Morphological and physiological aspects of the early phases of flower bud formation of apple

Promotor: dr. J. Tromp
hoogleraar in de tuinbouwplantenteelt, in het bijzonder de overblijvende gewassen

Morphological and physiological aspects of the early phases of flower bud formation of apple

F.A. Verheij

Proefschrift

ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr. C.M. Karssen,
in het openbaar te verdedigen
op maandag 15 januari 1996
des namiddags te vier uur in de Aula
van de Landbouwuniversiteit te Wageningen

ISBN: 9789022

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Verheij, F.A.

Morphological and physiological aspects of the early phases of flower bud formation of apple / F.A. Verheij. - [S.l. : s.n.]. Fig., Tab.
Thesis Wageningen. - With ref. - With summary in Dutch.
ISBN 90-5485-484-7
Subject headings: flower bud formation; apple.

Stellingen

1. Een korte plastochron en de aanwezigheid van een zeker minimum aantal bladvormingen in de knop zijn geen inducerende factoren in de bloemknopvorming van appel.

   Dit proefschrift

2. Het antagonisme tussen de groei en bloemknopvorming bemoeilijkt de bestudering van de bloemknopvorming bij appel in hoge mate.

   Dit proefschrift

3. Tussen het tijdstip van afsluiten van de groei, het begin van de bloemdifferentiatie en de bloemkwalitiet in het volgende voorjaar bestaat geen rechtstreeks verband.

   Dit proefschrift

4. Het feit dat polyaminen in alle tot nu toe onderzochte organismen zijn aangetroffen, en dat hun betrokkenheid in een groot aantal fysiologische processen is aangetoond, wijst er op dat polyaminen de ontwikkeling van planten beïnvloeden door middel van eenzelfde fundamenteel mechanisme in alle organismen.

5. Bij overblijvende gewassen verdient het dynamische karakter van de relatie tussen vorm en funktie meer aandacht.

6. Binnen populaties onderzoekers is statistische kennis doorgaans niet normaal verdeeld.

7. De in wervingsadvertenties veel gestelde eis van stress-bestendigheid wordt overbodig indien meer waarde wordt gehecht aan het plezier hebben in het werk.

8. Dat een natuurproduct, zoals mest, door toedoen van de mens op grote schaal het milieu bedreigt, toont aan hoezeer het evenwicht tussen mens en natuur is verstoord.

9. Het gebruik van de termen onderwijs"instituten" en onderzoeks"scholen" werkt verwarrend.

10. De vrees voor een teloorgang van regionale verschillen bij de éénwording van Europa is ongegrond zolang een snoei-advies aan Nederlandse fruittelers: "verenkelen om verkaling en verdikking tegen te gaan", door Belgische collega's wordt vertaald met: "ontdubbel om ontbloting en aanzetting te voorkomen".

    Van Kessel, T. 1995. Vuistregels bij de snoei van appelbomen niet klakkeloos toepassen. Fruitteelt, 85: 11-13.

11. Buitensporige mobiliteit blijkt een kwaad dat zichzelf straft: het spoor loopt dood.

12. Ondanks de toename van de gemiddelde levensduur en de verkorting van de gemiddelde arbeidsduur, wordt de roep dat men tijd tekort komt steeds luider.

Stellingen behorende bij het proefschrift:
'Morphological and physiological aspects of the early phases of flower bud formation of apple'

F.A. Verheij
Wageningen, 15 januari 1996

Abstract

Verheij, F.A. 1996. Morphological and physiological aspects of the early phases of flower bud formation of apple. Dissertation Wageningen Agricultural University, Wageningen, The Netherlands. 148 pp.; English and Dutch summaries.

For consistent yields in apple fruit production, knowledge of the factors affecting flower bud formation is required. The aim of this study was to gain more insight in the role of endogenous factors in flower bud formation of apple. The effects of temperature, applied gibberellin (GA_{4+7}), the presence of fruits, defoliation and bending on the plastochron (the time interval between formation of successive primordia by the meristem) and the number of appendages per bud at the start of floral differentiation were studied. Although most treatments distinctly affected flower bud formation, the plastochron was only affected by temperature and defoliation. Considerable variation was observed in the number of appendages at the start of floral differentiation, which varied between cultivars, between experiments with the same cultivar and between bud positions. It is concluded that the length of the plastochron and the number of appendages are not critical to the occurrence of flower bud formation.

Defoliation inhibited flower bud formation and caused an immediate reduction in the soluble sugar content of the shoot. Bending slightly enhanced flower bud formation, or had no effect, and did not affect the soluble sugar level in the shoot, but increased starch content. The poor correlation between the assimilate level of the shoot and the number of flower buds formed suggests that it is not a main regulatory factor in flower bud formation.

It was further investigated whether ammonium affects flower bud formation of apple through affecting endogenous levels of polyamines (PAs). Ammonium and applied PAs have previously been shown to stimulate flower formation of apple. However, due to a lack of effect of ammonium on flower bud formation in the present work, no conclusive evidence could be given. PA levels of buds did not greatly respond to treatments affecting flower bud formation (applied GA_{4+7} and shoot bending) despite the fact that both treatments did affect flower bud formation. PA levels did not show distinct changes during the supposed time of floral induction and floral differentiation. It is tentatively suggested that PAs are not major inductive stimuli of flower bud formation of apple.

Keywords: ammonium, apple, arginine, assimilates, bud morphology, defoliation, flower bud formation, gibberellin, *Malus domestica* Borkh., nitrate, plastochron, polyamines, shoot bending, shoot growth, temperature.

And pluck till time and times are done
The silver apples of the moon
The golden apples of the sun.

(Yeats)

Voorwoord

Aan de totstandkoming van dit proefschrift hebben veel mensen een bijdrage geleverd en daarvoor wil ik iedereen hartelijk bedanken. Een aantal mensen wil ik in het bijzonder noemen.

Allereerst ben ik mijn begeleider en promotor Prof. J. Tromp erkentelijk voor zijn stimulerende en kritische begeleiding, zowel tijdens de uitvoering van het onderzoek als bij het schrijven van het proefschrift. Zijn begeleiding heeft een grote bijdrage geleverd aan de goede afronding van dit proefschrift. Oege Borsboom was met zijn jarenlange praktijkervaring en grondige kennis van de teelt van appelbomen een waardevolle bron van informatie die ik veelvuldig heb geraadpleegd. Oege, je was bovendien een zeer gezellige kamergenoot. Arjen van de Peppel ben ik zeer dankbaar voor zijn expertise en hulp bij de vele chemische analyses. Dankzij jou kennis van de apparatuur is het niet bij malen gebleven. Ruud Lamers, Henk Meurs en het overige kaspersoneel wil ik bedanken voor de goede verzorging van de planten, George Martakis voor zijn hulp bij de statistische verwerking en Jan Peeters van Van Gaal voor de bemestingsadviezen. De studenten Hans de Wild, Marlijn Vos en Nicole van der Werf hebben op enthousiaste wijze meegewerkt aan dit onderzoek.

Het verrichten van promotie-onderzoek heb ik als zeer leerzaam ervaren, niet alleen op het inhoudelijke vlak, maar ook op veel andere terreinen. Ik heb met name geleerd dat een goede werksfeer enorm belangrijk is voor het plezier in je werk en daarmee de productiviteit. De vakgroepsmedewerkers wil ik bedanken voor de prettige werksfeer. Ik hoop in (een) volgende werkkring(en) weer zulke collega's aan te treffen!

Mede door het ontbreken van uitzicht op werk heb ik de afgelopen tijd ook als erg zwaar ervaren. Mw. L. Drees van de Geldergroep wil ik van harte bedanken voor het bieden van een luisterend oor, en de professionele hulp bij de (her)-oriëntatie op de arbeidsmarkt. Mijn ouders, Nicole en Alfred bedank ik voor hun betrokkenheid en steun, en ook mijn vrienden die voor de nodige hulp en afleiding hebben gezorgd. Tenslotte, Henk, bedankt voor je geduld. Je vormde thuis een waardevol rustpunt.

Contents

1. General introduction

Flowering, the first step of sexual reproduction of plants, is of paramount importance to agriculture, horticulture, and plant breeding as it is a major factor determining yield. Not only for economic, but also for scientific reasons, the dramatic morphological and physiological changes occurring during the transition of the meristem from vegetative to generative have been the topic of much research. In unravelling the mechanisms involved in the control of generative development, attention has mainly concentrated on herbaceous species, in particular those that flower in response to specific environmental stimuli such as day length or vernalization (Searle, 1965; Jackson and Sweet, 1972; Bernier *et al.*, 1993). This attribute enables the flowering response to be generated with a high degree of certainty and at a requested time. In most woody species, including apple, however, flowering is not triggered by a single environmental factor, but a wide range of factors, such as temperature, light, nutrition, water supply and growth regulators may all determine the response. Additionally, not all potential flowering sites (bud meristems) respond to the prevailing conditions in a similar manner, as only part of the meristems actually become generative. This inability to predict with certainty where and when a flower bud is likely to form has been a major handicap to better understanding flowering in trees (Crabbé, 1984; Marcelle, 1984). Furthermore, the flowering process from induction until anthesis is normally drawn out over a long period of time; in apple the entire process takes approximately a year. This means that the different stages of the flowering process occur under changing environmental conditions and coincide with other developmental activities occurring in the tree.

These factors combined make woody species a much more complex topic of research than herbaceous species. However, it is assumed that no fundamental difference exists in flowering of herbaceous and woody species, but that the mechanism determining which meristems will become floral is more intricate in woody species (Jackson and Sweet, 1972; Marcelle, 1984; Bernier, 1988). Despite this greater complexity considerable attention has nevertheless been devoted to reveal the mechanism by which flowering of woody species is controlled. A large part of this research has been done on temperate fruit trees and in particular on apple. Although the literature is extremely voluminous, many gaps still exist in the understanding of the mechanisms controlling flowering of apple. The work reported here is intended to fill some of these gaps. Before introducing this work, a

short review of the current views on the physiological control of flower bud formation is presented. The annual growth cycle of the apple tree, morphological aspects of (flower) bud formation and the terminology of flowering are also briefly discussed.

1.1 Morphological aspects

The annual growth cycle of the apple tree

As is characteristic of many temperate perennial species, the apple tree passes the winter in a state of 'rest', which requires a period of chilling before the meristems can respond to rising spring temperatures (Jackson and Sweet, 1972; Abbott, 1984; Gur, 1985). In the spring, when temperatures become sufficiently high to support growth, resting buds break to produce two different types of structures. Vegetative buds give rise to a leafy shoot, while generative buds, also known as flower buds, give rise to the flower cluster, i.e. a number of leaves and usually 5-6 pedicelled flowers borne on a short stem known as the bourse. A bud (the bourse bud) is usually found in the axils of one or two of the leaves subtending the inflorescence, and this bud may grow out to form a shoot (the bourse shoot) (Bijhouwer, 1924; Abbott, 1984). A number of vegetative and generative buds do not become active in the spring, and remain dormant or abort.

Following bud opening, shoots arising from vegetative buds or bourse buds may elongate considerably due to the extension of internodes and the production of new leaves. As leaves unfold, buds (known as axillary or lateral buds) are formed in the axils of each leaf. Shoots can cease growth at any time during the season. Internode elongation and the unfolding of leaves stop and a terminal bud is formed around the growing point. A large number of shoots scarcely elongate at all and only produce a rosette of a few leaves before a terminal bud starts to form. These short shoots are known as spurs and the terminal bud as spur buds. At a certain time during the season, all shoots still actively growing cease growth by forming terminal buds. This time, known as cessation of shoot growth, generally occurs in mid-summer but can vary considerably with age, culture conditions, climate and cultivar (Forshey and Elfving, 1989). The ability to form buds at any time during the season enables the tree to respond rapidly to changing conditions.

With the formation of terminal buds, the active growth period of the tree ceases. The buds are in a state of summer dormancy, which seems to be imposed

by other parts of the tree, since growth can resume by treatments such as pruning and defoliation (Abbott, 1984). Despite apparent quiescence in the tree, physiological processes, such as photosynthesis, fruit development and root growth still continue. There may also be considerable activity inside the buds, as during this period the morphological differentiation of the inflorescence takes place (Bijhouwer, 1924; Zeller, 1955, 1960). In autumn, as temperatures decrease, physiological activity declines. The rate of developmental activity in the buds is also reduced, but is never completely arrested and continues throughout the winter period (Zeller, 1955, 1960). Summer dormancy gradually changes into winter dormancy, which requires a period of low temperatures to overcome. Chilling requirements are usually satisfied by mid-winter, but bud break is prevented by the prevailing cold weather, a condition known as imposed dormancy (Abbott, 1984). As temperature rises in the spring, bud activity again increases, and the annual cycle is complete.

The morphology of the bud

Morphologically, buds consist of a greatly shortened stem bearing a number of modified leaf forms (appendages) (Bijhouwer, 1924; Abbott, 1970). Bud formation starts with the withering and eventual abscission of the lamina and petiole of a leaf primordium at some distance from the apical meristem (Abbott, 1970). Simultaneously, the base of the primordium swells and the stipules fuse with it, thereby forming the outer scale of the bud. The laminae, stipules and petioles of successively smaller primordia similarly wither, and also become bud scales. Some time after the start of bud formation, leaf primordia are first transformed into transition leaves before becoming scales. Transition leaves have greatly enlarged stipules in relation to the lamina and are green and hairy. A number of leaf primordia ("true" leaves) do not change, i.e. the stipules remain small as compared with the lamina. During bud opening in the following spring, the "true" leaves give rise to the leaves of the shoot or cluster, whereas the bud scales and transition leaves abscise (Bijhouwer, 1924). The apex of vegetative buds remains unchanged and resumes vegetative growth in the following spring. The initial phase of bud development of generative buds is similar to that of vegetative buds, and they only become distinguishable from vegetative buds during floral differentiation (see next section).

Flower bud formation

The terminology of flowering is confusing. In apple, flower bud formation is generally used to describe the entire sequence of events leading to the formation of flower buds. It can be divided into three successive developmental processes, each of these steps being amenable to independent regulation (Bubán and Faust, 1982).

1. Floral induction occurs during the period of active growth.
2. Floral differentiation starts around the time of cessation of shoot growth and continues until anthesis.
3. Anthesis (flowering, bloom) occurs in the following spring.

These terms will be used in the context described below. The term flower bud initiation, although frequently encountered in the literature, is avoided, as by different authors it is used to describe different stages of the flowering process.

Floral induction

In apple, floral induction denotes the process by which the meristem becomes committed to form flower buds. Unlike herbaceous species, in woody species the physiological events occurring during induction have not been extensively studied (Marcelle, 1984). However, similar to many herbaceous species it does not involve visible modification of the meristem, but only changes at the (cyto)biochemical or genetic level (Bernier, 1971, 1988; Bubán, 1981; Bubán and Faust, 1982). Although during induction bud meristems become committed to flower, they do not appear to be competent to express that determination, which may be due to the presence of some inhibiting influence (Lord and Eckard, 1987).

In herbaceous species the terminology as introduced by Evans (1969) is frequently used. Induction denotes the perception of the photoperiodic stimulus in the leaves resulting in the production of a chemical signal which is transported to the shoot apex. Floral evocation marks the arrival of the stimulus at the apex which commits the plant to the subsequent formation of flower primordia. In apple, perception of a signal by the leaves and translocation of this signal to meristems has not been demonstrated, and thus no distinction is made in perception of the floral stimulus in the leaves or by the meristem.

Floral differentiation

Floral differentiation refers to the morphological transformation of the apex leading to the formation of an inflorescence. During this process previously repressed genetic information is translated to form reproductive structures (Searle, 1965; Wellensiek, 1977; Bernier, 1988). There are many similarities in early floral differentiation in apple and in herbaceous plants (Bernier, 1988). A change in the meristem from the predominantly flat- to a dome-shape marks the beginning of generative development. This coincides with a distinct change in zonation typical of the vegetative apex (Gifford and Corson, 1971; Bubán, 1981) and an increase in mitotic activity and cell division (Bernier, 1971, 1988; Bubán and Faust, 1982). The rate of primordia production by the apex increases (Langer and Bussel, 1964; Luckwill and Silva, 1979) and bracts (leaf-forms in which the lamina and stipules are entirely suppressed) are formed. In the axils of the uppermost 2-3 leaves and 2-3 bracts flowers develop, and the apex is transformed in the apical or "king" flower (Bijhouwer, 1924; Pratt, 1988). Whorls of sepals, petals, stamens and carpels are initiated in rapid succession, and differentiation then proceeds at a slower rate throughout the winter period (Zeller, 1955). Just before and during bud opening in the spring the last developmental processes, i.e. development of pollen sacs and ovules take place (Zeller, 1960; Bergh, 1985). The start of floral differentiation can be seen under the microscope around the time of cessation of shoot growth (Goff, 1899; Davis, 1957; Bubán and Faust, 1982); in spur buds it generally occurs several weeks earlier than in lateral buds (Gibbs and Swarbrick, 1930; Zeller, 1960; Bubán and Faust, 1982).

Anthesis

Anthesis is the culminating stage of the flowering process, during which the stamens produce pollen and the pistil is receptive to pollination and fertilization. It occurs in the spring of the year following induction, when the temperature becomes sufficiently high to support growth activity. Leaves and flower parts in the bud expand, resulting in bud opening. The sepals and petals of the flower enlarge and move apart to expose the stigmas and stamens. After pollination the flowers may set fruit. Flowers which do not set fruit abscise.

Much experimental work on the application of various treatments at different times during the season has revealed that floral induction occurs early in the

season, i.e. from around the time of bloom until several weeks after full bloom (Luckwill, 1975; Bubán and Faust, 1982; Tromp, 1984). Thus the period from the start of the flowering process (induction in spring or summer) until expression (anthesis occurring in the following spring) usually takes nearly a year. It is not yet clear to what extent induction is a reversible process, but it is generally assumed that once a meristem has reached the differentiation phase it is irreversibly committed to produce flowers (Jackson and Sweet, 1972).

As was discussed, buds may be formed in three different positions on the tree, namely in the axils of leaves (lateral buds), terminally on shoots (terminal buds) or terminally on spurs (spur buds). It is generally assumed that any bud on the tree is capable of becoming floral. However, only part of the total number of buds actually do so. The tendency of buds to become floral depends on a complex of factors, and it may be affected by both external factors, such as temperature, light, water supply and nutrition, and internal factors such as hormones and resource levels. It also varies with morphological aspects of bud development (see later, pg. 12) and bud position. Spur buds have a high tendency to become floral, while this tendency varies with cultivar, age and vigour of the tree in terminal buds of shoots and lateral buds (Bubán and Faust, 1982; Forshey and Elfving, 1989). Along the shoot there is also a gradient in flowering potential. Middle buds have a higher tendency to become floral than basal or distal buds (Zeller, 1960; Bubán and Faust, 1982). In some cases, particularly after many flowers are damaged by frost, inflorescences may also be formed directly on vegetatively growing shoots, i.e. without prior bud formation. This is known as second flowering (Schupp and Ferree, 1987). It thus seems that in apple every growing point is capable of flowering and at any time of the year, but at most times this is prevented by unfavourable internal and/or external conditions (Luckwill, 1970a).

1.2 Physiological aspects

Extensive investigation of the factors affecting flower bud formation of apple has led to a large number of theories concerning its possible control. Before the discovery of plant hormones, flowering of apple was explained on a nutritional basis (particularly the C/N ratio) following a similar explanation for the fruitfulness of the tomato by Kraus and Kraybill (1918). A relatively high C/N ratio was considered to be promotive to floral induction, whereas a relatively low C/N ratio was regarded to promote vegetative growth and inhibit floral induction. Many

responses of apple trees could be explained by this theory, as conditions or treatments favouring carbohydrate accumulation (high C/N ratio) generally promote flower bud formation, while conditions causing carbohydrate depletion, or applications of N in the spring (low C/N ratio) inhibit it (Jackson and Sweet, 1972). However, despite substantial supporting evidence, experimental proof for this theory was never provided (Kobel, 1954; Davis, 1957). With the discovery of hormones from the 1930's onwards, the nutrient theory was largely abandoned in favour of a hormone theory. At first attention concentrated on the search for a specific flower promoting hormone ("florigen") believed to be synthesized in the leaves. In woody species the hypothesis of a single factor promoting flower bud formation has never been popular (Jackson and Sweet, 1972; Marcelle, 1984), and also in herbaceous species, despite considerable efforts, a single inductive hormone has not been isolated. A complex picture of control has gradually emerged, in which the previous nutrient and hormone theories are reconciled, as both groups of factors are thought to be essential for the flowering process. As will be discussed in more detail later, part of the aim of the present work was to study the possible interaction between nutrients and endogenous growth factors. However, first current concepts on the physiological regulation of flower bud formation of apple will be discussed in more detail.

Nutrients and assimilates

In plants in general much controversy exists as to the precise regulatory role of nutrients and assimilates in the flowering process, and three different views are regularly encountered (Bodson and Bernier, 1985). In the first view assimilates and nutrients only have a supportive role, functioning as substrates for metabolic activity. In the second view, commonly referred to as the "nutrient diversion" theory (Sachs, 1977), assimilates and nutrients are the primary controlling factors. According to this theory, a supply of assimilates greater than that required for vegetative growth is the signal for induction, i.e. induction will not occur if a target tissue receives less than some threshold level of substrates. In the third view assimilates and nutrients are part of a complex controlling system including both growth substances and substrates (the theory of "multifactorial control") (Bodson and Bernier, 1985; Bernier, 1988).

In apple, although there is little doubt that sufficient nutrients and assimilates are required for flower bud formation (Jackson and Sweet, 1972; Landsberg and Thorpe, 1975; Faust, 1989), it is generally not assumed that a change in assi-

milate concentration at the meristem is sufficient to trigger induction (Harley *et al.*, 1942; Kobel, 1954; Jackson and Sweet, 1972). The current thought is that assimilates are part of a complex of factors involved in the regulation of flowering, similar to that of "multifactorial control".

Hormones

A wealth of data obtained with exogenous applications of hormones and synthetic growth regulators suggest that endogenous hormones also have some regulatory role. However, much of this work has been empirical, and still little is known on the precise role of endogenous factors in the regulation of flowering (Gur, 1985). Current notions on the role of the five recognized groups of hormones are presented below.

Gibberellins

Of the hormones, gibberellin (GA) has most frequently been associated with flower bud formation of apple. Application of GA was first shown to inhibit flower bud formation of apple by Guttridge (1962) and Marcelle and Sironval (1963) and on numerous occasions since. Applied GA also inhibits flower bud formation in a wide range of other fruit crops, such as pear (Griggs and Iwakiri, 1961; Huet, 1973), peach (Hull and Lewis, 1959), almond, apricot, plum, cherry (Bradley and Crane, 1960), and *Citrus* (Monselise and Halevy, 1964; Goldschmidt and Monselise, 1972). Although it is often claimed that GA represses flowering by enhancing shoot growth (see later section), it is generally assumed that the effect of GA is direct, as GA may inhibit flower bud formation without a large effect on growth (Marino and Greene, 1981; Tromp, 1982; Greene, 1989).

The observation that exogenous application of GA inhibits flower bud formation of apple, has prompted the suggestion that endogenous GA similarly has a regulatory role (Luckwill, 1970a; 1975; Hoad, 1980). However, the evidence is largely circumstantial. Actively growing shoots and young seeded fruits are rich sources of GA and can both inhibit flower bud formation of apple (see later section). Applications of chemical growth retardants which may interfere with GA-action or biosynthesis, such as daminozide (Alar, SADH), chlormequat chloride (CCC) and paclobutrazol generally promote flower bud formation of apple, suggesting that induction of flowering may require low levels of endogenous GA (Luckwill, 1975; Hedden, 1990). However, the results with applications of growth

retardants have been highly variable, and enhanced flowering after applications with chemical growth retardants is not always associated with lower endogenous levels of GA (Grausland, 1972; Ramirez and Hoad, 1981).

Cytokinins

Cytokinins have almost exclusively been associated with promotion of flower bud formation. Zeatin or benzyladenine (BA) introduced into the transpiration stream promoted flowering of apple (Ramirez and Hoad, 1981; Gur and Sarig, 1983; Skogerbø, 1992). Spraying apple trees with BA soon after anthesis resulted in an increase in return bloom (McLaughlin and Greene, 1984; Greene and Autio, 1989; Unrath, 1989). Luckwill and Whyte (1968) and Grochowska and Karaszewska (1978) showed that the concentration of cytokinins emanating from the roots was highest at full bloom, when buds are most responsive to induction, and the levels subsequently decrease to a low level in August. However, as for GA, conclusive evidence for a promotive role for endogenous cytokinins is still lacking. Luckwill (1970a, 1975) and Hoad (1984) assumed that a balance of cytokinins, as floral promoters, and GA, as floral inhibitor is critical to flower bud formation. Late in the season, when endogenous levels of GA are low, high cytokinin levels would promote flower bud formation by ensuring sufficient meristematic activity for the differentiation of flower parts.

Auxins, abscisic acid and ethylene

The effect of auxins and abscisic acid (ABA) is not clear, as both promotion and inhibition of flowering have been reported (Luckwill, 1970a; Hoad, 1980; Ramirez and Hoad, 1981; Bubán and Faust, 1982; Gur, 1985; Bangerth *et al.*, 1986). Ethylene generally enhances flower bud formation, although the effect may be indirectly mediated through reduced shoot growth or increased fruit thinning (Walsh and Kender, 1982; Miller, 1988).

The importance of shoot growth, leaves, fruits and roots

Many years of research and observation have established that in fruit trees an antagonistic relationship exists between shoot growth and flowering (Forshey and Elfving, 1989). Treatments which stimulate vegetative growth often reduce flowering, while conversely, treatments curbing growth vigour tend to enhance it

(Luckwill, 1970a; Miller, 1988; Forshey and Elfving, 1989). The duration of growth may also affect flower bud formation. Treatments leading to early cessation of shoot growth tend to stimulate flower bud formation, while prolonged vegetative growth inhibits it (Swarbrick, 1929; Luckwill, 1970a). The inhibitory effect of excessive shoot growth on flower bud formation is often ascribed to the production of GA in terminal regions of rapidly elongating shoots. Young leaves and the upper internodes are known to be a major source of GA in the tree (Kato and Ito, 1962; Grausland, 1972). Early cessation of shoot growth may be conducive to flowering by enabling floral differentiation to proceed at a time when cytokinin levels are still sufficiently high (Luckwill, 1970a). However, despite their frequent association, flowering may be affected without a simultaneous effect on growth, suggesting that each developmental process is independently controlled (Luckwill, 1970a; Forshey, 1989).

Not only excessive vegetative development, but also too weak vegetative development may inhibit flower bud formation. The inhibitory effect of treatments such as defoliation, shading and leaf injury have long established that flower bud formation requires the presence of a sufficiently large and viable leaf area (Harley *et al.*, 1942; Davies, 1958; Fulford, 1966b; Jackson and Palmer, 1977; Cordes, 1987). The requirement for leaves has been explained both in terms of their acting as a source of assimilates and of hormones (Jackson and Palmer, 1977). Ramirez and Hoad (1981) showed that the inhibitory effect of leaf removal on flower bud formation could be reversed by application of cytokinins, which suggests that cytokinins may be the promotive factors emanating from the leaves. Leaves may also be of indirect importance by sustaining the transpiration stream, thereby ensuring that promotive substances produced elsewhere in the plant reach the sites of flower formation (Luckwill, 1970a; Hoad, 1980).

The presence of a large crop is also well known to have an inhibitory effect on flower bud formation of apple (Hoad, 1980, 1984; Bubán and Faust, 1982; Faust, 1989). Initially this was thought to be due to competition for assimilates as fruits constitute a large "sink" for carbohydrates at the time when the decisive steps for flower bud formation occur (Hoad, 1980). However, Chan and Cain (1967) demonstrated that de-seeded fruits (unpollinated) did not reduce flower number, indicating that the seeds of the fruits were the source of inhibition. The attention subsequently turned from carbohydrates to hormones. Developing seeds were shown to be rich sources of GA_4 and GA_7 which appear 4-5 weeks after full bloom and reach a maximum after 9 weeks (Dennis and Nitsch, 1966; Luckwill *et al.*, 1969; Ramirez and Hoad, 1981). Many other GA-types have been identified

in apple seeds (Hoad, 1978; Lin *et al.*, 1991) and the seeds of other plants have also been shown to be rich sources of hormones (Pharis and King, 1985; Graebe, 1987).

Strong competition exists between all other growth centres in the tree (Faust, 1989; Forshey and Elfving, 1989). Fruiting suppresses both shoot and root growth (Forshey and Elfving, 1989). Conversely, excessive shoot growth during the period of fruit development may cause fruit abscission (Forshey and Elfving, 1989). A viable root system is essential to flower formation, perhaps by ensuring a supply of cytokinins, as the roots are a major source of cytokinins in the plant (Van Staden and Davey, 1979). Root pruning or impaired root growth may benefit flower bud formation (Geisler and Ferree, 1984), but this may be related to the concurrently occurring reduction of shoot growth.

Environmental factors

Flowering of apple is not triggered by a single environmental factor such as day length or temperature, but many environmental factors may nevertheless affect the response (Gur, 1985). Flowering is enhanced by high levels of light in comparison with low levels, and by moderately low temperatures rather than high temperatures (Jackson and Sweet, 1972; Tromp, 1976, 1984; Gur, 1985). A low relative air humidity (RH) and conditions leading to moisture stress (excess moisture or drought) generally enhances flowering (Tromp, 1984; Gur, 1985). How the effect of environmental factors is mediated is still largely unknown, but it is often associated with altered shoot growth.

1.3 Relevance and aim of the thesis

The object of fruit tree cultivation is the consistent harvest of a high yield of good quality fruits. This requires the annual production of an optimum number of strong flower buds and favourable conditions for pollination and fruit set. Regulation of the number of flower buds has long been a central consideration in commercial fruit production, and already in the earliest writings recommendations for manipulations to maximize flower formation and increase productivity are found (see refs. in Davis, 1957). From this accumulated experience and through the contribution of research, the grower has at his disposal several cultural techniques to influence the number of flower buds. These treatments, such as grafting

on dwarfing rootstocks, application of growth regulators, bending of branches, ringing and root pruning, aim to promote flowering by suppressing vegetative growth. However, such techniques may be time consuming, difficult to manage or have undesirable side effects on fruit set, fruit quality or the life-span of the tree. Furthermore, the success of these methods is rather unpredictable, not only varying between cultivars or with different growing conditions, but also from tree to tree. Inconsistent response has been a major problem in successful regulation of flower bud formation of apple. Insight into the endogenous factors controlling floral induction of apple would clarify the problems of inconsistent response, and would enable the grower to more effectively apply cultural practices (Hoad, 1980). It would also minimize the reliance on artificial growth regulators, which may take away public concern for the use of chemicals in food production (Miller, 1988).

The aim of the present research is to contribute to a clearer understanding of endogenous control of flower bud formation of apple. In the first part of this study the importance of morphological aspects of bud development to the occurrence of flower bud formation is described. In the second part interest was focused on the interaction between nutrients and growth substances. It was investigated whether the distinct developmental effect induced by nutrients (such as ammonium) is mediated by modifying internal levels of endogenous growth factors (such as polyamines). These aims are discussed in more detail below.

The importance of morphological aspects of bud development to flower bud formation

In the literature two different views concerning regulation of flower bud formation of woody species are encountered. The first is that flower formation requires the synthesis of (a) specific flower-inducing substance(s). However, despite considerable efforts, the existence of such substances or the chemical nature has not been established with certainty. The second, based on the substantial evidence pointing to the participation of inhibitors in the regulation of flowering, assumes that induction of flowering is due to the removal of factors inhibiting reproductive development (Fulford, 1966b; Goldschmidt and Monselise, 1972; Luckwill, 1975). The latter view suggests that flowering is an autonomous event, which will always occur provided it is not inhibited. Thus, in apple, it has been suggested that a bud will become floral providing GA levels are sufficiently low.

However, another factor which may inhibit autonomous flower bud forma-

tion is insufficient bud development. The importance of bud development, and in particular the plastochron (the time interval between the formation of successive primordia by the meristem), to flower bud formation of apple was demonstrated by the elaborate work of Fulford (1965, 1966a,b,c), who by periodic dissection of spur buds showed that the occurrence of flower bud formation was related to the length of the plastochron. If the plastochron was 7 days flowers formed, while if it was 18 days or more the buds remained vegetative. A plastochron of less than 7 days early in the season caused buds to grow out in a second flush of growth. Treatments which inhibited flower bud formation, such as defoliation and the presence of fruits, simultaneously lengthened the plastochron (Fulford, 1965, 1966b,c).

The results of Fulford were subsequently used by several authors as a possible explanation of how treatments affect flower bud formation. Many factors which affect flower bud formation of apple are most effective early in the season, i.e. several weeks after full bloom (Davis, 1957; Luckwill, 1975; Bubán and Faust, 1982; Tromp, 1984; Greene, 1989). Since this is well before the morphological differentiation of flower parts, it has been suggested that factors which promote or inhibit flowering in apple exert their effects during early bud development by modifying the plastochron, rather than directly upon the floral induction process itself. Thus, the effect of hormones (Luckwill, 1975), defoliation (Fulford, 1965), the presence of fruits (Fulford, 1966c), fertigation (Dencker and Hansen, 1994) and temperature (Tromp, 1976, 1980, 1984) have been explained in terms of an effect of modifying the plastochron. For example, Tromp (1976, 1980, 1984) suggested that the inhibitory effect of a high temperature on flower formation was an indirect result of increased growth activity, whereby an increased production of GA by the shoots results in a lengthening of the plastochron. Whether temperature affects flower bud formation through the plastochron has not been investigated. A few studies have tested the effect of applied growth regulators on the plastochron and have shown that growth regulators, such as GA, affect flower bud formation without a simultaneous effect on the plastochron (Luckwill and Silva, 1979; McLaughlin and Greene, 1991a, b). However, these authors did find that flower bud formation generally did not occur before a certain number of appendages had been formed in the bud, suggesting that flower bud formation requires a specific level of complexity, i.e. the presence of a minimal number of appendages (Landsberg and Thorpe, 1975; Luckwill, 1975; Abbott, 1977). Thus, a short plastochron may be of indirect importance by ensuring that a minimal number of appendages is reached at a time before bud activity slows due to

unfavourable external conditions.

Morphological aspects of plant development are often ignored by physiologists, which is unfortunate, as this information may give some insight into the physiological control (Chailakhyan, 1968; Bernier, 1988). In this thesis the importance of morphological aspects of bud development (the plastochron and the number of appendages per bud) to flower bud formation is investigated.

The regulatory role of polyamines in flower bud formation

As was discussed, the flowering response may be determined by an interaction between (nutrient and/or carbohydrate) resources and endogenous growth factors. Nitrogen plays a central role in plant physiology (Trewavas, 1985), and particularly the form in which nitrogen is applied to the roots (as ammonium or as nitrate) may induce a wide range of physiological responses (Barker and Mills, 1980). These responses may be mediated by altered hormonal levels, as the mineral supply has been shown to affect the hormone status of the plant (Marschner, 1983; Moorby and Besford, 1983; Tromp, 1989). However, the influence of nutrients on the synthesis and levels of endogenous hormones remains insufficiently studied (Abbott, 1986).

In apple, ammonium instead of nitrate nutrition has been shown to markedly affect the vegetative/generative relationship. Flower bud formation was greatly increased in apple trees fed with ammonium as compared with those fed only nitrate, and flower formation was increased without an effect on growth (Grasmanis and Leeper, 1965; Grasmanis and Edwards, 1974; Rohozinski *et al.*, 1986). Following the application of ammonium, the asparigine and arginine level of tissues in the tree was increased (Grasmanis and Leeper, 1965; Rohozinski *et al.*, 1986). Arginine is a major component of the soluble nitrogen fraction in the xylem sap of apple (Hill-Cottingham and Cooper, 1970; Tromp and Ovaa, 1979) and is a precursor of polyamines (PAs) (Slocum *et al.*, 1984). PAs are involved in a wide range of growth and developmental processes in plants (Evans and Malmberg, 1989), and in recent years a particular role has been implicated in floral induction and development (Kakkar and Rai, 1993). Rohozinski *et al.* (1986) found that exogenous application of PAs to cut pedicels increased the number of flower buds to the same degree as ammonium did. PAs applied as spray also enhanced flower bud formation of apple (Costa and Bagni, 1983). These observations led Rohozinski *et al.* (1986) to suggest that the effect of ammonium on flowering was mediated through elevated internal concentrations of PAs.

Outline of the thesis

In the first part of this thesis the importance of the plastochron and the number of appendages to flower bud formation is investigated in detail and particular attention is directed toward the effect of temperature. In Chapter 2.1 the effect of temperature, application of GA and the presence of fruits on the plastochron and number of appendages of spur buds is presented. In the second part of this chapter (Chapter 2.2) the effects of these treatments is related to morphological characteristics of the flower cluster as seen during anthesis in the following spring. In Chapter 3 the importance of treatments such as bending (conducive to flower bud formation) and defoliation (inhibitory to flower bud formation) and the role of assimilates to development of spur and lateral buds and flower bud formation is described.

The hypothesis that the effect of ammonium on flowering may be mediated through elevated internal concentrations of PAs and the importance of PAs to flower bud formation of apple is investigated in the second part of this thesis. In Chapter 4 the effect of form of nitrogen (ammonium or nitrate) is studied on PA levels of various tissues and related to the occurrence of flower bud formation. In Chapter 5, the effect of treatments known to influence flowering of apple on the level of PAs in buds is analyzed.

In Chapter 6, in the general discussion, the results of the previous chapters are integrated and discussed.

2.1 The effect of temperature, applied gibberellin and the presence of fruits on shoot growth and flower bud formation. I. The effect on appendage formation of spur buds

Summary

The effect of temperature, application of gibberellin (GA_{4+7}) and the presence of fruits on the plastochron of spur buds was studied in relation to flower bud formation of two apple cultivars, Jonagold and Cox's Orange Pippin. Shoot growth was increased in trees placed at a higher temperature or after spraying with GA, while it was reduced in trees bearing fruits. A higher temperature slightly decreased flower bud formation in Jonagold, but increased it in Cox's Orange Pippin. Application of GA and the presence of fruits were inhibitory to flower bud formation. The extent in which GA affected growth and flower bud formation varied with temperature, and with bud position on the tree. Temperature affected the plastochron of spur buds, while GA and fruits had little or no effect. No relationship was found between the plastochron in the period preceding floral differentiation and the occurrence of flower bud formation. Furthermore, no support could be found for the suggestion that flower bud formation requires a certain minimum number of appendages in the bud. The rate of appendage formation tended to decline at constant temperatures, indicating that other (intrinsic) factors play a role. Assimilates or hormonal factors may be involved, both in determining the rate of appendage formation, and in the time when floral differentiation starts. Physiological implications of the results for flower bud formation of apple are discussed.

Introduction

The initial phase of bud development of apple is similar irrespective of whether buds remain vegetative or become generative and is characterized by an increase in the number of bud scales, transition leaves and true leaves in the bud (Bijhouwer, 1924). At some stage during bud development the meristem is induced to become floral (floral induction), which eventually results in the transformation of the meristem into an inflorescence (floral differentiation). Although the physiological factors involved in flower bud formation of apple have received much

attention, the precise nature of these factors or the mechanism of action remains obscure. Changes in the balance of hormones (Luckwill, 1975; Hoad, 1984) or changes in the distribution of assimilates to the meristem (Abbott, 1986) are thought to be involved.

It has also been suggested that a basic precondition for flower bud formation of apple is a short plastochron (time interval between the formation of successive primordia by the meristem) in the period preceding floral differentiation. Extensive research carried out by Fulford (1965, 1966a,b,c) showed that if the plastochron of spur buds was 7 days flowers formed, while if it was 18 days the buds remained vegetative. Defoliation and the presence of fruits lengthened the plastochron and reduced the number of flower buds (Fulford, 1965, 1966c). Fulford (1966a) postulated that the plastochron is regulated by hormones produced by the older developing leaves or fruits, and that both a gibberellin (GA)-like factor, as growth promoter, and a growth inhibitor might be involved. In other plants a relationship between plastochron and flower formation has also been found. For example, in many photoperiodic sensitive species an early response to floral induction is a shortening of the plastochron (Langer and Bussell, 1964; Lyndon and Battey, 1985).

