Axillary bud development in rose

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Proefschrift

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Stellingen

1. Bloemknopaanleg bij de roos vindt pas plaats na opheffen van de apicale dominantie.

   Dit proefschrift

2. Effecten van teeltcondities tijdens de aanleg van okselknoppen bij de roos op de uitgroei van die knoppen komen voornamelijk tot stand via beïnvloeding van het overige gedeelte van de plant.

   Dit proefschrift

3. Het aantal okselknoppen aan de basis van de rozestruik is niet beperkend voor de vorming van grondscheuten.

   Dit proefschrift

4. Door bij de vermeerdering gebruik te maken van dubbelstekken in plaats van enkelstekken wordt de uniformiteit en groeikracht van het plantmateriaal sterk bevorderd.

   Marcelis-van Acker, C.A.M. & Leutscher, K.J., 1993. Effect of type of cutting on heterogeneity and growth of *Rosa hybrida* cv. Motrea and *Schefflera arboricola* cv. Compacta. Sci. Hortic. 54: 59-67.

5. Bostrack gaat bij het verklaren van de waargenomen anatomische en morfologische verschillen tussen scheuten van diverse hoogten uit de boom voorbij aan mogelijke verschillen in leeftijd van de knoppen die tot deze scheuten uitgroeien.

   Bostrack, J.M., 1993. The relationship between size of shoot apices and morphological features of mature leaves and stems of four species of angiosperm trees. Ann. Bot. 72: 341-347.

6. Hooggekwalificeerde medewerkers vormen geen garantie voor succes zolang de teamgeest ontbreekt.

7. Gezien de veelal lange wachttijd voor professionele medische hulp bij een ongeval dient een ieder zich te bekwamen in het verlenen van Eerste Hulp Bij Ongevallen.

8. Een proefschrift schrijven is gewoon werk.

9. Door betere communicatie in de keten zal de Nederlandse boer meer brood gaan zien in baktarwe.

10. Het steeds frequenter in de publiciteit brengen van premature onderzoeksresultaten brengt het wetenschappelijk onderzoek in diskrediet.

11. Bij dalende werkgelegenheid wordt er door werkenden en werklozen harder gewerkt.

12. Het ambivalente van de Nederlander (m/v) ten aanzien van emancipatie is enerzijds het niet begrijpen waarom een vrouw een proefschrift schrijft en anderzijds het pas voor vol aanzien van een vrouw als ze gepromoveerd is*).

*) Intermediair, 10-1-1992: Gepromoveerde vrouwen zijn betrouwbaar.

13. Het hebben van een niet-alledaagse naam in combinatie met het spreken met een accent verhoogt de herkenbaarheid.

14. De LUW dient zich te realiseren dat met de voorgestelde uitholling van haar 'groene' richtingen/activiteiten haar bestaansrecht als zelfstandige (landbouw?)universiteit in het geding komt.

15. Soms moet je ver gaan om dichtbij te kunnen zien.

Stellingen behorende bij het proefschrift:
'Axillary bud development in rose'

C.A.M. Marcelis - van Acker
Wageningen, 18 november 1994

Abstract

Marcelis-van Acker C.A.M., 1994. Axillary bud development in rose. Dissertation Wageningen Agricultural University, Wageningen, The Netherlands. 131pp; English and Dutch summaries.

Axillary buds form the basis of flower production of a rose crop. Within a rose crop there exists an undesired large variation in shoot number and size, which affects flower yield. Part of this variation may be traced back to early variation in axillary buds. The aim of the research reported in this thesis was to enlarge the knowledge and insight in the development of axillary buds. It was investigated to what extent the growth of an axillary bud into a shoot can be influenced during axillary bud formation and to what extent during actual outgrowth into a shoot. Factors studied were bud age, bud position, assimilate supply and temperature. Growth potential of the buds was studied both *in situ* and in isolation (grafted or *in vitro*), enabling to distinguish between direct effects on the buds and indirect effects via the parent plant.

An axillary bud contains the lower part of the future shoot. The axillary buds which are most likely to form the first basal shoots are already present as secondary buds in the bud which is used for propagation. Later formed basal shoots usually develop from basal axillary buds of the basal shoots. Each basal shoot was shown to be connected to only a segment of the root xylem. Later formed basal shoots may restrict the growth of the older basal shoots by limiting the xylem serving the older basal shoots.

Axillary buds needed a certain developmental stage to be able to break. Bud break also required release from correlative inhibition. As long as axillary buds were correlatively inhibited, they remained in the vegetative stage. They were not dormant, but continued to grow although at a low rate. When released from inhibition their developmental programme (bud break, leaf initiation and flower initiation) was already set to a large extent. However, they displayed a high degree of plasticity in their development into a shoot, in response to ambient conditions in which they were growing. Number of leaves preceding the flower appeared to be determined during axillary bud formation and increased with increasing bud age, decreasing position along the shoot, increasing assimilate supply and decreasing temperature. Rate of bud break increased with increasing position, increasing temperature during bud formation and increasing temperature after release from inhibition. Shoot diameter correlated with pith diameter. Number of pith cells in the axillary bud reflects the potential diameter of the subsequent shoot. Final pith diameter was dependent on cell expansion after bud break and was reached soon after start of shoot growth. Increased assimilate supply and decreasing temperature positively affected expansion of the pith cells and as a result the pith diameter. Length and weight of the shoot at harvest and growth period were largely dependent on the assimilate supply and the temperature after release from inhibition.

Key words: age, anatomy, assimilate supply, axillary bud, basal shoots, bottom breaks, bud break, cell expansion, correlative inhibition, determination, developmental programme, dyes, flowering, *in vitro*, leaf initiation, morphology, ontogeny, pith, position, primordium, *Rosa hybrida*, rose, shoot growth, temperature, xylem pathways.

We must all be morphologists
before we can be biologists of any sort.

(E.W. Sinnott, 1960)

Aan mijn ouders

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Account

The Chapters 2-5 have been submitted for publication in international journals. The following chapters have already been published or accepted for publication:

Chapter 2.1: Marcelis-van Acker C.A.M., 1994. Ontogeny of axillary buds and shoots in roses: Leaf initiation and pith development. Scientia Horticulturae 57: 111-122.

Chapter 2.2: Marcelis-van Acker C.A.M., 1993. Morphological study of the formation and development of basal shoots in roses. Scientia Horticulturae 54: 143-152.

Chapter 2.3: Marcelis-van Acker C.A.M., Keijzer C.J. & Van de Pol P.A., 1993. Xylem pathways in rose plants in relation to basal shoot development. Acta Botanica Neerlandica 42: 313-318.

Chapter 4.1: Marcelis-van Acker C.A.M., 1994. Development and growth potential of axillary buds in roses as affected by bud age. Annals of Botany (in press).

Chapter 4.3: Marcelis-van Acker C.A.M., 1994. Effect of assimilate supply on development and growth potential of axillary buds in roses. Annals of Botany 73: 415-420.

1. General introduction

In the Netherlands the rose is the most important glasshouse cut flower having in 1993 a production area of 898 ha (Anonymous 1993) and an auction turnover of 781 million Dutch guilders (Anonymous 1994).

Rose is a perennial woody shrub. Glasshouse roses continuously form new shoots. Each shoot has the potential to form a terminal flower. After harvesting a flowering shoot the most distal axillary buds will sprout and develop into shoots. Flower quality declines with increasing plant age and after 4 to 7 years plants are grubbed and replaced by young ones.

Rose plants are usually propagated vegetatively. Several methods of vegetative propagation are employed: Cutting, stenting (Van de Pol & Breukelaar 1982), root grafting, bench grafting, budding and *in vitro* culture. For all these methods, an axillary bud is the start for the above-ground part of the plant. This bud will sprout and develop into a shoot, the so-called primary shoot. At the base of this shoot, strongly growing shoots develop, the so-called basal shoots or bottom breaks. These basal shoots form the frame of a rose plant and their number, diameter and degree of branching mainly determine the potential flower production (Zieslin *et al.* 1973; De Vries & Dubois 1983). Little is known about the morphological origin of basal shoots, but it is assumed that they originate from buds in the scale-axils of axillary buds (Khayat & Zieslin 1982).

Much research on rose has been focused on the effects of various scion-rootstock combinations (De Vries 1993; Fuchs 1994), harvesting procedures (Zieslin 1981; Kool & Van de Pol 1993), growth regulators (Mor & Zieslin 1987) and environmental factors (Van den Berg 1987; Roberts *et al.* 1993) on flower yield. Although considerable technical improvements and possibilities to control glasshouse climate have been developed, undesired large differences in shoot number and size still occur, among as well as within plants.

Little attention has been paid to axillary buds, although these buds are important in four aspects during the life-time of a rose plant:

(1) When propagated vegetatively, an axillary bud gives rise to the above-ground part of a new rose plant;

(2) Axillary buds are assumed to give rise to basal shoots (Khayat & Zieslin 1982), which determine the potential flower production (De Vries & Dubois 1983);

(3) The degree of branching of shoots depends on the growth of axillary buds (Wilkins 1988), which also is an important determinant of potential flower production;

(4) Axillary buds give rise to the flowering shoots, which determine the actual flower production.

Axillary bud formation

An axillary bud is an unextended, partly developed shoot, located in the axil of a leaf. Axillary buds are formed by axillary meristems, which are located just above each leaf

primordium (Mauseth 1988). An axillary meristem is in fact the apical meristem of each shoot.

Different concepts have been used to describe the organization of the shoot apical meristem (reviewed by Van Harten 1978; Medford 1992). In the tunica-corpus concept the shoot apical meristem consists of two distinct zones, the outer tunica and the inner corpus, which are distinguished on the basis of the orientation of cell divisions. The cells of the tunica divide anticlinally, although periclinal divisions also may occur, whereas the cells of the corpus divide in any direction (Mauseth 1988). The number of tunica layers ranges from one to five, most species having a two-layered tunica (Steeves & Sussex 1989). The corpus is composed of three zones (Fig. 1): An uppermost zone of central mother cells, below this the pith-rib meristem, and the whole surrounded by the peripheral zone (Mauseth 1988). The central mother cells are enlarged, rather highly vacuolated and divisions seem to occur more or less equally in all planes. The cells of the peripheral zone are small and densely cytoplasmic, and divide predominantly anticlinally, giving rise to rows of cells. Occasionally occurring periclinal divisions enlarge the number of rows, and the thickness of the peripheral zone increases towards its base. The cells of the pith-rib meristem are small and vacuolated. Divisions mainly occur in such a way that new cells are aligned vertically, and rows of cells result. Longitudinal divisions can also occur, increasing the number of rows (Esau 1977; Mauseth 1988). The rib meristem has two possible functions: it forms cells for the centre of the stem and it may act as an organizing centre for the shoot (Medford 1992).

The other concept of meristem organization is that of layers (Medford 1992). Based on studies of periclinal chimeras it has been concluded that the apical meristem contains three independent layers of initials, indicated by L1, L2 and L3: L1 is the outermost layer and produces the epidermis, L2 produces the one to several layers just below the epidermis and L3, the innermost layer, produces the interior portion of organs and stems, which forms the bulk of the shoot axis tissues. However, occurrence of such an organization is supposed to be scarce (Mauseth 1988). In *Rosaceae* the tunica-corpus concept was found suitable to describe the organization of the shoot apex (Rouffa & Gunckel 1951a,b).

Axillary buds are usually formed slightly later than the subtending leaf primordium (Esau 1977). Usually they arise in the leaf axil when one or two leaf primordia above this leaf are already formed by the apex, although considerable variation between species has been found (Romberger 1963). The cells in the axils of the leaf primordia do not undergo enlargement and vacuolation like the cells around them, but remain as detached meristems. These detached meristems are left behind by continued growth of the shoot apex and, therefore, enlarge and become organized into shoot apices with leaf primordia (Steeves & Sussex 1989). According to Esau (1965) the term axillary is somewhat inaccurate, be-

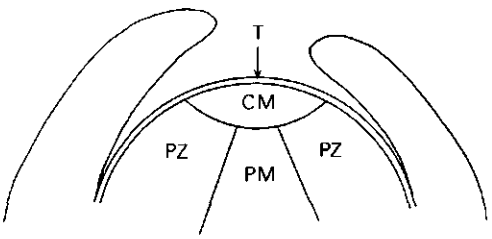


Fig. 1. Schematic representation of zonation in apical meristems (After Mauseth 1988).
T: tunica; CM: central mother cells; PZ: peripheral zones; PM: pith-rib meristem.

cause buds generally arise on the stem but become displaced closer to the leaf base or even onto the leaf itself by subsequent growth readjustments. Michno-Zatorska *et al.* (1986) distinguished three types of axillary meristems based on the position of the meristems, 'caulinar' (when arising on the stem), 'foliar' (when arising on a supporting leaf base) or exactly 'axillar'. Most authors, however, do not make that distinction and just use the term 'axillary'.

Correlative inhibition

Axillary buds usually are correlatively inhibited. Depending on the species, inhibited or quiescent buds may be fairly rudimentary or may be well-formed with readily discernible leaves, internodes and even floral primordia (Hillman 1984). The term dormancy is usually not applied to buds held under correlative inhibition, since correlative inhibited buds show growth although barely perceptible. For example in pear new leaf primordia were formed (Young *et al.* 1974) and in pea non-growing lateral buds were metabolically active (Tepper 1993) and incorporated labeled amino acids (Stafstrom & Sussex 1988). Several factors can contribute to the correlative inhibition of axillary buds, e.g. mature leaves both above and subtending the bud (Zieslin & Halevy 1976; McIntyre & Hsiao 1990; Suzuki 1990), stem tissue above the bud (Peterson & Fletcher 1975; Zieslin & Halevy 1976), relative position of the bud along the branch (Zieslin & Halevy 1976), but the most well-known contribution is from the apex. Apical dominance is the control exerted by the shoot apex (and the youngest leaves) over the outgrowth of axillary buds (Hillman 1984; Cline 1991). Apical dominance is expressed in three main ways: inhibition of axillary bud break, inhibition of axillary shoot growth and control of branch angles of axillary shoots (Zieslin & Halevy 1976).

Most studies on apical dominance concern herbaceous plants, whereas relatively little attention has been paid to apical dominance in woody perennials (Hillman 1984). Several theories have been advanced to explain the phenomenon of apical dominance, which have focused on inhibition by nutrient (mineral nutrients and/or sugars) deprivation and on hormonal inhibition (Phillips 1975; Hillman 1984; Cline 1991; 1994): According to the 'nutritive' theory a sufficient availability of nutrients in the vicinity of the axillary bud is assumed the primary requirement for its outgrowth. The apical meristem is assumed to consume all available nutrients resulting in nutrient deprivation and lack of outgrowth of the axillary buds. Hence, apical dominance is controlled by the internal competition between buds for nutrients. However, this theory has never been unequivocally proved or disproved. It seems more likely that the correlative signal in apical dominance has a hormonal control. In the 'direct auxin action' theory auxin produced in the terminal bud migrates down the stem and into the axillary buds, where it directly suppresses axillary bud outgrowth. Inhibition of buds decreases towards the base of the shoot. However, according to Stafstrom (1993), a direct role for auxin would require auxin content being higher in inhibited buds than in growing buds, which model is difficult to reconcile with the fact that terminal buds synthesize high levels of auxin and yet grow at the same time. There is, however, sufficient evidence that auxins play at least an indirect role in apical dominance. In the 'correlative inhibitor' theory, an inhibitor may be formed, as a result of auxin action, which moves into lat-

eral buds and prevent them from outgrowth. In the 'nutrient-diversion' theory, a modification of the nutrient theory, endogenous or exogenous auxin creates a flow of nutrients towards the point of auxin production (the active apex), such that the axillary buds become starved. In the 'vascular connection' theory, vascular connections between the lateral bud and the main stem are assumed to be necessary for the initiation of bud outgrowth. Auxin and/or a correlative inhibitor are thought to prevent the entry of factors into the buds by an effect on the vascular connections between bud and stem. This theory does not have much support (Rubenstein & Nagao 1976; Tepper 1993). In the 'hormonal balance' theory, a balance of hormonal factors controlling the inhibition and stimulation of bud development has been suggested. Cytokinins generally promote axillary bud outgrowth and are likely to play a secondary interacting role with auxins so that a decrease in auxin concentration in the bud following decapitation might induce an increase in cytokinin concentration. Auxin has also been suggested to play an indirect role in apical dominance via induction of secondary inhibitors such as ethylene or abscisic acid.

Despite the numerous studies on apical dominance, the action mechanism of this phenomenon is still unclear (Cline 1994). Hormones likely play an important role, although Trewavas (1981;1991) emphasized the importance of changes in tissue sensitivity to hormones in the control of developmental responses rather than changes in hormone concentration.

Relationship between the axillary bud and the subsequent shoot

As mentioned before, axillary buds are the start of each flower in rose. The large variation in flower production might be traced back to early variation in axillary buds. Sinnott (1921) concluded from experiments on bean and rock maple that the size of any given organ depends to a great extent upon the size of the growing point out of which it has been formed. A similar conclusion was drawn for poplar by Pieters (1974). In *Pinus*, bud size was shown to be a good indicator of shoot growth potential (Kozlowski *et al.* 1973). For leaves, fruits and grains the potential organ size was often found to be set largely during the early phase of development (Patrick 1988). Zamski *et al.* (1985) also suggested that in rose growth of an axillary shoot may be influenced by growth conditions before the axillary bud sprouted into that shoot.

One of the sources of variation in a rose crop was shown to be the pruning position on the shoot (Byrne & Doss 1981). Bud age, bud position as well as assimilate supply may interact in that effect.

Shoot diameter is an important parameter for flower shoot quality. Differences in shoot diameter might be related to differences in size of the pith. In several woody plants, shoot diameter was correlated with pith diameter (Sinnott 1936; Bostrack 1993). The pith is entirely primary in its origin. It persists indefinitely in all plants and in old stems it is present in size, shape and structure as it was in the young twig (Eames & MacDaniels 1947). In rock maple, the cross-sectional area of the pith of the internode below the attachment of an organ was found to represent the size of the growing point of that organ (Sinnott 1921). Furthermore, pith cells may be important for storage purposes (Glerum 1980).

Aim of the thesis

The aim of the present research was to enlarge the knowledge and insight in the development of axillary buds. Furthermore, it was investigated to what extent the growth of an axillary bud into a shoot can be influenced before release from correlative inhibition, i.e. during axillary bud formation, as well as after release from correlative inhibition, i.e. during actual outgrowth. More knowledge of the ontogeny and development of axillary buds and of the internal structure of rose plants will lead to a better understanding of growth and development of a rose crop and to better methods to manipulate and control the growth. Variation in shoot size and growth period may be reduced and flower production and shoot quality improved.

Outline of the thesis

In *Chapter 2* the ontogeny of axillary buds and shoots is described. *Chapter 2.1* focuses on axillary buds that form the start of harvestable flower shoots and *Chapter 2.2* on axillary buds at the base of the shoot, which grow into basal shoots. To get a better insight in the internal structure of a rose plant, xylem pathways and root-shoot connections in relation to basal shoot development are unravelled in *Chapter 2.3*.

In *Chapter 3* an *in vitro* model system is developed to grow buds isolated from the parent shoot enabling to study the growth potential of the axillary bud in the absence of the influences from the parent shoot or plant.

During growth and development of axillary buds two periods can be distinguished: (1) Formation of axillary buds and their development as long as they are inhibited correlatively and (2) Development of axillary buds when released from correlative inhibition, which includes bud break and outgrowth of the bud into a (flower) shoot.

Chapter 4 focuses on the effect of a number of factors on the development and the growth potential of the buds, when imposed during axillary bud formation (before bud break). Factors studied are bud age (*Chapter 4.1*), bud position along the shoot (*Chapter 4.2*), assimilate supply (varied by the number of leaves, *Chapter 4.3*) and temperature (*Chapter 4.4*). It was studied which parameters of shoot growth are determined by the axillary bud. Shoot growth after release from correlative inhibition was followed when the buds sprouted attached to the parent shoot as well as when sprouted in isolation. The first method represents the situation in a crop; the second makes it possible to separate bud effects from plant effects. Growth potential of axillary buds in isolation was studied by culturing the buds *in vitro* according to the system described in Chapter 3 or by grafting the buds onto cuttings.

Chapter 5 describes the effects of assimilate supply (*Chapter 5.1*) and temperature (*Chapter 5.2*) on growth and morphology of the shoot, when imposed after release from correlative inhibition. Bud age and bud position are not studied, since these characters are intrinsic to the bud and therefore their effects are described in Chapter 4. Comparing results of Chapter 4 and 5 shows to what extent shoot growth can be influenced during axillary bud formation and to what extent during shoot growth.

In *Chapter 6* in a general discussion an attempt is made to integrate results of the previous chapters.

2. Development of axillary buds and shoots

2.1. Ontogeny of axillary buds and shoots: Leaf initiation and pith development

Marcelis-van Acker C.A.M., 1994. Ontogeny of axillary buds and shoots in roses: Leaf initiation and pith development. Scientia Horticulturae 57: 111-122.

Abstract. The ontogeny of an axillary bud (in the middle region of a shoot) from initiation up to flowering of the subsequent shoot was studied. The first secondary buds appeared in the axillary bud (primary bud) when the leaf subtending the primary bud unfolded. By that time, the primary bud contained seven leaves, including leaf primordia. During the development of the parent shoot to the harvestable stage, the number of leaves and secondary buds in the primary bud increased to 11 and four, respectively. The primary bud appeared to contain the lower part of the future shoot. After release from correlative inhibition all the leaves and a flower bud had been formed within 10 days. No internodal elongation occurred between the scale-like leaves.
Pith diameter was found to be correlated with the shoot diameter and was about 50% of the diameter of harvestable shoots. In the primary bud the cells of the pith were isodiametric and equal in size; they were vital and contained starch and sugars. Two weeks after release of the bud, the final pith diameter was reached at the base of the shoot. Two types of cells had differentiated: small, vital cells and large, dead cells. The small cells, which appeared to form a network throughout the pith, could contain starch and sugars, the large cells were filled with air. The number of cells on a diameter line of the pith was constant after bud break and slightly decreased from the base towards the top of the shoot.

Introduction

The rose is an important glasshouse cut flower. The plants are considered as self-inductive for flower initiation, since this process is not obviously regulated by the environment (Halevy 1972), and the plants exhibit year-round recurrent flowering with terminal flowers on lateral shoots (Zieslin & Moe 1985). A harvestable shoot is cut just above a node. The most distal axillary buds will then sprout and develop into flowering shoots, the next flush of blooms.

Axillary buds are important at four stages during the development of a rose plant: (1) When propagated vegetatively, an axillary bud forms the aerial part of a new rose plant; (2) Axillary buds give rise to basal shoots (Chapter 2.2), which determine the potential flower production (De Vries & Dubois 1983); (3) The degree of branching depends on the growth of axillary buds (Wilkins 1988); (4) Axillary buds give rise to the flowering

shoots, which form the harvestable product. Despite the value of axillary buds for flower production, little attention has been paid to their ontogeny. Horridge & Cockshull (1974) studied the growth of axillary buds after release from inactivity, but restricted themselves to the development of the flower bud. Rouffa & Gunckel (1951a,b) made a comparative study of vegetative shoot apices in the Rosaceae, while Zamski *et al.* (1985) compared the anatomy of axillary buds along a harvestable shoot. Although it has been observed that part of the future shoot is already formed in the axillary bud (Zamski *et al.* 1985), a detailed analysis of the development of axillary buds from initiation until flowering of the subsequent shoot is still lacking.

Large differences in shoot diameter occur in a rose crop. In practice it was observed that these differences seemed to be related to the size of the pith. Eames & MacDaniels (1947) reported that the pith persists indefinitely in nearly all plants and that in old stems it is present in size, shape and structure exactly as it was in the young twig. However, apart from studies carried out about a century ago, little attention has been paid to pith tissue.

The object of the present study was to analyse the ontogeny of an axillary bud from initiation up to flowering. To gain better insight into the internal structure of a rose shoot, the development of the pith was studied during shoot growth. The relationship between pith and shoot diameter was also investigated.

Materials and Methods

For all experiments, double node cuttings, as described previously (Marcelis-van Acker & Leutscher 1993), of *Rosa hybrida* cv. Sweet Promise were used. In order to study the structure of an axillary bud and the formation of leaves and secondary buds, only one cutting was excised from the middle part of each flower shoot. To study the development of the pith during outgrowth of the bud into a shoot, two cuttings were excised from the middle region of each flower shoot. After the cuttings had rooted in a mixture of sand and peat (1:1 by volume), they were potted in 15 cm diameter plastic pots containing commercial potting compost, and pruned just above the axillary bud of the lower leaf.

Structure of axillary bud and formation of leaves and secondary buds

After the cuttings had rooted, 120 cuttings were grown in a heated glasshouse at 20°C. When the shoot formed by the axillary bud of the cutting had reached the harvestable stage (sepals reflexing), it was cut back just above the fourth five-leaflet leaf. The bud in the axil of this fourth leaf was allowed to sprout, whereas shoots developed from buds in the axils of lower positioned leaves were removed as soon as they appeared. From cutting onwards, two or three plants chosen randomly were collected every other day. Shoot length, the number of compound leaves visible without dissection and the number of unfolded compound leaves were recorded. Depending on the shoot length, the sprouting bud, the shoot tip or the bud in the axil of the fourth five-leaflet leaf was fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) at room temperature, rinsed in phosphate buffer, dehydrated in a series of tertiary butyl alcohol and embedded in Paraplast. Transverse sections were cut at a thickness of 7µm and stained in safranin-fast green. The number of leaves and buds in both the bud of the cutting and the bud in the axil of the fourth five-leaflet leaf of the shoot developed from the cutting,

were recorded. Curves of shoot length, number of buds and number of leaves in relation to time were fitted by Richards' growth functions (Richards 1959). Fresh, unfixed axillary buds were studied by use of a Jeol JSM scanning electron microscope (SEM) at 15 kV, either intact or after removal of bud scales and leaf primordia.

Development of pith

After 90 cuttings had been potted they were transferred to a climate chamber at 21°C, daylength of 16 h, light intensity of 25 W m^{-2} photosynthetically active radiation (PAR) provided by Philips fluorescent tubes and a relative air humidity of about 70%. At intervals of 3-7 days, six plants were chosen at random and thin transverse sections were made by hand at four positions: 1 cm above the base of the shoot, 1 cm below the lowermost five-leaflet leaf, 1 cm below the middle five-leaflet leaf and 1 cm below the uppermost five-leaflet leaf. Some sections were stained with IKI (for starch), dimethylthiazol (for mitochondria, as a viability test) or Fehlings reagent (for sugars). For each transverse section the diameters of the shoot and the pith were measured using an ocular micrometer and the number of pith cells passed in traversing a diameter line of the shoot was recorded. This was repeated for three diameter lines per section. As soon as the largest cells reached a size twice that of the small cells, two categories of cells were discerned (large and small cells). Curves of the number of cells and diameters of pith and shoot in relation to time were fitted by Richards' growth functions (Richards 1959).

To study the relationship between pith and shoot diameter, shoots of various diameters, grown in a climate chamber (21°C, daylength 16 h, light intensity 40 W m^{-2} PAR provided by high pressure sodium and high pressure mercury lamps, relative air humidity 70%), were collected when they reached the harvestable stage. Pith and shoot diameters were measured at 1 cm from the base of the shoot.

Results

Structure of axillary bud

In rose, one axillary bud is present in each leaf axil. Eleven leaves had already been formed in an axillary bud from the middle region of a harvestable shoot. The lowermost seven leaves were scale-like, the upper ones were compound leaves (Figs. 1A, 1B). An axillary bud was found in the axils of the lowermost four leaves. The most basal two buds were the biggest and each contained six or seven leaves (Figs. 1C, 1D). In the axils of the fifth and sixth leaf, only the meristems of axillary buds could be detected. Following Garrison (1949), axillary buds of a shoot will henceforth be referred to as primary buds and the buds that are developing within a primary bud as secondary buds.

