the terminal bud over lateral buds (Faust *et al.*, 1995; Williams *et al.*, 1979), specifically distally-located lateral buds (Jacobs *et al.*, 1981). However, adequately chilled areas also have primigenic dominance of the terminal, and this does not restrict budbreak (Cook *et al.*, 1998). In addition, apple genotypes have different levels of correlative inhibition by the terminal as evidenced by their differing degrees of sylleptic branching and acrotony (De Wit *et al.*, 2000; De Wit *et al.*, 2002; Lauri, 2007). This means that they may either differ in response to primigenic dominance of the terminal bud or differ in which bud (lateral or terminal) has primigenic dominance in areas with inadequate chilling (warm areas).

The aim of our study was to determine which bud (lateral or terminal) has primigenic dominance in the shoots of two cultivars, 'Granny Smith' and 'Golden Delicious' on two sites differing in their degree of winter chilling. The second aim of our study was to determine what influence the position of primigenic dominance has on lateral budbreak and outgrowth.

Materials and Methods

Plant Material and Sites – The trial was carried out on two apple cultivars with differing growth habits and architectural features: Golden Delicious on M793, and Granny Smith on seedling rootstock, in the 2006-2007 season. These cultivars have different shoot architectures, types III and IV, respectively, according to the typology of Lespinasse and Delort (1986).

The cultivars were selected in commercial orchards located in areas with both a lower (Warm Bokkeveld; 33º20' S, 19º19' E, 496m, 1119 PCU) and higher (Koue Bokkeveld; 33º12' S, 19º19' E, 1045m, 1698 PCU) chilling unit accumulation historically. In each area, one commercial farm was used. Hereafter the Koue Bokkeveld and Warm Bokkeveld will be

referred to as the cool area and warm area, respectively. Meteorological data was collected from the orchard used for the trial or the local area of the orchard. The net chilling units were calculated according to the daily positive chill unit model (PCU; Linsley-Noakes *et al.*, 1994).

In the cool area, 'Golden Delicious' trees were planted in 1997 at a spacing of 4.5 x 2.5 m; and 'Granny Smith' trees were planted in 1933 at a spacing of 5.6 x 5.6 m. In the warm area, the 'Golden Delicious' trees were planted in 1995 and the 'Granny Smith' trees were planted in 1995 at a spacing of 5.0 x 2.5 m. All trees were in full production in 2007.

All trees received standard irrigation and fertilization. No trees received any chemical restbreaking. The flowers and fruit were neither hand nor chemically-thinned. Flowers were open pollinated by honey bees and there were no pollination problems during the time of this trial.

For all cultivar/area combinations, shoots were randomly selected in June 2006 at shoulder height from 10-20 trees within one row. Number of shoots used for each cultivar/area combination shown in Table 1. Selected shoots started growing in September (spring) 2005, and therefore were comprised of a one-year-old shoot (hereafter referred to as main axis) with lateral buds, and a terminal bud. The shoots were approximately horizontal (but not below horizontal) when selected, and a representative sample of other shoots on the tree.

Branch Measurements - Nodes were numbered along the main axis of the studied branches from the proximal to distal end. The main axis was equally partitioned into four quadrants based on node number, with quadrant 1 being the most proximal and quadrant 4 the most distal. At the end of the season, the studied branch consisted of lateral shoots emanating from the laterally-located nodes, a terminal shoot, and a main axis (Figure 1).

Starting in early September, the studied branches were monitored every day. The position and date of the first bud to break on the main axis was recorded. Days between lateral and terminal budbreak, or vice versa, were calculated for each branch. Terminal and lateral types (reproductive, vegetative, latent, and dead) were also recorded for each node at the end of the season. Percent budbreak was calculated as the number of growing nodes (reproductive and vegetative combined) divided by the total number of nodes along either the main axis (total percent budbreak) or within a quadrant within the axis (percent budbreak per quadrant).

After harvest, lengths and proximal and distal diameters (D) (diameter taken 2 cm from the ring scars at the proximal and distal end, respectively, of the shoot or main axis) of

the main axis, terminal shoot, and lateral shoots were measured. In the case of outgrowth from reproductive buds, the length and diameters were considered to be those of the longest bourse shoot. Lateral shoot lengths were either used in analyses as actual lengths or put in length categories with accepted physiological differences (Costes *et al.*, 2006; Lespinasse & Delort, 1993): spurs (<5 cm); brindles (≥5 and <20 cm); and long shoots (≥20 cm). Basal diameter was used to calculate cross-sectional area (CSA) for each of the lateral shoots using the following formula: $CSA = \Pi * D^2/4$. For each branch, lateral shoot CSA was summed for both (a) all the lateral shoots along the main axis and (b) all the lateral shoots within each quadrant.

Shoot slenderness is an important selection criteria for branching as it is related to regular bearing (Lauri *et al.*, 1997) and we wanted to determine if they also influenced budbreak and lateral outgrowth in this study. Shoot slenderness was calculated as: main axis length divided by the average of the proximal and distal diameters of the main axis.

According to Corner's rules (Hallé *et al.*, 1978), two principles can be used to mathematically define architecture: (1) growth of lateral shoot on an axis is related to the main axis size, ie. the larger the diameter of the main axis, the larger and more complicated its appendages (lateral shoots, leaves, inflorescences); and (2) appendage size is related to the number of growing appendages, ie. the greater the number of lateral shoots along a main axis, the smaller the lateral shoots. Implied in this concept is that the total outgrowth of laterals is related to the axis size, and that differences may occur in the number, position and size of laterals. In a previous study on apple, the relationship between the laterals and the main axis (in that case, the ratio between the trunk CSA and the CSA of all branches summed) is genotype dependant, but not influenced by rootstock (Maguylo & Lauri, 2007). In our study, we similarly summed the CSAs of the lateral shoots along the main axis. The sum of lateral CSAs divided by the main axis CSA for each shoot is reported as 'Lateral CSA sum/Main Axis CSA'.

Leaves were destructively harvested for each node after fruit harvest and actual area of the leaf was determined using the LI-3100C (Li-Cor, Inc.). Leaf area was then summed for each node.

Data Analysis – For 'Granny Smith' and 'Golden Delicious' grown in both the cool and warm areas, the proportion of axes that reached green tip in the terminal position first (terminal budbreak precedence (BBP)), lateral position first (lateral BBP), and both in the lateral and terminal positions simultaneously (no budbreak positional precedence, or no

precedence) was determined. For the remaining analyses, only the 'Granny Smith' axes were used since there was either no (in the case of the cool area) or very little (in the case of the warm area) lateral BBP occurring in 'Golden Delicious' in our study. They were analyzed according to site and budbreak precedence (position, terminal or lateral, of the first bud to break on the shoot). The 'no precedence' category was not used in further analyses due to the low numbers of shoots in this category.

Percent budbreak was analyzed non-parametrically using the Kruskal-Wallis H test with the multiple means comparison test used to test the differences between factors. For total percent budbreak along the axis, individual site-budbreak precedence combinations were used as factors. Positional data was analyzed with position (quadrants 1-4) as the factor.

Main axis characteristics (lengths, diameters and number of nodes), ratio of lateral CSA sum to main axis CSA, and leaf data (number and area) were considered normally distributed and analyzed using the factorial ANOVA (Fisher F test) followed by Newman-Keuls test. Location (warm area and cool area) and budbreak precedence (lateral BBP and terminal BBP) were used as the factors. When sum of the lateral CSA/main axis CSA was analyzed for each site-budbreak precedence combination using position as the factor, a one-way ANOVA (Fisher F test) was used followed by the Newman-Keuls. All statistical analyses were done using StatSoft (2008)

Results

Observed Budbreak Patterns- Of the 26 'Granny Smith' shoots studied in the warm area, 58% had terminal BBP, while only 27% had lateral BBP (Table 1). About 15% of the axes had no BBP (ie. both the terminal and at least one lateral bud reached green tip on the same day). In the warm area, 43% of the shoots had terminal BBP and 38% had lateral BBP. 18% of the 'Granny Smith' axes in the warm area had no BBP. In 'Golden Delicious' all of the axes had terminal BBP with the exception of approximately 18% of the shoots in the warm area, of which half had lateral BBP and half had no BBP.

In 'Granny Smith' shoots with lateral BBP, the final length of the first shoot to grow in the lateral position was plotted against the relative position of that shoot along the axis (Fig 2). Although the first bud to break on lateral BBP shoots occurred in different positions, the few and only lateral shoots to exceed 10 cm occurred in the distal quarter of the shoot (greater than 0.75 on the x-axis).

In our study 45% and 34% lateral buds broke for 'Golden Delicious' in the cool area and in the warm area, respectively (with terminal BBP). 'Granny Smith' with lateral BBP in

both locations had a percent budbreak (34%) similar to what Costes and Guédon (2002) found viz. 39%. With terminal BBP, however, lateral budbreak was much lower (18% and 6% lateral budbreak in the cool area and the warm area, respectively).

On terminal BBP axes, 'Granny Smith' had a mean of 3.7 (warm area) and 6.4 (cool area) days between the terminal reaching green tip and the first lateral reaching green tip (Table 2). On lateral BBP axes, 'Granny Smith' had a mean of 2.8 (cool area) and 3.1 (warm area) days between the first lateral reaching green tip and the terminal reaching green tip. 'Golden Delicious' terminal BBP axes had a mean of 6.0 (cool area) and 19.9 (warm area) days between the terminal reaching green tip and at least one lateral reaching green tip.

Shoot Characteristics with Different BBP and in Different Locations -Terminal BBP in the warm area was characterized by a reproductive terminal bud in all cases (Table 3) and about 50% reproductive buds breaking laterally along the axis (Table 4), although this accounts for only a few buds since not many buds burst along the axis (Table 4, Fig 3). Terminal BBP axes in the cool area were characterized by a vegetative bud in the terminal position (Table 3) and only vegetative lateral buds (Table 4).

Lateral BBP in the warm area is characterized by mostly vegetative buds in the terminal position (only 11% reproductive buds) (Table 3), and mostly vegetative buds (~88%) in lateral position (Table 4). Lateral BBP in the cool area is characterized by a vegetative terminal bud in all cases (Table 3), and mostly (92%) vegetative lateral buds (Table 4). In contrast, in 'Golden Delicious', 63% of the terminal buds were reproductive in the cool area and 92% in the warm area (Table 3). Very few lateral buds were reproductive in 'Golden Delicious' with terminal BBP (8.4% in the cool area and 0% in the warm area). In the few shoots of 'Golden Delicious' with lateral BBP in the warm area, about 46% of the lateral buds were reproductive (Table 4).

Shoots were not significantly different between sites in length, base cross-sectional area (CSA), node number, or slenderness (Tables 5 and 6). However, shoots with terminal BBP were both more slender (Table 6) and had a lower base CSA (Table 5) than shoots with lateral BBP. Terminal length did not differ between sites or BBP and there was no interaction between site and BBP (Table 5).

Lateral Growth - Budburst is equal between sites in axes with lateral BBP, while lower in the warm area with terminal BBP compared to axes with lateral BBP (Fig 3). In the cool area, the ratio between the sum of lateral CSA and the main axis CSA is maintained, regardless of

which bud breaks first on the shoot (0.46 and 0.49 for lateral and terminal BBP, respectively) (Table 7). In the warm area, lateral CSA is increased relative to the main axis CSA when a lateral bud breaks first, and decreased when the terminal bud breaks first. In addition, since lateral BBP shoots have a higher base diameter (Table 5), the total CSA of lateral outgrowth is even greater than if both lateral and terminal BBP shoots had equal slenderness and base diameter. Likewise, since terminal BBP shoots have a smaller base diameter (Table 5), the sum of lateral CSA in these shoots is even smaller.

Leaf Area - Leaf area in lateral position did not differ between sites but was greater in shoots with lateral BBP than in shoots with terminal BBP, which resulted in a higher total leaf area in shoots with lateral BBP than terminal BBP (Table 8). Terminal leaf area per shoot did not differ by site or BBP. Due to the high variability in number of spurs per shoot between sites and BBP (Fig 4 A - D), there was a high variability in number of spur leaves per shoot and so the values were not significantly different (Table 8).

Lateral Outgrowth by Position -The percent of growing buds along the shoot is either higher in the distal half of the shoot as compared to the proximal half of the shoot (terminal BBP in the cool area) or does not differ with shoot position (lateral BBP in the cool area, and both lateral and terminal BBP shoots in the warm area) (Fig 5 A and B).

In the cool area, lateral CSA is highest in quadrants 3 and 4 (although quadrant 4 does not significantly differ from quadrants 1 and 2) with terminal BBP and does not differ by position in lateral BBP shoots (Fig 6A). The majority of this CSA is due to laterals less than 5 cm long (Fig 4 A). With lateral BBP, about 20% of the total buds are less than 5 cm in the distal 3 quadrants. No laterals were present in the most proximal quadrant (Fig 4 A). In terminal BBP shoots, a few brindle length shoots occur in quadrant 3. All other laterals are spurs and occur mostly in the greatest proportion in the distal half of the shoot (Fig 4 A).

In the warm area, lateral CSA sum is greatest in the most distal position in shoots with lateral BBP (Fig 6 B). This great increase in the most distal quadrant in CSA is due in part to the relatively higher proportion of both shoots between 5 and 20 cm and greater than 20 cm in this quadrant (Fig 4 B). Shoots in the warm area with terminal BBP have almost no budburst (Fig 3), and the laterals, with the exception of the most proximal position (which has some laterals greater than 20 cm), are less than 5 cm long (Fig 4 B), so the sum of lateral CSA in each position is very low and equal for all positions along the axis (Fig 6 B).

Discussion

Budbreak Precedence and Differential Dormancy Depth and Progression of Buds Along the One-Year-Old Shoot - A higher percentage of lateral BBP was observed in the warm area (as compared to the cool area) for both 'Granny Smith' and 'Golden Delicious' (Table 1). On axes with lateral BBP, the lateral bud must have a higher growth potential than the terminal bud at a point in time. This is most likely during the time when the terminal bud is deeply dormant. Lateral BBP, however, may also be due to the loss of correlative inhibition by the terminal over the laterals. One of the problems with using bud growth potential, or mean time to budburst, to determine depth of dormancy is that it is an indirect method of measuring dormancy and, in an intact shoot, doesn't discriminate between the different types of bud dormancy: para-, endo-, and eco-dormancy, as defined by (Lang *et al.*, 1985), especially when more than one type is occurring simultaneously (Faust *et al.*, 1995; Olsen, 2003).

Because dormancy progression in lateral buds during late winter is mainly due to para- or eco-dormancy (Mauget & Rageau, 1988; Williams *et al.*, 1979), specifically when the terminal is deeply endodormant, then lateral buds can be expected to have a higher propensity to burst first, as seen in our study (Table 1). As the terminal bud emerges from endodormancy and it's growth potential increases (Cook *et al.*, 1998; Hauagge & Cummins, 1991b; Mauget & Rageau, 1988), it has an increased ability to break before the laterals. This was observed in our study (Table 1). This is probably not a sudden shift in position of BBP, but a progressive and continuous shift from lateral BBP to terminal BBP. Due to the higher amount of lateral BBP in the warm rather than the cool area, the increase in lateral BBP can be attributed to a decrease in chilling unit accumulation. In our study, there was an increase in relative amount of terminal BBP in both 'Golden Delicious' and 'Granny Smith' from the warm area to the cool area (Table 1). Although 'Granny Smith' and 'Golden Delicious' have been shown to have similar chilling requirements (Hauagge & Cummins, 1991a), they, by definition, do not have an equal re-establishment of primigenic dominance of the terminal bud in our study as the terminal bud did not always break first (Table 1).

Faust et al (1995) attributes some of lateral bud dormancy to paradormancy, specifically apical dominance, and so these cultivars may have different levels of apical dominance. Chilling accumulation is therefore more of a continuous process that involves not only the ability of the terminal or lateral buds to break after a certain degree of chilling, but may also involve re-establishment of primigenic dominance of the terminal. Even though they have a similar chilling requirement, 'Granny Smith' was shown to have more variation in measurable chilling requirement depending on the year than 'Golden Delicious' (Hauagge &

Cummins, 1991a), and this may, in part, be due to other factors such as re-establishment of the terminal bud to break first (terminal BBP).

In areas with adequate chilling, the laterals may indeed by under ecodormancy after endodormancy (Williams *et al.*, 1979), preventing outgrowth when the auxin transport system is impaired due to cold temperatures (Morris, 1979). In warm winter areas, lateral buds may be under correlative inhibition by the terminal, as indicated by Cook and Jacobs (1999).

In addition to the differential dormancy depth and progression between the terminal and lateral buds, reproductive buds have a lower chilling requirement than vegetative buds in both apple (Naor *et al.*, 2003) and peach (Erez & Couvillon, 1987). In both warm and cool areas they will burst before the vegetative buds which have a higher chilling requirement (Erez & Couvillon, 1987; Naor *et al.*, 2003). This was evident in our study, and can explain the differences in BBP observed in the warm area, as all of terminal BBP 'Granny Smith' shoots were terminated by a reproductive bud while 89% of the lateral BBP shoots were terminated by a vegetative bud (Table 3). A reproductive bud in the terminal position, then, may predispose a shoot to terminal BBP in inadequately chilled areas. Since development of a reproductive bud in the terminal position is related to shoot slenderness (Lauri *et al.*, 1997), it may also be that a shoot's slenderness would predispose the shoot to have a reproductive bud, which would then predispose the shoot to have terminal BBP in areas of inadequate chilling. Not only would this result in both a primigenic and positional dominance over bud burst and growth of the laterals, but due to the energy required initially for the reproductive bud (flowering, fruit set), may limit the amount of energy that can be used for lateral outgrowth initially, possibly resulting in the reduced ability of the lateral buds to break and grow. In our study, for both 'Granny Smith' and 'Golden Delicious', the warm area had a higher proportion of shoots terminated by a reproductive bud than the cool area (Table 3).

Positional Dominance by the Terminal - Regardless of whether the terminal or the lateral breaks first on the Granny Smith shoots, the terminal length is not affected (Table 5). It is possible that the length of one annual shoot and that of the succeeding annual shoot is genetically regulated (Lauri & Trottier, 2004), and/or related to the ability of the terminal bud to retain its reserves while the laterals readily distribute them to surrounding laterals (Hansen, 1969). The terminal bud's positional dominance maintains that the terminal length will not be influenced, even when the laterals break first (i.e., laterals have primigenic dominance).

Primigenic Dominance by the Terminal and Budbreak - When the terminal had primigenic dominance, it suppressed both lateral budbreak (proportion of growing laterals) and lateral growth in 'Granny Smith' in the warm area. This may be a piece of a larger picture about the relationship between dormancy progression of the terminal and dominance of the terminal over the lateral buds.

In our study, 'Granny Smith' with lateral BBP had a percent budbreak (34%) and this is similar to what (Costes & Guédon, 2002) found (39%) in 'Granny Smith' in France (a cold winter climate). When the terminal had primigenic dominance, however, lateral budbreak decreased in the warm area.

The common consensus is that the delayed or absent budbreak as a result of delayed foliation, or prolonged dormancy syndrome, is a symptom of the terminal breaking first and establishing dominance over the laterals (Saure, 1985). Our data agrees with this. However, one exception is that we also observed the terminal losing primigenic dominance in the warm area (i.e., with less chilling accumulation) so that relatively fewer shoots had the terminal bud breaking first (Table 1). This may be due to the shoot length selected for our study (ie. different shoot lengths may respond differently to chilling or require different amounts of chilling to produce terminal BBP).

Re-Establishment of Primigenic Dominance of the Terminal and Lateral Bud Growth - Once the terminal has emerged from endodormancy enough to re-establish its primigenic dominance, lateral budbreak is suppressed. However, in areas with adequate chilling, when the terminal buds break first it does so without controlling lateral budbreak. In our study, it appears that on terminal BBP shoots the laterals may progressively regain the ability to break with an increase in chilling (i.e., lost .

Before the terminal bud has completed endodormancy, lateral buds have a higher growth potential than the terminal, or are not under correlative inhibition by the terminal. They then grow out first and establish primigenic dominance (as evidenced by the budbreak percent that is almost equal to that of 'Granny Smith' grown in an adequately chilled area (Costes & Guédon, 2002)). Regardless of chilling accumulation then, lateral budbreak does not suffer when at least one lateral bud has primigenic dominance over the terminal. Once the terminal bud does re-establish a higher potential to grow, it still has correlative inhibition over the laterals. At some point later, this correlative control must subside so that lateral budbreak can meet a genetically dictated maximum (Costes & Guédon, 2002; Lauri *et al.*, 2006). This is exemplified by the 34% and 45% lateral budbreak observed on terminal BBP 'Golden

Delicious' shoots for the warm area and the cool area, respectively, and the 6% and 18% lateral budbreak observed on terminal BBP 'Granny Smith' shoots in the warm area and the cool area, respectively.

Lateral growth and budbreak precedence - In the warm area, when lateral BBP does occur, the distal laterals attempt replacing the terminal as evidenced by the increase in long (>20cm) shoots in the distal quadrant. This does not happen in the cool area, even though percent budbreak and spur number in the distal section is greater than in the proximal sections. This might be due to a deeper dormancy of the terminal in the warm area than the cool area, or an increase in the control over the distal lateral bud becoming a replacement for the terminal (terminal replacement axis) in the cool area. This further substantiates the research of Faust et al. (1995), in which, if the terminal is removed the lateral buds become active (as determined by an increase in conversion of bound to free water), and then the distal-most lateral becomes dominant, replacing the terminal, and re-establishes the correlative inhibition over the budbreak of the proximally situated laterals. Cook and Bellstedt (2001) also found that during most of the progression of dormancy, the distal lateral bud has a higher growth potential than the terminal when the terminal has been removed. In our study, the distal-most lateral behaves as if the terminal had been removed, and the terminal, based on its length, behaves as in the other, adequately chilled shoots. In the cool area, the terminal, once it sprouts, may be recognized by the lateral buds in the distal section as evidenced by the lack of a terminal replacement axis, whereas, in the warm area, the terminal may not be recognized by the distally located buds, as evidenced by the presence of a terminal replacement axis. Due to this strong growth of the laterals in the distal section of lateral BBP 'Granny Smith' shoots in the warm area, the balance between lateral growth and main axis size is disrupted. The lateral CSA sum includes what is assumed to be the replacement axis. Either, in the warm area, total lateral growth is decreased relative to the annual shoot size with terminal BBP (due to a decrease in budbreak and subsequent outgrowth) or increased relative to the shoot axis size with lateral BBP (possibly due to the laterally located shoots replacing the main shoot and becoming dominant). In the cool area, the balance between total lateral outgrowth and main axis size is maintained.

Acrotony and BBP - Acrotonic budburst can be defined as primigenic dominance of the terminal over the laterals, as well as the precedence of the distal buds over the proximal buds within an annual shoot (Cook & Bellstedt, 2001). In our study, acrotonic budburst in regards

to the first definition is less in the warm area than the cool area for both 'Granny Smith' and 'Golden Delicious' (Table 1). However, acrotony, as defined by the increase in lateral size and organogenesis moving distally along an annual shoot (Lauri, 2007) takes place on a number of levels, including organogenesis that occurs in the preceding autumn, differential dormancy progression along an annual shoot, the differential budburst time along the shoot, and outgrowth of the laterals in regards to their position relative to other laterals. In our study, even though lateral BBP is considered as a loss of acrotonic budburst according to the definition of Cook and Bellstedt (2001), the length of the first bud to break was clearly related to its position. If the first bud to break on lateral BBP shoots was in the proximal section, it never grew longer than spur size, and shoots progressively got longer moving distally along the shoot when they grew out first (Fig 2).

In terms of lateral budbreak and size as related to position, acrotony was not always evident. In the warm area, very little growth occurred with terminal BBP shoots, leading to an equal lateral growth along the shoot (although very little).