Many factors which affect flower bud formation of apple are most effective early in the season, i.e. several weeks after full bloom (Bubán and Faust, 1982). Since this is well before the microscopical appearance of flower parts, it has been suggested that these factors may indirectly affect flower bud formation through an effect on the plastochron (Abbott, 1970; Luckwill, 1975; Tromp, 1976, 1984). The few studies carried out to test this hypothesis have particularly focused on the effect of applied growth regulators and have shown that growth regulators, such as GA, affect flower bud formation without a simultaneous effect on the plastochron (Luckwill and Silva, 1979; McLaughlin and Greene, 1991a,b). However, these authors did find that flower bud formation generally did not occur before a certain number of appendages had been formed in the bud, adding support to the suggestion that the presence of a minimal number may be critical to flower bud formation of apple (Landsberg and Thorpe, 1975; Luckwill, 1975; Abbott, 1977). Thus, a short plastochron may be of indirect importance by ensuring that a minimal number of appendages is reached at a time before bud activity slows due to unfavourable external conditions.

The morphological sequence of early bud development of apple has not been studied under controlled environmental conditions. Fulford (1965) and Schmidt and Hofmann (1988) found that under orchard conditions the plastochron

was characterized by constant rates, suggesting that the external environment has little effect on the activity of the bud meristem. In contrast, Abbott (1977) found that the plastochron varied between warm and cold seasons, and also during the season the plastochron was not constant, suggesting that it may be influenced by temperature or crop load.

The present study was undertaken to study how bud development is affected by temperature under otherwise constant environmental conditions. To further elucidate the importance of the plastochron and the presence of a minimum number of appendages to flower bud formation, the effect of application of GA_{4+7} and the presence of fruits, was studied on early bud development. The effect of the above treatments on cluster quality and cluster characteristics as seen during bloom in the following spring was also determined, and is reported in part 2 of this chapter.

Materials and Methods

From 1990-1993 3 experiments with apple cv. Jonagold on rootstock M.9 and 1 experiment with cv. Cox's Orange Pippin on rootstock M.9 were carried out in climate rooms. The trees grew in containers (16 l) filled with soil suitable for growing fruit trees. Two types of climate rooms differing in their light source were used. In artificial light (AL) climate rooms light was supplied by a mixture of high pressure sodium and mercury lamps (SON-T and HPI-T lamps, Philips), giving a light intensity of approx. 40 W/m^2 just above the trees at the start of the experiments. The day length was 16 h. In day light (DL) climate rooms light intensity and duration were dependent on natural day light (50% light from outside transmitted under diffuse day light conditions). In both types of climate rooms temperature could be controlled, and day and night temperature were the same. The relative humidity (RH) was 70% $\pm$ 5%, and was not adjusted to temperature so that differences in vapour pressure deficit existed.

Experiment 1: The effect of temperature on the plastochron and flower bud formation of Jonagold

From April to September 1990, 20 3-year-old trees were placed at 13°C and 21°C (10 trees per temperature) in AL climate rooms. Fruits (natural set) were not removed. In September the trees were placed outside, and in January 1991 they were transferred to DL climate rooms (21°C) to assess the effect of temperature on flowering.

Experiment 2: The effect of temperature and GA_{4+7} on the plastochron and flower bud formation of Jonagold

From August 1990 to February 1991 36 3-year-old trees, from the same batch of trees as used in experiment 1, were placed at 13°C and 27°C (18 trees per temperature) in DL climate rooms. For practical reasons, the winter rest of the trees was prolonged by keeping the trees at 1°C from February 1990. The activity of the buds was not completely arrested as evidenced by the green, swollen buds at the start of the experiment, but no cold damage was seen. At each temperature half the number of trees were sprayed with GA_{4+7} (500 ppm) at full bloom, with a repeat spray 3 weeks later. Fruits (natural set) were not removed. From December additional illumination was provided by 4 high pressure mercury lamps (HPI-T, Philips, 400 Watt, 16 hrs/day from 06:00 to 22:00) placed just above the trees. In February, the temperature in both climate rooms was gradually reduced, the trees were defoliated by hand and moved to a dark room at 1°C. In May 1991, the trees were transferred to a greenhouse (20°C) to assess the effect of treatments on flowering.

Experiment 3: The effect of temperature and fruits on the plastochron and flower bud formation of Jonagold

From February to July 1993 40 6-year-old trees were placed at 21°C and 27°C (20 trees per temperature) in AL climate rooms. At each temperature half of the trees were de-blossomed during bloom (control), the rest were hand-pollinated (pollen from cvs. Cox's Orange Pippin and Summerred) and allowed to set fruit. From the end of June the temperature in both climate rooms was gradually decreased; the trees were hand-defoliated and placed at 1°C in the dark until the end of November 1993, after which flowering was assessed in a greenhouse (15°C).

Experiment 4: The effect of temperature and GA_{4+7} on the plastochron and flower bud formation of Cox's Orange Pippin.

From full bloom (11-5-1992) until September 1992 40 2-year-old trees were placed at 13°C and 24°C (20 trees per temperature) in AL climate rooms. At full bloom the trees were deblossomed and half the number of trees in each room were sprayed with GA_{4+7} (500 ppm), with a repeat spray 3 weeks later. In September the trees were placed outside. Flowering was assessed in a greenhouse (20°C) in February-March 1993.

In all experiments care was taken to acclimatize the trees to their temperatures over a period of 2-4 weeks prior to or upon removal from the climate or cold rooms. When placed outside during winter, frost damage was avoided by placing the trees in a well-ventilated plastic tunnel. From experiment 1 and 2, 8 spur buds were randomly collected weekly in such a manner that an equal number had been taken from each tree by the end of the season. From experiment 3 and 4 each week 1 spur bud per tree was randomly collected. The buds were collected from the time of full bloom until 16-20 weeks after, by which time trees of all treatments had ceased growth and floral differentiation had started in most treatments. The buds were immediately dissected under a microscope, or stored in formalin-acetic-alcohol (F.A.A.) until dissection. The number of bud scales, transition leaves and true leaves was counted. The change from the flat- to the dome-shaped meristem was considered as the start of generative development, after which time the number of bracts was also counted. Primordia which were too small to be identified were counted as leaves until they could be distinguished as bracts.

In each treatment the time of cessation of shoot growth ($\pm$ 90% of the shoots >20 cm forming a terminal bud) and, at the end of the experiment, total shoot growth per tree were recorded. In the following spring the number of flower buds and total number of buds (to assess percentage of flower buds) per bud position on the tree, i.e. spur buds, terminal buds of shoots and lateral buds, were counted. Buds that failed to open were dissected to see whether they were floral or vegetative.

Statistical analysis

Since only 1 climate room was available per temperature, independent replication was not possible, and the effect of temperature could not be statistically analyzed. Within the climate rooms, trees were placed in blocks with 1 tree per treatment per block. The effect of GA and the presence of fruits on shoot growth, flower bud number, and flower percentage was analyzed with regression analysis, and significance of differences was analyzed with Student's *t*-test. Data on the increase in the number of appendages per bud of individual trees were fitted with negative exponential curves of the type $Y = A\text{-}BR^X$, where Y is the number of appendages per bud, X is the time, A is the asymptotic maximum value of the curve, A-B is the intercept with the Y-axis and R is the rate of increase towards the asymptote (Oude Voshaar, 1994). The effect of GA and the presence of fruits on the rate of increase was analyzed by fitting the data for individual trees and

subjecting the average R- and A-values to Student's *t*-test. Differences were considered significant at $P<0.05$.

Results

Shoot growth

In experiments 2 and 3 (Jonagold) and particularly in experiment 4 (Cox's Orange Pippin) shoot growth was stimulated at the higher temperature (Table 2.1). Cox's Orange Pippin at 13°C may have suffered from cold stress, as browning of leaves occurred from the middle of June, which increased as the season progressed and later resulted in the shedding of leaves. These symptoms were not observed at 24°C, or with Jonagold. Surprisingly, in experiment 1 (Jonagold), shoot growth was reduced at the higher temperature. This could not be attributed to increased fruit set, as fruit set was higher at 13°C than at 21°C, although the individual fruits were smaller (Table 2.2). The weak shoot growth at the higher temperature of experiment 1 was associated with the formation of a lower number of shoots (>5 cm) (Table 2.1). In the other experiments the number of shoots tended to be higher at the higher temperature. In general, shoot growth of trees of experiment 1 and 2 was characterized by the formation of many but short shoots (5-20 cm), while in experiment 3 and 4 fewer but longer (>20 cm) shoots were formed.

Application of GA stimulated shoot growth in both cultivars (experiments 2 and 4, Table 2.1). Compared to unsprayed controls GA treatment of Jonagold was more effective at the lower than at the higher temperature, and shoot growth at 13°C even exceeded that at 27°C. In Cox's Orange Pippin, the effect of GA on growth was only slightly more pronounced at the lower temperature. GA significantly increased the number of shoots at the low temperature, but not at the high temperature.

Although the final number of fruits per tree in experiment 3 was rather low (Table 2.2), the presence of fruits significantly reduced shoot growth, and this was partly due to the formation of fewer shoots (Table 2.1). The effect of fruits was slightly larger at the lower temperature, where more, larger and heavier fruits were formed, and the fruits also contained more seeds (Table 2.2). In experiment 2 fruit set was very low in all treatments (data not shown), and could hardly have affected growth.

Table 2.1: Effect of temperature, application of GA_{4+7} and presence of fruits on total shoot growth and number of shoots per tree, time to cessation of shoot growth, and time to appearance of the first dome shaped meristem in spur buds. JG = Jonagold, COP = Cox's Orange Pippin, AL = Artificial light, DL = Day light.

Exp. no. and cv.	Temp. and type of climate room	Treatment	Total growth of shoots >5 cm	Number of shoots >5 cm	Time to cessation of shoot growth	Time to first dome-shaped meristem in spur buds
			(m)		(weeks after full bloom)	
1, JG	13°C, AL	-	3.60	25	13	14
	21°C, AL	-	2.10	14	10	9
2, JG	13°C, DL	Control	3.00 a	25 a	10	13
		GA_{4+7}	8.60 b	50 b	14	18
	27°C, DL	Control	4.20 a	32 a	6	9
		GA_{4+7}	6.40 b	36 a	7	15
3, JG	21°C, AL	- fruits	4.00 b	16 b	11	9
		+fruits	2.30 a	12 a	11	9
	27°C, AL	- fruits	5.40 b	18 b	11	12
		+fruits	4.10 a	14 a	11	12
4, COP	13°C, AL	Control	2.70 a	13 a	16	15
		GA_{4+7}	4.30 b	18 b	16	*
	24°C, AL	Control	7.20 a	19 a	10	12
		GA_{4+7}	8.20 b	20 a	10	13

Per experiment and temperature, numbers within each column followed by different letters differ significantly at $P < 0.05$.
* No generative buds seen.

At a higher temperature the time to cessation of shoot growth of both cultivars was generally reduced (Table 2.1), although no effect was found in experiment 3. It must be noted that in this experiment and at 27°C of experiment 2, the exact time of cessation of growth was difficult to ascertain, as a few upright

Table 2.2: The effect of temperature on fresh weight per fruit, fruit diameter, and on the number of fruits per tree and the number of seeds per fruit, determined 18 (experiment 1) and 17 (experiment 3) weeks after full bloom. Data are the mean of 10 replicates (±SE). JG = Jonagold, AL = Artificial light, nd = not determined.

		Fruits			Seed
Exp., cv., climate room	Treatment	Fresh weight (g)	Diameter (cm)	Number	Number
1, JG, AL	13°C	nd	5.2 ± 0.3	22.2 ± 3.6	2.0 ± 0.1
	21°C	nd	7.5 ± 0.2	9.2 ± 1.0	2.1 ± 0.1
3, JG, AL	21°C	224 ± 15	8.2 ± 0.2	16.3 ± 2.2	4.5 ± 1.2
	27°C	163 ± 11	7.3 ± 0.2	12.8 ± 2.0	3.7 ± 0.6

shoots in the top of the tree continued growth, and some even failed to form a terminal bud by the end of the experiment. At the lower temperature and in Cox's Orange Pippin growth cessation of individual shoots occurred more synchronously. Application of GA postponed cessation of growth in Jonagold but not in Cox's Orange Pippin. The presence of fruits did not affect the time of cessation of shoot growth. The first signs of generative development in spur buds were generally not observed long before cessation of shoot growth, but could be postponed for a considerable time after (up to 8 weeks in experiment 2) (Table 2.1). Extra sampling of terminal and lateral buds of shoots showed that floral differentiation started at any time from 2 weeks after floral differentiation of spur buds until the end of the observation period, and treatments had a similar effect on the start of floral differentiation as in spur buds (data not given).

Flowering

Due to the large effect of treatments on growth, the data for total flowering per tree is expressed both in absolute number as in percentage of total buds formed (Table 2.3). At the higher temperature total number and percentage of flower buds of Jonagold tended to be reduced. The reverse was seen in Cox's Orange Pippin, where both flower number and flower percentage were higher at 24°C than at 13°C. Table 2.3 also shows the percentage of flower buds per bud position on the tree. The effect of temperature depended on bud position. In

Table 2.3: Effect of temperature, application of GA_{4+7} and presence of fruits on total number of flower buds per tree and total flower bud percentage for the tree as a whole and flower bud percentage for the different bud positions separately. Flower bud percentage calculated from the total number of buds. For abbreviations see Table 2.1.

Exp. no. and cv.	Temp. and type of climate room	Treatm.	Total no. per tree	Percentage			
				Total per tree	Term. buds of spurs	Term. buds of shoots	Lat. buds of shoots
1, JG	13°C, AL	-	214	57.4	92.6	99.2	49.0
	21°C	-	84	48.9	64.1	94.3	40.0
2, JG	13°C, DL	Control	114 b	42.1 b	69.7 b	92.7 b	33.1 b
		GA_{4+7}	64 a	11.7 a	28.0 a	54.6 a	7.3 a
	27°C, DL	Control	86 a	33.1 b	60.2 b	76.6 a	25.4 b
		GA_{4+7}	48 a	13.2 a	24.7 a	80.1 a	7.2 a
3, JG	21°C, AL	- fruits	187 b	68.4 b	84.7 b	86.5 a	63.9 b
		+fruits	93 a	41.7 a	59.3 a	90.2 a	31.5 a
	27°C, AL	- fruits	205 b	54.6 b	89.9 b	76.2 a	49.0 b
		+fruits	95 a	30.9 a	48.2 a	73.0 a	25.3 a
4, COP	13°C, AL	Control	105 b	29.2 b	74.2 b	74.2 a	3.5 b
		GA_{4+7}	21 a	4.7 a	12.6 a	59.6 a	0.3 a
	24°C, AL	Control	235 b	41.6 b	84.4 b	77.4 a	30.7 b
		GA_{4+7}	149 a	24.0 a	61.4 a	78.4 a	15.6 a

Per experiment and per temperature, numbers within each column followed by different letters differ significantly at $P < 0.05$.

experiment 1, a high temperature particularly inhibited flower formation in spur buds. In the unsprayed trees of experiment 2 the terminal buds of shoots were particularly inhibited by the higher temperature, but for trees treated with GA an opposite effect of temperature was seen. In de-fruited trees of experiment 3 lateral buds were particularly inhibited by the higher temperature. In Cox's Orange Pippin, the lower temperature nearly completely inhibited flower formation in lateral buds, which may be related to the observed cold damage.

In both cultivars application of GA inhibited total flower bud formation at both the high and the low temperature (Table 2.3). In Jonagold the negative effect of GA was independent of temperature, whereas in Cox's Orange Pippin the negative effect was more pronounced at the low than at the high temperature. Application of GA tended to reduce flowering in all positions, although the effect was smallest or absent on terminal buds of shoots. The presence of fruits inhibited flower bud formation at both temperatures. Like GA, fruits had least effect on terminal buds of shoots.

The plastochron prior to the shift to generative development.

Figs. 2.1-2.4 show the effect of the treatments on the rate of appendage formation in spur buds of experiments 1-4. In general the rate of appendage formation progressively decreased to reach an asymptote from around the time when the first generative bud was seen. The first indication of buds becoming generative was a change in the meristem from being predominantly flat to a domed shape. From, and including, the appearance of the dome-shaped meristem, a distinction was made between vegetative (closed symbols) and generative buds (open symbols) (Figs. 2.1-2.4). Data of vegetative buds were fitted by negative exponential curves of the type $Y = A - BR^X$, where A is the asymptotic maximum of the curve and R indicates the rate at which the asymptote is reached. As R approximates 1, the increase towards the asymptote is slower and approaches linearity. Average values of the parameters R and A after fitting data for individual trees and significant effects of applied GA and the presence of fruits are shown in Table 2.4. Due to heterogeneity in the data of individual trees it was not always possible to fit an exponential curve through the data; the number of successful cases is shown in brackets.

In general the increase in the number of appendages shifted from a more linear relationship with time at the low temperatures ($R \approx 1$) to a more curvilinear relationship at the higher temperatures ($R < 1$) (Figs. 2.1-2.4 and Table 2.4). In experiment 1 a negative exponential curve did not fit the data for the 13°C treatment, and a straight line was fitted instead. In experiments 1, 2 and 4 a faster increase towards the asymptote was found in trees at the higher temperature, but in experiment 3 the reverse was seen. Irrespective of temperature, GA did not significantly affect the rate of appendage formation in Jonagold and Cox's Orange Pippin (Table 2.4 and Figs. 2.2 and 2.4). The presence of fruits seemed to slightly reduce the number of appendages per bud from 4 weeks after full bloom both

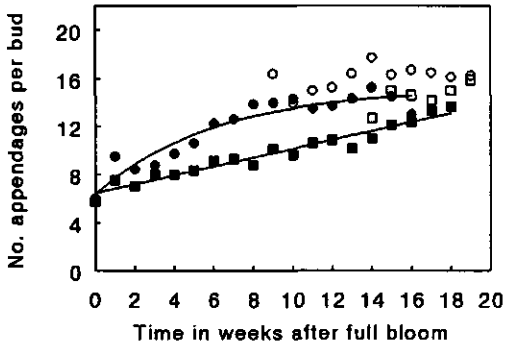

Figure 2.1: The effect of temperature on the number of appendages of spur buds of Jonagold (experiment 1). ■: 13°C, R^2=0.97 (linear), ●: 21°C, R^2=0.88 (exponential). Closed symbols: no visible generative development. Open symbols: visible generative development.

at a high and low temperature (Figs. 2.3A and B), but the effect on rate of appendage formation was not significant (Table 2.4).

From the time when generative buds were first seen, appendage formation of buds remaining vegetative generally stopped (had reached the asymptote A,

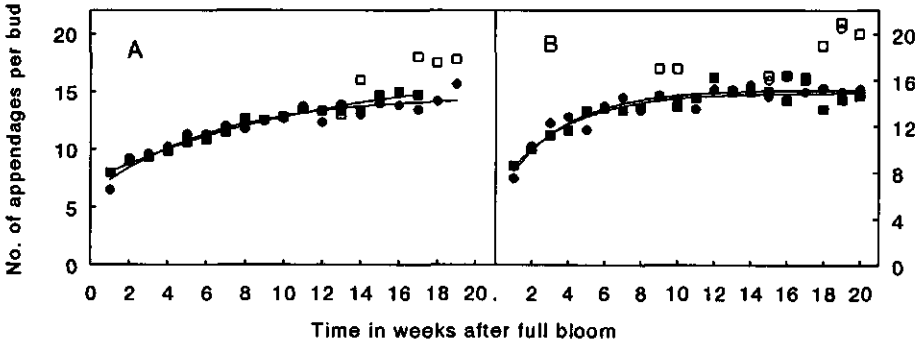

Figure 2.2: The effect of temperature and GA_{4+7} on the number of appendages of spur buds of Jonagold (experiment 2). A: 13°C, ■ unsprayed (R^2=0.97), ● GA_{4+7} (R^2=0.91). B: 27°C, ■ unsprayed (R^2=0.86), ● GA_{4+7} (R^2=0.88). Closed symbols vegetative buds. Open symbols generative buds.

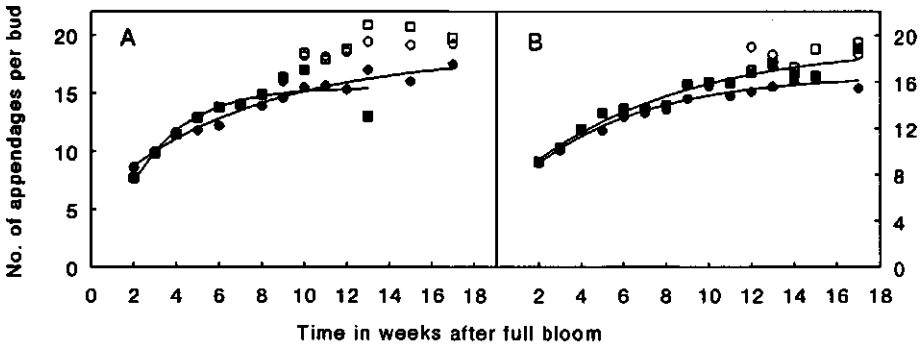

Figure 2.3: The effect of temperature and the presence of fruits on the number of appendages of spur buds of Jonagold (experiment 3). A: 21°C, ■ without fruits, (R^2=0.80), ● with fruits, (R^2=0.96). B: 27°C, ■ without fruits (R^2=0.95), ● with fruits, (R^2=0.96). Closed symbols vegetative buds. Open symbols generative buds.

Table 2.4) or continued slowly towards the asymptote (Figs. 2.1-2.4). The value of A varied from around 15 appendages per bud in experiments 1 and 2, to 17-18 in experiment 3 and 18-20 in experiment 4. No significant effects of applied GA and the presence of fruits were found.

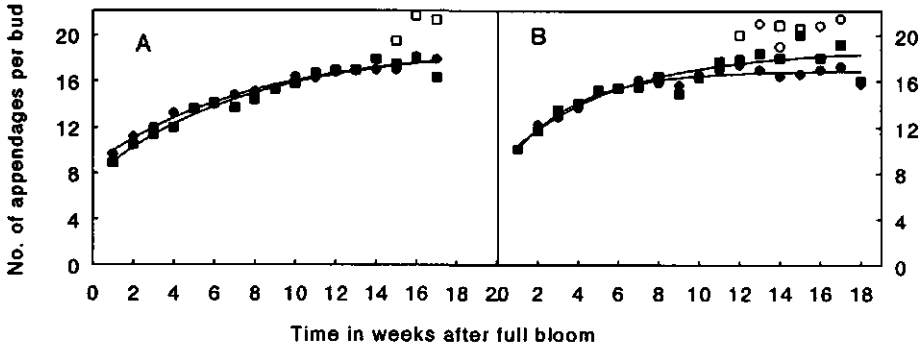

Figure 2.4: The effect of temperature and GA_{4+7} on the number of appendages of spur buds of Cox's Orange Pippin (experiment 4). A: 13°C, ■ unsprayed (R^2=0.95), ● GA_{4+7} (R^2=0.99). B: 24°C, ■ unsprayed (R^2=0.84), ● GA_{4+7} (R^2=0.94). Closed symbols vegetative buds. Open symbols generative buds.

Table 2.4: The effect of temperature, GA_{4+7} and the presence of fruits on parameters R and A of the exponential curve. The parameters are the averages of curve-fits of individual trees. The number of trees on which the average is based is shown in brackets. For abbreviations see Table 2.1.

Experiment number and cultivar.	Temperature and type of climate room	Treatment	R	A	Number of trees
1, JG	13°C, AL	-	-	-	-
	21°C	-	0.79	15.3	(6)
2, JG	13°C, DL	Control	0.93 a	16.0 a	(7)
		GA_{4+7}	0.87 a	14.8 a	(7)
	27°C, DL	Control	0.72 a	14.8 a	(8)
		GA_{4+7}	0.75 a	15.3 a	(7)
3, JG	21°C, AL	- fruits	0.78 a	16.3 a	(8)
		+fruits	0.83 a	16.9 a	(8)
	27°C, AL	- fruits	0.87 a	18.5 a	(10)
		+fruits	0.84 a	17.1 a	(8)
4, COP	13°C, AL	Control	0.87 a	18.9 a	(10)
		GA_{4+7}	0.86 a	19.6 a	(9)
	24°C, AL	Control	0.78 a	17.9 a	(7)
		GA_{4+7}	0.75 a	17.2 a	(10)

Per experiment and per temperature, numbers within each column followed by different letters differ significantly at $P < 0.05$.

The plastochron following the shift to generative development.

Approximately 1 week after appearance of the dome shape, flower initials could be distinguished in the axils of the uppermost 5-6 primordia, and again about a week later, the apex was transformed into the apical flower (sepal initials appeared). From this time, but not before, the uppermost 2-4 primordia could be distinguished as bracts. Soon after the start of floral differentiation the number of appendages in generative buds exceeded that of vegetative buds. In some experiments the switch to generative development occurred rather synchronously in all

buds and thus the higher number was reached abruptly. In other experiments, however, the switch to generative development was spread out over several weeks, and here the higher number of appendages was reached more gradually. In all experiments except experiment 3 generative development started earlier in trees at a higher temperature (Table 2.1 and Figs. 2.1-2.4).

In the 3 experiments carried out with Jonagold differences were found in the number of appendages in spur buds at the start of generative development (Figs. 2.1-2.3). It ranged from 11 at 13°C (experiment 1) to 17 at 27°C (experiment 3). In Jonagold, the number of appendages per bud at the start of generative development seemed to increase with temperature, but in buds of Cox's Orange Pippin little difference was seen between the two tested temperatures (Fig. 2.4). Buds of Cox's Orange Pippin contained more appendages (18) at the start of generative development than buds of Jonagold (11-17). Fruits slightly decreased the number of appendages present at the start of floral differentiation, whereas GA had no effect.

Type of appendages in the bud.

The treatments only slightly affected the proportion of bud scales, transition leaves, true leaves and bracts in the buds (data not shown). In experiment 1, only a very low number of bud scales was formed (4-5, compared to 6-7 in experiments 2 and 3 (Jonagold) and 7-8 in experiment 4 (Cox's Orange Pippin). In experiment 1 the higher temperature slightly increased the number of all types of appendages per bud, but in the other experiments, it only increased the number of leaves, while the number of bud scales, transition leaves and bracts was equal to that at the lower temperature. GA and the presence of fruits did not affect the proportion of the different types of appendages.

Discussion

The effect of temperature on growth, flowering and bud development.

In apple, an increase in temperature increases shoot growth while flower formation is either not affected or reduced (Tromp, 1976, 1980, 1984). With two exceptions, similar effects of temperature were found in the current experiments. In all experiments except experiment 1, the higher temperature enhanced extension

growth, as seen by the formation of more and longer shoots, which formed in a shorter period of time. In Jonagold, but not in Cox's Orange Pippin, the higher temperature tended to reduce flower bud formation. In apple, the antagonistic relationship between vegetative growth and generative development is well established (Forshey and Elfving, 1989), and thus the inhibitory effect of temperature on flower bud formation may have been indirectly mediated through enhanced shoot growth. However, the observations of experiment 1 (Jonagold), where both shoot growth and flowering were reduced by the higher temperature, and experiment 4 (Cox's Orange Pippin), where they were simultaneously enhanced at a higher temperature, suggest that the effect of temperature on flower formation may be direct and independent of an effect on growth. The data for Cox's Orange Pippin may be at variance with the general rule due to the poor condition of trees at 13°C, indicating that in this cultivar 13°C may have been too low to allow normal development. Why Jonagold behaved differently in experiment 1 than in experiments 2 and 3 is more difficult to explain. It may be related to differences in the vapour pressure deficit, which, at a similar RH, is greater in trees at a higher temperature. A greater vapour pressure deficit tends to reduce shoot growth (Tromp, 1984), thereby opposing the effect of temperature on growth vigour. Although in the other experiments the RH was also not adjusted, and similar differences in water-deficit will have existed, in these experiments direct temperature effects may have prevailed over those of vapour pressure deficit.

How temperature affects flower bud formation is not known. Tromp (1976, 1980, 1984), by dividing the growth season into periods of 4-9 weeks and varying the temperature in the different periods, showed that temperature had the greatest effect on flower bud formation during the first period, i.e. during the early vegetative phase of bud development. He suggested that the effect on flower formation was mediated through the plastochron by two opposing effects, namely a direct and an indirect effect. The direct effect of temperature would decrease the plastochron due to the general tendency of temperature to increase the rate at which physiological processes, such as leaf formation, occur (Landsberg, 1975; Terry *et al.*, 1983). The indirect effect would be due to enhanced shoot growth, whereby the increased activity of growth centres elsewhere in the tree inhibits bud development, i.e. lengthens the plastochron. This mechanism of regulation of the plastochron by the activity of growth centres elsewhere in the tree is similar to that postulated by Fulford (1965, 1966a). However, the current data show that an increase in temperature both stimulated shoot growth and shortened the plastochron in spur buds (Figs. 2.1-2.4), indicating that direct temperature effects

prevail in determining the activity of the bud meristem.

Abbott (1977), working under orchard conditions, also found that the rate of appendage formation in apple spur buds depended on temperature, as higher rates occurred in a warm than in a cool season. It is generally assumed that the relationship between rate of leaf formation and temperature follows a parabola, i.e. an optimum temperature exists at which rate of leaf formation is highest, and above and below which an increase in temperature again results in a lengthening of the plastochron (Dale and Milthorpe, 1983). Results with the three tested temperatures for Jonagold implied a similar relationship; when compared within one experiment the rate of appendage formation at 21 °C was higher than at 13 °C or 27 °C (Figs. 2.2 and 2.3). However, the large difference in the R-value found at 27 °C in experiments 2 and 3 (Table 2.4) indicates that other factors than temperature alone are involved in determining the rate of appendage formation.

Similarly, involvement of other factors than environmental factors in controlling the rate of appendage formation is implied by the observed increase in the plastochron in time at constant environmental conditions. In plants it is generally assumed that the rate of leaf formation of shoots remains constant at constant environmental conditions (Dale and Milthorpe, 1983), and the observed decrease may therefore be characteristic of buds. Abbott (1977) and Luckwill and Silva (1979) in apple spur buds also found that the plastochron increased with time. In contrast, Fulford (1965) found that the plastochron of apple spur buds remained constant for long periods of the growing season. However, these authors worked under orchard conditions, where changes in environmental conditions may have affected appendage formation. The current results, showing how bud development of apple proceeds under controlled environmental conditions, indicate that the observed increase in plastochron must be attributed to changes in intrinsic factors.

Such intrinsic factors may be nutritional or hormonal in nature. The progressive decline in the rate of appendage formation during the season may be attributed to reduced availability of assimilates or nutrients (Landsberg and Thorpe, 1975). Lakso (1994) suggested that in the spring, when reserves are plentiful, temperature may be limiting to growth activity, while later in the season resource availability becomes the limiting factor. This is supported by the present observations. Early in the season a higher temperature increased the rate of appendage formation, but this was followed by an earlier and more pronounced slowing later in the season, perhaps due to earlier depletion of assimilates as a result of more vigorous shoot growth and/or higher demands for maintenance respiration. It is not known whether assimilates are important in determining the rate of leaf

formation. In cucumber, it was suggested that the assimilate supply to the apex may be involved in the control of primordia production (Milthorpe, 1959; Marcelis, 1993). However, in tomato and green pepper, treatments influencing assimilate supply such as light intensity, plant density and fruit and leaf pruning did not affect the rate of leaf formation (Heuvelink and Marcelis, 1995). In the current work appendage formation in vegetative buds did not resume when competition for available assimilates ceased following cessation of shoot growth, suggesting that at this time regulation of appendage formation is under hormonal control.

Accumulation of a growth inhibitor or decreasing levels of a growth promoter during the season could also explain the observed effect. As buds develop, the meristem becomes progressively more isolated from the young, expanding leaves due to the formation of bud scales. Young developing leaves and bud scales can affect the activity of the meristem, since removal of these appendages causes buds to grow out as a shoot (Fulford, 1965; Abbott, 1970). Abscisic acid (ABA) has frequently been associated with growth inhibition and rest induction, and this may be the inhibitor which accumulates in bud scales and slows growth activity of the meristem (Abbott, 1970; Wareing and Saunders, 1971; Robitaille and Carlson, 1976). Auxins may also be involved, as it was shown that resumption of growth of the bud after leaf removal could be prevented by application of auxin to the petioles (Fulford, 1970b). Auxins have frequently been associated with inhibition of bud growth induced by apical dominance (Cline, 1991; Wang *et al.*, 1994).

The effect of GA and the presence of fruits on growth, flowering and bud development.

In agreement with a wealth of other data for apple (Bubán and Faust, 1982; Hoad, 1984), application of GA enhanced shoot growth and reduced flower bud formation, while the presence of fruits inhibited both shoot growth and flowering. The effect of GA on shoot growth and flower bud formation depended on temperature; GA was generally less effective when applied at the higher temperature. The reduced responsiveness to GA at the higher temperature may be due to impaired GA-uptake, -action or enhanced metabolic conversion to inactive products. In the present work, temperature did not seem to affect GA-uptake, since in experiment 2 flower formation of spur and lateral buds was inhibited to a similar degree at a high and low temperature. Steffens and Hedden (1992), working with dwarf trees of apple, also found reduced responsiveness to applied GA at a high temperature, and this was associated with alterations in GA metabolism. In the current work

GA did not inhibit flower bud formation of terminal buds of shoots of trees at a higher temperature, i.e. buds formed late in the season, which may suggest that a higher temperature enhances GA-deactivation. At a low temperature GA was also less inhibitory to flower bud formation of terminal buds of shoots as compared with other buds. Again, this may be related to the time of bud formation, since previous results have shown that GA most effectively inhibits buds present at the time of GA application or formed shortly after, with less effect on buds formed considerably later (Tromp, 1987; Chapter 5).

The presence of fruits had a pronounced effect on growth and flower bud formation. Like GA, fruits had least inhibitory effect on flower bud formation of terminal buds of shoots, which would favour the suggestion that the inhibitory action of fruits on flower bud formation is mediated by seed-derived GA (Chan and Cain, 1967; Bubán and Faust, 1982; Hoad, 1984).

The fact that GA and the presence of fruits profoundly affected shoot growth of the entire tree (Table 2.1), while the rate of appendage formation in spur buds was little affected (Figs. 2.1-2.4) may suggest that leaf formation of buds and of actively growing shoots is not regulated in the same manner. However, it should be realized that total shoot growth is not only determined by the rate of leaf formation, but also by differences in the number of shoots, internode length, and time to cessation of shoot growth. For example, the profound effect of GA on shoot growth of experiment 2 was due to the formation of more shoots (> 5cm), and to the fact that growth cessation was postponed in comparison with unsprayed trees. Fruits (experiment 3) lowered both the number of shoot and shoot length. Thus, it remains possible that treatments had similar effects on the rate of leaf formation of actively growing shoots and of spur buds.

The lack of effect of GA on appendage formation supports the observations of Luckwill and Silva (1979) who found no effect of applied GA_3 in apple spurs. McLaughlin and Greene (1991b) found that GA_{4+7} lengthened the plastochron of apple buds only in the presence of fruits. In contrast to the large inhibitory effect of fruits on appendage formation as found by Fulford (1966c), McLaughlin and Greene (1991a) observed only a slight inhibitory effect, occurring at about 20-25 days after full bloom. Similarly, in the present work, a slight inhibitory effect due to the presence of fruits was observed from around 4 weeks after full bloom, which, interestingly coincides with the time when seeds start producing GA (Luckwill *et al.*, 1969; Ramirez and Hoad, 1981). Developing apple seeds are rich sources of GA, and many different GA-types are produced, including GA_4 and GA_7 (Dennis and Nitsch, 1966; Lin *et al.*, 1991). Since applied GA did not affect

appendage formation, it may be illogical to ascribe the inhibitory effect of fruits to seed-derived GA. However, perhaps a continuous supply of GA to the apex is effective in inhibiting appendage formation, whereas incidental applications are not. Seeds also produce auxins (Luckwill, 1957; Gruber and Bangerth, 1990), and generally a greater inhibitory role on bud activity is ascribed to auxins than to GA (Fulford, 1970b; Cline, 1991).

No support was found for the suggestion of Fulford (1965, 1966b) that a shortening of the plastochron in the period preceding floral differentiation is critical to the occurrence of flower bud formation. For example, despite the large effect of GA and the presence of fruits on flower bud formation, they did not affect the plastochron substantially. Similar conclusions were reached by Luckwill and Silva (1979) and McLaughlin and Green (1991a,b). In Fulford's (1965, 1966b) work lengthening of the plastochron in buds remaining vegetative and a shortening of the plastochron in buds destined to become floral occurred late in the season, i.e. end of July-beginning of August, at the time when flower differentiation generally occurs. In the present work a long plastochron in buds remaining vegetative, and a shortening of the plastochron in buds undergoing floral development, was also observed at this time. If the correlation between plastochron and flower bud formation is indeed limited to the period of actual floral differentiation, it must be considered as a consequence of flower bud formation and not as a causal event determining it.

Morphology and physiology of bud development following the shift to generative development

The start of generative development was characterized by a rapid increase in appendage formation, similar to that found by Abbott (1977) and Luckwill and Silva (1979). Appearance of the dome-shaped meristem did not occur in all buds destined to become floral at the same time but was spread out over several weeks, which accounts for the often large heterogeneity in the number of appendages in samples of generative buds. However, per bud the events occurring after the appearance of the dome shape were rapid; generally two weeks after its appearance axillary flower initials were observed and the apex had been transformed into the apical flower. In many herbaceous species, floral differentiation also involves elongation of the shoot apex, stimulation of primordia production and formation of axillary buds, and it has been suggested that these phenomena are universal steps in the morphological sequence by which flower initials are produced in plants

(Langer and Bussell, 1964).

The difference in number of appendages between vegetative and flower buds corresponded with the number of bracts (2-4), and the rapid decrease in plastochron thus coincided with the period of bract formation. In apple, flowers form in the axils of bracts and of the youngest 2-3 leaves. Thus, after the time when the meristem becomes dome shaped, flowers are formed both in the apical direction, i.e. in the axils of newly formed bracts, and in the basipetal direction, i.e. in the axils of the previously formed leaf primordia.

Importance of a minimal number of appendages for flower bud formation

Considerable variation was observed in the number of appendages at the start of floral differentiation. Differentiation started at a higher number in Cox's Orange Pippin than in Jonagold. Cultivar differences in the number of appendages at which flower formation occurs have been observed previously (Luckwill and Silva, 1979; Abbott, 1977; McLaughlin and Greene, 1991a). However, the current work shows that also within a cultivar considerable variation may occur. The greatest variation was found between experiments 1 (11) and experiment 2 (17), but slight variations also occurred within experiments. In experiments 1 and 2, the higher temperature slightly increased the number of appendages at which flowers were formed. However, despite the presence of more appendages, flower bud formation was reduced. Large differences in the number of appendages at the start of generative development also occur in buds in different positions on the tree, e.g. it is lower in lateral buds and terminal buds of shoots than in spur buds (Dheim and Browning, 1988; Dencker and Hansen, 1994; Chapter 3). In conclusion, the large variation in the number of appendages between treatments and between different bud positions, and the lack of correlation between the number of appendages and the degree of flower bud formation do not support the suggestion that a certain minimal number of appendages is critical for flower bud formation of apple, in contrast to what has been suggested (Landsberg and Thorpe, 1975; Luckwill, 1975; Abbott, 1977).

The relationship between cessation of shoot growth and start of generative development.