Formation of leaves and secondary buds

As long as the primary bud of the double node cutting was not released from correlative inhibition, bud length and number of leaves and secondary buds did not increase. As soon as the primary bud was released from correlative inhibition by pruning above the bud, sprouting started (Fig. 2A). The scale-like leaves, which were already present in the primary bud at the time of release from correlative inhibition, remained at the base of the sprouting bud. Internodal elongation did not occur between the scale-like leaves, but did occur between the compound leaves. Increased activity of the axillary bud was reflected in initiation of new

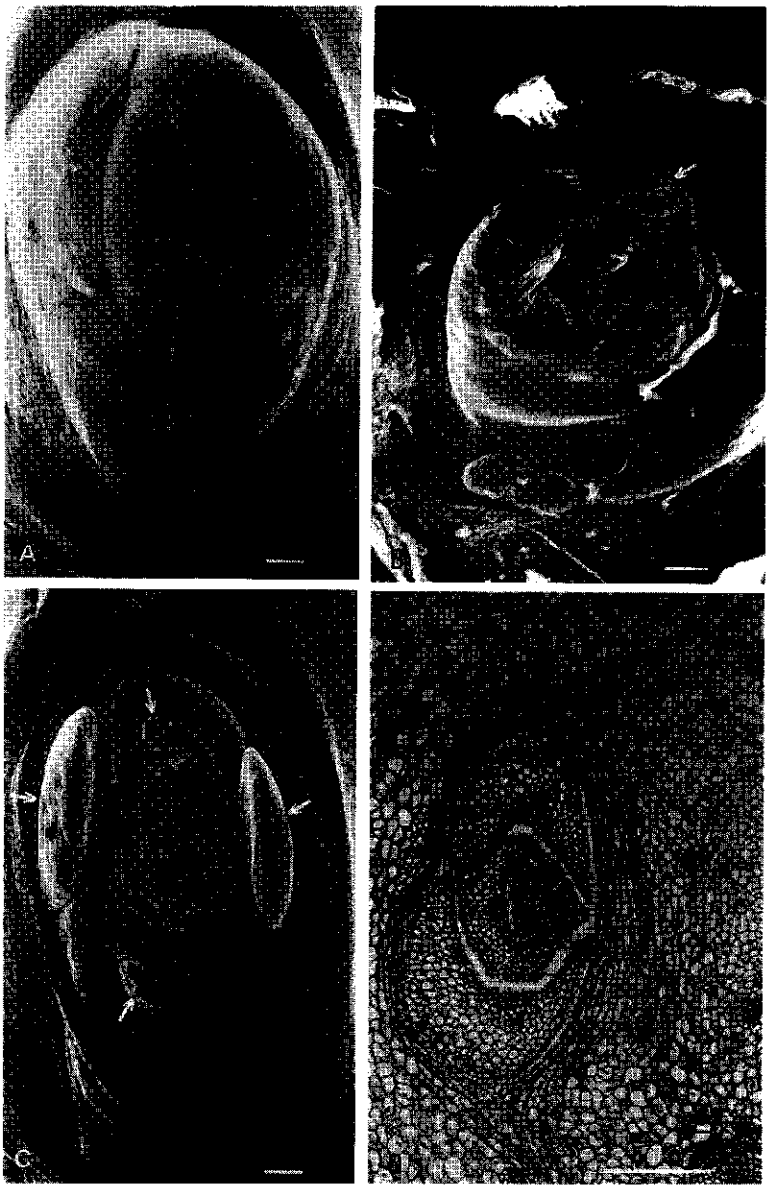


FIG. 1. Structure of an axillary bud. (A) Outermost, scale-like leaves (SEM). (B) Inner, compound leaves. The scale-like leaves have been removed. Arrow indicates leaflets of a compound leaf (SEM). (C) Secondary buds (indicated by arrows) in the axils of the outer scale-like leaves. All leaves have been removed (SEM). (D) Transverse section of a secondary bud containing six leaves. Bar, 200 μm.

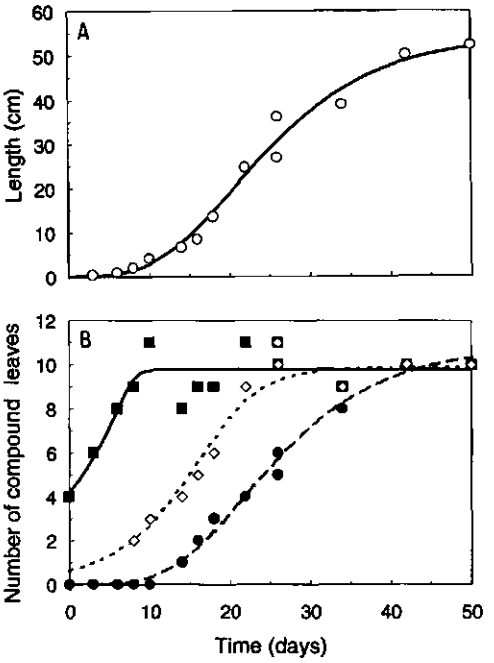


FIG. 2. Primary bud development after release from correlative inhibition in relation to time. Curves were fitted by Richards' growth function. (A) Length of the primary shoot, $r^2 = 0.98$.
(B) Number of compound leaves formed (■, $r^2 = 0.75$), visible without dissection (◇, $r^2 = 0.95$) and unfolded (●, $r^2 = 0.99$).

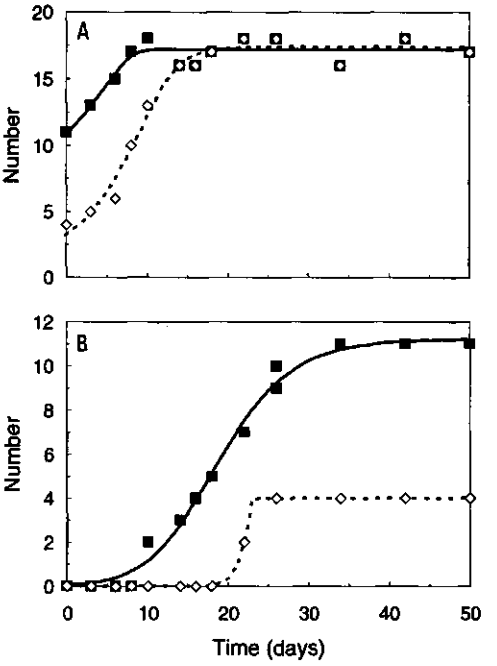


FIG. 3. Number of leaves (■) and secondary buds (◇) of the primary bud (A) and the axillary bud in the axil of the fourth five-leaflet leaf (B) in relation to time. Curves were fitted by Richards' growth function.
(A) r^2 (leaves) = 0.82, r^2 (secondary buds) = 0.97.
(B) r^2 (leaves) = 0.99, r^2 (secondary buds) = 1.0.

leaf primordia. About 10 days after the release of the primary bud, all leaves and a terminal flower bud had been formed (Fig. 2B) and about 2 weeks later, all compound leaves were visible without dissection. It was another 2 weeks before all the compound leaves had unfolded (Fig. 2B). The secondary buds appeared above the middle vein of the subtending leaf. Although bud meristems were present at an earlier stage, the appearance of the first leaves of the secondary buds was taken as the first indication of secondary bud development. By the time all of the leaves and the flower bud of the primary bud had been formed, secondary bud formation had occurred up to leaf 13 i.e. the fifth leaf axil below the flower bud (Fig. 3A). During the growth of the primary bud into the primary shoot, the secondary buds became primary buds. In the axils of the leaves of these buds a new generation of secondary buds appeared (sensu strictu tertiary buds). In the axil of the fourth five-leaflet leaf of the primary shoot (counted upward), secondary buds appeared when the subtending leaf unfolded. At that time, this fourth bud contained seven leaves, including leaf primordia (Fig. 3B). When the flower of the primary shoot was harvestable, the number of leaves in

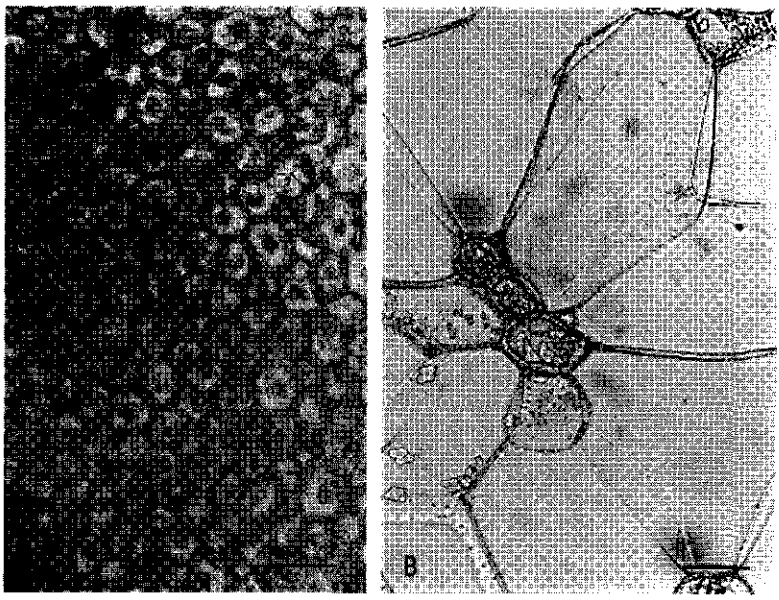


FIG. 4. Transverse section of the pith. (A) Axillary bud, the cells are uniform in size. (B) Mature shoot; two types of cells occur: small cells, which are vital and contain starch and large cells, which are dead and filled with air. Bar, 50 µm.

the fourth primary bud had increased to 11, whereas the number of secondary buds had increased to four (Fig. 3B). When the primary bud in the axil of the fourth five-leaflet leaf was released from correlative inhibition, by harvesting the flower shoot above, the cycle of sprouting and formation of leaves and secondary buds started again.

Development of pith

In the primary bud the cells of the pith were isodiametric and equal in size (Fig. 4A). The cells were vital, as indicated by staining with dimethylthiazol, and contained starch and sugars, as indicated by staining with IKI and Fehlings reagent, respectively. During pith development two types of cells differentiated (Fig. 4B): small cells (20-80 µm in diameter), having thick walls with many pits and large cells (at least 80 µm in diameter), characterised by thin walls and few pits. The small cells appeared to form a network throughout the

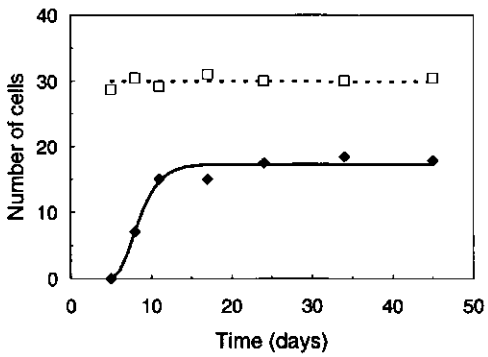


FIG. 5. Development of pith in the primary bud or shoot after release from correlative inhibition. The total number of cells (□) and the number of large cells (◆) on a diameter line are presented. The curve of the large cells was fitted by Richards' growth function: $r^2 = 0.93$.

pith that was connected with the pith rays. They were vital and often accumulated starch (Fig. 4B) and sugars. However, the large cells died and became filled with air. During primary shoot growth the number of cells, measured on a diameter line, was constant (Fig. 5). In a different experiment, carried out in a greenhouse (temperature set at 20°C), the number of pith cells in an axillary bud when released from inhibition was found to be equal to the number in the subsequent shoot (30.7±2.7 and 29.0±1.1 cells in the bud and in the internode below the middle five-leaflet leaf of the shoot, respectively). The number of cells slightly decreased from the base towards the top of the shoot (data not shown). Observations after release of the primary bud from correlative inhibition revealed that soon after the bud sprouted the final number of small and large cells was reached (Fig. 5). Cell enlargement seemed to be accompanied by loss of starch. Final pith diameter was reached two weeks after release from correlative inhibition at the base of the shoot (Fig. 6A), and 1 week later at 1 cm below the uppermost five-leaflet leaf (Fig. 6B). The fraction of the pith in the total diameter of a harvestable flower shoot decreased during shoot development. In shoots at the same developmental stage (flower harvestable), the diameter of the shoot was found to be correlated with the diameter of the pith (Fig. 7).

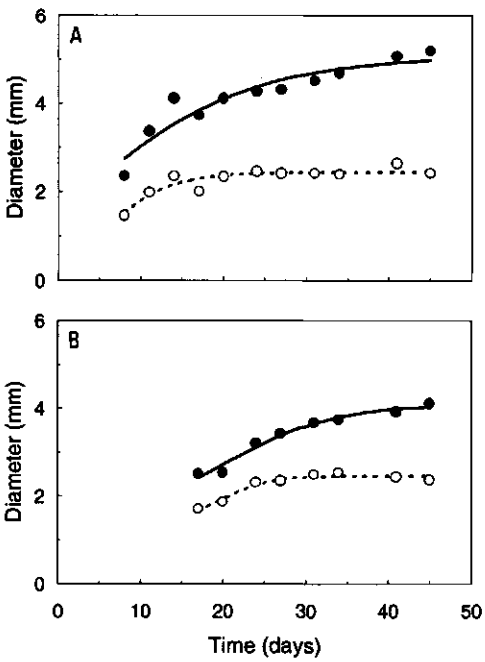


FIG. 6. Time course of diameter of pith (○) and diameter of shoot (●) 1 cm above the base of the shoot (A) and 1 cm below the uppermost five-leaflet leaf (B). Curves were fitted by Richards' growth function. (A) r^2 (shoot) = 0.84, r^2 (pith) = 0.77; (B) r^2 (shoot) = 0.95, r^2 (pith) = 0.92.

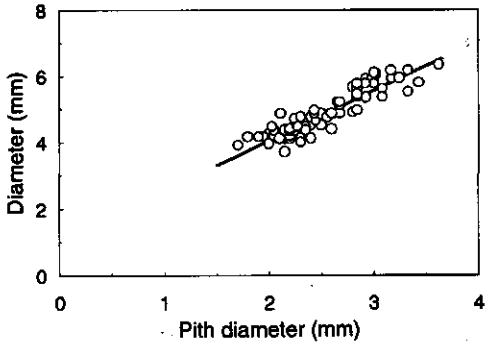


FIG. 7. Relation between diameter of the pith and diameter of the shoot of flower shoots in the harvestable stage: $r^2 = 0.83$.

Discussion

Axillary buds form the basis for the potential flower production of a rose plant. Knowledge of the development and internal structure of an axillary bud may lead to a better understanding of the growth of a plant. Furthermore, it may contribute to elucidate effects of cultural practices.

An axillary bud of a flowering shoot in the harvestable stage already contained the lower

part of the future shoot, which is in accordance with the results of Cockshull & Horridge (1977) and Zamski *et al.* (1985). Soon after the primary bud sprouted, all the leaves and the flower bud of the primary shoot had been formed, although it took some weeks before all the leaves had unfolded. According to the terminology of Steeves & Sussex (1989), most of the leaves of the primary shoot were found to be preformed (already formed in the primary bud), whereas most of the secondary buds were neoformed (formed after release of the primary bud from correlative inhibition). When the primary bud developed into the primary shoot, internodal elongation only occurred between the compound leaves, while the scale-like leaves remained at the base of the shoot. Steeves & Sussex (1989) reported that a leaf has an effect on the elongation of the internode below it. In *Populus* and *Ginkgo*, preformed leaves seemed to be associated with an absence of internodal elongation and neoformed leaves with extensive internodal elongation. However, in *Larix*, internodes belonging to early preformed leaves did not elongate, while those subtending later preformed leaves did elongate (Steeves & Sussex 1989). The latter situation may also hold for rose. It is not clear why internodes belonging to preformed leaves do not elongate (Steeves & Sussex 1989). Barlow (1970) reported for apple that internodal elongation was promoted by gibberellins produced by leaves at an early stage of development; since bud scales did not produce gibberellins, a condensed axis resulted.

In many woody plants, secondary buds are initiated and become well-formed in a primary bud (Romberger 1963; Zimmermann & Brown 1971). However, in *Araucaria*, axillary meristems only develop into buds when released from apical dominance (Burrows 1986). In rose the secondary buds belonging to the scale-like leaves were also already present in the primary bud, either as a bud or a meristem. According to Zamski *et al.* (1985), rose buds in the scale-axils develop to a lesser extent than buds in the axils of compound leaves. Prevailing growth conditions in successive stages of the primary bud development or competition for assimilates or nutrients may be responsible for this phenomenon. The buds in the axils of the lowermost scale-like leaves will give rise to the basal shoots and are already present in the axillary bud used for propagation (Chapter 2.2). Growth conditions during axillary bud development before propagation may affect growth potential of the basal shoots.

A primary bud containing 11 leaves and four secondary buds seems to be ready for sprouting as soon as the correlative inhibition is released, since these numbers did not increase apparently until the correlative inhibition was released.

The sub-apical meristemic region is the major site of cell multiplication and elongation contributing to stem elongation (Sachs 1965). The pith differentiates from the derivatives of the pith meristem. In the meristem the cells divide transversely so that the derivatives of individual cells form vertical files (Rouffa & Gunckel 1951b; Esau 1977; Mauseth 1988). Less frequently, longitudinal divisions occur (Popham & Chan 1950; Esau 1977; Mauseth 1988). Increase in pith diameter after release from correlative inhibition was mainly the result of cell enlargement, as was also found by Kassner (1884) for woody plants. Soon after the bud sprouted, the final size of the pith was reached and the large cells died. The occurrence of two types of cells in the pith of a mature shoot was also reported by Gris (1872) and Kassner (1884). Since the small

vital cells could accumulate starch and sugars and formed a network throughout the pith, this suggests that they have a function in accumulation and distribution of stored substances. Glerum (1980) also reported that pith cells are able to store reserves and remain alive for a long time in many tree species. He suggested that the pith cells are important for storage purposes, especially during the early stages of plant development when the proportion of living pith cells is relatively large. Eames & MacDaniels (1947) mentioned that in woody plants the living cells of the pith serve as storage cells in resting periods and become filled with starch and fatty substances.

The diameter of a rose shoot forms a criterion for the quality of the shoot. Shoot and pith diameters were found to be correlated. A similar correlation between the diameter of the pith and the vascular tissue was reported for *Pinus* (Ladell 1963). Although as a result of secondary growth by the cambium the fraction of the pith in the total shoot diameter decreased, it was still 50% in a harvestable flowering shoot. The potential diameter of the pith is determined by the number of cells produced at the axillary bud stage. Since the final pith diameter is reached a few weeks after bud break, a large portion of the final shoot diameter can only be influenced during early stages of shoot growth. Environmental factors either during bud development or during early shoot growth may affect the diameter of the pith and subsequently the diameter of the shoot.

2.2. Morphological study of the formation and development of basal shoots

Marcelis-van Acker C.A.M., 1993. Morphological study of the formation and development of basal shoots in roses. Scientia Horticulturae 54: 143-152.

Abstract. Basal shoots are the vigorous shoots at the base of the plant. In roses, basal shoots determine the potential flower production of the plant. Although many attempts have been made to promote the formation of basal shoots for commercial production, little attention has been paid to the origin and development of these shoots. The present study addresses this by following the development of a rose plant, raised from a cutting. Basal shoots only originated from basal axillary buds and not from adventitious buds. The first basal shoot of a plant emerged from one of the two most basal axillary buds of the primary shoot. The second basal shoot also emerged from an axillary bud of the primary shoot or, sometimes, from an axillary bud of the first basal shoot. If a third basal shoot occurred, it originated from an axillary bud of a basal shoot. The buds, which became the first and second basal shoot, were already present as secondary buds in the axils of the scales of the axillary bud when used for propagation. During the development of this primary bud into the primary shoot the secondary buds continued to initiate new leaf primordia, but did not sprout until the growth of the primary shoot slowed down. Removal of these two secondary axillary buds in the primary bud resulted in less basal shoots per plant and the basal shoots developed from buds number 3, 4 or 7.

Introduction

In a number of woody plant species, including roses, a common feature is the formation of vigorous shoots at the base of the plant. These basal shoots often have juvenile characteristics such as vigorous growth, delayed flower formation and easy rooting. In contrast to many woody plants, the formation of basal shoots in roses is desirable, since their number, diameter and degree of branching determine the potential flower production of the plant (Zieslin *et al.* 1973; De Vries & Dubois 1983). The basal shoots emerge during the first year after vegetative propagation, after which new basal shoots are rare.

The formation of basal shoots in roses can be promoted by pinching (Zieslin *et al.* 1976b), lateral bud removal (Zieslin & Mor 1981), low air temperature (Khayat & Zieslin 1982), high irradiance (Fisher & Kofranek 1949), application of cytokinins (Parups 1971; Carpenter & Rodriguez 1971; Okhawa 1979) and ethephon (Zieslin *et al.* 1972).

Vigorous shoots in woody plants can originate from inhibited buds in the scale-axils of axillary buds (Church & Godman 1966), from adventitious buds (Fink 1983) or from sphaeroblasts (Baldini & Mosse 1956). Little is known about the morphological origin of basal shoots in roses. Zieslin *et al.* (1976b) mentioned that the source of the basal shoots in roses is unknown and might be adventitious buds or sphaeroblasts, but later Khayat & Zieslin (1982) assumed that the basal shoots

originate from buds in the scale-axils of axillary buds.

The aim of the present study was to elucidate the origin of the basal shoots in roses and to follow their development, in order to get a better understanding of basal shoot formation.

Materials and Methods

Plants used in all experiments were grown in a heated glasshouse (day/night temperature set at 20/17°C).

To show the origin of basal shoots (Experiment 1), 40 plants of *Rosa hybrida* cv. Ruby, rootstock *Rosa canina* cv. Inermis, were harvested 7 months after grafting. At that time they had one, two or three basal shoots and the date of appearance of each basal shoot was recorded. The plants were cut at the root collar and immediately placed in an aqueous solution of 1% acid fuchsin (Merck AG-Darmstadt no. 42685) to stain the xylem, which was then clearly distinguishable from the pith. After 1 day, the basal parts of the plants were split longitudinally through the basal shoots. The origin of the shoot could be determined by following the pith of the basal shoot downwards.

To find out more precisely which buds become basal shoots and to follow the development of these buds, two further experiments (Experiments 2 and 3) were carried out. For both experiments, cuttings, bearing two leaves and two axillary buds, were excised from the middle part of harvestable flower shoots of *Rosa hybrida* cv. Sweet Promise and were allowed to root for 3 weeks. Those still having a quiescent bud in the axil of the lowermost leaf were potted and pruned approximately 1 cm above the lowermost leaf. The bud in the axil of this leaf formed the primary shoot. To study the developmental stage of the axillary buds at the moment of cutting, some buds were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) at room temperature, rinsed in phosphate buffer, dehydrated in a series of tertiary butyl alcohol and embedded in Paraplast. Transverse sections were cut at a thickness of 7 µm and stained in safranin-fast green.

Experiment 2 started in February 1990. After the plants were potted the following treatments, in 16 replicates, were applied to the bud in the axil of the lowermost leaf: (1) Control; (2) Outermost (i.e. most basal) two bud scales removed; (3) Outermost (i.e. most basal) two bud scales removed and the buds in the axils of these scales marked with nail polish; (4) Outermost (i.e. most basal) two bud scales and the buds in the axils of these scales removed.

In accordance with commercial practice, 2 months after potting the primary shoot was bent in the direction of the cutting leaf, to promote the formation of basal shoots. For each basal shoot the time until flowering was recorded. The number of basal shoots per plant was determined at the end of the experiment (23 weeks after potting).

Experiment 3 started in March 1991. Twice a week the length of the primary shoot was measured and the two most basal axillary buds of the primary shoot of eight plants were dissected under a dissecting microscope (x50); the number of leaves and leaf primordia was recorded. The layout for both experiments was a randomized block design with four blocks. The data were analysed by means of analysis of variance and the significance of differences determined by Student's *t*-test (P=0.05).

Results

Longitudinal dissections of basal parts of 7-month-old 'Ruby' plants (Experiment 1) showed that basal shoots emerged from basal axillary buds, since the pith of a basal shoot was connected to the pith of the older shoot (Fig. 1). The first basal shoot, and usually also the second one, originated from basal axillary buds of the primary shoot, but the second basal shoot sometimes emerged from a basal axillary bud of the first basal shoot, as was always the case for the third basal shoot (Table 1).

In the case of vegetative propagation, an axillary bud of a rose shoot at the harvestable stage (sepals reflexing) forms the starting material for the aerial parts of a new rose plant. In this so-called 'primary bud' of cv. Sweet Promise approximately 11 leaves and leaf primordia were present (Fig. 2). The outermost six or seven leaves of the axillary bud were scales. In the axils of the outermost four scales a bud was present, which is called a 'secondary axillary bud' (Fig. 2). By the time the primary bud of a cutting had developed into the so-called 'primary shoot', the secondary axillary buds had become primary axillary buds of that shoot. At that time about seven axillary buds were found at the base of the primary shoot without internodal elongation between the buds. Subsequently, strong growing shoots emerged at the base of the primary shoot. Basal shoots are defined as the shoots arising from the region at the base of the plant without internodal elongation.

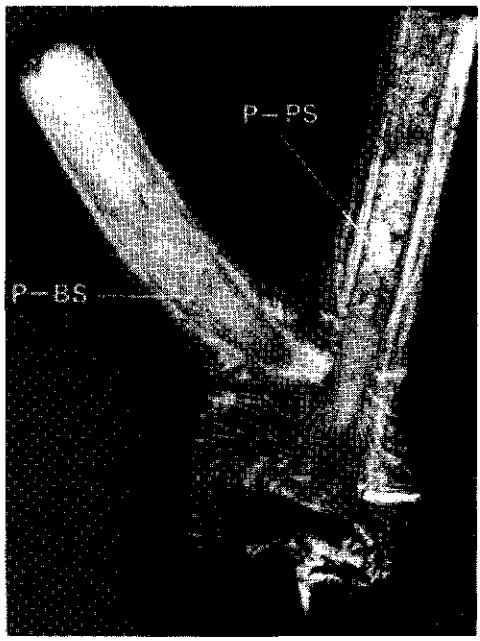


FIG. 1. Longitudinal dissection of the basal part of a rose plant cv. Ruby. The pith of the basal shoot (P-BS) is connected to the pith of an older shoot (P-PS). The xylem has been stained with acid fuchsin.

To clarify which of the seven basal axillary

TABLE 1. Origin of basal shoots in rose plants cv. Ruby with one, two or three basal shoots[1)].

No. of basal shoots per plant	No. of plants	1 AB PS (%)	2 AB PS (%)	1 AB PS 1 AB BS (%)	3 AB PS (%)	2 AB PS 1 AB BS (%)	1 AB PS 2 AB BS (%)
1	11	100					
2	20		80	20			
3	9				0	100	0

[1)] AB = axillary bud; PS = primary shoot; BS = basal shoot.

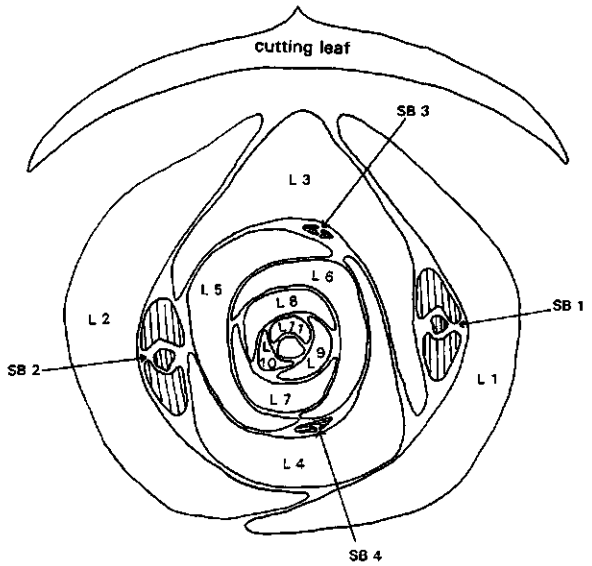


FIG. 2. Transverse section of an axillary rose bud cv. Sweet Promise. The bud is composed of a total of 11 leaves and leaf primordia (L). The outermost leaves of the bud will become the lowermost leaves of the shoot. Secondary buds (SB) are present in the axils of the outermost four leaves.

buds of the primary shoot are most likely to sprout into basal shoots, the two most basal axillary buds of the primary bud (i.e. in the axils of the outermost two scales) were marked with nail polish when the plants were potted (Experiment 2). Remains of nail polish were found on the emerging basal shoots. Removal of the bud scales and marking of the secondary buds did not affect the plant habit, the number of basal shoots nor the time to flowering (Table 2).