Conclusion **-** Correlative inhibition of the lateral buds by the terminal begins during shoot growth in the preceding year when the buds are formed. This type of paradormancy continues until a point during dormancy when bud growth potential of the lateral buds is greater than that of the terminal, whether this is due to degree of endodormancy, paradormancy (in the lateral buds), or both. During this time, the lateral buds also go through endodormancy and are not able to burst due to physiological factors. After this, however, if temperatures are warm enough, then the lateral buds will break before the terminal (being less dormant, not under strong correlative inhibition by the terminal, and not endodormant). In cold winter areas, lateral buds are most likely ecodormant while the chilling requirement of the terminal is being completed. However, in warm winter areas, lateral bud dormancy is maintained via correlative inhibition by the terminal. This is different for cultivar though, as 'Golden Delicious' maintains terminal budbreak precedence and lateral outgrowth, while 'Granny Smith' loses terminal BBP and when the terminal does break first, it limits lateral budbreak. As the terminal bud is exiting dormancy, it re-establishes its correlative inhibition over the laterals. At some point after the terminal has accumulated enough chilling, the terminal releases dominance over the lateral buds. Perhaps, the chilling requirement for the terminal bud to release control over the laterals is longer than the chilling requirement to release the terminal bud from endodormancy in some cultivars. Either way, if the chilling requirement is

met, the terminal should have primigenic dominance and yet also a reduced correlative inhibition over the laterals.

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Table 1. Relative frequencies of shoots with lateral budbreak precedence, terminal budbreak precedence, or no budbreak precedence. Budbreak precedence refers to the position of the first bud on the shoot to reach green tip. One-year-old shoots of two cultivars, Granny Smith and Golden Delicious, grown in two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld) were used. Lateral precedence refers to at least one lateral bud to reach green tip before the terminal bud; terminal budbreak precedence refers the terminal reaching green tip before any of the laterals; and no precedence refers to both the terminal and at least one lateral bud on the shoot reaching green tip simultaneously. 'n' is the total number of shoots used the study for each cultivar/site combination.

Genotype	Site	Budbreak Precedence			n
		Lateral	No precedence	Terminal	
'Granny Smith'	Cool Area	26.9 %	15.4 %	57.7 %	26
	Warm Area	38.1 %	18.0 %	42.9 %	21
'Golden Delicious'	Cool Area	0 %	0 %	100 %	18
	Warm Area	9.1 %	9.1 %	81.8 %	22

Table 2. Mean ± SE of days between either the terminal and the lateral bud breaking on terminal budbreak precedence apple shoots, or the lateral and terminal breaking on lateral budbreak precedence apple shoots. Shoots were 'Granny Smith' or 'Golden Delicious' one-year-old apple shoots grown in two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld), and having two types of budbreak precedence, lateral and terminal.

Cultivar (Site)	Number of days
Terminal Budbreak Precedence	
'Granny Smith' (Cool Area)	6.4 ± 3.0
'Granny Smith' (Warm Area)	3.7 ± 0.9
'Golden Delicious' (Cool Area)	6.0 ± 1.3
'Golden Delicious' (Warm Area)	19.9 ± 1.3
Lateral Budbreak Precedence	
'Granny Smith' (Cool Area)	2.8 ± 1.1
'Granny Smith' (Warm Area)	3.1 ± 0.7

Table 3. Percentage of total growing terminal buds that are reproductive on 'Granny Smith' and 'Golden Delicious' one-year-old apple shoots grown in two locations with differing amounts of winter chilling, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld), and with different budbreak precedences. Lateral precedence refers to at least one lateral bud to reach green tip before the terminal bud; terminal budbreak precedence refers the terminal reaching green tip before any of the laterals; and no precedence refers to both the terminal and at least one lateral bud on the shoot reaching green tip simultaneously. Total number of growing buds is in parentheses.

Genotype	Site	Budbreak Precedence		
		Lateral	No precedence	Terminal
'Granny Smith'	Cool Area	0% (7)	25% (4)	0% (15)
	Warm Area	11% (8)	83% (4)	100% (9)
'Golden Delicious'	Cool Area			63% (18)
	Warm Area			92% (18)

Table 4. Percentage of total growing lateral buds that are reproductive on 'Granny Smith' and 'Golden Delicious' one-year-old apple shoots grown in two locations with differing amounts of winter chilling (a cool area, Koue Bokkeveld, and a warm area, Warm Bokkeveld) and different budbreak precedences. Lateral precedence refers to at least one lateral bud to reach green tip before the terminal bud; terminal budbreak precedence refers the terminal reaching green tip before any of the laterals; and no precedence refers to both the terminal and at least one lateral bud on the shoot reaching green tip simultaneously. Total number of growing buds is in parentheses.

Genotype	Site	Budbreak Precedence		
		Lateral	No precedence	Terminal
Granny Smith	Cool Area	8% (13)	43% (21)	0% (34)
	Warm Area	12% (42)	84% (19)	50% (8)
Golden Delicious	Cool Area			8% (131)
	Warm Area	46% (13)	0% (13)	0% (77)

Table 5. Mean (± SE) for length, base cross-sectional area, and total number of nodes of the one-year-old shoot axis, and length of the terminal for *n* number of 'Granny Smith' one-year-old axes grown in two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld), and having two types of budbreak precedence, lateral and terminal. For each column a factorial analysis was performed, and Fisher F stat and corresponding p-value are shown. No interaction among site and budbreak precedence was found for any of the variables so only main effects are shown.

Cultivar (Site)	Budbreak Precedence	n	Main Length	Main CSA	Main Node Number	Terminal Length
'Granny Smith'		38	16.0 ± 1.8	24.4 ± 3.0	12.4 ± 0.9	18.2 ± 2.2
Cool Area		21	14.3 ± 2.4	23.3 ± 4.4	11.6 ± 1.1	14.1 ± 3.0
Warm Area		17	17.2 ± 2.5	25.2 ± 4.2	13.0 ± 1.4	21.9 ± 3.7
	Lateral BBP	15	15.6 ± 3.3	35.3 ± 5.9	13.6 ± 2.0	24.1 ± 5.5
	Terminal BBP	23	16.3 ± 2.0	17.4 ± 1.8	11.6 ± 0.9	15.3 ± 2.4
		df	*p*	*p*	*p*	*p*
	Site	*1*	*0.3045*	*0.6383*	*0.4865*	*0.0853*
	Budbreak Precedence	*1*	*0.5397*	*0.0037*	*0.5385*	*0.3365*
	Site x Budbreak Precedence	*1*	*0.3691*	*0.9904*	*0.4400*	*0.1419*

Table 6. Mean (± SE) for slenderness of the one-year old axis of 'Granny Smith' apple shoots grown in two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld), and having two types of budbreak precedence, lateral and terminal. For each column a factorial analysis was performed and Fisher F stat and corresponding p-value are shown. No interaction among site and budbreak precedence was found for any of the variables so only main effects are shown.

Cultivar (Site)	Budbreak Precedence	n	Slenderness
Granny Smith		38	4.1 ± 0.3
Cool Area		21	4.0 ± 0.4
Warm Area		17	4.1 ± 0.4
	Lateral BBP	15	3.2 ± 0.3 b
	Terminal BBP	23	4.7 ± 0.4 a
		Df	*p*
	Site	*1*	*0.4731*
	Budbreak Precedence	*1*	*0.0066*
	Site x Budbreak Precedence	*1*	*0.8318*

Table 7. Mean ± SE for ratio of 'Sum Lateral CSA/Main CSA' of 'Granny Smith' one-year-old axes grown in two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld), and having two types of budbreak precedence, lateral and terminal. A factorial analysis was performed, and the Fisher F stat and corresponding *P*-value are shown. Since interaction was significant at p<0.10, values for site/budbreak precedence combinations are shown. Different letters represent significant differences (at $P < 0.05$) using Newman-Keuls multiple mean comparison.

Cultivar (Site)	Budbreak Precedence	Sum Lateral CSA / Main CSA
'Granny Smith'		0.48 ± 0.08
Cool Area		0.48 ± 0.12
Warm Area		0.48 ± 0.11
	Lateral BBP	0.70 ± 0.12
	Terminal BBP	0.35 ± 0.09
Interactions		
Cool Area	Lateral BBP	0.46 ± 0.31 ab
	Terminal BBP	0.49 ± 0.13 ab
Warm Area	Lateral BBP	0.79 ± 0.12 a
	Terminal BBP	0.18 ± 0.11 b
		df	*p*
	Site	*1*	0.9604
	Budbreak Precedence	*1*	0.0735
	Site x Budbreak Precedence	*1*	0.0502

Table 8. Mean (± SE) for total leaf area in lateral position, total leaf area in terminal position, total leaf area per shoot, and total number of leaves in spur positions for one-year-old 'Granny Smith' apple shoots grown in two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld), and having two types of budbreak precedence, lateral and terminal. For each column a factorial analysis was performed, and Fisher F stat and corresponding p-value are shown. Site/budbreak precedence combination means (± SE) are shown. Different letters represent significant differences (at $P < 0.05$) using Newman-Keuls multiple mean comparison.

Cultivar (Site)	Budbreak Precedence		Leaf Area in Lateral Position	Leaf Area of Terminal	Total Leaf Area per Shoot	Total Number of Leaves on Spurs
Granny Smith			440 ± 95	383 ± 58	823 ± 116	11.7 ± 2.4
Cool Area			408 ± 129	424 ± 121	832 ± 213	11.6 ± 3.1
Warm Area			461 ± 136	355 ± 53	817 ± 136	11.8 ± 3.7
	Lateral BBP		680 ± 168	465 ± 108	1144 ± 207 a	16.0 ± 4.1
	Terminal BBP		248 ± 80	318 ± 55	566 ± 84 b	8.7 ± 2.7
		df	*p*	*p*	*p*	*p*
	Site	*1*	*0.8591*	*0.2942*	*0.6880*	*0.8858*
	Budbreak Precedence	*1*	*0.0466*	*0.0899*	*0.0152*	*0.1542*
	Site x Budbreak Precedence	*1*	*0.2164*	*0.0957*	*0.8896*	*0.2046*

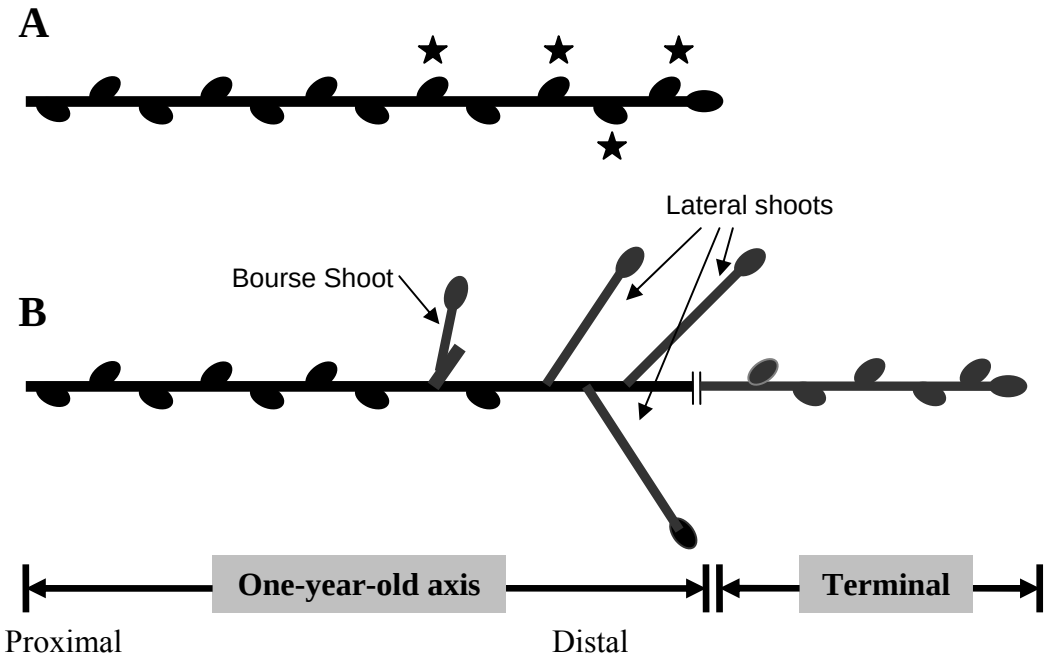


Figure 1. Representative one-year-old (1YO) axis at (A) the beginning of data collection and (B) the end of the season. Budburst at the beginning of the season and is indicated by stars. Resultant lateral shoots from either reproductive buds (bourse shoots) or vegetative buds are indicated by arrows.

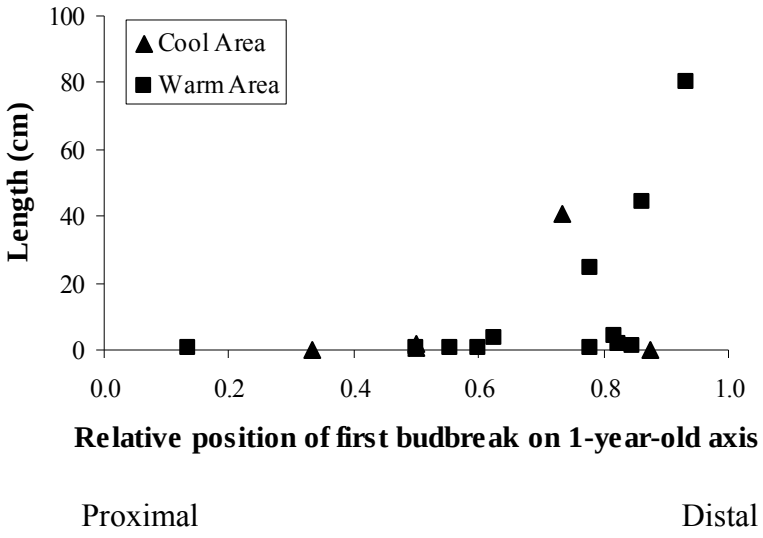


Figure 2. Final length and relative position of the first bud to reach green tip on 'Granny Smith' one-year-old apple shoots with lateral budbreak precedence. Squares represent shoots in the warm area (Warm Bokkeveld) and triangles represent shoots in the cool area (Koue Bokkeveld). Relative position is based on node number and calculated as the node number of the first bud to reach green tip (counting from the proximal end) divided by the total number of nodes on the one-year-old shoot section.

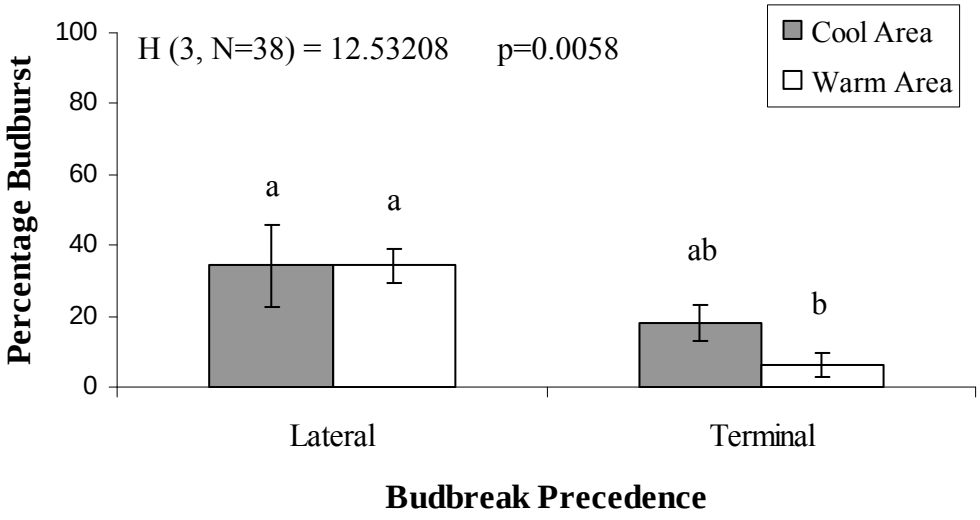
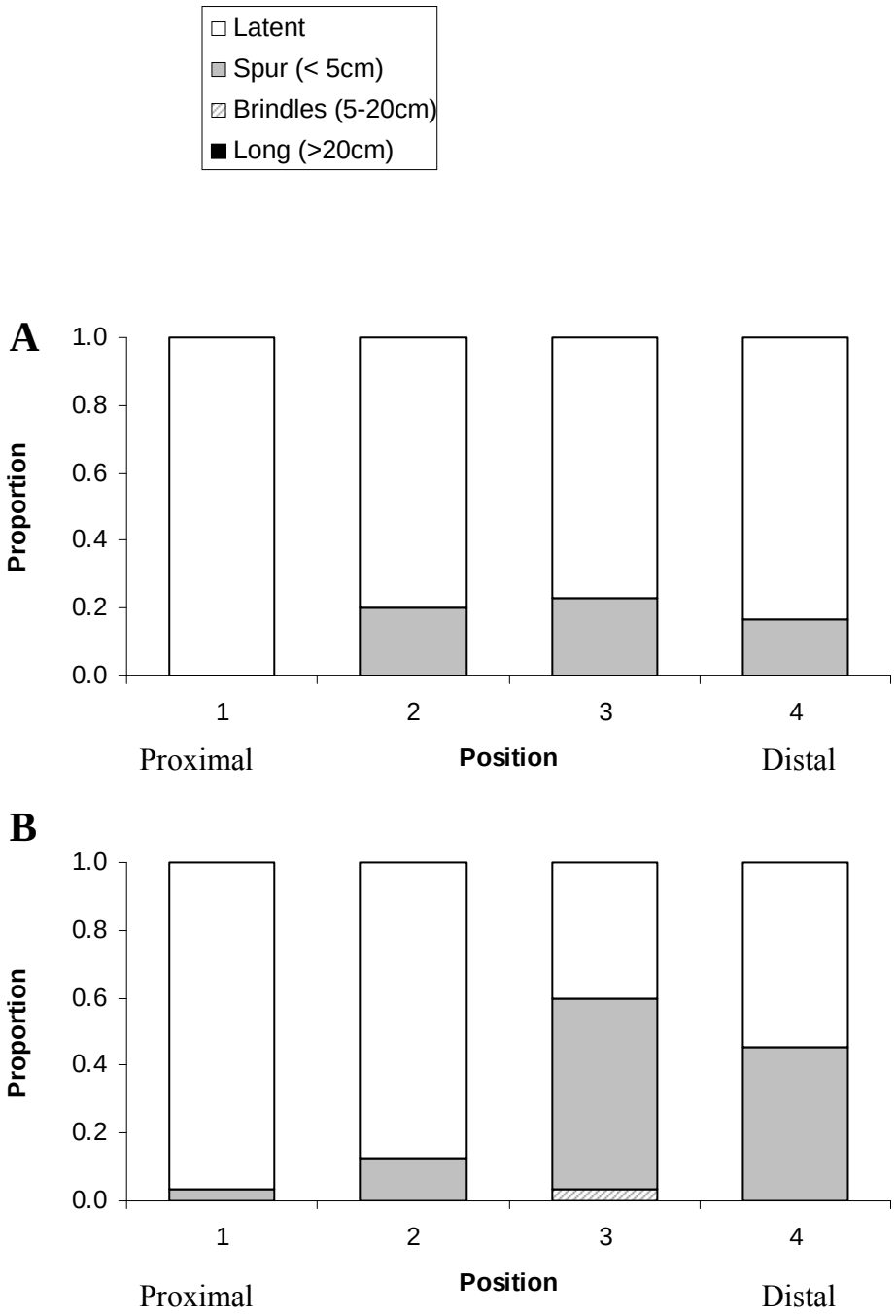


Figure 3. Percentage budbreak along the main axis of the one-year-old shoots of 'Granny Smith' apple trees with either lateral or terminal budbreak precedence in two sites, a cool area (Koue Bokkeveld), and a warm area (Warm Bokkeveld). Percentage budbreak was calculated using the amount of growing nodes (reproductive and vegetative) divided by total nodes along the shoot and multiplied by 100. Letters signify significant differences at α=0.05 using mean separation of the Kruskal-Wallis test between individual site/budbreak precedence combinations.


Latent
Spur (< 5cm)
Brindles (5-20cm)
Long (>20cm)
A
Proportion
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
Proximal
Position
Distal
B
Proportion
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
Proximal
Position
Distal

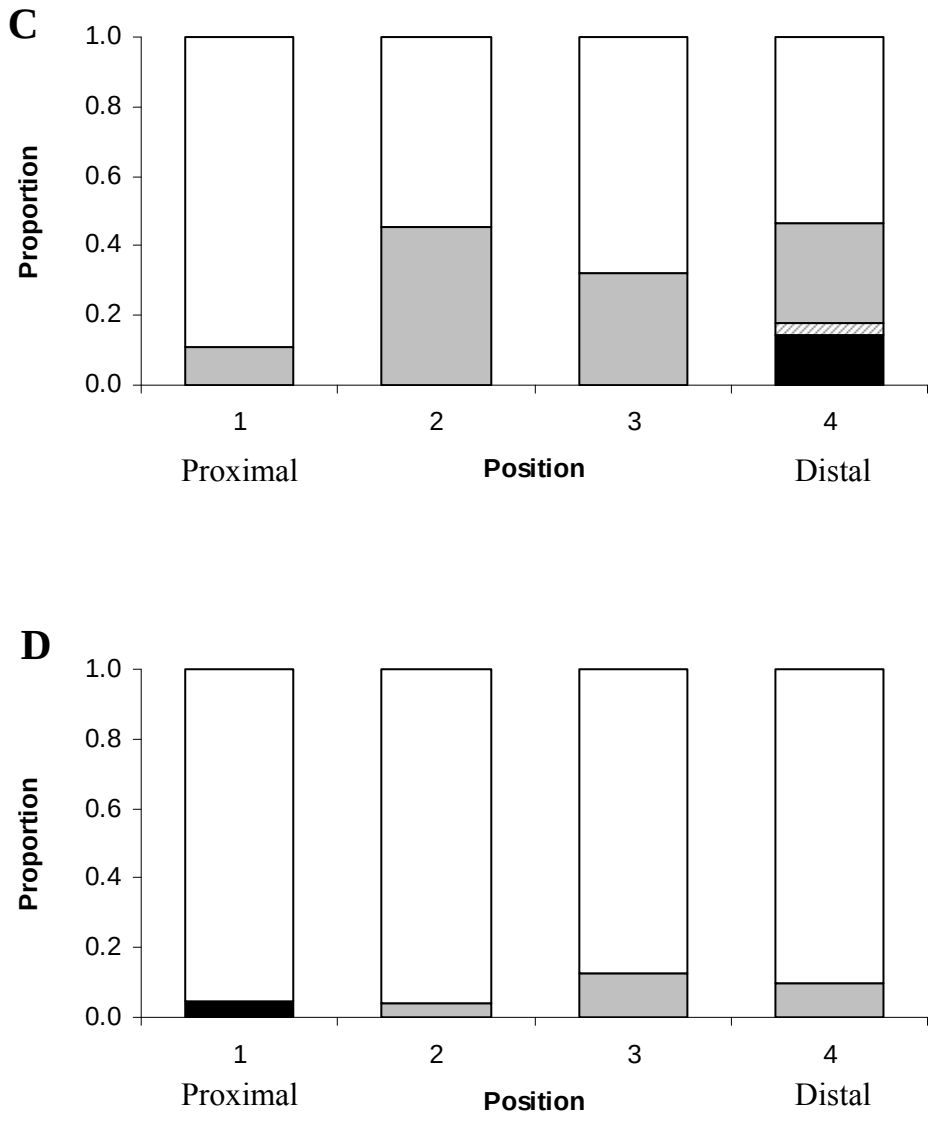


Figure 4. Proportion of laterals in different length classes in the one-year-old axis of 'Granny 'Smith' apple shoots. Relative position (quadrant) within the one-year-old axis (1=proximal and 4=distal) was divided according to equal distribution of node numbers within each quadrant. Treatments were as follows: (A) grown in a cool area (Koue Bokkeveld) with Lateral Budbreak Precedence (BBP); (B) grown in a cool area with Terminal BBP; (C) grown in a warm area (Warm Bokkeveld) with Lateral BBP; and (D) grown in a warm area with terminal BBP. Length classes observed were: latent, or no length; spurs (< 5 cm); brindles (5-20 cm); and long shoots (> 20 cm).