A correlation between the of start of generative development and the time of cessation of shoot growth has been shown on many occasions (Bubán and Faust,

1982), which suggests that these processes are physiologically related. Hormones produced by actively growing shoots are thought to be responsible for preventing the shift to generative development (Luckwill, 1970a, 1975). In most experiments in the present work correlation was also fairly good; the first signs of floral differentiation generally occurred from 1-2 weeks before until 1-3 weeks after cessation of shoot growth. However, in GA-treated trees of experiment 2 the start of generative development was postponed for quite some time after cessation of shoot growth, which suggests that the release from inhibition from substances produced by active shoot growth is not sufficient, but that floral differentiation requires intervention of another flower-inducing factor. Experiment 2 differed from the other experiments in that the growth season was delayed and trees grew in day light climate rooms at a time of year when natural day light (both intensity and day length) were low (autumn and winter). This may indicate that favourable assimilate levels are required for the shift to generative development to occur. In apple, it was suggested that cytokinin levels may be critical at this time; cytokinin levels must be sufficiently high to enable the enhanced metabolic activity associated with floral differentiation (Luckwill, 1970a). Experimental evidence supporting this suggestion is lacking. As has been noted earlier, in some treatments the exact time of cessation of shoot growth was difficult to establish, which may also account for some of the dissimilarities in the observed time to cessation of shoot growth and flower formation.

Concluding remarks.

It can be concluded from the present results that both environmental factors (temperature) and endogenous factors are involved in regulating appendage formation of apple spur buds. The effect of treatments on flower bud formation was not mediated through effects on bud development, such as the plastochron or the number of appendages per bud. Studying flower bud formation under constant environmental conditions, and varying temperature to alter the vegetative-generative relationship may be a useful tool to gain more information on how flower bud formation of apple is regulated. However, to fully understand the mechanism involved in regulation of vegetative and generative development of apple, simultaneous studies on hormonal relations are needed.

2.2 The effect of temperature, applied gibberellin and the presence of fruits on shoot growth and flower bud formation. II. The effect on morphological characteristics of the flower cluster

Summary

The effect of temperature, application of gibberellin (GA_{4+7}) and the presence of fruits during the growing season of apple trees was studied on the morphological characteristics of the flower cluster as seen during anthesis in the following spring. The number of leaves of the cluster was higher at a higher temperature (21 °C, 27 °C) than at a low temperature (13 °C), while the number of flowers was slightly reduced. Application of GA tended to increase the number of leaves and lower the number of flowers at the low temperature; at a high temperature GA did not have a significant effect. The presence of fruits did not largely affect flower cluster morphology. The number of bourse buds/shoots per cluster was positively related with the number of leaves in the cluster. Variations in the number of leaves are explained in terms of treatment effects on rate of appendage formation and on time when floral differentiation started in the season preceding anthesis. No relation existed between the total number of flower buds per tree and the number of flowers per cluster, suggesting that these factors are independently regulated. The slight variations in the number of flowers per cluster under rather diverse experimental conditions further indicate that this number is mainly genetically determined. Cluster quality (i.e. the number of well-developed flowers per cluster) varied greatly, and seemed dependent on a wide range of factors.

Introduction

Flower bud formation of apple starts in the year preceding anthesis with floral induction, which is followed by the transformation of the meristem into an inflorescence (floral differentiation). Floral differentiation starts in mid-summer, around the time of cessation of shoot growth and continues at a reduced rate throughout the winter period (Zeller, 1955). It is first seen in spur buds and several weeks to several months later in terminal and lateral buds of shoots (Zeller, 1960). Just before and during bud opening in the spring the final stages of flower development are completed. Upon bud opening, flower buds give rise

to a rosette of leaves and a number of pedicelled flowers (the flower cluster). In the axils of one or more leaves a bud (the bourse bud) is usually found which can grow out to form a shoot (the bourse shoot) (Bijhouwer, 1924; Abbott, 1984).

The quantity and quality of flower buds reaching anthesis in the spring are important factors determining yield. The number of flower buds reaching anthesis is only a part of the total number formed, as many buds abscise before reaching full development (Zeller, 1960). Flower quality is generally defined in terms of fertility, i.e. the capacity of flowers to respond to fruit setting stimuli. Good quality flowers are those in which stigmas remain receptive and the embryo sac stays viable for a long period of time, thereby increasing the chance for successful pollination (Williams, 1965). Several studies have shown that the ability of flowers to set fruit was related to morphological features of the flower cluster. A "strong" cluster has many flowers with short stalks, and a high tendency to set fruit, while a "weak" blossom has few flowers on long stalks which set fruit poorly (Rudloff and Feucht, 1957; Williams, 1965). Abbott (1970, 1984) showed that such morphological features were associated with the time in the season that flower buds formed. Strong blossoms were "old", i.e. they were formed early in the previous season, while a weak blossom was "young", i.e. formed late in the season.

While much information is available on how various treatments affect the number of flower buds of apple, simultaneous information on morphological characteristics is scarce, although this information may lead to a better insight in regulation of generative development. The present paper reports on the effects of temperature, application of gibberellin (GA_{4+7}) and the presence of fruits during early bud development on final morphological features of the bud seen during anthesis in the following spring. The main aim of this work was to study how these treatments affect early bud development, i.e. the rate of appendage formation and type of appendages formed. These results have been reported in part 1 of this chapter.

Materials and Methods

From full bloom until 19-22 weeks after, trees of the apple cvs. Jonagold on M.9 and Cox's Orange Pippin on M.9 growing in containers were placed in climate rooms. A detailed description of treatments and the experimental conditions in the climate rooms is given in Chapter 2.1. Several weeks after floral differentiation had started in spur buds (as observed during weekly dissections to study

early bud development), the trees were transferred from the climate rooms to cold conditions (description part 1). The main object of the cold treatment was to break dormancy in a short period of time, and not to simulate natural winter conditions. After the cold treatment the trees were transferred to a mildly heated greenhouse, and the total number of buds and the number of flower buds were counted. Buds that failed to open were dissected to see whether they were floral or vegetative. Of the emerged flower buds the total number of flowers, the number of leaves and the number of bourse buds or shoots (no distinction made) in the axils of leaves of the cluster were recorded. Cluster quality was determined by counting the number of clusters with 4 or more (Jonagold) or 5 or more (Cox's Orange Pippin) well-developed flowers and was expressed as a percentage of total number of flower buds. A flower was considered well-developed if pedicel and flower parts were expanded, and no malformed petals or stamens were seen.

Statistical analysis

Since only 1 climate room was available per temperature, independent replication was not possible, and the effect of temperature could not be statistically analyzed. Within the climate rooms, trees were placed in blocks with 1 tree per treatment per block. The effect of GA_{4+7} and the presence of fruits on morphological characteristics of the flower cluster was analyzed with regression analysis, and significance of differences between these treatments was analyzed with Student's *t*-test. Differences were considered significant at $P < 0.05$.

Results

Weekly dissections of spur buds during the growing season revealed that treatments greatly affected the time when generative development commenced (Table 2.5). In all experiments except experiment 3 floral differentiation started earlier in trees placed at the higher temperature. Application of GA postponed the start of floral development in Jonagold but not in Cox's Orange Pippin. The presence of fruits had no effect on the time when floral differentiation started. Extra sampling of terminal and lateral buds of shoots showed that floral differentiation started at any time from 2 weeks after floral differentiation of spur buds until the end of the observation period, and treatments had a similar effect on the start of floral differentiation as in spur buds (data not given).

Table 2.5: The effect of temperature, application of GA_{4+7} and presence of fruits on the time of cessation of shoot growth, and time to appearance of the first dome shaped meristem in spur buds in the season preceding anthesis. JG = Jonagold, COP = Cox's Orange Pippin, AL = Artificial light, DL = Daylight.

Experiment number and cultivar	Temperature and type of climate room	Treatment	Duration of temperature treatment	Time to cessation of shoot growth	Time to first dome-shaped meristem in spur buds
			Weeks after full bloom		
1, JG	13°C, AL	-	18	13	14
	21°C, AL	-	19	10	9
2, JG	13°C, DL	Control	22	10	13
		GA_{4+7}	22	14	18
	27°C, DL	Control	19	6	9
		GA_{4+7}	19	7	15
3, JG	21°C, AL	- fruits	17	11	9
		+fruits	17	11	9
	27°C, AL	- fruits	17	11	12
		+fruits	17	11	12
4, COP	13°C, AL	Control	20	16	15
		GA_{4+7}	20	16	*
	24°C, AL	Control	17	10	12
		GA_{4+7}	17	10	13

* No generative buds observed during weekly dissections.

Although the winter period was generally much shorter than under natural conditions, in all cases winter rest was sufficiently broken, and bud break appeared to occur in a normal fashion. Bud break of trees growing at the higher temperature in the previous season was delayed in comparison with trees previously growing at the lower temperature. The effect of treatments on the total number and percentage of flower buds as determined during anthesis was presented in Table 2.3, part 1 (pg. 25). Briefly, in Jonagold the number and percentage of flower buds tended to decrease at the higher temperatures. In Cox's Orange Pippin

Table 2.6: Effect of temperature, application of GA_{4+7} and presence of fruits on number of leaves per flower cluster as seen during anthesis. For abbreviations see Table 2.5.

Experiment number and cultivar	Temperature and type of climate room	Treatment	Terminal buds of spurs	Terminal buds of shoots	Lateral buds
1, JG	13°C, AL	-	4.7	5.9	2.8
	21°C	-	5.4	6.9	4.4
2, JG	13°C, DL	Control	6.4 a	7.1 a	6.4 a
		GA_{4+7}	7.7 b	8.0 b	7.0 a
	27°C, DL	Control	8.9 a	9.8 a	6.6 a
		GA_{4+7}	9.5 a	10.2 a	4.9 a
3, JG	21°C, AL	- fruits	6.9 a	9.8 b	4.6 a
		+fruits	7.1 a	8.1 a	5.9 a
	27°C, AL	- fruits	10.0 a	11.1 a	6.8 a
		+fruits	10.6 b	11.2 a	7.7 a
4, COP	13°C, AL	Control	7.9 a	10.4 a	8.1
		GA_{4+7}	9.4 b	9.5 a	*
	24°C, AL	Control	8.5 a	10.6 a	9.2 a
		GA_{4+7}	8.3 a	10.5 a	8.8 a

Per temperature and experiment, numbers within each column followed by a different letter differ significantly at $P<0.05$.
* Flower bud number too low to determine cluster characteristics.

the reverse effect of temperature was seen with increased flower bud number and percentage at the higher temperature. In both cultivars and at both high and low temperatures GA significantly inhibited flower bud formation of spurs and lateral buds, while in terminal buds of shoots it was only inhibitory at the low temperature. The presence of fruits inhibited flower bud formation of spurs and lateral buds at both temperatures, but did not affect flower bud formation of terminal buds of shoots.

The number of leaves per cluster was generally higher at a higher temperature (Table 2.6). The effect was more pronounced in Jonagold than in Cox's Orange Pippin. In Jonagold application of GA tended to increase the number of

Table 2.7: Effect of temperature, application of GA_{4+7} and presence of fruits on number of shoots/buds per flower cluster seen during anthesis. For abbreviations see Table 2.5.

Experiment number and cultivar	Temperature and type of climate room	Treatment	Terminal buds of spurs	Terminal buds of shoots	Lateral buds
1, JG	13°C, AL	-	0.4	0.4	0.1
	21°C	-	0.6	0.8	0.5
2, JG	13°C, DL	Control	0.6 a	0.7 a	0.3 a
		GA_{4+7}	0.4 a	1.2 b	0.5 a
	27°C, DL	Control	0.9 a	1.3 b	0.7 a
		GA_{4+7}	0.9 a	1.0 a	0.6 a
3, JG	21°C, AL	- fruits	0.8 a	1.8 b	0.6 a
		+fruits	0.9 b	0.9 a	0.6 a
	27°C, AL	- fruits	0.9 a	1.8 a	0.6 a
		+fruits	1.1 b	1.8 a	1.0 a
4, COP	13°C, AL	Control	1.0 a	1.6 a	0.9
		GA_{4+7}	1.4 b	1.8 a	*
	24°C, AL	Control	1.1 a	2.0 a	1.2 a
		GA_{4+7}	1.0 a	2.0 a	1.3 a

Per temperature and experiment, numbers within each column followed by different letters differ significantly at $P < 0.05$.
* Flower bud number too low to determine cluster characteristics.

leaves in the cluster, but the effect was only significant at the low temperature. In Cox's Orange Pippin, GA only increased the number of leaves of spur buds at the low temperature. The presence of fruits generally increased the number of leaves in the cluster, although in terminal buds of shoots at the lower temperature the effect of fruits was reversed. Generally, the number of leaves per cluster was highest in the terminal buds of shoots and lowest in lateral buds, with spur buds taking an intermediate position.

In experiments 1 and 2, more bourse buds and shoots per cluster were found at the high temperature, but the effect was less clear in experiments 3 and 4 (Table 2.7). No consistent effect of GA or the presence of fruits was found on

Table 2.8: Effect of temperature, application of GA_{4+7} and presence of fruits on number of flowers per flower cluster seen during anthesis. For abbreviations see Table 2.5.

Experiment number and cultivar	Temperature and type of climate room	Treatment	Terminal buds of spurs	Terminal buds of shoots	Lateral buds
1, JG	13°C, AL	-	6.6	6.0	5.4
	21°C	-	5.7	5.7	4.8
2, JG	13°C, DL	Control	6.2 a	5.8 b	5.6 a
		GA_{4+7}	6.3 a	4.8 a	5.2 a
	27°C, DL	Control	5.2 a	6.0 a	5.0 a
		GA_{4+7}	5.6 a	6.4 a	4.6 a
3, JG	21°C, AL	- fruits	5.7 a	6.3 b	5.1 a
		+fruits	5.8 a	6.0 a	5.0 a
	27°C, AL	- fruits	5.8 a	6.3 a	5.0 a
		+fruits	5.9 a	6.1 a	5.2 a
4, COP	13°C, AL	Control	6.5 a	6.4 b	5.6
		GA_{4+7}	6.2 a	4.5 a	*
	24°C, AL	Control	5.5 a	6.0 a	5.8 a
		GA_{4+7}	5.3 a	6.1 a	5.9 a

Per temperature and experiment, numbers within each column followed by different letters differ significantly at $P < 0.05$.
* Flower bud number too low to determine cluster characteristics.

the number of bourse buds and shoots. Terminal buds of shoots contained a higher number of bourse buds and shoots than spur buds or lateral buds.

Variation in the number of flowers per cluster (Table 2.8) was less than that for leaves. In experiment 1, the number of flowers per cluster was reduced at the higher temperature. The same trend was seen in experiments 2 and 4, but the effect was not consistent for all types of buds. In experiment 3 no effect of temperature was seen. Application of GA and the presence of fruits only significantly lowered the number of flowers in terminal buds of shoots and only at the lower temperature. Lateral buds tended to have the lowest number of flowers in the cluster, while no consistent difference was found between spur buds and terminal

Table 2.9: Effect of temperature, application of GA_{4+7} and presence of fruits on cluster quality (i.e. the number of flower clusters with 4 or more (Jonagold) or 5 or more (Cox's Orange Pippin) well-developed flowers in the cluster expressed as a percentage of total emerged flower buds). For abbreviations see Table 2.5. nd = not determined.

Experiment number and cultivar	Temperature and type of climate room	Treatment	Terminal buds of spurs	Terminal buds of shoots	Lateral buds
1, JG	13°C, AL	-	83.7	94.0	19.2
	21°C	-	65.6	91.0	45.5
2, JG	13°C, DL	Control	79.7 b	72.5 b	50.5 b
		GA_{4+7}	51.8 a	21.0 a	20.1 a
	27°C, DL	Control	85.7 a	81.1 a	60.1 a
		GA_{4+7}	93.1 a	97.1 a	42.9 a
3, JG	21°C, AL	- fruits	82.4 a	56.3 a	nd
		+fruits	85.2 a	67.2 a	nd
	27°C, AL	- fruits	54.4 a	23.2 a	nd
		+fruits	65.6 b	33.1 a	nd
4, COP	13°C, AL	Control	70.7 b	22.5 a	16.4
		GA_{4+7}	18.7 a	14.8 a	*
	24°C, AL	Control	50.3 a	16.3 a	19.2 a
		GA_{4+7}	62.9 a	19.1 a	22.7 a

Per temperature and experiment, numbers within each column followed by different letters differ significantly at $P < 0.05$.
* Flower bud number too low to determine cluster quality.

buds of shoots.

Abnormalities in morphological features of the individual flowers, such as variation in the number of petals and stamens, intermediate forms between stamens and petals, fusion of 2 or more flower stalks or branched flower stalks were frequently observed. In a few cases, a number of flowers in the cluster remained sessile, showing no expansion of flower parts. The number of well-developed flowers per cluster, i.e. those flowers without the above abnormalities were counted and used as an indicator of cluster quality (Table 2.9). In experiment 1 and 2 cluster quality of lateral buds appeared to be improved at a higher temperature.

In experiment 3 and 4 cluster quality of spur and terminal buds tended to be reduced at the higher temperature. In both cultivars, application of GA reduced cluster quality of buds at the low temperature, whereas at the high temperature GA may have improved cluster quality slightly, although the effect was not significant. The presence of fruits slightly improved cluster quality, but the effect was only significant for spur buds at a high temperature.

Cluster quality varied with bud position; in general it was relatively poor in lateral buds. In experiment 3 and 4 cluster quality of terminal buds of shoots was also poor. Cluster quality also depended on time of bud emergence; more abnormalities were seen in buds emerging later (data not shown). Bourse length and pedicel length were also larger in buds emerging later, and in lateral and terminal buds in comparison with spur buds. In general, variation in cluster quality was larger between buds in different positions on the tree and between buds opening early or late than between treatments.

The cold stress observed in trees of Cox's Orange Pippin at 13°C did not adversely affect cluster quality of the control trees, but in conjunction with GA application it resulted in very poor quality. The leaves of these flower clusters expanded normally, but the flowers did not fully develop, remained yellow and shrivelled before opening.

Discussion

Factors determining the number of leaves per cluster

Early bud development of apple is characterized by an increase in the number of appendages, i.e. bud scales, transition leaves and "true" leaves in the bud (Bijhouwer, 1924). During floral differentiation the apex usually produces 2-4 bracts, and 5-7 lateral flowers form in the axils of these bracts and the uppermost 2-3 leaves. The apex itself is transformed into the apical flower, whereby production of appendages ceases. As a consequence, the number of leaves per cluster is determined by the rate at which appendages are formed in the period preceding floral differentiation, and by the time in the season that floral differentiation starts.

This reasoning is in line with the observations made during early bud development of spur buds (Chapter 2.1). The increased number of leaves in the cluster at the higher temperature was associated with an increased rate of appendage formation in the early part of the previous season (Figs. 2.1-2.4, part 1, pgs. 27-28),

leading to more appendages in the bud at the start of floral differentiation. This was so despite the fact that at a high temperature floral differentiation occurred earlier than at a low temperature, i.e. appendage formation occurred over a shorter period of time. The increase in leaf number by GA at the low temperature may be due to the fact that GA postponed the start of floral differentiation (Table 2.5), allowing appendage formation to occur over a longer period of time. Although GA also postponed floral differentiation at a high temperature, this happened at a time when appendage formation had stopped, so that it could not affect the number of leaves. The effect of fruits of slightly enhancing the number of leaves at anthesis could not be explained in terms of effects on early bud development, as fruits slightly lowered the number of appendages per bud and did not affect the time of floral differentiation. The presence of fruits may have slightly affected the proportion of the various types of appendages in the bud. Although only spur buds were periodically dissected in the previous year, it may be assumed that in terminal and lateral buds of shoots the number of leaves per cluster was also determined by the rate of appendage formation, and/or start of floral differentiation.

An increase in the number of leaves per cluster induced by treatments was associated with a higher number of bourse buds and shoots, thereby strengthening the vegetative character of buds. The naturally high number of bourse buds and shoots in terminal buds of shoots confirms the importance of these sites for future extension growth (Abbott, 1984; Forshey and Elfving, 1989).

The number of leaves per cluster may be an important aspect of cluster quality, as they are required for fruit set. Removal of cluster leaves before or after bloom severely reduced fruit set or increased fruit drop (Arthey and Wilkinson, 1964; Llewelyn, 1968; Ferree and Palmer, 1982). Removal of leaves from shoots did not have a similar negative effect (Llewelyn, 1968). Fruit set particularly depends on the leaves on their own spur (Ferree and Palmer, 1982). The presence of the bourse shoot reduced early set, but increased final yield due to the formation of larger fruits (Ferree and Palmer, 1982).

Factors determining the number of flowers per cluster

In comparison to the effect of the treatments on the number of leaves, the effect on the number of flowers per cluster was very small. At a high temperature the number of flowers per cluster tended to be reduced in all positions, while application of GA or the presence of fruits only lowered the number of flowers per cluster of terminal buds of shoots and only at the low temperature. A relationship

existed between the number of leaves in the cluster and the number of flowers; where treatments increased the number of leaves, the number of flowers was reduced. Comparing different bud positions such a relationship did not exist. However, Rudloff and Feucht (1957) showed that if leaf area is considered an antagonistic relationship between vegetative and generative parts also exists in buds on different positions; lateral buds contained fewer flowers and had a larger leaf area than spur buds. In grape vine (Mullins, 1968), tomato (Kinet, 1977; Abdul and Harris, 1978) and *Bougainvillea* (Tse *et al.*, 1974) removal of young leaves just prior to floral development increased inflorescence development, and it was suggesting that leaves and flowers may compete for cytokinins or assimilates. Application of cytokinin increased the number of flowers in the inflorescence (Mullins, 1968; Kinet, 1977), and both defoliation and applied cytokinin increased the level of assimilates in the shoot tip (Tse *et al.*, 1974). In apple, applications of cytokinin also increased the number of flowers in the cluster (McLaughlin and Greene, 1984).

Alternatively, rather than compete for the same substrate, leaves may reduce the number of flowers by the production of an inhibitor. In tomato, Abdul and Harris (1978) found that the number of flowers in the inflorescence was reduced by a higher temperature and by application of GA, similar results to those obtained here. Leaves from plants growing at the higher temperature regime yielded greater amounts of GA-like substances than leaves grown at low temperatures, suggesting that the reduced number of flowers in the inflorescence was due to high endogenous levels of GA.

In the present work the effect of application of GA on morphological features of the cluster depended on temperature; GA had a larger effect at a low than at a high temperature. A similar response was seen for flower bud number and shoot growth (Chapter 2.1), and confirms that responsiveness to applications of GA is dependent on temperature.

No positive relationship existed between the effect of treatments on total number of flower buds per tree and the number of flowers per cluster, suggesting that these factors are controlled independently. Furthermore, the slight variations in the number of flowers per cluster at rather extreme temperatures, and the minimal effects of GA and the presence of fruits, suggests that the number of flowers per cluster is mainly genetically determined rather than sensitive to environmental or hormonal conditions.

Factors determining cluster quality

Abbott (1970, 1984) showed that the morphology of the flower cluster varied greatly with the time in the season that floral differentiation occurred. Transferring trees shortly after the start of floral differentiation to winter conditions, produced "young" flower buds, which in the following spring produced clusters with long flower pedicels and bourses, a reduced number of flowers and well-developed leaves in comparison with the flowers. These buds were of poor quality, as they set fruit poorly. In contrast, when transfer to cold conditions occurred late in the season "old" buds resulted, which after bud opening showed short bourses and flower pedicels, advanced flower development as compared with the leaves and numerous flowers which set fruit well. Rudloff and Feucht (1957) also observed clusters with such different characteristics, but found that they were more dependent on time of bud opening in the spring, and on bud position; spur buds flowered early and had a typically "old" appearance, while lateral buds flowered late and had a typically "young" appearance.

Since in the current work treatments profoundly affected the time of floral differentiation, morphological features typical of "young" and "old" buds could have been expected. However, although such buds were frequently observed, the occurrence was not related to treatments, but, in accordance with observations of Rudloff and Feucht (1957), more dependent on time of bud opening. Buds which emerged early (i.e. spur buds) had the morphology typical of "old" buds and were of better quality than buds emerging later (i.e. lateral buds). Terminal buds occupied an intermediate position. Poor quality of lateral buds is a well-known phenomenon and is generally explained by the fact that the initial developmental stages occur under more adverse (sometimes winter) conditions, resulting in slower and incomplete floral development (Zeller, 1960). Since spur buds form flowers earlier in the season than lateral buds (Zeller, 1955, 1960), the observed differences in cluster quality between bud positions may, after all, be related to the time when floral differentiation started in the previous season, similar to that found by Abbott (1970, 1984). However, as far as treatment effects are concerned no consistent relationship was found between earlier floral differentiation and improved cluster quality. For example, a higher temperature generally advanced the start of floral differentiation, while cluster quality was either improved, not affected or reduced. Similarly, although application of GA delayed floral differentiation at both temperatures, cluster quality was only reduced at the lower temperature. It therefore appears that a complex of factors, including the conditions

prevailing prior to or during early floral differentiation and physiological differences existing between buds on different positions may determine cluster quality.

Little is known about which endogenous factors affect cluster quality. In a number of studies evidence was presented that the nutritive status of trees during floral differentiation is important, as summer and autumn application of nitrogen (N) improved flower quality, while application in spring or no application had no effect (Williams, 1965; Delap, 1967; Hill-Cottingham and Williams, 1967). Since leaf senescence and abscision were postponed by late applications of N, it was suggested that the promotive effect of N may be mediated through enhanced levels of assimilates (Hill-Cottingham and Williams, 1967). Alternatively, enhanced production of cytokinins in the roots after applications of N may be responsible for enhanced flower quality (Hill-Cottingham and Williams, 1967).

Concluding remarks

Although a first prerequisite for a large crop is the presence of a sufficient number of flower buds at the start of the season, the quality of the flower cluster may be equally important. The present results show how morphological features of the cluster can be affected by a number of treatments. Morphological features of the cluster, such as the number of leaves and (well-developed) flowers, show poor correlation with factors determining flower bud formation. The number of leaves is determined by events occurring during the previous season, i.e. by the rate of appendage formation and the start of floral differentiation; processes not directly involved in regulation of flower bud formation (Chapter 2.1 and 3). Despite the fact that all treatments had profound effects on flower bud formation, the number of flowers per cluster varied little. The great heterogeneity in response in cluster morphology to treatments, for example between buds in different positions on the tree or with time of bud emergence, at present only confirms the complexity of regulation of generative development, but contributes little to clarify its mechanism of control.

3. The effect of defoliation and bending on shoot growth, bud development and on assimilate levels of shoots in relation to flower bud formation

Summary

In two experiments carried out with one-year-old 'Cox's Orange Pippin' apple trees on rootstock M.9 the effect of defoliation and bending applied singly or in combination was studied in relation to flower bud formation. In the first experiment, only branches and newly formed shoots were bent horizontally, while in the second experiment the entire tree was placed in the horizontal position. To study whether the effect depended on the time of treatment, the treatments were applied at different times during the season (i.e. middle of May, middle of June and middle of July). In order to determine whether treatments affected flower bud formation through an effect on the plastochron, the number of appendages of spur and lateral buds was determined at regular intervals during the season. Furthermore, in the second experiment the effect of treatments was studied on the assimilate levels of one-year-old shoots.

Bending of shoots or placing trees in the horizontal position reduced shoot growth. Flower bud formation was only enhanced if the entire tree was placed horizontally, and only if it occurred in July. On all dates, defoliation reduced shoot growth and inhibited flower bud formation. The suppression of flower bud formation was progressively more severe the later in the season leaves were removed, and inhibition was nearly complete if leaf removal occurred in July. Bending did not greatly affect the rate of appendage formation of buds, while defoliation caused a temporary stagnation. Effects of treatments on flower bud formation could not be explained through effects on the plastochron, or on the number of appendages in the bud at the start of floral differentiation. The soluble sugar content of shoots was not affected by a horizontal tree position, while starch was increased. Defoliation caused an immediate reduction in the soluble sugar level of the shoot, particularly on early defoliation dates. Starch was not affected. A relationship could not be demonstrated between the effect of treatments on flowering and assimilate levels of the shoot. A negative relationship existed between the level of growth and starch level of the shoot. It is concluded that a horizontal shoot position and defoliation likely affect flower bud formation through altering hormonal relations in the shoot and possible mechanisms of action are discussed.

Introduction

The importance of a sufficiently large and viable leaf area to flower bud formation of apple has long been recognized, as defoliation, leaf injury or shading have repeatedly been shown to reduce flowering (Harley *et al.*, 1942; Davies, 1959; Fulford, 1966b, 1970a; Hennerty and Forshey, 1971; Jackson and Palmer, 1977; Cordes, 1987). The mechanism by which leaves affect flower bud formation is obscure. Hoad (1980) suggested that cytokinins may be involved, as the inhibitory effect of leaf removal on flowering could be reversed by petiole application of cytokinins (Ramirez and Hoad, 1981). Leaves of apple are rich in cytokinins (Greene, 1975), and applications of cytokinins can enhance flower bud formation of apple (McLaughlin and Greene, 1984; Unrath, 1989; Skogerbø, 1992). However, leaves may also be important as producers of assimilates, or to sustain an active transpiration stream, thus ensuring that promotive substances produced elsewhere in the plant, or nutrients taken up by the roots reach the sites of flower formation (Luckwill, 1975; Hoad, 1980).

Another way in which leaf removal may affect flower bud formation is by interfering with early bud development. By removing all leaves from mature trees of apple at different times during the season, Fulford (1965) showed that an immediate response to defoliation was a shortening of the plastochron (the time interval between the formation of two successive primordia by the meristem) of apple spur buds, which was followed by a lengthening of the plastochron later in the season. Fulford (1966b) further showed that the occurrence of flower bud formation was associated with the length of the plastochron. If the plastochron was short (approximately 7 days) flowers formed, while if it was considerably longer (18 days) buds remained vegetative. The inhibitory effect of leaf removal on flower bud formation was explained in terms of its effect of lengthening the plastochron. Removal of leaves also caused buds to grow out in a second flush of growth, thereby delaying the time in the season when new buds formed and reducing the number of appendages in the bud (Fulford, 1960, 1970a). It has been suggested that floral differentiation of apple requires the presence of a certain "critical" number of appendages (Landsberg and Thorpe, 1975; Luckwill, 1975; Abbott, 1977) and the adverse effect of leaf removal on flower bud formation may therefore be related to its tendency to reduce the number of appendages per bud.

Bending branches horizontally or placing whole trees in the horizontal position generally reduces vegetative growth and enhances flower bud formation of apple (Wareing and Nasr, 1958; Longman *et al.*, 1965; Tromp, 1968, 1970b,

1972). The precise mechanism by which bending enhances flowering is not known, but both accumulation of carbohydrates in horizontal shoots and alterations in the distribution of endogenous hormones have been put forward as possible explanations (Kato and Ito, 1962; Longman *et al.*, 1965).

To gain more insight in the mechanism of control of flower bud formation of apple, treatments of defoliation (inhibitory to flower bud formation) and bending (conducive to flower bud formation) were applied singly or in combination to young apple trees and related to assimilate levels of one-year-old shoots. To investigate whether treatments affect flower bud formation via an effect on the plastochron, the number of appendages of spur and lateral buds was determined at regular intervals during the season. Lastly, to study whether the effect depended on the time of treatment, the treatments were applied at different times during the season (i.e. middle of May, middle of June and middle of July).

Materials and Methods

In May 1992, 2 experiments were simultaneously carried out, each with 100 one-year-old trees of cv. Cox's Orange Pippin on rootstock M.9. The first experiment consisted of branched trees, i.e. trees which had formed laterals in the year in the nursery. The second experiment consisted of unbranched trees, i.e. trees without laterals at the start of the experiment. The trees were potted in December 1991 in 16 l containers filled with soil suitable for growing fruit trees. In the beginning of May, 2 weeks before full bloom (FB), the trees were placed at the experimental site outside. To prevent fruit set flowers were removed. The trees were regularly fertigated. The following treatments were applied:

Branched trees

Shoot bending: At 3 different times during the growing season (in the middle of May, middle of June and middle of July) a group of 10 trees was bent by training the branches and newly formed shoots horizontally (at an angle of 90° with the main stem). Prior to bending all branches were trained upright along the main stem, to enhance contrast with branches already bent.

Defoliation of vertical shoots: In the middle of May all branches and newly formed shoots of 30 trees were trained vertically along the main stem. At 3

different times during the growth season (i.e. immediately, in the middle of June and in the middle of July) a group of 10 trees was defoliated. Defoliation meant manually removing all leaves except those still rolled around the growing point. After defoliation new leaves were allowed to grow.

Defoliation of horizontal shoots: In the middle of May all branches and newly formed shoots of 30 trees were trained horizontally (at an angle of 90° with the main stem). At 3 different times during the growth season (i.e. immediately, in the middle of June and in the middle of July) a group of 10 trees was defoliated.

Control: Ten trees were not treated in any way, i.e. the branches and newly formed shoots were not defoliated and allowed to grow at their natural angle.

Unbranched trees

Treatments and treatment times were the same as described above, but in the bending treatments the entire tree was placed in the horizontal position. At the start of the experiment, the main stem was topped at a height of 80-85 cm above the pot to enhance formation of laterals. To enhance differences in shoot orientation between vertical and horizontal trees, all newly formed shoots were trained in line with the stem. The horizontal trees were rotated 180° around the axis twice weekly to avoid growth to the upright position. A small hole was drilled in 2 opposite sides of the pot to facilitate fertigation and drainage. The horizontal trees were put upright again in October.

During winter all trees were kept in a well-ventilated plastic tunnel. They were transferred to a greenhouse (temperature 20°C) from February to April to evaluate the effect of treatments on flowering.

Shoot growth and flowering

Time of cessation of shoot growth and final length of all shoots were recorded. During the following spring the number of flower buds and total number of buds (to determine flower bud percentage) were determined. Buds that failed to open were dissected to see whether they were floral or vegetative. In order to ascertain whether the effect of treatment depended on the stage of bud development, flower bud formation on one-year-old shoots of branched trees was separate-

ly evaluated in bottom, middle and top thirds. In unbranched trees flower bud formation of every 5th bud along the one-year-old shoots, counting from the shoot base, was separately recorded to relate data of bud dissections (see later) to tendency of the bud to become floral. Cluster quality was evaluated by counting the number of clusters having 5 or more well-developed flowers (flower stalk expanded, no malformed stamen or petals) and expressing this as a percentage of total flower buds formed.

Bud dissection

At 2-weekly intervals from FB to 16-18 weeks after, spur buds (branched trees) and lateral buds (unbranched trees) were randomly collected. Per sampling date 8 spur buds per treatment were taken from 4 branched trees (2 per tree). In the unbranched trees, 1 newly formed shoot each from 6 trees per treatment was selected and every 5th lateral bud (counting from the shoot base) was cut off just below the bud scales. The buds were immediately dissected under a microscope to count the number of appendages within the bud, or stored in formalin-acetic-alcohol (F.A.A.) until later dissection. The change from the flat- to the dome-shaped meristem was considered as the start of generative development, after which time the number of bracts was also counted. Primordia which were too small to be identified were counted as leaves until they could be distinguished as bracts.

Carbohydrate analysis

Carbohydrate content in shoots of unbranched trees was determined. At 4-weekly intervals starting from FB, 1 newly formed shoot was randomly cut off from each of 6 trees per treatment. The leaves were removed, and after weighing the shoot was divided into sections of 8-10 cm. From the middle of each section 1 cm of stem was cut and the cut pieces were combined to form the sample. Since on the first collection date (FB) the shoots had generally not yet reached a length of 8-10 cm, the entire shoot was taken as sample on this date. The samples were weighed (fresh weight), freeze-dried for 1 week, weighed again (dry weight), and stored at -20°C until analysis. Approximately 15 mg of ground, dried material was used for analysis. Soluble sugars were extracted 3 times with 3 ml ethanol (96%) at 80°C (15 min) and centrifuged for 5 min. The supernatants (soluble sugars) and the pellet (starch) were dried under a stream of air at 40°C. The soluble sugars

fraction was re-suspended in 5 ml of water, mixed for 3 min (ultrasonically) and centrifuged for 15 min. The mixture was filtered before analysis of soluble sugars by high performance liquid chromatography (HPLC). After adding 2 ml of water the pellet was placed at 100°C for 1 hour and thereafter centrifuged for 15 min; 0.5 ml was used for the enzymatic hydrolysis of starch to glucose by amyloglucidase (Boehringer, Mannheim Germany). Amyloglucidase (5 mg) was dissolved in 6 ml citric buffer (50 mM, pH 4.6) and 0.5 ml of this solution was added to 0.5 ml sample, placed at 60°C for 15 min, and centrifuged for 15 min. Glucose was determined by HPLC.

The chromatographic system consisted of a LKB 2248 pump (Pharmacia, Uppsala Sweden), a Marathon autosampler (Spark, Holland) a CHO-620 column with guard column (Interaction, San Jose U.S.A.), a LKB 2155 Column Oven (Pharmacia, Uppsala Sweden) and a 410 Refractive Index Detector (Waters Assoc., Milford U.S.A). Carbohydrates were eluted from the column at 90°C at a flow rate of 0.5 ml/min with solvent water. Detector output was collected and analyzed using a 740 integrator (Waters Assoc., Milford U.S.A).

Statical analysis

Branched trees and unbranched trees were placed in separate plots according to a complete randomized block design, with 1 tree per treatment per block. Shoot growth, flower number and flower percentage were analyzed with regression analysis. Significance of differences between pairs of treatments was determined with Student's *t*-test. Differences were considered significant at $P < 0.05$.

Results

Shoot growth

Branched trees: The effect of treatments on growth depended on their timing (Table 3.1). All treatments reduced shoot growth relative to the control. Bending reduced total shoot growth most if done late in the season, whereas defoliation both of vertical and horizontal shoots was more effective early in the season. The effect of treatments on average shoot length was less pronounced but tended to be similar to the effect on total shoot growth.

Table 3.1: The effect of bending shoots (branched trees) or placing entire trees horizontally (unbranched trees) (Hor) and defoliation (Def) at three different times in the season on total shoot growth per tree, average shoot length and time to cessation of shoot growth. T=0 (middle of May), T=1 (middle of June) and T=2 (middle of July): timing of treatments.

Treatment	Branched trees			Unbranched trees	
	Total growth per tree (in m)	Average shoot length (in cm)	Cessation of shoot growth in weeks after full bloom (date)	Average shoot length (in cm)	Cessation of shoot growth in weeks after full bloom (date)
Control	12.70 e	42 cd	17 (7 Sept)	93 e	22 (12 Oct)
Hor, T=0	11.15 cd	39 bc	14 (17 Aug)	28 b	17 (7 Sept)
Hor, T=1	10.85 bcd	38 bc	14 (17 Aug)	47 c	17 (7 Sept)
Hor, T=2	9.10 a	34 ab	14 (17 Aug)	68 d	16 (31 Aug)
Vertical shoots				Vertical trees	
Def, T=0	9.40 ab	41 cd	21 (5 Oct)	77 d	22 (12 Oct)
Def, T=1	10.40 abcd	38 bc	21 (5 Oct)	72 d	22 (12 Oct)
Def, T=2	11.35 de	45 d	21 (5 Oct)	66 d	21 (5 Oct)
Horizontal shoots				Horizontal trees	
Def, T=0	9.15 a	32 a	14 (17 Aug)	18 a	17 (7 Sept)
Def, T=1	9.65 abc	35 ab	14 (17 Aug)	22 ab	17 (7 Sept)
Def, T=2	10.50 abcd	36 ab	17 (7 Sept)	25 b	16 (31 Aug)

Per column, numbers followed by different letters differ significantly at $P < 0.05$.

Unbranched trees: As the number of shoots removed for carbohydrate analyses in unbranched trees depended on the start of treatment (middle of May, June or July), Table 3.1 only shows the effect of treatments on the final average shoot length, not total growth per tree. Similar to branched trees all treatments reduced shoot growth relative to the control, but the effects were more pronounced. Horizontal placement of trees reduced shoot growth more if done early in the season, while defoliation of vertical trees was more effective if carried out later, opposite trends to those observed in branched trees. Similar to branched trees, defoliation of horizontal trees was most detrimental early in the season, and gave very poor

shoot growth.

Treatments had a large effect on time to cessation of shoot growth (Table 3.1). Control trees terminated shoot growth late in the season. Placing shoots or trees in the horizontal position (both undefoliated and defoliated) advanced cessation of shoot growth by 3 weeks (branched trees) or by 5-6 weeks (unbranched trees). In defoliated vertical trees cessation of shoot growth was postponed by 4 weeks in branched trees, while it was more or less equal to the control in unbranched trees.