Periodic dissection and analysis of the two most basal axillary buds during development of the primary shoot (Experiment 3) showed that the number of leaf primordia increased continuously at the same rate. When the growth of the primary shoot slowed down, usually one of the two buds sprouted into a basal shoot. In that case a further increase in number of leaf primordia occurred, whereas the initiation of leaf primordia in the buds which had not sprouted stopped almost com-

TABLE 2. Effect of manipulation of secondary axillary buds on number of basal shoots and time from potting until flowering of the basal shoots of rose plants cv. Sweet Promise.

Treatment	Number of basal shoots	Time (days)
1. Control	1.6	93.9
2. Outermost two bud scales removed	1.6	94.0
3. Outermost two bud scales removed and axillary buds marked	1.4	94.1
4. Outermost two bud scales and axillary buds removed	1.1	110.7
Least significant difference (P=0.05)	0.23	9.9

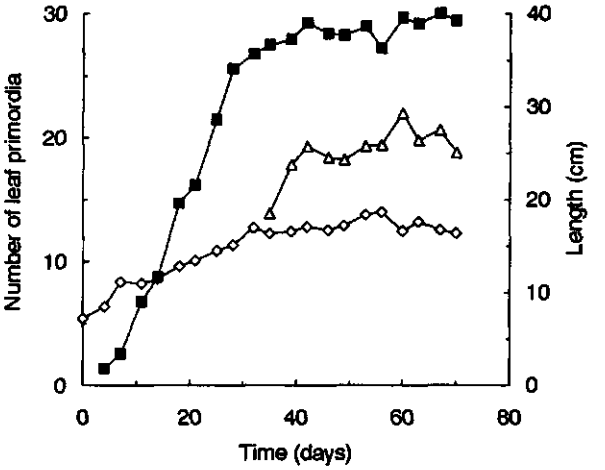


FIG. 3. Time course of the length of the primary shoot (■) and the total number of leaves and leaf primordia in the two most basal axillary buds (not sprouted (◇) and sprouted (△)) in rose plants cv. Sweet Promise. Values are means of eight replicates.

pletely (Fig. 3).

When the two most basal secondary axillary buds of a primary bud (defined as buds number 1 and 2) were removed (Experiment 2), the bud in the axil of the third, fourth or seventh scale (from the base) developed into a basal shoot (Fig. 4). These buds were located in the basal stem region of the primary shoot where no internodal elongation had occurred. Sprouting of buds positioned higher on the primary shoot (bud number 8 and higher) was also observed (on average 0.5 shoot per plant). Removal of the two most basal secondary axillary buds resulted in less basal shoots per plant and an increase in the time to harvest (Table 2). This increase in time was caused by both a delayed sprouting and a lower growth rate (data not shown).

Discussion

The longitudinal dissections (Experiment 1) and the 'marking' treatment (Experiment 2) indicate that basal shoots of rose plants originate from basal axillary buds of the primary shoot as suggested by Khayat & Zieslin (1982). The first two basal shoots usually emerge from the two most basal axillary buds of the primary shoot. These buds are already present as secondary buds in the axils of the scales of the primary shoot when this primary shoot is still at the bud stage. If a third basal shoot occurs, it originates from an axillary bud of a basal shoot. In the plants studied, the third basal shoot emerged some time after the first two basal shoots. When three basal shoots emerge at the same time, which may occur in very vigorous plants, the third basal shoot will originate from an axillary bud of the primary shoot.

Removal of the bud scales (Experiment 2), which was necessary to mark the secondary buds, did not affect their sprouting. This was somewhat unexpected since removal of bud scales accelerated bud burst in apple (Swartz *et al.* 1984). Pukacki *et al.* (1980) reported that bud scales have a light-filtering function,

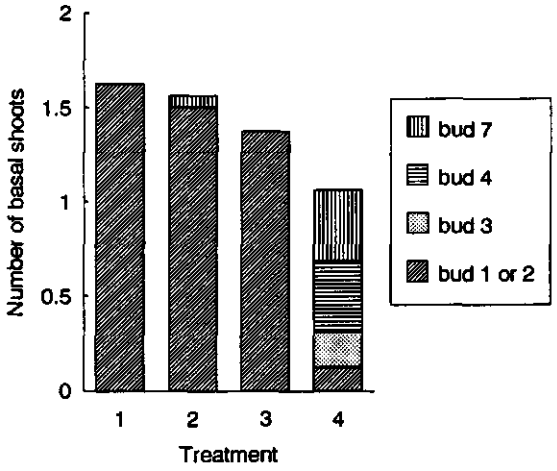


FIG. 4. Effect of manipulation of secondary axillary buds on the formation and origin of the basal shoots in rose plants cv. Sweet Promise. Buds 1 and 2 were the lowermost axillary buds.
Treatments: (1) Control; (2) Outermost two bud scales removed; (3) Outermost two bud scales removed and axillary buds marked; (4) Outermost two bud scales and axillary buds removed.

and light can influence branching (Wilkins 1988). Kauppi *et al.* (1987) observed that bud scales are important for maintaining the activity of the bud. There is no indication in the present experiment that nail polish had any effect on bud sprouting. The observation in Treatment 4 (Experiment 2) that basal buds numbers 5 and 6 never and number 3 hardly ever sprouted when the two most basal buds (numbers 1 and 2) were removed could be due to our practice of bending the primary shoot. In that case, buds number 3, 5 and 6 were oriented downwards and buds number 4 and 7 oriented upwards; the position of buds number 1 and 2 was not influenced. Mullins (1965) showed for apple, and Zieslin & Halevy (1978) for rose, that downward oriented axillary buds were highly inhibited, whereas in buds facing upwards the inhibition was minimal, probably as a result of a downward gravitational movement of an inhibitory factor. The observation that in a few cases buds numbers 1 or 2 developed into a basal shoot might be due to incomplete removal of the buds.

Removal of the two most basal secondary buds resulted in a lower number of basal shoots. As well as a basal bud, an axillary bud positioned higher on the primary shoot often sprouted, resulting in a shoot positioned at some distance from the base of the plant. In a commercial crop, basal shoots are preferred over these higher positioned shoots.

Usually the two most basal axillary buds of the primary shoot develop into basal shoots. These buds seem to show an advanced development and/or to have a dominant position. If for any reason these buds are unable to sprout, other basal axillary buds or axillary buds positioned higher on the shoot will then sprout. The number of axillary buds present in the basal region of the primary shoot (i.e. the region with no internodal elongation) appeared to be related to the position on the parent shoot and the age of the primary bud when used for propagation (Chapter 4). Furthermore, each basal shoot has several

axillary buds at its base and such basal buds may branch, as described for *Betula* (Kauppi *et al.* 1987), resulting in a cluster of axillary buds at the base of the plant. All these buds have the potential of sprouting into a basal shoot, so the number of basal axillary buds probably does not limit the formation of the basal shoots.

As long as the primary shoot grew, the basal axillary buds did not sprout, but continued to initiate new leaf primordia (Experiment 3). This result corresponds with data on roses from Cockshull & Horridge (1977), who studied the development of the bud in the axil of the fourth five-leaflet leaf from the base of the stem. Zamski *et al.* (1985) divided the buds along a rose shoot into three groups, based on their anatomical differences. They found the two most basal buds on a shoot comparable to the bud in the axil of the lowermost five-leaflet leaf. The number of leaves and leaf primordia in this bud did not increase until the upper part of the stem was removed, which seems in contrast to our results. This discrepancy may indicate a difference between the bud in the axil of the lowermost five-leaflet leaf and the basal axillary buds, or may be a result of the experimental methods: Zamski *et al.* (1985) studied buds on 3-year-old plants, while in the experiment reported here the primary bud was used for propagation, which might result in a different level of correlative inhibition of the buds.

2.3. Xylem pathways in relation to basal shoot development

Marcelis-van Acker C.A.M., Keijzer C.J. & Van de Pol P.A., 1993. Xylem pathways in rose plants in relation to basal shoot development. Acta Botanica Neerlandica 42: 313-318.

Abstract. Application of dyes to roots or shoots is an easy way to visualize xylem pathways in plants. It was shown that upward transport was sectorial straight. A main root appeared to contribute to the transport to several basal shoots. Application of dyes to shoots showed that each basal shoot is supplied by only a part of the root xylem. It is supposed that at the moment of appearance of a new basal shoot, the root xylem becomes enveloped by a new xylem cylinder, resulting in a limited area of root xylem serving the former developed shoot. The large variation in shoot diameter in a rose crop is discussed in relation to xylem systems.

Introduction

In a rose crop, considerable variation in number and diameter of shoots occurs, resulting in variation in shoot yield. Little attention has been paid to water pathways in rose plants, apart from some work on cut rose flowers (Mayak *et al.* 1974; Dixon *et al.* 1988; Darlington & Dixon 1991). In several trees the pattern of water transport has been studied in relation to insect and disease control (Vité & Rudinsky 1959; Kozlowski & Winget 1963).

Vité & Rudinsky (1959) described five types of water-conducting systems in sapwood of conifers: (1) spiral ascent, turning right; (2) spiral ascent, turning left; (3) interlocked ascent; (4) sectorial, winding ascent; (5) sectorial, straight ascent. Kozlowski & Winget (1963) found no clear and consistent differences in patterns of water ascent between gymnosperms and angiosperms.

The xylem transport pathway and as a result the pathway of water movement in plants can simply be followed by using water coloured by dyes (Zimmermann 1978; Fisher & Ewers 1992). The movement of the dyes should not be hampered by the pathway construction and the concentration of dye should be sufficient so that any reactions which may take place along the pathway have no significant effect on the transport. Acid fuchsin has often been used for this purpose (Vité & Rudinsky 1959; Kozlowski & Winget 1963; Altus & Canny 1985), but also alcian blue, eosin and fast green (Altus & Canny 1985), toluidine blue-O (Shigo 1985), safranin and crystal violet (Fisher & Ewers 1992) appeared useful.

In the present study xylem pathways in rose plants were visualized to show: (1) the type of the water conducting system; (2) which shoots are connected to one root; and (3) which roots are connected to one shoot. This information may contribute to the explanation of the large variation in shoot diameters within plants and between plants in a rose crop.

Materials and Methods

In a commercial rose crop, plants are propagated vegetatively by cutting or grafting a piece of stem bearing one leaf and an axillary bud. When propagated, the axillary bud grows into the so-called primary shoot. Later at the base of this shoot basal buds sprout, which become strong growing shoots. These so-called basal shoots form the main frame of the plant. The part of the stem between the basal shoots and the roots, which was the piece of stem used for propagation, will be referred to as basal stem.

For our experiments we used more than 60 plants of *Rosa hybrida* cv. Sweet Promise and *Rosa hybrida* cv. Madelon, grown in a heated glasshouse for 1.5 years, having a primary shoot and one or two basal shoots. Plants were propagated by cutting or by stenting, i.e. grafting a piece of stem bearing a leaf and an axillary bud onto an internode as described by Van de Pol & Breukelaar (1982). To show both the type of the water conducting system according to the classification of Vité & Rudinsky (1959) and which shoots were connected to one particular root, one main root (diameter at least 1 mm) was immersed in a dye solution in a vial. The remaining roots were kept in water or soil. As soon as the dye became visible in the veins of the leaves (after several hours), the experiment was terminated and the bark of the stem was removed to study the pathway of the dye.

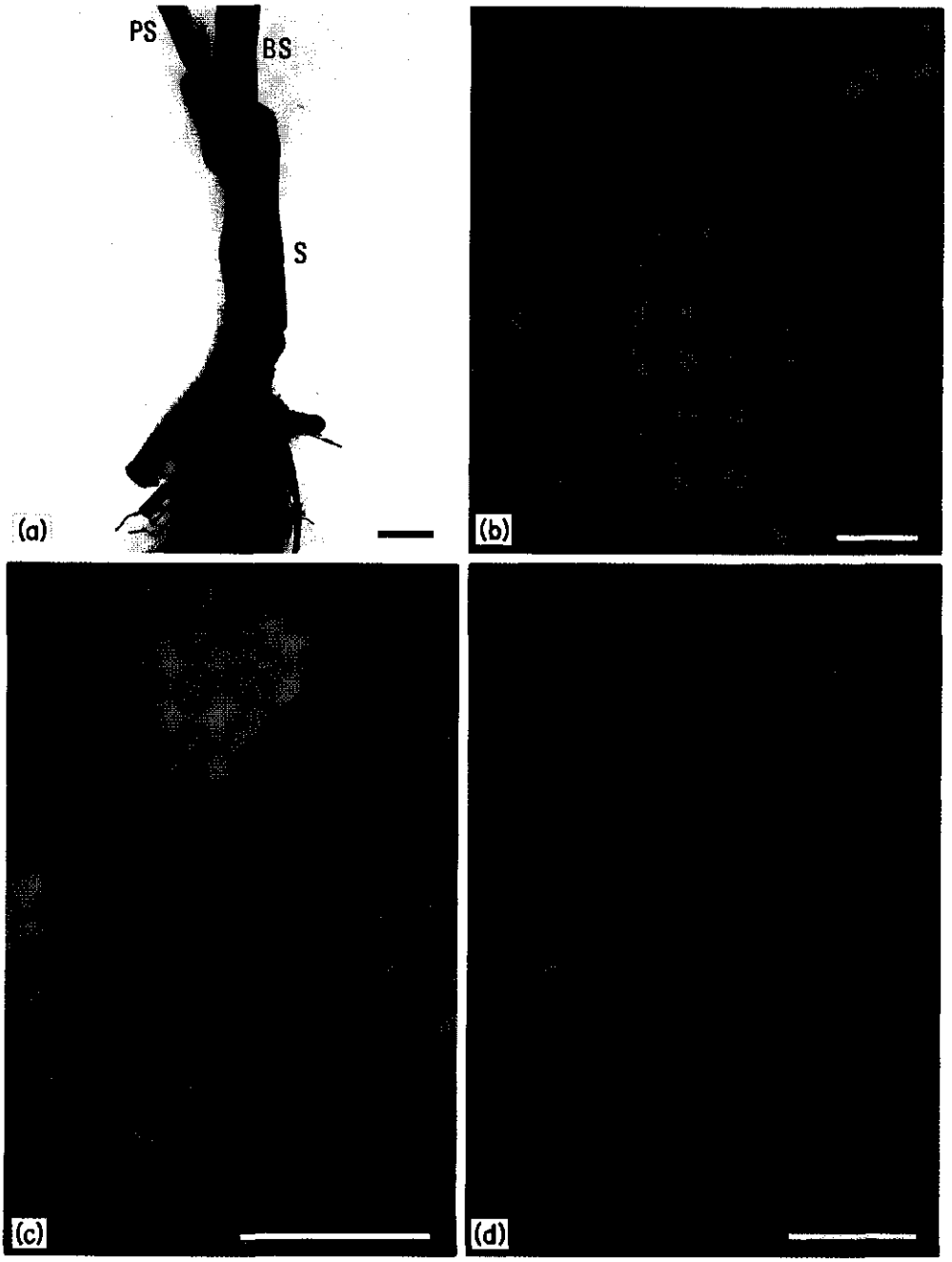
To determine which main roots were connected to one shoot, the two or three shoots were cut transversely and vials, each containing a different coloured dye solution, were placed upside down on top of them (Van de Pol & Marcelis 1988). Soil between the roots was removed as far as possible to promote evaporation. To distinguish effects of gravity, plants were held either upright or upside down. In some trials the roots were wrapped in aluminium foil in order to prevent them from drying out. As soon as the roots coloured, the experiment was terminated. The main roots were cut transversely and the basal stem was split longitudinally to show the pathway of the dyes.

Aqueous solutions of 1% acid fuchsin (Merck AG- Darmstadt no. 42685, colour red), 1% light green yellow (Merck AG-Darmstadt no. 1315, colour green) and 0.5% trypan blue (Merck Darmstadt no. 23850, colour blue) were used.

Results

Dyes applied to both roots and shoots coloured only the xylem. Positioning of the plant (upright or upside down) did not affect the distribution of the dye. However, when roots

FIG. 1. (A) Sectorial dye pattern in the basal stem when red dye (acid fuchsin) was applied to a root. A slight bend in the path of the dye occurs at the grafting area, half-way up the basal stem. The bark was removed when the experiment was finished. PS: primary shoot; BS: basal shoot; S: basal stem, i.e. piece of stem used for propagation. Bar = 1 cm. (B) Dye pattern in a radial longitudinal section of the basal stem, and (C) in a transverse section of a main root. The plant had a primary shoot and one basal shoot. Red dye (acid fuchsin) was applied apically to the primary shoot and blue dye (trypan blue) to the basal shoot. The pith and a part of the xylem of the basal stem were not involved in dye transport. Large vessels in the root appear as dark dots. (B) Bar = 1 mm; (C) bar = 0.5 mm. (D) Dye pattern in a transverse section of a main root of a plant having a primary shoot and two basal shoots. Green dye (light green yellow) was applied apically to the primary shoot, red dye (acid fuchsin) to the first basal shoot and blue dye (trypan blue) to the second basal shoot. Bar = 1 mm.


PS
BS
S
(a)
(b)
(c)
(d)

were wrapped in aluminium foil, dye applied to the shoot did not move further than the root collar. Dye applied to a main root showed a sectorial, straight upward transport, even through the grafting area (Fig. 1A). Also, in transverse sections of the basal stem, the dye was found in a sector of the xylem. No deviations from a straight path were observed. A main root appeared to contribute to the transport to several basal shoots. However, when dye was applied to a sector of a main root, dye was found only in a sector of the xylem of a shoot, corresponding with the pathways found when dyes were applied to shoots (data not shown).

When dye was applied to both shoots of plants with a primary shoot and only one basal shoot, both dyes were found in the xylem of the basal stem (Fig. 1B). The outer (younger) xylem contained dye coming from the basal shoot, and the inner (older) xylem dye from the primary shoot. Sometimes the dyes were clearly separated, in other cases there was also a transitional region. Similar dye patterns were found in the main roots (Fig. 1C). Smaller roots were stained by the dye applied to the primary shoot, or by that applied to the basal shoot, or by both.

When dye was applied to all three shoots of plants with a primary shoot and two basal shoots, the inner (oldest) xylem of the basal stem and the main roots contained only dye applied to the primary shoot. In the outer xylem of the basal stem and the root the situation varied. In the basal stem the outer xylem contained the dye from the nearest basal shoot, i.e. from the shoot at the same side of the plant. In the root it usually contained only dye coming from one of the basal shoots. However, in a few cases (out of 20) three different coloured concentric zones were visible in the xylem of the basal stem and the root (Fig. 1D). The dye in the central part of the root xylem then originated from the oldest shoot (which is the primary shoot). The outer, youngest, zone contained the dye applied to the youngest shoot (which is the second basal shoot). The dye in the middle zone came from the first basal shoot.

Discussion

By feeding a dye solution to a main root it has been shown that the type of water conducting system in rose is sectorial, straight ascent, according to the classification of Vité & Rudinsky (1959). A main root was found to contribute to the transport to several shoots. A shoot was found to be connected to a part of each main root. Comparison of the pathways visualized when dye was applied to (a part of) a main root and those visualized when dye was applied to shoots, support the assumption that upward and downward pathways are similar.

Kozlowski & Winget (1963) found that in trees a straight vertical pathway for water transport is not so common. Rudinsky & Vité (1959) noted that the most complete distribution of water in the plant was achieved by spiral ascent and the least effective distribution by vertical ascent. Dye was transported with little tangential spreading, which was also found in *Cayratia* by Fisher & Ewers (1992).

It is assumed that the discovered pathways represent xylem connections between shoot and root and show the preferred water pathways, which is in accordance with Fisher & Ewers (1992) who mentioned that dye flow indicates the fastest and most efficient pathway for water movement, although it is not the only one possible in the intact plant. Canny (1990), however, argued that due to diffusion

the movement and distribution of dye through a plant does not always correctly reveal pattern of water flow. In these experiments the movement of the dye stopped when roots were wrapped in aluminium foil, indicating that the movement was not due to diffusion but due to water flow driven by evaporation from the roots. As position of the plant did not affect the distribution of the dye, movement of dye was not achieved by gravity. It was shown that the pith and the cortex tissues are not the preferred pathways for water transport through the stem, which is in accordance with Darlington & Dixon (1991).

In roses the first basal shoot emerges from a basal axillary bud of the primary shoot, while the second basal shoot emerges either from a basal axillary bud of the primary shoot or from a basal axillary bud of the first basal shoot (Chapter 2.2). Application of dyes to shoots indicates that obviously each (basal) shoot is supplied with water through a distinct zone of the basal stem xylem and root xylem. This fits in with work done by Sachs (1970) who found that buds growing on an intact shoot were connected to the roots by vascular strands which ran parallel to the vascular system of the stem. Neeff (1914) showed that large tree branches also have a relatively independent vascular system. Eames & MacDaniels (1947) stated that at the region where tissues of the main stem and a branch meet, their conducting tissues remain more or less distinct. This means that the vasculature of the branch is not directly connected with that of the stem above the branch.

The dye patterns found in rose plants suggest that the area of xylem in the basal stem and root, which supplies the primary shoot, becomes fixed soon after the appearance of the first basal shoot (a lateral shoot of the primary shoot). The basal shoot is expected to induce new formation of vascular tissue downwards, since growth of lateral shoots resulted in an increase in diameter of its parent shoot (Marcelis-van Acker, unpublished data). Zimmermann & Brown (1971) also reported for trees that growing shoots have a definite influence on the amount of radial growth in the stem beneath. The formation of this so-called branch collar (Shigo 1985) starts at the side of the growing basal shoot and subsequently envelops the entire basal stem xylem. Also, the root xylem becomes enveloped by new vascular tissue.

The dye pattern found in plants with two basal shoots was not always the same. Observation of many plants led to the following hypothesis. Where a basal shoot is the result of the outgrowth of an axillary bud of the latest developed shoot, basal stem and root are composed of separated xylem cylinders, each cylinder connected to one shoot. Where two basal buds on opposite sides of the primary shoot sprout simultaneously, branch tissue differentiations start from each side and will meet half-way around the stem. If these two basal buds do not sprout exactly simultaneously, a more complicated pattern will occur. Where successive sprouting of basal buds of any shoot is interrupted by a long time interval, xylem of basal stem and root will be composed of separated concentric zones, each zone connected to one shoot.

Growth of the primary shoot and the first basal shoot is assumed to become limited due to a restricted xylem system. The xylem system becomes restricted because it will be enveloped by xylem serving a younger shoot. Shigo (1985) reported for trees that a branch is structurally attached to the trunk by a series of trunk and branch collars. Every growing season a branch collar is formed, which is enveloped by a trunk collar. However, we did

not observe this in roses. Moreover Kool *et al.* (1991) found for rose that the diameter increase of the first basal shoot was restricted when a new basal shoot emerged, while the growth of this new basal shoot was not influenced by the older basal shoot. These observations support the hypothesis that the xylem system serving a single shoot becomes fixed and will be limited after some time.

The variation in xylem capacity of basal stem and root probably influences the growth of future shoots. The addition of new xylem induced by a shoot depends on the time elapsed until a new shoot is formed. When a new shoot is formed the xylem supplying the former shoot will become fixed. As xylem ages it becomes less functional in conduction (Milburn 1979), so its capacity will decrease. The effects of fixing and ageing combined with differences in the length of the time interval in basal shoot formation will result in a variability of xylem capacity for individual shoots. This capacity may partly be expressed in the shoot diameter.

3. Growth of axillary buds: An *in vitro* model system

Marcelis-van Acker C.A.M. & Scholten H.J., 1994. Growth of axillary buds of rose: an in vitro model system. (submitted).

Abstract. An *in vitro* system has been developed to grow axillary buds, excised with a small fragment of stem and petiole, into shoots, which are morphologically comparable to those grown *in vivo*. In the development of the shoot three stages can be distinguished: sprouting of the bud, unfolding of leaves already present in the bud, and new formation of leaves and a flower bud. A low concentration of BA, sugar (preferably glucose), and a cultivar-dependent concentration of a suitable agar was necessary to obtain a complete shoot. The size of the *in vitro* shoot positively correlated with the size of the explant. The presence of the petiole inhibited the outgrowth of lateral buds, which were already present in the inoculated bud. Using this *in vitro* system the growth potential of axillary buds, apart from influences of other plant parts, can be studied. Furthermore, this system can be used for micropropagation by single-node culture.

Abbreviations: MS=Murashige and Skoog; BA=Benzyladenine; NAA=Naphthaleneacetic acid; IAA=Indole-3-acetic acid; GA=Gibberellic acid

Introduction

Flower yield of roses depends on the willingness of axillary buds to sprout and their subsequent growth into flowering shoots (Zieslin *et al.* 1973). The development of axillary buds on intact plants depends on three partly interdependent factors, i.e. the intrinsic growth potential of the buds, their position on the plant (Zieslin *et al.* 1976a) and influences of other plant parts (Zieslin & Halevy 1976). Especially this latter point makes it difficult to assess the growth potential of the buds themselves in intact plants. *In vitro* culture proved useful to study bud and shoot growth, isolated from the interactions the buds are subjected to in the intact plant. Moreover, it offers a technique to study the physiology and requirements for growth and development of isolated organs (Dutcher & Powell 1972; Halim *et al.* 1988; Nadel *et al.* 1991).

In each leaf axil of a rose shoot an axillary bud is present. A quiescent axillary bud of a flowering shoot contains the lower part of the future shoot, i.e. 6 to 7 scale-like leaves and 4 to 5 compound leaves. When the correlative inhibition of the axillary bud is released by removing the stem part above the bud, the bud will sprout and approximately 7 new leaves and a flower will be formed (Chapter 2.1).

In vitro culture of rose has been described previously, e.g. micropropagation by Jacobs *et al.* (1969), Hasegawa (1979), and Bressan *et al.* (1982), and somatic embryogenesis and adventitious shoot formation by Tweddle *et al.* (1984). The studies on *in vitro* culture of

rose have been reviewed by Short & Roberts (1991). All methods of micropropagation are based on the system of multiple shoot formation, starting with axillary buds or shoot tips, which are regularly subcultured. In most cases a standard MS medium (Murashige & Skoog 1962) is used with a rather high BA concentration. The shoot proliferation may decline after a number of subcultures at a high BA concentration, as reported by Norton & Norton (1986). Furthermore, *in vitro* derived plants often show more branching after hardening than *in vivo* plants (Dubois *et al.* 1988; Vijaya & Satyanarayana 1991), which might be a carry-over effect of the relatively high BA concentration applied in the multiple shoot system. In order to study the growth potential of axillary buds *in vitro*, isolated from the correlative influences in the intact plant, the multiple shoot system is not suitable.

The aim of the present study was to develop an *in vitro* model system for physiological studies on development of axillary rose buds. In this system axillary shoots of rose are grown as single elongated shoots at low cytokinin concentration. The effects of medium components (agar, plant growth regulators, and sugars), presence of petiole, and explant size on bud and shoot development are evaluated.

Materials and Methods

From two cultivars of *Rosa hybrida*, 'Sweet Promise' and 'Motrea', about 1 cm long stem segments bearing a quiescent axillary bud including a petiole stump of 1 cm were cut from the middle part of flower stems in the harvestable stage (sepals reflexing). Flower stems were used immediately after harvest. The explants were surface-sterilized in 70% alcohol for a few seconds, followed by 20 min in 1% NaOCl. Explants were then washed three times with sterile water. Before inoculation the buds were excised, leaving a few mm of stem and petiole tissue attached.

The basic culture medium consisted of MS salts with 37.5 mg l^{-1} NaFeEDTA. Unless stated otherwise, 45 g l^{-1} glucose, 0.1 mg l^{-1} BA and 5 g l^{-1} agar (MC 29; Lab M, U.K.) were used. Preliminary results showed that, in contrast to 'Sweet Promise', 'Motrea' required the addition of MS vitamins and glycine. In some experiments different commercially available agars were used, i.e. Daichin (Brunschwig Chemie, The Netherlands), Difco Bacto (Difco, U.S.A.), MC 29 (Lab M, U.K.), BD (Becton Dickinson, U.S.A.) grade A, BD granulated, and BD purified. The pH was adjusted to 5.8. Culture tubes were filled with 15 ml medium, closed with a cotton plug, and autoclaved for 20 min. Tubes with an explant were sealed with Vitafilm (Good Year) and incubated in a culture room at 23 °C with a 16h photoperiod (5-7 W m^{-2}, Philips TL 54).