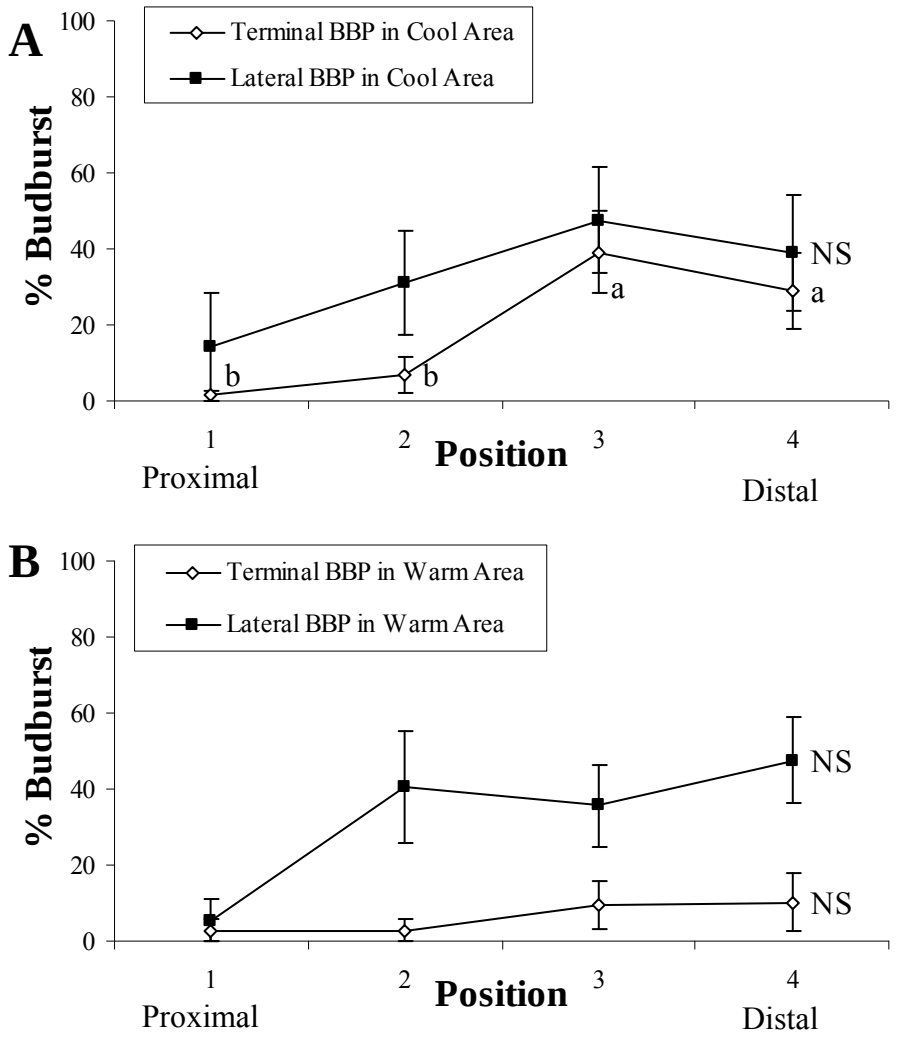


Figure 5. Relationship between percent budburst and relative position (quadrant) along the shoot (1=proximal and 4= distal) according to node number within the one-year-old axis. Percent budbreak was calculated as the number of growing nodes (reproductive and vegetative combined) divided by the total number of nodes within each quadrant of the axes (percent budburst per position). Data were collected from both a (A) cool area and (B) area for 'Granny Smith'. Within each site, shoots were further separated according to budbreak precedence (lateral and terminal). Means and standard errors were calculated for each position within each site/budbreak precedence combination. Letters signify significant differences at α=0.05 using mean separation of the Kruskal-Wallis test (K-W) between relative position within a single site-budbreak precedence combination. NS = nonsignificant.

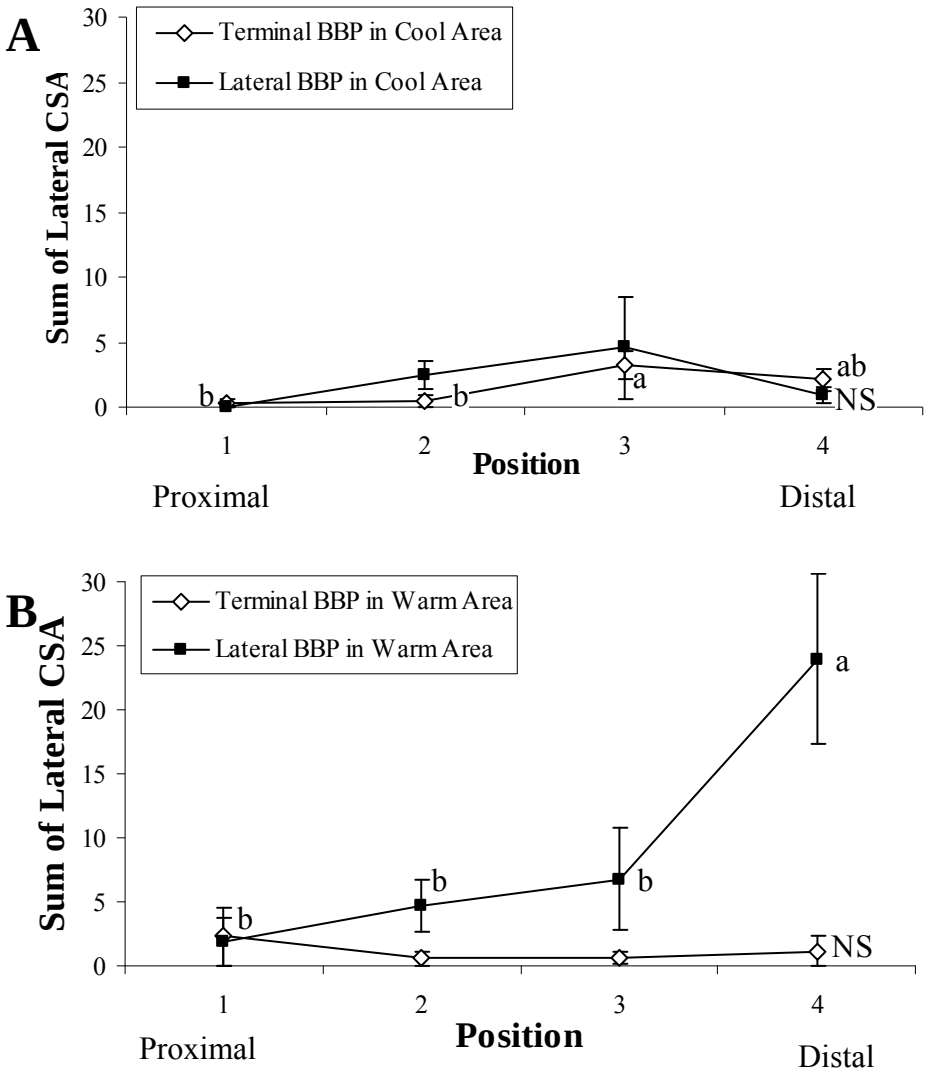


Figure 6. Relationship between the 'Sum of Lateral Cross-Sectional Area (CSA)' and relative position (quadrant) along the shoot (1=proximal and 4= distal) according to node number within the one-year-old axis. The 'Sum of Lateral CSA' was calculated by summing the CSA's of all the lateral shoots within a single relative position within the one-year-old axis. Data were collected from both a (A) cool area and (B) a warm area for 'Granny Smith' shoots. Within each site, shoots were further separated according to budbreak precedence (BBP) (lateral and terminal). Means and standard errors were calculated for each position within each site/budbreak precedence combination. Letters signify significant differences at α=0.05 using mean separation of the Newman-Keuls test (N-K) between relative position within a single site-budbreak precedence combination. NS = nonsignificant.

PAPER 3. Primigenic dominance and the development of acrotony in 'Granny Smith' and 'Golden Delicious' apple (*Malus x domestica* Borkh.) branches grown in areas with inadequate winter chilling

Abstract

This study was conducted to determine the dynamics of apple (*Malus x domestica* Borkh.) branch architecture in two areas with different degrees of winter chilling. Apple branches were grown with adequate (cool area) and inadequate (warm area) winter chilling. In the warm area, apple branches exhibited prolonged dormancy syndrome, which is characterized by a decreased, prolonged and protracted budburst. Acrotony is a major aspect of branch architecture in apple. Even though some aspects of acrotony (i.e., acropetal increase in lateral length and basipetal increase in lateral abortion) were clearly lost in warm areas, results show that some aspects of acrotony (i.e., acropetal increase in number of growing laterals) were maintained. However, it was clear that other aspects of acrotony were lost (i.e., increase in length of laterals moving distally along the axis of the shoot). This loss of acrotonic branching may be more related to factors other than budburst; acrotonic budburst being indirectly related to the development of acrotony.

Introduction

Branching in apple is the result of positional or temporal competitions among meristems within an annual shoot (Bell, 1991). Competitions among meristems can be observed along annual shoots in many ways, such as the differential length, size, and organogenesis of laterals (Champagnat, 1978; Lauri, 2007). Buds can also display a difference in the ability to become active (budburst or flowering) (in reference to acrotony, Champagnat, 1978; Cook *et al.*, 1998; Hallé *et al.*, 1978). Therefore, meristems within an annual shoot may have differences in time of activity, or primigenic dominance (dominance of a structure based on its time of development) (Bangerth, 1989; Bangerth & Ho, 1984; Cook *et al.*, 1998). Another indication of competition between meristems is the differential ability to maintain and/or re-establish growth relative to surrounding laterals on a growth unit or axis (such as the ability to retain fruit after fruit-set (Racskó *et al.*, 2008; Paper 3)) .

Acrotony is a type of dominance that describes morphological relationships among proleptic buds and lateral shoots within a growth unit or axis (Champagnat, 1978). Typically it is defined as "the increase in vigor (length, diameter, number of leaves) of the vegetative proleptic shoots (from dormant buds) from the bottom to the top position of the parent growth unit" and has more recently included in its definition architectural features and bud organogenesis (Lauri, 2007). However, acrotony can be, and has been, defined on different levels.

Some researchers (Champagnat, 1978; Hallé *et al.*, 1978) define acrotony as a greater ability of lateral shoots to occur in the distal position of the axis implying an increase in budburst in this position irrespective of the lengths of the resultant lateral shoots. A recent review of plant architecture (Barthélémy & Caraglio, 2007) supports this idea that acrotony is the increase in number of growing laterals moving distally along the axis of the shoot (further indicating that the increase in length of the lateral shoots should be referred to as acrotonic branching (Barthélémy & Caraglio, 2007) as budburst and outgrowth are regulated differently (Borchert, 2000; Costes & Guédon, 2002; Erez, 2000)). In fact, to many researchers, this greater ability to burst in the distal section of the shoot is, if not the only, at least a part of the idea of acrotony (e.g., as an increase in percentage of growing laterals in the distal position of an axis (Lauri, 2007) or as an increase in the budburst potential of the terminal bud and/or lateral buds in the distal position (Cook *et al.*, 1998)).

Whether defined as acrotony or acrotonic budburst, etc., there is further evidence of positional dominance of the distal part of the shoot. Powell (1995) observed that organogenesis occurs in a basipetal direction along an axis in *Picea* spp. so that there is an

increase in organ number in the buds moving distally along an axis, or an acrotonic organogenic activity. This is also observed in apple as an increase in preformed spur leaf number in the distal part of one-year-old shoots (Costes, 2003; Lauri, 2007), as well as an acropetal increase in the percent of buds that are reproductive (Lauri, 2007).

The phase of acrotony that occurs after budburst (acrotonic branching to Barthélémy and Caraglio (2007) and Cline and Harrington (2007)) exemplifies the positional dominance that distally located laterals have over more proximally located laterals and/or correlative dominance among laterals within the same axis (Cline & Harrington, 2007). This results in the acrotonic habit that is typical of branches on trees such as apple which is also characterized by a basipetal increase in lateral death (lateral abortion) (Lauri, 2007). Lateral abortion is genetically-regulated (i.e., related to the architectural development of branches) (Lauri, 2009) and implicated in the development of acrotony in apple shoots (Lauri, 2007).

While both correlative dominance and positional dominance play roles in the increase in length and size of laterals moving distally along the shoot as indicated when all buds burst at the same time (in reference to acrotony, Cline & Harrington, 2007; in reference to apical control, Wilson, 2000), it is also hypothesized that primigenic dominance plays a role in the development, and therefore loss, of acrotony when budburst is differential along an axis (Cook *et al.*, 1998; Lauri, 2007).

Differences in time of budburst within an axis are often observed in areas with inadequate winter chilling, and are referred to as ‘delayed foliation’ or ‘prolonged dormancy syndrome’ (Black, 1952; Saure, 1985; Strydom *et al.*, 1971). In apple, this prolonged period of budburst influences the acrotonic budburst tendency along the shoots (Cook & Jacobs, 1999; Jacobs *et al.*, 1981). This is due to the fact that lateral buds differentially enter and exit endodormancy. Within a shoot, there is an initial basitonic bursting tendency that becomes acrotonic as dormancy progresses (Champagnat, 1983; Cook *et al.*, 1998; Crabbé & Barnola, 1996; Jacobs *et al.*, 1981). More proximally-located buds have a higher growth rate and are less dormant than more distally-located buds from autumn to mid-winter (Champagnat, 1983; Cook *et al.*, 1998; Crabbé, 1981). This basitonic bursting tendency, specifically in areas where endodormancy is not completed, may result in primigenic dominance of the first bud to be active, and therefore, a loss of acrotonic branching. Although the loss of an acrotonic budburst tendency is well-documented and the symptoms (prolonged and decreased budburst) of ‘delayed foliation’, or ‘prolonged dormancy syndrome’, are evident in areas of inadequate winter chilling, the specific relationship between loss of acrotonic budburst tendency and outgrowth of the laterals is not well-documented.

Since acrotony is understood on different levels, the objective of our study is to characterize the loss of acrotony that occurs in areas with inadequate winter chilling in terms of position, type, and number of lateral buds that burst. Since buds of apple branches subjected to inadequate winter chilling are known to burst and die (by not elongating) (Erez, 2000), another objective is to characterize type and rate of lateral bud abortion. The next objective is to relate the loss of acrotonic branching in these areas to position of budburst or time of budburst in order to determine the involvement of both primigenic and positional dominance in the loss of acrotonic branching tendency. Since architecture of apple trees is dependant on percent budburst and lengths of resulting lateral shoots, this may also be a step in understanding what is variant and invariant in apple branch architecture.

Materials and Methods

Plant Material and Sites – The trial was carried out on two apple cultivars with differing growth habits and architectural features: Golden Delicious on M793, and Granny Smith on seedling rootstock, in the 2006-2007 season. These cultivars have different shoot architectures, types III and IV, respectively, according to the typology of Lespinasse and Delort (1986).

The cultivars were selected in commercial orchards located in areas with both a lower (Warm Bokkeveld; 33º20' S, 19º19' E, 496m, 1119 PCU) and higher (Koue Bokkeveld; 33º12' S, 19º19' E, 1045m, 1698 PCU) chilling unit accumulation historically. According to Hauagge and Cummins (1991), 'Golden Delicious' has a chilling requirement of 1050 ± 15 chill units (CU) and 'Granny Smith has a chilling requirement of 1049 ± 151 CU. In each area, one commercial farm was used. Hereafter the Koue Bokkeveld and Warm Bokkeveld will be referred to as the cool area and warm area, respectively. Meteorological data was collected from the orchard used for the trial or the local area of the orchard. The net chilling units were calculated according to the daily positive chill unit model (PCU; Linsley-Noakes *et al.*, 1994).

In the cool area, 'Golden Delicious' trees were planted in 1997 at a spacing of 4.5 x 2.5 m; and 'Granny Smith' trees were planted in 1933 at a spacing of 5.6 x 5.6 m. In the warm area, the 'Golden Delicious' trees were planted in 1995 and the 'Granny Smith' trees were planted in 1995 at a spacing of 5.0 x 2.5 m. Trees were trained to a central leader. All trees were in full production in 2007.

All trees received standard irrigation and fertilization. No trees received any chemical restbreaking. Trees were not pruned during the trial. The flowers and fruit were neither hand

nor chemically-thinned. Flowers were open pollinated by honey bees and there were no pollination problems during the time of this trial.

Branch measurements **–** For all cultivar/area combinations, 20 branches were randomly selected in June 2006 at shoulder height from 10-20 trees within one row. Selected branches started growing in September (spring) 2004, and therefore were comprised of a two-year-old axis, one-year-old axis and a terminal bud (Fig 1). Since acrotony is evident within a single growth unit (Lauri, 2007), selection was based on the two-year-old axis of branches having only one growth unit. The branches were oriented approximately horizontally when selected.

On each annual shoot (hereafter referred to as axis), laterals were considered as either growing when the axis was 1YO or 2YO (laterals being Y1 and Y2, respectively) (Figure 1). Y1 laterals subtend Y2 laterals on 2YO axes (Fig 1).

Total number of nodes was counted per axis. Nodes were numbered along both the one- and two-year-old (1YO and 2YO, respectively) axes of the selected branches from the proximal to distal end. Each annual axis was partitioned into four equal quadrants based on node number, with quadrant 1 being the most proximal and quadrant 4 being the most distal.

Starting in early September 2006, the branches were monitored every day. Date of budburst and type of bud were recorded for each node along the axis. Node types were considered to either be growing (G) or non-growing (Lauri *et al.*, 1995). Non-growing types are either dormant or latent (L) or dead, i.e., scars (S) and growing lateral types are reproductive (R) and vegetative (V).

One of the objectives was to characterize the differences (e.g., length, lateral abortion) between the first, second, third and fourth buds to burst along an axis. Since the date of the first bud within an axis to burst was not the same across all axes, the budburst within each axis was considered sequentially. The first bud to burst along a single axis was first in the sequence of budburst (sequence 1), the second bud to burst was sequence 2, and so forth. If two buds along the same axis burst on the same day, then they were both considered to have the same sequence number.

After harvest, diameters (D) at the proximal end (approximately 2 cm from the ring scars) and lengths of the 1YO and 2YO axes and terminal shoot were measured. In the case of reproductive terminal and lateral shoots, the length and diameters were considered to be those of the longest bourse shoot. Lateral shoot lengths were either used in analyses as actual lengths or put in length categories with accepted physiological differences (Costes *et al.*,

2006; Lespinasse & Delort, 1993): spurs (<5 cm); brindles (≥5 and <20 cm); and long shoots (≥20 cm).

Axis slenderness and conicity (degree of conical shape) are related to the architectural development of apple branches (Lauri *et al.*, 1997) and were calculated for the 1YO and 2YO axes. Shoot slenderness was calculated as: main axis length divided by the average of the proximal and distal diameters of the main axis. Conicity was calculated as: (distal diameter of the main axis minus proximal diameter of the main axis) divided by the main axis length.

For each axis, two calculations concerning proportion of lateral types were calculated: (a) percent growing laterals, and (b) percent reproductive within growing laterals. Percent growing laterals was calculated as the number of growing laterals (reproductive and vegetative combined) divided by the total number of nodes (including latent); percent reproductive within growing laterals was calculated as the number of reproductive laterals divided by the total number of growing (reproductive and vegetative) laterals. Both were calculated for the axis as a whole, as well as for quadrant (1-4) within the axes. Reproductive within growing was additionally calculated for each budburst sequence.

Lateral abortion, or transition from a growing bud in Y_n to a scar in Y_{n+1} was calculated for both position and budburst sequence for each cultivar-area-axis age combination using the following formula of (Lauri, 2007): $[(G_{Y1} \rightarrow S_{Y2})/ G_{Y1}$; where G = growing laterals and S = scars]. The type of bud (vegetative or reproductive) that aborted was also recorded and reported as proportion of lateral abortion due to vegetative (V) lateral abortion $[(V_{Y1} \rightarrow S_{Y2})/(G_{Y1} \rightarrow S_{Y2})$; where G = growing laterals and S = scars]. Since lateral abortion occurs at the end of the season (Lauri & Térouanne, 1995) (i.e., via terminal death of vegetative buds or non-production of bourse shoots on reproductive buds), lateral abortion was calculated for $(Y_1 \rightarrow Y_2)$ on the 1YO axes and for $(Y_2 \rightarrow Y_3)$ on the 2YO axes.

Data Analysis – Discrete and continuous data (length, base diameter, etc.) were analyzed parametrically using the Fisher *F* test. Newman-Keuls multiple mean comparison test was used to test differences at α=0.05 between sites within a specific cultivar-axis age combination.

In order to determine the influence of position and relative time of budburst on type of bud and architectural characteristics of the laterals, percent reproductive within growing laterals and lateral abortion were analyzed by both quadrant and sequence number. Percent growing laterals was analyzed per position (quadrant number). Axes were pooled, when necessary, to increase the minimum number of nodes to a minimum of five. Only relative

frequencies with a minimum value of 5 were considered in these analyses. Proportions of laterals and lateral abortion were then analyzed as non-parametric data with the Kruskal-Wallis H test, using the β-approximation when necessary. Multiple means comparison test was used to test differences between either quadrants or sequence of budburst (Kruskal and Wallis 1952). Differences at $\alpha=0.05$ were considered significantly different.

All statistical analyses were done using Statsoft (2008) with the exception of the p-values of the β approximation for the non-parametric data, which was calculated by hand using the tables in Kruskal-Wallis (1952).

Results

Axis characteristics – Within each cultivar, the mean axis length did not differ between the cool and warm area with the exception of the one-year-old (1YO) axis of 'Granny Smith', in which the axes of trees cultivated in the cool area had a slightly significant shorter length (10.1 cm) than the warm area (18.0 cm) (Table 1). Diameter and slenderness differed between areas for the 1YO and 2YO axes of 'Golden Delicious', with the warm area having more slender axes with a smaller base diameter. In addition, the terminal shoot of 'Granny Smith' was also more slender in the warm area. The only axis that differed in conicity was the 1YO axis of 'Golden Delicious' which more cone-shaped in the cool area.

The shorter length of the 2YO axis of 'Granny Smith' in the cool area coincided with both a greater number of nodes (21.1 vs. 16.7) and growing laterals (8.7 vs. 5.8), and a lower relative frequency of reproductive laterals among growing ones (0.39 vs. 0.75) of the 2YO axis (Table 2). Number of growing laterals was also greater in the cool area for the 1YO axis of 'Golden Delicious', even though the frequency of reproductive laterals did not differ.

The mean total number of budburst sequences observed per axis ranged from 2.5 ± 1.4 on the 1YO axis of 'Granny Smith' in the cool area to 5.4 ± 1.6 on the 2YO axis of 'Golden Delicious' in the cool area (Table 3). There were no apparent differences between areas.

Budbreak Pattern – Budburst sequence one (first bud to burst on the axis) never exceeded 50% for any of the four quadrants on any cultivar, site, or axis age (Tables 4 and 5). On both axes however, the majority of buds broke in the distal half of the axis (quadrants 3 and 4 combined).

In all axes that had sufficient reproductive buds, reproductive budburst took temporal precedence over vegetative budburst in both 'Golden Delicious' and 'Granny Smith' except for the 1YO axes of 'Granny Smith' in the cool area and the 1YO axes of 'Golden Delicious'

in the warm area (Fig 2 A and B). 2YO axes of 'Golden Delicious' in the cool area had a distinct reproductive budburst period (majority from 9/7 to 9/17 with a few reproductive buds bursting later) (Fig 3B). In the warm area, reproductive bud burst is more protracted with the majority of reproductive buds bursting from 9/7 to 9/27 (10 days longer than in cool area). The beginning of vegetative budburst on the 2YO axes of 'Golden Delicious' coincides with the latter part of reproductive budburst in both areas (9/14 in the cool area and, because reproductive budburst is protracted in the warm area, starting 9/24 in warm area). Vegetative budburst is more protracted in the warm area also, as evidenced by the slower and more gradual increase in cumulative vegetative budburst as compared to the steeper slope in the cool area (Fig 3 C and D)).

Reproductive budburst on the 2YO axes of 'Granny Smith' in the cool area occurs from 9/2 to 9/10. While budburst begins at the same time in the warm area, it lasts until 9/20 (Fig 3 F). Vegetative budburst on the 2YO axes of 'Granny Smith' in the cool area occurs mainly during the time of reproductive budburst (9/2 – 9/10), with a later vegetative budburst period just after reproductive budburst (9/11 – 9/27) and a few buds bursting around 10/11. In the warm area, there are only a few vegetative buds (25% of the growing laterals (Table 2)), and they burst after the reproductive buds and over a long period of time (9/23 – 10/23) (Fig 3 H).