Flowering

Branched trees: Bending shoots horizontally did not affect the percentage of flower buds on one-year-old shoots, while defoliation of both vertical and horizontal shoots had a pronounced negative effect (Table 3.2). Inhibition of flower bud formation was greater if trees were defoliated later in the season, and it was nearly complete when trees were defoliated in July. A similar effect of treatments was found in spur buds, but defoliation in July did not show the same drastic inhibition observed on shoots, and it even was less inhibitory compared to defoliation in June. The effects of treatment on cluster quality were not very pronounced. Bending tended to enhance the percentage of flower buds with 5 or more well-developed flowers slightly, while defoliation in July tended to lower it.

Unbranched trees: Percentage bloom was enhanced relative to the control on trees placed horizontally in July, while in May or June it had no effect. In all defoliation treatments flowering was reduced. Defoliation of horizontal trees depressed flower formation more than defoliation of vertical trees. Similar to one-year-old shoots of branched trees, defoliation of vertical and horizontal trees in the middle of July suppressed flower formation nearly completely. Horizontal placement enhanced, and defoliation of vertical trees decreased the percentage of flower buds with 5 or more well-developed flowers. In defoliated horizontal trees the number of flower buds at $T=1$ and $T=2$ was too low to draw definite conclusions.

Due to differences in time of bud formation, buds along newly formed shoots will have been in very divergent stages of development at the time of treatment applications. In order to ascertain whether the effect of treatment depended on the stage of bud development, flower bud formation on one-year-old shoots of branched trees (as shown in Table 3.2), was studied in more detail by separately recording flower bud formation on the basal, middle and top sections (of equal

Table 3.2: The effect of bending shoots (branched trees) or placing entire trees horizontally (unbranched trees) (Hor) and defoliation (Def) at three different times in the season on flowering of one-year-old shoots and spurs. Flower bud percentage calculated from the total number of buds formed. T=0 (middle of May), T=1 (middle of June) and T=2 (middle of July): timing of treatments.

Treatment	Branched trees				Unbranched trees	
	One-year-old shoots		Spurs		One-year-old shoots	
	Percentage flower buds				Percentage flower buds	
	of total buds	with ≥5 well-developed flowers	of total buds	with ≥5 well-developed flowers	of total buds	with ≥5 well-developed flowers
Control	59.0 d	25.1 b	87.3 e	45.1 b	62.8 de	21.1 b
Hor, T=0	56.5 d	29.6 c	89.9 e	54.3 bc	52.5 d	29.7 bc
Hor, T=1	54.0 d	26.5 bc	88.5 e	52.6 bc	71.4 ef	52.0 d
Hor, T=2	53.7 d	28.4 bc	85.4 e	51.4 bc	77.5 f	34.9 c
Vertical shoots					Vertical trees	
Def, T=0	35.9 c	16.6 a	56.3cd	52.1 bc	33.6 c	3.6 a
Def, T=1	12.4 b	27.2 bc	13.6 a	68.0 c	21.9 b	4.7 a
Def, T=2	0.9 a	15.2 abc	46.4 c	15.1 a	0.4 a	0.2 a
Horizontal shoots					Horizontal trees	
Def, T=0	36.7 c	24.7 bc	64.2 d	52.9 bc	22.3 bc	31.1 bcd
Def, T=1	11.7 b	26.9 bc	23.9 b	46.0 b	3.8 a	42.0 bcd
Def, T=2	0.7 a	15.6 abc	33.9 b	25.6 a	0.1 a	0.5 a

Per column, numbers followed by different letters differ significantly at $P < 0.05$.

length) and in the apical bud (Table 3.3). In general, flower percentage was highest in the apical bud, while along the shoot flower bud percentage decreased in the order middle, upper, basal section. Bending did not affect flower formation in the different sections. Defoliation of both vertical and horizontal branches in May particularly affected flower formation in the basal section, whereas later defoliation affected buds in all positions.

Table 3.3: The effect of bending shoots in the horizontal position (Hor) and defoliation (Def) at three different times in the season on percentage of lateral flower buds on different sections along one-year-old shoots of branched trees. Flower bud percentage calculated from the total number of buds formed. T=0 (middle of May), T=1 (middle of June) and T=2 (middle of July): timing of treatments.

Position		Bending			Defoliation					
					Vertical shoots			Horizontal shoots		
	Control	Hor T=0	Hor T=1	Hor T=2	Def T=0	Def T=1	Def T=2	Def T=0	Def T=1	Def T=2
Basal	44.3	39.6	41.8	40.2	18.9	1.7	2.0	20.7	4.0	1.0
Middle	72.5	72.4	71.2	72.8	62.0	18.9	0.8	54.8	15.7	0.7
Top	58.8	55.5	48.5	48.3	31.8	11.4	0.2	36.6	13.4	0.2
AB	91.7	88.5	83.4	84.8	60.3	43.8	0.0	67.5	54.7	6.6

In order to relate the data of bud dissections of every 5th bud along the shoot in unbranched trees (see later) to their tendency to become floral, flower bud formation of these buds was recorded separately (Table 3.4). On control trees buds on positions 10-30 all became generative, whereas buds near the top (45-50) and the apical bud itself remained vegetative. Irrespective of time of treatment, horizontal placement enhanced flower formation of the apical bud. Horizontal placement also slightly enhanced flower bud formation in position 5, but tended to decrease it in positions 10-30. The effect of defoliation on flower formation per bud position depended on timing in the season. Defoliation of vertical trees particularly affected buds present at the time of defoliation. For example, at T=0 (middle of May) only bud 5 was present and flower formation in this position was completely inhibited. Bud 10 may have been present in the axils of leaves still rolled around the growing point, and flower bud formation in this position was also affected to a great extent, but inhibition was not complete. Buds 15-30 were affected to a lesser degree. At T=1 (middle of June) buds 5, 10 and 15 were present. Inhibition of bud 5 and 10 was complete, 15 and 20 were greatly inhibited, and buds on higher positions were still affected to a large degree. At T=2 (middle of July) buds 5-25 were present, but flower formation in all buds, also in buds formed later, was completely inhibited. The effect of defoliating horizontal trees in May on flower bud formation was comparable with that on vertical trees, but later defoliation gave much greater (complete) inhibition.

Table 3.4: The effect of placing entire trees horizontally (Hor) and defoliation (Def) at three different times of the season on percentage of flower buds on different positions (counting from the shoot base) along one-year-old shoots of unbranched trees. Flower bud percentage calculated from the total number of buds formed. T=0 (middle of May), T=1 (middle of June) and T=2 (middle of July): timing of treatments. AB= apical bud.

Bud Position		Horizontal placement			Defoliation					
					Vertical trees			Horizontal trees		
	Control	Hor T=0	Hor T=1	Hor T=2	Def T=0	Def T=1	Def T=2	Def T=0	Def T=1	Def T=2
5	40.0	45.5	62.5	50.0	0.0	0.0	0.0	16.7	0.0	0.0
10	100.0	30.0	75.0	73.7	22.2	0.0	0.0	33.3	0.0	0.0
15	100.0	55.6	85.7	90.0	75.0	20.0	0.0	75.0	0.0	0.0
20	100.0	87.5	100.0	100.0	77.8	11.1	0.0	66.7	0.0	0.0
25	100.0	0.0	75.0	75.0	66.7	30.0	0.0	0.0	0.0	0.0
30	100.0	0.0	50.0	80.0	42.9	50.0	0.0			0.0
35	66.7	0.0	0.0	60.0	33.3	37.5	0.0			
40	22.2		0.0	0.0	28.6	14.3	0.0			
45	0.0				0.0	0.0	0.0			
50	0.0				0.0		0.0			
AB	0.0	88.9	96.2	95.8	0.0	0.0	0.0	100.0	100.0	3.3

The effect of treatments on the plastochron

Spur buds (branched trees):

Shoot bending: Fig. 3.1A shows the effect of bending shoots at different times in the season on the increase in number of appendages in spur buds. Bending did not affect the rate of appendage formation until 8 weeks after FB when slight but inconsistent differences became apparent. Irrespective of time of treatment the first sign of generative development (dome-shaped meristem) occurred around the same time as in the control, and coincided with cessation of shoot growth (week 14, Table 3.1); in control spur buds it preceded cessation of shoot growth (week 17, Table 3.1) by 3 weeks.

Defoliation of vertical shoots: Defoliation at T=0 and T=1 caused a temporary stagnation in the increase in appendages of spur buds (Fig. 3.1B). This was less apparent at T=2, probably because the increase in appendages in control buds had also slowed appreciably by this time. After the stagnation in appendage formation a recovery occurred and the curves ran parallel to that of control spur buds. As a consequence, at the time of generative development the buds of defoliation treatments T=0 and T=1 had fewer appendages than found in control buds. Generative buds were first seen in weeks 14-18 after FB, i.e. considerably prior to cessation of shoot growth, which occurred 21 weeks after FB (Table 3.1).

Defoliation of horizontal shoots: Bud development (Fig. 3.1C) was very similar to that seen on vertical defoliated branches (Fig. 3.1B). Defoliation caused a temporary stagnation in appendage formation. However, subsequently the number of appendages continued to increases over a longer period of time than in the control buds, so that at the time of generative development a similar number of appendages was found in all treatments. Generative buds were first seen in weeks 14-18 after FB, while cessation of shoot growth occurred 16-17 weeks after FB (Table 3.1).

Lateral buds (unbranched trees):

Horizontal placement of trees: Fig. 3.2 shows the effect of placing trees in the horizontal position on the increase in the number of appendages with time of buds 5, 10, 15 and 20, counting from the base of the shoot. (Due to too few data points, data for buds 25 and above are not shown). Placing trees horizontally seemed to slow appendage formation slightly, but the effect was only pronounced in trees placed horizontally at T=0. Bending also reduced shoot growth, which will have resulted in buds at higher positions being younger and therefore less developed. In week 16 generative buds were observed on positions 10, 15 and 20 of horizontal trees (not shown in the Figs.). This time coincided with the time of cessation of shoot growth (week 16-17, Table 3.1). During the weekly dissections no generative buds were observed in the control trees.

Defoliation of vertical trees: Defoliation of vertical trees tended to give a temporary stagnation of appendage formation (Fig. 3.3), both in buds already present at the time of treatment (e.g. bud 5 at T=1) and buds formed after treatment (e.g. buds 10 and 15 at T=0). As shoot growth was affected only to a

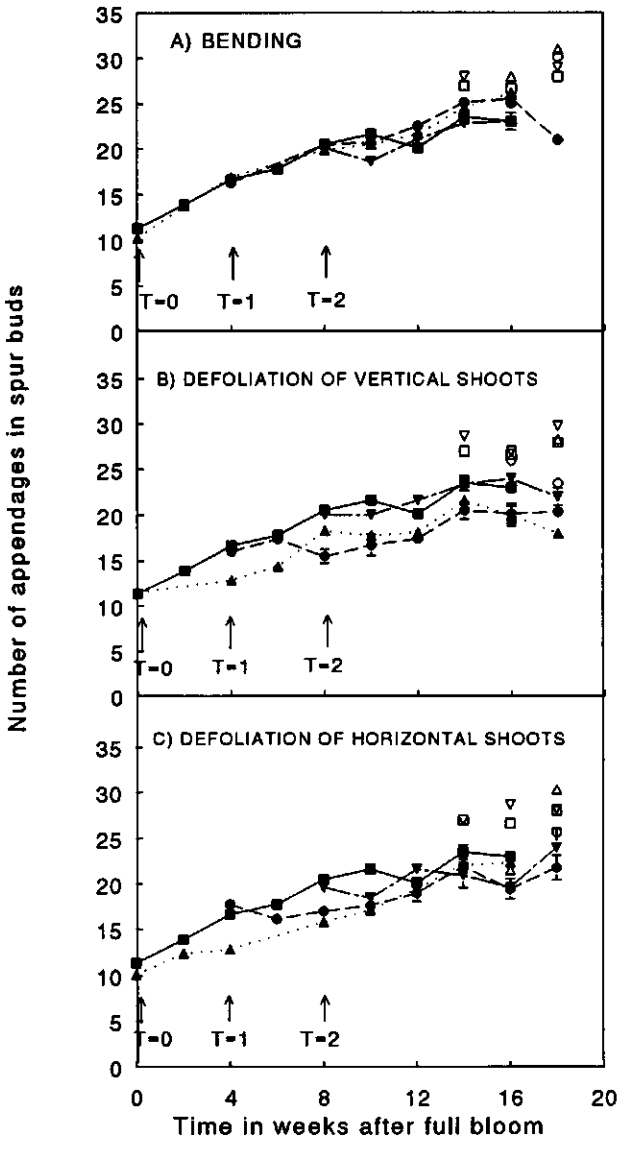

Figure 3.1: The effect of A) bending shoots horizontally, B) defoliation of vertical shoots and C) defoliation of horizontal shoots of branched trees on the number of appendages in spur buds. ■: Control, ▲: treatment at T=0, ●: T=1 and ▼: T=2. Closed symbols: no visible generative development. Open symbols: visible generative development. Data are the mean of 8 replicates ±SE. Where SE is not shown, it falls within the size of the symbol.

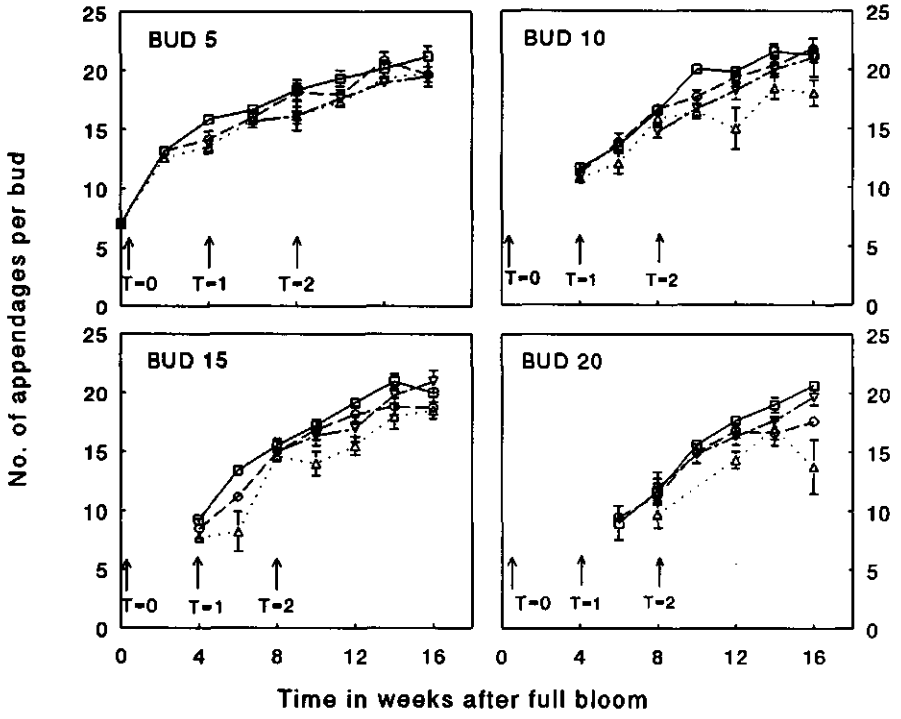

Figure 3.2: The effect of placing trees horizontally on the number of appendages in lateral buds of unbranched trees on positions 5, 10, 15 and 20, counting from the shoot base. □: Control, △: treatment at T=0, ○: T=1 and ▽: T=2. Data are the mean of 6 replicates ±SE. Where SE is not shown, it falls within the size of the symbol.

slight degree, differences in bud age in this case cannot have caused the differences in plastochron. No generative buds were observed during the weekly dissections.

Defoliation of horizontal trees: The effect of defoliation of horizontal trees on the number of appendages in bud 5 and 10 resembled that of defoliated vertical trees, but the effect in bud 15 and 20 was more pronounced leading to substantially lower numbers of appendages than in control buds (Fig. 3.4). Since defoliation of horizontal trees greatly reduced shoot growth, differences in appendage number may also be due to differences in bud age. No generative buds were observed during the weekly dissections.

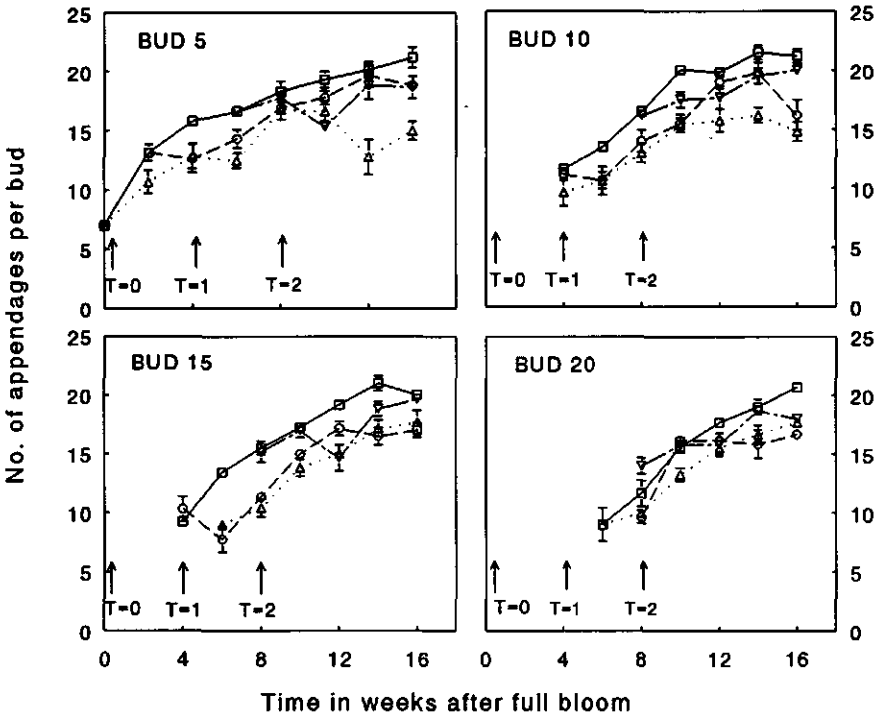

Figure 3.3: The effect of defoliation of vertical trees on the number of appendages in lateral buds of unbranched trees on positions 5, 10, 15 and 20, counting from the shoot base. □: Control, △: treatment at T=0, ○: T=1 and ▽: T=2. Data are the mean of 6 replicates ±SE. Where SE is not shown, it falls within the size of the symbol.

The effect of treatments on the carbohydrate levels of the shoot

Fig. 3.5 shows the effect of treatments on the soluble sugar (sucrose, glucose, fructose, sorbitol) and on the starch content in stem tissue of newly formed shoots of unbranched trees. Placing trees in a horizontal position did not affect soluble sugar content of the shoots, while starch increased several fold relative to the control. This increase commenced 4 weeks after FB in trees placed horizontally at T=0, and immediately after horizontal placement at T=1 and T=2. At the end of the season, the highest level of starch was found in trees placed horizontally at T=0. In contrast to bending, defoliation of vertical shoots had a pronounced effect on content of soluble sugars and hardly any effect on starch. It is important to note here that the samples were actually taken 2-4 days after the

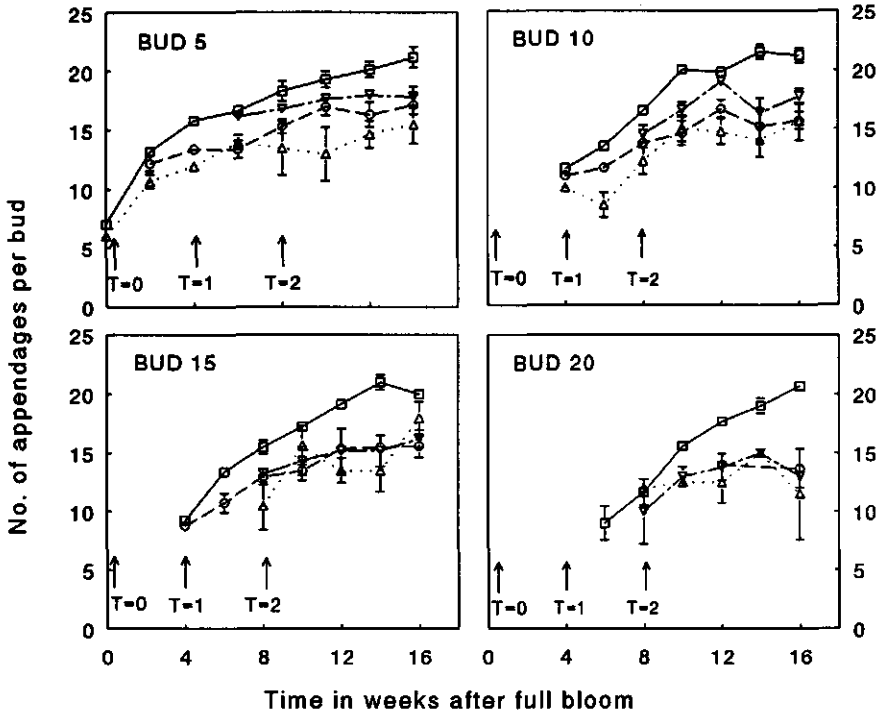

Figure 3.4: The effect of defoliation of horizontal trees on the number of appendages in lateral buds of unbranched trees on positions 5, 10, 15 and 20, counting from the shoot base. □: Control, △: treatment at T=0, ○: T=1 and ▽: T=2. Data are the mean of 6 replicates ±SE. Where SE is not shown, it falls within the size of the symbol.

treatments were applied, which accounts for why already on the first sampling dates, large treatment effects could occur. Defoliation caused an immediate reduction in all soluble sugars at T=0 and T=1, but at T=2 the effect was less marked, absent or postponed. At 4-8 weeks after the drop, sugar levels had again attained the level of the control. Glucose and fructose tended to increase to a level above that of the control 4 weeks after defoliation at T=1 and T=2, then decreased again to the control level (T=1) or remained higher (T=2). The level of sucrose increased more gradually to reach the level of the control. With regard to soluble sugars, generally the same trends were seen in vertical and horizontal defoliated trees, with somewhat more pronounced changes in glucose and fructose content of horizontally defoliated trees. Large differences were seen when comparing starch content. In horizontal trees increases in starch were seen from 8 weeks

after FB, except for trees defoliated at T=2 which showed a sharp decrease in starch content from week 8-12, then again increased from week 12-16 to above the control level. At the end of the season, starch levels of horizontal trees defoliated at T=0 and T=1 were higher than undefoliated horizontal trees.

Discussion

The effect of treatments on shoot growth and flowering

Shoot orientation and defoliation had profound effects on growth and flowering. The effects depended on timing of the treatments, and to some extent also on type of plant material used (branched or unbranched trees). Marked seasonal responses of shoot growth and flower bud formation to bending and defoliation are well known. Bending branches away from the vertical generally decreases shoot growth (Wareing and Nasr, 1958; Kato and Ito, 1962; Mika, 1969; Greene and Lord, 1978; Tromp, 1968, 1970b, 1972), and can stimulate flowering (Wareing and Nasr, 1958; Longman *et al.*, 1965; Tromp, 1968, 1970b, 1972). However, in several experiments a horizontal shoot position failed to promote flowering (Longman *et al.*, 1965; Mullins, 1965; Mika, 1969; Greene and Lord, 1978). Tromp (1970b) and Longman *et al.* (1965) found that placing entire trees horizontally was more effective in stimulating flower formation than if only shoots were bent. Similarly, in the present work flowering was only increased when the entire tree was placed in the horizontal position (Table 3.2). A horizontal tree position also alters the position of the roots, and as was pointed out by Tromp (1972), flower bud formation may as likely be affected by altered root activity as by gravitational responses of the shoot system.

Flower bud formation was only enhanced if trees were placed horizontally in July. In contrast, Tromp (1968, 1970b) showed that the effect was independent on time of treatment, since placing trees horizontally as late as after cessation of shoot growth was equally effective as when done early in the growing season. In Chapter 5 (Table 5.3, pg. 100), it was shown that a horizontal tree position particularly stimulated flower bud formation on the upper part of the shoot, an effect also reported by Longman *et al.*, (1965) and Tromp (1987). In the present work, the horizontal tree position greatly increased flower bud formation of the terminal buds (Table 3.4), but the distribution of flower buds along the shoot was not affected.

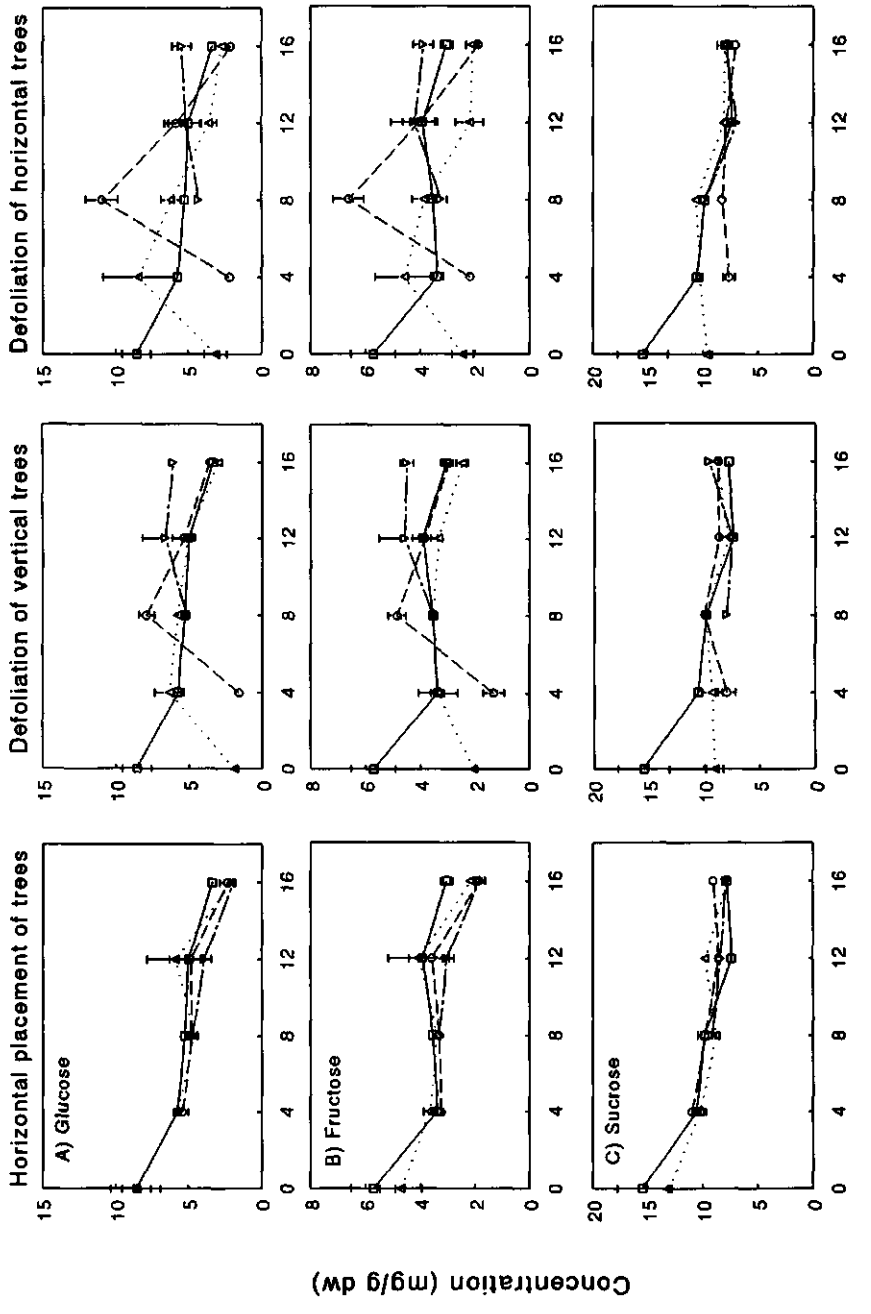

Figure 3.5: continued next page.

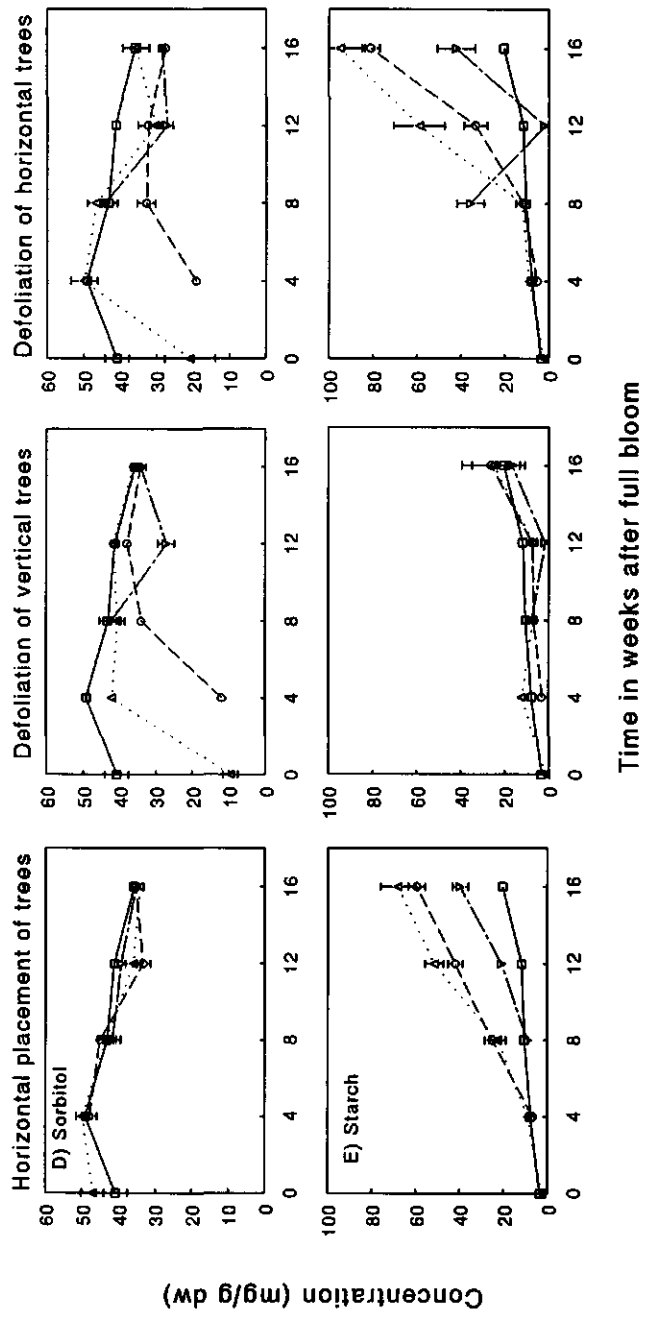

Figure 3.5: The effect of placing trees horizontally, defoliation of vertical and defoliation of horizontal trees on A) glucose, B) fructose, C) sucrose, D) sorbitol and E) starch content (mg/g dw) of shoots of unbranched trees. □: Control, △: treatment at T=0, ○: T=1 and ▽: T=2. Data are the mean of 6 replicates ±SE. Where SE is not shown, it falls within the size of the symbol.

Although a common response to defoliation is regrowth of buds (Harley *et al.*, 1942; Kato and Ito, 1962; Fulford, 1962, 1965, 1970a), defoliation reduced shoot growth. In branched trees shoot growth was reduced somewhat more if it occurred early in the season, but vertical unbranched trees showed no difference in response to time of treatments. The inhibitory effect of leaf removal on flower bud formation was progressively more severe the later it was carried out, results which agree with those obtained by Davies (1959), Fulford (1966b) and Cordes (1987). Similar to the current results, Cordes (1987) found nearly complete inhibition of flower bud formation when trees were defoliated in August.

In apple, the antagonistic relationship between vegetative and generative development is well established (Forshey and Elfving, 1989). Treatments promoting vegetative growth are generally associated with reduced flower bud formation, while treatments limiting growth vigour frequently promote flowering. In the present work, however, the effect of treatments on flower bud formation appeared to be direct, i.e. independent from an effect on shoot growth. For example, in unbranched trees the strongest stimulation of flower formation was found in the trees placed horizontally latest, which had least reduction of shoot growth. Defoliation simultaneously inhibited flower bud formation and shoot growth, ruling out the possibility that flowering was reduced due to stimulation of shoot growth.

Not only excessive vegetative growth, but also prolonged growth may inhibit flower bud formation (Swarbrick, 1929; Luckwill, 1970a). In apple, the histological differentiation of flower parts (floral differentiation) generally occurs around the time when active shoot growth ceases (Bubán and Faust, 1982). Early cessation of shoot growth is thought to be conducive to flowering both in quantity and quality, as it allows the initial steps of floral differentiation to occur under favourable (summer) conditions (Zeller, 1960; Abbott, 1970). Thus, the promotive effect of bending and the inhibitory effect of defoliation on flower bud formation may be associated with their general effect of respectively enhancing (Tromp, 1968) and delaying (Fulford, 1960, 1970a) termination of shoot growth. In the present work, a relationship between earlier cessation of shoot growth and enhanced flower bud formation was only observed in horizontal undefoliated unbranched trees (Table 3.1 and 3.2), and the profound stimulation of flower bud formation of the terminal buds may possibly be a result of the early growth termination (Table 3.4). However, in branched trees, flowering was not stimulated by earlier cessation of growth induced by a horizontal shoot position. Defoliation only delayed cessation of growth in branched trees but not in unbranched trees, and only in vertical and not in horizontal shoots (Table 3.1). However, all defoliation treat-

ments were inhibitory to flower bud formation. Thus, the inhibitory effect of defoliation on flower bud formation is not related with a prolonged period of growth.

In apple, floral differentiation of spur buds may occur several weeks before termination of growth (Bubán and Faust, 1982; Forshey and Elfving, 1989). In control trees and trees with vertical shoots, floral differentiation of spur buds could be seen well prior to cessation of shoot growth (3-7 weeks). Spur floral differentiation in trees with horizontal shoots generally coincided with the time of cessation of shoot growth. Floral differentiation of lateral buds (when observed during the periodic dissections) also coincided with the time of cessation of shoot growth.

It was concluded in Chapter 2.2, that cluster quality is not related to the time in the season when differentiation starts, and a similar conclusion is reached here. In branched trees, cluster quality was adversely affected by defoliation in July, while floral differentiation occurred at the same time as in the control (Fig. 3.1). In horizontal unbranched trees, the only treatment in which the dissections revealed flower buds, improved cluster quality of lateral buds was associated with earlier floral differentiation. However, this may also be a direct effect of the horizontal shoot position. Tromp (1970b) also found that a horizontal shoot position improved flower quality and this was independent of the time of cessation of shoot growth.

The effect of treatments on early bud development

The general observation that defoliation causes buds to grow out in a flush of growth suggests that leaves are involved in control of primordia production of bud apices. Fulford (1965) observed that an initial response to defoliation was a temporary shortening of the plastochron of spur buds. In contrast, in the present work defoliation caused a temporary lengthening of the plastochron. A major difference between the present work and that of Fulford (1965) is that his observations included buds which burst into a new flush of growth, i.e. buds likely to have a short plastochron. Such buds were excluded in the current experiments, and only buds which did not leaf-out were observed. Thus it appears that defoliation can differentially affect the plastochron of buds. These effects may be related. Defoliation removes the inhibition exerted on bud meristems, the plastochron shortens and growth activity increases. As a result of this increased growth activity, inhibition exerted on other buds increases, the buds do not sprout and the plastochron lengthens.

How leaves control the rate of appendage formation is not understood.

Fulford (1965) suggested that the rate of appendage formation is controlled by negative feedback mechanisms, whereby the foliage outside the bud controls the rate of development of younger primordia in the bud, which in turn control the growth activity of the apex. Fulford (1966a) hypothesized that this effect was mediated through interaction of three types of growth regulators, i.e. auxins, GA and a growth inhibitor. Evidence that hormones control the rate of appendage formation of buds is scarce. Indirect evidence for a role for auxins was given by Fulford (1962, 1970b), who showed that regrowth of buds was greater if the young leaves (rich in auxins) rather than the old leaves were removed, and after leaf removal regrowth could be prevented if lanolin paste containing auxins was applied to the petiole stubs. Application of GA did not affect the rate of appendage formation (Luckwill and Silva, 1979; Chapter 2.1), suggesting that GA is not involved in regulation of appendage formation. However, a continuous supply of GA to the apex from endogenous sources may have a different effect on appendage formation than incidental exogenous applications.

In Chapter 2.1 it was suggested that the availability of assimilates may influence the rate of appendage formation of apple buds, and thus leaves may affect appendage formation through the assimilate supply. In the present work, a relationship was found between the rate of appendage formation of lateral buds and the level of soluble sugars in the shoot. For example, bending did not greatly affect the plastochron of lateral buds (Fig. 3.2), nor did it have much effect on the soluble sugar content of shoots (Fig. 3.5). The temporary lengthening of the plastochron in response to defoliation (Figs 3.3 and 3.4) coincided with a temporary reduction in the sugar content (Fig. 3.5), and the plastochron again resembled that of control buds when sugar levels had been restored to that of the control.

The increase in the number of appendages with time showed a pattern similar to that found by other workers (Abbott, 1977; Luckwill and Silva, 1979), and as has been described in more detail in Chapter 2.1. In accordance with results reported previously (Luckwill and Silva, 1979; McLaughlin and Greene, 1991a,b) and those reported in Chapter 2.1, the rate of appendage formation in the period preceding floral differentiation was not correlated with the tendency of the bud to become floral. For example, placing unbranched trees horizontally in July did not greatly affect the plastochron of lateral buds (Fig. 3.2), but the percentage of flower buds was higher. Similarly, defoliation in July suppressed flower bud formation of lateral buds far more than earlier defoliation, but the plastochron was only little affected (Fig. 3.3). Treatments also did not seem to affect flower bud formation through the number of appendages per bud. For example, the number

of appendages in buds 5-20 of vertical unbranched trees defoliated in July was only slightly below that of the control (Fig. 3.3), while flowering was nearly completely inhibited (Table 3.2). Conversely, a reduced number of appendages induced by treatments did not always result in reduced flowering. Thus in accordance with previous results (Chapter 2.1), it is concluded that the presence of a short plastochron and a minimal number of appendages is not a critical factor in flower bud formation of apple, in contrast to what has frequently been suggested (Landsberg and Thorpe, 1975; Luckwill, 1975; Abbott, 1977; McLaughlin and Greene, 1991a).

The effect of treatments on the assimilate levels of the shoot

In apple, enhanced flower bud formation is generally associated with conditions favouring carbohydrate accumulation (Harley *et al.*, 1942; Jackson and Sweet, 1972; Landsberg and Thorpe, 1975). In this view the stimulatory effect of the horizontal shoot position on flower bud formation may be due to a higher level of assimilates in the branch, both as a result of reduced growth and reduced assimilate export. In the current work the horizontal tree position did not affect the soluble sugar content of the shoot, but starch levels were increased. Similar results were reported by Kato and Ito (1962) and Hansen (1972). However, the increase in starch was not related to enhanced flower bud formation, as the highest level was found in trees placed horizontally earliest, which did not have a higher percentage of flower buds than the control (Table 3.2). Thus the current results imply that the effect of the horizontal position on flowering is mediated via another mechanism than via accumulation of carbohydrates.

Similarly, removal of leaves may inhibit flower bud formation through lowering the level of carbohydrates in the shoot. In agreement with results of Hansen (1972), removal of leaves resulted in a rapid reduction in soluble sugar content of the shoot. Since more leaves were removed on later dates of defoliation, the observation that the effect on sugar level of the shoot was lower with later defoliation dates was somewhat surprising. This may be due to the fact that shoot growth activity also decreases as the season progresses. However, the fact that the soluble sugar content was only slightly affected by late leaf removal while flower bud formation was nearly completely inhibited (Table 3.1 and 3.2) suggests that the effect of defoliation is mediated through other factors than the carbohydrate status. Although Hennerty and Forshey (1971) reached a similar conclusion, these workers measured carbohydrate content in the following spring, i.e. nearly a year

after defoliation. The present work shows that the effect on sugar content is immediate, but that it may be restored to the level of the control soon after defoliation. In *Citrus*, although treatments enhancing flower formation frequently increase carbohydrate levels, it was also concluded that carbohydrates are not a major regulatory factor in flower formation (Goldschmidt *et al.*, 1985; Garcia-Luis *et al.*, 1995).