Routinely, the effects of the treatments on growth and development of the axillary buds into shoots were evaluated after 4 weeks by determining length, weight and number of compound leaves of the main shoot, and number of lateral shoots. The experiment on cytokinin type was evaluated after 5 weeks. For calculation of the means only sprouted buds were taken into account.

The effects of medium components and explant size were investigated in one or two replicate experiments. Per treatment 12, 18, or 24 replicate explants were used. When one experiment was performed the mean and the standard error of the mean (SE) were calculated per treatment. In the case of two experiments analysis of variance was applied and the

significance of differences was determined by Student's *t*-test (P=0.05).

Results

Morphology

In general, at least 90% of the axillary buds inoculated *in vitro* sprouted (stage I). On most media, leaves and internodes which were already in the bud, unfolded (stage II). When cultured under optimal conditions, additional leaves and internodes and a flower were formed (stage III). Comparative studies revealed that this developmental process under optimal conditions *in vitro* is very similar to that *in vivo*, resulting in a miniature version of the *in vivo* plant. The number of leaves preceding the flower and the leaf form (number of leaflets per leaf) of *in vitro* grown shoots were similar to those of *in vivo* grown shoots.

Medium components

Plant growth regulators: For both cultivars the presence of a cytokinin was necessary for prolonged shoot growth of axillary buds; without cytokinin, axillary buds did sprout, but did not develop further than stage II, since little shoot elongation occurred and only a few leaves were formed (Table 1). A concentration of 0.1 mg l^{-1} BA (0.44 µM) resulted in elongated shoots with a flower bud, whereas higher concentrations induced a cluster of shoots. At high concentrations weight and length of the main shoot decreased (Table 1). Comparing equimolar concentrations (0.44 µM) of the cytokinins BA, zeatin, zeatin riboside, 2iP and IPA showed that all cytokinins, except kinetin, stimulated the growth of axillary buds into shoots of 'Sweet Promise' (Fig. 1). BA, zeatin and zeatin riboside resulted in the longest shoots with the highest weight (Fig. 1). Increasing the concentration of kinetin to 2 mg l^{-1} had no effect on weight, length and number of leaves of 'Motrea' (data not shown).

In preliminary experiments NAA, IBA and GA_3 were shown to have no positive effects on shoot growth (data not shown).

Agar: Six different commercially available agars at a concentration of 5 g l^{-1} were tested (Fig. 2). Both cultivars appeared to be sensitive to the agar brand. In several experiments the agars Daichin (agar 1), MC 29 (agar 3), and BD purified (agar 6) gave the best results. In Fig. 2 a representative experiment is shown. Since MC 29 showed least symptoms

TABLE 1. Effect of BA concentration on axillary shoot development from single node explants of rose 'Sweet Promise'. Values are the mean of 48 plants.

BA conc. (mg l^{-1})	No. of leaves	Length (cm)	Weight main shoot (g)	No. of laterals	Total weight (g)
0	6.0	1.0	0.24	0.1	0.24
0.01	6.9	1.2	0.23	0.1	0.23
0.05	10.1	2.3	0.25	0.5	0.25
0.1	11.3	3.4	0.30	1.2	0.34
0.2	11.6	3.0	0.28	2.3	0.37
0.5	11.5	1.7	0.24	2.7	0.46
LSD (P=0.05)	0.9	0.6	0.06	0.9	0.08

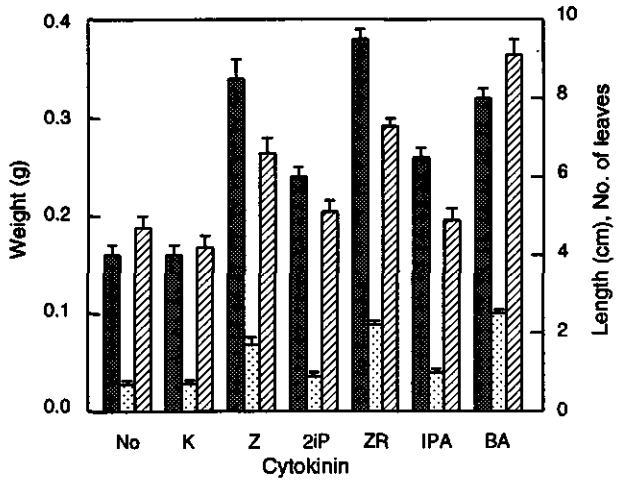


FIG. 1. Effect of cytokinin type (0.44 µM) on number of leaves ( ), length ( ) and weight ( ) of axillary shoots from single node explants of rose 'Sweet Promise'. Values are the mean of 18 explants. Bars indicate SE.
No: no cytokinin added, K: kinetin, Z: zeatin, ZR: zeatin riboside.

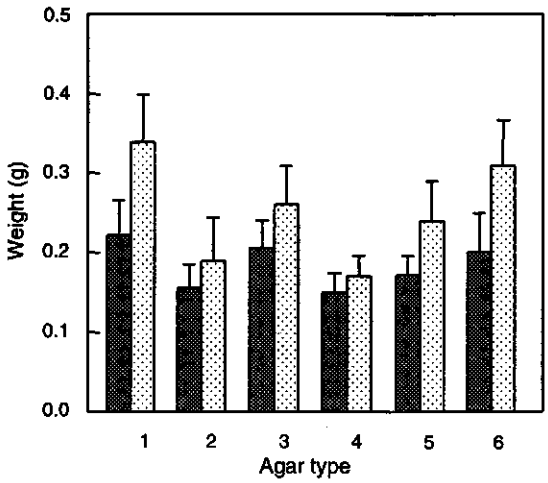


FIG. 2. Effect of the agar brand on weight of axillary shoots from single node explants of rose 'Motrea' ( ) and 'Sweet Promise' ( ) . Values are the mean of 12 plants. Bars indicate SE.
Agar 1: Daichin; agar 2: Difco Bacto; agar 3: MC 29; agar 4: BD grade A; agar 5: BD granulated; agar 6: BD purified.

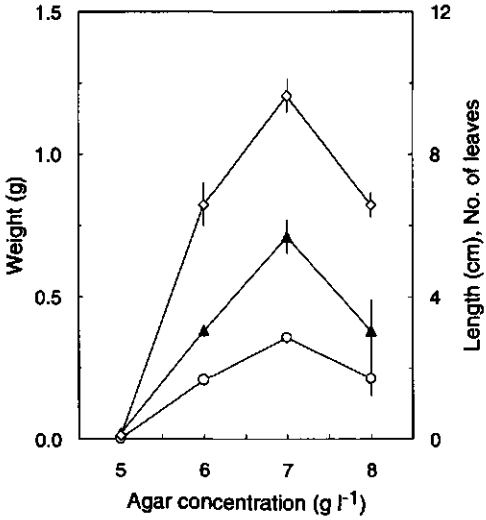


FIG. 3. Effect of agar (MC 29) concentration on weight (▲), length (○) and number of leaves (◇) of axillary shoots from single node explants of rose 'Motrea'. Values are the mean of 12 plants. Bars indicate SE.

of necrosis of the apex, this agar was routinely used.

With respect to the optimal agar concentration large differences between the cultivars were observed. 'Motrea' preferred high concentrations of agar. At 7 g l^{-1} completely developed shoots were formed (Fig. 3). 'Sweet Promise' on the other hand showed the best results with extremely low concentrations (4 g l^{-1}) or when grown on small rockwool plugs with liquid medium (data not shown).

Sugar: For both cultivars glucose gave better growth than sucrose (Table 2). For 'Motrea', development of the buds was more enhanced with glucose in the medium. The time of addition of 45 g l^{-1} sucrose, before or after autoclaving the medium, did not make any difference. Chemical analysis with HPLC showed, that after autoclaving only 3% of the sucrose was decomposed while glucose could be recovered completely. With respect to the concentration of glucose, length and weight of the main shoot were lower at 35 g l^{-1} than at 45 or 55 g l^{-1} (Table 3). No effect on the developmental stage was found, but the number of laterals decreased with increasing glucose concentration.

Explant factors

For 'Sweet Promise' the effect of stem length (when petiole length constant) and the effect of petiole length (when stem length constant) on shoot growth were studied. Shoot growth increased with increasing stem tissue, but no effect on developmental stage was found (Table 4). Absence of the petiole induced

TABLE 2. Effect of sugar type at 45 g l^{-1} on axillary shoot development from single node explants of rose 'Sweet Promise' and 'Motrea'. Values are the mean of 48 plants.

Cultivar	Sugar	No. of leaves	Length (cm)	No. of laterals	Total weight (g)
Sweet Promise	Sucrose	10.3	2.5	1.6	0.25
	Glucose	10.8	3.4	1.6	0.33
LSD (*P*=0.05)		1.3	0.4	0.7	0.03
Motrea	Sucrose	8.9	0.5	0	0.10
	Glucose	14.0	1.9	0	0.27
LSD (*P*=0.05)		1.9	0.6	--	0.08

TABLE 3. Effect of glucose concentration on axillary shoot development from single node explants of rose 'Sweet Promise'. Values are the mean of 48 plants.

Concentration ($g\ l^{-1}$)	No. of leaves	Length (cm)	Weight main shoot (g)	No. of laterals	Total weight (g)
35	11.4	2.1	0.26	1.6	0.32
45	11.3	3.3	0.31	0.8	0.33
55	10.7	3.7	0.31	0.3	0.32
LSD (P=0.05)	1.6	0.9	0.03	1.0	0.04

growth of lateral shoots, whereas the main shoot remained short (Fig. 4). The pattern of the effect of BA concentration on explants was not influenced by the presence of the petiole, but the optimum concentration was lower in its absence (0.05 mg l^{-1} instead of standard 0.1 mg l^{-1}). An increase in petiole length resulted in a slight increase in both shoot length and shoot weight, while the numbers of leaves and laterals were not substantially affected (Fig. 4).

Discussion

No specific medium composition was required for bud break *in vitro*, since no large differences in sprouting were recorded between treatments. Axillary buds are not dormant but correlatively inhibited by upper plant parts (Zieslin & Halevy 1976). As soon as the inhibition is released *in vivo*, by pruning the stem part above the bud, the bud will sprout and develop into a shoot. The medium requirements for unfolding of preformed stem parts *in vitro* also appeared not very specific. As the sprouting bud is a strong sink for assimilates (Mor & Halevy 1979), carbohydrates were supplied to the bud by addition of sugar to the medium. The effect of different sugars on growth of rose *in vitro* has not been reported before. Commonly sucrose is supplied, only Ghashghaie *et al.* (1992) used glucose. The present study shows that glucose induces more vigorous growth and especially for 'Motrea' use of glucose should be strongly advised.

TABLE 4. Effect of explant size and position of the bud, on axillary shoot development from single node explants of rose 'Sweet Promise'. Values are the mean of 48 plants.

Explant	No. of leaves	Length (cm)	No. of laterals	Total weight (g)
Axillary bud	8.8	1.7	0.9	0.22
Stem slice of 0.5 cm	8.3	2.0	0.6	0.26
Stem slice of 1 cm, bud 'proximal'	8.8	2.7	0.6	0.40
Stem slice of 1 cm, bud 'distal'	8.7	2.3	0.3	0.39
Stem slice of 2 cm, bud 'middle'	8.4	2.5	0.7	0.44
LSD (P=0.05)	0.7	0.4	0.6	0.02

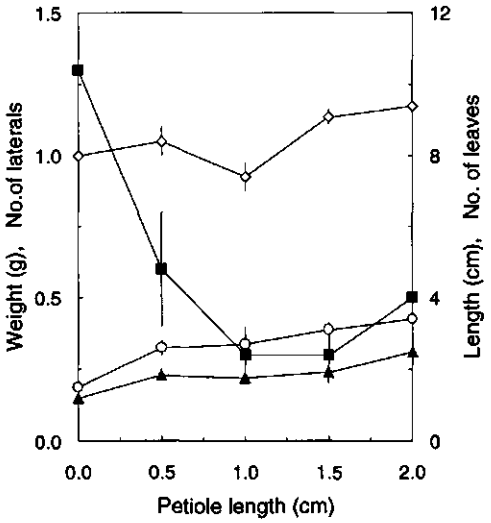


FIG. 4. Effect of petiole length on weight (▲), number of laterals (■), length (○) and number of leaves (◇) of axillary shoots from single node explants of rose 'Sweet Promise'. Values are the mean of 24 plants. Bars indicate SE.

In vivo, the apical meristem completes its developmental programme, after release from correlative inhibition, by forming several additional leaves and a terminal flower bud. *In vitro*, the composition of the medium determines which developmental stage is reached. Addition of cytokinin to the culture medium appeared necessary for the bud to complete its developmental programme isolated from the intact plant, whereas auxin and gibberellin were not a prerequisite. Accordingly, axillary buds of pea did not form new leaves and grew only slightly on a medium without cytokinin (Gould *et al.* 1987). Excised floral buds are also reported to require cytokinin for the growth and development of floral organs (Rastogi & Sawhney 1989). *In vivo*, cytokinin will primarily be supplied by the roots, that are absent *in vitro*. Of the several cytokinins tested, BA appeared most suitable, although it should be noted that the optimum concentration might be dependent on the cytokinin used. Kinetin did not stimulate growth of axillary buds into shoots, which is in accordance with results of Hasegawa (1979) on multiple shoot formation in rose. The BA concentration applied in the multiple shoot system is supra optimal for the single shoot system, since also the buds, which are already present in the axils of the bud scales (Chapter 2.1), were released from inhibition resulting in a cluster of shoots. Outgrowth of the buds was accompanied by a smaller main shoot (Table 1), indicating that the main shoot experienced competition of the axillary shoots.

The agar brand may affect the growth and development of *in vitro* plants (Debergh 1983). Rose also appeared to be very sensitive to agar brand. Several explanations have been given for the agar effect on *in vitro* cultures, e.g. availability of water or nutrients (Debergh 1983; Bornman & Vogelmann 1984; Scherer *et al.* 1988; Ghashghaie *et al.* 1991). In case of rose the nutrient availability may be a likely explanation. For, a reduced nutrient supply at higher agar concentration agrees very well with the observation that 'Sweet Promise', in contrast to 'Motrea', is not able to grow at lower nutrient concentration than the standard MS medium (Scholten, unpublished results). Moreover, addition of liquid medium to the agar medium after three weeks of culture (Maene & Debergh 1985) had a positive effect on growth of 'Sweet Promise'.

The effect of explant size on growth of the axillary bud might be nutritional and/or hormonal. The data on the effect of stem tissue attached to the excised axillary bud (Table 4) suggest a nutritional effect of the stem tissue. In contrast to intact plants, in which the stem tissue above the axillary bud is known to inhibit growth of the bud (Zieslin & Halevy 1976), in excised buds *in vitro* the stem tissue,

even when located above the bud, stimulated growth of the bud. The effect of the petiole might also be hormonal, since absence of the petiole induced release of the buds in the axils of the bud scales of the inoculated bud.

The single shoot system is suitable for physiological studies on axillary bud development. Furthermore, it offers possibilities for micropropagation by single-node culture. In this way carry-over effects, after transfer to *in vivo* conditions, due to the high cytokinin concentration in the culture medium, may be reduced.

4. Development and growth potential of axillary buds as affected before release from correlative inhibition

4.1. Effect of bud age on development and growth potential of axillary buds

Marcelis-van Acker C.A.M., 1994. Development and growth potential of axillary buds in roses as affected by bud age. Annals of Botany (in press).

Abstract. The effect of axillary bud age on the development and potential for growth of the bud into a shoot was studied in roses. Age of the buds occupying a similar position on the plant varied from 'subtending leaf just unfolded' up to one year later. With increasing age of the axillary bud its dry mass, dry-matter percentage and number of leaves, including leaf primordia, increased. The apical meristem of the axillary bud remained vegetative as long as subjected to apical dominance, even for one year.
The potential for growth of buds was studied either by pruning the parent shoot above the bud, by grafting the bud or by culturing the bud *in vitro*. When the correlative inhibition (i.e. domination of the apical region over the axillary buds) was released, additional leaves and eventually a flower formed. The number of additional leaves decreased with increasing bud age and became more or less constant for axillary buds of shoots beyond the harvestable stage, while the total number of leaves preceding the flower increased. An increase in bud age was reflected in a greater number of scales, including transitional leaves, and in a greater number of non-elongated internodes of the subsequent shoot. Time until bud break slightly decreased with increasing bud age; it was long, relatively, for one year old buds, when they sprouted attached to the parent shoot. Shoot length, mass and leaf area were not clearly affected by the age of the bud that developed into the shoot. With increasing bud age the number of pith cells in the subsequent shoot increased, indicating a greater potential diameter of the shoot. However, final diameter was dependent on the assimilate supply after bud break. Axillary buds obviously need a certain developmental stage to be able to break. When released from correlative inhibition at an earlier stage, increased leaf initiation occurs before bud break.

Introduction

In rose plants axillary buds are the source of flowers. Axillary buds are correlatively inhibited by the apical portions of the shoot. After harvesting a flowering shoot, the most distal axillary buds sprout and develop into the next generation of flowering shoots. In commercial practice, a wide variation in rapidity of bud sprouting and growth vigour can be observed.

Several factors are responsible for the variation, both environmental and correlative, e.g. mature leaves and stem tissue above the bud, (Zieslin & Halevy 1976; Zieslin *et al.* 1976a) and factors intrinsic to the axillary buds (Zamski *et al.* 1985). In *Citrus*, Halim *et al.* (1988) showed that the variation in bud burst was intrinsic to the bud.

The flowering shoots of roses can be cut "upward", i.e. above 1 or 2 five-leaflet leaves of the flowering shoot, or "downward", i.e. below the place of insertion of the flowering shoot on its parent shoot (Zieslin 1981). The downward method was found to result in production of fewer flowers than the upward method. This may be caused by the lower position or greater age of the buds that develop into flowering shoots or by the age of the parent stem (Zieslin 1981). In the older stem tissues an accumulation of inhibitory factors may occur (Zieslin *et al.* 1978). Moreover, the amount of stored carbohydrates may change (Glerum 1980; Kozlowski 1992).

So far effects of bud age on axillary bud development in rose have only been investigated for relatively young stages, i.e. before the flower bud of the parent shoot became visible, when the flower was enclosed in the sepals and when the flower was open (Cockshull & Horridge 1977; Zamski *et al.* 1985). No information is available on the development and growth potential of axillary buds, which are subjected to apical dominance for a long period and give the new flowers when the downward method of harvesting is applied.

In the present study the effect of age on axillary bud development has been investigated. At various stages, varying from 'subtending leaf just unfolded' up to one year later, development and potential for growth of axillary buds from a similar position on the plant were studied. The number of leaves and leaf primordia in the buds was assessed at the moment of release from inhibition. Shoot growth of an axillary bud after release from inhibition, is defined as the bud growth potential and was investigated with the bud attached to the parent plant, by pruning the plant above the axillary bud, as well as in isolation, by culturing the axillary bud *in vitro* (young stages) or by grafting the axillary bud (old stages).

Materials and Methods

For all experiments plants of *Rosa hybrida* cv. Sweet Promise were raised from double node cuttings, as described by Marcelis-van Acker & Leutscher (1993). By using double node cuttings uniform and vigorous plants were obtained. One shoot, the so-called primary shoot, was allowed to develop on each cutting. In five experiments (Exp. 1-5) various stages of relatively young buds of the primary shoot, varying from 'subtending leaf just unfolded' until 'parent shoot harvestable (sepals reflexing)', were studied. In one further experiment (Exp. 6) stages of old buds, up to one year beyond 'parent shoot harvestable', were studied. In this experiment buds of a basal shoot were used for study.

Experiment 1. On five occasions during the development of the primary (parent) shoot, fresh and dry mass of the bud in the axil of the middle five-leaflet leaf were determined (15 replicate plants per developmental stage).
Experiment 2. Four times during the development of the primary (parent) shoot the number of leaves and leaf primordia in the axillary bud of the third five-leaflet leaf

(counted from the base of the shoot) was counted under a dissecting microscope (x 50; 4 replicate plants). At each stage 12 plants were pruned just above the third leaf with at least five leaflets. The 4 treatments (developmental stages) were arranged in a randomized block design with 4 blocks. Three times a week all sprouting buds, except for the uppermost one, were removed. When the uppermost bud had grown into a harvestable shoot (sepals reflexing), length, diameter (at 1 cm from the base of the shoot), fresh mass, number of leaves and leaf area were determined.

Experiment 3. This experiment was similar to Exp. 2, except for bud position: the bud in the axil of the fourth five-leaflet leaf was studied. Five treatments (developmental stages) were arranged in a randomized block design with 4 blocks. Thin transverse hand cut sections were made 1 cm from the base of shoots. On each transverse section the diameter of the pith was measured microscopically using an ocular micrometer and the number of pith cells crossed by on three diameter lines of the shoot was recorded. The pith was considered as a parameter for primary growth.

Experiment 4. On four occasions during the development of the primary (parent) shoot the bud in the axil of the third five-leaflet leaf was cultured *in vitro* (16 replicate plants per developmental stage) on a medium containing Murashige & Skoog (1962) salts (except Fe) at full strength, 37.5 mg l^{-1} NaFeEDTA, 45 g l^{-1} glucose, 0.1 mg l^{-1} benzyladenine and 5 g l^{-1} MC 29 agar (Lab M, U.K.). Cultures were grown at 23° C (day and night), a day length of 16 h and 23-32 µmol m^{-2} s^{-1} PAR provided by Philips fluorescent tubes (TL 54/36W). After 45 days shoot length, fresh mass and number of compound leaves were recorded.

Experiment 5. The development of very young axillary buds after release from correlative inhibition was studied. Primary shoots were pruned above the third five-leaflet leaf as soon as this leaf unfolded. Four plants were selected at random each day and length and number of leaves and leaf primordia of the axillary bud in the axil of this third five-leaflet leaf were recorded during a ten-day period.

Experiment 6. In order to promote basal shoot formation the primary shoot was bent horizontally seven weeks after the buds on the cuttings sprouted. One basal shoot per plant was allowed to grow. Date of appearance of the basal shoots was recorded. Eight treatments (bud ages) were arranged in a randomized block design with 9 blocks, according to the date of appearance of the basal shoot. The buds in the axils of the third up to sixth five-leaflet leaf (counted from the base of the shoot) of the basal shoot were selected for study. To prevent sprouting of the selected axillary buds, the basal shoot in the harvestable stage (sepals reflexing) was pruned at the uppermost five-leaflet leaf. Age of the selected axillary buds was expressed in weeks after sprouting of the basal shoot (parent shoot): 5, 8, 11, 15, 20, 30, 43 and 62 weeks. The age '5 weeks' corresponded with the harvestable stage of the parent shoot. At each of the eight bud ages, nine plants were pruned at the third five-leaflet leaf. The bud in the axil of this leaf was allowed to grow, while lower positioned sprouting buds were frequently removed. The buds from the axil of the fourth and fifth five-leaflet leaf were grafted in between the two leaves of double node cuttings. The buds were grafted by means of an inverted T-incision and tied with tape (Ribon; Mauritz, Bussum, The Netherlands). The cuttings were dipped into talcum powder with 0.4% indole butyric acid to promote rooting.

When rooted, the budding tape was removed and the cuttings were pruned just above the grafted bud. The bud in the axil of the sixth five-leaflet leaf was used to determine the number of leaves and leaf primordia using a dissecting microscope (x 50). As soon as the selected buds (buds from third, fourth and fifth five-leaflet leaves of basal shoot) had grown into shoots with the flower at the 'sepals reflexing' stage, they were harvested. Length, diameter (at 1 cm from the base of the shoot), fresh mass, number of leaves and leaf area were recorded. Thin transverse hand cut sections were made 1 cm from the base of the shoot. Diameter of the pith and number of pith cells were determined as described for Exp. 3.

Plants were grown in a climate chamber at a temperature of 21°C (day and night), a relative air humidity of approximately 70% and at 200±20 μmol m^{-2} s^{-1} photosynthetically active radiation provided by high pressure sodium lamps (SON/T) and metal halide lamps (HPI/T) of 250W and 400W (Exp. 1,2 and 5) or at 115±10 μmol m^{-2} s^{-1} photosynthetically active radiation (Philips fluorescent tubes, TLD 84 HF/50W; Exp. 4 and 6). Plants of Exp. 3 were grown in a heated greenhouse (day/night temperature set at 20/17°C).

Data were analysed with Genstat 5 by one-way (Exp. 4) or two-way (Exp. 2, 3 and 6) analysis of variance. The significance of differences was determined by Student's *t*-test (P=0.05).

Results

Axillary buds are correlatively inhibited, i.e. they do not sprout due to apical dominance. When the inhibition of the buds is released, by pruning the shoot above the buds, they will sprout and grow into a shoot. The development and potential for growth of axillary buds during growth of the shoot bearing the buds, the so-called parent shoot, was studied. Although sprouting of the axillary buds was inhibited by apical dominance during growth of the parent shoot, dry mass and dry-matter percentage of the buds increased with age (Fig. 1). The rate of increase slowed with age.

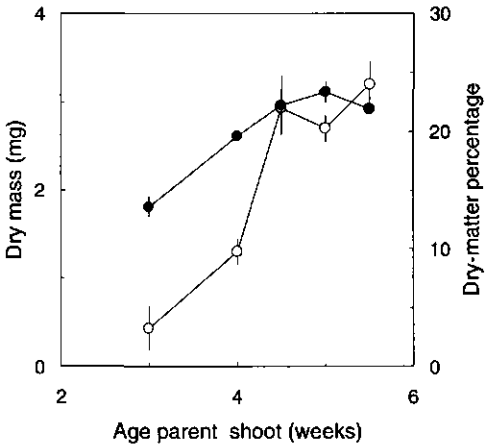


FIG. 1. Effect of axillary bud age on dry mass (○) and percentage dry-matter (●) of buds of rose 'Sweet Promise' (Exp. 1). Vertical bars indicate standard error of mean, when larger than symbols.

Several times during the development of the parent shoot, up to one year after the parent shoot was harvestable, the number of leaves and leaf primordia in the axillary bud of the third five-leaflet leaf (counted from the base of the shoot) was determined. Total number of leaves, including leaf primordia, in the axillary bud increased with bud age (Fig. 2). The increase was most pronounced for young buds, up to the harvestable stage of the parent shoot (Exp. 2), later on the increase was only slight (Exp. 6). No floral primordia were detected, even in the one year old buds.

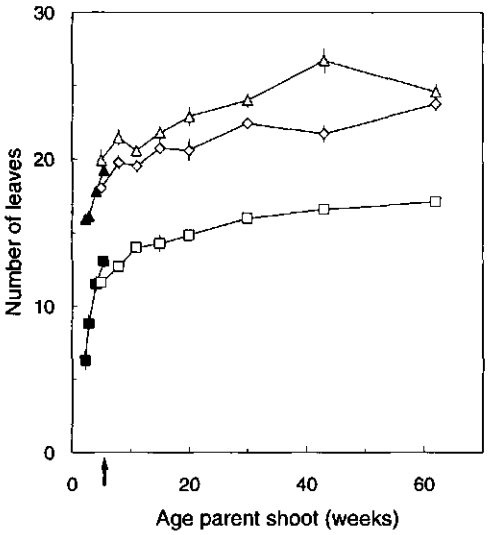


FIG. 2. Effect of axillary bud age on number of leaves including leaf primordia of the bud (■, □) and total number of leaves preceding the flower of the subsequent shoot (◇, △) of rose 'Sweet Promise'. Axillary buds sprouted when grafted (◇) or attached to the parent shoot (▲, △). The difference between the number of leaves in the axillary bud and that preceding the flower, represents the number of additional leaves formed after release from apical dominance by pruning the stem above. Arrow indicates the harvestable stage (sepals reflexing) of the parent shoot. Vertical bars indicate standard error of mean, when larger than symbols.
Closed symbols: young stages of axillary bud (Exp. 2).
Open symbols: old stages of axillary bud (Exp. 6).

When the correlative inhibition of the bud was released by pruning the parent shoot just above the bud, additional leaves and eventually a flower bud were formed. Buds sprouted either attached to the parent plant or in isolation after being grafted on a cutting. Buds attached to the parent plant and grafted buds behaved similarly: The number of additional leaves, formed after release from correlative inhibition, decreased with increasing bud age and became more or less constant for bud ages beyond the harvestable stage of the parent shoot, while the total number of leaves preceding the flower increased (Fig. 2).