All of the 1YO axes had a high percentage of vegetative buds, with the exception of 'Granny Smith' in the warm area, which had few growing buds overall (Table 2), and a high percentage of reproductive buds, which had a distinct reproductive budburst period (9/5 to 9/9) (Fig 3 C). The vegetative budburst period was mainly from 9/7 to 10/3. 1YO axes of 'Granny Smith' in the cool area had a low reproductive budburst, and vegetative budburst period mainly from 9/7 to 9/24 (Fig 3 E). On 1YO axes of 'Golden Delicious', the duration of budburst did not differ between the warm (9/23 – 10/12) and cool (9/29 – 10-19) areas, but in the warm area is began one week later (Fig 3 A).

In 'Golden Delicious' cultivated in both the warm and cool areas, there is a distinct difference in time between beginning of budbreak of the 2YO axis and that of the 1YO axis. The 1YO axes burst approximately two and three weeks later than the 2YO axes in the cool and warm areas, respectively (Fig 3 A – D))). In 'Granny Smith' in both areas, budburst on the 1YO axis began only a few days after that on the 2YO axis (Fig 3 E – H).

Temporal and positional aspects of budbreak and lateral outgrowth – In 'Golden Delicious', the percentage of growing laterals increased from quadrants 1 to 3 and then was decreased in

quadrant 4 in both areas and both axis ages (Fig 4 A). This trend was also evident in 1YO and 2YO axes of 'Granny Smith' in the cool area. In the warm area, 'Granny Smith' had a higher proportion of growing laterals in quadrants two through four than in quadrant one, although quadrants two through four did not differ from each other in the 2YO axis and four was greater than two and three in the 1YO axis (Fig 4 B).

Number of growing buds was insufficient to determine percent reproductive in quadrant 1 of 1YO axes of 'Granny Smith' in both areas, as well as all 'Golden Delicious' axes with the exception of 2YO axes in the warm area. One-year-old axes had a low relative frequency of reproductive buds for all quadrants, with the exception of 'Granny Smith' one-year-old axes in the warm area which had a higher frequency of reproductive buds in quadrants three and four while quadrant two was relatively low (Fig 5 B). Two-year-old axes of 'Golden Delicious' had a high relative frequency of reproductive buds in quadrants two through four in both areas (always greater than 50%). 2YO axes of 'Granny Smith' had a high frequency of reproductive buds in quadrant one in both areas (~80%). In the warm area, quadrants two through four maintained the relative frequency of reproductive buds at approximately 75%, and in the cool area, quadrants two through four had a relative frequency of reproductive buds of approximately 50% (Fig 5 B).

Spurs were the dominant length classes observed in all quadrants of all axes with sufficient growing laterals (Fig 6 A – H) (quadrant one having few growing laterals overall (Fig 4 A and B)). In 'Golden Delicious' in the cool area, brindles and long shoots were located in the distal two quadrants on both 1YO and 2YO axes as well as quadrant 2 of the 2YO axis (Fig 6 A and B). In the warm area, brindles and long shoots were evident in all quadrants of 2YO axes, and although no long shoots were observed along 1YO axes of 'Golden Delicious' in the warm area, brindles were observed in the two distal quadrants (Fig 6 C and D). 'Granny Smith' in the cool area had some long shoots in quadrant 3 and only a few brindles along 1YO axes (quadrants 3 and 4); both brindles and long shoots were observed in quadrants 2 and 4 of 2YO axes, although in a lower percentage in quadrant 2 (Fig 6 E and F). In the warm area, 'Granny Smith' 1YO axes had a few long shoots in quadrants 1, 3, and 4, and a few brindles in quadrant 4. 2YO axes had mostly spurs with very few brindles observed in quadrants 2 through 4, and long shoots in quadrants 2 and 4 (Fig 6 G and H).

Long shoot production occurred only during the first two budburst sequences in all axes that produced long shoots (Fig 7 A - H). In 'Golden Delicious' 2YO axes in both areas, brindles were produced regardless of what sequence the bud broke along the axis relative to the other buds, although they were the preferential lateral length when they burst first (Fig 7 B

and D). In 1YO axes, there was no clear trend, although the highest percentage of brindles occurred when the bud burst second in the cool area, and when the bud broke first in the warm area (Fig A and C). 'Granny Smith' grown in the cool area could produce brindles in 2YO axes regardless of the budburst sequence of the individual bud indicating a positional dominance in the cool area since brindles occurred primarily in the most distal quadrant (Fig 6 F). In 1YO axes of 'Granny Smith' in the cool area, there were only a few sequences, and very few brindles or long shoots, both of which were produced in the first two budburst sequences (Fig 7 E). In the warm area, no brindles were produced on 1YO axes, only long shoots in the first two sequences and then only spurs. Brindles were produced in sequences 1, 2, and 4 on 2YO axes of 'Granny Smith' in the warm area (Fig 7 G).

Lateral abortion – Overall proportion of laterals aborting in 'Granny Smith' ranged from 0.03 for the $Y_2 \rightarrow Y_3$ transition in the cool area to 0.13 for the $Y_2 \rightarrow Y_3$ transition in the warm area. 'Golden Delicious' lateral abortion ranged from 0.06 for the $Y_1 \rightarrow Y_2$ transition in the warm area to 0.12 for the $Y_2 \rightarrow Y_3$ transition in the cool area (Table 6).

Growing buds in quadrant one were insufficient to determine lateral abortion. Lateral abortion was low and equal for $Y_1 \rightarrow Y_2$ transition across quadrants two through four in 'Golden Delicious' in both areas and for all 'Granny Smith' axes in the cool area (Fig 8 A and B). $Y_2 \rightarrow Y_3$ lateral abortion transition decreased moving distally along the axes of 'Golden Delicious', although not significantly in the cool area. In 'Granny Smith' grown in the warm area, lateral abortion ($Y_1 \rightarrow Y_2$ transition) only occurred in quadrant 4 and was higher in quadrant 3 for the $Y_2 \rightarrow Y_3$ transition with the than either quadrant 2 or 4 (Fig 8 B).

In both the $Y_1 \rightarrow Y_2$ and $Y_2 \rightarrow Y_3$ transitions of 'Golden Delicious' and 'Granny Smith' in the cool area, lateral abortion did not significantly differ according to either sequence of budburst (Fig 9 A and B) or the type (vegetative or reproductive) of lateral that aborted (vegetative in all cases except for budburst sequences 1 and 2 of 'Golden Delicious' for the $Y_1 \rightarrow Y_2$ and $Y_2 \rightarrow Y_3$ transitions, respectively (Table 7). In the warm area, the fifth group of buds in the budburst sequence has a higher probability of aborting for both axes of 'Golden Delicious' (Fig 9 A), and there was an increasing tendency for lateral abortion to be due to vegetative buds aborting with an increase in budburst sequence number (Table 7). Lateral abortion of 'Granny Smith' buds did not differ according to budburst sequence for 1YO and 2YO axes in the cool area or 1YO axes in the warm area but is considerably increased in sequences 3 and 4 for 2YO axes in the warm area (Fig 9 B). This lateral abortion

was entirely due to reproductive buds that aborted in budburst sequences 2 and 3 and due to reproductive buds 67% of the time in sequence four (Table 7).

Discussion

Budburst Patterns

In the warm area in our study, both the reproductive and vegetative budburst was prolonged (Fig 3 C, D, G, and H), indicative of 'prolonged dormancy syndrome', or 'delayed foliation'. This was not measured previously, although previously observed, with the exception of the low percentage of budburst known to occur in apple grown in warm areas (Petri & Leite, 2004). Cook and Jacobs (1999) attributed this to the low growth potential of lateral buds in areas with inadequate winter chilling.

There was a distinct vegetative budburst pattern and a distinct reproductive budburst pattern. In our study, the reproductive budburst phase preceded the vegetative budburst. This supports the findings of Naor et al. (2003) in which reproductive buds had a lower chilling requirement than vegetative. There was disjunction in time between reproductive and vegetative budburst phases and this was increased in the warm area (Fig 3 C, D, G, and H) further supporting the idea that vegetative buds have a higher chilling requirement. In addition, both the reproductive and vegetative budburst phases were protracted and prolonged in the warm areas relative to the cool areas; and vegetative budburst was less protracted in the one-year-old axis than the two-year-old axis, as indicated by the steeper slope of cumulative budburst in one-year-old axes as compared to the two-year-old axes.

Temporal and Positional Aspects of Acrotony

Growing laterals - It is understood that there is an inherent, genetic basis for branch architecture, and that there is also an inherent way of altering branch development when environmental conditions are not ideal (Hallé *et al.*, 1978). Acrotony, by any of the definitions, is a characteristic of branching in apple. Part of the idea of researching the dynamics of budburst in reference to branch development, specifically in areas of inadequate winter chilling, was to help determine what characteristics actually define acrotony in apple, how these characteristics occur (i.e., is there involvement of primigenic/temporal dominance, and/or only positional), and subsequently begin to understand what is invariant in branch architecture and what varies with environment (in this case, in terms of inadequate accumulation of chilling).

Even though ‘delayed foliation’ was clearly evident as protracted budburst in the warm areas as compared to the cool areas, and regardless of the whether the first bursting bud was in the distal section or not, the acrotonic gradient of number of growing laterals (% growing) was maintained from quadrants 1 to 3 and the exception being a lower percent of growing laterals in quadrant 4 as compared to quadrant 3 in most cases (Fig 4 A and B). The difference between the areas was evident in the actual percent growing in quadrants 3 and 4 (higher in the cool area) and not in the percent growing in each quadrant as compared to the others. Therefore, by the definition of Barthélémy and Caraglio (2007) and Hallé et al. (1978) (i.e., acropetal increase in percent growing laterals), acrotony was maintained in both areas of our study.

Acrotonic budburst potential (i.e., greater percentage of the first bud to burst in the most distal quadrant (Tables 4 and 5)) was not completely clear. There was a relatively high tendency for buds in the distal half of the axis (quadrants 3 and 4 combined) as well as in the distal quadrant of the axis to burst first, and this was more evident in the warm area in 1YO axes (Tables 4 and 5). This may be related to the lack of paradormancy of the terminal over the distal-most laterals, as hypothesized in Paper 2 of this dissertation. Cook et al. (1998) observed that distal lateral buds have a higher potential to burst than the more proximally-located lateral buds in the later parts of dormancy, and a higher potential to burst than the terminal just before the terminal bud rapidly exits dormancy. This agrees with our results as the distal-most lateral buds have a higher tendency to burst than the more proximally-located lateral buds. In addition, it was shown in Paper 2 of this dissertation that in the warm area as compared the cool area, the laterals have an increased ability to burst before the terminal. This may explain why, in the two-year-old axes, there was very little difference in the percent of buds that burst first in the distal half of the axis between the warm and cool areas, while in the one-year-old axes there was a clear difference in percent of buds breaking first in the distal half of the axes between the warm and cool areas (warm area having a greater percent of buds bursting in the distal half first). Overall, this means that not only is primigenic dominance of the distal-most buds not required for the acropetal increase in number of growing buds from quadrant 1 to 3 (decrease in quadrant 4), but that the bursting of buds in the distal half of the axis first may either negatively impact this gradient by decreasing the number of growing buds or at least be an early indicator of decreased budburst along the axis. Since the terminal bud has a decreased budburst potential as compared to the distal-most lateral buds before its rapid exit from dormancy (Cook *et al.*, 1998), this earlier budburst of

the distal-most laterals may be an attempt of these lateral buds to replace the main axis in the event that the still partially-dormant terminal does not regain it ability to burst.

The one aspect of acrotony that was altered in warm areas was the position of long shoots and brindles in lateral positions. The increase in length of laterals moving distally along the axis (acrotonic branching) was maintained in the cool area. Primigenic dominance was implicated in the development of long shoots as the long shoots were produced only in the first one or two budburst sequences for any of the treatments. Brindle length shoots on the other hand were produced throughout the sequences, and with the exception of a strong production in the first budburst sequence of two-year-old 'Golden Delicious' axes, in the first budburst sequence of one-year-old axes in the warm area, and the second budburst sequence on one-year-old axes in the cool area (Fig 7 B and D), almost appeared to be produced at random times. This is an indication that acrotonic branching is related to amount of chilling and not to primigenic dominance of the distal laterals since brindle length shoots were produced in almost all budburst sequences and yet were only located in the distal half of the axes for both 'Golden Delicious' and 'Granny Smith' in the cool area. In the warm area, 'Golden Delicious' produced brindle length lateral shoots in many of the budburst sequences and they were spread through out the axis, indicating a loss of acrotonic branching. In 'Granny Smith' grown in the warm area, there were very few overall brindle length lateral shoots and there was also not an acrotonic branching tendency as they were spread more equally along the axis. For 'Granny Smith' in the warm area there was low overall budburst and a relatively high proportion of long shoots which may be a result of low competition in time of budburst. These long shoots may represent reiterations (Hallé *et al.*, 1978; Lauri *et al.*, 2009), and reiterations may be a result of low competition at the time of budburst.

Lateral abortion - Lateral abortion is considered to be a major component of branch architecture (i.e., elimination of growing points) and linked to autonomy of the remaining and neighboring laterals (Lauri, 2009). Previous studies have shown that lateral abortion may occur in growing laterals up to 50% of the time in 'Granny Smith' and 18% of the time in 'Golden Delicious' (Lauri *et al.*, 1995), so overall lateral abortion was relatively low in our study. Abortion of a lateral may either create an acrotonic tendency that is not produced by growing laterals (in the case of a increase in lateral abortion moving proximally on the axis of the shoot (Lauri, 2007)) or may decrease the acrotonic tendency by aborting laterals in the more distal sections of the axis. In our study, the two-year-old axes of 'Golden Delicious' had this trend of increasing lateral abortion moving proximally along the axis, which is

consistent with establishing an acrotonic number of growing laterals (Lauri, 2007). In previous studies, the majority of lateral abortion was due to non-production of bourse shoots on reproductive laterals (viz. 97% in 'Golden Delicious' and 99% in 'Granny Smith') (Lauri *et al.*, 1995). In our study, lateral abortion in 'Golden Delicious' was most due to lateral abortion of reproductive buds in the early sequences in the warm area (Y2-Y3 transition) but may be increasingly due to vegetative buds as budburst sequence number increases. This increase in lateral abortion with the later sequences in the warm area is almost entirely, if not completely, due to vegetative lateral abortion. In 'Granny Smith', the majority of lateral abortion occurs in quadrant 3 (as compared to quadrants 2 and 4) in the warm area (Y2-Y3 transition) (Fig 8 B), implying a loss of acrotony. Lateral abortion in 'Granny Smith' was linked to sequence number (Y2-Y3 transition) in the warm area; and the type of lateral aborting for the Y2-Y3 transition also differed by sequence number in the warm area (50% being vegetative for budburst sequence 1, and 100% reproductive buds aborting for sequences 2 and 3) (Table 7). This means abortion of laterals in warm areas differed from the way that laterals abort in cool areas; specifically in Granny Smith in which there was a higher percentage of reproductive buds aborting (Y2-Y3 transition; 2YO axes) in the warm area as compared to the cold area (Table 7).

Conclusion

Apple axes in our study had an inherent ability to maintain the acropetal increase in number of growing laterals with the exception of quadrant 4, indicative of acrotony for many researchers, regardless of cultivar or area. Other characteristics associated with acrotony [basipetal increase in lateral abortion (with the exception of Y2-Y3 transition in 'Golden Delicious' in the warm area), acropetal increase in length of growing laterals], however, were not maintained in the warm area.

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Table 1. Mean (± SE) for length, base diameter, slenderness and conicity of the two-year-old (2YO), and one-year-old (1YO) axes, as well as the terminal, of 'Golden Delicious' and 'Granny Smith' apple branches grown in two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld). Within each cultivar and axis age an analysis of variance was performed to determine difference between sites. The p-values for Fisher F-test are shown in italics.

	'Golden Delicious'			**'Granny Smith'**		
	Cool Area	**Warm Area**	*p*	**Cool Area**	**Warm Area**	*p*
Length						
2YO	31.2 ± 1.8	29.3 ± 1.7	*0.4391*	34.5 ± 3.5	27.1 ± 2.0	*0.0712*
1YO	22.2 ± 3.1	25.8 ± 1.6	*0.2863*	10.1 ± 2.0	18.0 ± 2.4	*0.0151*
Terminal	17.3 ± 2.8	17.7 ± 2.2	*0.8887*	18.6 ± 3.4	22.0 ± 3.2	*0.4776*
Diameter						
2YO	8.9 ± 0.5	7.0 ± 0.3	*0.0040*	8.9 ± 0.5	7.8 ± 0.5	*0.1046*
1YO	5.8 ± 0.5	4.3 ± 0.2	*0.0062*	5.5 ± 0.3	5.3 ± 0.4	*0.8211*
Terminal	4.0 ± 0.3	3.5 ± 0.2	*0.1055*	4.9 ± 0.3	4.3 ± 0.4	*0.2139*
Slenderness						
2YO	3.5 ± 0.2	4.3 ± 0.2	*0.0156*	3.6 ± 0.2	3.7 ± 0.2	*0.8184*
1YO	4.3 ± 0.2	6.4 ± 0.3	$<10^{-7}$	3.8 ± 0.3	4.3 ± 0.3	*0.3072*
Terminal	6.3 ± 0.3	6.9 ± 0.3	*0.1372*	4.9 ± 0.3	6.4 ± 0.4	*0.0175*
Conicity						
2YO	-0.0085 ± 0.0006	-0.0073 ± 0.00005	*0.1135*	-0.0077 ± 0.0005	-0.0071 ± 0.0007	*0.4702*
1YO	-0.0072 ± 0.0005 a	-0.0048 ± 0.0004 b	*0.0003*	-0.0053 ± 0.0015	-0.0043 ± 0.0017	*0.6706*
Terminal	-0.0089 ± 0.0012	-0.0076 ± 0.0007	*0.3160*	-0.0054 ± 0.0012	-0.0046 ± 0.0019	*0.7507*

Table 2. Mean (± SE) for number of nodes, number of growing laterals, and relative frequency of reproductive laterals within growing laterals of the two-year-old (2YO) and one-year-old (1YO) axes of 'Golden Delicious' and 'Granny Smith' apple branches grown in two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld). Within each cultivar and axis age a non-parametric Kruskal-Wallis and Fisher F-tests were performed for non-parametric and parametric data, respectively. The p-values for these tests are shown in italics.

	'Golden Delicious'			**'Granny Smith'**		
	Cool Area	**Warm Area**	*p*	**Cool Area**	**Warm Area**	*p*
Node Number						
2YO	16.9 ± 0.9	15.5 ± 0.6	*0.2232*	21.1 ± 1.2	16.7 ± 0.7	*0.0028*
1YO	14.6 ± 1.6	13.0 ± 0.5	*0.3000*	10.3 ± 0.9	12.8 ± 1.2	*0.0969*
Number of Growing Laterals						
2YO	8.3 ± 0.5	7.3 ± 0.5	*0.1646*	8.7 ± 0.8	5.8 ± 0.6	*0.0062*
1YO	7.2 ± 1.0	4.7 ± 0.5	*0.0264*	3.1 ± 0.7	3.3 ± 0.7	*0.8332*
Proportion of Reproductive Laterals						
2YO	0.58 ± 0.05	0.66 ± 0.08	*0.1801*	0.39 ± 0.08	0.75 ± 0.09	*0.0106*
1YO	0.08 ± 0.03	0.08 ± 0.06	*0.4932*	0.19 ± 0.19	0.18 ± 0.18	*1.0000*

Table 3. Means (± standard deviations) for the total number of budburst sequences observed on one-year-old (1YO), and two-year-old (2YO) axes of 'Golden Delicious' and 'Granny Smith' apple branches grown in two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld).

Budburst sequences		**Site**	
Cultivar	**Axis Age**	**Cool Area**	**Warm Area**
'Golden Delicious'	2YO	5.4 ± 1.6	4.9 ± 1.5
	1YO	4.9 ± 1.7	3.9 ± 1.7
'Granny Smith'	2YO	4.3 ± 1.8	3.4 ± 1.1
	1YO	2.5 ± 1.4	2.6 ± 1.9

Table 4. Percentage of buds from each budburst sequence (1-5) that occurred in a particular position (quadrants 1-4) along the two-year-old axes of 'Golden Delicious' and 'Granny Smith' apple cultivars grown at two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld).

Cultivar	Site	Budburst Sequence	Position → 1 (Proximal)	2	3	4 (Distal)
'Golden Delicious'	Cool Area	**1**	0%	21%	46%	33%
		2	0%	18%	41%	41%
		3	4%	7%	48%	41%
		4	0%	13%	67%	20%
		5	6%	29%	12%	53%
	Warm Area	**1**	0%	20%	36%	44%
		2	4%	24%	44%	28%
		3	4%	39%	26%	30%
		4	6%	29%	41%	24%
		5	8%	42%	25%	25%
'Granny Smith'	Cool Area	**1**	6%	19%	35%	39%
		2	0%	13%	42%	46%
		3	10%	38%	19%	33%
		4	0%	25%	50%	25%
		5	0%	36%	36%	27%
	Warm Area	**1**	4%	15%	31%	50%
		2	7%	41%	34%	17%
		3	0%	26%	32%	42%
		4	0%	36%	18%	45%
		5	0%	25%	25%	50%

Table 5. Percentage of buds from each budburst sequence (1-5) that occurred in a particular position (quadrants 1-4) along the one-year-old axes of 'Golden Delicious' and 'Granny Smith' apple cultivars grown at two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld).

Cultivar	Site	Budburst Sequence	Position → 1 (Proximal)	2	3	4 (Distal)
'Golden Delicious'	Cool Area	**1**	0%	33%	38%	29%
		2	0%	22%	48%	30%
		3	0%	26%	58%	16%
		4	6%	31%	44%	19%
		5	0%	58%	33%	8%
	Warm Area	**1**	0%	14%	43%	43%
		2	0%	14%	52%	33%
		3	0%	16%	74%	11%
		4	0%	36%	36%	29%
		5	0%	75%	13%	13%
'Granny Smith'	Cool Area	**1**	4%	26%	43%	26%
		2	6%	11%	61%	22%
		3	0%	14%	57%	29%
		4	0%	0%	33%	67%
		5	0%	67%	0%	33%
	Warm Area	**1**	4%	11%	37%	48%
		2	10%	35%	35%	20%
		3	0%	67%	0%	33%
		4	17%	67%	17%	0%
		5	0%	0%	33%	67%

Table 6. Proportion of lateral buds aborting along one-year-old (transition from Y1 → Y2) and two-year-old (transition from Y2 to Y3) axes of two *Malus x domestica* cultivars, 'Golden Delicious' and 'Granny Smith', on two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld). Lateral abortion was considered as a transition from a growing lateral in one year to a scar in the following year. Proportion of lateral abortion due to vegetative buds aborting (relative amount of vegetative lateral abortion) was also recorded for these axes.

	'Golden Delicious'		**'Granny Smith'**	
Lateral Abortion	**Cool Area**	**Warm Area**	**Cool Area**	**Warm Area**
Y1 → Y2	0.08	0.06	0.06	0.04
Y2 → Y3	0.08	0.12	0.03	0.13
Relative amount of vegetative lateral abortion				
Y1 → Y2	0.90	0.83	1.00	1.00
Y2 → Y3	0.64	0.44	1.00	0.23

Table 7. Percentage of lateral abortion due to vegetative buds aborting. Lateral abortion was determined for position (quadrants 1-4) and budbreak sequence (1-5 or 1-3, depending on number of sequences observed). Data was collected from one- (1YO) and two- (2YO) year-old axes of two *Malus x domestica* cultivars, Golden Delicious and Granny Smith, grown at two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld). Dashes indicated that no lateral abortion occurred at these positions or sequences.

Vegetative Lateral Abortion (rel. freq.)