The high level of sorbitol in comparison with the other sugars in shoots and its rapid decrease following defoliation confirm that in apple this sugar is a major end product of photosynthesis (Bieleski, 1969; Oliveira and Priestly, 1988). Besides a decrease in sorbitol, defoliation also decreased the level of glucose and fructose, while sucrose was less affected. It is not clear why subsequently glucose and fructose levels in defoliated shoots increased above the level in control shoots, nor why this increase was more pronounced in horizontal than in vertical trees. It may indicate that these sugars are particularly produced by young, newly formed leaves, although it is generally assumed that sucrose is the major end product of young leaves (Bieleski and Redgewell, 1985).

The starch level of the shoot was negatively correlated with shoot growth. The trees with shortest shoots (early horizontal placement) had highest starch levels. In defoliated horizontal shoots higher starch levels were found than in horizontal trees, and this may be due to the extremely low levels of growth observed in these trees.

The mechanism of control of flower bud formation

From the foregoing it can be concluded that the effects of bending and defoliation on flowering cannot be satisfactory explained in terms of effects on bud development or assimilate levels. A number of studies have shown that the horizontal shoot position can alter hormone levels of the shoot. Kato and Ito (1962) for apple and Ryugo and Sansavini (1972) for cherry found less diffusible GA in the apical parts of horizontal than in upright shoots. In Japanese pear, increased flower bud formation in horizontal shoots was associated with lower levels of GA, and a marked increase in cytokinin content in lateral buds (Banno *et al.*, 1985). Since GA is often associated with inhibition of flower bud formation, while cytokinins may promote it (Luckwill, 1975; Hoad, 1980), lower levels of GA and higher levels of cytokinins in horizontal shoots could account for enhanced flower bud formation.

It is generally assumed that defoliation inhibits flower bud formation due to

the removal of promoters of flowering (Luckwill, 1975). It is not yet clear whether leaves themselves produce the promotive factors, or that leaves are required to sustain the transpiration stream, thereby allowing promotive substances produced elsewhere to reach the buds. Some evidence in favour of leaves being the sites of synthesis comes from ringing experiments. In ringed apple branches fewer leaves were required for flower bud formation than in unringed branches (Harley *et al.*, 1942), which suggests that ringing prevents a factor necessary for flower bud formation from moving out of the branch. In apple, evidence is accumulating suggesting that cytokinins may be the promotive factors produced by the leaves (Hoad, 1980). Leaves may be important sites of cytokinin synthesis (Greene, 1975). Ramirez and Hoad (1978) showed that applying cytokinin to the petioles after removal of the lamina, reversed the adverse effects on flower bud formation, and exogenous applications of cytokinins may enhance flower bud formation of apple (McLaughlin and Greene, 1984; Unrath, 1989; Skogerbø, 1992). As it is well known that cytokinins are also formed in the roots, leaves may also be important in ensuring a supply of root-derived cytokinins via the transpiration stream (Luckwill, 1975; Hoad, 1980).

If leaves enhance flower bud formation by producing or ensuring a supply of promotive factors, the present results suggest that buds need that promotive factor for a large part of their development. Otherwise it would not be clear why in lateral buds, absence of leaves for a short time during early or late development, irreversibly inhibited flower bud formation (Table 3.4). For example, early defoliation (in May) inhibited flower bud formation of basal buds, despite the formation of new leaves soon after defoliation. On the other hand, late defoliation (in July) also inhibited flower bud formation of basal buds, despite the fact that leaves were present for the first 2 months of their development. Thus, in lateral buds, a continuous influence of the leaves appears to be necessary to keep the flowering commitment of the buds. However, in spur buds defoliation was less inhibitory to flower bud formation, and flower bud formation was not completely inhibited by a short absence of leaves. Under conditions of limited cytokinin supply i.e. due to the removal of leaves, spur buds may have a higher competitive potential for cytokinins produced in the roots, due to closer proximity, or better vascular connections than lateral buds.

Because it is somewhat difficult to envisage how a short interruption in the supply of promotive factors by leaf removal can irreversibly inhibit flower bud formation, an alternative view is that defoliation, rather than removing factors essential to flower bud formation, is inhibitory by producing a negative factor, as

was suggested by Williams (1973). Since a typical response of defoliation is regrowth of buds, and GA is produced in young expanding leaves (Kato and Ito, 1962), defoliation may increase endogenous GA levels. The inhibitory effect of GA on flower bud formation is widely recognized (Luckwill, 1975; Hoad, 1980; Tromp, 1982; Chapter 2.1). Indeed, Taylor *et al.* (1984) found an increase in GA-like substances in buds following late defoliation (after floral differentiation had started). However, several observations argue against involvement of GA. For example, in the present work defoliation was particularly inhibitory late in the season, and all buds (i.e. buds in very divergent stages of development) were inhibited (Tables 3.2-3.4). In contrast, application of GA is particularly inhibitory when applied early in the season, i.e. during the early stages of bud development (Luckwill, 1975; Tromp, 1972, 1982, 1987; Chapter 5). It is also possible that the effect of defoliation is due to a combined effect of lowering promotive (cytokinins) and increasing inhibitory factors (GA) levels. Another possibility is that early leaf removal particularly prevents floral induction, perhaps due to the formation of inhibitors by new leaves, while late removal particularly inhibits floral differentiation, possibly by lowering levels of promoters (Cordes, 1987).

Concluding remarks

The current results amply illustrate the complexity of regulation of flower bud formation. Bending and defoliation do not appear to affect flower bud formation through the length of the plastochron or the number of appendages in the bud, nor through the carbohydrate level of the shoot. Clearly, to fully understand how flower bud formation is regulated and how it is affected by treatments such as defoliation and bending, more information is required on how hormone levels of the shoot are affected. Simultaneous investigations on levels of hormones originating from the roots may provide valuable additional information.

4. The effect of ammonium- and nitrate-nutrition on tissue arginine and polyamine content in relation to shoot growth and flowering

Summary

Bud grafts of 'Cox's Orange Pippin' on M.9 during the first season after budding were supplied with nitrate (NO_3^-) as their sole nitrogen (N) source or received part of their N as ammonium (NH_4^+). The effect of NO_3^- and NH_4^+ on arginine and free polyamine (PA) content in stem, leaves, buds, shoot top and roots was determined, and related to growth and flower bud formation. Trees receiving part of their N in the form of NH_4^+ had less total extension growth due to shorter laterals, and slightly enhanced flower bud formation. NH_4^+-nutrition increased the arginine, putrescine and spermidine content of most tissues, while spermine content was not affected. Per tissue no close correlation was found between changes in arginine and PA content. Arginine content was generally higher than PA content. Different ratios of putrescine, spermidine and spermine were found in the different tissues. The possible role of arginine and PAs in growth and flowering of apple is discussed.

Introduction

The role of nitrogen (N) in the growth and development of apple trees has received considerable attention (Titus and Kang, 1982). Depending on time and rate of application, N may affect shoot growth, floral induction, flower development, fruit set and fruit maturation. Also the form in which N is supplied may produce distinct developmental effects. In apple, NO_3^- generally promotes shoot growth, whereas NH_4^+ favours flower bud formation (Grasmanis and Leeper, 1965, 1967; Grasmanis and Edwards, 1974; Manolakis and Lüdders, 1977a; Gao *et al.*, 1992).

Despite its central role in plant nutrition, little is known about the precise mode of action of N and its products within plants (Titus and Kang, 1982). Growth reduction by NH_4^+ has been explained in terms of a higher demand for available assimilates, as NH_4^+ accumulation is toxic to the cell and thus needs to be rapidly converted to amino acids, while NO_3^- may be stored in the vacuole (Givan, 1979; Miflin and Lea, 1980; Salsac *et al.*, 1987; Kafkafi, 1990). Efficient

use of assimilates in plants fed with NH_4^+ is reflected in synthesis of compounds with a high N:C ratio (Miflin and Lea, 1977). For example, in apple trees supplied with NH_4^+, increased levels of asparagine (2N:4C) and/or arginine (4N:6C) have frequently been reported (Grasmanis and Leeper, 1965; Tromp and Ovaa, 1979; Rohozinski *et al.*, 1986).

It has also been suggested that the developmental response invoked by NH_4^+ may not be merely nutritional, but may be mediated through increased levels of hormones (Moorby and Besford, 1983; Marschner, 1986). Bubán *et al.* (1978) and Gao *et al.* (1992) measured increased cytokinin activity in apple xylem sap after NH_4^+ nutrition. Enhanced cytokinin production could account for the observed effects of NH_4^+ on growth and flowering, as cytokinins tend to stimulate the formation of short laterals and flower bud formation of apple (Skogerbø, 1992).

NH_4^+ may also affect flowering by increasing the level of polyamines (PAs), possibly via an effect on arginine synthesis (Rohozinski *et al.*, 1986). Arginine is a major component of the soluble N fraction in the xylem of apple (Hill-Cottingham and Cooper, 1970; Tromp and Ovaa, 1979) and a precursor of PAs (Slocum *et al.*, 1984). PAs are involved in a wide range of growth and developmental processes in plants (Evans and Malmberg, 1989), and in recent years a particular role has been implicated in floral induction and development (Kakkar and Rai, 1993). Rohozinski *et al.* (1986) working with young apple trees, found that NH_4^+ nutrition enhanced flowering and increased the arginine content of the stem, and that exogenous application of PAs to cut pedicels increased the number of flower buds to the same degree as NH_4^+ did. The authors suggested that the effect of NH_4^+ on flowering was mediated through elevated internal concentrations of PAs. PAs applied as spray enhanced flower bud formation of apple (Costa and Bagni, 1983).

The hypothesis that NH_4^+ increases flowering of apple by enhancing levels of PAs has not been investigated. The present work was undertaken to study the effect of NO_3^- or NH_4^+ nutrition on the level of arginine and PAs in various tissues of young Cox's Orange Pippin trees in an attempt to relate differences to effects on growth and flowering.

Materials and Methods

Beginning of April 1992, 200 bud grafts of 'Cox's Orange Pippin' on M.9 rootstocks in 10 l pots filled with clay granules were placed in a mildly heated greenhouse to activate growth. Early in May, when the sprouting buds had

reached an average length of 25 cm, 180 trees were selected for uniformity and moved to the experimental site outside. From May 13th to September the trees were supplied with culture solution. Half the trees received all N as NO_3^- (8 mmol/l), the other half received part in the form of NH_4^+ (3.25 mmol/l NO_3^- + 4.75 mmol/l NH_4^+). To maintain a similar ionic balance in the 2 culture solutions changes in other components (i.e. in SO_4^-, Cl^-) were necessary (Table 4.1).

The pH and EC of both solutions were 6.2 and 1.5 ($mScm^{-1}$), respectively. The culture solution was applied 6 to 8 times daily (90 ml per application). Pots were placed on 3 cm deep plates to ensure adequate solution availability between applications. In spite of frequent trickling an increase in the pH occurred in the plates of trees receiving only NO_3^-. Lowering the pH of the solution from 6.2 to 5.6 did not prevent this shift; the pH increased in time from 6.5 in May to 8.2 in September. The pH was more constant in the plates of trees receiving NH_4^+, a decrease in pH to 5.2 only occurring in September. From September to October the concentration of the solution was slowly reduced to stimulate cessation of extension growth. From October only water was given.

Table 4.1: Concentrations of mineral elements in the two culture solutions.

Element	Concentration (per litre)	
	NO_3^-	NO_3^- + NH_4^+
NO_3^-	8.0 mmol	3.25 mmol
NH_4^+	-	4.75 mmol
PO_4^-	1.5 mmol	1.5 mmol
SO_4^{2-}	1.0 mmol	3.0 mmol
K^+	2.5 mmol	2.5 mmol
Mg^{2+}	2.0 mmol	2.0 mmol
Ca^{2+}	2.25 mmol	2.25 mmol
Cl^-	-	5.00 mmol
Fe^{3+}	160.0 μmol	160.0 μmol
Mn^{2+}	29.6 μmol	29.6 μmol
Bo^{3-}	29.4 μmol	29.4 μmol
Zn^{2+}	1.2 μmol	1.2 μmol
Mo^{6-}	0.8 μmol	0.8 μmol
Cu^{2+}	0.4 μmol	0.4 μmol

Sampling

At frequent intervals from May to September, 4 trees (replicates) per treatment were randomly taken for analysis of arginine and PAs. The stem was divided into segments of equal length (8-10 cm), and from the middle of each segment, a leaf, the adherent bud and 1 cm of stem were taken. The buds, leaves and stem from all segments were combined to form bud, leaf and stem samples. Shoot top samples comprised all tissue including and upwards from the last leaf still folded around the growing point. From the roots a sample of tissue was taken. At first, the samples consisted only of old roots, but in time the share of young roots in the sample increased, until on the last 5 dates the samples consisted entirely of young roots.

In the middle of June an additional 2 trees per treatment were sampled to determine whether a gradient in arginine and PA content exists along the stem. The stem was divided into segments of 10 cm each, and stem material and leaves of alternating segments analyzed for arginine and PAs. Arginine and PA content of young and old roots was also determined.

The fresh weight of the samples and of the remaining tissue was determined. The samples were immediately immersed in liquid N, freeze-dried, and thereafter kept at -20°C until analysis. Before analysis the samples were ground. The dry weight of the remaining tissue was determined after drying at 70°C.

As a further check of the effect of the applied N in the tree, mineral content (total-N, P, K, Ca, Mg and NO_3^-) of the leaves was determined on the last 4 sampling dates. All leaves of 4 trees per treatment were dried at 70°C and ground. Sub-samples of 300 mg were digested using a mixture of concentrated sulphuric acid + Selenium + salicylic acid (Novozamsky *et al.*, 1983). In the digest, N and P were measured colorimetrically, Ca and K by flame emission and Mg by atomic absorption spectroscopy. NO_3^- was measured colorimetrically after reduction to NO_2^-. To investigate whether a difference in uptake occurred by trees receiving both NO_3^- or NH_4^+, the concentration of both ions was measured colorimetrically in samples taken from the plates just before solution application. Both ions decreased to 1.5-0.1 mmol/l, but no preference for either ion could be demonstrated (data not shown).

Per treatment 32 trees were separately evaluated for growth and flowering. Shoot growth (from the stock union) was measured after cessation of shoot growth (middle of October). Flowering, expressed as number and percentage of flower buds of total buds per tree was determined in the following spring. The effect of

treatment on flower quality was also assessed by counting the number of well-developed flowers per cluster, but since no difference between treatments was seen the data is not presented.

Arginine and polyamine analysis

Arginine and PAs were extracted from approximately 15 mg freeze dried material in 1 ml 0.2N perchloric acid (PCA) according to Smith and Davies (1985b). For analysis of PA conjugates, the PCA insoluble fraction and 0.5 ml of the supernatant fraction were hydrolysed for 16 hours in 6N HCl at 110°C under vacuum. The hydrolysates were dried at 80°C under a stream of air and re-suspended in 0.5 ml 0.2N PCA. The PCA supernatant containing the free PAs, the hydrolysed PCA supernatant and the hydrolysed pellet containing the PAs liberated from the conjugates were prepared for high performance liquid chromatography (HPLC) analysis as described by Smith and Davies (1985b) with the following modifications. Following dansylation the PAs were extracted with 2 ml ethylacetate; the organic phase was separated from the aqueous phase and evaporated to dryness. After a clean-up step with 5M methanolic KOH to remove interfering peaks (Seiler and Deckardt, 1975), the dansylamines were again extracted with 2 ml ethylacetate, separated from the aqueous phase and evaporated to dryness. The residue was dissolved in 1 ml methanol for analysis by HPLC. For arginine analyses the PCA soluble fractions were dansylated according to Tapuhi *et al.* (1981).

A Waters (Waters Assoc., Milford U.S.A) chromatographic system was used, consisting of a 600E solvent delivery system, a 700 WISP autosampler, and a 470 fluorescence detector. The system was equipped with a 125-4 LiChrospher 100 RP-18 (5 μm) column with guard column (Merck, Darmstadt F.R.G). For fluorescence the excitation and emission wavelengths were 365 nm and 510 nm respectively. Detector output was collected and analyzed using a Baseline 810 workstation (Dynamic Solutions). PAs were eluted from the column at a flow rate of 1 ml min^{-1} with solvent A (95% methanol : 5% 0.01M Tris-HCl buffer pH=7.7) and solvent B (25% methanol : 75% 0.01M Tris-HCl buffer pH=7.7) according to the following gradient programme (% solvent A:B): 0 to 19 min linear gradient from 66:34% to 100:0% and 19 to 20 min 100:0%. Arginine was eluted at the same conditions with the following gradient programme (% solvent A:B): 0 to 7 min linear gradient from 21:79% to 30:70%, 7 to 9 min linear gradient from 30:70% to 50:50% and 9 to 10 min 50:50%. Chemicals were obtained from Janssen Chimica (Geel, Belgium), Sigma (St. Louis, U.S.A.) and Merck

(Darmstadt F.R.G.).

Statistical analysis

Plants were arranged in a complete random design. The data for shoot growth and flowering was analyzed with regression analysis, using the statistical programme GENSTAT. Due to over-dispersion the probability distribution was not exactly binomial or Poisson, so the mean deviance ratio instead of Pearson's chi-square was used to analyze for significance of differences. Differences were considered significant at $P < 0.05$.

Results

Growth and flowering

Although growth of the main axis of trees receiving NH_4^+ lagged somewhat during July, the form of N supplied did not affect final length reached (Table 4.2). During the experiment many buds along the main stem sprouted to form laterals (sylleptic shoots). Trees receiving only NO_3^- produced slightly more shoots (>5 cm) with a greater total length. NH_4^+ nutrition produced more spurs (<5 cm). The total number of laterals was not affected.

Total flowering of trees receiving NH_4^+ was slightly enhanced when expressed as percentage (Table 4.3). In absolute number no significant difference was seen between treatments. On the main stem (buds used for analysis) a slightly higher absolute number of flower buds were formed in trees receiving NH_4^+; flower percentage was not significantly affected.

Dry weight increment of the tissues was generally slow during May and June, but became more rapid from July onwards (data not shown). Dry weight increment did not significantly differ between trees receiving NH_4^+ or NO_3^-. For this reason the data for arginine and PA levels is only expressed as content (μmol/g dw) and not as total amount per tissue as well.

Table 4.2: Effect of form of nitrogen on vegetative growth.

Treatment	Growth per tree (cm)			Number of laterals per tree	
	Total	Main axis	Laterals	< 5 cm	> 5 cm
NO_3^-	274 a	123 a	151 a	3.3 a	5.4 a
NO_3^- + NH_4^+	218 b	122 a	96 b	4.7 b	3.1 b

Values within columns followed by a different letter differ significantly at $P<0.05$.

Gradient

Arginine: Table 4.3 shows changes in arginine content from base to top in the stem and leaves, and the difference in content between old and young roots. In the stem and leaves arginine content was lowest in the lower (older) stem sections and leaves, and increased towards the top (younger tissue). Highest arginine content in the stem was found in the section below the top, in leaves it was found in the top section. An opposite trend was observed in the roots, as by far the highest arginine content was found in the old roots, while the level in young roots was low. Coincidentally, the stems of trees receiving NH_4^+ were shorter, and thus had fewer segments, making direct comparison of segments between treatments difficult. However, arginine content was generally higher in trees receiving NH_4^+.

Table 4.3: Effect of form of nitrogen on total number of buds (vegetative + flower buds), number of flower buds and % of flower buds per tree and on the stem only.

Treatment	Tree			Stem		
	Total Number	Flower buds Number	%	Total Number	Flower buds Number	%
NO_3^-	129.2 a	44.5 a	34.4 a	44.0 a	23.3 a	53.0 a
NO_3^- + NH_4^+	106.7 b	47.4 a	44.4 b	47.0 b	26.5 b	56.4 a

Values within columns followed by a different letter differ significantly at $P<0.05$.

Table 4.4: Gradient in arginine content in the stem and leaves of different stem segments and of old and young roots estimated in June. Data are the means of 2 replicates ± SE.

Treatment	Stem length (cm)	Arginine content (μmol/g dw)			
		Distance from stock union:			
		0-10 cm	20-30 cm	40-50 cm	60-70 cm
Stem					
NO_3^-	66.5	1.44 ±0.80	6.55 ±0.16	11.11 ±0.51	7.45 ±1.05
$NO_3^-+NH_4^+$	55.0	4.29 ±1.78	17.94 ±0.88	12.15 ±2.93	
Leaves					
NO_3^-	66.5	0.03 ±0.02	0.08 ±0.01	1.04 ±0.11	1.81 ±0.18
$NO_3^-+NH_4^+$	55.0	0.04 ±0.02	0.23 ±0.07	4.64 ±0.22	
Roots		Old		Young	
NO_3^-		94.93 ±18.55		0.63 ±0.14	
$NO_3^-+NH_4^+$		97.47 ±11.28		1.43 ±0.52	

Total polyamines: Only very low levels of conjugated PAs (< 5% of the free PAs) were measured in all tissues, and no changes could be measured in time, and so subsequent reference to PAs concerns only free PAs (putrescine, spermidine and spermine). Like arginine, PA content of stem and leaves increased from the base to the top (old to young tissue), but in contrast to arginine, PA content was higher in the young rather than old roots (Table 4.4). Again, direct comparison of segments between treatments is difficult, but PA content seemed little affected by the type of fertilizer.

Arginine and polyamine content of the various tissues

Arginine: Arginine content was high in all plant parts early in the season, and initially decreased (Fig. 4.1). The decrease was particularly sudden in leaves, buds and shoot tops, while in the stem the decrease was more gradual. In roots, the sharp drop in June coincided with young roots becoming predominant in the samples. Arginine content was higher in roots and stem (perennating plant parts) than in leaves and buds, and by far the highest content was found in the old roots. Arginine contents tended to rise somewhat towards the end of the growing season.

Table 4.5: Gradient in total polyamine content in the stem and leaves of different stem segments and of old and young roots estimated in June. Data are the mean of 2 replicates ± SE.

Treatment	Stem length (cm)	Polyamine content (μmol/g dw)			
		Distance from stock union:			
		0-10 cm	20-30 cm	40-50 cm	60-70 cm
Stem					
NO_3^-	66.5	0.43 ±0.02	0.53 ±0.04	0.75 ±0.02	2.25 ±0.06
$NO_3^-+NH_4^+$	55.0	0.54 ±0.03	0.63 ±0.00	1.36 ±0.28	
Leaves					
NO_3^-	66.5	0.78 ±0.02	1.10 ±0.13	1.27 ±0.04	2.62 ±0.19
$NO_3^-+NH_4^+$	55.0	1.16 ±0.12	1.36 ±0.05	2.24 ±0.30	
Roots		Old		Young	
NO_3^-		0.21 ±0.02		0.73 ±0.08	
$NO_3^-+NH_4^+$		0.38 ±0.06		1.01 ±0.01	

On several sampling dates, especially during the latter part of the growing season significantly higher contents of arginine were found in the ammonium-fed trees.

Polyamines: Slightly higher putrescine (Fig. 4.2A) and spermidine (Fig. 4.2B) contents were found in stem, leaves, buds and shoot tops of trees receiving NH_4^+, particularly in the latter part of the growing season; however, differences were small. In roots no consistent difference was seen between treatments. The content of putrescine and spermidine differed according to tissue and stage of development. In stem, buds, and particularly, leaves spermidine was the predominant PA, in roots and shoot tops both putrescine and spermidine were present in high amounts. In stem, leaves and buds putrescine and spermidine content dropped from relatively high, fluctuating levels early in the season, to lower, more constant levels later in the season. In the shoot tops spermidine gradually decreased with time, whereas putrescine, after an initial decrease, increased until June and decreased again thereafter. In the roots both putrescine and spermidine content increased until mid-July and then again decreased. Throughout the season, PA content was highest in the shoot tops. Spermine content was not affected by the type of fertilizer and remained rather constant throughout the season, and is

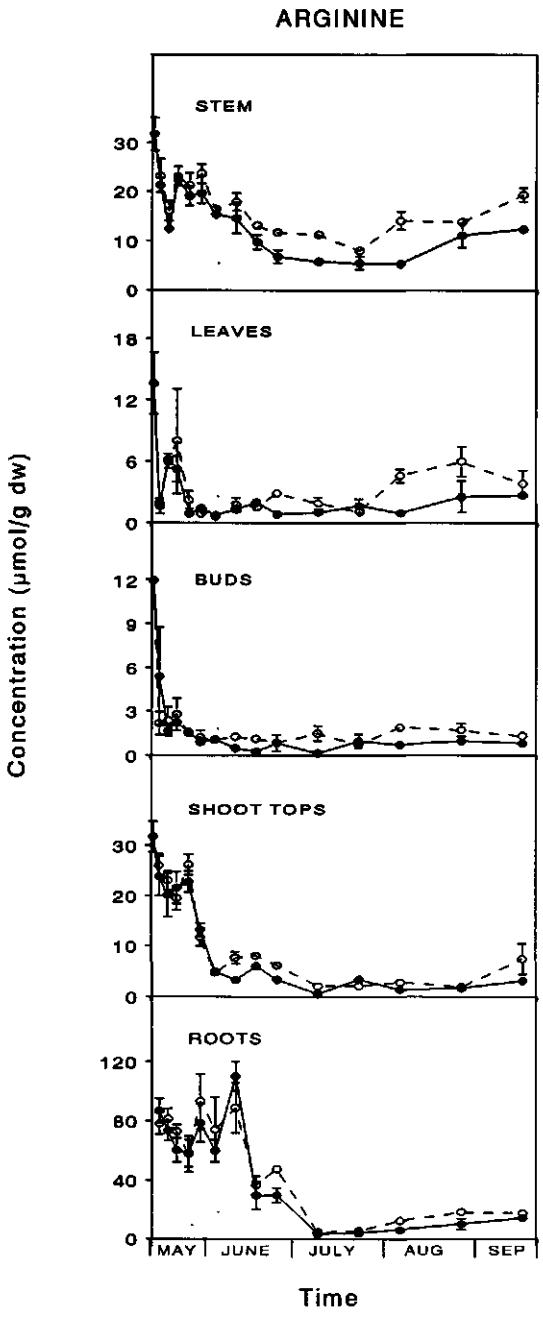

Fig. 4.1: The effect of nitrate (●) and ammonium (○) nutrition on arginine content (µmol/g dw) of various tissues. Data are the mean of 4 replicates ± SE. Where SE is not shown, it falls within the size of the symbol.

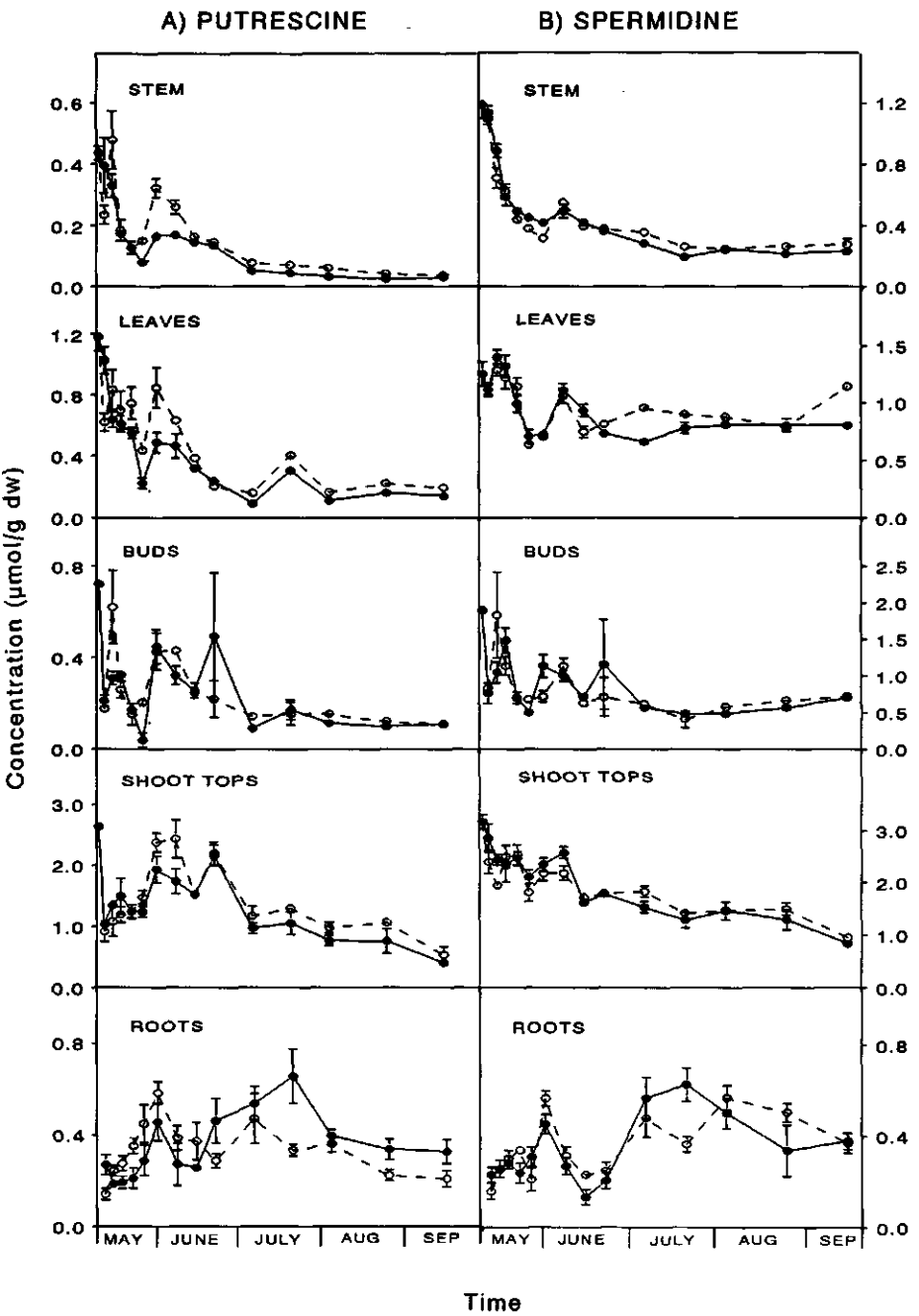

Fig. 4.2: The effect of nitrate (●) and ammonium (○) nutrition on putrescine (A) and spermidine (B) content (µmol/g dw) of various tissues. Data are the mean of 4 replicates ± SE. Where SE is not shown, it falls within the size of the symbol.

Table 4.6: Effect of nitrate and ammonium nutrition on total-N and P-content (mmol/g dw) of the leaves on the last 4 sampling dates.

Sampling date	Total-N		P	
	NO_3^-	NO_3^- + NH_4^+	NO_3^-	NO_3^- + NH_4^+
22-7	1.83 a	2.21 b	0.07 a	0.09 b
5-8	1.59 a	1.98 b	0.05 a	0.08 b
26-8	1.66 a	1.86 a	0.06 a	0.07 a
16-9	1.67 a	1.79 a	0.06 a	0.08 b

Per element, values within rows followed by a different letter differ significant at $P<0.05$.

therefore not included in the Figure. Average tissue concentrations of spermine were 0.15 μmol/g dw in the stem, 0.19 μmol/g dw in the leaves, 0.35 μmol/g dw in the buds and 0.42 μmol/g dw in the shoot tops. In the roots spermine was not detected until the last 5 sampling dates (sampling of young roots only) when only very low levels (0.07 μmol/g dw) were found.

Mineral content of the leaves

Leaf sampling in July, August and September revealed higher total-N and P contents in trees fed with NH_4^+ (Table 4.6). Levels of K (range 0.44-0.49 mmol/g dw), Ca (0.24-0.36 mmol/g dw) and Mg (0.09-0.14 mmol/g dw) were not affected by treatments. NO_3^- content of leaves was too low to be measured. NH_4^+ content of leaves was not determined as in apple all NH_4^+ is converted in the roots.

Discussion

Grasmanis and Edwards (1974) and Rohozinski *et al.* (1986), working with similar material (bud grafts of 'Jonathan' on M.M.104 or M.M.105) and with similar ion concentrations (8 mmol NO_3^- or 4 mmol NO_3^- + 4 mmol NH_4^+) as in the present experiment, showed that application of NH_4^+ doubled the number of flower buds formed during the first growth season. The effect of NH_4^+ was independent on the timing or duration of application, and no effect was observed on growth. The profound effect on flowering could not be reproduced here; NH_4^+

only slightly enhanced flowering, while growth was reduced. In a preliminary experiment with one-year-old Cox Orange Pippin trees on M.9, a short period of NH_4^+ applied 1 week following full bloom and 1 week at the end of July did not affect flowering or growth (data unpublished). Manolakis and Lüdders (1977a,b) in a 3 year trial with Cox's Orange Pippin on M.9, found reduced shoot growth after NH_4^+ application with no effect on flowering. Gao *et al.* (1992) showed that the growth and flowering response of apple to NH_4^+ was related to the vigour-controlling potential of the rootstock, and this effect seemed to be mediated through cytokinins. NH_4^+ reduced shoot growth and increased flower bud formation and cytokinin-like activity in the xylem only on dwarfing rootstocks. The variable results with NH_4^+ application in apple suggest that NH_4^+ does not affect flowering directly, but likely acts through other metabolites, the formation of which may vary under different experimental conditions.

Arginine and PA content in the tissues seemed dependent on tissue age, as was seen by the gradient in arginine and PA content in stem and leaf tissue along the stem, and by the difference in levels in young and old roots. Since the samples consisted of both young and old tissue, these differences must be taken into account. Highest arginine levels occurred in the youngest leaves and stem sections and when young tissue predominated in the samples (early in the season), while levels decreased as tissues matured, suggesting that young assimilating tissues use arginine as a substrate for growth. Although it is still a matter of debate whether new growth mainly draws on new uptake of N or on N reserves (Titus and Kang, 1982), the continued high arginine content of old roots during May and June shows that during this period of rapid growth, arginine reserves in the roots were not used up to any great extent or were immediately replenished by new N uptake. The sharp decrease seen in the roots from June to July is more likely due to the sampling of younger roots, than to an actual decrease in arginine content of the old roots. The increase in arginine content of the stem from July, and the high arginine content of old roots support findings that these woody, perennating tissues are major storage sites of arginine (Hill-Cottingham and Cooper, 1970; Tromp, 1970a; Titus and Kang, 1982).

NH_4^+ slightly enhanced arginine and PA content of most tissues; arginine was affected to a larger extent than PA content. No close association between changes in arginine and PA content in relation to time were found per tissue, and thus little can be concluded to what extent arginine functioned as a precursor to PAs under the present conditions. In contrast, Wang and Faust (1993) found that variations in PA content of apple shoots paralleled arginine content, suggesting that shoot PA

levels may be determined by arginine levels.

The slightly higher arginine and PA content in trees receiving NH_4^+ was correlated with slightly enhanced flowering. However, since the promotive effect on flowering was small, it is difficult to draw definite conclusions on whether this effect was mediated through elevated levels of PAs. The PA content of buds was generally high early in the growing season, i.e. several weeks after full bloom, which is when flower induction is thought to occur in apple (Bubán and Faust, 1982). However, as cell division is also active in all tissues at this time, high PA levels may also be correlated with high cell dividing activity. Involvement of PAs in cell division is supported by the observation that shoot tops were most abundant in PAs (Fig. 4.2), and that highest PA levels were found in the youngest tissues (Table 4.5). High levels of PAs in actively growing tissues have been found on numerous occasions (Slocum *et al.*, 1984). In apple shoots high levels of PAs were found during spring (Wang and Faust, 1993) and in apple flowers and fruits during and shortly after full bloom (Biasi *et al.*, 1988). Furthermore, NH_4^+ increased the PA levels of stem, leaves and shoot tops, but not specifically at the sites where flower formation occurs, the buds (Fig. 4.2). Microscopically visible flower parts in buds on the main axis were only seen on the last sampling date (middle of September). The morphogenetic events occurring at the meristem at this time, involving rapid cell division, were not paralleled by increased PA levels in buds.

NH_4^+ reduced shoot growth by reducing the length of the laterals. Decreased total shoot growth in apple trees receiving NH_4^+ has been found on numerous occasions (Grasmanis and Leeper, 1965; Manolakis and Lüdders, 1977a; Bubán *et al.*, 1978). Since in apple the negative relationship between vegetative growth and flower bud formation is well known (Forshey and Elfving, 1989), NH_4^+ may have enhanced flowering indirectly by reducing extension growth. Similarly, increased arginine content in trees receiving NH_4^+ may have been the result of reduced growth. In plants there is little negative feedback control for N uptake during periods of reduced growth or reduced protein synthesis (Miflin and Lea, 1980). Growth reduction in trees receiving NH_4^+ thus would result in N-excess, as is supported in the present experiment by the observed higher total-N content in the leaves of trees receiving NH_4^+. Under conditions of N-excess arginine may be preferentially formed above other amino acids (Hill-Cottingham and Cooper, 1970; Tromp and Ovaa, 1979), which is generally attributed to the efficient use of carbon (Miflin and Lea, 1977). NH_4^+ application has also been shown to elevate PA levels in several plants, and it has been suggested that increased arginine and PA synthesis are mechanisms whereby toxic effects of elevated levels of NH_4^+

may be avoided (Rabe and Lovatt, 1986; Slocum and Weinstein, 1990; Sagee and Lovatt, 1991; Altman and Levin, 1993).

Differences in ratio of putrescine, spermidine and spermine in the various tissues has been reported previously (Perdrizet and Prevost, 1981; Palavan and Galston, 1982), which suggests that the activity of biosynthetic enzymes and the functions of the individual PAs differ per tissue. Little information is available as to what extent the individual PAs induce tissue-specific morphogenetic effects. It has been suggested that the ratio of putrescine to spermidine is a measure of the degree of tissue maturation, with putrescine being relatively more abundant in differentiated cells, whereas spermidine is abundant in undifferentiated meristematic tissue (Dumortier *et al.*, 1983; Shen and Galston, 1985). For example, Shen and Galston (1985) found that the putrescine:spermidine ratio rose with increasing age and elongation of pea internodes and corn coleoptiles, suggesting that a block in the conversion of putrescine to spermidine may be an important step in the change from cell division to elongation. The relatively high share of putrescine found in shoot tops (young, unelongated cells) and of spermidine in leaves, bud and stem (differentiating tissue) in this experiment (Fig. 4.2) does not support these findings, and rather suggests the converse to be true. In tobacco thin cell layers, bud formation coincided with increases in putrescine and spermidine (Torrigiani *et al.*, 1987), while the appearance of floral buds was particularly correlated with a rise in spermidine (Kaur-Sawhney *et al.*, 1988; Tiburcio *et al.*, 1988). In the present experiment no specific PA or alterations in the ratio of PAs were implicated in bud development or floral differentiation.

The absence of spermine in old roots and its appearance only in young roots, suggests a particular role for spermine in root formation. Several studies have associated PAs in general, and spermine in particular, with enhanced rooting. The induction of roots and increase in root weight of apple were associated with an increase in PAs levels (Wang and Faust, 1986). In *Phaseolus* exogenous applications of spermine increased the number of roots (Jarvis *et al.*, 1983; Kakkar and Rai, 1987). High free spermine levels have been found at the root apex of several species (Dumortier *et al.*, 1983; Shen and Galston, 1985).

Although conjugates have previously been found in apple tissues (Wang *et al.*, 1986; Biasi *et al.*, 1988), only very low levels were found in this study. Due to the unavailability of conjugate standards, it could not be ascertained whether this was due to the analytic procedure followed. Pronounced changes particularly of conjugates have frequently been observed during developmental processes of plants. Hence the possibility that in the present experiment formation of conjugates

masked the true relationship between levels of PAs and developmental responses cannot be ruled out.

Concluding remarks

In conclusion, elevated levels of N-containing compounds, such as arginine and free PAs in trees receiving NH_4^+ may have been a result of internal conditions of N-excess due to decreased growth or more efficient N uptake and/or assimilation. Since NH_4^+ failed to have a profound effect on flowering no conclusive statement can be made as to whether flowering was affected directly by elevated levels of PAs, or indirectly via reduced growth. The different ratio of the individual PAs per tissue suggests that they have tissue specific functions. However, as to their precise biological role in growth and development, both as a group of substances and individually, much remains to be discovered.