According to Zamski *et al.* (1985), three groups of leaves may be distinguished along a shoot: scales and transitional leaves below the lowermost five-leaflet leaf, five-leaflet leaves (i.e. leaves with at least five leaflets), and upper leaves above the uppermost five-leaflet leaf. For young buds the number of scales, including transitional leaves, and five-leaflet leaves of the subsequent shoot increased with increasing bud age (Fig. 3A). For old buds the number of five-leaflet leaves and the number of upper leaves remained fairly constant, whereas the number of scales including transitional leaves increased with increasing bud age

TABLE 1. Effect of bud age on subsequent shoot growth characteristics of rose 'Sweet Promise'. At several developmental stages during the growth of the parent shoot (PS), the axillary bud was released from inhibition by pruning the parent shoot above the bud. (Exp. 2).

Stage PS	Time from pruning		Shoot length (cm)	Shoot mass (g)	Leaf area (cm^2)	Shoot diameter (mm)
	until bud break (d)	until harvest (d)				
SL* unfolded	5.5	38.1	56.9	28.1	821	5.4
2nd leaf above SL unfolded	4.8	37.3	56.2	27.5	853	5.2
Flower bud pea-sized (6-8 mm)	3.3	37.1	58.6	28.0	867	5.7
Sepals reflexing	1.5	37.5	54.0	25.0	770	5.7
LSD (P=0.05)	1.3	1.9	5.1	3.6	109	0.4

* SL=subtending leaf.

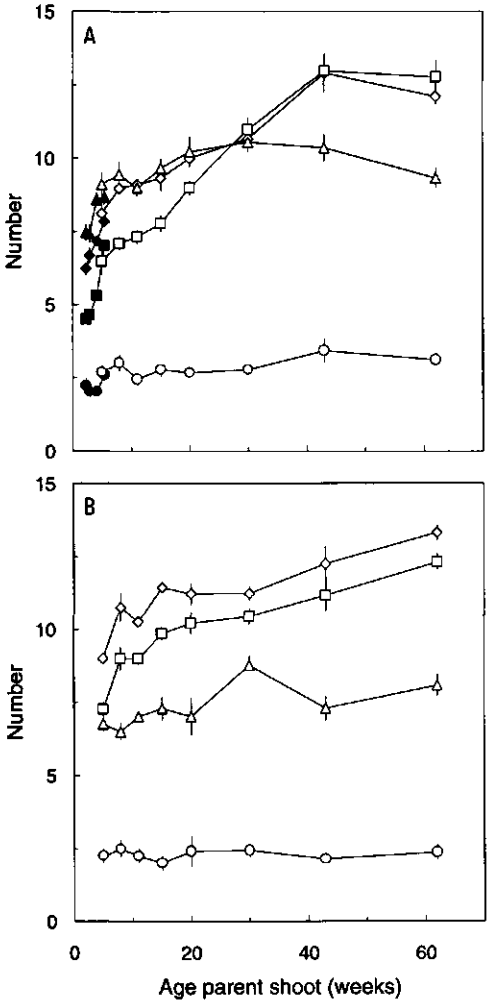


FIG. 3. Effect of axillary bud age on number of scales including transitional leaves (◆◇), number of five-leaflet leaves (▲, △), number of upper leaves (●, ○) and number of non-elongated internodes (■, □) of the subsequent shoot of rose 'Sweet Promise'. Vertical bars indicate standard error of mean, when larger than symbols.
A. Shoots were grown from buds attached to the parent shoot (Exp. 2 and 6);
B. Shoots were grown from buds after being grafted on a cutting (Exp. 6).
Closed symbols: young stages of axillary bud (Exp. 2);
Open symbols: old stages of axillary bud (Exp. 6).

(Figs. 3A,B); there was no essential difference in this respect between shoots formed by grafted buds and shoots formed by buds attached to the parent shoot. The increase in number of scales was accompanied by an increase in number of non-elongated internodes (Figs. 3A,B). Shoots formed by grafted buds had slightly more scales, including transitional leaves, but fewer five-leaflet leaves than shoots formed by buds attached to the parent shoot.

The effect of age of the axillary bud on subsequent shoot growth was small. For young buds (up to the stage 'parent shoot harvestable', Exp. 2) final shoot length, shoot mass and leaf area were not clearly influenced by the age of the axillary bud, but shoot diameter slightly increased (Table 1). Time until bud break decreased with increasing bud age, whereas the total growth period was not significantly affected. In a comparable experiment carried out in the greenhouse (Exp. 3) similar results were obtained (not shown). Pith diameter and number of pith cells on a line across the diameter of the shoot were not obviously affected by the age of the axillary bud, while the diameter of the shoot slightly increased with increasing bud age (Table 2). When the potential for growth of buds was studied by culturing the buds *in vitro* (Exp. 4), the youngest buds (subtending leaf just unfolded) showed a very poor growth; no significant differences were found between the older stages (Table 3).

Axillary buds, which were released from inhibition at a very young stage (subtending leaf just unfolded), needed more time to break and formed more additional leaves prior to the formation of the flower bud than axillary buds released from inhibition on a harvestable shoot. When buds were released from inhibition at the time the subtending leaf had just

TABLE 2. Effect of bud age on diameters of shoot and pith and on number of pith cells of the subsequent shoot of rose 'Sweet Promise'. At several developmental stages during the growth of the parent shoot (PS), the axillary bud was released from inhibition by pruning the parent shoot above the bud. The number of pith cells was determined in transverse sections of the shoot by counting the number of cells passed traversing the diameter. (Exp. 3)

Stage PS	Shoot diameter (mm)	Pith diameter (mm)	Pith no. of cells
SL* unfolded	4.9	2.9	29.4
Flower bud rice-sized (3-5 mm)	4.8	2.8	28.9
Flower bud pea-sized (6-8 mm)	5.3	3.1	30.4
Sepals reflexing	5.2	2.9	29.3
Flower open	5.2	2.9	29.5
LSD (*P*=0.05)	0.3	0.14	1.5

* SL=subtending leaf.

unfolded, its number of leaf primordia immediately increased, whereas the length of the bud increased markedly only four days later (Fig. 4). The rate of leaf formation was greater than for buds which remained under correlative inhibition (1.2 day^{-1} and 0.4 day^{-1} respectively).

When old buds (beyond the stage 'parent shoot harvestable', Exp. 6) were forced to grow into a shoot by pruning the stem above the bud, shoot mass and leaf area of the subsequent shoot were not clearly affected by bud age; shoot length seemed to be slightly lower for buds older than 11 weeks, whereas diameters of shoot and pith and number of pith cells increased with increasing bud age (Table 4). The time until bud break slightly decreased with increasing bud age, except for week 62, where a long time was required. The total growth period was not obviously affected by the age of the axillary bud (Table 4). When buds were forced to grow into a shoot after being grafted onto a cutting, time until bud break, shoot length, mass, leaf area and growth period were not clearly affected by the bud age (Table 4). Shoot and pith diameter slightly increased and number of pith cells significantly increased with increasing bud

TABLE 3. Effect of bud age on growth of buds of rose 'Sweet Promise' into shoots *in vitro*. At several developmental stages during the growth of the parent shoot (PS), the axillary bud was excised and cultured *in vitro*. (Exp. 4)

Stage PS	Shoot length (cm)	Shoot mass (g)	No. of compound leaves
SL* unfolded	0.6	0.10	10.9
2nd leaf above SL unfolded	2.2	0.24	12.0
Flower bud pea-sized (6-8 mm)	2.4	0.22	13.0
Sepals reflexing	2.6	0.27	12.8
LSD (*P*=0.05)	0.8	0.07	1.1

* SL=subtending leaf.

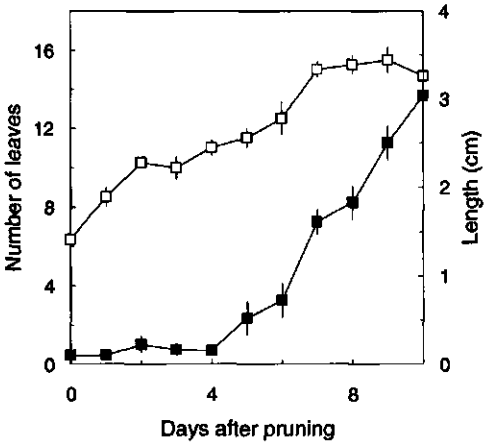


FIG. 4. Length (■) and number of leaves including leaf primordia (□) of axillary buds of rose 'Sweet Promise' after release from inhibition by pruning the stem above. Inhibition was released when the subtending leaf was just unfolded (Exp. 5). Vertical bars indicate standard error of mean, when larger than symbols.

age. Buds grafted onto a cutting formed smaller shoots with a lower number of pith cells than buds attached to the parent shoot, although the growth period was similar (Table 4).

Discussion

Horridge & Cockshull (1974) proposed the hypothesis that axillary apical meristems in rose are not competent to respond to flower initiation as long as they are subjected to apical dominance. This hypothesis is supported by the present study, which showed that the apical meristem of the axillary bud remained vegetative while subject to apical dominance, even during a period of one year. During this inhibition the buds were active in forming new leaf primordia (Fig. 2), which is in accordance with results of Young *et al.* (1974) on pear. Also Hillman (1984) reported that correlatively inhibited buds do show growth, although barely perceptible, and for that reason the term "dormancy" is not usually applied to buds held under correlative inhibition. The increase in number of leaf primordia in the bud was mainly reflected in an increase in the number of scales on the subsequent shoot (Fig. 3), indicating that leaf primordia became scales, in agreement with Cockshull & Horridge (1977). With increasing age of the bud, the number of scales and the number of non-elongated internodes of the subsequent shoot increased (Fig. 3). Zimmermann & Brown (1971) also reported that in trees of the temperate zone the internodes between bud scales seldom elongate to any appreciate extent. A relation between (early) preformed leaves and internodal elongation was also found in *Larix*, *Populus* and *Ginkgo* (Steeves & Sussex 1989). Since bud age affects the number of non-elongated internodes, it will also affect the number of basal buds, which may form basal shoots when the bud is used for propagation by cutting or grafting (Chapter 2.2).

Concerning buds of shoots younger than the harvestable stage, the number of additional leaf primordia formed after release from inhibition, decreased with bud age (Fig. 2), which corroborates results of Cockshull & Horridge (1977) on rose and results of Singer *et al.* (1992) on tobacco; however, for older buds the number of additional leaf primordia was more or less the same. Our data suggest that the degree of inhibition of an axillary bud determines number and form of the leaves of the subsequent shoot.

The total number of leaves preceding the flower increased with increasing bud age (Fig. 2), which is in accordance with results of Rylski & Halevy (1972) on pepper and Cockshull & Horridge (1977) on rose, al-

TABLE 4. Effect of bud age on subsequent shoot growth characteristics of rose 'Sweet Promise' after release from inhibition. At several developmental stages during the growth of the parent shoot (PS), the axillary bud was released from inhibition by pruning above the bud. Buds sprouted attached to the parent shoot (pruned) or grafted on a cutting (grafted). Age of the parent shoot is expressed in weeks after it started sprouting. (Exp. 6)

Age PS (weeks)	Time from pruning until bud break (d)	Time from pruning until harvest (d)	Shoot length (cm)	Shoot mass (g)	Leaf area (cm^2)	Shoot diameter (mm)	Pith diameter (mm)	Pith no. of cells
Pruned								
5	7.4	44.7	54.6	18.6	678	5.0	2.8	34.0
8	7.5	42.7	55.7	21.5	763	5.3	3.2	35.1
11	7.0	41.1	54.4	23.5	829	5.2	3.2	36.0
15	7.0	43.7	50.0	23.0	839	5.1	3.1	37.6
20	6.3	44.7	44.6	21.3	785	5.5	3.1	37.1
30	6.2	43.2	49.0	24.8	774	5.9	3.3	39.6
43	6.8	38.7	52.7	26.6	831	6.4	4.0	43.2
62	9.9	42.3	46.0	23.8	649	6.3	4.0	41.1
LSD (P=0.05)	2.2	3.4	6.4	4.1	136	0.5	0.4	3.2
Grafted								
5	6.5	38.5	36.3	10.3	387	4.0	2.4	28.7
8	5.2	43.5	28.8	8.6	278	3.7	2.2	31.2
11	6.3	42.4	31.8	10.2	366	3.7	2.2	30.8
15	3.4	39.7	34.8	11.4	387	4.4	2.9	32.4
20	7.5	42.0	29.6	9.7	335	3.8	2.3	32.1
30	4.6	40.9	38.3	14.2	464	4.2	2.5	35.7
43	3.5	41.1	34.3	12.2	364	4.2	2.5	36.2
62	4.9	39.3	34.1	12.7	374	4.6	2.7	37.7
LSD (P=0.05)	2.1	3.2	3.4	1.5	48	0.3	0.2	2.6

though these two studies concerned only young developmental stages. The slight difference in total number of leaves preceding the flower between grafted buds and buds attached to the parent plant (Fig. 2) can be explained by the position of the bud on the parent shoot, since grafted buds originated from a higher position.

The effect of bud age on time until bud break was small for buds of shoots beyond the harvestable stage (Table 4). Halim *et al.* (1988) also reported for *Citrus* that the age of the buds does not appear to be a major factor in determining the rate of bud bursting. The slow sprouting of the buds of 62 week old plants, when attached to the parent plant, might be caused by an accumulation of inhibitory factors in the plant as suggested by Zieslin *et al.* (1978).

An increase in bud age had a positive effect on (final) shoot diameter (Table 4). This held especially for buds attached to the parent shoot, and might be due to the increase in parent shoot diameter with increasing age, since the diameter of the daughter shoot was found to be related to the diameter of the parent shoot (Byrne & Doss 1981; Chapter 4.3). In shoots, grown from grafted buds as well as from buds attached to the parent shoot, the number of cells in the transverse section of the

pith increased with bud age (Table 4). Since the number of pith cells remains constant after bud break (Chapter 2.1) the increase in its number must have occurred in the bud stage indicating that buds, although quiescent, remain active when inhibited by upper plant parts. The difference between number of pith cells of shoots, grown from buds attached to the parent plant, and shoots, grown from grafted buds was small but consistent (Table 4) and might be explained by the difference in assimilate supply during bud sprouting. Assimilates for the grafted buds were from only one leaf (the cutting leaf), whereas the buds attached to the parent shoot could use the assimilates including stored reserves, of the parent plant.

When buds were isolated from the parent plant in a young stage (subtending leaf just unfolded), subsequent growth *in vitro* was poor (Table 3) compared to older ages; however, when such buds developed into a shoot when still attached to the parent shoot, no effect of bud age on shoot growth and number of pith cells was found, except that the young buds needed a longer time to break (Table 1). For older buds (beyond parent shoot harvestable) bud age appeared to affect the number of pith cells (Table 4). This may indicate that the young buds were not fully developed at the time of release from inhibition. It seems that buds in stages younger than 'parent shoot harvestable' had not reached a developmental stage enabling them to break. When released from inhibition by pruning the stem above, their development was accelerated, shown by increased leaf initiation (Fig. 4). When buds were released from inhibition when the subtending leaf has just been unfolded, it took only a few more days to initiate a total of 11 leaf primordia, whereas when buds remained under apical dominance on the parent shoot, it took two weeks (Figs. 4 versus 2). The developmental stage required for sprouting might be associated with a minimum number of leaf primordia and/or a minimum number of pith cells.

In conclusion, axillary buds continue growth, although at a low rate, during inhibition by upper plant parts, but they remain vegetative as long as they are inhibited from sprouting. Axillary buds need a certain developmental stage to be able to break. The potential diameter of a shoot is determined during axillary bud development, by means of the number of pith cells. However, the final diameter of a shoot is largely affected by the assimilate supply during shoot growth. Length, mass, leaf area and growth period of a shoot are not clearly affected by the age of the bud that develop into the shoot, but are largely dependent on the growth conditions during bud sprouting and shoot growth.

4.2. Effect of bud position on development and growth potential of axillary buds

Marcelis-van Acker C.A.M., 1994. Anatomy, morphology and growth potential of axillary buds in roses as affected by position along the shoot. (submitted).

Abstract. The effect of position along the shoot on anatomy and morphology and on growth potential of axillary buds (both *in situ* and *in vitro*) was studied. Weight of the buds increased and dry matter percentage decreased towards the stem apex. Buds in the axils of the uppermost three leaves of the shoot, commonly the leaves with less than five leaflets, often were generative and contained only a few leaves and a flower bud. Buds occupying a lower position along the shoot were in the vegetative stage. Considering the buds in the axils of the five-leaflet leaves, the number of leaves and leaf primordia in the bud increased towards the apex for cv. Motrea; for cv. Sweet Promise this number was not affected by bud position, except for the uppermost bud, in which it was reduced; for both cultivars the proportion of leaf primordia which were reduced to scales decreased towards the apex. Buds in the axils of the middle leaves of a shoot contained most pith cells and the highest sugar and starch content. The bud in the axil of the uppermost five-leaflet leaf appeared to be best vascularly connected to the stem.
Pruning position on the shoot affected the growth potential of the buds. Bud position, bud age and assimilate supply contribute to the effects of pruning position. Rate of bud break increased and total growth period decreased towards the apex. These effects were due to effects of bud position. The total number of leaves preceding the flower of the shoot grown from the bud decreased with increasing bud position. The number of non-elongated internodes also decreased towards the apex. The effects of pruning position on final size of the subsequent shoot were largely the result of differences in assimilate supply during shoot growth.

Introduction

The greenhouse rose is a perennial crop, where every shoot forms a terminal flower bud. The flower bud, however, may abort under unfavourable conditions. Flower production depends on the ability of sprouting of axillary buds and their growth into flowering shoots. Despite attempts to control flower production by harvesting procedures, growth regulators and by varying environmental factors (Zieslin 1981; Mor & Zieslin 1987; Van den Berg 1987) a large variation in shoot number, shoot size and degree of branching still remains. One of the sources of that variation was shown to be the pruning position on the shoot (Byrne & Doss 1981). The effects of pruning position may be due to differences in assimilate supply, bud age or bud position.

Assimilate supply during shoot growth was shown to affect growth rate, duration of growth and shoot size at harvest to a large extent (Chapter 4.3). Bud age affected the potential diameter of the subsequent shoot via an effect on the number of pith cells (Chapter 4.1).

In several crops effects of bud position have been studied in relation to propagation and significant effects of bud position on adventitious rooting and axillary bud break of

cuttings have been reported (Wang & Boogher 1988; Hansen 1989; De Vries & Dubois 1992). In pea (Gould *et al.* 1987) and in *Betula* (Marks & Myers 1992) developmental potential of explants *in vitro* varied with their previous position on the stock plant.

Outgrowth of axillary buds along a rose shoot is controlled by correlative inhibition. This inhibition can be exerted by the terminal bud as well as by the leaves and stem above the axillary bud and its subtending leaf, as was reported for rose by Zieslin & Halevy (1976) and for mulberry by Suzuki (1990). The degree of inhibition increased from top to base of a shoot (Zieslin *et al.* 1976a). In several plant species correlative inhibition is suggested to be due to lack of vascular connections between axillary buds and the main stem (Gregory & Veale 1957; Sorokin & Thimann 1964), although it has also been reported that vascular connections are not a prerequisite for bud break (Goodwin 1967; Peterson & Fletscher 1973; Richards & Larson 1981).

In rose 'Baccara', bud position was found to affect the developmental stage of the bud (Zamski *et al.* 1985). Zamski *et al.* (1985) distinguished three groups of leaves along the shoot: Lower leaves (i.e. scales and transitional leaves below the lowermost five-leaflet leaf), five-leaflet leaves (i.e. leaves with at least five leaflets), and upper leaves (i.e. leaves above the uppermost five-leaflet leaf). Based on their anatomical structure the axillary buds could be divided similarly into three groups. Buds in the axils of five-leaflet leaves are advanced in their development as compared with lower or upper positioned buds. Even within the group five-leaflet leaves a slight gradient of axillary bud growth could be shown (Zamski *et al.* 1985).

Diameter of a flower shoot is correlated with the diameter of the pith (Chapter 2.1). The pith tissue persists as primary tissue in all plants and keeps its size, shape and structure during the life-time of the plant (Eames & MacDaniels 1947). Its cells may be important for storage purposes (Glerum 1980). Size of the pith is dependent on both number and size of the cells. After bud break cell number (on cross section) of the pith does not change considerably (Chapter 2.1). Zamski *et al.* (1985) mentioned that slight differences in pith size could be observed between buds of different positions along the shoot.

In the present study we investigated the effect of position on bud morphology and anatomy in two rose cultivars, 'Motrea' and 'Sweet Promise'. Furthermore by pruning at various heights growth potential of the axillary buds was evaluated. In order to unravel the various factors contributing to the effect of pruning position, the study included experiments on *in situ* buds, i.e. on the intact plants, as well as on isolated buds, i.e. *in vitro* experiments.

Materials and Methods

Morphology and anatomy of axillary buds

Harvestable shoots (sepals reflexing) of *Rosa hybrida* cv. Motrea and cv. Sweet Promise having a similar number of five-leaflet leaves were selected. Shoots were taken from plants grown in a heated glasshouse (day/night temperature set at 20/17°C) or grown in a climate chamber at 21°C, a relative air humidity of approximately 70% and 16 h light, 40 W m^{-2} photosynthetically active radiation (PAR) provided by high pressure sodium lamps (SON/T) and metal halide lamps (HPI/T) of 250W and 400W. Shoots were formed at double node cuttings (Marcelis-van Acker & Leutscher 1993) or at mature plants.

Number of leaflets per leaf and leaf area of all leaves along the shoot were measured and the number of leaves and leaf primordia present in the buds in the axils of the five-leaflet leaves was recorded using a dissecting microscope (x50).

Fresh and dry weight of buds, which were carefully excised under a dissecting microscope, was determined. Dry weight was measured after drying for 3 d at 80°C.

Axillary buds were observed using a Jeol JSM-35C scanning electron microscope (SEM) at 15 kV. Fresh unfixed buds were used (Nell & Rasmussen 1979). Buds were studied either intact or after removal of their leaf primordia.

For the sake of pith measurements, longitudinal median hand cut sections were made of the buds in the axils of the five-leaflet leaves of shoots grown from double node cuttings. The number of pith cells on a diameter line at the base of the bud and at right angles to the length axis of the bud was recorded.

To study the vascular connections between bud and stem, axillary buds were excised including a part of the stem tissue and were cleared according to Herr (1971) and thereafter stored in a saturated chloral hydrate solution. Since the bark of the stem and the scales of the cleared bud contained crystals (probably calcium oxalate; Esau 1965), hampering the observation of the vascular connections between stem and bud, the bark was removed showing the vascular connections as white strings.

To determine total sugar and starch content, axillary buds of the middle and lowermost five-leaflet leaf of harvestable shoots (sepals reflexing) were excised and freeze-dried. Per position 3 samples of 5 buds each were used. The dried material was dissolved in alcohol. Total sugar content was analysed according to the Anthron method (Yem & Willis 1954) and starch content was measured according to the Nelson/Somoghi method (Nelson 1944).

Growth potential of axillary buds

Four experiments (Exp. 1 to 4) were undertaken to study the effect of bud position on growth potential *in situ*, i.e. attached to the parent shoot. In a further experiment (Exp. 5) the effect of bud position on growth potential was studied in isolated buds, i.e. *in vitro*. For Experiments 1 to 4 plant material consisted of double node cuttings (Marcelis-van Acker & Leutscher 1993), of *Rosa hybrida* cv. Sweet Promise (Exp. 1 and 3) and cv. Motrea (Exp. 2 and 4). Only one shoot (the primary shoot) was allowed to grow on each cutting. In Experiments 1 and 2, treatments were applied to this primary shoot, but in Experiments 3 and 4 (older plants) the lateral shoot emerging after pruning the primary shoot just above the fourth five-leaflet leaf was used. Treatments included pruning the shoots at three levels, i.e. just above the uppermost, the middle or the lowermost five-leaflet leaf when the flower was at stage 4 (Exp. 1 and 2) or at stage 2 (Exp. 3 and 4). The developmental stage of the flower was assessed according to the scale used by Halevy & Zieslin (1969), in which 2=flower bud pea-sized; 3=sepals closed and colour of the flower not yet visible; 4=sepals closed, but colour of the flower visible; 5=sepals reflexing. To achieve plants with the same number of leaves, in Experiment 2, in addition, immediately after applying the various pruning treatments all leaves except the uppermost one were removed. After pruning, only the bud in the axil of the uppermost leaf was allowed to grow into a shoot; other emerging lateral shoots were removed at appearance. Bud break (defined as 0.5 cm long

buds) was recorded. When the flower of the shoot had reached stage 5, the shoot was harvested and its length, diameter (at 1 cm from the shoot base), fresh weight, number of leaves and leaf area measured. In addition, in Experiment 3 and 4 thin transverse hand cut sections were made at 1 cm from the base of the shoots. Per transverse section the number of pith cells on a diameter line was recorded. This was repeated for three diameter lines per shoot.

Experiments 1 and 2 were carried out in a climate chamber at 21°C, a relative air humidity of approximately 70% and a day length of 16 h, 25 W m^{-2} PAR provided by fluorescent tubes (Philips TLD 84 HF/50W). In Experiment 1 the treatments were arranged in a randomized block design with 6 replicate plants arranged in two blocks. In Experiment 2 the treatments were arranged in a randomized block design with ten replicate plants arranged in ten blocks.

Experiments 3 and 4 were carried out at 17, 21 or 25°C, a relative air humidity of approximately 70% and a day length of 16 h, 40 W m^{-2} PAR provided by high pressure sodium lamps (SON/T) and metal halide lamps (HPI/T) of 250W and 400W. The treatments were applied after the plants were transferred to a chamber at 21°C. The treatments were arranged in a split-plot design with temperature at the whole-plot level and pruning position at the sub-plot level. There were 10 replicate plants per treatment. As the effects of pruning position on growth potential showed similar trends for all three temperatures (no significant interaction between temperature and pruning position at the 5% level), the results were averaged over the three temperatures.

In Experiment 5, buds in the axils of five-leaflet leaves of shoots cv. Sweet Promise (flower at stage 5) grown in a heated glasshouse were excised, surface-sterilized for 20 min in 1% NaOCl (v/v) with a few drops of Tween 20 and rinsed in sterile tap water. The buds were cultured *in vitro* on a medium containing Murashige & Skoog (1962) salts (except Fe) at full strength, 37.5 mg l^{-1} NaFeEDTA, 45 g l^{-1} glucose, 0.1 mg l^{-1} benzyladenine and 5 g l^{-1} MC29 agar (Lab M, U.K.). Cultures were grown at 23°C, at a day length of 16 h and 5-7 W m^{-2} PAR provided by fluorescent tubes (Philips TL 54/36W). Six weeks after inoculation, shoot length, number of leaves and fresh weight were determined. Treatments were arranged in a randomized block design with 24 replicate plants arranged in 4 blocks.

Statistical analysis

In all experiments data were analysed by analysis of variance and the significance of differences was determined by Student's *t*-test (P=0.05).

Results

Morphology and anatomy of axillary buds

The number of leaflets and leaf area per leaf showed an optimum halfway along the shoot (Fig. 1). Leaves of 'Motrea' were smaller than those of 'Sweet Promise'. Within the group five-leaflet leaves fresh and dry weight of the axillary bud increased towards the stem apex, whereas dry-matter percentage decreased (Table 1).

Buds in the axils of the uppermost leaves (above the uppermost five-leaflet leaf) were smaller than those in the axils of five-leaflet leaves. They usually contained only three to four leaves and a flower bud (Fig. 2). For 'Sweet Promise' this could also be the case for

TABLE 1. Effect of position along the shoot on bud characteristics of rose 'Sweet Promise' and 'Motrea', i.e. fresh and dry weight, dry-matter percentage, total number of leaves (including leaf primordia and scales), number of leaves reduced to scales and number of pith cells on a diameter line at the base of axillary buds. Position 1 is the bud in the axil of the lowermost and position 8 or 9 the bud in the axil of the uppermost five-leaflet leaf.