	'Golden Delicious'				**'Granny Smith'**			
	Cool Area		**Warm Area**		**Cool Area**		**Warm Area**	
	1YO	**2YO**	**1YO**	**2YO**	**1YO**	**2YO**	**1YO**	**2YO**
Position								
1								
2	50	100	100	50	-	100	-	33
3	100	67	75	29	100	100	-	14
4	100	0	100	100	100	100	100	50
Budbreak Sequence								
1	100	0	-	0	100	100	100	50
2	50	50	-	0	100	-	-	0
3	100	-	0	0	-	100	100	0
4	100	100	100	50		100		33
5	100	100	100	80		-		

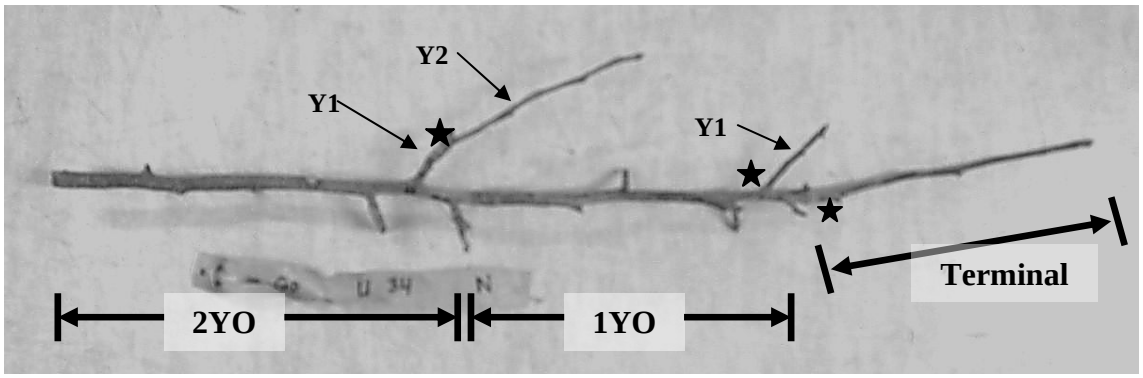


Figure 1. Diagram of representative branch used for this study. The branch was composed of a two-year-old axis (2YO), one-year-old axis (1YO), and terminal extension shoot (terminal) at the end of the study. Laterals that grew when the axis was one-year-old are designated as Y1 laterals, and laterals that grew when the axis was two-years-old are designated as Y2 laterals. Examples of Y1 and Y2 laterals on the 1YO and 2YO axes are indicated. At the beginning of the study, budburst occurred at the terminal bud (to produce the terminal), from lateral buds on the 1YO axis (to produce Y1 laterals), and on Y1 laterals on the 2YO axis (to produce Y2 laterals). Examples of where budburst occurred is indicated with a star.

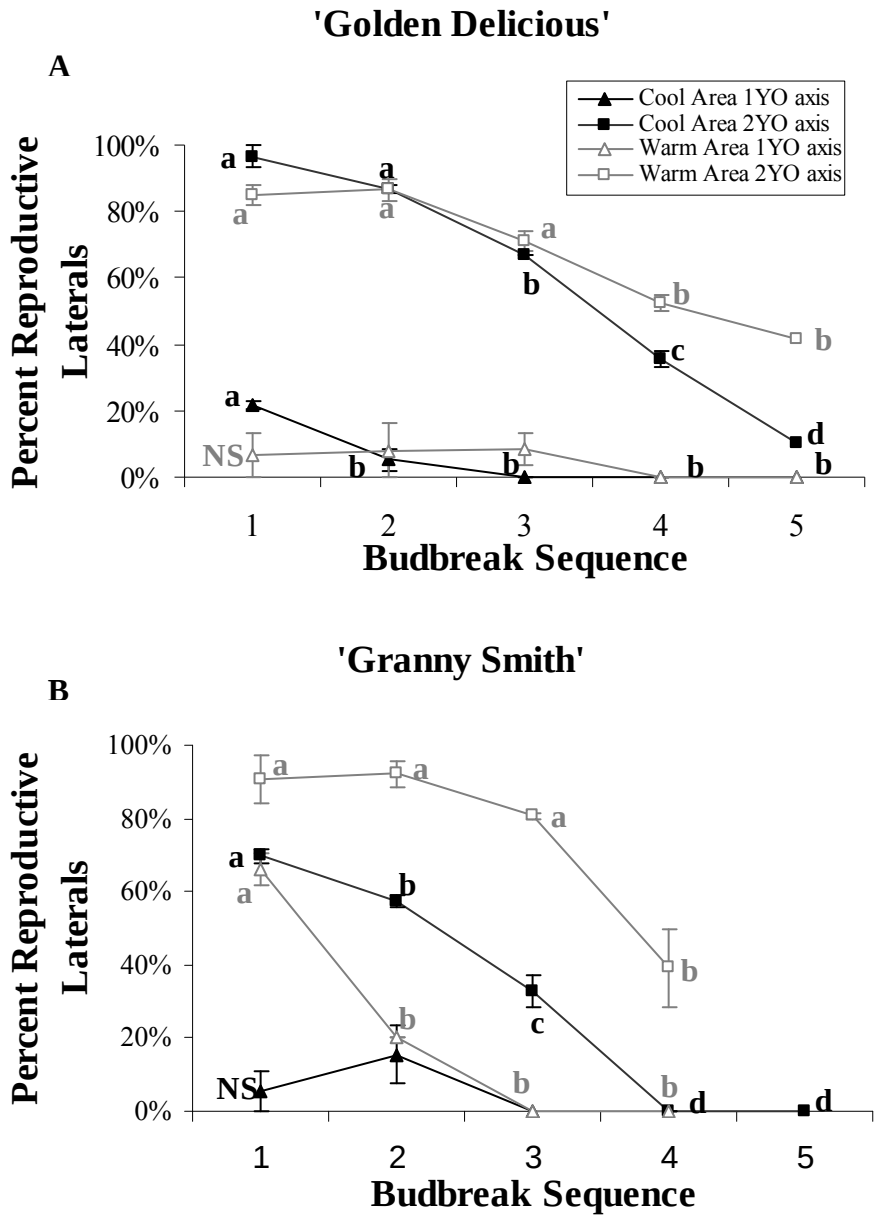
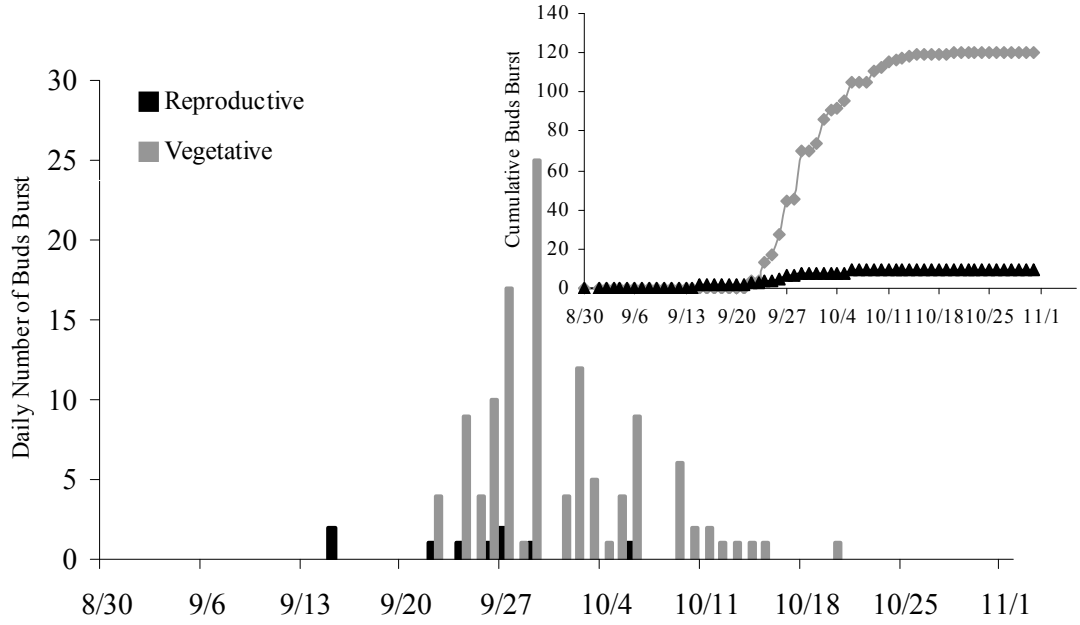
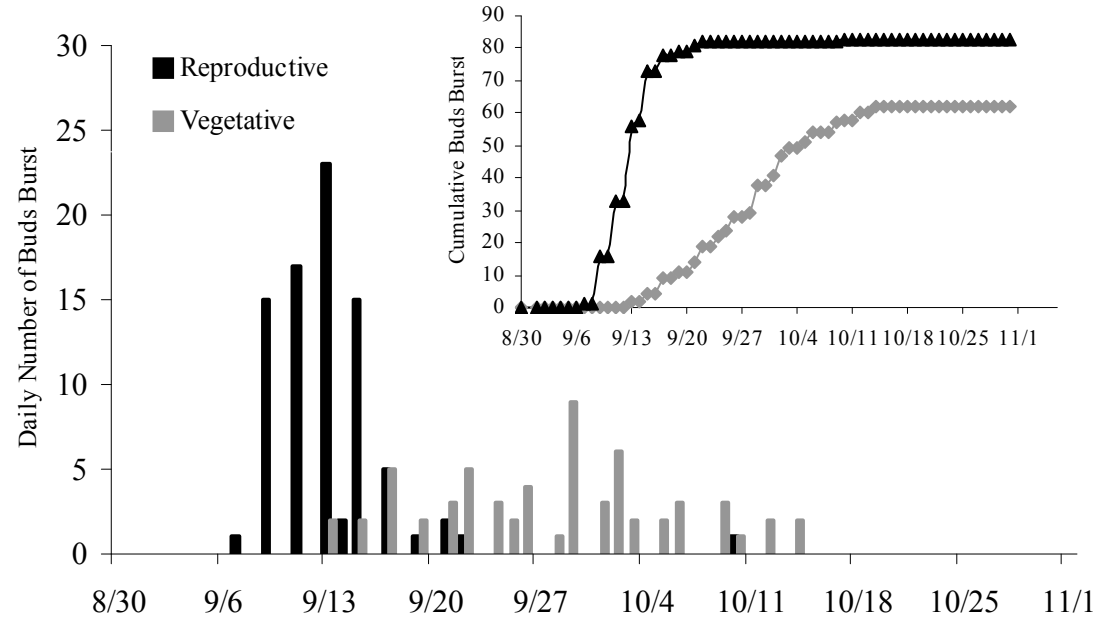


Figure 2. Relationship between the percent of growing buds that are reproductive and their budbreak sequence (1 = first bud to reach green tip along the axis and 2 = second to reach green tip along the axis, etc.) within one- (1YO) and two- (2YO) year-old axes. Percent reproductive within growing laterals was calculated as the number of reproductive laterals divided by the number of growing laterals within each position. Data were collected from both the cool area (Koue Bokkeveld) and the warm area (Warm Bokkeveld) for (A) 'Golden Delicious' and (B) 'Granny Smith' apple cultivars. Means and standard errors were calculated for each position within each cultivar-site-axis age combination. Letters signify differences at α=0.05 using mean separation of the Kruskal-Wallis test (K-W) among budburst sequences within a single cultivar-site-axis age. NS = nonsignificant.

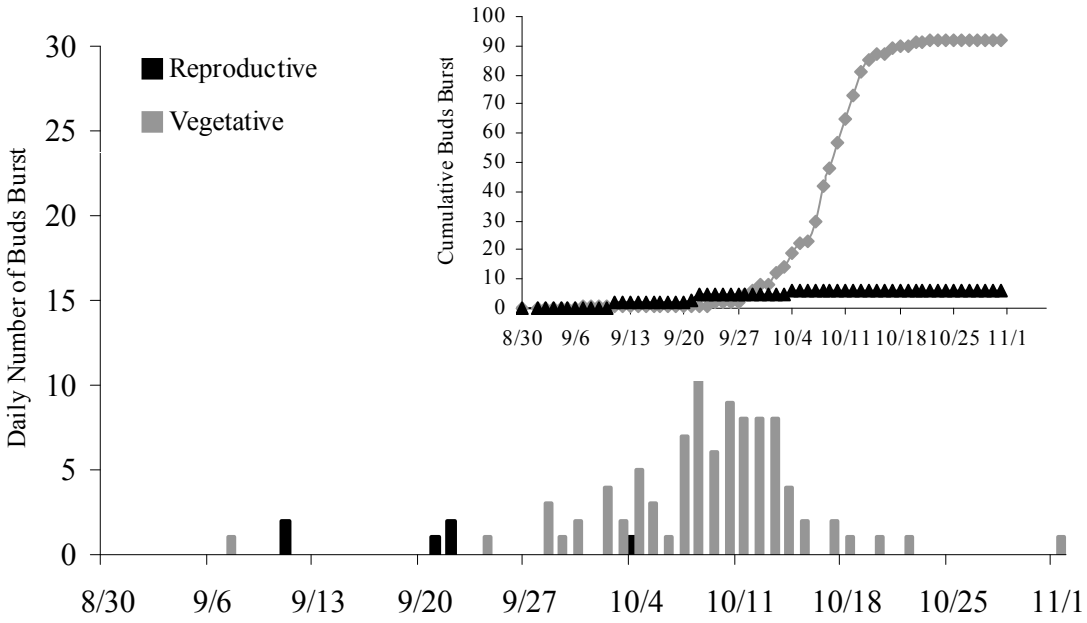
A. GD/Cool Area/1YO axis



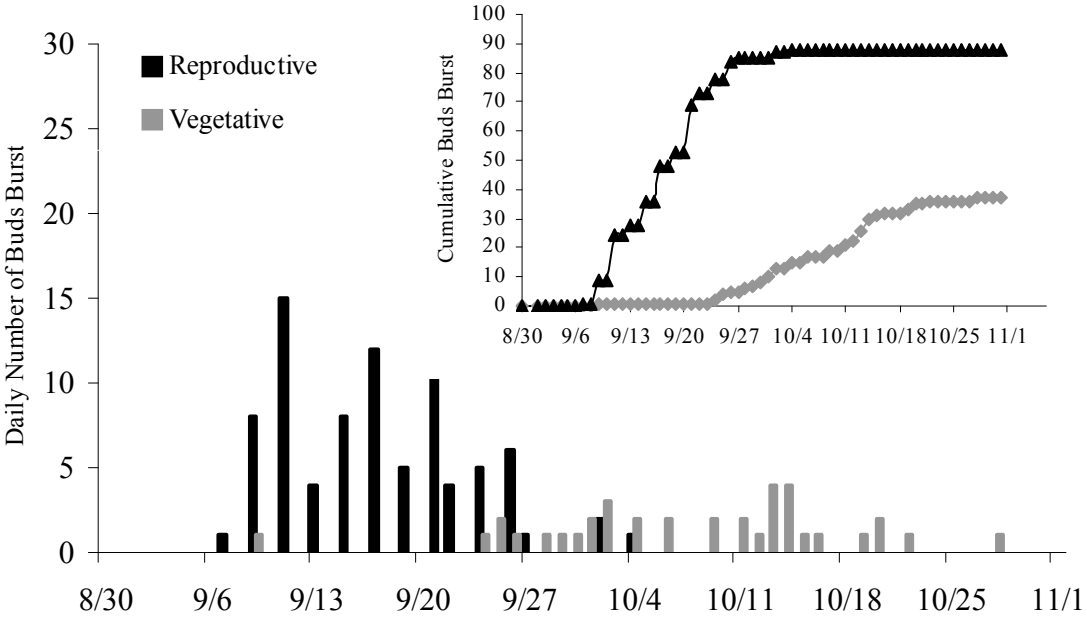
B. GD/Cool Area/2YO axis



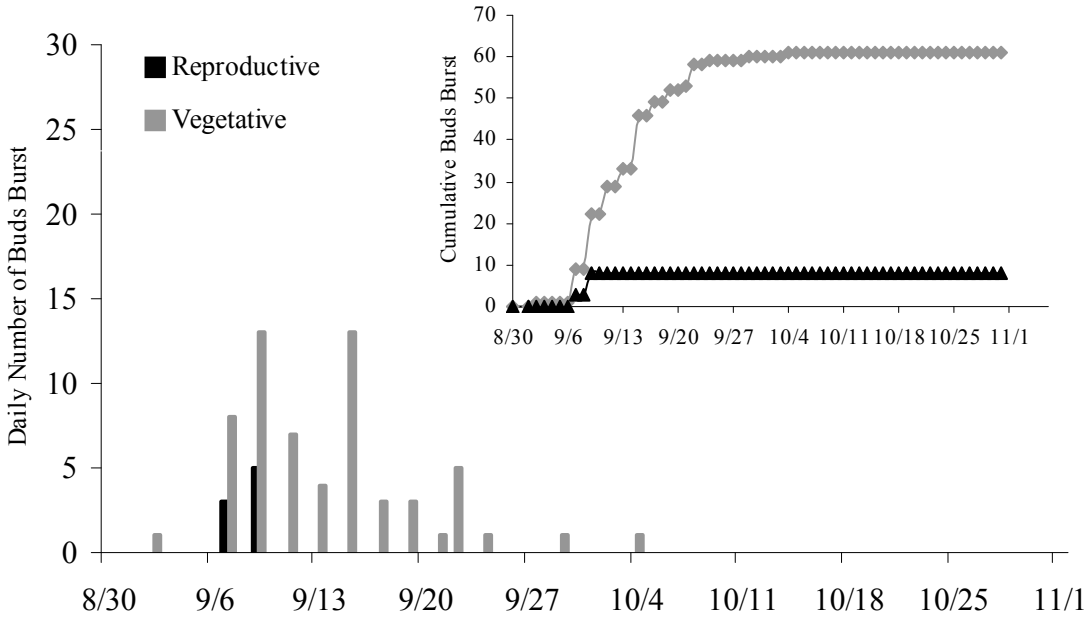
C. GD/Warm Area/1YO axis



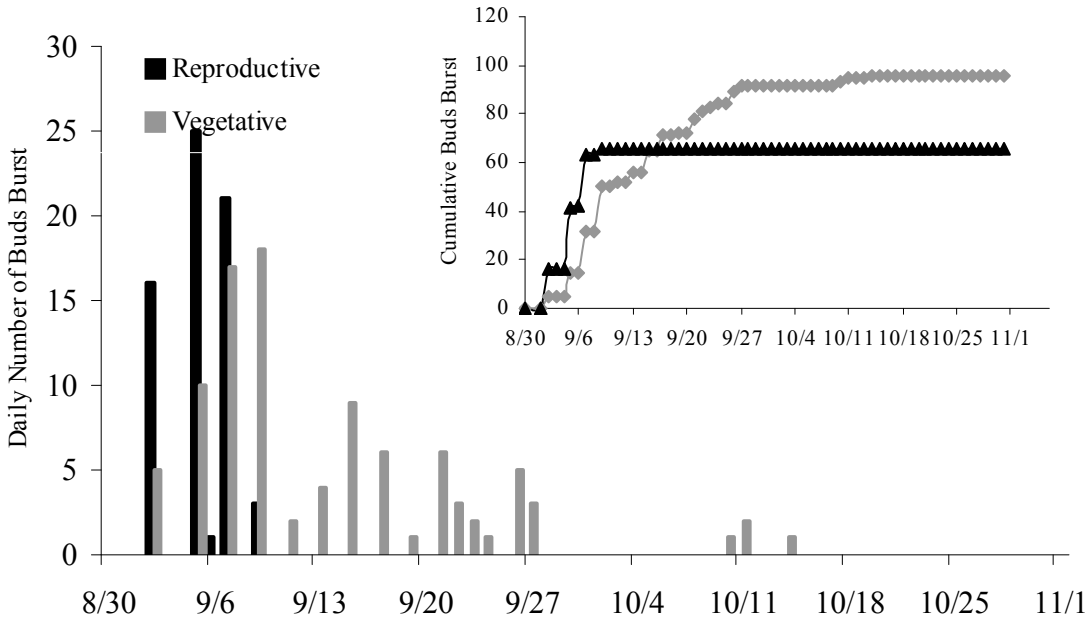
D. GD/Warm Area/2YO axis



E. GS/Cool Area/1YO axis



F. GS/Cool Area/2YO axis



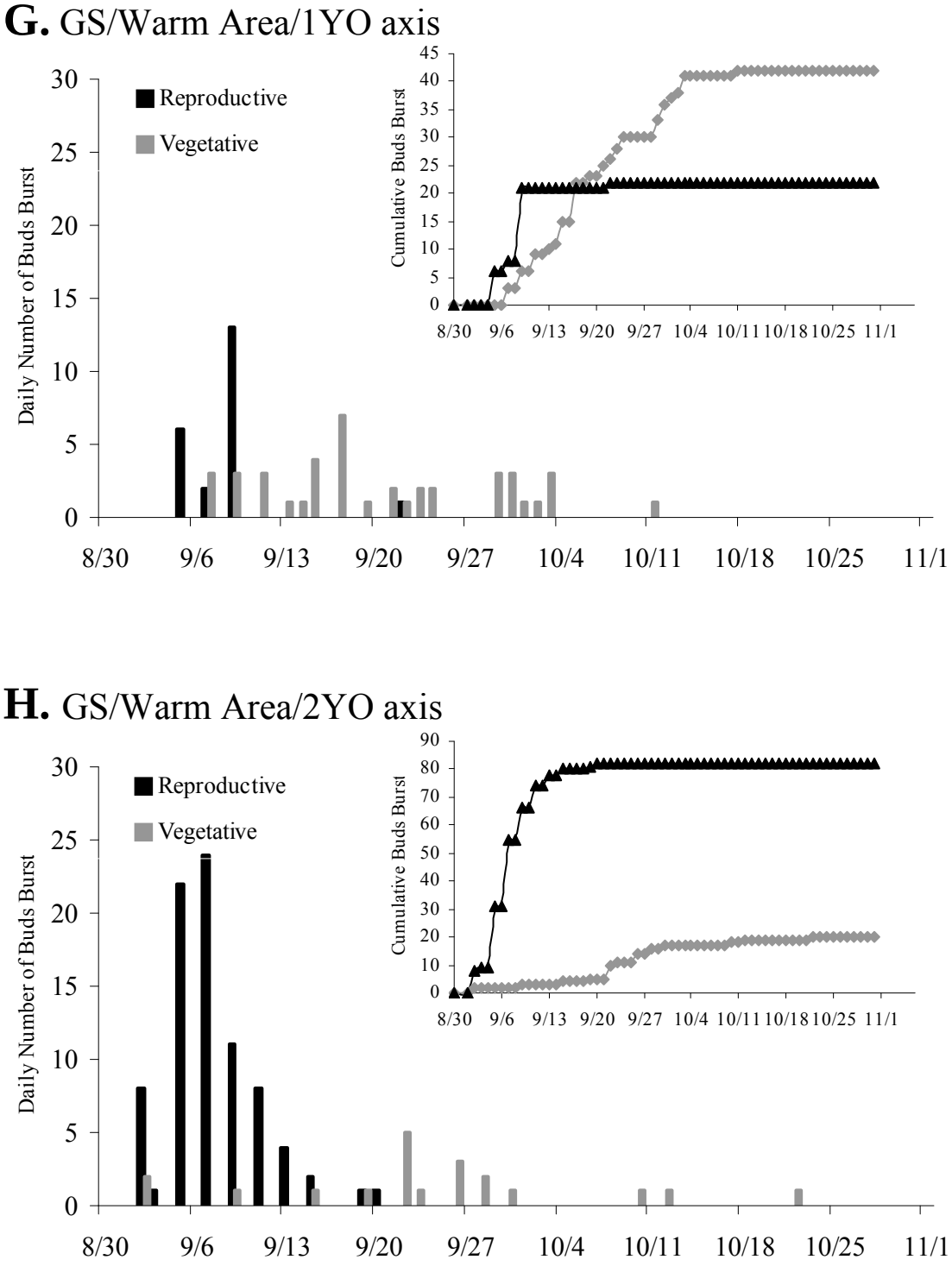


Figure 3. Frequency distribution of number of reproductive (black) and vegetative (grey) buds burst on each day for (A,C,E,G) one (1YO)- and (B,D,F,H) two (2YO)- year-old Golden Delicious (GD) (A-D) and Granny Smith (GS) (E-H) axes grown at two sites, (A,B,E,F) a cool area (Koue Bokkeveld) and (C,D,G,H) a warm area (Warm Bokkeveld). The cumulative distribution of vegetative and reproductive bud burst is shown in the inset graph.