5. The role of arginine and polyamines in the process of flower bud formation

Summary

To investigate the role of polyamines (PAs) in the process of flower bud formation of apple, one-year-old 'Cox's Orange Pippin' trees on M.9 were treated with gibberellin (GA) and branches were bent to affect the number of flower buds formed. The effect of these treatments on flowering was related to levels of arginine (a precursor of PAs) and free PAs in buds. Although treatments had a profound effect on flowering, little difference was found in bud arginine and PA content. This was the case both during the expected time of floral induction and floral differentiation. A positive correlation was found between flower bud formation and total amount of PAs per bud, however this was related to greater dry weights of buds likely to flower. Changes in PA content during bud development were best explained in terms of changes in cell division. No close correlation was found between bud arginine and PA content; arginine content was generally higher and increased during bud development, while PA content decreased.

Introduction

Numerous studies involving a wide variety of species and diverse experimental conditions have shown that increases in polyamines (PAs) and their biosynthetic enzymes are associated with active cell division and growth (Slocum *et al.*, 1984). The mechanism through which PAs regulate cellular processes is generally ascribed to their capacity to bind with sub-cellular components such as nucleic acids, proteins and membranes (Tiburcio *et al.*, 1993). In plants, it is yet unclear to what extent PAs may induce developmental responses similar to those of plant hormones. However, the use of PA inhibitors has shown that PAs are required during several developmental processes, including embryogenesis, root formation and fruit set and development (Evans and Malmberg, 1989).

In recent years, considerable evidence has accumulated suggesting a role for PAs in floral induction and development (Kakkar and Rai, 1993). In tissue cultures of thin cell layers of tobacco the formation of adventitious flower buds coincided with a marked increase in spermidine (Kaur-Sawhney *et al.*, 1988; Tiburcio *et al.*, 1988). Inhibition of spermidine synthesis prevented the formation of floral buds while exogenously applied spermidine could induce flower bud formation in

cultures which would otherwise form only vegetative buds (Kaur-Sawhney *et al.*, 1988). However, later studies showed that other factors were clearly required as spermidine was not able to induce flower buds in the absence of cytokinin, nor was it able to induce flower formation on tissue material derived from non-flowering tobacco plants (Kaur-Sawhney *et al.*, 1990). Similar to the results obtained with tobacco, in vivo flowering of *Spirodela punctata* was inhibited by inhibitors of PA synthesis, while exogenous spermidine cancelled the inhibition, but spermidine could not promote or induce flowering itself (Bendeck de Cantú and Kandeler, 1989). In several species which depend on environmental stimuli for floral induction, endogenous levels of PAs change during floral induction (Dai and Wang, 1987; Fiala *et al.*, 1988; Harkess *et al.*, 1992).

PAs may be conjugated to hydroxycinnamic acids (HCAs) and in many species these substances occur mainly in the reproductive parts (Martin-Tanguy *et al.*, 1978; Martin-Tanguy *et al.*, 1985). A specific role for conjugates of PAs in flower formation of plants has also been suggested (Martin-Tanguy *et al.*, 1985; Filner, 1987). HCAs accumulated in apical parts during floral induction of tobacco (Martin-Tanguy *et al.*, 1985). However, in vitro inhibition of HCA formation in tobacco explants did not inhibit the formation of flowers (Wysz-Benz *et al.*, 1990).

In apple, Rohozinski *et al.* (1986) showed that ammonium nutrition enhanced flowering and increased the arginine content of young apple trees. Arginine is a major component of the soluble nitrogen fraction in apple tissues (Tromp and Ovaa, 1979) and is a precursor of PAs (Slocum *et al.*, 1984). Rohozinski *et al.* (1986) showed that exogenous applications of PAs increased the number of flower buds to a similar degree as ammonium did. The authors suggested that ammonium-enhanced flowering of apple may be mediated through elevated levels of PAs via an increase in arginine. Stress-induced flowering of *Citrus* trees was associated with increased ammonium content of the leaves, and it was suggested that enhanced flowering may be mediated through increased arginine and PA synthesis (Lovatt *et al.*, 1988). Costa and Bagni (1983) also showed that exogenous application of PAs increased the number of flower buds of apple. However, in later work these results could not be reproduced (Costa *et al.*, 1986). It has been suggested that exogenous applications may only be effective when endogenous concentrations are low (Bagni *et al.*, 1981).

The present work was undertaken to study the role of free PAs during floral induction and early stages of floral differentiation of apple. The number of flower buds formed on young apple trees was affected by spraying trees with GA_{4+7} (generally inhibitory to flower formation) or by bending branches in the horizontal

position (generally conducive to flower formation). An attempt was made to correlate the effect of these treatments on flowering to bud arginine and PA content.

Material and methods

Eighty one-year-old trees of 'Cox Orange Pippin' on M.9 in 16 l containers filled with soil suitable for growing fruit trees were placed in a phytotron where irradiance (16h, SON-T and HPI-T lamps (Philips) at $\pm$ 40 W/m^2 measured above the trees), temperature (constant day and night temperature of 13°C) and the relative humidity (70%) were controlled. Forty trees were branched (had formed laterals in the nursery), the other 40 were unbranched. Half of the number of branched and unbranched trees were sprayed with GA_{4+7} (500 ppm) to drip point at full bloom (FB), with a repeat spray 3 weeks after FB. In the unbranched trees a further treatment was applied; 7 weeks after FB half the number of shoots of every tree were bent horizontally, the other half vertically. Time of cessation of shoot growth, and final shoot length were recorded. In the following spring the effect of treatment on the number of vegetative and generative buds was determined.

Sampling

Buds were collected weekly during 20 weeks following FB. From the branched trees, 2 spur buds (1 sample) from 4 trees (4 replicates) were randomly collected weekly. The spur bud was cut from the spur just below the bud scales. From the unbranched trees 1 vertical and 1 horizontal newly formed shoot per treatment were collected at random. Each shoot was divided into 3 equal sections (basal, middle and top), and from each section 4 lateral buds were cut: the 4 lowermost buds from the basal section, the middle 4 buds from the middle section, and the top 4 buds from the top section. To avoid damaging the buds, a little stem material was included when cutting the buds from the shoots. The 4 buds from each section formed 1 sample. The samples were weighed (fresh weight), freeze dried, again weighed to determine dry weight and stored at -20°C until analysis.

Arginine and polyamine analysis

Arginine and PAs were extracted from approximately 15 mg freeze dried material in 1 ml 0.2N perchloric acid (PCA) (Smith and Davies, 1985b). PAs present in the PCA soluble fraction were dansylated and prepared for high performance liquid chromatography (HPLC) analysis as described by Smith and Davies (1985b) with the following modifications. After dansylation the PAs were extracted with 2 ml ethylacetate, the organic phase was separated from the aqueous phase and evaporated to dryness. After a clean up step with 5M methanolic KOH to remove interfering peaks (Seiler and Deckardt, 1975), the dansylamines were again extracted with 2 ml ethylacetate, separated from the aqueous phase and evaporated to dryness. The residue was dissolved in 1 ml methanol before analysis by HPLC. For arginine analysis the PCA soluble fractions were dansylated according to Tapuhi *et al.*, (1981).

A Waters (Waters Assoc., Milford U.S.A) chromatographic system was used, consisting of a 600E solvent delivery system, a 700 WISP autosampler, and a 470 fluorescence detector. The system was equipped with a 125-4 LiChrospher 100 RP-18 (5 μm) column with guard column (Merck, Darmstadt F.R.G). For fluorescence the excitation and emission wavelengths were 365 nm and 510 nm, respectively. Detector output was collected and analyzed using a Baseline 810 workstation (Dynamic Solutions). PAs were eluted from the column at a flow rate of 1 ml min^{-1} with solvent A (95% methanol : 5% 0.01M Tris-HCl buffer pH=7.7) and solvent B (25% methanol : 75% 0.01M Tris-HCl buffer pH=7.7) according to the following gradient programme (% solvent A:B): 0 to 19 min linear gradient from 66:34% to 100:0% and 19 to 20 min 100:0%. Arginine was eluted at the same conditions with the following gradient programme (% solvent A:B): 0 to 7 min linear gradient from 21:79% to 30:70%, 7 to 9 min linear gradient from 30:70% to 50:50% and 9 to 10 min 50:50%. Chemicals were obtained from Janssen Chimica (Geel, Belgium), Sigma (St. Louis, U.S.A.) and Merck (Darmstadt F.R.G.).

Statistical analysis

Treatments were randomly assigned to and within the trees according to a complete random design with a split for branch orientation in unbranched trees (split-plot). The data for shoot growth and flowering was statistically analyzed with regression analysis using the statistical programme GENSTAT. Due to over-

dispersion the probability distribution was not exactly binomial or Poisson, so the mean deviance ratio instead of Pearson's chi-square was used to analyze for significance of differences. Differences were considered significant at $P < 0.05$.

Results

Shoot growth and flowering

Treatment with GA had a different effect on shoot growth and flowering in branched and unbranched trees. In branched trees (Table 5.1), GA significantly increased shoot growth, and decreased both the number and percentage of flower buds on new growth (one-year-old shoots) and spurs, although the effect was only significant for spurs. In unbranched trees (Table 5.2), GA slightly decreased shoot growth, total number and percentage of flower buds, but the effects were not significant. Bending shoots horizontally only slightly decreased shoot growth, but greatly increased percentage and number of flower buds. Along the shoot a clear difference in tendency to form flowers was seen (Table 5.3). The tendency was highest in the middle section, less high in the upper section, and lowest in the basal section. The effect of GA and bending on the percentage of flower buds varied with bud position. GA lowered flower percentage of basal and middle buds, whereas in top buds, although the effect was not significant, GA slightly enhanced flowering. Bending did not significantly affect flower bud percentage of basal buds, but greatly increased it in middle and top buds.

Table 5.1: Effects of GA_{4+7} on branched trees: growth per tree, number of flower buds and flower bud percentage of one-year-old shoots and spur buds per tree.

Treatment	Growth	Flower buds			
		One-year-old shoots		Spurs	
	(m per tree)	Number	Percentage	Number	Percentage
Control	4.22 a	92.3 a	34.7 a	31.1 a	81.0 a
GA_{4+7}	4.83 b	84.0 a	25.4 a	17.9 b	48.1 b

Values per treatment separated by a different letter differ significantly at $P < 0.05$.

Table 5.2: Effects of GA_{4+7} and shoot orientation on unbranched trees: growth per tree, total number of flower buds and percentage of flower buds per tree.

Treatment	Shoot orientation	Growth m per tree	Flower buds Number	Percentage
Control	Vertical	1.64	28.0	26.7
GA_{4+7}		1.50	18.7	21.9
Control	Horizontal	1.46	43.9	47.5
GA_{4+7}		1.35	35.0	45.3
C vs GA_{4+7}		NS	NS	NS
Branch orientation		S	S	S
Interaction		NS	NS	NS

S= significant, NS= not significant at $P<0.05$.

Arginine and polyamine levels

Branched trees (spur buds): Dry weight increase of spur buds treated with GA was reduced in comparison with untreated spur buds (Fig. 5.1). Arginine content and amount were also lowered in trees treated with GA (Fig. 5.2A and B). In both unsprayed as GA-treated trees arginine content and total amount per bud increased

Table 5.3: Effect of GA_{4+7} and shoot orientation on percentage of flower buds per shoot section (basal = lower 1/3, middle = middle 1/3, top = top 1/3) of unbranched trees.

Treatment	Shoot orientation	Flowering (%) Basal	Middle	Top
Control	Vertical	17.0	56.7	18.4
GA_{4+7}		5.4	38.3	23.8
Control	Horizontal	23.5	77.8	50.2
GA_{4+7}		4.7	69.8	57.6
C vs GA_{4+7}		S	S	NS
Branch orientation		NS	S	S
Interaction		NS	NS	NS

S= significant, NS= not significant at $P<0.05$.

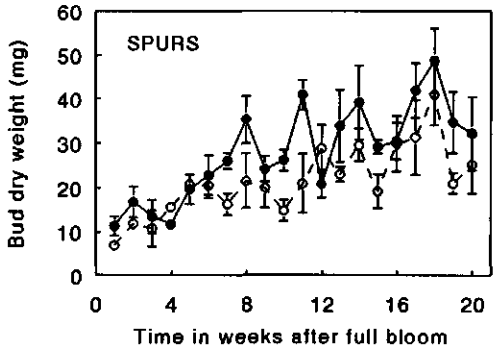

Fig. 5.1: Effect of GA_{4+7} on dry weight (mg) of spur buds on branched trees. Control (●), GA_{4+7} (○). Data are the mean of 4 replicates ±SE.

with time. PA content in spur buds varied little between treatments (Fig. 5.3A). It was slightly higher in GA-treated buds in the period that GA was applied (0-3 weeks after full bloom), and slightly lower from about week 11. In both treatments PA content decreased until week 11 remaining more constant thereafter. Larger differences between treatments were found when the data is expressed as total amount per bud (Fig. 5.3B), with higher PA amounts in control spur buds. However, these higher amounts are mainly due to a higher bud dry weight of control buds (Fig. 5.1).

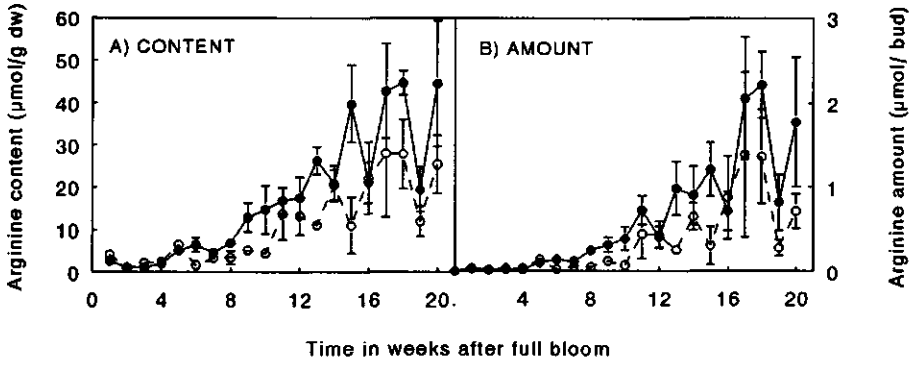

Fig. 5.2: The effect of GA_{4+7} on arginine content (A, µmol/g dw) and amount (B, µmol/bud) of spurs on branched trees. Control (●), GA_{4+7} (○). Data are the mean of 4 replicates ±SE.

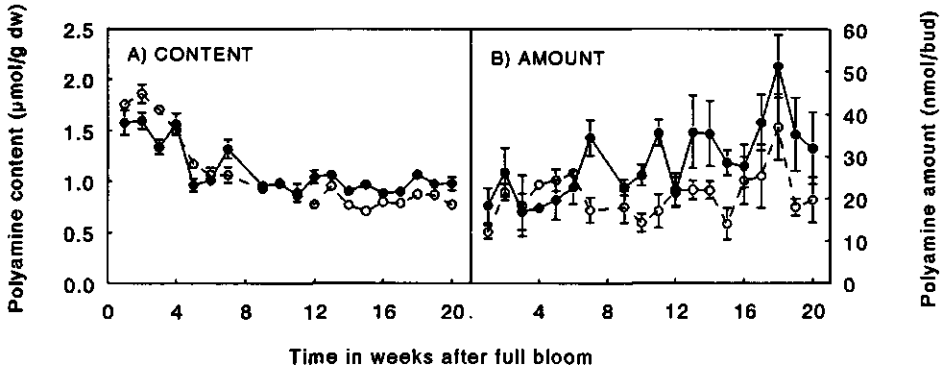

Fig. 5.3: The effect of GA_{4+7} on polyamine content (A, µmol/g dw) and amount (B, nmol/bud) of spurs on branched trees. Control (●), GA_{4+7} (○). Data are the mean of 4 replicates ±SE.

Unbranched trees (lateral buds): Bud dry weight and arginine and PA content were not affected by bending or by GA application. Larger differences were seen when comparing buds along the stem (basal, middle, top). The data for the different treatments (bending and GA) have therefore been combined, and only the data for the different bud positions is shown. Fig. 5.4 shows bud dry weights per bud position. Middle buds had a higher dry weight than basal and top buds. Arginine in lateral buds was lower than in spurs (compare Fig. 5.5A and B with Fig. 5.2A

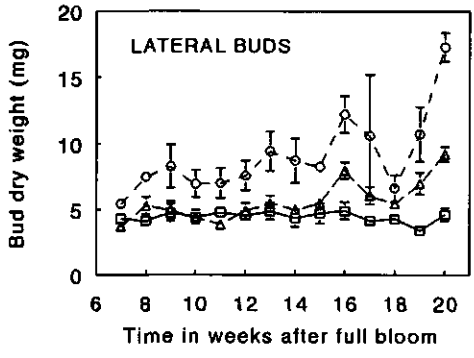

Fig. 5.4: Effect of bud position on dry weight (mg) of lateral buds on unbranched trees. □: Basal, ○: Middle, △: Top. The data for GA-treatment and bending has been combined. Data are the mean of 4 replicates ±SE.

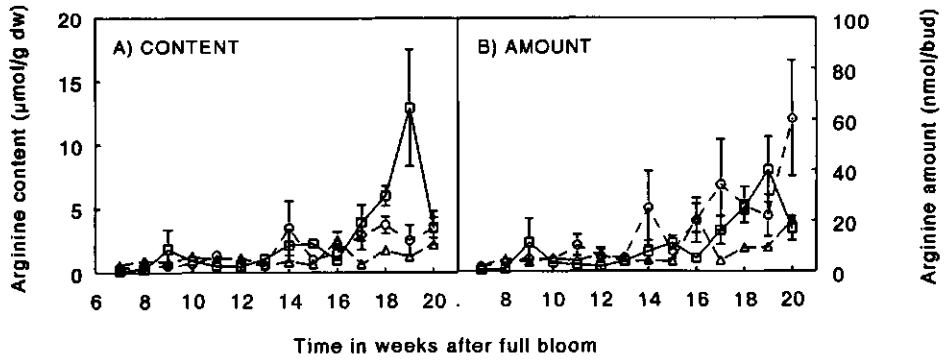

Fig. 5.5: The effect of bud position on arginine content (A, µmol/g dw) and amount (B, nmol/bud) of lateral buds on unbranched trees. □: Basal, ○: Middle, △: Top. The data for GA-treatment and bending has been combined. Data are the mean of 4 replicates ±SE.

and B). It slightly increased with time in all bud positions. A peak in arginine content occurred in basal buds in week 19. In contrast to arginine content, PA content in lateral buds was as high as in spurs (compare Fig. 5.6A and B with Fig. 5.3A and B). PA content was initially highest in top lateral buds, lower in middle buds and lowest in basal buds (Fig. 5.6A). After an initial decrease in top and middle buds, the content was similar in all positions and remained more or less

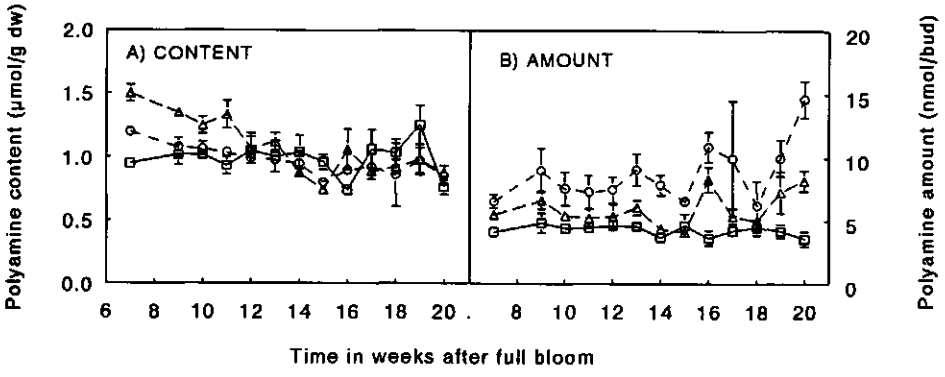

Fig. 5.6: The effect of bud position on polyamine content (A, µmol/g dw) and amount (B, nmol/bud) of lateral buds on unbranched trees. □: Basal, ○: Middle, △: Top. The data for GA-treatment and bending has been combined. Data are the mean of 4 replicates ±SE.

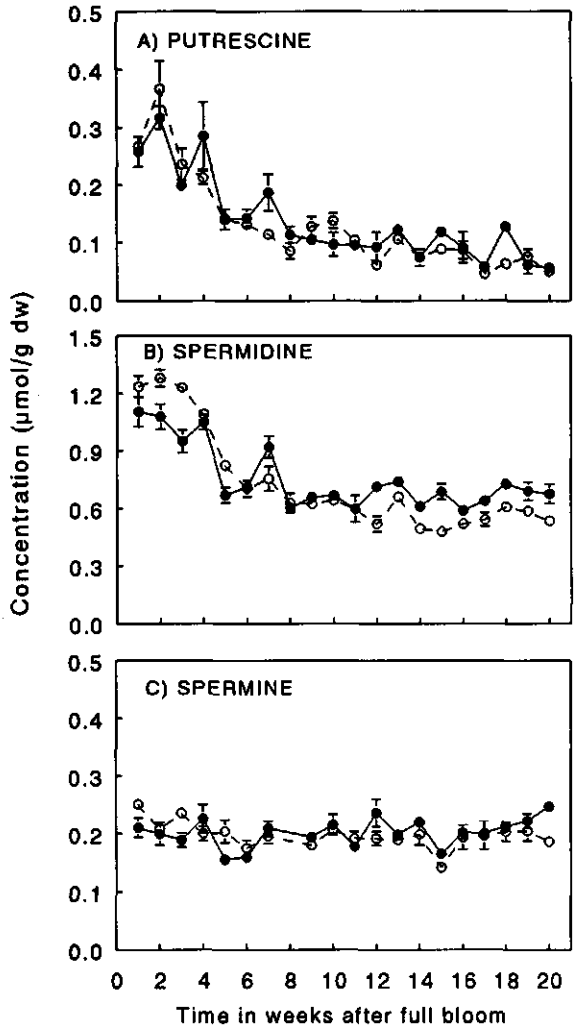

Fig. 5.7: The effect of GA_{4+7} on putrescine (A), spermidine (B) and spermine (C) content (µmol/g dw) of spur buds on branched trees. Control (●), GA_{4+7} (○). Data are the mean of 4 replicates ±SE.

constant. Larger differences were seen when the data is expressed as total amount per bud (Fig. 5.6B). PA levels now decreased in the order of middle, top and basal buds. This pattern closely resembled bud weights (Fig. 5.4).

The previous data refers to total PAs (putrescine + spermidine + spermine). Fig. 5.7A-5.7C (spur buds) and Fig. 5.8A-5.8C (lateral buds) show changes in

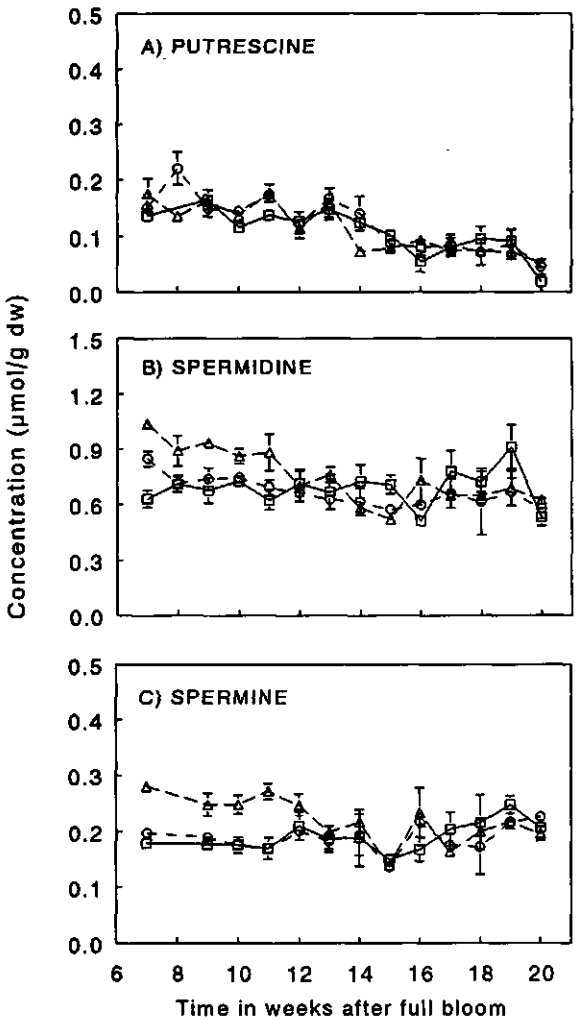

Fig. 5.8: The effect of bud position on putrescine (A), spermidine (B) and spermine (C) content (µmol/g dw) of lateral buds on unbranched trees. □: Basal, ○: Middle, △: Top. The data for GA-treatment and bending has been combined. Data are the mean of 4 replicates ±SE.

content of the individual PAs. In both bud types, spermidine was the major PA present, and thus mainly determined the pattern of total PAs. In time, putrescine showed a more pronounced decrease than spermidine, whereas spermine remained more constant. The higher level of total PAs found during GA application (0-3

weeks after FB) was due to an increase in spermidine content, while the lower level from week 11 was due to a lower spermidine content. The higher level of total PAs in lateral top buds 7-12 weeks after FB was due to higher content of spermidine and spermine.

Discussion

In apple, flower bud formation involves two distinct phases, namely floral induction, during which the meristem is programmed to become generative, and floral differentiation, which involves the morphological modification of the meristem, resulting in microscopically visible flower parts (Bubán and Faust, 1982). In apple, much experimental data on timing of treatments affecting the number of flower buds has shown that floral induction is mainly affected early in the season, but depending on the treatment and bud position it can also be affected later, until after the time cessation of shoot growth (Bubán and Faust, 1982). Floral differentiation generally starts around the time of cessation of shoot growth (Bubán and Faust, 1982; Forshey and Elfving, 1989).

In the present experiment, GA_{4+7} applied during or 3 weeks after FB and bending 7 weeks after FB affected the number of flower buds in comparison to untreated controls. Treatment with GA particularly inhibited flower formation of buds present during treatment (i.e. spurs and basal buds), while bending particularly affected flower formation of buds formed after treatment (i.e. top buds). Thus, floral induction was influenced during most of the period of active growth. During the time in which GA affected the number of flowering spur buds, PA content was high in both treated and untreated spur buds; however, no difference was found in PA content of buds more (untreated) or less (GA-treated) likely to become floral. Bending and GA-treatment also affected flower bud formation of lateral buds, but a correlation between the degree of floral induction and PA content was also not found. These results are in contrast to results obtained with several other plants, where changes in PA levels were shown to coincide with critical phases of induction. For example, in *Pharbitis nil* putrescine levels declined during photoperiodic induction (Dai and Wang, 1987), while declining spermidine levels were found during high temperature floral induction of *Iris hollandica* (Fiala *et al.*, 1988). In *Rudbeckia hirta* an increase in putrescine and spermidine occurred during photoperiodic floral induction (Harkess *et al.*, 1992).

Floral differentiation generally starts around the time of cessation of shoot

growth (Bubán and Faust, 1982; Forshey and Elfving, 1989). In the present experiment cessation of shoot growth occurred at 12-14 weeks after FB. At this time, a slightly higher PA content in control than in GA-treated spur buds was found, and since more flower buds formed in the control spurs, this may be related to more active floral differentiation. However, in lateral buds, a higher PA content in middle than basal or top buds was not observed during the supposed time of floral differentiation, in spite of the fact that middle buds formed more flower buds. Moreover, during the time of floral differentiation PA content was generally low, similar to what was found with cherry (Wang and Faust, 1985) and in previous experiments with apple (Wang and Faust, 1994; Chapter 4).

Thus, the observed changes in PA content do not correlate well with the occurrence of floral induction and differentiation, but rather seem to reflect changes in cell dividing activity. The high PA content in the early stages of bud development coincided with the period of active cell division, while the decrease in time coincided with a decrease in cell division as buds mature. A similar relationship between PA content and cell dividing activity of buds was found in pea (Smith and Davies, 1985a). In the present study the higher PA content initially found in top buds (younger tissues), and in spur buds of GA-treated trees (stimulation of growth), also point to involvement of PAs in cell division. GA application particularly increased spermidine content of the buds, as was found by Smith and Davies (1985a), who suggested that spermidine might be required for GA-promoted growth. High levels of PAs and their biosynthetic enzymes in tissues undergoing active cell division have been found on numerous occasions (Slocum *et al.*, 1984).

When PA levels were expressed as total amount per bud, a positive correlation was found with flower bud formation. However, higher PA amounts were strongly correlated with a higher dry weight of buds likely to form flowers. A similar positive correlation between PA levels and bud size was reported for pea (Smith and Davies, 1985a). However, since whole buds were measured, small localized changes in PA levels at the meristem may have escaped detection. It is not possible to say whether the increase in PAs occurred generally throughout the bud, or whether it occurred at specific sites, e.g. at the meristem. A higher level of PAs at the meristem well before the appearance of flower parts and the accompanying increase in cell division and metabolic activity may indicate involvement in the induction process. In *Rudbeckia hirta* increases in putrescine and spermidine during floral induction were shown to occur at the meristem just prior to the first histological changes (Harkess *et al.*, 1992).

The greater increase in dry weight in treatments with increased flowering was seen well before floral differentiation, which poses the question whether higher assimilate levels are involved in floral induction. It has been suggested that floral induction is essentially determined by the supply of assimilates to the meristem, and that hormones affect flower formation mainly by affecting the assimilate supply (Sachs, 1977). The observed effects of GA of both decreasing dry weight and inhibiting flowering would support this hypothesis. In basal buds GA inhibition of flower formation was also correlated with lower bud dry weights, but in middle and top buds GA inhibited flower bud formation without an effect on bud dry weight (data not shown). Thus GA may affect flowering without a simultaneous effect on assimilate supply. In general no correlation was found between changes in bud arginine and PA levels, as bud arginine content generally increased while PA content decreased during bud development. This is in contrast to results obtained by Wang and Faust (1993), who in shoots of apple found similar changes in arginine and PA content in time, which suggested that PA levels were mainly determined by arginine levels. From the current results no conclusions can be drawn as to what extent arginine acted as a precursor for PAs.

In apple a negative relationship between shoot growth and flower bud formation is well established (Forshey and Elfving, 1989), and the effect of treatments on flower bud formation is sometimes explained in terms of their effect on shoot growth. In unbranched trees, GA-treatment and bending affected flowering without an effect on growth. Similar effects were found by Tromp (1970b). Tromp (1970b, 1972) showed that bending enhanced flower bud formation even after cessation of shoot growth, indicating that increased flower bud formation on horizontal shoots is a direct effect of shoot orientation. How shoot orientation can affect flower formation is not known, but it is generally assumed to be mediated through altered hormonal relations (Longman *et al.*, 1965). In the present experiment, the inhibitory effect of GA was most pronounced in basal buds, i.e. buds present during GA application, and it persisted in the shoot for some time after, as seen by inhibition of flower formation in middle buds. During formation of top buds an inhibitory effect was no longer evident, and flowering even seemed to be slightly enhanced. This slight enhancement of GA_{4+7} on flowering of top buds is interesting, and may be attributed to the presence of GA_4 in the mixture, as GA_4 has been shown to be able to enhance flower formation of apple (Looney *et al.*, 1985). However, Looney *et al.* (1978) showed that GA_4 is rapidly broken down in apple tissue, and it therefore does not seem likely that it could have persisted in the branch long enough to account for the enhanced flower formation of top

buds. However, the differential effects of GA on buds on different positions places doubt on the role of GA as mere antagonists of flower bud formation, similar to other recent suggestions (Looney *et al.*, 1985; Greene, 1989).

Similar to the results of Tromp (1987), GA particularly inhibited flower formation on the lower half of branches, whereas bending had greatest stimulatory effects on top buds, and had less or no effect on middle and basal buds. Tromp (1972) suggested that GA inhibits the early stages of flower bud formation, while bending affects later stages. GA seems to block flower formation in an irreversible manner (Tromp, 1972), and the current results support this, as bending of shoots was not able to alleviate GA inhibition of basal buds. However, buds formed after GA application (middle buds) were inhibited to a lesser degree on horizontal branches than on vertical branches, suggesting that the horizontal position may counteract part of the negative effect of GA in newly formed buds, perhaps due to reduced GA transport. The upright position also seemed to inhibit flower bud formation in an irreversible manner, as bending did not significantly enhance flower formation of buds formed prior to bending (basal buds). This result is in contrast to those of Tromp (1970b, 1972) who found that bending stimulated flower bud formation even if it was done after cessation of shoot growth, i.e. it enhanced flower bud formation of buds formed prior to bending.

Concluding remarks

As can be concluded from the above, a complex of factors, both positive and negative may regulate flower formation of apple. However, the results obtained here suggest that, in apple, changes in PA levels may not be involved in flower bud formation, as opposed to results obtained with several other species. However, these and other studies, have shown that more dramatic changes may occur in levels of PA conjugates than in free PAs, and the observation that the former accumulate specifically in reproductive parts whereas the latter may occur anywhere in the plant, suggests that PA conjugates may play a more important role in the flowering process (Filner, 1987). Only very low levels of conjugates were found in apple tissues in a previous experiment (< 5% of the free PAs) (Chapter 4), which is in contrast to other work with apple (Wang and Faust, 1986; Biasi *et al.*, 1988) and with plants in general. More work involving activities of biosynthetic enzymes, the use of biosynthetic inhibitors, and research into the physiological role of PA conjugates is required to clarify the importance of PAs in the flowering process.

6. General discussion

As was reviewed in the introduction, regulation of flower bud formation of apple is a complex process which may be affected by a wide range of internal and external factors, and both promoters or inhibitors may be involved. The results reported in this thesis amply illustrate this. A variety of factors, such as temperature, gibberellin (GA), and cultural treatments (the presence of fruits, shoot orientation and defoliation) were shown to affect flower bud formation in positive or negative ways. In some cases, growth vigour was also affected, and because of the usually antagonistic relationship between shoot growth and flowering the treatments may have indirectly affected flowering through growth vigour. However, as will be discussed, in most experiments a direct effect on flower bud formation could be established. It was further stated in the introduction that variable response is a major factor limiting insight of regulation of flower bud formation, as was also encountered in the present work. For example, while a profound stimulation of flower bud formation by ammonium has been reported in the literature, only a slight stimulation was found in the present study. In Chapter 5, bending was shown to stimulate flowering, while in Chapter 3 the stimulatory response was much less clear. Such inconsistencies emphasize that flowering of apple is not regulated by a single factor in a simple all or nothing fashion, but that a complex of factors interact to determine the response.

Although variable response may confuse interpretation on the short term, consistent responses recurring in the long term can lead to formulation and testing of new hypotheses, through which a greater understanding of regulation of flowering will be achieved. As was aptly stated by Luckwill (1970b): "Hypotheses are our stepping stones across the flood of knowledge - we progress from one to the other, and only when we have no hypothesis are we in danger of drowning in the confusion of apparently unrelated and conflicting facts." The aim of this thesis was to test the validity of two hypotheses frequently encountered in the literature. The first of these was that morphological events of bud development (the plastochron and the number of appendages per bud) may be critical to the occurrence of flower bud formation, and the second was that nutrients, such as ammonium, may affect flower bud formation through altering the level of endogenous growth factors, such as, polyamines (PAs). In this final chapter the results and implications of these hypotheses will be summarized and discussed. Some general features of regulation of flower bud formation and the relationship between vegetative and generative development will also be considered.

6.1 Morphological events.

Plastochron

Interest in the plastochron of apple buds was initiated by the work of Fulford (1965, 1966a,b,c), who provided convincing evidence that the occurrence of flower bud formation of apple was related to the length of the plastochron. Floral differentiation only occurred in buds with a plastochron of 7 days. A shorter plastochron caused buds to break out into a flush of growth, while buds with a longer plastochron remained vegetative. Since many treatments which promote or inhibit flowering in apple are most effective early in the season, i.e. when buds are in their early stages of development, it was thought that they may affect flower bud formation through the plastochron. Treatments which enhance flower bud formation could do so by shortening the plastochron, while inhibitory treatments could lengthen it (Fulford, 1965, 1966c; Luckwill, 1975; Tromp, 1976, 1984).

In the present work, the effect of several treatments known to promote or inhibit flower bud formation of apple on the plastochron was studied (Chapter 2.1 and 3). It could convincingly be shown that the length of the plastochron was independent of the occurrence of flower bud formation. Temperature, application of GA_{4+7}, the presence of fruits, defoliation and shoot orientation influenced flower bud formation but only defoliation and temperature affected the plastochron. A higher temperature initially increased the rate of appendage formation, but not in all experiments did this higher rate coincide with increased flower bud formation (Chapter 2.1). Defoliation both lengthened the plastochron and inhibited flower bud formation (Chapter 3). However, the plastochron was least (if at all) affected at the time when it inhibited flowering most (defoliation in July).

From the lack of a clear link between the occurrence of flower bud formation and the plastochron, it is concluded that a specific rate of appendage formation during early bud development is not critical to flower formation of apple. Similar conclusions were reached by other authors studying the effect of growth regulators and the presence of fruits (Luckwill and Silva, 1979; McLaughlin and Greene, 1991a,b). However, it is important to note, as was already mentioned in Chapter 2.1, that the close correlation between the plastochron and flower bud formation as found by Fulford (1965, 1966b) occurred late in the season and thus may have coincided with the period of actual floral differentiation, rather than with the earlier phase of floral induction. In apple, as in many herbaceous species, the appearance of flower initials is associated with an increase in the rate of primordia

production, i.e. a shortening of the plastochron (Langer and Bussell, 1964; Abbott, 1977; Luckwill and Silva, 1979; Lyndon and Battey, 1985), while in buds remaining vegetative the plastochron remains long. This was also found in the present work. *It may therefore be concluded that a shortening in the plastochron, often claimed to be a regulatory mechanism in flower bud formation of apple, may be a consequence of the process rather than a causal event determining it.*

Number of appendages per bud

Several authors have found that the number of appendages in flower buds of apple is rather constant, and that floral differentiation does not occur before a certain number of appendages has been formed in the bud. This has led to the suggestion that the presence of a minimal number of appendages is a prerequisite for floral differentiation (Landsberg and Thorpe, 1975; Luckwill, 1975; Abbott, 1977). In other species a close connection between the vegetative development of buds or shoots and floral differentiation has also been demonstrated. In tobacco (McDaniel *et al.*, 1989), rose (Marcelis-van Acker, 1994) and black currant (Tinklin *et al.*, 1970) shoots a minimum number of leaf primordia must be present before flower formation can occur. In buds of grape (Buttrose, 1970) and Japanese pear (Banno *et al.*, 1986; Higashiuchi *et al.*, 1990) a close correlation exists between the number of appendages per bud and the tendency of the bud to become floral. The mechanism of this relationship is not clear (McDaniel *et al.*, 1989).

In apple, it was suggested that a possible way in which treatments affect flower bud formation proceeds through the number of appendages per bud, i.e. if treatments increase the number of appendages above a critical value, flower formation occurs, whereas if buds fail to reach this number before the onset of dormancy flower formation is prevented. According to this reasoning, a short plastochron, rather than being of direct importance as postulated by Fulford (1965), is of indirect importance, by ensuring that a minimal number of appendages is reached at a time before bud activity slows due to unfavourable external conditions.