Position	Motrea					Sweet Promise					
	Fresh weight (mg)	Dry weight (mg)	Dry-matter percentage (%)	Total no. of leaves	No. of scales	Fresh weight (mg)	Dry weight (mg)	Dry-matter percentage (%)	Total no. of leaves	No. of scales	No. of cells
1	2.8	0.8	27.3	8.6	4.6	2.1	0.5	25.7	11.0	6.6	27.9
2	3.9	0.8	23.9	9.3	4.7	2.9	0.7	23.9	12.0	6.7	32.1
3	3.8	0.9	23.1	9.3	4.6	3.3	0.9	23.1	12.0	6.0	33.0
4	3.9	0.7	18.9	9.7	4.4	4.5	1.0	23.2	11.6	5.7	34.1
5	5.0	1.0	20.4	10.4	4.4	4.5	1.0	22.8	12.0	5.2	32.2
6	5.8	1.1	19.4	10.1	4.4	5.0	1.1	22.7	11.1	4.2	29.6
7	5.9	1.1	18.8	10.6	4.6	4.9	1.1	21.4	10.9	3.8	28.7
8	7.8	1.4	18.2	10.9	3.3	5.4	1.2	21.9	8.4	3.2	28.4
9	10.8	1.9	17.9								
LSD (P=0.05)	1.0	0.2	4.1	0.5	0.6	1.0	0.2	1.6	1.3	0.6	2.4

Motrea: Weight and number of leaves were recorded on shoots with 9 respectively 8 five-leaflet leaves. Data are means of 7 replicate shoots.
Sweet Promise: Measurements were done on shoots with 8 five-leaflet leaves. Data of weight and number of leaves are means of 9 replicate shoots; data on number of pith cells are means of 7 replicate shoots.

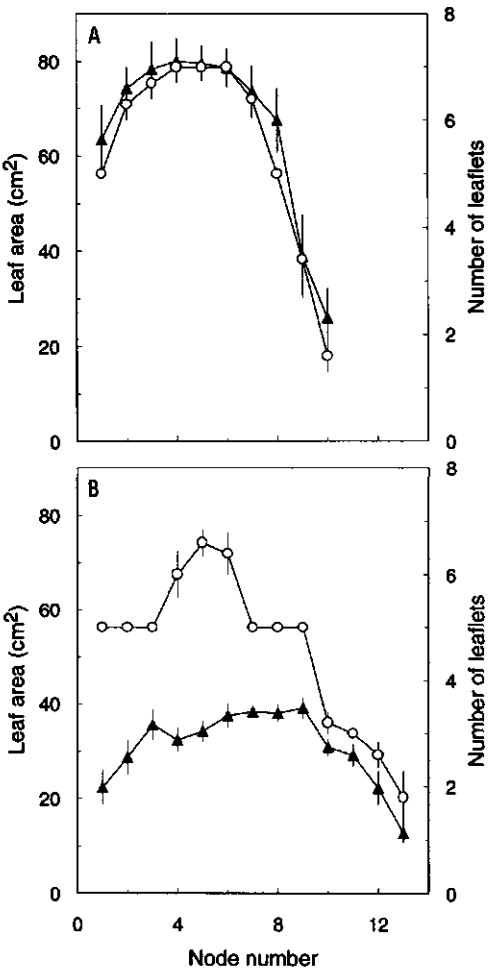


FIG. 1. Effect of position along the shoot on number of leaflets (○) and leaf area (▲) per leaf of rose 'Sweet Promise' (A) and 'Motrea' (B). Position is numbered acropetally.

the bud in the axil of the uppermost five-leaflet leaf. Buds in the axils of lower positioned leaves along the shoot were always vegetative and contained leaves and leaf primordia, of which the lowermost (outermost) were reduced to scales. Within the group of buds in the axils of five-leaflet leaves, the number of leaves and leaf primordia in the bud increased towards the apex for 'Motrea', while for 'Sweet Promise' no effect was found except for the uppermost bud which had a lower number (Table 1). For both cultivars the number of scales as a fraction of the total number of leaves and leaf primordia decreased towards the apex. In each axil of the lowermost three to four leaves of a bud a secondary bud was present. Size of these secondary buds and the angle between bud and stem decreased towards the apex (Fig. 2).

It could be seen that within the group of buds in the axils of the five-leaflet leaves the middle buds contained the highest number of pith cells (Table 1). The number of vascular connections between bud and stem increased towards the apex (Fig. 3).

Buds from the middle five-leaflet leaf contained 592±227 mg total sugar/g and 31.6±8.0 mg starch/g dry weight, against 284±104 mg/g and 7.1±4.6 mg/g respectively for the buds from the lowermost five-leaflet leaf.

Growth potential of axillary buds

Since experiments were carried out on small plants (primary shoot) and on larger plants (lateral shoots of primary shoots), interaction of pruning height and plant size could be studied.

For both cultivars the time period from pruning until bud break and from bud break until harvest (flower at stage 5) increased with severity of pruning (Table 2 and 3). This effect of pruning position was neither dependent on number of leaves left on the plant after pruning nor on plant size, although it was larger in small plants than in larger plants (Table 2 and 3). Also when buds were cultured *in vitro*, shoot elongation started sooner with increasing bud position (data not shown).

The total number of leaves (including scales) preceding the flower decreased with height of bud position. The effect was inde-

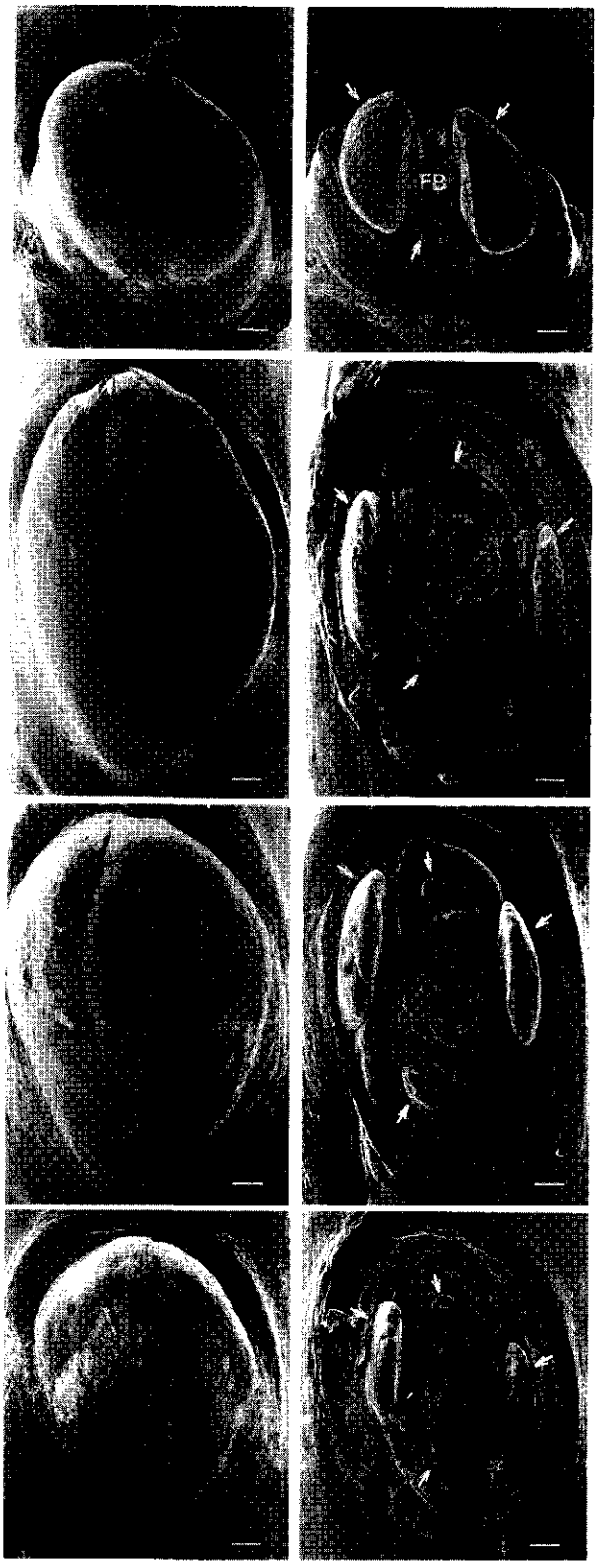


FIG. 2. Effect of position along the shoot on size of the axillary bud of rose 'Sweet Promise'. Bud position is numbered acropetally. Buds were studied by use of SEM both intact (left) and after removal of the leaves and leaf primordia present in the bud (right); in the latter case the secondary buds (indicated by arrows) can be detected. In the bud in the axil of the uppermost leaf a flower bud (FB) is present.
From top to base: position 10, 7, 5, 2. Bud no. 2 is the bud in the axil of the second five-leaflet leaf, whereas bud no. 10 is the bud in the axil of the leaf (with 3 leaflets) immediately below the flower. Bar, 200 μm.

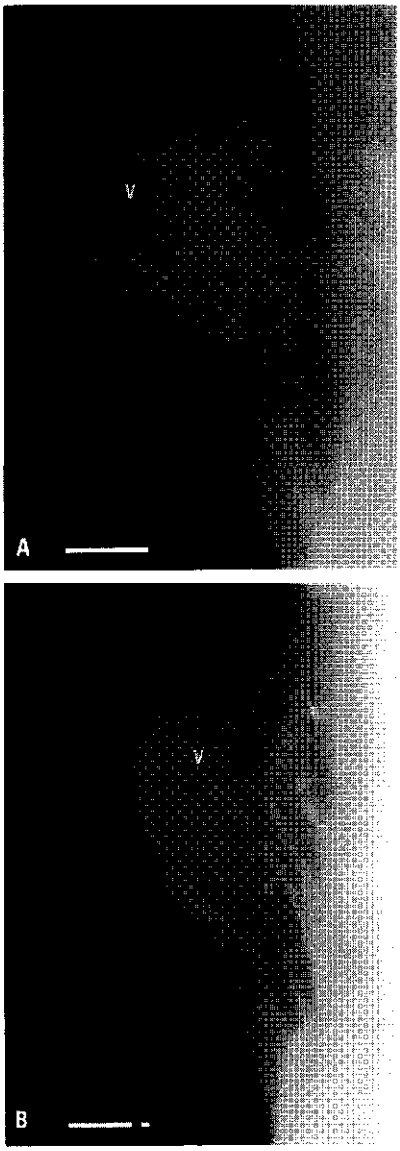


FIG. 3. Cleared axillary buds of rose 'Sweet Promise'. The bark of the stem has been removed. The bud contains crystals (white dots) and is vascularly connected (V) to the stem. Bud position is numbered acropetally. Bar, 500 µm.
A. Bud no. 7, which is the bud in the axil of the second five-leaflet leaf below the flower;
B. Bud no. 5, which is the bud in the axil of the fifth five-leaflet leaf.

pendent on the number of leaves left on the plant or the plant size (Table 2 and 3). Even when buds were grown *in vitro*, i.e. without influences of other plant parts, a similar effect of bud position on the total number of leaves was found (Table 4). The number of five-leaflet leaves decreased towards the apex for 'Sweet Promise' (Table 2) and was not clearly affected for 'Motrea' (Table 3). For both cultivars the number of non-elongated internodes decreased towards the apex.

Pruning severity affected the size of the subsequent shoot. For 'Sweet Promise' pruning just above the uppermost five-leaflet leaf yielded shorter shoots than lower pruning positions, irrespective of plant size (Table 2). When treatments were applied to the lateral shoot of the primary shoot, weight, leaf area and diameter responded similarly to pruning position, but when treatments were applied to the primary shoot these characteristics were smallest for shoots grown from the bud in the axil of the lowermost five-leaflet leaf (Table 2). When buds were cultured *in vitro*, bud position affected shoot length only for the uppermost bud, while weight of the main shoot was not affected (Table 4).

For 'Motrea' the effect of pruning position on shoot growth was dependent of plant size (Table 3); when treatments were applied to the primary shoot, shoot weight and leaf area increased with increasing pruning height, while length was maximal for shoots from the middle position and diameter was smallest for shoots from the lowermost position. When only one leaf was retained on the parent shoot, the effect was reduced but still noticeable. However, when treatments were applied to the lateral shoot of the primary shoot, the effect of pruning position on shoot size was rather small; shoot length decreased while

TABLE 2. Effect of pruning position (lower, middle or upper five-leaflet leaf) on shoot characteristics of rose 'Sweet Promise'. Treatments were applied to the primary shoot (Exp. 1) or to a lateral shoot of the primary shoot (Exp. 3).

Position	Time from pruning until		Total no. of leaves	No. of 5-leaflet leaves	No. of non-elongated internodes	Length (cm)	Weight (g)	Leaf area (cm^2)	Diameter (mm)	No. of pith cells
	bud break (d)	harvest (d)								
Pruning of primary shoot										
Lower	5.8	41.0	18.8	10.1	6.3	50.4	16.6	628	4.7	---
Middle	4.5	33.5	16.5	7.3	5.2	50.6	23.0	803	5.2	---
Upper	3.2	31.5	13.5	7.2	2.5	40.7	24.0	730	5.2	---
LSD (P=0.05)	1.3	3.5	1.0	0.8	0.8	5.3	3.2	164	0.7	---
Pruning of lateral shoot										
Lower	5.6	33.6	18.4	8.5	5.6	53.0	25.9	739	5.9	33.3
Middle	5.0	33.2	17.0	7.8	4.8	55.7	26.0	661	5.7	34.0
Upper	4.0	31.2	12.9	6.4	2.2	38.0	18.9	464	4.9	30.1
LSD (P=0.05)	0.4	0.8	0.5	0.6	0.3	2.8	1.7	49	0.2	1.1

Pruning of primary shoot: Data are means of 6 replicate shoots.
Pruning of lateral shoot: Data are means of 10 replicate shoots.

TABLE 3. Effect of pruning position (lower, middle or upper five-leaflet leaf) and number of five-leaflet leaves on the parent shoot (PaS) on shoot characteristics of rose 'Motrea'. Treatments were applied to the primary shoot (Exp. 2) or to a lateral shoot of the primary shoot (Exp. 4).

Position	No. of leaves PaS	Time from pruning until bud break (d)	Time from pruning until harvest (d)	Total no. of leaves	No. of 5-leaflet leaves	No. of non-elongated internodes	Length (cm)	Weight (g)	Leaf area (cm^2)	Diameter (mm)	No. of pith cells
Pruning of primary shoot											
Lower	1	5.4	40.6	17.5	7.9	4.4	25.4	7.1	309	3.2	---
Middle	5	4.9	35.2	17.3	7.0	4.0	30.8	11.2	445	3.8	---
	1	5.2	37.8	17.0	6.8	4.1	26.7	8.1	336	3.2	---
Upper	9	2.9	30.4	14.0	7.8	2.3	29.1	13.2	509	3.8	---
	1	2.9	33.6	14.7	8.1	2.0	24.7	8.7	366	3.2	---
LSD (P=0.05)		0.5	1.1	0.7	0.7	0.3	1.4	0.7	30	0.1	---
Pruning of lateral shoot											
Lower		5.4	37.7	17.4	6.9	4.2	31.2	15.9	441	4.3	29.9
Middle		5.1	36.1	16.8	6.8	3.8	30.8	16.9	441	4.4	31.2
Upper		4.4	34.5	14.5	6.5	2.2	28.3	17.0	423	4.6	30.2
LSD (P=0.05)		0.4	0.7	0.4	0.5	0.3	1.1	0.9	24	0.1	0.7

Data are means of 10 replicate shoots.

TABLE 4. Effect of bud position along the shoot on shoot growth *in vitro* of rose 'Sweet Promise'. Position 1 is the bud in the axil of the lowermost and position 9 the bud in the axil of the uppermost five-leaflet leaf. Shoot growth was measured 6 weeks after inoculation.

Position	Total no. of leaves	Length (cm)	Fresh weight (g)
1	19.4	2.5	0.30
2	20.4	2.7	0.30
3	19.5	3.1	0.31
4	19.1	2.9	0.31
5	18.5	2.8	0.31
6	18.3	2.8	0.33
7	18.0	2.3	0.29
8	17.6	2.7	0.33
9	17.2	2.1	0.29
LSD (P=0.05)	1.1	0.5	0.04

Data are means of 24 replicate plants.

weight and diameter increased with increasing pruning height.

For both cultivars the number of pith cells in the shoot was slightly greater for the middle position than for the lower and upper positions (Table 2 and 3).

Discussion

Bud inhibition

Several bud characteristics such as bud size and rate of bud break indicated that the degree of axillary bud inhibition in rose increases towards the basis of the shoot, which is in accordance with conclusions of Zieslin *et al.* (1976a) and Tamas (1987). Buds in the axils of the leaves above the uppermost five-leaflet leaf were already generative and contained only a few leaf primordia. De Vries & Dubois (1992) proposed that these buds are part of the inflorescence.

As also known for fruit trees (Tromp *et al.* 1976), branch angles in rose plants were found to decrease with increasing position. Zieslin & Halevy (1976) suggested that control of branch angle is an expression of apical dominance. Jacobs *et al.* (1980) reported that a large angle between an axillary bud and the stem internode below (i.e. a small branch angle) correlates with a high sink activity of the bud. Moreover, the observed high content of sugar and starch, rate of bud break and amount of vascular connections of high positioned buds may indicate a high sink activity of these buds.

In quiescent buds vascular connections to the stem were present, as was also found in *Citrus* by Halim *et al.* (1988). Therefore, inhibition of bud break due to apical dominance is not caused by an absence of vascular connections.

Bud age decreased towards the apex. However, the effect of position on bud characteristics as number of leaf primordia, number of pith cells and size of the bud cannot be explained by bud age, since they were shown to increase with increasing age of the bud (Chapter 4.1). Furthermore, the extent of vascular differentiation is expected to increase with increasing age as was shown for *Populus* by Richards & Larson (1981). The less enhanced development of the lower positioned buds might be caused by inhibitory factors which, as reported by Zieslin *et al.* (1978), accumulate in lower plant parts. Moreover, lower positioned buds may have been initiated at a low level of assimilates, which was shown to affect bud size (Chapter 4.3).

Factors contributing to the effect of pruning position

Dependent of the parameter of bud or shoot development studied, the effects of pruning position were mediated primarily via effects of bud position, bud age or number of leaves on

the parent shoot. The effect of leaf number was assumed to be primarily an effect of assimilate supply. By comparing the effects of pruning position in small and large plants (Table 2 and 3) and with and without leaf removal (Table 3), it can be studied which of the factors are most important. The effect of pruning position on rate of bud break was mediated via effects of bud position. Therefore, rate of bud break seems to be intrinsic to the bud, which fits in with the view of Zieslin & Halevy (1978) and Halim *et al.* (1988). The effects of pruning position on the growth period from bud break until harvest depended primarily on bud position, although the effect was strengthened by differences in assimilate supply (Table 3). The number of leaves preceding the flower on the newly developed shoot was determined by the position and the age of the bud, but not by the assimilate supply during shoot growth. Effects of pruning position on size of the shoot at harvest were mainly effectuated by the differences in assimilate supply during shoot growth. However, in 'Sweet Promise' the bud in the axil of the uppermost five-leaflet leaf yielded a smaller shoot than lower positioned buds, despite the higher assimilate supply provided by the higher number of leaves left on the parent shoot after pruning. It seemed that the growth potential of the bud in the axil of the uppermost five-leaflet leaf depends on the distance from the flower. In 'Sweet Promise' commonly one to three leaves appear above the uppermost five-leaflet leaf, whereas in 'Motrea' this number is three to five. As suggested by De Vries & Dubois (1992), the uppermost axillary buds are part of the inflorescence. For 'Sweet Promise' the uppermost five-leaflet leaf may also be part of the inflorescence.

Although our study indicated that effects of pruning position on bud and shoot development are the result of differences in bud position, bud age and assimilate supply, the effects of pruning position on final size of the shoot were largely the result of differences in assimilate supply during shoot growth.

Future shoot in relation to axillary bud

In many plants the characteristic shape of a leaf is established early in its ontogeny and changes in leaf shape are therefore probably related to factors operating at the apex (Bernier *et al.* 1981). In rose, leaf area and number of leaflets per leaf were largest halfway along the shoot (Fig. 1). Since the lower leaves were already present in the axillary bud, these leaves may have been inhibited during early stages of development. Moreover, they may have unfolded at a low assimilate supply. The reduced size of the upper leaves, which is a common feature in many plant species (Bernier *et al.* 1981), might be due to the proximity or simultaneous development of the flower bud, which according to Mor & Halevy (1979) may act as a strong sink. The leaves in the middle part of the shoot may experience less competition and therefore reach the largest size.

The number of pith cells was highest in buds in the middle part of the shoot, indicating a higher potential shoot diameter for shoots grown from middle buds. Length of growth period until the sepals reflexing stage decreased towards the apex, which is in accordance with results of Zieslin *et al.* (1976a) and Byrne & Doss (1981) on rose. However, Rylski & Halevy (1972) did not find any effect of pruning position on number of days to anthesis in pepper.

Although the number of leaves and leaf primordia already present in the axillary bud

was not affected by bud position ('Sweet Promise') or increased towards the apex ('Motrea'), the total number of leaves (including scales) of the subsequent shoot decreased towards the apex. Similar results were found by Rylski & Halevy (1972) for pepper and by McDaniel (1978) and McDaniel *et al.* (1989) for tobacco. However, Byrne & Doss (1981) observed in rose an influence of bud position on the number of nodes on the developing shoot only above the tenth compound leaf bearing node, and Zamski *et al.* (1985) did not find any difference in leaf number between shoots from the uppermost and lowermost five-leaflet leaf. For both cultivars in our study the number of additional leaves (leaves formed after release of the bud from correlative inhibition) decreased towards the apex.

When buds are used for propagation, basal buds in the region with non-elongated internodes at the base of the subsequent shoot will form basal shoots (Chapter 2.2). As a result of the shorter growth period, buds from a higher position will form basal shoots faster than lower positioned buds. However, the number of basal buds and hence the number of potential basal shoots will be lower since the number of non-elongated internodes decreased with increasing position of the bud. Moreover, this number is related to the age of the bud at the time of release (Chapter 4.1). Flower yield was found to correlate positively with time of emergence and number of basal shoots (De Vries & Dubois 1992).

In conclusion, the final number of leaves on a shoot, the potential shoot diameter, the shape of the lower leaves and the growth period appeared to be determined as early as in the axillary bud stage.

4.3. Effect of assimilate supply on development and growth potential of axillary buds

Marcelis-van Acker C.A.M., 1994. Effect of assimilate supply on development and growth potential of axillary buds in roses. Annals of Botany 73: 415-420.

Abstract. The effect of assimilate supply on axillary bud development and subsequent shoot growth was investigated in roses. Differences in assimilate supply were imposed by differential defoliation. Fresh and dry mass of axillary buds increased with increased assimilate supply. The growth potential of buds was studied either by pruning the parent shoot above the bud, by grafting the bud or by culturing the bud *in vitro*. Time until bud break was not clearly affected by assimilate supply during bud development. Increase in assimilate supply slightly increased the number of leaves and leaf primordia in the bud; the number of leaves preceding the flower on the shoot grown from the axillary bud substantially increased. No difference was found in the number of leaves preceding the flower on shoots grown from buds attached to the parent shoot and those from buds grafted on a cutting, indicating that at the moment of release from inhibition the bud meristem became determined to produce a specific number of leaves and to develop into a flower. Assimilate supply during axillary bud development increased the number of pith cells, but the final size of the pith in the subsequent shoot was largely determined by cell enlargement, which was dependent on assimilate supply during shoot growth. Shoot growth after release from inhibition was affected by assimilate supply during axillary bud development only when buds sprouted attached to the parent shoot, indicating that shoot growth is, to a major extent, dependent on the assimilate supply available while growth is taking place.

Introduction

Axillary buds form the basis of production of a rose crop. The number that actually sprout determines the number of structural shoots, the degree of branching and the potential number of harvested flowers per plant. However, little attention has been paid to axillary buds in rose research. In *Citrus*, Furr *et al.* (1947) demonstrated that the physiological and morphological status of the buds was not changed when they were grafted on rootstocks, and Halim *et al.* (1988) reported that the variation in bud burst was intrinsic to the bud. For leaves, fruits and grains the potential organ size was found to be set largely during the early phase of development (Patrick 1988). Seed size, germination characteristics, subsequent growth rate and morphology of soybean and tomato plants were also found to be affected by the environment in which seeds had been developed (Paul *et al.* 1984; Caulfield & Bunce 1991). In *Pinus*, bud size was shown to be a good indicator of shoot growth potential (Kozlowski *et al.* 1973). The shoot growth of an axillary bud after release from inhibition is defined as the bud growth potential. The growth potential of axillary buds may be influenced by growth conditions during axillary bud development, i.e. during growth of the parent shoot (which is the shoot bearing the axillary buds), as was suggested by Zamski *et al.* (1985). Moe (1971) observed that rose plants, which were kept at low tem-

peratures throughout the season, formed significantly more malformed flowers in the second flush of flowers than in the first flush and suggested that the temperature during axillary bud formation might have an effect on subsequent growth.

Assimilate supply during shoot growth might be expected to influence the size of the shoot at harvest; the more assimilates the bigger the shoot. In rose, the size of axillary buds seems to be related to the diameter of the parent shoot (Byrne & Doss 1981). Meristem activity might be limited by assimilate supply (Patrick 1988) and assimilate supply during axillary bud development might affect the subsequent growth potential of the axillary bud when released from inhibition.

In the present study the effect of assimilate supply on development and growth potential of axillary buds of roses was investigated. The assimilate supply was varied by changing the number of leaves. Growth potential of axillary buds was studied when the bud sprouted attached to the parent shoot and also when it sprouted isolated from the parent shoot. The first method mimics the situation in a commercial crop, the second makes it possible to separate the effects on the parent shoot from those on the bud itself. Furthermore, since the pith contributes to a large extent to the shoot diameter (Chapter 2.1), it was investigated whether the effect of assimilate supply on growth potential of axillary buds was mediated by effects on cell number or cell size of the pith.

Materials and Methods

Two experiments were carried out (Experiments 1 and 2), using double node cuttings of *Rosa hybrida* cv. Motrea as described previously (Marcelis-van Acker & Leutscher 1993). One shoot, the so-called primary shoot, was allowed to develop on each cutting. The developmental stage of the flower was assessed according to the scale used by Halevy & Zieslin (1969), in which 1 represents flower bud visible without dissection; 4 denotes sepals closed, but colour of flower visible; 5 indicates sepals reflexing. Treatments were applied to the primary shoot, when its flower was at stage 4. At the start of the treatments the cutting leaf was removed

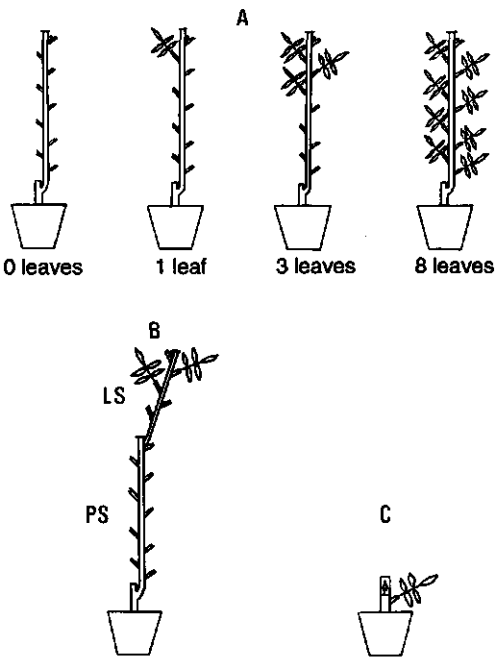


FIG. 1. Schematic representation of the rose plants used in Experiment 1: (A) The primary shoot (PS) was pruned at the uppermost five-leaflet leaf and 0, 1, 3 or 8 leaves were retained. After pruning, only the uppermost axillary bud was allowed to grow into a lateral shoot (LS). (B),(C) When the LS was almost flowering (stage 4), it was pruned at the middle five-leaflet leaf. After pruning, the uppermost bud was allowed to grow into a shoot either attached to the LS (also called parent shoot; B) or after being grafted onto a cutting (C). When the bud stayed attached to the LS, all leaves except 2 were removed (B).

and the shoot was pruned. Only the bud in the axil of the uppermost leaf was allowed to grow into a lateral shoot, while other emerging lateral shoots were continually removed. When the flower of this lateral shoot was at stage 4, the axillary buds of this shoot were studied. Plants were grown in a climate chamber at a temperature of 21°C (day and night), a relative humidity of approximately 70%, 115 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) provided by fluorescent tubes (Philips TLD 84 HF/50W) and a day length of 16 h.