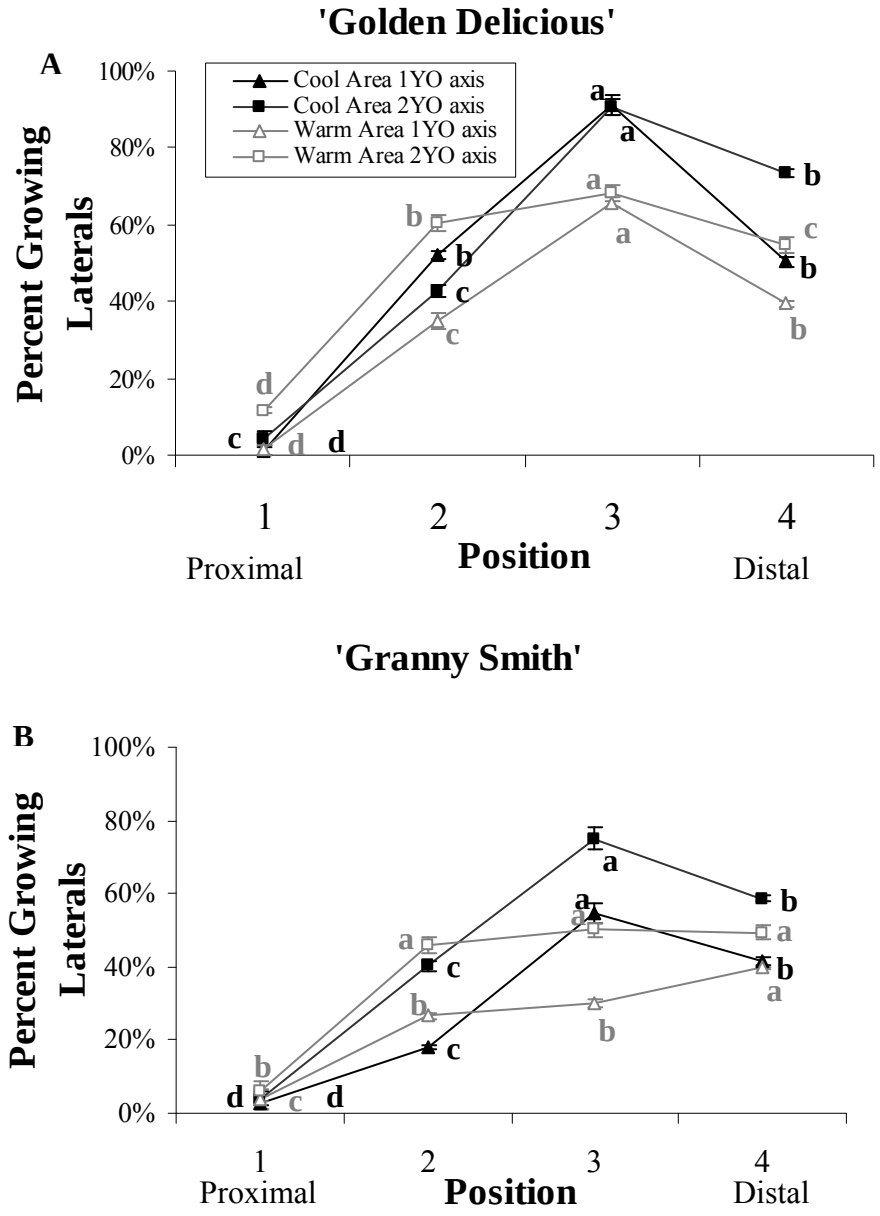


Figure 4. Relationship between percent growing laterals and relative position (quadrant) along one- (1YO) and two- (2YO) year-old axes. Relative position was determined according to equally distribution of node number within each axis (1 = proximal and 4 = distal). Percent growing laterals was calculated as the number of growing laterals divided by the total number of nodes (including latent) within each position. Data were collected from both a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld) sites for (A) 'Golden Delicious' and (B) 'Granny Smith'. Means and standard errors were calculated for each position within each site-cultivar-axis age combination. Letters signify differences at α=0.05 using mean separation of the Kruskal-Wallis test (K-W) among positions within a single cultivar-site-axis age age combination. NS = nonsignificant.

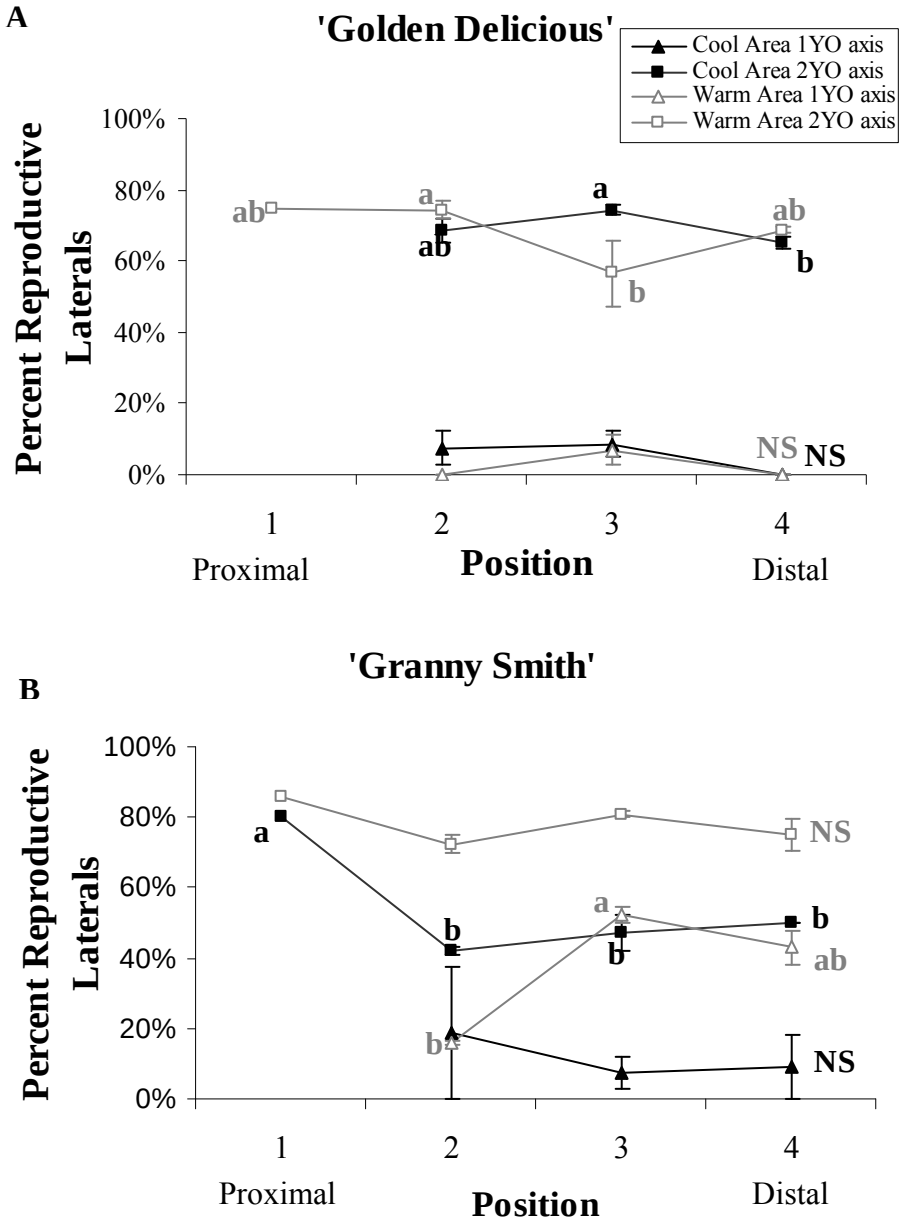
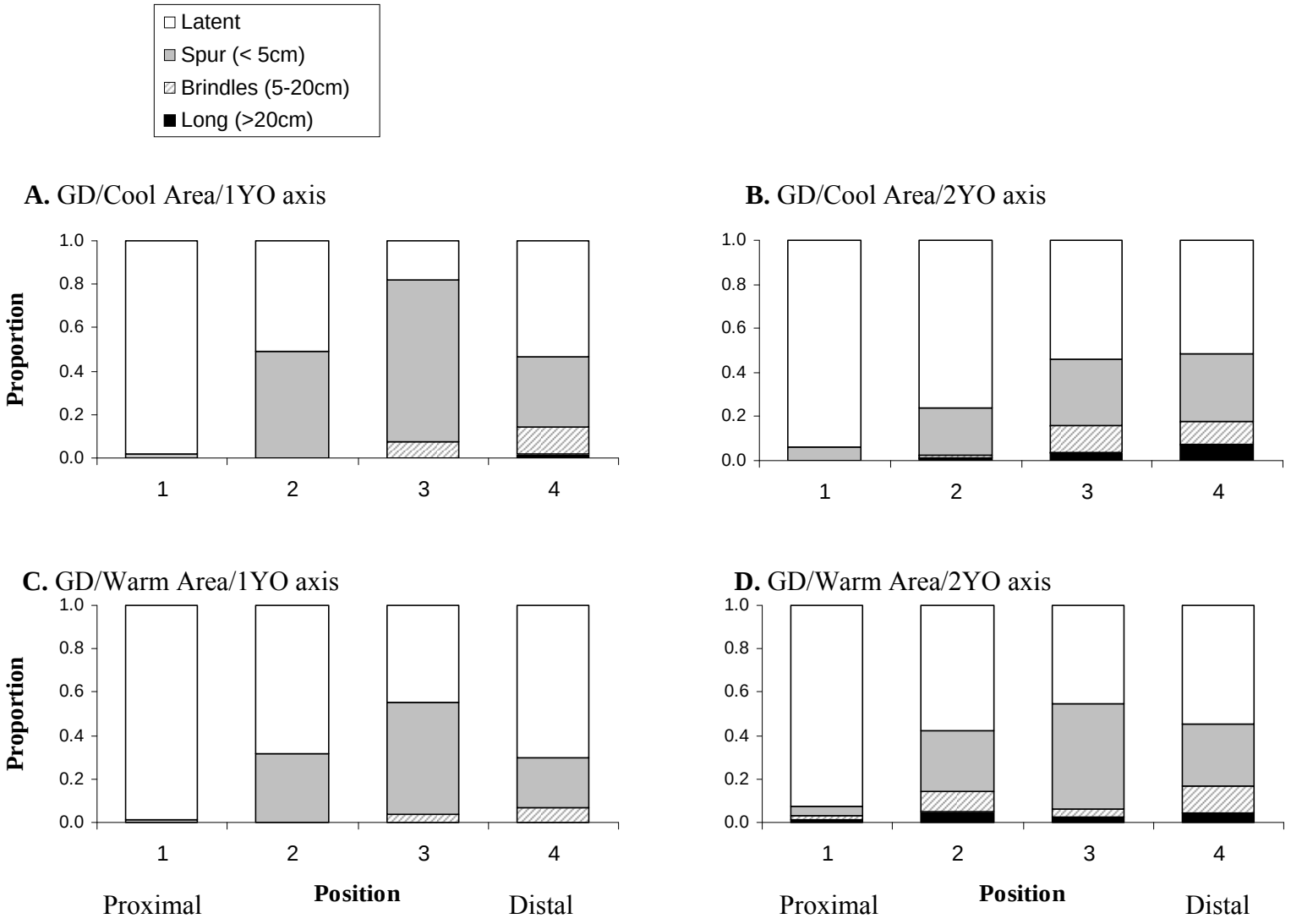


Figure 5. Relationship between percent of growing laterals that were reproductive and relative position along one- (1YO) and two- (2YO) year-old axes within the shoot. Relative position was determined according to node number within each axis (1 = proximal and 4 = distal). Percent of reproductive laterals was calculated as the number of reproductive laterals divided by the number of growing (reproductive and vegetative) laterals within each position. Data were collected from both the Koue Bokkeveld (KB) and Warm Bokkeveld (WB) sites for (A) 'Golden Delicious' (GD) and (B) 'Granny Smith' (GS). Means and standard errors were calculated for each position within each cultivar-site-axis age combination. Letters signify significant differences at α=0.05 using mean separation of the Kruskal-Wallis test (K-W) among positions within a single cultivar-site-axis age combination. NS = nonsignificant.


Latent
Spur (< 5cm)
Brindles (5-20cm)
Long (>20cm)
A. GD/Cool Area/1YO axis
B. GD/Cool Area/2YO axis
C. GD/Warm Area/1YO axis
D. GD/Warm Area/2YO axis
Proportion
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
Proximal
Position
Distal

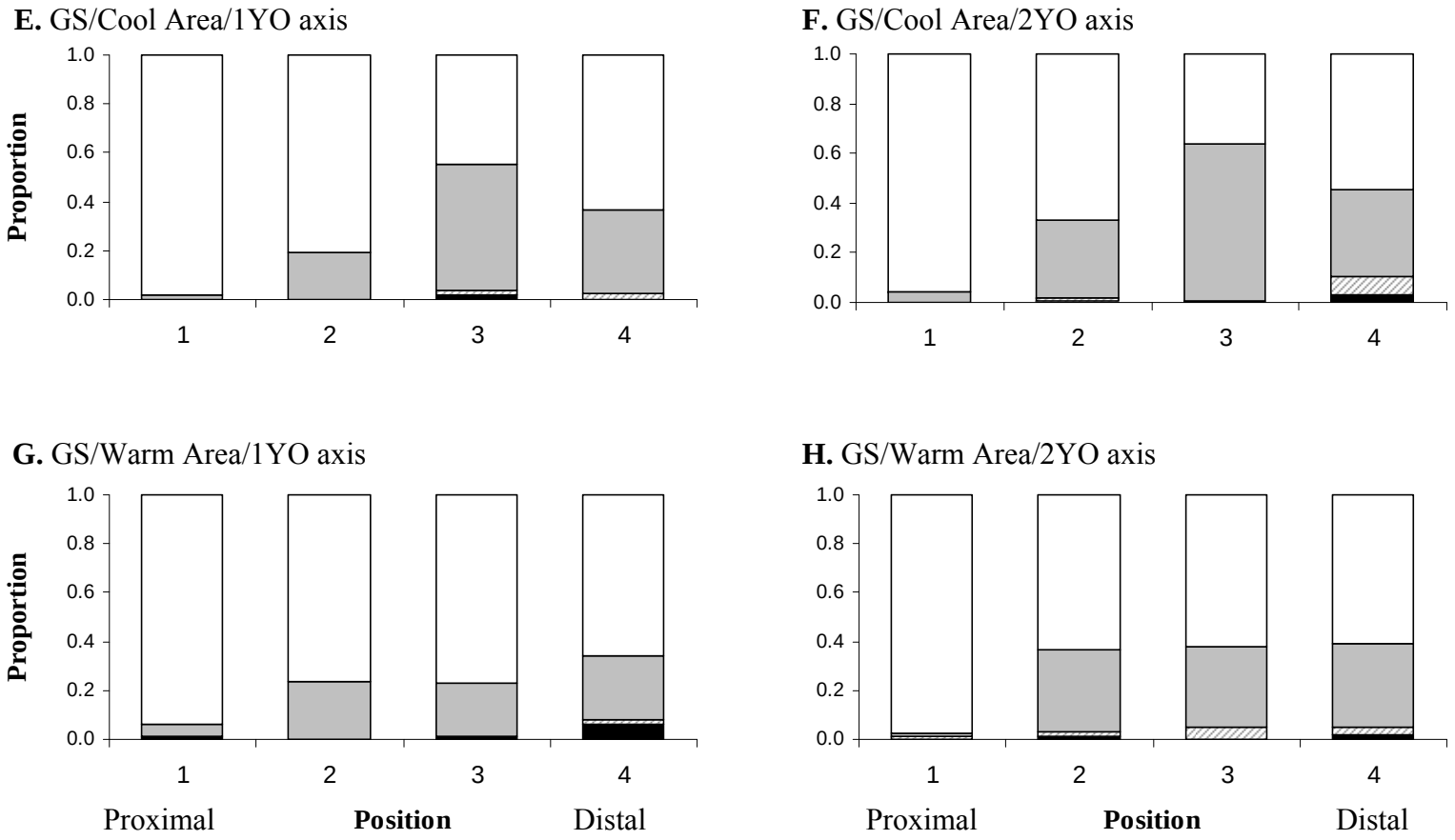
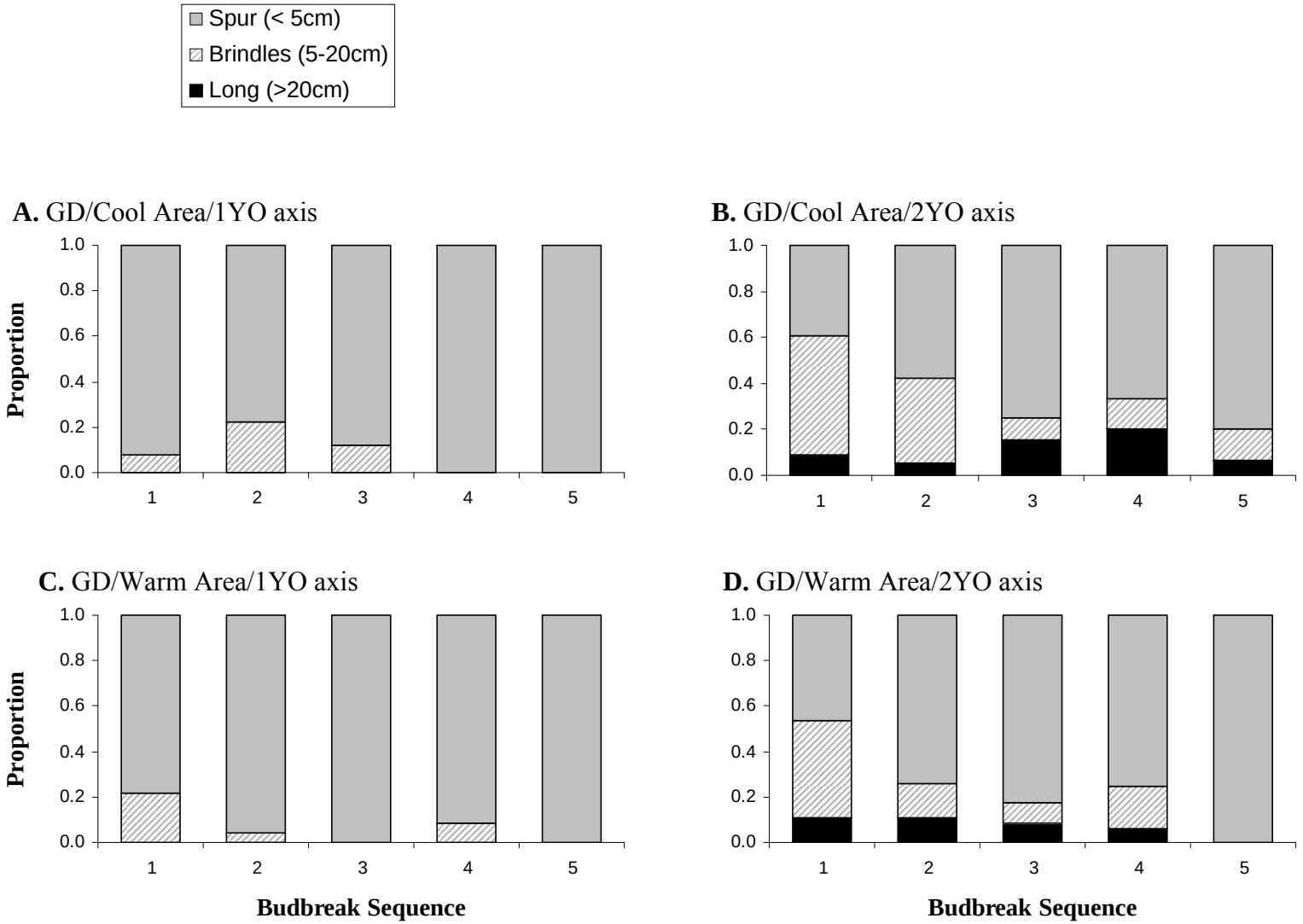


Figure 6. Proportion of laterals in different length classes in the (A,C,E,G) one (1YO)- and (B,D,F,H) two (2YO)- year-old axes according to relative position (quadrant) (1=proximal and 4=distal). Data was collected from 'Golden Delicious' (GD) (A-D) and 'Granny Smith' (GS) (E-H) apple trees grown in two sites, (A,B,E,F) a cool area (Koue Bokkeveld) and (C,D,G,H) a warm area (Warm Bokkeveld). Length classes observed were: latent, spurs (< 5cm), brindles (5-20 cm), and long shoots (> 20cm).


Spur (< 5cm)
Brindles (5-20cm)
Long (>20cm)
A. GD/Cool Area/1YO axis
B. GD/Cool Area/2YO axis
C. GD/Warm Area/1YO axis
D. GD/Warm Area/2YO axis
Proportion
Budbreak Sequence
0.0
0.2
0.4
0.6
0.8
1.0
1
2
3
4
5

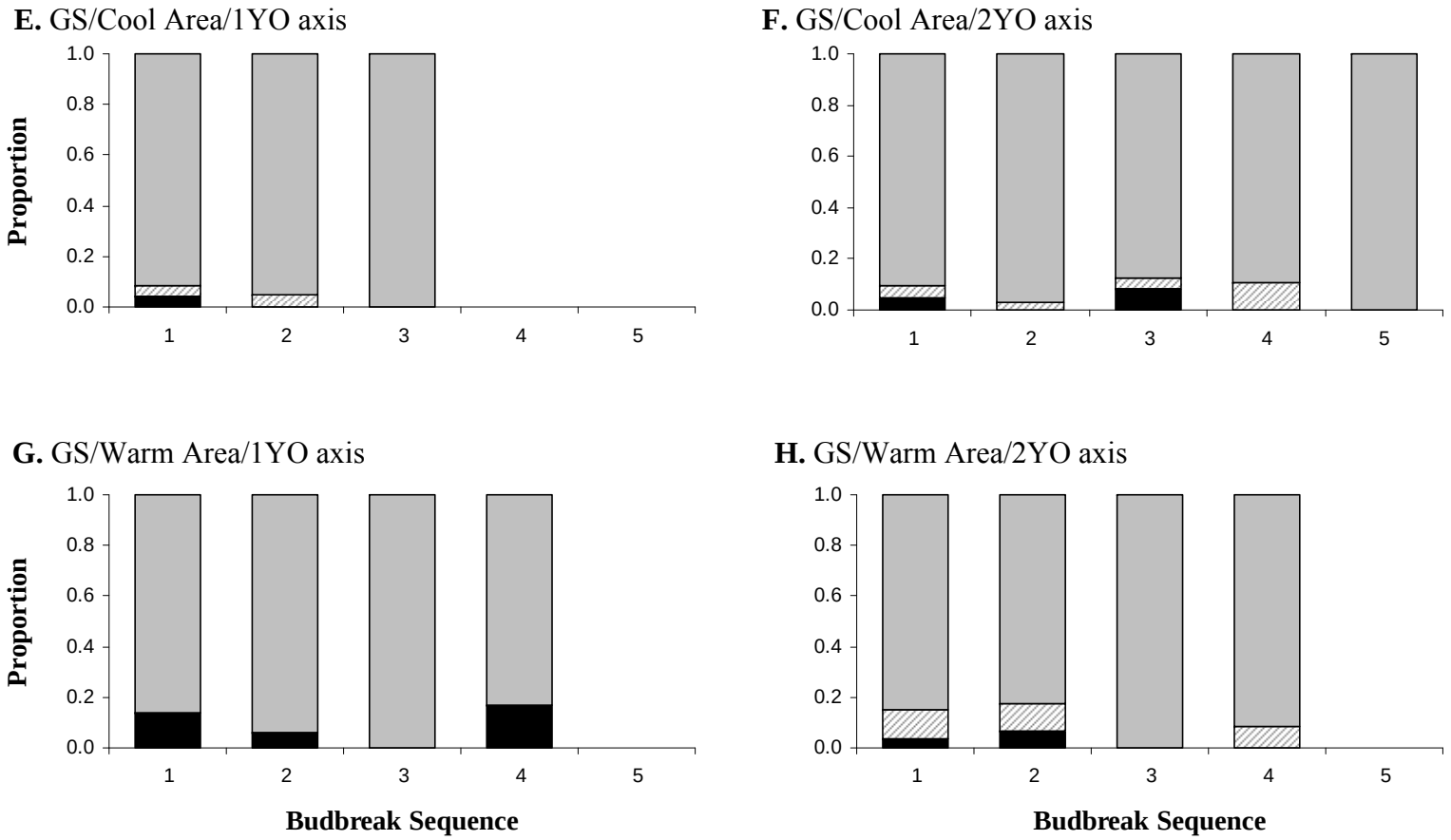


Figure 7. Proportion of laterals in different length classes in the (A,C,E,G) one (1YO)- and (B,D,F,H) two (2YO)- year-old axes according to budburst sequence (1=first bud to reach green tip along the axis and 2= second to reach green tip along the axis, etc.). Data was collected from (A-D) 'Golden Delicious' (GD) and (E-H) 'Granny Smith' (GS) apple trees grown in two sites, (A,B,E,F) a cool area (Koue Bokkeveld) and (C,D,G,H) a warm area (Warm Bokkeveld). Length classes observed were: spurs (< 5cm), brindles (5-20 cm), and long shoots (> 20cm).