However, in the present work no indication was found that flower bud formation depends on the presence of a specific number of appendages per bud. The number of appendages at the start of floral differentiation varied within a bud sample and also between cultivars (Cox's Orange Pippin and Jonagold), experiments and bud positions. In experiments 1 and 2 of Chapter 2.1, the higher temperature slightly increased the number of appendages at which flowers formed, but

flower bud formation was nevertheless reduced. Application of GA and the presence of fruits inhibited flower bud formation without significantly lowering the number of appendages below that of the control. Defoliation (Chapter 3) simultaneously lowered the number of appendages and inhibited flower bud formation. However, at the time that it most effectively inhibited flower bud formation (July) it did not significantly lower the number of appendages. *From the large variation in the number of appendages per bud at the start of floral differentiation and the lack of correlation between the number of appendages and the occurrence of flower bud formation it is concluded that a certain minimal number of appendages is not critical for flower bud formation of apple.*

Nevertheless, in apple it is often observed that buds with a limited number of appendages generally remain vegetative. For example, basal buds on shoots show little bud development, have fewer appendages per bud and a lower potential to become generative than middle buds or spur buds. This relationship between the number of appendages per bud and flower bud formation may simply reflect a non-causal correlation, as they may both be a under control of apical dominance. It is well known that apical dominance inhibits bud growth (Cline, 1991), and in apple, as has been shown for several other plants, it has been suggested that flower formation requires the release from apical dominance before buds reach a dormant state (Luckwill, 1970a). As buds age they gradually pass into a state of dormancy. Basal buds are under strong apical dominance, which may prevent the accumulation of appendages and flower bud formation. By the time apical dominance ceases due to cessation of shoot growth, dormancy may have proceeded too far for generative development to proceed, while middle, top and terminal buds are still sufficiently active to enable the enhanced metabolic activity associated with floral differentiation. Several experiments support the suggestion that both the rate of appendage and flower bud formation are under apical control. In pear, sprays of paclobutrazol, thought to reduce apical dominance, increased the number of appendages per bud as well as flower bud formation (Dheim and Browning, 1988). In apple, Dencker and Hansen (1994) found that fertigation both increased the number of flower buds and the number of appendages per bud. It was suggested that this was due to fertigation preventing a shortage of nutrients, thereby enhancing meristematic activity. Escobedo and Crabbé (1989) observed that treatments which promote flower bud formation, markedly increased appendage formation of buds. If they were applied too early, buds broke out into shoots. However, if applied later, such treatments could cause a complete shift to flower formation. It was concluded that these treatments enhanced flower bud formation by increa-

sing the activity of bud meristems. Thus, although it was concluded earlier that a specific rate of appendage formation or a specific number of appendages per bud are not primary inductive stimuli in flower bud formation of apple, they may nevertheless reflect a high potential for flowering.

The mechanism of control of appendage formation of buds

The rate of appendage formation in buds decreased as the season progressed; a pattern similar to that found by other workers (Abbott, 1977; Luckwill and Silva, 1979). In contrast Fulford (1965) and Schmidt and Hofmann (1988) found that the plastochron of buds was characterized by constant rates. This was the case despite large seasonal fluctuations of orchards conditions, suggesting that appendage formation of buds is largely independent of the environment. Fulford (1965) proposed that a control mechanism in the bud compensates for changes in the environment, possibly through some form of feed-back control. An increase in, for example, temperature increases the activity of the meristem but simultaneously increases the inhibition exerted by young primordia resulting in a stable rate of appendage formation.

However, the current experiments showed the plastochron of buds to be dependent of temperature (Chapter 2.1). At a higher temperature the initial rate of appendage formation was higher and slowing in appendage formation started earlier and was more pronounced than at a lower temperature. From the current observations that the rate of appendage formation changes at constant environmental conditions, it can be concluded that endogenous factors must be involved in the regulation of appendage formation. In Chapter 3 leaf removal was shown to affect the rate of appendage formation, which suggests that these endogenous factors may be products of leaf metabolism. Fulford (1966c) postulated that the plastochron was regulated by the interaction of several hormones, produced by the developing leaves, fruits and buds. The inhibitory effect of foliage on primordia production was thought to be due to auxin produced by the leaves. GA produced in the bud was thought to counteract the inhibitory effect of auxin. Later in the season a growth inhibitor accumulating in the bud scales was considered to be responsible for a more permanent change in the pattern of bud development (Fulford, 1966a).

Whether these hormones are really involved in bud development of apple remains to be discovered. Information on the endogenous control of appendage formation of plants is scarce. A stimulation of the rate of primordia production by

GA has been reported in a few cases (Wardlaw and Mitra, 1958: Bernier *et al.*, 1964; Maksymowych and Maksymowych, 1973; Lord and Eckard, 1987). Some evidence suggests that auxins produced in the leaves may inhibit appendage formation. Young leaves are major sources of auxin (Goodwin and Erwee, 1983), and growth of lateral buds have often been shown to be inhibited by applications of auxins (Cline, 1991). In apple, Fulford (1962) showed that regrowth of buds was greater if the young leaves (rich in auxins) rather than the old leaves were removed, and after leaf removal regrowth could be prevented if lanolin paste containing auxins was applied to the petioles (Fulford, 1970b). Fulford *et al.* (1968) showed that terminal bud formation as evidenced by the appearance of bud scales could be enhanced by auxins and growth retardants, and prevented by GA.

The temperature experiments (Chapter 2.1) and defoliation experiments (Chapter 3) may be interpreted as assimilates playing a role in appendage formation. A higher temperature may initially enhance primordia production by increasing the rate at which physiological processes occur, such as assimilate production, but demands for maintenance respiration also increase with temperature, which could account for the earlier slowing of appendage formation. Lakso (1994) similarly suggested that in the spring growth activity of apple is particularly limited by temperature, while later in the season resource availability may become the limiting factor. In Chapter 3 a correlation was found between the rate of appendage formation of buds and the soluble sugar content of the shoots. It is well known that leaf production of plants is generally more rapid under high irradiance, but it is not known whether this is due to higher assimilate levels or to some other photomorphogenetic response (Dale and Milthorpe, 1983). In tomato and green pepper a higher level of assimilates induced by treatments such as light intensity, plant density and fruit and leaf pruning did not result in an increased leaf appearance rate (Heuvelink and Marcelis, 1995).

6.2 The role of ammonium and polyamines in flower bud formation of apple.

It is well known that the form in which nitrogen (N) is applied to plants can induce marked developmental effects; however, the mechanism through which these effects are achieved is still unclear (Barker and Mills, 1980). In the present work (Chapter 4), application of ammonium markedly reduced growth, a common response of plants to ammonium nutrition. This has been explained in terms of

acidification of the nutrient solution, a higher demand for oxygen or available assimilates and toxicity. The latter factor is not likely to have played a major role in the current work, as no symptoms of toxicity were observed. Furthermore, plants such as apple that incorporate inorganic ammonium-N into organic N in the roots, are more tolerant to ammonium than those which transport ammonium freely to the shoots (Barker and Mills, 1980).

In apple, substantial promotion of flower bud formation following application of ammonium has been reported in a number of studies (Grasmanis and Leeper, 1965, 1967; Shear and Faust, 1971; Grasmanis and Edwards, 1974; Rohozinski *et al.*, 1986). However, in agreement with the current work (Chapter 4), in the work of Manolakis and Lüdders (1977b) ammonium failed to have a profound promotive effect. The disparities in the literature concerning the response to applications of ammonium may be regarded as evidence that the effect of ammonium is indirect. The response to ammonium may be mediated through hormones, as it has frequently been shown to alter the hormone status of plants (Moorby and Besford, 1983; Tromp, 1989). In apple, Bubán *et al.* (1978) and Gao *et al.* (1992) found increased cytokinin activity in xylem sap after ammonium nutrition. High endogenous levels of cytokinins could account for the observed effects on growth and flowering, as applied cytokinins tend to stimulate the formation of short laterals and flower bud formation of apple (Unrath, 1989; Skogerbø, 1992). The presence of ammonium in the nutrient solution can also affect the ratio in which other cations and anions are taken up, and it particularly increases the phosphorous concentration of plant tissues (Barker and Mills, 1980; Salsac *et al.*, 1987). In apple, high tissue phosphorous levels and increased flowering are often related (Bould and Parfitt, 1973; Neilsen *et al.*, 1990). Ammonium may also only stimulate flower bud formation if the general level of N is low. In the present work (Chapter 4), the high level of arginine in the old roots suggests that the grafts were well supplied with N at the start of the experiment.

Rohozinski *et al.* (1986) suggested that a possible mechanism through which ammonium enhances flower bud formation, was through elevating levels of polyamines (PAs). These authors found a profound stimulation of flower bud formation in apple trees receiving ammonium and ammonium also enhanced the arginine content of apple tissues. Arginine is a precursor of PAs, and applications of PAs to cut petioles (Rohozinski *et al.*, 1986) or applied as spray (Costa and Bagni, 1983) enhanced flower bud formation of apple. Stress-induced flowering of *Citrus* trees is associated with higher ammonium content of the leaves (Lovatt *et al.*, 1988) and increased arginine synthesis (Sagee and Lovatt, 1991) and it was sug-

gested that the higher levels of endogenous PAs were responsible for enhanced flower formation (Lovatt *et al.*, 1988). Ammonium nutrition has been shown to enhance tissue levels of PAs in several studies. PAs may act as buffer under conditions of excess protons, thereby maintaining cellular pH (Altman and Levin, 1993). In the current work ammonium nutrition only slightly enhanced the level of PAs in tissues and only slightly enhanced flower bud formation (Chapter 4). *Due to the lack of a distinct effect of ammonium on both flower bud formation and PA levels, it could not conclusively be shown whether PAs are involved in enhanced flowering induced by ammonium.*

In Chapter 5, by the application of GA and bending of shoots in the horizontal position, large differences in flower bud formation were created. However, application of GA inhibited and bending promoted flowering without altering levels of PAs in buds. Thus, no correlation existed between conditions inducing or inhibiting flower bud formation and the levels of PAs in buds. Furthermore, no pronounced changes in PA content of buds or other tissues occurred during the supposed time of flower induction and/or differentiation (Chapter 4 and 5). In contrast, in a number of other plants changes in PA content and/or metabolism occurred at critical stages of flowering (Dai and Wang, 1987; Fiala *et al.*, 1988; Kaur-Sawhney *et al.*, 1988; Kushad *et al.*, 1990; Harkess *et al.*, 1992). It must be stressed that the current evidence is insufficient to completely discount a regulatory role for PAs in flower bud formation of apple, as the effect of PAs on plant development, just as holds for growth substances, not only depends on their concentration, but also on their rates of supply or synthesis, tissue sensitivity, and removal or degradation (Trewavas, 1982; Evans and Malmberg, 1989). Thus, conclusive proof discounting a role for PAs in floral induction can only be provided if the rate of turnover of PAs is measured. Another possible objection to the current work is that whole buds were measured, and that small, localized changes in PA levels at the meristem will have escaped detection.

The regulatory role of PAs in plant development is still controversial. Although the use of PA inhibitors has shown that normal PA metabolism is necessary for several plant developmental processes, including several aspects of generative development, the present view is that PAs play important but essentially secondary roles (Evans and Malmberg, 1989). As was pointed out by Tiburcio *et al.* (1993) *from the fact that PAs have been detected in all tissue and organisms studied, and their implication in a wide range of physiological processes, it seems probable that PAs modulate plant development through a fundamental and common mechanism in all living organisms.* Such a fundamental and common mechanism

may be maintenance of cell division and general cell viability. At a physiological pH, free PAs are protonated which enables them to bind to macromolecules and cellular structures, such as nucleic acids, cell wall components, phospholipids, or certain proteins. Several of their possible biological functions may be explained by their interaction with these sub-cellular components (Tiburcio *et al.*, 1993). Significant changes in PA content, in the activity of their biosynthetic enzymes and in the incorporation of several precursors during cell division has been reported on numerous occasions (Slocum *et al.*, 1984). In the current work, the observed changes in PA content in various tissues are consistent with a role for PAs in cell division, as PA content was high early in the season, and in young tissues as compared with older tissues.

6.3 The relationship between vegetative and generative development.

Table 6.1 and 6.2 summarize the general effect of the treatments investigated in this study on growth and development of apple. Table 6.1 shows the results of the experiments described in Chapter 2.1 and 2.2. Table 6.2 shows the effect of treatments described in Chapters 3, 4 and 5. From these tables the validity of several frequently encountered claims regarding the relationship between vegetative and generative development of apple can be evaluated:

1) Vegetative and generative development are antagonistic, i.e. vigorous and prolonged shoot growth inhibit floral induction.
2) Active shoot growth prevents floral differentiation.
3) Early floral differentiation is conducive to flower quality.

1) Antagonism between vegetative and generative development

In apple, as in many other fruit trees and other higher plants, it is well established that an antagonistic relationship exists between shoot growth and flowering (Forshey and Elfving, 1989). Treatments which induce vigorous shoot growth generally reduce flower production, while treatments decreasing vegetative shoot growth generally enhance it. This has led to the suggestion that active growth directly inhibits floral induction perhaps due to the production of GA in growing shoots. Shoot tips and young leaves have high GA-like activity (Kato and Ito, 1962; Grausland, 1972; Goodwin and Erwee, 1983), and the negative effect of GA on flower bud formation of apple is widely recognized (Luckwill,

Table 6.1: Summary of the effect of temperature, application of GA_{4+7} and the presence of fruits on several aspects of growth and flower bud formation of Jonagold and Cox's Orange Pippin (results of Chapter 2). + = increased, – = reduced, +/– = unaffected, var = effect variable.

Response	High temperature	Application of GA_{4+7}	The presence of fruits
Total shoot growth	+ *	+	–
Time to cessation of shoot growth	–	+ **	+/–
Appendage formation in spur buds	+	+/–	–
Flower bud formation	– **	–	–
Time of flower differentiation	–	+	+/–
Flower quality	var	var	+/–

* Not in experiment 1
** Not in Cox's Orange Pippin.

1970a; Hoad, 1980; Tromp, 1982; Chapter 2.1). However, these processes, while frequently associated, may be independently controlled, as flowering is frequently affected with no or little effect on growth (Batjer *et al.*, 1964; Luckwill, 1970a; Tromp, 1970b, 1972; Volz and Knight, 1986; Greene and Lord, 1978; Forshey, 1989).

Nevertheless, when interpreting the effect of treatments on flowering the possibility of an indirect effect via shoot growth should be taken into account. It can be concluded from Table 6.1 and 6.2 that *in most cases treatments directly affected flower bud formation, ruling out the possibility that flowering was affected through shoot growth*. In Chapter 2.1 (Table 6.1) application of GA generally increased shoot growth, while flower bud formation was reduced, confirming the antagonistic relationship between these processes. However, in Chapter 5, application of GA inhibited flower bud formation without increasing shoot growth, implying a direct inhibitory effect on flower bud formation. A higher temperature generally enhanced shoot growth, but had different effects on flower bud formation (Chapter 2.1). In two instances temperature affected flower bud formation and shoot growth in the same direction. The presence of fruits (Chapter 2.1) and defoliation (Chapter 3) simultaneously inhibited shoot growth and flower bud formation. Lastly, the response to ammonium nutrition (Chapter 4) or bending (Chapter 3) demonstrated that shoot growth may be reduced without an increase

Table 6.2: Summary of the general effect of application of GA_{4+7}, bending, defoliation and application of NH_4^+ on different aspects of growth and flowering of Cox's Orange Pippin (results of Chapters 3, 4 and 5). + = enhanced, - = reduced, +/– = unaffected. nd = not determined.

Response	Bending			Defoliation		GA_{4+7}	NH_4^+
	Ch. 3		Ch. 5	Ch. 3		Ch. 5	Ch. 4
	Br.	Unbr.		Br	Unbr.		
Total shoot growth	–	–	+/–	–	–	+/–	–
Time to cessation of shoot growth	–	–	nd	+	+/–	nd	+/–
Appendage formation in spur buds	+/–	+/–	nd	–	–	nd	nd
Flower bud formation	+/–	+	+	–	–	–	+
Time of flower differentiation	+/–	–	nd	+/–	+/–	nd	nd
Flower quality	+/–	+	+	+/–	–	+/–	+/–

in flowering.

Floral induction may not only be inhibited by vigorous growth during the season but also by the time that growth ceases. Thus treatments or conditions inducing early cessation of shoot growth (such as environmental stress or shoot bending) frequently result in increased flowering (Swarbrick, 1929; Luckwill, 1970a; Forshey, 1989). It has been suggested that floral induction, besides requiring low GA levels, also requires an adequate supply of cytokinins, as cytokinins may be required to induce the enhanced metabolic activity associated with floral development (Luckwill, 1970a). According to Luckwill and Whyte (1968) both GA and cytokinins reach a maximum in the xylem sap early in the season, and decrease as the season progresses. Actively growing extension shoots provide a continuing, though likely declining, supply of GA until extension growth ceases. Early cessation of growth may be conducive to flowering by enabling floral differentiation to proceed when cytokinin levels are still sufficiently high.

However, in the current work (Tables 6.1 and 6.2) *no clear relationship existed between the time of cessation of shoot growth and degree of flower bud formation.* High temperature advanced cessation of shoot growth but had a variable effect on flower bud formation (Chapter 2.1), suggesting that early cessation of shoot growth does not automatically stimulate flower bud formation. In the case of bending and defoliation no clear relationship between cessation of shoot growth and flower bud formation emerged (Chapter 3). Only as concerns application of GA a relationship between cessation of shoot growth (postponed) and flower bud formation (inhibited) was found (Chapter 2.1). This may be due to a direct effect of GA, rather than associated with the time of cessation of shoot growth.

2) Inhibition of floral differentiation by active shoot growth

Goff (1899) was the first to investigate the time when floral differentiation of several fruit species started and observed that it began after the termination of active growth. Later investigations with apple have repeatedly confirmed this relationship, as factors which result in early cessation of shoot growth also result in the early appearance of flower primordia. However, some controversy exists as to whether active growth only prevents the differentiation of lateral buds, or also of spur buds. Most authors maintain that active growth only inhibits floral differentiation of buds on shoots (lateral and terminal buds), while spur buds may differentiate flowers several weeks before cessation of shoot growth (Bubán and Faust,

1982; Forshey and Elfving, 1989). Others claim that no relationship exists between growth cessation and flower bud differentiation of apple (Benko, 1967; Faust, 1989).

Table 6.1 and 6.2 may explain some of the differences in opinions, as *no simple relationship emerged between the time of cessation of shoot growth and the onset of floral differentiation.* In the experiments reported in Chapter 2.1, a close correlation between cessation of shoot growth and floral differentiation in spur buds was seen, as floral differentiation generally did not occur long before cessation of shoot growth. However, floral differentiation in spur buds could be postponed for quite some time after shoot growth cessation. Conversely, in Chapter 3, it was found that floral differentiation in spur buds could occur at a time well prior to cessation of shoot growth. Lateral bud differentiation, when observed in Chapter 3, coincided with the time of cessation of shoot growth.

The differences between timing of spur bud differentiation found in the current work may be related to differences in growth vigour. It has been suggested that hormones, particularly GA, produced by the actively growing shoots not only suppress floral induction (as discussed in previous section) but also prevent the expression of floral differentiation in already induced flower buds (Luckwill, 1970a). The young trees used in the experiments of Chapter 3 continued growth for a long time into the season, while the somewhat older trees of experiments of Chapter 2.1 ceased growth much sooner. Early, synchronous cessation of shoot growth, as found in Chapter 2.1, may cause an abrupt lowering in GA levels, allowing synchronous floral differentiation and hence the close association between cessation of shoot growth and differentiation. Delayed, heterogenous cessation of shoot growth, as found in Chapter 3, may cause a steady decline in the level of GA below a threshold level, permitting floral differentiation in some buds before all shoots have ceased growth.

3) Early floral differentiation enhances flower quality

The quality of the flower cluster at the start of the season may be equally important to tree productivity as the quantity of flower buds. In general, the factors affecting flower quality have not received much attention. It is thought that flower quality depends on the time of floral differentiation, perhaps as it allows the early steps of differentiation to occur under favourable internal (hormonal) and external (summer) conditions (Zeller, 1960; Williams, 1965; Abbott, 1970). From the results obtained here, this hypothesis could not be verified, as *early floral*

differentiation did not always correspond with improved flower quality (Tables 6.1 and 6.2).

6.4 Concluding remarks.

The present series of experiments, largely carried out under constant conditions, confirms several aspects of growth and development of apple obtained with data from field experiments. Due to the large number of possible confounding factors, hypotheses concerning the regulation of flower bud formation of apple are difficult to conclusively prove or disprove. The value of the present work mainly lies in disclaiming the validity of several hypotheses frequently encountered in the literature. However, this thesis also emphasizes the limitations of our knowledge on the endogenous regulation of flowering and makes clear that much must be resolved before we will understand this complex, but crucial process to reproductive development of apple.

References

Abbott, D.L. 1970. The role of budscales in the morphogenesis and dormancy of the apple fruit bud. In: Physiology of tree crops (L.C. Luckwill and C.V. Cutting, eds.). Academic Press, London, pp. 65-82.

Abbott, D.L. 1977. Fruit-bud formation in Cox's Orange Pippin. Rep. Long Ashton Res. Sta. for 1976, pp. 167-176.

Abbott, D.L. 1984. The apple tree: Physiology and management. Grower Books, London, 90 pp.

Abbott, D.L. 1986. A tree physiologist's view of growth regulators. Acta Hort. 179: 293-299.

Abdul, K.S. and Harris, G.P. 1978. Control of flower number in the first inflorescence of tomato (*Lycopersicon esculentum* Mill.): The role of gibberellins. Ann. Bot. 42: 1361-1367.

Altman, A. and Levin, N. 1993. Interactions of polyamines and nitrogen nutrition in plants. Physiol. Plant. 89: 653-658.

Arthey, V.D. and Wilkinson, E.H. 1964. The effect of pre-blossom defoliation on the cropping of Cox's Orange Pippin apple. Hort. Res. 4: 22-26.

Bagni, N., Serafini-Fracassini, D. and Torrigiani, P. 1981. Polyamines and growth in higher plants. In: Advances in polyamine research, Vol. 3 (C.M. Caldarera, V. Zappia and U. Bachrach, eds.). Raven Press, New York, pp. 377-388.

Bangerth, F., Freimüller, M. and El-Mahdy, R.K. 1986. Effects of growth regulators on endogenous hormones in apple shoot tips and possible relations to flower formation. Acta Hort. 179: 271-272.

Banno, K., Hayashi, S. and Tanabe, K. 1985. Effects of SADH and shoot-bending on flower bud formation, nutrient components and endogenous growth regulators in Japanese pear (*Pyrus serotina* Rehd.). J. Jpn. Soc. Hort. Sci. 53: 365-376. (In Japanese with English summary).

Banno, K., Hayashi, S. and Tanabe, K. 1986. Morphological and histological studies on flower bud differentiation and development in Japanese pear (*Pyrus serotina* Rehd.). J. Jpn. Soc. Hort. Sci. 55: 258-265.

Barker, A.V. and Mills, H.A. 1980. Ammonium and nitrate nutrition of horticultural crops. Hort. Rev. 2: 395-423.

Batjer, L.P., Williams, M.W. and Martin, G.C. 1964. Effects of N-dimethyl amino succinamic acid (B-nine) on vegetative and fruit characteristics of apples, pears and sweet cherries. Proc. Amer. Soc. Hort. Sci. 85: 11-16.

Bendeck de Cantú, L. and Kandeler, R. 1989. Significance of polyamines for flowering in *Spirodela punctata*. Plant Cell Physiol. 30: 455-458.

Benko, B. 1967. Morphological differentiation of flower buds in apple trees. Biol. Plant. 9: 263-269.

Bergh, O. 1985. Morphogenesis of *Malus domestica* cv. Starking flower buds. S. Afr. J. Plant Soil 2: 187-190.

Bernier, G. 1971. Structural and metabolic changes in the shoot apex in transition to flowering. Can. J. Bot. 49: 803-819.

Bernier, G. 1988. The control of floral evocation and morphogenesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 39: 175-219.

Bernier, G., Bronchart, R. and Jacqmard, A. 1964. Action of gibberellic acid on the mitotic activity of the different zones of the shoot apex of *Rudbeckia bicolor* and *Perilla nankinensis*. Planta 61: 236-244.

Bernier, G., Havelange, A., Houssa C., Petitjean, A. and Lejeune, P. 1993. Physiological signals that induce flowering. Plant Cell 5: 1147-1155.

Biasi, R., Bagni, N. and Costa, G. 1988. Endogenous polyamines in apple and their relationship to fruit set and fruit growth. Physiol. Plant. 73: 201-205.

Bieleski, R.L. 1969. Accumulation and translocation of sorbitol in apple phloem. Aust. J. Biol. Sci. 22: 611-620.

Bieleski, R.L. and Redgewell, R.J. 1985. Sorbitol versus sucrose as photosynthesis and translocation products in developing apricot leaves. Aust. J. Plant Physiol. 12: 657-668.

Bijhouwer, J. 1924. De periodiciteit van de knopontwikkeling bij den appel. Meded. Landb. Hoogesch. Wageningen 27: 1-64.

Bodson, M. and Bernier, G. 1985. Is flowering controlled by the assimilate level? Physiol. Vég. 23: 491-501.

Bould, C. and Parfitt, R.I. 1973. Leaf analysis as a guide to the nutrition of fruit crops. X. Magnesium and phosphorus sand culture experiments with apple. J. Sci. Fd. Agric. 24: 175-185.

Bradley, M.V. and Crane, J.C. 1960. Gibberellin-induced inhibition of bud development in some species of *Prunus*. Science 131: 825-826.

Bubán, T. 1981. Histological and cytochemical aspects of flower bud formation in apple. Acta Hort. 120: 113-117.

Bubán, T. and Faust, M. 1982. Flower bud induction in apple trees: Internal control and differentiation. Hort. Rev. 4: 174-203.

Bubán, T., Varga, A., Tromp, J., Knegt, E. and Bruinsma, J. 1978. Effects of ammonium and nitrate nutrition on the levels of zeatin and amino nitrogen in xylem sap of apple rootstocks. Z. Pflanzenphysiol. 89: 289-295.

Buttrose, M.S. 1970. Fruitfulness in grapevines: Development of leaf primordia in buds in relation to bud fruitfulness. Bot. Gaz. 131: 78-83.

Chailakhyan, M.K. 1968. Internal factors of plant flowering. Annu. Rev. Plant Physiol. 19: 1-36.

Chan, B.G. and Cain, J.C. 1967. The effect of seed formation on subsequent flowering in apple. Proc. Amer. Soc. Hort. Sci. 91: 63-68.

Cline, M.G. 1991. Apical dominance. Bot. Rev. 57: 318-358.

Cordes, D. 1987. Vegetatives und generatives Verhalten von Apfelbäumen nach Entblätterung. Erwerbsobstbau 29: 245-249.

Costa, G. and Bagni, N. 1983. Effects of polyamines on fruit-set of apple. HortSci. 18: 59-61.

Costa, G., Biasi, R. and Bagni, N. 1986. Effect of putrescine on fruiting performance of apple (cv. Hi Early). Acta Hort. 179: 355-361.

Crabbé, J. 1984. Vegetative vigor control over location and fate of flower buds in fruit trees. Acta Hort. 149: 55-63.

Dai, Y.R. and Wang, J. 1987. Relation of polyamine titer to photoperiodic induction of flowering in *Pharbitis nil*. Plant Sci. 51: 135-139.

Dale, J.E. and Milthorpe, F.L. 1983. General features of the production and growth of leaves. In: The growth and functioning of leaves (J.E. Dale and F.L. Milthorpe, eds.). Cambridge Univ. Press, Cambridge, pp. 151-178.

Davies, M.H.E. 1959. Some trials of the effect of defoliation upon cropping of apples. Rep. East Malling Res. Sta. for 1958, pp. 53-59.

Davis, L.D. 1957. Flowering and alternate bearing. Proc. Amer. Soc. Hort. Sci. 70: 545-556.

Delap, A.V. 1967. The effect of supplying nitrate at different seasons on the growth, blossoming and nitrogen content of young apple trees in sand culture. J. Hort. Sci. 42: 149-167.

Dencker, I. and Hansen, P. 1994. Flower initiation and node development of axillary buds, growth partitioning, and bleeding sap production of young apple trees as affected by fertigation. J. Hort. Sci. 69: 869-876.

Dennis, F.G., Jr. and Nitsch, J.P. 1966. Identification of gibberellins A_4 and A_7 in immature apple seeds. Nature 211: 781-782.

Dheim, M.A. and Browning, G. 1988. The mechanism of the effect of (2RS, 3RS)-paclobutrazol on flower initiation of pear cvs Doyenne du Comice and Conference. J. Hort. Sci. 63: 393-405.

Dumortier, F.M., Flores, H.E.; Shekhawat, N.S. and Galston, A.W. 1983. Gradients of polyamines and their biosynthetic enzymes in coleoptiles and roots of corn. Plant Physiol. 72: 915-918.

Escobedo, J, and Crabbé, J. 1989. Correlative control of early stages of flower bud initiation in 'bourse' shoots of apple (*Malus x domestica* Borkh. cv. Golden Delicious). Ann. Sci. For. 46: 44s-46s.

Evans, L.T. 1969. The nature of flower induction. In: The induction of flowering (L.T. Evans ed.). MacMillan and Co., Melbourne, pp. 457-480.

Evans, P.T. and Malmberg, R.L. 1989. Do polyamines have roles in plant development? Annu. Rev. Plant Physiol. Plant Mol. Biol. 40: 235-269.

Faust, M. 1989. Physiology of temperate zone fruit trees. John Wiley and Sons, New York, 338 pp.

Ferree, D.C. and Palmer, J.W. 1982. Effect of spur defoliation and ringing during bloom on fruiting, fruit mineral level and net photosynthesis of 'Golden Delicious' apple. J. Amer. Soc. Hort. Sci. 107: 1182-1186.

Fiala, V., Le Nard, M., Querou, Y. and Jolivet, E. 1988. La spermidine, marqueur moléculaire de l'induction florale chez le bulbe d'*Iris hollandica*. C.R. Acad. Sci. Paris 306: 579-582.

Filner, P. 1987. Candidate for a sexual reproduction specific pathway in plants: putrescine to GABA via hydroxycinnamic amide intermediates. NATO A.S.I. Ser., Ser. A 140 (Plant Mol. Biol.): 243-251.

Forshey, C.G. 1989. Measuring the effects of growth regulators on the vegetative growth - fruiting relationship in apple trees. Acta Hort. 239: 211-219.

Forshey, C.G. and Elfving, D.C. 1989. The relationship between vegetative growth and fruiting in apple trees. Hort. Rev. 11: 229-287.

Fulford, R.M. 1960. The use of defoliating sprays for the control of biennial bearing in apple. J. Hort. Sci. 35: 202-213.

Fulford, R.M. 1962. The development of apple spur buds in relation to leaves and fruits. Proc. 16th Int. Hort. Congr. 3: 343-346.

Fulford, R.M. 1965. The morphogenesis of apple buds. I. The activity of the apical meristem. Ann. Bot. 29: 167-179.

Fulford, R.M. 1966a. The morphogenesis of apple buds. II. The development of the bud. Ann. Bot. 30: 25-38.

Fulford, R.M. 1966b. The morphogenesis of apple buds. III. The inception of flowers. Ann. Bot. 30: 207-219.

Fulford, R.M. 1966c. The morphogenesis of apple buds. IV. The effect of fruit. Ann. Bot. 30: 597-606.

Fulford, R.M. 1970a. The effect of chemical defoliation on the development of apple spurs. Ann. Bot. 34: 1079-1088.

Fulford, R.M. 1970b. Leaves, fruit and flower initiation. Proc. 18th Int. Hort. Congr. 4: 143-150.

Fulford, R.M., Quinlan, J.D., Lacey, H.J. and Barlow, H.W.B. 1968. The acropetal movement of growth substances from young leaves on woody shoots. In: Biochemistry and physiology of plant growth substances (F. Wightman and G. Setterfield, eds.). Runge Press, Ottawa, pp. 1187-1203.

Gao, Y.P., Motosugi, H. and Sugiura, A. 1992. Rootstock effects on growth and flowering in young apple trees grown with ammonium and nitrate nitrogen. J. Amer. Soc. Hort. Sci. 117: 446-452.

García-Luis, A., Fornes, F. and Guardiola, J.L. 1995. Leaf carbohydrates and flower formation in *Citrus*. J. Amer. Soc. Hort. Sci. 120: 222-227.

Geisler, D. and Ferree, D.C. 1984. Response of plants to root pruning. Hort. Rev. 6: 155-188.

Gibbs, M.A. and Swarbrick, T. 1930. The time of differentiation of the flower-bud of the apple. J. Pom. Hort. Sci. 8: 61-67.

Gifford, E.M., Jr and Corson, G.E., Jr. 1971. The shoot apex in seed plants. Bot. Rev. 37: 143-229.

Givan, C.V. 1979. Metabolic detoxification of ammonia in tissues of higher plants. Phytochemistry 18: 375-382.

Goff, E.S. 1899. The origin and early development of the flowers in the cherry, plum, apple and pear. Wisconsin Agr. Exp. Sta. Ann. Rep. 16: 289-303.

Goldschmidt, E.E., Aschkenazi, N., Herzano, Y., Schaffer, A.A. and Monselise, S.P. 1985. A role for carbohydrate levels in the control of flowering in *Citrus*. Scientia Hort. 26: 159-166.

Goldschmidt, E.E. and Monselise, S.P. 1972. Hormonal control of flowering in *Citrus* and some other woody perennials. In: Plant Growth Substances, 1970 (Carr, D.J. ed.). Springer, Berlin, pp. 758-766.

Goodwin, P.B. and Erwee, M.G. 1983. Hormonal influences on leaf growth. In: The growth and functioning of leaves. (J.E. Dale and F.L. Milthorpe, eds.). Cambridge Univ. Press, Cambridge, pp. 207-232.

Graebe, J.E. 1987. Gibberellin biosynthesis and control. Annu. Rev. Plant Physiol. 38: 419-465.

Grasmanis, V.O. and Edwards, G.R. 1974. Promotion of flower initiation in apple trees by short exposure to the ammonium ion. Aust. J. Plant Physiol. 1: 99-105.

Grasmanis, V.O. and Leeper, G.W. 1965. Ammonium versus nitrate in culture solutions. Agrochimica 10: 54-63.

Grasmanis, V.O. and Leeper, G.W. 1967. Ammonium nutrition and flowering of apple trees. Aust. J. Biol. Sci. 20: 761-767.

Grausland, J. 1972. Gibberellins in diffusates from shoots of apple trees. Physiol. Plant. 27: 65-70.

Greene, D.W. 1975. Cytokinin activity in the xylem sap and extracts of MM 106 apple rootstocks. HortSci. 10: 73-74.

Greene, D.W. 1989. Gibberellins A_{4+7} influence fruit set, fruit quality and return bloom of apples. J. Amer. Soc. Hort. Sci. 114: 619-625.

Greene, D.W. and Autio, W.R. 1989. Evaluation of benzyladenine as a chemical thinner on 'McIntosh' apples. J. Amer. Soc. Hort. Sci. 114: 68-73.

Greene, D.W. and Lord. W.J. 1978. Evaluation of scoring, limb spreading and growth regulators for increasing flower bud initiation and fruit set on young 'Delicious' apple trees. J. Amer. Soc. Hort. Sci. 103: 208-210.

Griggs, W.H. and Iwakiri, B.T. 1961. Effects of gibberellin and 2,4,5-trichlorophenoxypropionic acid sprays on Bartlett pear trees. Proc. Amer. Soc. Hort. Sci. 77: 73-89.

Grochowska, M.J. and Karaszewska, A. 1978. A possible role of hormones in growth and development of apple trees and a suggestion on how to modify their action. Acta Hort. 80: 457-464.

Gruber, J. and Bangerth, F. 1990. Diffusible IAA and dominance phenomena in fruits of apple and tomato. Physiol. Plant. 79: 354-358.

Gur, A. 1985. Rosaceae - deciduous fruit trees. In: CRC Handbook of flowering, Vol. 1 (A.H. Halevy, ed.). CRC Press, Boca Raton, Florida, pp. 355-389.

Gur, A. and Sarig, P. 1983. Application of growth regulators to peach and apple trees growing under stress conditions in shallow and stony soils. Acta Hort. 137: 153-168.

Guttridge, C.G. 1962. Inhibition of fruit-bud formation in apple with gibberellic acid. Nature 196: 1008.

Hansen, P. 1972. Some effects of ringing, defoliation, sorbitol-spraying and bending of shoots on rate of translocation, on accumulation, and on growth in apple trees. Tidsskr. Planteavl. 76: 308-315.

Harkess, R.L., Lyons, R.E. and Kushad, M.M. 1992. Floral morphogenesis in *Rudbeckia hirta* in relation to polyamine concentration. Physiol. Plant. 86: 575-582.

Harley, C.P., Magness, J.R., Masure, M.P., Fletcher, L.A. and Degman, E.S. 1942. Investigations on the cause and control of biennial bearing of apple trees. U.S.D.A. Tech. Bull. No. 792: 1-58.

Hedden, P. 1990. The action of plant growth retardants at the biochemical level. In: Plant Growth Substances, 1988 (R.P. Pharis and S.B. Rood, eds.). Springer-Verlag, Heidelberg, pp. 322-332.

Hennerty, M.J. and Forshey, C.G. 1971. Effects of defruiting, scoring, defoliation and shading on the carbohydrate content of 'Golden Delicious' apple trees. J. Hort. Sci. 46: 153-161.

Heuvelink, E. and Marcelis, L.F.M. 1995. Influence of assimilate supply on the leaf appearance rate in greenhouse fruit vegetable crops. J. Hort. Sci. (submitted).

Higashiuchi, Y., Kido, K., Ichii, T., Nakanishi, T., Kawai, Y. and Ozaki, T. 1990. The effect of gibberellins and growth retardants on in vitro flowering of the vegetative apex of Japanese pear. Scientia Hort. 41: 223-232.

Hill-Cottingham, D.G. and Cooper, D.R. 1970. Effect of time of application of fertiliser nitrogen on the distribution and identity of the nitrogenous constituents of young apple trees. J. Sci. Fd. Agr. 21: 172-177.

Hill-Cottingham, D.G. and Williams, R.R. 1967. Effect of time of application of fertilizer nitrogen on the growth, flower development and fruit set of maiden apple trees, var. Lord Lambourne, and on the distribution of total nitrogen within the trees. J. Hort. Sci. 42: 319-338.

Hoad, G.V. 1978. The role of seed derived hormones in the control of flowering in apple. Acta Hort. 80: 93-103.

Hoad, G.V. 1980. Growth regulators, endogenous hormones and flower initiation in apple. Rep. Long Ashton Res. Sta. for 1979, pp. 199-206.

Hoad, G.V. 1984. Hormonal regulation of fruit-bud formation in fruit trees. Acta Hort. 149: 13-23.

Huet, J. 1973. Floral initiation in pear trees. Acta Hort. 34: 193-198.

Hull, J., Jr. and Lewis, L.N. 1959 Response of one-year-old cherry and mature bearing cherry, peach and apple trees to gibberellin. Proc. Amer. Soc. Hort. Sci. 74: 93-100.

Jackson, J.E. and Palmer, J.W. 1977. Effects of shade on the growth and cropping of apple trees. II. Effects on components of yield. J. Hort. Sci. 52: 253-266.

Jackson, D.I. and Sweet, G.B. 1972. Flower initiation in temperate woody plants. Hort. Abstr. 42: 9-24.

Jarvis, B.C., Shannon, P.R.M. and Yasmin, S. 1983. Involvement of polyamines with adventitious root development in stem cuttings of mung bean. Plant Cell Physiol. 24: 677-683.

Kafkafi, U. 1990. Root temperature, concentration and the ratio NO_3^-/NH_4^+ effect on plant development. J. Plant Nutr. 13: 1291-1306.

Kakkar, R.K. and Rai, V.K. 1987. Effects of spermine and IAA on carbohydrate metabolism during rhizogenesis in *Phaseolus vulgaris* L. hypocotyl cuttings. Indian J. Exp. Biol. 25: 476-478.

Kakkar, R.K. and Rai, V.K. 1993. Plant polyamines in flowering and fruit ripening. Phytochemistry 33: 1281-1288.

Kato, T. and Ito, H. 1962. Physiological factors associated with the shoot growth of apple trees. Tohuko J. Agr. Res. 13: 1-21.

Kaur-Sawhney, R., Kandpal, G., McGonigle, B. and Galston, A.W. 1990. Further experiments on spermidine-mediated floral-bud formation in thin-layer explants of Wisconsin 38 tobacco. Planta 181: 212-215.