In Experiment 1 the primary shoot was pruned just above the uppermost five-leaflet leaf and the uppermost leaf was removed. Four levels of defoliation (considered equivalent to four increasing amounts of assimilate supply) were applied by retaining none, one, three or eight leaves on the primary shoot (Fig. 1A). Each treatment was applied to 36 plants, arranged in six blocks. The effect of assimilate supply on the bud in the axil of the middle five-leaflet leaf of the lateral shoot was studied by determining the number of leaves including leaf primordia in the bud of six plants per treatment using a dissecting microscope (x50) and measuring fresh and dry mass of the bud (six plants per treatment). Dry mass was measured after drying for 3 d at 80°C. The growth potential of the bud was determined in two different ways (Fig. 1B,C): (*a*) by pruning the lateral shoot just above this bud (twelve plants per treatment). All leaves except the two just below the uppermost leaf were removed and all but the uppermost lateral shoot were continually removed; and (*b*) by grafting the bud in between the two leaves of a double node cutting, excised from the middle part of a 'Motrea' shoot at stage 4 (twelve plants per treatment). The bud was grafted by means of an inverted T-incision and

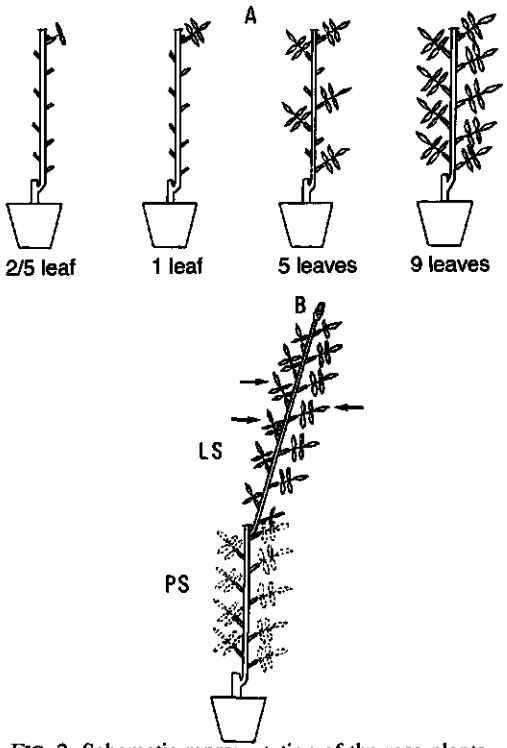


FIG. 2. Schematic representation of the rose plants used in Experiment 2: (A) The primary shoot (PS) was pruned at the uppermost five-leaflet leaf and 2/5, 1, 5 or 9 leaves were retained. After pruning, only the uppermost axillary bud was allowed to grow into a lateral shoot (LS). (B) When this LS (also called parent shoot) was almost flowering (stage 4), the buds from the axils of the middle three five-leaflet leaves (arrows) were cultured *in vitro*.

tied with tape (Ribon; Mauritz, Bussum, The Netherlands). The cuttings were dipped into talcum powder with 0.4% indole butyric acid to promote rooting. When rooted, cuttings were pruned just above the grafted bud and the budding tape was removed. Also the bud in the axil of the leaf immediately below the middle five-leaflet leaf of the lateral shoot was grafted.

In order to quantify the effect of defoliation on shoot growth, the lateral shoots from plants which were used for measuring bud

mass and number of leaf primordia in the bud or for grafting the bud, were harvested and length, diameter (at 1 cm from the base of the shoot), fresh mass, leaf area and growth period were recorded.

Bud break (defined as released buds with a length of at least 0.5 cm) was recorded and the time course of shoot length was determined for both pruned and grafted plants by measuring shoot length three times a week. The data were smoothed by calculating moving averages of three consecutive data points per plant and subsequently averaging over the plants. The shoots were harvested when the flower of the shoot was at stage 5. Length, diameter (at 1 cm from the base of the shoot), fresh mass, number of leaves and leaf area were determined. Thin transverse hand cut sections were made at 1 cm from the base of the shoots grown from grafted buds. Per transverse section the diameter of the pith was measured using an ocular micrometer and the number of pith cells passed on three diameters of the shoot was recorded.

In Experiment 2 the primary shoot was pruned just above the uppermost five-leaflet leaf and nine leaves (all leaves), five leaves (every other leaf), one leaf (uppermost leaf) or two fifths of a leaf (two out of five leaflets of the uppermost leaf) were retained (Fig. 2). Buds in the axils of the middle three leaves of the lateral shoot were excised, surface-sterilized for 20 min in 1% NaOCl (v/v) with a few drops of Tween 20 and rinsed in sterile tap water for 5, 10 and 10 min. The buds were cultured *in vitro* on a medium containing Murashige & Skoog (1962) salts (except Fe) at full strength, 37.5 mg l^{-1} NaFeEDTA, 45 g l^{-1} glucose, 0.1 mg l^{-1} benzyladenine and 5 g l^{-1} MC 29 agar (Lab M, U.K.). Cultures were grown in a climate chamber at 23°C (day and night), a day length of 16 h and 23-32 µmol m^{-2} s^{-1} PAR provided by fluorescent tubes (Philips TL 54/36W). Five weeks after inoculation, shoot length, number of leaves and fresh mass were determined.

Data were analysed by analysis of variance and the significance of differences was determined by Student's *t*-test (P=0.05).

Results

An increase in the number of leaves from none to three, and thus, it is assumed, in assimilate supply, resulted in an increase in fresh and dry mass of the axillary buds. A further increase in number of leaves did not affect bud mass

TABLE 1. Effect of number of leaves (on the primary shoot) during axillary bud development on mass and number of leaves and leaf primordia in axillary buds of rose cv. 'Motrea' when the flower of the lateral shoot bearing the axillary buds was almost flowering (stage 4). Data are means of six replicate buds. Treatments as shown in Fig. 1.

No. of leaves on primary shoot	Axillary bud		
	Fresh mass (mg)	Dry mass (mg)	No. of leaves and leaf primordia
0	1.8	0.4	8.2
1	3.5	0.8	9.0
3	6.3	1.3	8.7
8	6.2	1.3	9.3
LSD (P=0.05)	1.4	0.2	0.9

TABLE 2. Effect of number of leaves (on the primary shoot) on growth of the lateral shoot of rose cv. 'Motrea'. Growth characteristics of the lateral shoot were measured when the flower was almost flowering (stage 4). Data are means of 24 replicate plants. Treatments as shown in Fig. 1.

No. of leaves on primary shoot	Time from pruning until harvest (d)	Length (cm)	Fresh mass (g)	Leaf area (cm^2)	Diameter (mm)
0	49.6	17.5	5.1	182	2.6
1	40.0	23.9	8.4	318	3.2
3	35.8	27.5	11.1	458	3.7
8	35.3	30.5	12.2	475	3.9
LSD (P=0.05)	2.2	1.7	1.3	60	0.2

(Table 1). The effect of the number of leaves able to form assimilates on the number of leaves and leaf primordia in the bud was small; only when all leaves were removed was the number of leaves and leaf primordia significantly reduced (Table 1). The size of the parent shoot (i.e. lateral shoot) was also increased by the area of leaf retained (Table 2). The leaf area and presumably assimilate supply during bud development did not clearly affect time until bud break after release from inhibition, irrespective of whether the bud remained on the parent shoot or was grafted, although grafted buds sprouted slightly faster than the buds on the parent shoot (Table 3). However, sprouting of grafted buds was variable and no consistent differences between treatments could be found. When the bud sprouted on the parent shoot, an increase in assimilate supply during bud development stimulated the subsequent growth of the bud into a shoot; the rate of elongation increased with a greater leaf area until about 20 d after bud break (Fig. 3A). At harvest, the length, diameter, mass, leaf area, total number of leaves and number of five-leaflet leaves had increased, whereas the growth period until harvest decreased with increasing leaf area retained (Table 3). When the bud sprouted isolated from the parent shoot, after being grafted on a double node cutting, there was little effect of size of the assimilate source during bud development on subsequent shoot growth (Fig. 3B, Table 3), except for an increase in the total number of leaves preceding the flower and the number of five-leaflet leaves. Survival of the grafted buds was also not affected by the number of leaves during axillary bud development. The number of leaves preceding the flower of shoots grown on the parent shoot was similar to that of shoots grown from grafted buds (Table 3). A larger leaf area retained during axillary bud development resulted in an increase in the number of pith cells of the subsequent shoots, but had no effect on the diameter of either the pith or the shoot (Table 4).

When buds, developed on plants with different numbers of leaves, were grown *in vitro* the total number of leaves increased with increasing leaf area and assimilate supply, while no effect on mass was found (Table 5). Length slightly decreased with increasing leaf area retained.

Discussion

Removal of leaves from the primary shoot reduced the mass of the lateral shoot while it

TABLE 3. Effect of number of leaves (on the primary shoot) during axillary bud development of rose cv. 'Motrea' on bud break and subsequent shoot growth of axillary buds after release from inhibition. Buds sprouted attached to the parent shoot (Pruned) or grafted on a cutting (Grafted). Growth characteristics of the shoots, grown from the buds, were measured when the flower was at stage 5 (sepals reflexing). Data are means of 12 (Pruned) or 16 (Grafted) replicate plants. Treatments as shown in Fig. 1.

No. of leaves on primary shoot	Time from pruning until bud break (d)	Time from pruning until harvest (d)	Length (cm)	Fresh mass (g)	Leaf area (cm^2)	Diameter (mm)	Total no. of leaves	No. of 5-leaflet leaves
Pruned								
0	5.7	46.3	20.8	5.9	191	2.8	13.8	5.7
1	4.8	42.3	22.3	8.1	265	3.3	16.2	6.3
3	5.1	41.0	25.5	10.5	335	3.6	17.3	7.7
8	5.5	41.3	27.6	11.9	371	3.7	17.8	7.8
LSD (P=0.05)	1.2	1.6	1.9	1.2	45	0.2	1.0	0.6
Grafted								
0	4.9	42.1	21.1	6.1	188	3.2	15.5	6.0
1	3.1	43.1	21.7	6.2	203	3.2	16.7	6.0
3	4.9	47.5	22.8	6.8	220	3.3	17.7	6.8
8	3.6	43.7	21.6	6.2	199	3.3	18.1	7.1
LSD (P=0.05)	1.6	3.6	1.5	0.8	27	0.2	0.8	0.8

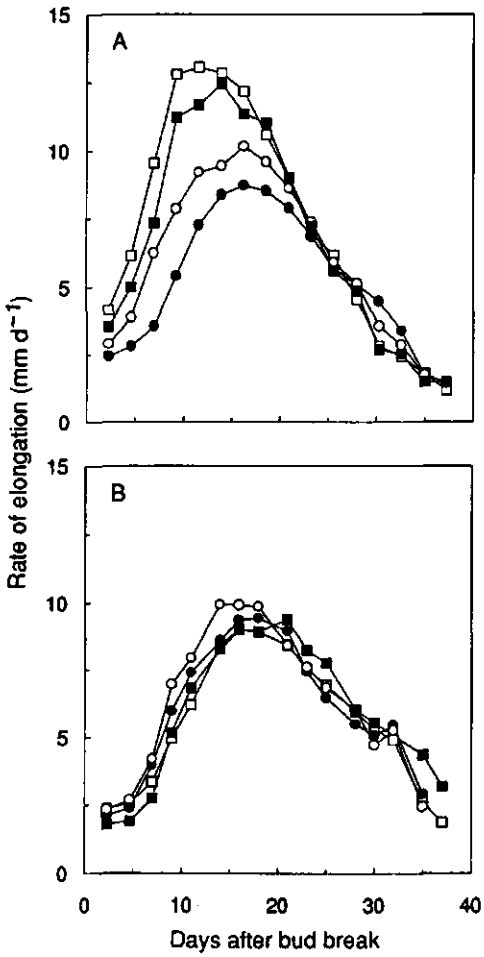


FIG. 3. Effect of number of leaves (●, 0 leaves; ○, 1 leaf; □, 3 leaves and, ■, 8 leaves on the primary shoot) during axillary bud development of rose cv. 'Motrea' on the rate of elongation after release from inhibition of axillary buds of the lateral shoot. Shoots grown from these buds were harvested when its flower was at stage 5 (sepals reflexing). Treatments as shown in Fig. 1. (A) Axillary buds sprouted when attached to the parent shoot (i.e. lateral shoot); s.e.m. did not exceed 0.07; (B) Axillary buds sprouted when grafted onto a cutting, s.e.m. did not exceed 0.07.

increased the growth period of the lateral shoot. These results justify the assumption that assimilate supply decreased due to defoliation.

Increasing the assimilate supply during axillary bud development increased the mass of the bud and slightly increased the number of leaves and leaf primordia in the bud. The size of the parent shoot (i.e. the lateral shoot) also increased with the greater assimilate supply. A positive relationship between parent shoot diameter and both the number of leaf primordia and diameter of the axillary buds was also reported by Byrne & Doss (1981). Despite the shorter growth period of the parent shoot and therefore of the axillary buds, the axillary buds became bigger with increased assimilate supply. As axillary buds are sinks for assimilates (Kozlowski 1992), an increase in assimilate supply might be expected to stimulate their growth. Although the effect of assimilate supply on the number of leaves and leaf primordia in the bud was only small, the effect on the number of leaves preceding the flower of the subsequent shoot was substantial. The difference in total number of leaves preceding the flower mainly affected the number of five-leaflet leaves, which are the leaves in the middle part of the shoot.

An axillary bud of rose, when released from apical dominance, produces a number of nodes before forming its terminal flower, as was also reported for tobacco (McDaniel *et al.* 1989). In tobacco, an axillary bud appears to 'count' the nodes below its apical meristem and employs this information to establish when to initiate flower development. This node counting process was found to be influenced by the physiological status of the plant and not by the number of leaves and leaf primordia in the bud at the time of release from apical dominance (McDaniel *et al.* 1989). The present study shows that also for rose the number of leaves and leaf primordia in the bud

TABLE 4. Effect of number of leaves (on the primary shoot) during axillary bud development of rose cv. 'Motrea' on diameters of shoot and pith and number of pith cells of shoots, grown from grafted buds. The number of pith cells was determined in transverse sections of the shoot by counting the number of cells passed traversing a diameter line. Data are means of 16 replicate plants. Treatments as shown in Fig. 1.

No. of leaves on primary shoot	Diameter shoot (mm)	Diameter pith (mm)	No. of cells
0	3.2	1.6	21.8
1	3.2	1.6	22.5
3	3.3	1.6	23.3
8	3.3	1.6	24.1
LSD (*P*=0.05)	0.2	0.1	0.9

seems not to determine the number of additional leaves.

According to McDaniel (1978) the number of nodes produced before differentiation into a flower represents the developmental potential of the axillary bud. Since, in rose, the total number of leaves preceding the flower was found to be equal for grafted buds and buds attached to the parent shoot, the bud meristem appeared to be or to become determined for a limited growth pattern, i.e. to produce a specific number of leaves and to develop into a flower, at the time of release from inhibition. Unlike tomato, where an increase in assimilate supply decreases the number of leaves preceding the first inflorescence (Dieleman & Heuvelink 1992), in rose the number of leaves below the flower increased under these conditions. In rose apical control is assumed to be involved in the transition of the apical meristem from the vegetative into the floral stage, since the transition occurs only after release from inhibition of the buds (Cockshull & Horridge 1977; Zieslin 1992; Chapter 4.1). Although the transition occurs several days after release from inhibition, the present results indicate that the meristem is already programmed at an earlier stage.

Time until bud break was not clearly influenced by the assimilate supply during bud de-

TABLE 5. Effect of number of leaves (on the primary shoot) during axillary bud development of rose cv. 'Motrea' on growth of axillary buds into shoots. Buds were inoculated *in vitro* and cultured for 5 weeks. Data are means of 21 replicate plants. Treatments as shown in Fig. 2.

No. of leaves on primary shoot	Length (cm)	Mass (g)	Total no. of leaves
2/5[x]	2.5	0.33	16.4
1	2.7	0.35	17.2
5	2.1	0.33	17.2
9	2.0	0.35	18.1
LSD (*P*=0.05)	0.5	0.04	1.0

[x] Three of the five leaflets of the leaf were removed.

velopment, indicating that assimilate supply did not affect the degree of inhibition of the bud. No explanation can be given for the variable sprouting of the grafted buds. Although to some extent assimilate supply affected the development of the axillary bud up to the stage that the parent shoot was harvestable, the growth period from pruning until flowering and the final size of the shoot grown from the bud, were mainly dependent on the assimilate supply during bud sprouting and subsequent shoot growth. Since the size of the parent shoot (i.e. lateral shoot) increased with an increase in assimilate supply during axillary bud development, subsequent shoot growth of axillary buds attached to the parent shoot will also be affected. This situation occurs in commercial practice. Byrne & Doss (1981) also found for rose a positive relationship between diameters of the parent and daughter shoots. The difference in rate of elongation of sprouting buds, developed under a different assimilate supply and sprouted into shoots attached to the parent shoot, is also probably the result of the differences in assimilate supply during shoot growth. The observation that the differences in rate of elongation disappeared after about three weeks, indicates that shoots became self supporting for assimilates at that time (Mor & Halevy 1979).

Assimilate supply during bud development did not affect bud break and subsequent shoot growth when the buds were separated from the parent shoot, either when the buds were grafted or when the buds were cultured *in vitro*. The effect of the axillary bud on the final size of the shoot was small and may only become apparent between buds from shoots that vary greatly in shoot diameter. When buds of shoots, varying in diameter between 3.5 and 5.5 mm, were grown *in vitro*, a slight positive relationship between shoot mass and diameter of the parent shoot was found (data not shown). Growth conditions during bud break and subsequent shoot growth clearly determine shoot size to a greater extent than does the axillary bud. Similar results were reported for cucumber fruits by Marcelis (1993a), who found that the early development of a fruit seemed not to be crucial for setting the growth potential of the fruit.

In the present study the pith was considered as a parameter of primary growth. In woody plants it persists in size, shape and structure exactly as it was in the young stem (Eames & MacDaniels 1947). In a flowering rose shoot, the pith contributes about 50-60% to the shoot diameter (Chapter 2.1) and shoot diameter is a parameter of shoot quality. Pith diameter is determined by cell number and cell size. Assimilate supply might affect cell division and cell enlargement. In rose, cell number across the stem diameter remained constant after bud break, so cell number may be affected during bud development only. In tomato, differences in final fruit size seem to be correlated to the cell number in the pre-anthesis ovary (Bohner & Bangerth 1988). Assimilate supply during axillary bud development was found to influence the cell number in the pith and as a result the potential pith diameter of the subsequent rose shoot. However, final pith diameter seems to be primarily affected by cell enlargement, as seems also the case for mature fruit size (Ho 1992). Since cell enlargement mainly takes place after bud break, the growing conditions during shoot growth largely influence the final diameter of the pith. The results indicate that a low cell number can be compensated to a great extent by a greater enlargement per cell, which is in accordance with the conclusion of Marcelis (1993a) for cucumber fruits.

In conclusion, the number of leaves and hence the assimilate supply during axillary bud development affects the size of the axillary bud and its developmental potential. However, except for the number of leaves, the growth of this bud into a shoot is only indirectly influenced by the assimilate supply during axillary bud development, via an effect on the parent shoot.

4.4. Effect of temperature on development and growth potential of axillary buds

Marcelis-van Acker C.A.M., 1994. Effect of temperature on development and growth potential of axillary buds in roses. (submitted).

Abstract. The effect of a temperature pre-treatment (i.e. temperature during axillary bud formation) on axillary bud development and subsequent shoot growth was investigated. Growth potential of the axillary buds was studied either *in situ*, by pruning the parent shoot above the bud, or in isolation, by grafting the bud or by culturing the bud *in vitro*. Although rate of leaf initiation increased with increasing temperature, the number of leaves and leaf primordia in the bud at the time of release from inhibition was not clearly affected. Time until bud break decreased with increasing pre-treatment temperature, as did the total growth period until harvest. The total number of leaves preceding the flower decreased with increasing pre-treatment temperature, while no difference in leaf number was found between shoots grown from buds *in situ* and shoots from buds in isolation. Shoot size at harvest (sepals reflexing) slightly decreased with increasing pre-treatment temperature, but effects were larger for isolated buds than for buds attached to the parent shoot. The ratio pith to shoot diameter was not affected by temperature and the number of pith cells in cross section only slightly decreased with increasing pre-treatment temperature.

Introduction

Flower production in rose depends on the ability of sprouting of axillary buds and their growth into flowering shoots. In a rose crop a large variation in number, size and growth period of shoots occurs, which is at least partially due to intrinsic factors of the axillary buds. Variation in bud burst in *Citrus* appeared to be intrinsic to the bud (Halim *et al.* 1988). For leaves, fruits and grains it has been reported that the potential organ size is set largely during the early phase of development (Patrick 1988). Environmental conditions during growth of parent plants may affect the growth of the daughter plants, both when propagated *in vivo* (Moe & Andersen 1988) and when propagated *in vitro* (Litz & Conover 1981; Cassels & Minas 1983). In soybean and tomato it was shown that the environment in which seeds had formed, affected the germination characteristics, subsequent growth rate and plant morphology (Paul *et al.* 1984; Caulfield & Bunce 1991). Zamski *et al.* (1985) suggested that also for rose the growth potential of axillary buds is influenced during the growth of the parent shoot.

Shoot growth of an axillary bud after release from inhibition will be referred to as the bud growth potential. As temperature has a pronounced effect on shoot growth of rose (Van den Berg 1987; Chapter 5.2), it might be one of the major environmental factors affecting growth potential of axillary buds. Accordingly, Moe (1971) suggested that temperature during axillary bud formation might affect subsequent shoot growth. In *Nephrolepis* a carry-over effect of temperature during growth of the parent plant on subsequent growth *in vitro* was found (Hveslof-

Eide 1991). In woody plants both the temperature during the year of bud formation and the temperature during the year of bud expansion into a shoot may influence shoot growth (Kozlowski *et al.* 1991).

In the present study the effect of temperature during axillary bud formation on development and growth potential of axillary buds of two rose cultivars was studied. Growth potential of the axillary buds was studied when the bud sprouted either attached to the parent shoot or isolated from the parent shoot. The first method represents the normal situation in a crop, the second method makes it possible to separate the temperature effects on the parent plant from those on the bud itself. Furthermore, since the pith, representing the primary growth of a shoot, persists in size, shape and structure as it was in the young stem (Eames & MacDaniels 1947) and contributes to a large extent to the shoot diameter (Chapter 2.1), it was investigated whether the effect of temperature on growth potential of axillary buds was mediated by effects on cell number or cell size of the pith.

Materials and Methods

Four experiments were undertaken, two with plants of *Rosa hybrida* cv. Motrea (Experiments 1 and 3) and two with plants of *Rosa hybrida* cv. Sweet Promise (Experiments 2 and 4). Plants were raised from double node cuttings, as described by Marcelis-van Acker & Leutscher (1993). After rooting, the cuttings were exposed in three growth chambers to a temperature of constant 17°C, 21°C or 25°C and a relative air humidity of approximately 70%. Day length was 16 h and light intensity 40 W m^{-2} photosynthetically active radiation (PAR) provided by Philips high pressure sodium lamps (SON/T) and metal halide lamps (HPI/T). One shoot was allowed to develop on each cutting. The developmental stage of the flower was assessed according to the scale used by Halevy & Zieslin (1969), in which 1=flower bud visible without dissection; 2=flower bud pea-sized; 3=sepals closed and colour flower not yet visible; 4=sepals closed, but colour of flower visible; 5=sepals reflexing. As soon as the flower of the shoot was at stage 5, the shoot was pruned just above the fourth (Exp. 1 and 2) or the third (Exp. 3 and 4) five-leaflet leaf counted from the base of the shoot. Only the bud in the axil of the uppermost leaf was allowed to grow into a lateral shoot, while other sprouting axillary buds were continually removed. The time of unfolding of the five-leaflet leaves of the lateral shoot was recorded in Experiments 3 and 4. When the flower of the lateral shoot was at stage 1 (Exp. 1), stage 2 (Exp. 2), or stage 4 (Exp. 3 and 4), length of growth period, shoot length and diameter (at 1 cm from the base) and number of leaves were measured. Then, all plants were transferred to 21°C; the lateral shoot was pruned just above the middle five-leaflet leaf. In this way the axillary buds had been formed at different temperatures (i.e. temperature pre-treatments), while outgrowth of the buds into shoots occurred at the same temperature of 21°C. Furthermore, in Experiments 3 and 4 at the end of the temperature pre-treatment, some plants were used to determine the number of leaves, including leaf primordia of the bud in the axil of the middle five-leaflet leaf using a dissecting microscope (x50). In order to study the growth potential of the buds in isolation, in Experiments 3 and 4 the middle three buds of several lateral shoots were either grafted or cultured *in vitro*.

-*Grafting*. The bud was grafted in between the two leaves of a fresh unrooted double node cutting of the same cultivar. The cutting was taken from the middle part of a shoot at stage 5, grown in the 21°C climate chamber. The bud was grafted by means of an inverted T-incision and tied with tape (Ribon; Mauritz, Bussum, The Netherlands). To promote rooting, the base of the cuttings were dipped into talcum powder with 0.4% indole butyric acid. When rooted, cuttings were pruned just above the grafted bud and the budding tape was removed.

-*In vitro culture*. Buds were cultured *in vitro* on a medium containing Murashige & Skoog (1962) salts (except Fe) at full strength, 37.5 mg l^{-1} NaFeEDTA, 45 g l^{-1} glucose, 0.1 mg l^{-1} benzyladenine and 5 g l^{-1} MC29 agar (Lab M, U.K.). Cultures were grown in a climate chamber at constant 23°C, a day length of 16 h and 5-7 W m^{-2} PAR provided by fluorescent tubes (Philips TL54/36W).

Time to bud break (time from pruning until buds had reached a length of 0.5 cm) was recorded in Experiments 3 and 4. In all four experiments, *in vivo* grown shoots were harvested when the flower was at stage 5. Length of growth period, shoot length and diameter (at 1 cm from the base of the shoot), shoot fresh weight, number of leaves and leaf area were determined. In Experiments 1 and 2, thin transverse sections were made by hand at 1 cm from the base of the shoot. In each transverse section the diameter of the pith was measured using an ocular micrometer and the number of pith cells on a diameter line was counted. This was repeated for three diameter lines per shoot.

In vitro cultured shoots of 'Sweet Promise' were harvested when the flower bud was visible without dissection. For 'Motrea', it was not possible to discern the flower bud when shoots were in the culture tubes. Therefore, shoots of 'Motrea' were harvested 5 weeks after inoculation. Length, fresh weight and number of leaves of the shoots were recorded.

Experimental set-up and statistical analysis

Experiments comprised 10 (Exp. 1 and 2) and 40 (Exp. 3 and 4) replicate plants at each of the temperature treatments. In Experiments 3 and 4, per temperature, buds of groups of 10

TABLE 1. Effect of temperature during development of the axillary bud and its parent shoot on growth characteristics of the parent shoot of rose 'Motrea' (Exp.1 and 3) and 'Sweet Promise' (Exp. 2 and 4). Data are means of two experiments.

Cultivar	Temperature (°C)	Length (cm)	Diameter (mm)	Total no. of leaves	No. of 5-leaflet leaves	Growth period (d)
Motrea	17	32.5	4.6	21.6	9.0	36.0
	21	29.7	4.1	19.4	8.2	24.2
	25	25.9	3.8	18.4	7.7	20.4
LSD (P=0.05)		3.3	0.5	2.1	1.9	2.0
Sweet Promise	17	48.6	5.6	18.6	8.6	33.7
	21	47.5	5.0	17.9	8.4	23.4
	25	40.8	4.7	17.6	8.4	19.3
LSD (P=0.05)		5.5	0.7	0.9	0.7	0.9

plants each were used for determining the number of leaves in the bud at the time of release from inhibition, for the *in vitro* study, for grafting and for growth observations of buds attached to the parent shoot.