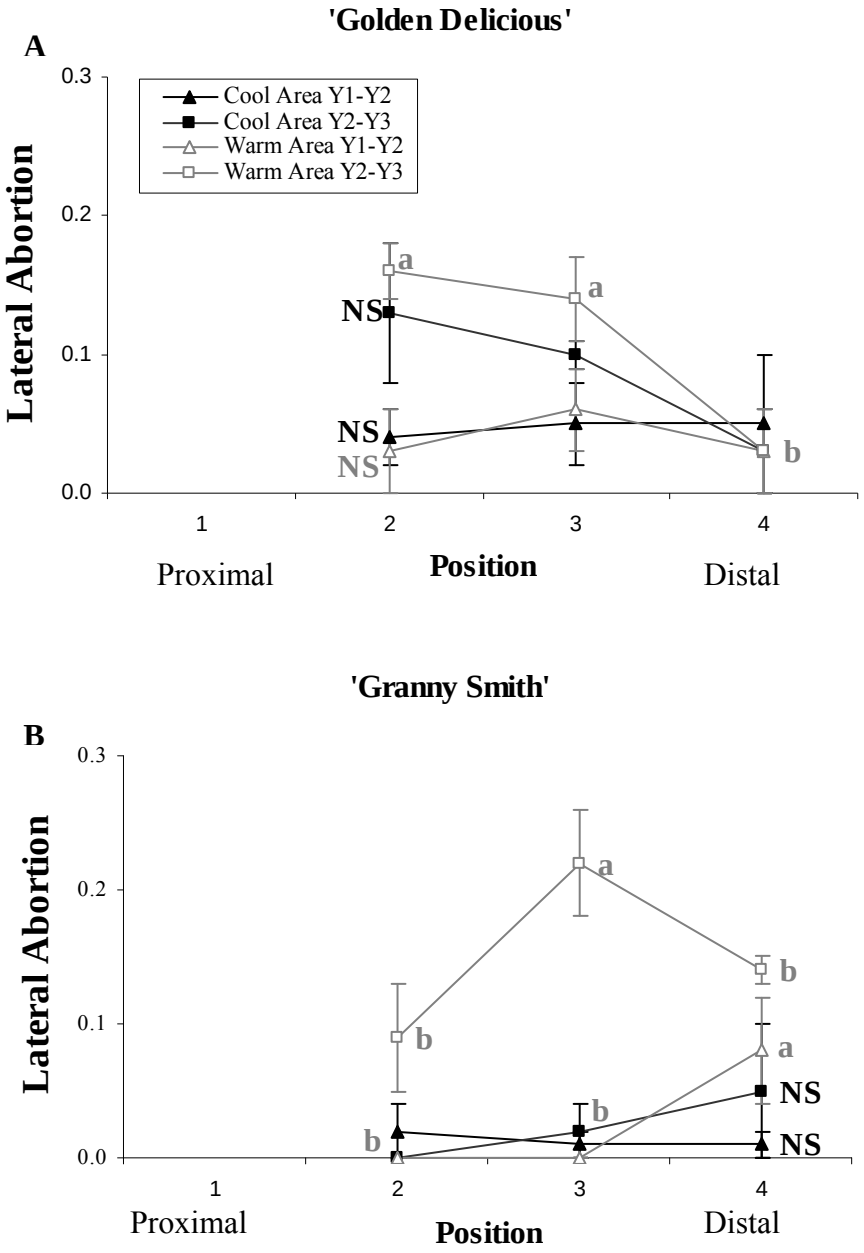


Figure 8. Relationship between lateral abortion of a bud and its position within an axis (1 = proximal and 4 = distal). Lateral abortion is considered as a transition from a growing lateral in one year to a scar in the following year. Lateral abortion was observed on the 1YO axis as a transition from growing Y1 lateral to a scar in year two (Y1-Y2), and on the 2YO axis as a transition from a growing Y2 lateral to a scar in year three (Y2-Y3). Data was collected from two *Malus x domestica* cultivars, (A) Golden Delicious and (B) Granny Smith, on two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld). Means and standard errors were calculated using pooled shoot data. Letters signify differences at α=0.05 using mean separation of the Kruskal-Wallis test (K-W) among relative positions within a single cultivar-site-axis age combination. NS = nonsignificant.

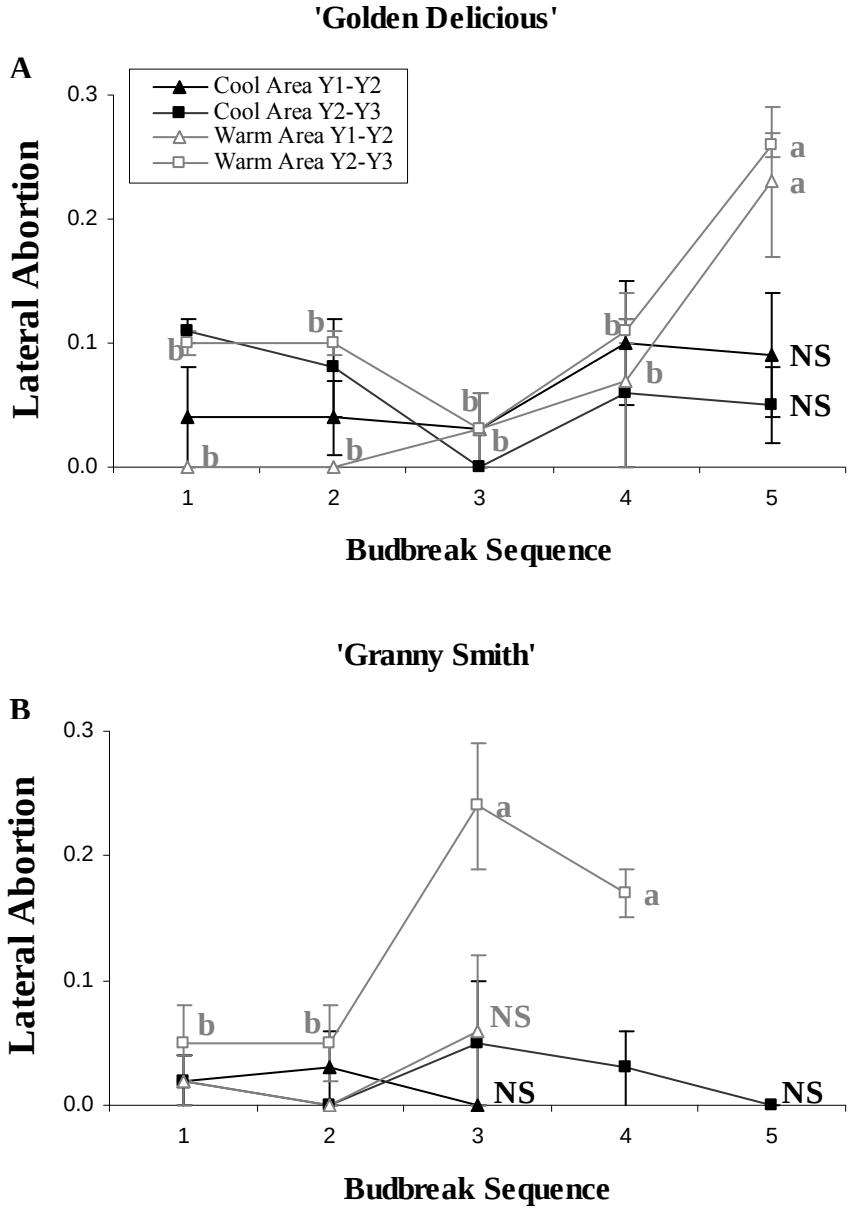


Figure 9. Relationship between lateral abortion of a bud and its budbreak sequence within an axis (1 = first bud to reach green tip along the axis and 2 = second to reach green tip along the axis, etc.). Lateral abortion is considered as a transition from a growing lateral in one year to a scar in the following year. Lateral abortion was observed on the one-year-old axis as a transition from a growing Y1 lateral to a Y2 scar (Y1 – Y2), and on the two-year-old axis as a transition from a growing Y2 lateral to a Y3 scar (Y2 –Y3). Data was collected from both: (A) 'Golden Delicious' and (B) 'Granny Smith', at two sites, a cool area (Koue Bokkeveld) and a warm area (Warm Bokkeveld). Means and standard errors were calculated using pooled shoot data. Letters signify differences at α=0.05 using mean separation of the Kruskal-Wallis test (K-W) among budbreak sequences within a single cultivar-site-axis age combination. NS = nonsignificant.

PAPER 4. Quantification of Branching Habit in a 'Telamon' X 'Braeburn' (*Malus x domestica* Borkh.) Mapped Population based on Vegetative Branching Variables

Abstract

Apple (*Malus x domestica* Borkh.) shoots can be classified qualitatively based on their branching habits according to Lespinasse typology. In our study, variation in branching habit was quantified using the two-year-old branches of progeny from a mapped 'Telamon' x 'Braeburn' population. Laterals on the two-year-old axes were grouped into length classes and sorted according to position within the axis. Branches could be successfully classified in branching habit groups, even though these were not related to the Lespinasse groups. This was due to the dominance of the columnar gene found in 'Telamon', as well as the influence of using 'Braeburn' as a parent, which resulted in overall high number of laterals less than 5 cm (spur length) and a low number of Type IV trees present in the progeny. However, spur density in the distal section of the axes was a key characteristic that could discriminate between the both the quantitative branching habit groups and qualitative Lespinasse types.

Introduction

Apple cultivars can be classified into ideotypes based on branching habits (Lespinasse, 1977). These types (Lespinasse Types I – IV) are based on growth (upright to weeping) and location and length of shoots within the canopy (polyarchic to hierarchic) and influence fruiting habit (Lespinasse & Delort, 1986). Type I trees (e.g., 'Telamon'), representing one side of the spectrum, are spurred and mainly fruit on two-year-old wood and older, while Type IV trees (e.g., 'Granny Smith' and 'Fuji') have longer branches with a weeping habit and fruit in distal and/or terminal positions on brindle length shoots. Types I and IV are characterized as biennial and regular-bearing, respectively. Types II (e.g., 'Oregon Spur Delicious' and 'Reine des Reinettes') and III (e.g., 'Golden Delicious', 'Braeburn', and 'Royal Gala') have intermediate branching and fruiting habits.

The Lespinasse types are essentially a way to characterize polyarchy and hierarchy among laterals which is, at least in part, genetically determined (Lauri *et al.*, 1995). Hierarchy, which establishes a strong central leader and a high proportion of growing laterals, most of which become spurs, is associated with an irregular bearing habit. Lespinasse Type II and III trees are typically hierarchic. Polyarchy, on the other hand, is related to autonomy of the laterals, a good balance between reproductive and vegetative growth, and regular bearing. Lespinasse Type IV trees are typically polyarchic.

Even though Lespinasse type is linked to such traits as regularity of bearing, this system of characterizing branching habit is qualitative, based on visual observation of the tree, and not quantitative. If tree growth and fruiting habit could be quantified and correlated to vegetative variables on a branch, then this can be used in the selection of new cultivars in apple breeding programs (Costes *et al.*, 2004). Previous studies on pear (du Plooy *et al.*, 2002) and apple (De Wit *et al.*, 2002) have discriminated between branching habits of genotypes.

Our objective is to quantify variation in branching habits of two-year-old branches and determine the relationship between these variables and final tree form (Lespinasse type). We used progeny of a 'Telamon' x 'Braeburn' cross because it's a mapped population (Kenis & Keulemans, 2005) and opens the door to develop quantitative trait loci (QTLs) in the future for fruiting habits in apple, further aiding in the selection of new cultivars. At the annual shoot level, genotypes can be differentiated using variables such as proportion of growing laterals (Costes & Guédon, 2002; Lauri *et al.*, 2006). Since these variables are not related to shoot length (Lauri *et al.*, 2006; Costes & Guédon, 2002), or tree size (Lauri *et al.*, 2006), differences among branches on trees in the breeding selection process can be indicative of

future growth of the branch, as well as branch habit when tree size differs (i.e., own-rooted trees vs. those grafted on M9) (Lauri *et al.*, 2006).

Materials and Methods

In May 1999, seedlings of a 'Telamon' x 'Braeburn' (*Malus x domestica* Borkh.) cross (each having it's own genotype) were planted on their own roots in a nursery near Rillaar, Belgium (52°N) at a distance of 30 x 40 cm. On 7 June 1999, the entire population was cut back to 20 cm high (because of damage by rabbits) and only the uppermost shoot was allowed to develop. The origin of the progeny was tested using microsatellites (Kenis *et al.*, 2001). After discarding outcrosses, dead plants, and trees with missing measured variables (case-wise deletion of missing data for the analyses), 222 seedlings remained.

After two growing seasons, during winter 2000-2001, these seedlings were replanted at a distance of 1 x 3 m, on their own roots. After growth cessation in 2003, one branch per tree was selected to be used in this trial. For each branch, the length of the branch (excluding the terminal bud) and position of the one-year-old lateral shoots on the two-year-old axis, and the length of the axis measured. Shoot position was measured as distance from the proximal end of the axis. The Lespinasse type (Lespinasse, 1977) for each tree was determined visually and recorded.

Shoot density and size class values were calculated according to the method described by du Plooy et al. (2002). Briefly, all one-year-old shoots were classified into 1 of 4 length classes: <1cm (A), 1-5 cm (B), 5-20 cm (C), and >20 cm (D). Shoot position was classified by dividing each two-year-old axis into 4 equal quadrants [Q1 (proximal), Q2, Q3, and Q4 (distal)]. For each two-year-old axis, the shoot density (number of shoots per cm of quadrant length) was determined for each of the 16 classes (eg. Q1A, Q1B, etc.).

The mean shoot density per class for each branch was submitted to a correlation matrix to confirm that they were not correlated and subsequently, to a k-means clustering analysis to form 4 distinct clusters with a minimum variability within clusters and maximum variability between clusters. Thereafter the clusters were submitted to forward stepwise and canonical discriminant function analyses in order to determine which variables were influential in forming the clusters.

The Lespinasse types were then used as the grouping variables in forward stepwise and canonical discriminant function analyses with the shoot density per class variables to determine which variables were the most influential in discriminating between the Lespinasse types.

To determine the best clustering method, many options were considered to determine the optimal technique and which variables to include. Initially, all variables (including number of shoots within each length class and 1- and 2-year-old shoot lengths) were used, but the results were very similar to the presented results. However, when all of these variables were included in the discriminant function analysis initially, 2-year-old shoot length was always one of the first discriminating factors (data not shown). Since the aim of this project is ultimately to assist in breeding selection, and the length of the 2-year-old shoot is dependant on more than genotype, these variables were removed from the cluster and discriminant function analyses. In order to analyze the data with comparable variables, only the mean shoot density per class variables were used.

To determine the most effective number of clusters to use, the mean shoot density per class variables were used to create from 2 to 8 clusters. In each number of clusters, the same variables came out of the stepwise discriminant function analyses as discriminating between groups. The relationship between any of these numbers of clusters and Lespinasse types was not changed, so the 4 clusters were used for this presentation. Combining Lespinasse type I and II into one group and Type III and IV into a second group, which increases the number of trees in each group, resulted in less variables selected by the stepwise discriminant function analysis and didn't relate better to any of the cluster numbers used.

For both the cluster groups and Lespinasse types, once the discriminant function analyses were completed, an analysis of variance was performed on the measured variables using the trees that comprised each of the respective groups. These variables included length of the 2-year-old axis and adjoining 1-year-old shoot (in the terminal position), and on the 2-year-old axis: total number of shoots (all classes combined), total number of spurs (Class A, <1 cm), total number of Class B shoots (1-5cm), and total number of shoots greater than 5 cm (this value is the combination of Class C and Class D shoots). Class C and Class D were combined due to the relatively low number of shoots in each class.

All statistical analyses were performed using Statistica (StatSoft, 2008).

Results

All of the 222 trees in the study were combined to determine the number of laterals in each length class on the 2-year-old axis (Fig. 1A). There were 5535 total growing laterals and of these, 4169 (75%) were Class A (spurs). 'Telamon' contains the Columnar gene and this high number of spurs in the progeny of the 'Telamon' X 'Braeburn' cross is due to the dominance of the Columnar gene, in addition to the influence of 'Braeburn' which is also known to have

spurs. Only 2% of the laterals were longer than 20 cm (Class D). Within Lespinasse Type I, the spurs comprised 96% of all lateral types, while in Type II, III, and IV, the spurs were only 54%, 63% and 63% of the total laterals, respectively (Fig. 1B, C, D, and E).

Clustering based on the mean shoot density per quadrant variables failed to make clusters that corresponded to Lespinasse types (Table 1). Even though Cluster 3 did contain only Type I trees (100%), this only amounted to 40% of the total Type I trees, with the majority actually in Cluster 2 (51%). Type III and IV trees were spread across Clusters 1, 2, and 4.

A forward stepwise discriminant function analysis for the clusters selected the following variables, in order of importance, as influential in discriminating between the clusters: Q4A, Q3B, Q2A, Q3A, Q4C, Q4B, Q4D, Q2C, Q2D, and Q1B (Table 2). The Wilkes Lambda value (and associated p-value) quantifies the cumulative discriminatory power of the model up to that step, with 1.0, signifying no discriminatory power and 0.0 having perfect discrimination (StatSoft, 2008). In the clusters, the Wilkes Lambda value begins at 0.12 and, with the addition of the remaining variables, improves discrimination between clusters up to a value of 0.04.

Three canonical roots described the discrimination between clusters (p-value for Chi-Square test was <0.0001 for the first 2 roots and 0.015 for the third root) (Table 2). The first root explained 94% of the discrimination, and Q4A was the most influential in the discrimination (standardized coefficient = -0.76538). Cluster 3 had the greatest density of Class A (spurs) in Q4 and Cluster 4 had the lowest density (Fig 2). The second root discriminated mainly using the variables Q3B (-0.640504), Q4A (-0.578809), Q4B (-0.534742), and Q4C (-0.509944) (Table 2). Cluster 4 had the highest Q3B and Q4C and Q4B values. The third root was significant at 0.015 and mainly discriminated on the basis of Q4C. When the 'observed' clusters (clusters formed via k-means clustering) were reclassified into predicted classifications based on the computed discriminant functions, there was at least 90% correct reclassification for each of the clusters (Table 3). The main exceptions were 6 of the 52 trees in Cluster 4 that were wrongly placed into Cluster 1, 3 trees from Cluster 3 that were placed into Cluster 2, and 1 tree from Cluster 1 that was placed into Cluster 4.

The forward stepwise discriminant function analysis for the Lespinasse types used Q4A, Q4B, Q3A, Q4C, Q2D, and Q1A in the model to discriminate between types (Table 4). The Wilkes Lambda value started at 0.40 and was only able to discriminate between types up to 0.34, so the variables in this model were not as strong at discriminating between Lespinasse types as the variables in the model for the discrimination between clusters. Two roots were

found to be significant ($p<0.0001$ and $p=0.022$, respectively, for Roots 1 and 2). For the first root, which explained 94% of the discrimination between types, the most important discriminating variable was Q4A (0.685586), which was greatest in Type I trees (Fig 3). For the second root, the most influential variables were Q4B (1.064773) and Q4A (0.533146). Q4B was lowest in Type I (Fig 3). When the visually observed Lespinasse types were reclassified based on the computed discriminant functions, none of the Type IV's were reclassified correctly, while 90% of the Type I's were reclassified correctly (Table 5). Type II and Type III were reclassified with 68% and 45% correct, respectively. 15 Type II trees were misclassified as Type III, and 23 of the Type III trees were misclassified as Type II.

After the discriminant function and cluster analyses, an ANOVA was performed on all the variables for both the clusters and the Lespinasse types. The mean shoot densities per class for Lespinasse types are in Fig 3, and the mean shoot densities per class for the clusters are in Fig 2. The means for the measured variables for Lespinasse types and clusters are in Table 6 and 7, respectively. All measured variables are different between Lespinasse types and all, except 1-year-old shoot length, are different between clusters. Lespinasse Type IV trees had the longest 2-year-old axes (94 cm) and the Type I's had the shortest (Table 6). Type I trees had the greatest amount of Class A shoots (25 / 2-year-old axis) and Type IV trees had the greatest amount of Class C and D shoots combined (3.7 / 2-year-old axis). Within the clusters, Cluster 4 had the lowest number of Class A shoots (5) and the greatest number of Class B and combination of Class C and D shoots (Table 7).

Discussion

Apple branches could be successfully classified into 4 different branching habit clusters based on vegetative branching variables, even though it was not possible to relate these groups to the final Lespinasse types. The development of models to predict classification of a branch into a final tree form requires an equal representation of the initial tree types, as clustering data attempts putting data in equally represented groups. This did not occur in our study due to dead trees and the columnar habit of the 'Telamon' parent. 'Telamon' is heterozygous for the Columnar gene, while 'Braeburn' does not contain this gene, so approximately 50% percent of the progeny were expected to be Type I. This was true in our study as 42% were Type I.

The columnar gene influence is apparent in the proportion of laterals that were spurs on all trees, as well as the influence of 'Braeburn' on the production of spurs (Fig 1A) (Tobutt, 1985). Type IV trees, although with considerably less spurs along the shoot than

Type I, had a relatively high proportion spurs (<1cm) (63%) relative to the total amount of growing laterals. Typically, polyarchic trees have longer laterals that rapidly become autonomous (Lauri *et al.*, 1995). In our case, polyarchy may be the result of the decrease in proportion of growing laterals in Type IV shoots. The number of laterals growing per shoot was maintained across Lespinasse types, even though Type I shoots (typically hierarchic) were almost half the length of the Type IV shoots (Table 6). This means that the number of laterals growing per unit length was much less in the Type IV shoots. Even though the laterals were very short, the stage has been set for autonomy by limiting competing laterals (Lauri & Terouanne, 1999; Hansen, 1969).

Spur density in the distal section of the shoots was important in discriminating among both Lespinasse types and clusters (Tables 2 and 4). Two of the clusters (2 and 3) contained the majority of Type I seedlings. Acrotony is evident in trees on a number of levels, one of which is the increase in proportion of growing laterals moving distally along the shoot (Lauri, 2007). In both Lespinasse Type I and Clusters 2 and 3, acrotony was evident in this increase in density of spur length laterals moving distally along the shoot. Even though Cluster 4 had longer shoots in the distal quadrant, longer shoots were also evident in quadrants 2 and 3, indicating that these shoots are more polyarchic. As Lespinasse Types I-IV had a relatively equal distribution of laterals, there is an indication that a degree of polyarchy exists. This also indicates however, that degree of polyarchy in the future branch is not always obvious in the early branch, specifically since, Lespinasse Type I branches differed from the other Types on all lateral growth characteristics, Types II-IV did not obviously differ among themselves (Table 6; Fig 3). Type I trees are easy to remove from the population based on vegetative branching variables, although these are generally easy to detect visually (high number of spurs, short internodes) (Tobutt, 1985). Finding easily detectable differences among one-year-old annual shoots of the other types would be beneficial but was not obvious in our study.

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Table 1. Classification of trees with specific Lespinasse types into cluster groups based on lateral shoot length and position variables.

		Cluster				
	Lespinasse	**1**	**2**	**3**	**4**	Row Totals
	Type I	**6**	**48**	**38**	**2**	**94**
Column %		10%	72%	100%	3%	
Row %		6%	51%	40%	2%	
	Type II	**25**	**12**	**0**	**35**	**72**
Column %		42%	18%	0%	60%	
Row %		35%	17%	0%	49%	
	Type III	**25**	**6**	**0**	**18**	**49**
Column %		42%	9%	0%	31%	
Row %		51%	12%	0%	37%	
	Type IV	**3**	**1**	**0**	**3**	**7**
Column %		5%	1%	0%	5%	
Row %		43%	14%	0%	43%	
	Column Totals	**59**	**67**	**38**	**58**	**222**

Table 2. Variables selected by forward stepwise discriminant analysis that discriminate between clusters and their respective Wilkes Lambda value. Standardized coefficients for the variables and results of the Chi-Square test for the successive roots are listed.