Kaur-Sawhney, R., Tiburcio, A.F. and Galston, A.W. 1988. Spermidine and flower-bud differentiation in thin-layer explants of tobacco. Planta 173: 282-284.

Kinet, J.M. 1977. Effect of defoliation and growth substances on the development of the inflorescence in tomato. Scientia Hort. 6: 27-35.

Kobel, F. 1954. Lehrbuch des Obstbaus auf physiologischer Grundlage. Springer-Verlag, Berlin, 348 pp.

Kraus, E.J. and Kraybill, H.R. 1918. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149: 1-90.

Kushad, M.M., Orvos, A.R. and Yelenosky, G. 1990. Relative changes in polyamines during *Citrus* flower development. HortSci. 25: 946-948.

Lakso, A.N. 1994. Apple. In: Handbook of environmental physiology of fruit crops. Vol. 1 Temperate crops. (B. Schaffer and P.C. Andersen, eds.). CRC Press, Boca Raton, Florida, pp. 3-42.

Landsberg, J.J. 1975. Temperature effects and plant response. In: Progress in biometeorology: The effect of weather and climate on plants, Vol. 1, Progress in plant biometeorology, Division C (Smith, L.P. ed.). Swets and Zeitlinger, Amsterdam, pp. 86-107.

Landsberg, J.J. and Thorpe, M.R. 1975. The mechanisms of apple bud morphogenesis: Analysis and a model. Ann. Bot. 39: 689-699.

Langer, R.H.M. and Bussell, W.T. 1964. The effect of flower induction on the rate of leaf initiation. Ann. Bot. 28: 163-167.

Lin, J.T., Stafford, A.E. and Steffens, G.L. 1991. Identification of endogenous gibberellins in immature apple seeds. Agric. Biol. Chem. 55: 2183-2185.

Llewelyn, F.W.M. 1968. The effect of partial defoliation at different times in the season on fruit drop and shoot growth in Lord Lambourne apple trees. J. Hort. Sci. 43: 519-526.

Longman, K.A., Nasr, T.A.A. and Wareing, P.F. 1965. Gravimorphism in trees. 4. The effect of gravity on flowering. Ann. Bot. 29: 459-473.

Looney, N.E., Kamienska, A., Legge, R.L. and Pharis, R.P. 1978. Metabolism of ^{3}H gibberellin A_4 in relation to flower initiation in apple. Acta Hort. 80: 105-114.

Looney, N.E., Pharis, R.P. and Noma, M. 1985. Promotion of flowering in apple trees with gibberellin A_4 and C-3 epi-gibberellin A_4. Planta 165: 292-294.

Lord, E.M. and Eckard, K.J. 1987. Shoot development in *Citrus sinensis* L. (Washington navel orange). II. Alteration of developmental fate of flowering shoots after GA_3 treatment. Bot. Gaz. 148: 17-22.

Lovatt, C.J., Zheng, Y., and Hake, K.D. 1988. Demonstration of a change in nitrogen metabolism influencing flower initiation in *Citrus*. Isr. J. Bot. 37: 181-188.

Luckwill, L.C. 1957. Studies of fruit development in relation to plant hormones. IV. Acidic auxins and growth inhibitors in leaves and fruits of the apple. J. Hort. Sci. 32: 18-33.

Luckwill, L.C. 1970a. The control of growth and fruitfulness of apple trees. In: Physiology of tree crops (L.C. Luckwill and C.V. Cutting, eds.). Academic Press, London, pp. 237-254.

Luckwill, L.C. 1970b. Progress in the control of flowering and fruiting. Proc. 18th Int. Hort. Congr. 4: 177-185.

Luckwill, L.C. 1975. A new look at the process of fruit bud formation in apple. Proc. 19th Int. Hort. Congr. 3: 237-245.

Luckwill, L.C. and Silva, J.M. 1979. The effects of daminozide and gibberellic acid on flower initiation, growth and fruiting of apple cv. Golden Delicious. J. Hort. Sci. 54: 217-223.

Luckwill, L.C., Weaver, P. and MacMillan, J. 1969. Gibberellins and other growth hormones in apple seeds. J. Hort. Sci. 44: 413-424.

Luckwill, L.C. and Whyte, P. 1968. Hormones in the xylem sap of apple trees. In: Plant Growth regulators, Soc. Chem. Ind. Monogr. No. 31: 87-101.

Lyndon, R.F. and Battey, N.H. 1985. The growth of the shoot apical meristem during flower initiation. Biol. Plant. 27: 339-349.

Maksymowych, R. and Maksymowych, A.B. 1973. Induction of morphogenetic changes and acceleration of leaf initiation by gibberellic acid in *Xanthium pennsylvanicum*. Amer. J. Bot. 60: 901-906.

Manolakis, E. and Lüdders, P. 1977a. Die Wirkung gleichmäßiger und jahreszeitlich abwechselnder Ammonium- und Nitraternährung auf Apfelbäume. I. Einfluß auf das vegetative Wachstum. Gartenbauwiss. 42: 1-7.

Manolakis, E. and Lüdders, P. 1977b. Die Wirkung gleichmäßiger und jahreszeitlich abwechselnder Ammonium- und Nitraternährung auf Apfelbäume. III. Einfluß auf das generative Wachstum. Gartenbauwiss. 42: 118-126.

Marcelis, L.F.M. 1993. Leaf formation in cucumber (*Cucumis sativus* L.) as influenced by fruit load, light and temperature. Gartenbauwiss. 58: 124-129.

Marcelis-van Acker, C.A.M. 1994. Axillary bud development in rose. Dissertation Agric. Univ. Wageningen, 131 pp.

Marcelle, R. 1984. The flowering process and its control. Acta Hort. 149: 65-69.

Marcelle, R. and Sironval, C. 1963. Effect of gibberellic acid on flowering of apple trees. Nature 197: 405.

Marino, F. and Greene, D.W. 1981. Involvement of gibberellins in the biennial bearing of 'Early McIntosh' apples. J. Amer. Soc. Hort. Sci., 106: 593-596.

Marschner, H. 1986. Mineral nutrition of higher plants. Academic press Ltd, London, 674 pp.

Martin-Tanguy, J. 1985. The occurrence and possible function of hydroxycinnamoyl acid amides in plants. Plant Growth Regul. 3: 381-399.

Martin-Tanguy, J., Cabanne, F., Perdrizet, E. and Martin, C. 1978. The distribution of hydroxycinnamic acid amides in flowering plants. Phytochemistry 17: 1927-1928.

McDaniel, C.N., Sangrey, K.A. and Singer, S.R. 1989. Node counting in axillary buds of *Nicotiana tabacum* cv. Wisconsin 38, a day-neutral plant. Amer. J. Bot. 76: 403-408.

McLaughlin, J.M. and Greene, D.W. 1984. Effects of BA, GA_{4+7} and daminozide on fruit set, fruit quality, vegetative growth, flower initiation, and flower quality of 'Golden Delicious' apple. J. Amer. Soc. Hort. Sci. 109: 34-39.

McLaughlin, J.M. and Greene, D.W. 1991a. Fruit and hormones influence flowering of apple. I. Effect of cultivar. J. Amer. Soc. Hort. Sci. 116: 446-449.

McLaughlin, J.M. and Greene, D.W. 1991b. Fruit and hormones influence flowering of apple. II. Effects of hormones. J. Amer. Soc. Hort. Sci. 116: 450-453.

Miflin, B.J. and Lea, P.J. 1977. Amino acid metabolism. Annu. Rev. Plant Physiol. 28: 299-329.

Miflin, B.J. and Lea, P.J. 1980. Ammonia assimilation. In: The biochemistry of plants, Vol. 5 (P.K. Stumpf and E.E. Conn, eds.). Academic Press, New York, pp. 169-203.

Mika, A.L. 1969. Effects of shoot-bending and pruning on growth and fruit bud formation in young apple trees. Hort. Res. 9: 93-102.

Miller, S.S. 1988. Plant bioregulators in apple and pear culture. Hort. Rev. 10: 309-401.

Milthorpe, F.L. 1959. Studies on the expansion of the leaf surface. I. The influence of temperature. J. Exp. Bot. 10: 233-249.

Monselise, S.P. and Halevy, A.H. 1964. Chemical inhibition and promotion of *Citrus* flower bud induction. Proc. Amer. Soc. Hort. Sci. 84: 141-146.

Moorby, J. and Besford, R.T. 1983. Mineral nutrition and growth. In: Encyclopedia of plant physiology, N.S. (A. Läuchli and R.L. Bieleski, eds.). Springer-Verlag, Berlin, 15B: 481-527.

Mullins, M.G. 1965. The gravitational responses of young apple trees. J. Hort. Sci. 40: 237-247.

Mullins, M.G. 1968. Regulation of inflorescence growth in cuttings of the grape vine (*Vitis vinifera* L.). J. Exp. Bot. 19: 532-543.

Neilsen, G.H., Hogue, E.J. and Parchomchuk, P. 1990. Flowering of apple trees in the second year is increased by first-year P fertilization. HortSci. 25: 1247-1250.

Novozamsky, I., Houba, V.J.G., Eck, R. van and Vark, W. van. 1983. A novel digestion technique for multi-element plant analysis. Commun. Soil Sci. Plant Anal. 14: 239-248.

Oliveira, C.M. and Priestley, C.A. 1988. Carbohydrate reserves in deciduous fruit trees. Hort. Rev. 10: 403-430.

Oude Voshaar, J.H. 1994. Statistiek voor onderzoekers. Wageningen Pers, Wageningen, 253 pp.

Palavan, N. and Galston, A.W. 1982. Polyamine biosynthesis and titer during various developmental stages of *Phaseolus vulgaris*. Physiol. Plant. 55: 438-444.

Perdrizet, E. and Prevost, J. 1981. Aliphatic and aromatic amines during development of *Nicotiana tabacum*. Phytochemistry 20: 2131-2134.

Pharis, R.P. and King, R.W. 1985. Gibberellins and reproductive development in seed plants. Annu. Rev. Plant Physiol. 36: 517-568.

Pratt, C. 1988. Apple flower and fruit: Morphology and anatomy. Hort. Rev. 10: 273-308.

Rabe, E. and Lovatt, C.J. 1986. Increased arginine biosynthesis during phosphorus deficiency. A response to the increased ammonia content of leaves. Plant Physiol. 81: 774-779.

Ramirez, H. and Hoad, G.V. 1981. Effects of growth substances on fruit-bud initiation in apple. Acta Hort. 120: 131-136.

Robitaille, H.A. and Carlson, R.F. 1976. Gibberellic and abscisic acid-like substances and the regulation of apple shoot extension. J. Amer. Soc. Hort. Sci. 101: 388-392.

Rohozinski, J., Edwards, G.R. and Hoskyns, P. 1986. Effects of brief exposure to nitrogenous compounds on floral initiation in apple trees. Physiol. Vég. 24: 673-677.

Rudloff, C.F. and Feucht, W. 1957. Funktionelle Tendenzen der reproduktiven Organe des Apfels. I. Beziehungen zwischen Anthese, Morphologie und Fruchtungsvermögen. Gartenbauwiss. 22: 26-42.

Ryugo, K. and Sansavini, S. 1972. Effect of succinic acid 2,2-dimethyl hydrazide on flowering and gibberellic acid contents of sweet cherry (*Prunus avium* L.). J. Hort. Sci. 47: 173-178.

Sachs, R.M. 1977. Nutrient diversion: An hypothesis to explain the chemical control of flowering. HortSci. 12: 220-222.

Sagee, O. and Lovatt, C.J. 1991. Putrescine content parallels ammonia and arginine metabolism in developing flowers of the 'Washington' navel orange. J. Amer. Soc. Hort. Sci. 116: 280-285.

Salsac, L., Chaillou, S., Morot-Gaudry, J.F., Lesaint, C. and Jolivet, E. 1987. Nitrate and ammonium nutrition in plants. Plant Physiol. Biochem. 25: 805-812.

Schmidt, S. and Hofmann, R. 1988. Zur Bildung von Blattanlagen in Knospen von *Malus domestica* (Borkh.). Arch. Gartenbau 36: 211-217.

Schupp, J.R. and Ferree, D.C. 1987. Deblossoming stimulates a second bloom in 'Golden Delicious' apple trees. HortSci. 22: 958-959.

Searle, N.E. 1965. Physiology of flowering. Annu. Rev. Plant Physiol. 16: 97-118.

Seiler, N. and Deckardt, K. 1975. Determination of amines and amino acids in sugar-containing samples by dansylation. J. Chromatogr. 107: 227-229.

Shear, C.B. and Faust, M. 1971. Nutritional factors influencing the mineral content of apple leaves. J. Amer. Soc. Hort. Sci. 96: 234-240.

Shen, H.J. and Galston, A.W. 1985. Correlations between polyamine ratios and growth patterns in seedling roots. Plant Growth Regul. 3: 353-363.

Skogerbø, G. 1992. Effects of xylem cytokinin application on flower bud development in apple (*Malus x domestica* Borkh.). Norw. J. Agric. Sci. 6: 473-483.

Slocum, R.D., Kaur-Sawhney, R. and Galston, A.W. 1984. The physiology and biochemistry of polyamines in plants. Arch. Biochem. Biophys. 235: 283-303.

Slocum, R.D. and Weinstein, L.H. 1990. Stress-induced putrescine accumulation as a mechanism of ammonia detoxification in cereal leaves. In: Polyamines and ethylene: Biochemistry, physiology, and interactions. (H.E. Flores, R.N. Arteca and J.C. Shannon, eds.). American Society of Plant Physiologists, Rockville, MD, pp. 157-165.

Smith, M.A. and Davies, P.J. 1985a. Manipulation of the polyamine content and senescence of apical buds of G2 peas. Plant Growth Regul. 3: 401-417.

Smith, M.A. and Davies, P.J. 1985b. Separation and quantitation of polyamines in plant tissue by high performance liquid chromatography of their dansyl derivatives. Plant Physiol. 78: 89-91.

Steffens, G.L. and Hedden, P. 1992. Effect of temperature regimes on gibberellin levels in thermosensitive dwarf apple trees. Physiol. Plant. 86: 539-543.

Swarbrick, T. 1929. Factors governing fruit bud formation. VIII. The seasonal elongation growth of apple varieties on some vegetative rootstocks; and its possible relation to fruit bud formation. J. Pomol. Hort. Sci. 7: 100-129.

Tapuhi, Y., Schmidt, D.E., Lindner, W. and Karger, B.L. 1981. Dansylation of amino acids for high-performance liquid chromatography analysis. Anal. Biochem. 115: 123-129.

Taylor, J.S., Pharis, R.P., Loveys, B., Notodimedjo, S. and Edwards, G.R. 1984. Changes in the endogenous hormones in apple during bud burst induced by defoliation. Plant Growth Regul. 2: 117-134.

Terry, N., Waldron L.J. and Taylor, S.E. 1983. Environmental influences on leaf expansion. In: The growth and functioning of leaves (J.E. Dale and F.L. Milthorpe, eds.). Cambridge Univ. Press, Cambridge, pp. 179-205.

Tiburcio, A.F., Campos, J.L., Figueras, X. and Besford, R.T. 1993. Recent advances in the understanding of polyamine functions during plant development. Plant Growth Regul. 12: 331-340.

Tiburcio, A.F., Kaur-Sawhney, R. and Galston, A.W. 1988. Polyamine biosynthesis during vegetative and floral bud differentiation in thin layer tobacco tissue cultures. Plant Cell Physiol. 29: 1241-1249.

Tinklin, I.G., Wilkinson, E.H. and Schwabe, W.W. 1970. Factors affecting flower initiation in the black current *Ribes nigrum* (L.). J. Hort. Sci. 45: 275-282.

Titus, J.S. and Kang, S.M. 1982. Nitrogen metabolism, translocation and recycling in apple trees. Hort. Rev. 4: 204-246.

Torrigiani, P., Altamura, M.M., Pasqua, G., Monacelli, B., Serafini-Fracassini, D. and Bagni, N. 1987. Free and conjugated polyamines during de novo floral and vegetative bud formation in thin cell layers of tobacco. Physiol. Plant. 70: 453-460.

Trewavas, A.J. 1982. Growth substance sensitivity: The limiting factor in plant development. Physiol. Plant. 55: 60-72.

Trewavas, A.J. 1985. A pivotal role for nitrate and leaf growth in plant development. In: Control of leaf growth (N.R. Baker, W.J. Davies, and C.K. Ong, eds.). Cambridge Univ. Press, Cambridge, pp. 77-91.

Tromp, J. 1968. Flower-bud formation and shoot growth in apple as affected by shoot orientation. Acta Bot. Neerl. 17: 212-220.

Tromp, J. 1970a. Storage and mobilization of nitrogenous compounds in apple trees with special reference to arginine. In: Physiology of tree crops. (L.C. Luckwill and C.V. Cutting, eds.). Academic Press, London, pp. 143-159.

Tromp, J. 1970b. Shoot orientation effects on growth and flower-bud formation in apple. Acta Bot. Neerl. 19: 535-538.

Tromp, J. 1972. Effects of growth-regulating substances and tree orientation on growth and flower-bud formation in apple. J. Hort. Sci. 47: 525-533.

Tromp, J. 1976. Flower-bud formation and shoot growth in apple as affected by temperature. Scientia Hort. 5: 331-338.

Tromp, J. 1980. Flower-bud formation in apple under various day and night temperature-regimes. Scientia Hort. 13: 235-243.

Tromp, J. 1982. Flower-bud formation in apple as affected by various gibberellins. J. Hort. Sci. 57: 277-282.

Tromp, J. 1984. Flower-bud formation in apple as affected by air and root temperature, air humidity, light intensity, and day length. Acta Hort. 149: 39-47.

Tromp, J. 1987. Growth and flower-bud formation in apple as affected by paclobutrazol, daminozide, and tree orientation in combination with various gibberellins. J. Hort. Sci. 62: 433-440.

Tromp, J. 1989. Interrelationships between plant growth regulators and fruit tree mineral nutrition. Acta Hort. 239: 399-408.

Tromp, J. and Ovaa, J.C. 1979. Uptake and distribution of nitrogen in young apple trees after application of nitrate or ammonium, with special reference to asparagine and arginine. Physiol. Plant. 45: 23-28.

Tse, A.T.Y., Ramina, A., Hackett, W.P. and Sachs, R.M. 1974. Enhanced inflorescence development in *Bougainvillea* 'San Diego Red' by removal of young leaves and cytokinin treatments. Plant Physiol. 54: 404-407.

Unrath, C.R. 1989. The influence of 6-benzyladenine on growth, flowering and fruiting of young bearing apple trees following treatment as non-bearing trees. Acta Hort. 239: 269-272.

Van Staden, J. and Davey, J.E. 1979. The synthesis, transport and metabolism of endogenous cytokinins. Plant Cell Environ. 2: 93-106.

Volz, R.K. and Knight, J.N. 1986. The use of growth regulators to increase precocity in apple trees. J. Hort. Sci. 61: 181-189.

Walsh, C.S. and Kender, W.J. 1982. Effect of cultivar, strain and growth regulator treatments on shoot development and ethylene evolution in apple trees. J. Amer. Soc. Hort. Sci. 107: 198-202.

Wang, S.Y., Faust, M. and Steffens, G.L. 1985. Metabolic changes in cherry flower buds associated with breaking of dormancy in early and late blooming cultivars. Physiol. Plant. 65: 89-94.

Wang, S.Y. and Faust, M. 1986. Effect of growth retardants on root formation and polyamine content in apple seedlings. J. Amer. Soc. Hort. Sci. 111: 912-917.

Wang, S.Y. and Faust, M. 1993. Comparison of seasonal growth and polyamine content in shoots of orchard-grown standard and genetic dwarf apple trees. Physiol. Plant. 89: 376-380.

Wang, S.Y. and Faust, M. 1994. Changes in polyamine content during dormancy in flower buds of 'Anna' apple. J. Amer. Soc. Hort. Sci. 119: 70-73.

Wang, S.Y., Faust, M. and Line, M.J. 1994. Apical dominance in apple (*Malus domestica* Borkh.): The possible role of indole-3-acetic acid (IAA). J. Amer. Soc. Hort. Sci. 119: 1215-1221

Wardlaw, C.W. and Mitra, G.C. 1958. Responses of a fern apex to gibberellic acid, kinetin and α-naphthaleneacetic acid. Nature 181: 400-401.

Wareing, P.F. and Nasr, T. 1958. Gravimorphism in trees. Effects of gravity on growth, apical dominance and flowering in fruit trees. Nature 182: 379-380.

Wareing, P.F. and Saunders, P.F. 1971. Hormones and dormancy. Annu. Rev. Plant Physiol. 22: 261-288.

Wellensiek, S.J. 1977. Principles of flower formation. Acta Hort. 68: 17-27.

Williams, M.W. 1973. Chemical control of vegetative growth and flowering of apple trees. Acta Hort. 34: 167-174.

Williams, R.R. 1965. The effect of summer nitrogen applications on the quality of apple blossom. J. Hort. Sci. 40: 31-41.

Wysz-Benz, M., Streit, L. and Ebert, E. 1990. Feruloylputrescine and caffeoylputrescine are not involved in growth and floral bud formation of stem explants from *Nicotiana tabacum* L. var Xanthi nc. Plant Physiol. 92: 924-930.

Zeller, O. 1955. Entwicklungsverlauf der Infloreszenzknospen einiger Kern- und Steinobstsorten. Angew. Bot. 29: 69-89.

Zeller, O. 1960. Entwicklungsgeschichte der Blütenknospen und Fruchtanlagen an einjährigen Langtrieben von Apfelbüschen. I. Entwicklungsverlauf und Entwicklungsmorphologie der Blüten am einjährigen Langtrieb. Z. PflZücht. 44: 175-214.

Summary

The dramatic morphological and physiological changes occurring during the transition of the meristem from the vegetative to the generative condition have been the topic of much research. For practical reasons much of this research has been with plants that use environmental cues to induce flowering, as these cues evoke predictable and synchronous flowering. In apple, however, as in many other woody species, there is no specific external factor responsible for the inception of flowers, and a wide range of factors may influence flowering. However, the response to a given treatment may be highly inconsistent, varying from year to year, or even from tree to tree. Moreover, not all bud meristems respond in the same way to the prevailing conditions, as not all buds become generative. Despite this greater complexity, considerable research has been devoted to how flowering of apple is regulated. Although this research has revealed much on how flower bud number can be manipulated by cultural treatments, the mechanism of endogenous control remains elusive. The aim of the present work was to gain more insight in how endogenous factors affect flower bud formation of apple.

In Chapter 1, in a review of the literature, morphological and physiological aspects of flower bud formation are discussed. The formation of buds and subsequent development of flowers is a lengthy and complex process. Upon bud opening in the spring two populations of buds can be distinguished; those containing inflorescences (generative buds) or only vegetative shoots (vegetative buds). The effect of treatments on flowering is usually evaluated during anthesis in the spring. However, flowering starts nearly a year earlier with floral induction, which commits the meristem to the formation of flowers. At the end of summer in the year preceding anthesis the histological differentiation of flower primordia starts, which continues throughout winter and the following spring.

Relatively little attention has been given to the importance of early morphological stages of bud development to flower bud formation. This aspect is investigated in the first part of this thesis (Chapter 2 and 3). It has been suggested that a prerequisite for flower formation is a short plastochron (time interval between the formation of successive primordia at the meristem) and the presence of a certain minimal number of appendages per bud. By periodic examination of buds under a dissecting microscope during the season the plastochron and the number of appendages per bud was monitored. In Chapter 2.1, the results are described of four experiments carried out under controlled conditions (phytotron) with two

apple cultivars, Jonagold and Cox's Orange Pippin. In these experiments the effect of temperature, applied gibberellin (GA_{4+7}) and the presence of fruits on the plastochron and the number of appendages per bud was investigated in relation to flower bud formation. A higher temperature, application of GA and the presence of fruits decreased the number of flower buds. Although all treatments profoundly affected flowering, only temperature affected the plastochron. The plastochron was not constant at constant environmental conditions, but increased in time. A higher temperature initially shortened the plastochron, but the shorter plastochron was not always associated with increased flowering.

In Chapter 2.2 the effect of the above treatments on flower cluster morphology seen during anthesis in the following spring is described. The number of leaves in the cluster varied with treatment and was dependent on the rate of appendage formation in the previous season, and on the time in the season when floral differentiation started. Treatments that accelerated the rate of appendage formation (high temperature) or postponed floral differentiation (application of GA) enhanced the number of leaves in the cluster. The number of flowers showed less variation than the number of leaves, while the number of bourse buds and shoots was positively related to the number of leaves in the cluster. Cluster quality, determined as the number of well-developed flowers per cluster, varied greatly, and did not appear to be dependent on treatments.

In Chapter 3 the effect of defoliation and shoot bending on appendage formation was studied in one-year-old trees of Cox's Orange Pippin growing outside in containers. Defoliation profoundly inhibited flower bud formation, particularly if it occurred in July, while bending was slightly promotive or had no effect. Defoliation simultaneously caused a lengthening of the plastochron, but it is concluded that the negative effect of defoliation on flower bud formation is not mediated through the plastochron. In both chapters (Chapters 2 and 3), a large variation was found in the number of appendages present at the time that the bud became floral. On the basis of the present results, it is concluded that a short plastochron and the presence of a minimal number of appendages are not critical to floral induction.

Nevertheless poor bud development and inhibition of flower bud formation are frequently associated. It is possible that factors which are preventing bud development (apical dominance) are also preventing flower bud formation. From the present results that the plastochron was not constant at a constant environment, but increased as buds developed, it can be deduced that not only exogenous factors, such as temperature, but also endogenous factors are involved in regulation of

appendage formation. The observation that leaf removal affected the plastochron (Chapter 3) suggests that the endogenous factors involved may be products of leaf metabolism.

In Chapter 3, besides investigating the effect on the plastochron, the effect of defoliation and bending on assimilate levels of shoots was also investigated. Defoliation, particularly in May or June, caused an immediate lowering of the soluble sugar content of the shoot, while starch was not affected. In contrast, bending had no effect on the soluble sugar content of the shoot, while starch content was increased. The assimilate level of the shoot correlated poorly to the number of flower buds formed, suggesting that assimilates are not a main regulatory factor in flower formation. Leaf removal and bending more likely affect flower formation by altering the hormone balance of the shoot, but the mechanism of action is still obscure. In lateral buds a continuous influence of the leaves is necessary to sustain the flowering commitment of the buds, as a short period without leaves during early or late development irreversibly inhibited flower bud formation.

Before the discovery of hormones from the 1930's, regulation of flower bud formation was thought to be controlled by the level of resources, in particular by the C/N ratio. With the discovery of plant hormones, this theory gave way to the theory that control is hormonal. In the second part of this thesis, an attempt is made to reconcile the hormonal theory with the older nutrient theory. In Chapter 4, the hypothesis is tested that stimulation of flower formation of apple by ammonium nutrition is due to enhanced levels of polyamines (PAs). In the literature applications of ammonium and PAs have been shown to enhance flower bud formation of apple. Ammonium simultaneously increased the arginine content of stem tissue, and since arginine is a precursor of PAs, it was suggested that ammonium may enhance flower bud formation by elevating levels of PAs. Using bud grafts of Cox's Orange Pippin growing on an inert substrate, the effect of ammonium nutrition on arginine and PA levels in various tissues was studied. Similar to the general effect on plants, ammonium reduced shoot growth, but in contrast to that reported in a number of studies with apple, flower bud formation was not profoundly enhanced. PA levels were only slightly increased in trees receiving ammonium. Due to the lack of a distinct effect of ammonium on both flower bud formation and PA levels it could not be concluded whether ammonium enhances flower bud formation through elevating tissue levels of PAs.

In Chapter 5 larger differences in flowering were created by treatments such as application of GA and bending. Two experiments were carried out with one-

year-old trees of Cox's Orange Pippin placed at controlled conditions. Although both treatments affected flower bud formation, PA content of buds was not altered. Pronounced changes in PA content during the time of floral induction and floral differentiation were also not observed. Although more evidence is needed regarding the role of PAs in floral induction, it is tentatively suggested, from the available literature and the present results, that they are not primary inductive stimuli in flower bud formation of apple.

In apple, as in many other fruit trees and other higher plants, it is well established that an antagonistic relationship exists between shoot growth and flowering, as vigorous and prolonged vegetative growth results in poor return bloom. However, it could be concluded from the present results that in most cases treatments *directly* affected flower bud formation, i.e. independent of an effect on shoot growth. No clear relationship existed between the time of cessation of shoot growth, the onset of floral differentiation and the degree of flower bud formation. The hypothesis that early differentiation is conducive to flowering could not be verified, as early floral differentiation did not always correspond with improved flower quality.

Regulation of flower bud formation is a complex phenomenon which may involve a balance of hormones which interact in a highly specific manner. The balance and interaction may be modified by external factors, such as nutrition and environment, and by internal factors, such as the level of substrates (nutrients and assimilates) or factors associated with bud position on the tree.

Samenvatting

De morfologische en fysiologische veranderingen die plaatsvinden als het meristeem overgaat van vegetatief naar generatief hebben de aandacht van veel onderzoekers getrokken. Om praktische redenen heeft het onderzoek zich voornamelijk gericht op planten waarbij omgevingsfactoren de bloei induceren. Deze factoren leiden namelijk tot een voorspelbare en synchrone bloei. De bloei-inductie bij appel, evenals bij veel andere houtige soorten, reageert niet op één specifieke omgevingsfactor; een groot aantal factoren kan de bloei beïnvloeden. Het effect van een bepaalde behandeling kan echter sterk variëren, zowel van jaar tot jaar als ook van boom tot boom. Bovendien reageren niet alle knopmeristemen gelijk op een gegeven behandeling, want slechts een deel van het totale aantal knoppen wordt generatief. Ondanks deze grotere complexiteit is er toch veel onderzoek verricht naar de regulatie van de bloei van appel. Dit onderzoek heeft belangrijke resultaten opgeleverd over hoe het aantal bloemknoppen door diverse teeltmaatregelen kan worden beïnvloed, maar het mechanisme van de endogene regulatie is daarmee niet opgehelderd. Het doel van het hier beschreven onderzoek was om meer inzicht te verwerven in het interne reguleringsmechanisme van de bloemknopvorming bij appel.

In Hoofdstuk 1, in een literatuur overzicht, worden morfologische en fysiologische aspecten van de bloemknopvorming besproken. De ontwikkeling van knoppen en de aanleg van bloemdelen is een lang en ingewikkeld proces. Als de knoppen in het voorjaar uitlopen kan men twee populaties onderscheiden: knoppen die bloemtrossen bevatten (generatieve knoppen) en knoppen met scheuten (vegetatieve knoppen). Het effect van behandelingen op het aantal bloemknoppen wordt meestal tijdens de bloeiperiode in het voorjaar gemeten. De bloemknopvorming begint echter al bijna een jaar eerder, namelijk met de bloei-inductie in de zomer voorafgaand aan de bloei. Daarop volgt de bloemdifferentiatie, die aanvangt aan het einde van de zomer, en doorgaat tot de knoppen in het volgende voorjaar uitlopen.

Aan het belang van de morfologische aspecten van de vroege knopontwikkeling voor de bloemknopvorming is relatief weinig aandacht besteed. In het eerste deel van dit proefschrift (Hoofdstuk 2 en 3) is dit aspect nader onderzocht. Volgens de literatuur is een korte plastochron (tijdsinterval tussen twee opvolgende bladafsplitsingen van het meristeem) en een bepaald minimum aantal bladvormingen per knop een voorwaarde voor de bloemknopvorming. Beide grootheden

zijn door middel van het periodiek prepareren van knoppen onder de microscoop bepaald. In Hoofdstuk 2.1 zijn de resultaten weergegeven van vier in een fytotron uitgevoerde proeven met twee appel cultivars, Jonagold en Cox's Orange Pippin. In deze proeven is het effect van de temperatuur, bespuiting met gibberellinen (GA_{4+7}) en de aanwezigheid van vruchten op de plastochron en het aantal bladvormingen per knop ten tijde van de omslag naar een generatief meristeem bestudeerd. Een hoge temperatuur, GA-bespuiting en de aanwezigheid van vruchten remden de bloemknopvorming, maar alleen de temperatuur beïnvloedde de plastochron. In een constante omgeving was de plastochron niet constant, maar nam toe in de tijd. Bij een hogere temperatuur was de plastochron aanvankelijk korter, maar de kortere plastochron resulteerde niet altijd in meer bloemknoppen.

In Hoofdstuk 2.2 wordt het effect van genoemde behandelingen op de morfologie van de bloemtros gedurende de bloei toegelicht. Het aantal bladeren per bloemtros varieerde per behandeling en was afhankelijk van de bladafsplitsingsnelheid in het voorgaande groeiseizoen en van het tijdstip waarop de bloemdifferentiatie begon. Behandelingen die de bladafsplitsing versnelden (hoge temperatuur) of die het tijdstip van de bloemdifferentiatie uitstelden (GA-bespuiting) resulteerden in meer bladeren per bloemtros. Het aantal bloemen per bloemtros vertoonde minder variatie dan het aantal bladeren, terwijl het aantal scheuten of knoppen op de beurs positief gecorreleerd was met het aantal bladeren per bloemtros. De bloemkwaliteit, bepaald als het aantal goed gevormde bloemen per bloemtros, varieerde sterk en leek niet afhankelijk van de behandelingen.

In Hoofdstuk 3 is het effect van ontbladering en buigen op de plastochron van één-jarige bomen van Cox's Orange Pippin bestudeerd. Ontbladering had een sterk negatief effect op de bloemknopvorming, vooral wanneer dit in juli plaatsvond. Buigen had een gering bevorderend of geen effect. Ontbladeren had een verlenging van de plastochron tot gevolg, maar het negatieve effect op de bloei was niet gecorreleerd met die verlenging. In alle proeven (Hoofdstuk 2 en 3) was de variatie in het aantal bladvormingen in de knop ten tijde van de bloemdifferentiatie groot. Op grond van deze resultaten wordt geconcludeerd dat een korte plastochron en de aanwezigheid van een zeker minimum aantal bladvormingen niet bepalend zijn voor het optreden van de bloemaanleg.

Niettemin zijn een geringe knopontwikkeling en remming van de bloemknopvorming vaak gecorreleerd. Het is denkbaar dat factoren die de knopontwikkeling remmen (apicale dominantie), ook de bloemknopvorming tegengaan. Het feit dat in een constante omgeving de plastochron niet constant was maar toenam tijdens de knopontwikkeling, duidt erop dat niet alleen externe factoren, zoals de

temperatuur, maar ook interne factoren de plastochron kunnen beïnvloeden. De waarneming dat ontbladeren de bladafsplitsing beïnvloedde (Hoofdstuk 3) wijst op een rol van produkten van de bladstofwisseling.

In Hoofdstuk 3 is, naast het effect op de plastochron, het effect van ontbladeren en buigen op het assimilatengehalte van de scheut bepaald. Ontbladeren, vooral in mei of juni, veroorzaakte een directe verlaging van het gehalte aan oplosbare suikers in de scheut, terwijl het zetmeelgehalte niet werd beïnvloed. Buigen had daarentegen geen effect op het gehalte aan oplosbare suikers, maar deed het zetmeelgehalte toenemen. Het gehalte aan assimilaten was niet gecorreleerd met het aantal gevormde bloemknoppen, wat erop wijst dat assimilaten geen belangrijke rol spelen in de bloei-inductie. Het is waarschijnlijker dat ontbladeren en buigen de bloemknopvorming beïnvloeden middels de hormoonhuishouding, maar het mechanisme is nog onbekend. In okselknoppen is een continue produktie van een bladfactor noodzakelijk, want een korte periode zonder blad gedurende de vroege of late knopontwikkeling had een onomkeerbare onderdrukking van de bloemknopvorming tot gevolg.

Vóór de ontdekking van hormonen in de dertiger jaren werd de bloemknopvorming van appel vooral verklaard via de voedingstoestand van de plant, met name de C/N verhouding. Veel effecten van teeltmaatregelen of omgevingsfactoren op appelbomen konden met deze theorie worden verklaard. Het experimentele bewijs voor deze theorie is echter nooit geleverd. Met de ontdekking van hormonen is de aandacht verschoven naar deze groep stoffen. In het tweede deel van dit onderzoek is getracht een verband te leggen tussen de moderne groeistoftheorie en de oudere voedingstheorie. In Hoofdstuk 4 is nagegaan in hoeverre de bloeibevorderende werking van ammonium- in plaats van nitraatvoeding, berust op een verhoging van het gehalte aan polyaminen (PAs) in verschillende weefsels. Eerder onderzoek heeft aangetoond dat zowel toediening van ammonium als PAs de bloemknopvorming van appel kunnen bevorderen. Toediening van ammonium verhoogde tevens het gehalte aan arginine in scheuten, een precursor van PAs, zodat ammonium de bloemknopvorming wellicht bevordert via verhoging van het endogene gehalte aan PAs. Het effect van de ammoniumvoeding op het gehalte aan arginine en PAs in verschillende weefsels is onderzocht in appeloculaties van Cox's Orange Pippin die groeiden op een inert substraat. Overeenkomstig resultaten in de literatuur, remde ammonium de groei, maar in tegenstelling tot eerdere onderzoeken, was het bloeibevorderende effect zeer gering. Omdat ook het gehalte aan PAs slechts weinig hoger was, kunnen geen conclusies worden getrokken betreffende de rol van PAs in de beïnvloeding van de bloemknopvorming door

ammoniumvoeding.

Hoofdstuk 5 behandelt twee proeven waarbij door middel van behandelingen als GA-bespuiting en buigen grotere verschillen in de bloemknopvorming zijn gecreëerd. De proeven werden uitgevoerd met één-jarige bomen van Cox's Orange Pippin onder constante condities van temperatuur en licht. Alhoewel beide behandelingen de bloemknopvorming beïnvloedden, gold dat niet of nauwelijks voor het gehalte aan PAs. Er waren ook geen duidelijke veranderingen in PA-gehalte tijdens de perioden van bloei-inductie en bloemdifferentiatie. Alhoewel meer informatie nodig is omtrent de rol van PAs in de bloei-inductie wordt, op grond van de huidige resultaten en resultaten uit de literatuur, geconcludeerd dat PAs geen primaire inductieve factoren zijn in de bloemknopvorming van appel.

In appel, evenals in veel andere houtige gewassen en hogere planten, is het bekend dat er een antagonisme bestaat tussen de scheutgroei en de bloemknopvorming. Sterke groei over een lange periode resulteert veelal in een geringe bloei. In het huidige onderzoek hadden de behandelingen echter meestal een *direct* effect op de bloei, d.w.z. onafhankelijk van effecten op de groei. Er was geen duidelijke relatie tussen het tijdstip van afsluiten van de groei, het begin van de bloemdifferentiatie en het aantal gevormde bloemknoppen. De hypothese dat een vroege bloemdifferentiatie de bloemkwaliteit bevordert ging ook niet altijd op.

De regulatie van de bloemknopvorming van appel is een ingewikkeld proces, waarbij een balans van hormonen, die op een zeer specifieke manier met elkaar interacties aangaan, een belangrijke rol lijkt te spelen. Die balans en interactie worden waarschijnlijk gemodificeerd door exogene factoren, zoals de minerale voeding en omgevingsfactoren, en door endogene factoren, zoals de gehalten aan voedingstoffen (nutrienten en assimilaten) en factoren samenhangend met de knoppositie aan de boom.

Curriculum vitae

Frances Anne Verheij werd geboren op 20 maart 1966 te Wageningen. Na het behalen van het diploma International Bacchalaureate aan het United World College of South East Asia te Singapore in 1984, begon zij in hetzelfde jaar haar studie Biologie aan de Landbouwuniversiteit te Wageningen. Tijdens de doctoraalfase werden als afstudeervakken Plantenfysiologie en *In vitro* cultuur van de hogere plant gekozen. Een stage Plantenfysiologie vervulde zij in Australië bij de Horticultural PostHarvest Group te Brisbane en het Queensland Agricultural College te Gatton. Het doctoraalexamen werd in januari 1990 behaald. Van februari 1990 tot februari 1994 verrichtte zij als assistent in opleiding bij de vakgroep Tuinbouwplantenteelt van de Landbouwuniversiteit het onderzoek dat beschreven is in dit proefschrift.