Step	**Variable**	**Lambda**	***Pr>f***	**Root 1**	**Root 2**	**Root 3**
1	Q4A	0.120228	*0.000001*	-0.76538	-0.578809	0.040204
2	Q3B	0.083448	*0.000001*	0.20001	-0.640504	0.264148
3	Q2A	0.065227	*0.000001*	-0.41835	0.070423	-0.512528
4	Q3A	0.059534	*0.000198*	-0.34429	-0.075421	-0.215437
5	Q4C	0.055115	*0.000877*	0.18037	-0.509944	-0.684903
6	Q4B	0.049867	*0.000088*	0.28152	-0.534742	-0.365658
7	Q4D	0.046456	*0.001772*	0.12401	-0.369946	-0.239043
8	Q2C	0.044634	*0.037321*	0.11991	-0.226094	0.111760
9	Q2D	0.043926	*0.338502*	-0.12881	-0.045370	0.148008
10	Q1B	0.043200	*0.321791*	-0.04371	-0.195233	0.156733
Cum. Prop				0.94243	0.992653	1.000000
Chi-Square Test (Pr>*f*)				*0.0001*	*0.0001*	*0.015*

Table 3. Classification matrix for the clusters determined by mean shoot density classes with percentage of correct predicted classifications for each cluster, and *a priori* classification probabilities (p).

		Predicted Classifications			
Observed Cluster Classifications	*Percent Correct*	**1** p=.09459	**2** p=.50450	**3** p=.20721	**4** p=.19369
1	98%	58	0	0	1
2	100%	0	67	0	0
3	92%	0	3	35	0
4	90%	6	0	0	52

Table 4. Variables selected by forward stepwise discriminant analysis that discriminate between Lespinasse types and their respective Wilkes Lambda value. Standardized coefficients for the variables and results of the Chi-Square test for the successive roots are listed.

Step	**Variable**	**Lambda**	***Pr>f***	**Root 1**	**Root 2**	**Root 3**
1	Q4A	0.400159	*0.0001*	0.685586	0.533146	-0.065827
2	Q4B	0.369910	*0.0007*	-0.154974	1.064773	0.063746
3	Q3A	0.355311	*0.0333*	0.291515	0.054718	0.629934
4	Q4C	0.347378	*0.1819*	-0.180089	0.041042	0.843231
5	Q2D	0.340017	*0.2040*	-0.131386	-0.319451	0.117908
6	Q1A	0.335059	*0.3711*	-0.138299	0.175631	0.310272
Cum. Prop				0.944515	0.992872	1.000000
Chi-Square Test (Pr>*f*)				*0.0001*	*0.021869*	*0.597248*

Table 5. Classification matrix for the Lespinasse types of 'Telamon' x 'Braeburn' progeny by mean shoot density classes with percentage of correct predicted classifications for each type, and a priori classification probabilities (p).

		Predicted Classifications			
Observed Lespinasse Classifications	*Percent Correct*	**Type I** p=.42342	**Type II** p=.32432	**Type III** p=.22072	**Type IV** p=.03153
Type I	90%	85	5	4	0
Type II	68%	8	49	15	0
Type III	45%	4	23	22	0
Type IV	0%	1	2	4	0

Table 6. Number of 'Telamon' x 'Braeburn' progeny apple seedlings in each Lespinasse type (n) and means of the measured (main branch lengths and lateral shoot lengths and numbers) variables.

	Lespinasse Type				
Variable	**I**	**II**	**III**	**IV**	***Pr>f***
n	94	72	49	7	
2-year-old axis length (cm)	56c	65bc	73b	91a	*0.0001*
1-year-old axis length (cm)	59a	56ab	50ab	45b	*0.0088*
Number of spurs (<1cm)	25a	12b	14b	16b	*0.0001*
No. of laterals from 1 to 5 cm	1b	8a	6a	6a	*0.0001*
Number of laterals > 5cm	0.2b	2.4a	2.9a	3.7a	*0.0001*
Total number of laterals	26	22	23	25	*0.0493*

Table 7. Number of 'Telamon' x 'Braeburn' progeny apple seedlings in each cluster (n) and means of the measured (main branch lengths and lateral shoot lengths and numbers) variables.

	Cluster				
Variable	**1**	**2**	**3**	**4**	***Pr>f***
n	59	67	38	58	
2-year-old axis length (cm)	72a	63a	47b	67a	*0.0001*
1-year-old axis length (cm)	55	57	60	52	*0.1874*
Number of spurs (<1cm)	17c	25b	28a	5d	*0.0001*
No. of laterals from 1 to 5 cm	4b	1c	0c	10a	*0.0001*
Number of laterals > 5cm	2.1b	0.3c	0.1c	3.7a	*0.0001*
Total number of laterals	23b	26ab	28a	19c	*0.0001*

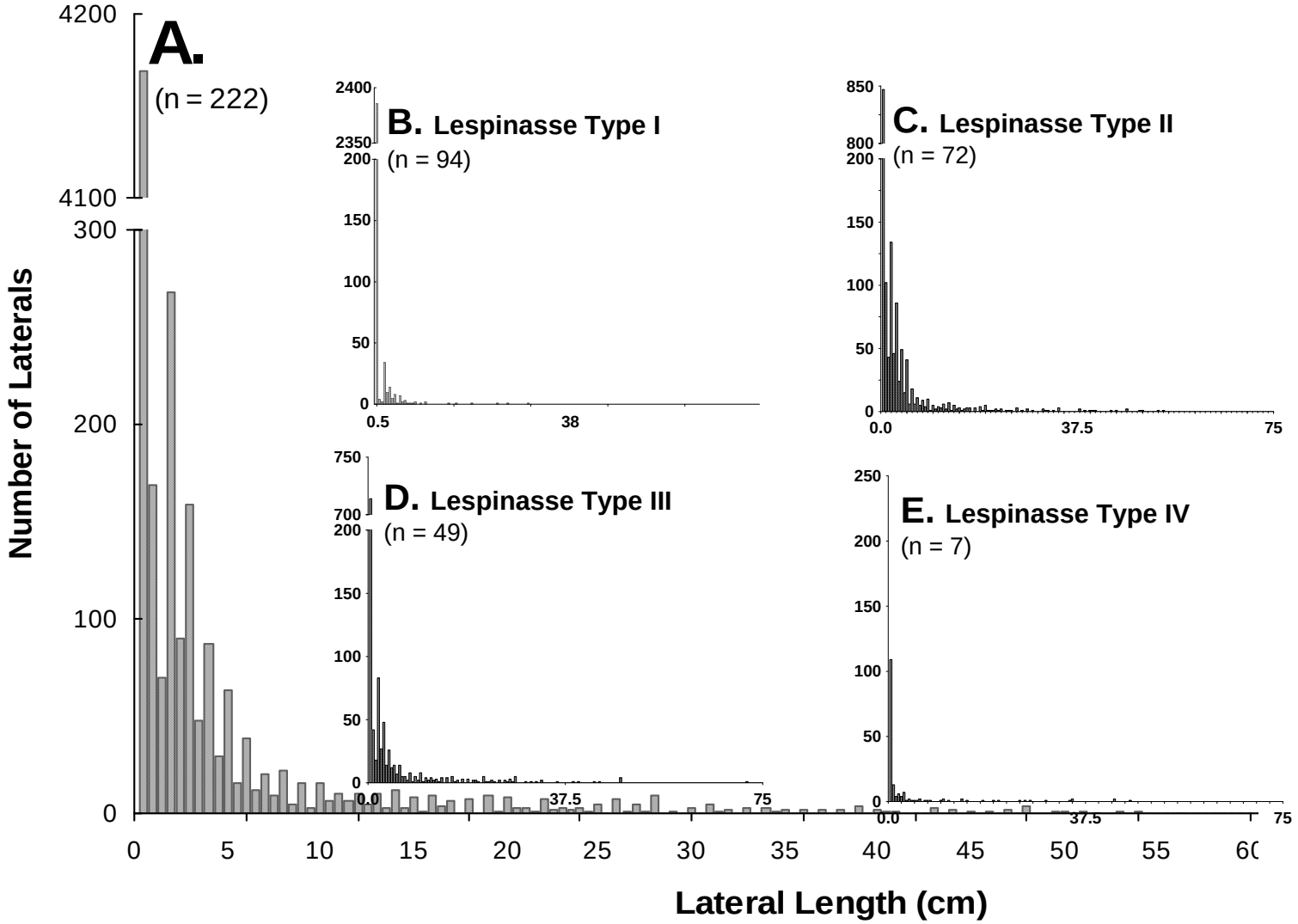

Figure 1. Frequency distribution of number of lateral lengths measured on the 2-year-old axis of 'Telamon' x 'Braeburn' crosses for either (A) all the trees combined; or only trees within (B) Lespinasse Type I, (C) Lespinasse Type II, (D) Lespinasse Type III, or (E) Lespinasse Type IV.

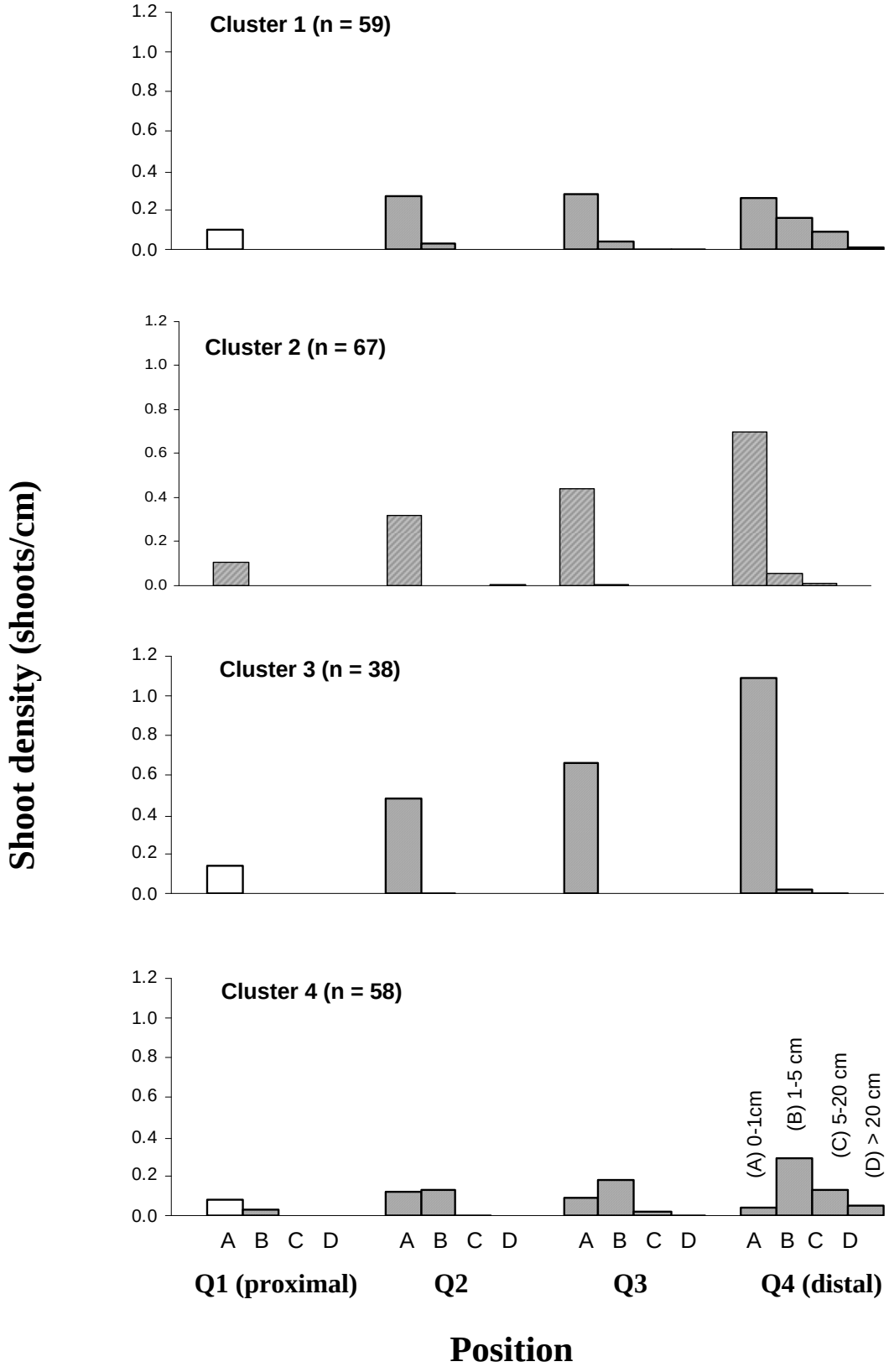

Figure 2. Shoot density of each length by position class for the four clusters. Shaded areas indicate significant differences between clusters for the specific variable at $\alpha = 0.05$.

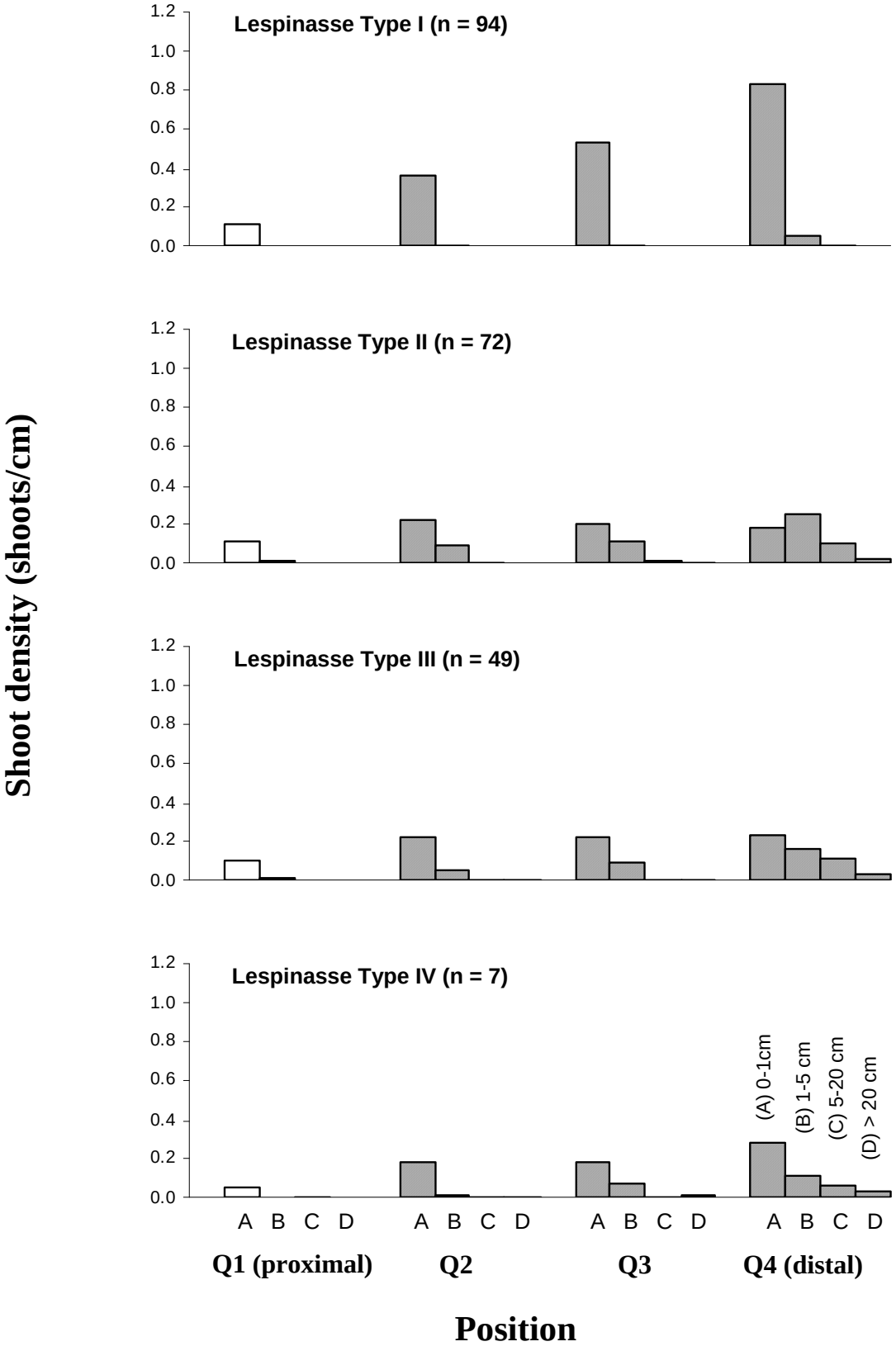

Figure 3. Shoot density of each length by position class for the four Lespinasse types. Shaded areas indicate significant differences between types for the specific variable at $\alpha = 0.05$.

General Discussion

Branching dynamics **-** The aim of this work was to better understand the dynamics underlying branch architecture. Branch architecture has been largely researched at single points in time and then dynamics of branch development are explained retroactively. In this dissertation, taking into account the position within the axis, the time of activity of buds and flowers were studied on a daily basis to provide an explanation for how dominance occurs within axes. Architectural characteristics were due to the relative time of activity and/or position of buds and laterals (positional and/or temporal competitions, respectively) within an axis. However, the type of competition differed between sites and therefore was most likely due to degree of chill unit accumulation. In the cool area in our study, there was a larger positional component (acrotony being more evident); in areas with a lower chilling unit accumulation there was a larger temporal component (primigenic dominance being evident).

While positional aspects of branch development are well-known in apple (Lauri, 2007), there were few studies on the temporal aspects; and while budburst potential of individual buds was known to differ through dormancy (Cook *et al.*, 1998a; Cook & Jacobs, 2000), there were few, if any, studies that detailed growth dynamics after budburst. This dissertation was a step in understanding the dynamics that precede the resulting architecture. Although the original idea for these studies was to characterize branch architecture in areas with inadequate winter chilling, it became increasingly clear that the majority of differences we observed were related to the gain or loss of acrotonic tendencies.

Even though organogenesis is known to occur basipetally within an axis (Lauri, 2007; Powell, 1995), all reproductive buds in our study had an equally high number of organs (spur leaves, flowers) indicating that there was sufficient time for all reproductive buds to proceed through organogenesis (Paper 1). This may be an adaptive strategy as reproductive buds will burst before vegetative buds (Paper 3) (assumed to be due to their lower chilling requirement (Naor *et al.*, 2003)), and areas with inadequate winter chilling are usually coupled with a more than sufficient autumn period (allowing organogenesis to proceed).

Within an axis (during forcing experiments), buds have an initially basitonic bursting tendency that becomes acrotonic as dormancy progresses (Cook *et al.*, 1998b; Jacobs *et al.*, 1981; Crabbé & Barnola, 1996; Champagnat, 1983). In our study, this was not evident as there was a higher budburst potential in the most distal quadrant in the warm area (less chill units accumulated) rather than in the cool area (more chill units accumulated) (Paper 3). The idea behind this involves the progression of both terminal and lateral bud dormancies relative to each other.

The terminal correlatively inhibited lateral budburst in the preceding season and continued until a point when the lateral buds have a greater growth potential than the terminal (i.e., progression of endodormancy of the terminal being longer than that of the laterals buds) (Paper 2). During this time, the lateral buds were also endodormant and therefore not able to burst due to physiological factors. After this, however, if temperatures are warm enough, then the lateral buds will burst before the terminal (being less dormant, not under strong correlative inhibition by the terminal, and not endodormant). In cold winter areas, lateral buds are most likely ecodormant while the chilling requirement of the terminal is being completed. However, although there were differences between 'Golden Delicious' and 'Granny Smith', generally in warm winter areas lateral bud dormancy is maintained via correlative inhibition by the terminal. As the terminal bud is exiting dormancy, it re-establishes its correlative inhibition over the laterals. At some point after the terminal has accumulated enough chilling, the terminal releases its dominance over the lateral buds. Perhaps, the chilling requirement for the terminal bud to release control over the laterals is longer than the chilling requirement to release the terminal bud from endodormancy in some cultivars.

If the chilling requirement is completely met, the terminal should have both primigenic dominance (i.e., it will burst first) and yet also a low correlative inhibition over the laterals (i.e., number of growing laterals is not reduced) (Paper 2). As reproductive buds are the first to burst, the increase in number of growing buds is due to an increase in the number of vegetative laterals (Paper 3). Therefore, these characteristics (both primigenic dominance of the terminal and low correlative inhibition by the terminal over the laterals), and not only days to budburst, should be taken into account when considering chilling requirement of a cultivar. This differs from previously research in which chilling requirements of 'Golden Delicious' and 'Granny Smith' were reported as similar (1050 and 1049 chill units, respectively) (Hauagge & Cummins, 1991a).

After budburst, there was a clear distinction between the warm and cool areas in the dynamics of bud outgrowth (Paper 3) and fruit set (Paper 1). In the warm area, there was a strong temporal component, and in the cool area there was a strong positional component to dominance within the axes. In the cool area, acrotonic branching (specifically brindle shoot development) and fruit set were not related to time of budburst and time of anthesis, respectively. However, in the warm area, the first buds to burst or flower had the greatest ability to grow longer (Paper 3) or set fruit (Paper 1), respectively, regardless of position in the shoot, insinuating a primigenic dominance effect with limited chilling (i.e., limited

reserves). Basically, in warm areas, there was a "first come, first serve" basis to allocating resources.

Even though there was the temporal component to resource allocation in the warm area, and positional one in the cool area, one characteristic was more innate in architecture than others and characteristic of both areas. Even with a loss of acrotonic budburst tendency and a decrease in the total number of buds that burst in the inadequately chilled area, there was the innate ability of axes to maintain the acropetal increase in number of growing laterals (higher percent of growing laterals in the distal half of the shoot as compared to the proximal half of the shoot) (Paper 3). This solely defines acrotony for a number of researchers (Hallé *et al.*, 1978; Barthélémy & Caraglio, 2007; Champagnat, 1978) and was true in our study as well.

Although time of activity (primigenic dominance) influences have been implicated in the development of acrotony (Bangerth, 1989), this has not been verified as far as we know. One of the main conclusions of this dissertation is that, with the exception of organogenesis in the season preceding growth, acrotonic tendencies (number of growing laterals, lateral length, fruit set) were not related to primigenic dominance of the distally located buds or flowers. This insinuates another, or an innate, acrotonic influence that occurs within the shoot (i.e., hormones, carbohydrates), and one that is independent of a bud's time of activity. In warm areas, both relative budburst and flowering time within an axis did depict the loss of acrotony.

A final part of this study was to quantify branch architecture and relate it to known qualitative apple branching ideotypes (Lespinasse types 1-4) (Paper 4). Even though it was possible to make clusters based on branching variables, it was not possible to relate these clusters to Lespinasse types. As it was possible to discriminate between Lespinasse types, the conclusion that can be made was that any genotype containing the columnar gene ('Telamon' was heterozygous for it in this study) should not be used in a cross to discriminate between branching types as it is dominant and therefore, produces a high percentage of progeny that are columnar. In order to accurately quantify architecture of progeny and relate them to Lespinasse types, there should be a somewhat equal representation of all 4 types in the progeny.

'Prolonged dormancy syndrome'- Even though this dissertation and research were designed specifically to characterize branch architecture in areas with inadequate winter chilling, it also characterized some of the symptoms of 'prolonged dormancy syndrome' (Paper 3). As far as I know, this was previously unmeasured with the exception of the low percentage of budburst

known to occur in apple grown in warm areas (Petri & Leite, 2004) which is attributed to the low growth potential of lateral buds in areas with inadequate winter chilling (Cook *et al.*, 1998b). In the warm area, the reproductive and vegetative budburst was prolonged, indicative of 'prolonged dormancy syndrome'. In addition, there was a distinct vegetative budburst pattern and a distinct reproductive budburst pattern (reproductive preceding the vegetative, supporting the findings of Naor et al. (2003)) in which reproductive buds had a lower chilling requirement than vegetative.

Conclusion

Apple architecture is mainly studied in areas with adequate chilling. In these areas, the development of architecture is controlled by positional competitions among buds. In areas with inadequate winter chilling, the architectural plasticity is very evident, as temporal competitions take precedence. Different mechanisms then are responsible for the development of architecture depending on the environment in which the tree grows.

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