For each of the two cultivars the data on growth potential of axillary buds attached to the parent plant were analysed by analysis of variance (ANOVA) based on 3 temperature treatments and 2 replicate experiments. The significance of differences was determined by Student's *t*-test (P=0.05).

The data on the number of leaves in the bud at the time of release from inhibition and on growth of the bud into a shoot after either grafting or *in vitro* culture, could not be analysed by ANOVA. In that case per treatment means and standard error of the means were calculated.

Results

Parent shoot and axillary buds

Length, diameter, number of leaves and length of growth period of the parent shoot, i.e. the shoot bearing the axillary buds, decreased with increasing temperature for both cultivars (Table 1). Although the age of the axillary bud, expressed as days from subtending leaf unfolded until transfer to 21°C, also decreased with increasing temperature, the number of leaves and leaf primordia in the axillary bud was not clearly affected by temperature (Table 2). Length, diameter and leaf area of the parent shoot and number of leaves in the axillary bud of 'Sweet Promise' exceeded that of 'Motrea'.

Growth potential of buds attached to the parent shoot

Both the time from pruning until bud break and the time from bud break until harvest decreased by a high temperature pre-treatment when buds remained attached to the parent shoot (Table 3). The values for the various growth parameters for 'Motrea' decreased with increasing temperature, although the differences were not always significant (Table 4). For 'Sweet Promise', however, shoot development was little affected by temperature, except for shoot length which was smallest at a pre-treatment temperature of 17°C (Table 4). Leaf area of the parent shoot, which remained after pruning above the middle five-leaflet leaf, predominated at 21°C (Table 4).

TABLE 2. Effect of temperature during axillary bud formation on age of the bud (days from subtending leaf unfolded) and on number of leaves and leaf primordia in the bud of rose 'Motrea' (Exp. 3) and 'Sweet Promise' (Exp. 4) when the parent shoot was at stage 4. Data are means ± SE.

Cultivar	Temperature (°C)	Bud age (d)	No. of leaves and leaf primordia
Motrea	17	13.4±0.2	10.7±0.2
	21	9.0±0.3	11.0±0.0
	25	7.8±0.3	10.3±0.2
Sweet Promise	17	12.9±0.5	12.1±0.2
	21	9.3±0.4	11.9±0.1
	25	7.3±0.4	11.3±0.2

TABLE 3. Effect of temperature during axillary bud formation on time from pruning until bud break and time from bud break until harvest (flower at stage 5) of rose 'Motrea' (Exp. 2) and 'Sweet Promise' (Exp. 4). Buds sprouted at 21°C, either attached to the parent shoot or after being grafted on a cutting. Data are means ± SE.

Cultivar	Temperature (°C)	Attached		Grafted	
		Time from pruning until bud break (d)	Time from bud break until harvest (d)	Time from pruning until bud break (d)	Time from bud break until harvest (d)
Motrea	17	5.2±0.1	40.3±0.4	5.8±0.8	42.9±0.8
	21	4.7±0.2	37.1±0.4	2.9±0.4	39.5±0.6
	25	4.5±0.2	36.2±0.5	2.6±0.5	38.6±0.5
Sweet Promise	17	5.9±0.2	33.7±0.4	6.2±1.0	31.9±0.6
	21	5.4±0.3	32.2±0.4	4.7±0.7	31.6±0.5
	25	4.0±0.4	31.5±0.6	3.9±0.7	29.6±0.7

The ratio of pith to shoot diameter was not affected by pre-treatment temperature (Table 5). The number of pith cells in cross section slightly decreased with increasing temperature for 'Motrea' and was at 25°C lower than at 17°C and 21°C for 'Sweet Promise'.

Growth potential of grafted buds

For grafted axillary buds of both cultivars, time from pruning of the cutting until bud break and time from bud break until harvest decreased with increasing pre-treatment treatment (Table 3). Total number of leaves (including scales) per shoot also decreased when temperature increased (Table 6) and was more or less similar to that for shoots attached to the parent shoot (Table 4). Leaf area, shoot weight and length slightly decreased with increasing temperature but shoot diameter was not clearly affected (Table 6).

TABLE 4. Effect of temperature during axillary bud formation on subsequent shoot growth of rose 'Motrea' (Exp. 1 and 3) and 'Sweet Promise' (Exp. 2 and 4). Buds sprouted after release from correlative inhibition attached to the parent shoot at 21°C. The subsequent shoots were harvested when the flower was at stage 5 (sepals reflexing). Data are the means of two experiments.

Cultivar	Temperature (°C)	Length (cm)	Diameter (mm)	Weight (g)	Total no. of leaves	No. of 5-leaflet leaves	Leaf area (cm^2)	Leaf area parent shoot* (cm^2)
Motrea	17	33.6	4.8	18.4	19.3	8.5	447	226
	21	31.2	4.8	17.2	17.7	7.1	428	253
	25	29.5	4.4	15.8	16.5	6.3	387	197
LSD (P=0.05)		3.8	0.2	3.5	0.4	1.8	144	75
Sweet Promise	17	48.1	5.4	23.7	17.3	8.0	557	284
	21	58.7	5.5	23.8	17.5	8.0	547	333
	25	55.4	5.6	25.6	16.7	7.1	598	308
LSD (P=0.05)		3.7	1.1	8.7	1.7	1.9	253	43

* Area of parent shoot after pruning.

TABLE 5. Effect of temperature during axillary bud formation on diameter of pith, number of pith cells on a diameter line and ratio of the pith diameter and the shoot diameter of shoots of rose 'Motrea' (Exp. 1) and 'Sweet Promise' (Exp. 3). Data are means ± SE.

Cultivar	Temperature (°C)	Diameter pith (mm)	Ratio pith/shoot (%)	Number of cells
Motrea	17	2.6±0.05	54.2±0.5	32.0±0.5
	21	2.6±0.04	54.3±1.2	31.6±0.3
	25	2.4±0.04	54.8±0.6	30.1±0.4
Sweet Promise	17	3.1±0.1	52.4±1.1	34.2±0.6
	21	3.3±0.1	54.3±0.8	35.1±0.7
	25	3.2±0.1	53.8±0.5	32.6±0.4

Growth potential of buds in vitro

Temperature during axillary bud formation affected the outgrowth of the buds *in vitro* (Table 7). Only a few shoots from 17°C-buds of 'Sweet Promise' reached the stage 'flower bud visible' and most of the shoots showed senescence before the flower bud was visible. No shoots from 17°C-buds of 'Motrea' reached this stage within the 5 weeks' culture period. For both cultivars number of leaves and weight of the shoots slightly decreased with increasing temperature, but shoot length was not clearly affected (Table 7).

Discussion

In agreement with the general response of plants to temperature (Terry *et al.* 1983), rate of development of the parent shoot bearing the axillary buds and rate of leaf initiation of the axillary bud meristem was accelerated at higher temperature. As a result, the age of the axillary buds at the end of the temperature treatment decreased with increasing temperature. However, temperature did not clearly affect the number of leaves including leaf primordia in the bud at the time of release from

TABLE 6. Effect of temperature during axillary bud formation on bud break and subsequent shoot growth of axillary buds of rose 'Motrea' (Exp. 2) and 'Sweet Promise' (Exp. 4). Buds sprouted, after being grafted on a cutting, at 21°C. The subsequent shoots were harvested when the flower was at the stage 'sepals reflexing'. Data are means ± SE.

Cultivar	Temperature (°C)	n	Length (cm)	Diameter (mm)	Weight (g)	Total no. of leaves	No. of 5-leaflet leaves	Leaf area (cm^2)
Morea	17	11	22.1±1.0	3.5±0.1	8.6±0.6	18.9±0.3	7.3±0.4	250±18
	21	23	21.6±0.5	3.7±0.1	8.5±0.3	18.1±0.2	6.8±0.2	222± 8
	25	25	18.4±0.6	3.6±0.1	7.3±0.3	16.9±0.2	4.8±0.2	184±10
Sweet Promise	17	12	34.1±0.8	4.2±0.1	17.0±0.5	16.6±0.3	6.4±0.3	463±13
	21	23	29.6±1.1	3.8±0.1	12.0±0.6	16.1±0.3	5.0±0.3	304±18
	25	10	30.1±1.5	3.8±0.2	11.3±0.8	15.6±0.2	5.3±0.4	291±24

TABLE 7. Effect of temperature during axillary bud formation on growth of axillary buds into shoots *in vitro* of rose 'Motrea' (Exp. 2) and 'Sweet Promise' (Exp. 4). Shoots of 'Motrea' were harvested 35 days after inoculation, shoots of 'Sweet Promise' were harvested when the flower bud was visible. Data are means ± SE.

Cultivar	Temperature (°C)	n	Length (cm)	Weight (g)	Total no. of leaves	Growth period (d)
Motrea	17	28	1.7±0.1	0.35±0.01	18.8±0.3	35
	21	30	2.3±0.1	0.36±0.01	18.7±0.2	35
	25	30	2.2±0.1	0.32±0.01	17.5±0.3	35
Sweet Promise	17	5	3.8±0.7	0.38±0.05	17.6±0.5	34.2±1.6
	21	20	4.0±0.2	0.28±0.01	15.6±0.2	32.7±0.9
	25	19	4.3±0.2	0.26±0.01	16.0±0.3	34.9±0.7

inhibition, i.e. at the time of pruning the parent shoot. As for all temperature treatments the parent shoot was pruned at a similar developmental stage, the physiological age of all buds was presumably the same. Therefore, the effect of temperature was not likely to be confounded with a developmental effect. The number of leaves initiated in the bud was shown to increase with increasing physiological age of the bud (Chapter 4.1). The positive relationship between parent shoot diameter and number of leaf primordia in the axillary bud reported by Byrne & Doss (1981) could not be confirmed in the current experiments, where the diameter of the parent shoot was varied by imposing different temperatures.

Although the number of leaves in the axillary bud was not affected by temperature during axillary bud formation, number of leaves preceding the flower of the subsequent shoot decreased with increasing temperature, irrespective of the cultivar. The number of leaves preceding the flower for buds which sprouted attached to the parent shoot was the same as for buds which were grafted on a cutting or cultured *in vitro*, indicating that the bud meristem developed autonomously. At the time of release from inhibition, the meristem apparently was already determined to produce a specific number of leaves before developing into a flower. A similar observation was reported for tobacco (McDaniel 1978). The number of additional leaves, i.e. leaves formed after release from inhibition, increased as the pre-treatment temperature decreased. At a low temperature carbohydrate content usually increases, because growth falls more than the rate of photosynthesis (Farrar 1988). The effect of low temperature on number of leaves may be mediated via an effect on the carbohydrate content, since it is similar to the effect of a high assimilate supply on number of leaves (Chapter 4.3).

Since the relative humidity was the same for the different temperatures, the vapour pressure deficit differed between the treatments. It cannot be ruled out that this might have affected the results, as reported by Hoffman (1979). However, Grange & Hand (1987) reported that humidities between 1.0 and 0.2 kPa vapour pressure deficit have little effect on the physiology and development of horticultural crops. The vapour pressure deficits at the temperatures in our experiments fell within this range.

The observation that bud break was hastened by increasing pre-treatment temperature, even when buds were isolated from the

parent plant, may indicate that temperature affects the degree of inhibition of the bud, an effect that is located inside the bud itself. In *Citrus*, buds produced under winter conditions required considerably more time before bud burst than those produced under summer conditions (Halim *et al.* 1988). The shorter growth period at higher pre-treatment temperature may also explain why the various growth parameters showed a decrease when temperatures increased.

Although a decrease in temperature might have reduced the rate of cell division (Francis & Barlow 1988), final number of pith cells in transverse section was not greatly affected by temperature during axillary bud formation. As the number of pith cells increased at increasing assimilate supply (Chapter 4.3), the positive effect of the supposed increase in carbohydrate content at low temperatures on cell division may counteract the negative effect of temperature on rate of cell division.

The effect of pre-treatment temperature on growth potential of the buds was less obvious for buds which sprouted attached to the parent shoot than for buds in isolation. Since the *in vitro* experiment on 'Motrea' buds was terminated at a fixed time, while the growth period seemed to decrease with increasing pre-treatment temperature, the effect of temperature might have been larger when shoots had been harvested in a similar developmental stage. When buds sprouted attached to the parent shoot, not only the bud but also the parent plant was affected by temperature, resulting in plants that differed in leaf area (Table 4), shoot diameter and length (Table 1) and likely in amount of stored assimilates. Furthermore, the rate of photosynthesis of the plants, when transferred to 21°C, might have been affected by the pre-treatment temperature, as suggested by Kozlowski *et al.* (1991). These factors might have interacted with the effect of temperature on the bud itself.

Differences in the parent shoot represent the effects of temperature both during the period the parent shoot was still an axillary bud and during subsequent shoot growth. Comparing the effects of temperature during axillary bud formation and shoot growth (Table 1) with the effects of temperature during axillary bud formation only (Table 4 and 6) indicates that the effect of temperature on final size of the parent shoot was largely the result of the temperature during shoot growth. Although within the range 17°C - 25°C, effects of temperature during axillary bud formation (Table 6) and effects during shoot growth (Chapter 5.2) were similarly directed, the effects during shoot growth were much larger than during axillary bud formation. However, the differences in number of leaves of a shoot are only determined by temperature during axillary bud formation.

In conclusion, we found growth conditions during axillary bud formation to affect the subsequent growth of the buds into shoots, which confirm suggestions of Moe (1971), Zamski *et al.* (1985) and Patrick (1988). When buds sprout attached to the parent shoot, which situation occurs in a commercial crop, the effect of a temperature pre-treatment on the bud growth potential is combined with an effect on the parent plant, which both affect subsequent shoot growth.

5. Bud break and shoot growth as affected after release from correlative inhibition

5.1. Effect of assimilate supply on bud break and shoot growth

Marcelis-van Acker C.A.M., 1994. Growth and morphology of the rose shoot as affected by assimilate supply. (submitted).

Abstract. The effect of assimilate supply, varied by retaining a different number of leaves or a different number of competing sprouting buds, on growth and development of rose shoots was studied. An increase in assimilate supply did not affect the rate of bud break, but shortened the subsequent growth period until harvest. Rate of elongation was stimulated until three weeks after start of the treatments, indicating that at that time shoots became self-supporting for assimilates. At harvest, shoot size was positively affected by an increase in assimilate supply; the increase in shoot diameter was mainly the result of an increase in pith diameter, caused by more cell enlargement. Assimilate supply did not influence the number of leaves preceding the flower, indicating that the axillary bud meristem was already determined to produce a specific number of leaves and to develop into a flower when the treatments were started.

Introduction

In a rose crop a large variation exists in plant types (Kool *et al.* 1991) and flower production (Zieslin *et al.* 1973). Flower production per plant depends on the number of structural shoots, the number of sprouting buds and the number of blind shoots (Zieslin *et al.* 1973). Not only the number of flowering branches, but also their length and diameter, which are important parameters for shoot quality, vary largely between individual plants. Another source of variability is the length of time elapsing between one harvest and the next. Differences between plants increase with age of the plants, resulting in a crop with a number of different developmental stages (varying from sprouting buds to harvestable shoots) at the same time.

One of the variable factors in a crop is the assimilate supply for a single sprouting bud. Experiments in which the effect of assimilate supply on development and growth potential of axillary buds was studied, indicated that shoot growth is to a major extent dependent on the assimilate supply available while growth is taking place (Chapter 4.3). However, quantitative data on the effect of assimilate supply on shoot growth are scarce. In order to study interrelationships between

several independent environmental factors in their effect on growth and development of flowering shoots of rose attempts have been made to develop a simulation model (Hopper & Hammer 1991; Lieth & Pasian 1991). To develop and validate such a simulation model quantitative data on shoot growth are necessary.

When an axillary bud is released from inhibition, by pruning the part of the shoot above it, the bud is still vegetative (Chapter 2.1). After the bud has started sprouting, the bud meristem becomes generative (Horridge & Cockshull 1974; Chapter 2.1). McDaniel (1978) defined in tobacco the number of leaves produced by an axillary bud meristem before differentiation into a flower as its developmental potential. In a number of plant species assimilate supply is assumed to affect flower initiation (Sachs & Hackett 1969). Assimilate supply might affect the developmental potential of axillary buds in rose.

In the present study the effect of assimilate supply on growth and morphology of axillary buds after release from inhibition was investigated. The assimilate supply was varied by changing the number of leaves or the number of competing lateral shoots. Since the pith represents the primary growth of a shoot and contributes to a large extent to the shoot diameter (Chapter 2.1), it was investigated whether the effect of assimilate supply on shoot diameter was mediated by effects on cell number or on cell size of the pith.

Materials and Methods

Three experiments were carried out. For all experiments double node cuttings, as described elsewhere (Marcelis-van Acker & Leutscher 1993), of *Rosa hybrida* cv. Motrea and *Rosa hybrida* cv. Sweet Promise were used. One shoot (the primary shoot) was allowed to develop on each cutting. Developmental stage of the flower was assessed according to the scale used by Halevy & Zieslin (1969), in which 1 represents flower bud visible without dissection; 2 denotes flower bud pea-sized; 3 indicates sepals closed and colour of flower not visible; 4 represents sepals closed but colour of flower visible; 5 indicates sepals reflexing. Treatments were applied to this primary shoot when its flower was at developmental stage 3 (Experiment 1) or stage 4 (Experiments 2 and 3). At the start of the treatments the cutting leaf was removed. Lateral shoots were harvested when their flower was at developmental stage 4 (Experiment 1) or stage 5 (Experiments 2 and 3).

The assimilate supply was varied by retaining a different number of leaves on the primary shoot in Experiment 1 (cv. Motrea) and in Experiment 2 (cv. Sweet Promise) or by retaining a different number of lateral shoots on the primary shoot in Experiment 3 (cv. Sweet Promise). Plants of Experiments 1 and 2 were grown in a climate chamber at a temperature of 21°C, a relative air humidity of about 70% and 25 W m^{-2} photosynthetically active radiation (PAR) provided by Philips fluorescent tubes, and a day length of 16 h; plants of Experiment 3 were grown in a greenhouse (temperature day/night set at 20/17°C).

In Experiment 1 shoots were pruned just above the uppermost five-leaflet leaf and nine leaves (all leaves; 280±30 cm^2 leaf area), five leaves (every other leaf; 164±10 cm^2), one leaf (uppermost leaf; 48±5 cm^2) or two fifth of a leaf (two out of five leaflets of the uppermost leaf; 22±3 cm^2) were retained. In Experiment 2 shoots were pruned at the fifth five-leaflet leaf and five leaves (all leaves),

three leaves (every other leaf) or one leaf (uppermost leaf) were retained. In both experiments the bud in the axil of the uppermost leaf was allowed to grow into a shoot, while other emerging lateral shoots were continually removed.

In Experiment 3 shoots were pruned at the fourth five-leaflet leaf and the bud in the axil of the uppermost leaf, the uppermost two or uppermost three leaves were allowed to grow into a shoot, while other emerging lateral shoots were continually removed. Growth of the uppermost shoot was studied.

The treatments were arranged in a randomized block design, with ten replicate plants arranged in ten blocks in Experiment 1, twelve replicate plants arranged in three blocks in Experiment 2 and eight replicate plants arranged in four blocks in Experiment 3. Bud break (defined as buds with a length of 0.5 cm) was recorded and the time course of shoot length was measured. At harvest length, diameter (at 1 cm from the base of the shoot), fresh weight, number of leaves and leaflets (per leaf) and leaf area of the shoots were determined. In Experiment 1 thin transverse hand cut sections were made at 1 cm from the base of the shoot. Diameter of the pith was measured using an ocular micrometer and number of pith cells was counted on three diameter lines per section. Data were analysed by analysis of variance and the significance of differences determined by Student's *t*-test (P=0.05).

Results

For both cultivars an increase in assimilate supply, imposed by varying the number of leaves, did not affect the time until bud break, but shortened the subsequent growth period until harvest (Tables 1 and 2). An increase in assimilate supply stimulated the rate of elongation until about 20 days after start of the treatments, resulting in an increase in shoot length (Fig. 1 and 2). The time until the flower bud was visible without dissection was recorded in Experiment 1 and appeared to decrease with increasing assimilate supply (Fig. 1). At harvest length, diameter, weight and leaf area of the shoot were positively influenced by an increase in assimilate supply (Tables 1 and 2), whereas the number of leaves and number of leaflets per leaf were not significantly affected (Fig. 3 and 4) for both

TABLE 1. Effect of number of leaves on the parent shoot (PS) on shoot characteristics of rose 'Motrea'. Shoots were harvested when the flower was at the stage 'sepals closed, but colour of flower visible'. Data are means of 10 replicate plants (Experiment 1).

No. of leaves PS	Time from pruning until bud break (d)	Time from pruning until harvest (d)	Length (cm)	Fresh weight (g)	Leaf area (cm^2)	Total no. of leaves	Diameter shoot (mm)	Diameter pith (mm)	No. of pith cells
2/5[x]	3.1	35.2	23.0	7.7	339	14.4	3.0	1.8	27.2
1	2.9	33.6	24.7	8.7	366	14.0	3.2	2.0	27.9
5	3.1	31.0	26.7	11.0	460	14.6	3.5	2.2	27.9
9	2.9	30.4	29.1	13.2	509	14.7	3.8	2.4	28.6
LSD (P=0.05)	0.8	0.9	1.4	0.7	30	0.7	0.1	0.2	1.3

[x] three of the five leaflets of the leaf were removed

cultivars. The increase in shoot diameter with increasing assimilate supply was to a major extent the result of an increase in pith tissue (Table 1) and to a minor extent also of growth of remaining tissues (xylem, phloem and cortex; data not shown). The number of pith cells was not clearly affected by the assimilate supply, indicating that the increase in pith tissue was achieved by an increase in cell size (Table 1). It can be calculated that the relative area of the pith on transverse section increased with increasing assimilate supply,

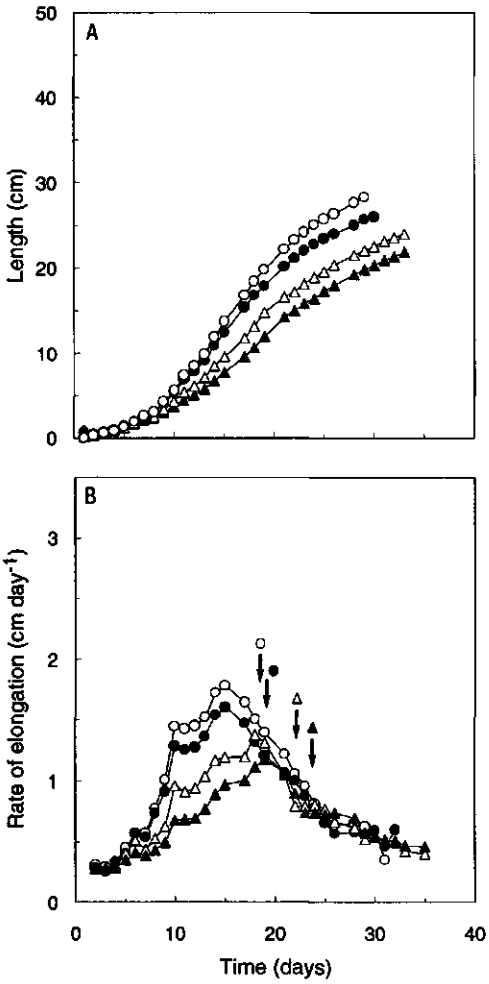


FIG. 1. Effect of number of leaves on the parent shoot on shoot growth (A) and rate of elongation (B) of rose 'Motrea'. Shoots were harvested when the flower was at the stage 'sepals reflexing'. Arrows indicate the time that the flower bud became visible without dissection. Data are means of 10 replicate plants (Experiment 1).
(▲) 2/5 leaf: three of the five leaflets removed; (△) 1 leaf; (●) 5 leaves; (○) 9 leaves.

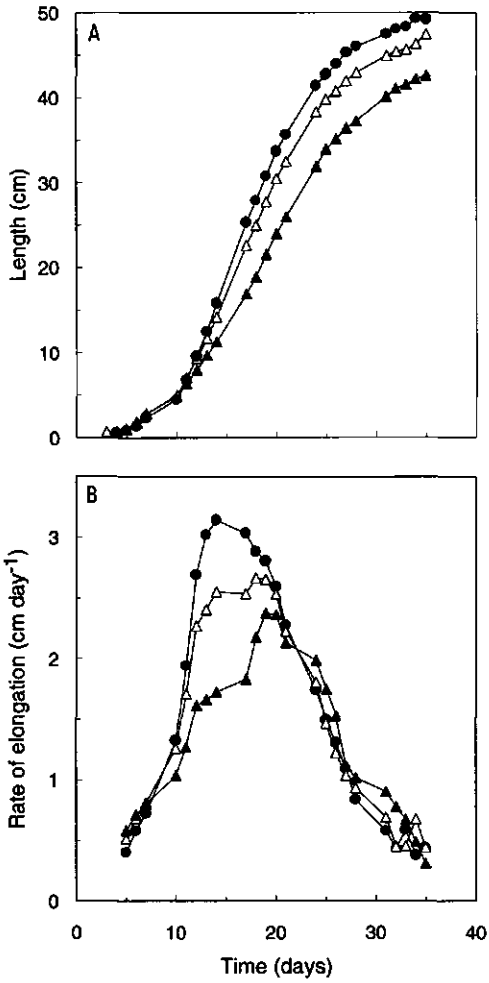


FIG. 2. Effect of number of leaves on the parent shoot on shoot growth (A) and rate of elongation (B) of rose 'Sweet Promise'. Shoots were harvested when the flower was at the stage 'sepals reflexing'. Data are means of 12 replicate plants (Experiment 2).
(▲) 1 leaf; (△) 3 leaves; (●) 5 leaves.

TABLE 2. Effect of number of leaves on the parent shoot (PS) on shoot characteristics of rose 'Sweet Promise'. Shoots were harvested when the flower was at the stage 'sepals reflexing'. Data are means of 12 replicate plants (Experiment 2).

No. of leaves PS	Time from pruning until bud break (d)	Time from pruning until harvest (d)	Length (cm)	Fresh weight (g)	Leaf area (cm^2)	Total no. of leaves	Diameter shoot (mm)
1	4.5	37.4	43.1	18.6	698	16.5	4.5
3	4.3	34.8	46.1	20.5	756	15.5	4.9
5	4.2	34.3	48.9	22.9	815	16.1	5.3
LSD (*P*=0.05)	0.2	2.3	5.1	2.6	79	1.9	0.4

while the absolute areas of both pith and secondary tissues also increased (data not shown). The effect of assimilate supply imposed by the number of competing sprouting buds (Experiment 3) was comparable to the effect of changing the number of leaves (Table 3).

Discussion

Assimilate supply did not influence the rate of bud break, which is in accordance with results of Zieslin *et al.* (1976a). This indicates that bud break is an intrinsic characteristic of the buds, as was reported for *Citrus* by Halim *et al.* (1988). Assimilate supply affected the rate of elongation during the first three weeks of shoot growth; by that time the shoots had reached the developmental stage that the flower bud was visible without dissection. Probably at that time the new shoot became self-supporting for assimilates. Results of Mor & Halevy (1979) support this hypothesis; they found for rose cv. Marimba that a shoot with a length of 16-20 cm and a terminal flower bud of 4 mm in diameter was no longer a sink for the assimilates produced by the mature leaves on the lower branch; until that time most of the assimilates were directed from the mature leaves on the lower branch to the young shoot.

Length, diameter, weight and leaf area of the shoot at harvest appeared to be dependent on conditions during shoot growth. When more than one bud were allowed to grow out

TABLE 3. Effect of competing lateral shoots (CLS) on shoot characteristics of rose 'Sweet Promise'. Shoots were harvested when the flower was at the stage 'sepals reflexing'. Data are means of 8 replicate plants (Experiment 3).

No. of CLS	Time from pruning until bud break (d)	Time from pruning until harvest (d)	Length (cm)	Fresh weight (g)	Leaf area (cm^2)	Total no. of leaves	Diameter shoot (mm)
0	5.5	36.1	49.7	25.2	615	14.9	6.1
1	4.6	37.1	47.4	19.6	521	15.3	5.3
2	5.9	38.1	44.4	15.6	437	15.5	4.7
LSD (*P*=0.05)	1.2	1.3	3.0	1.9	51	1.2	0.5

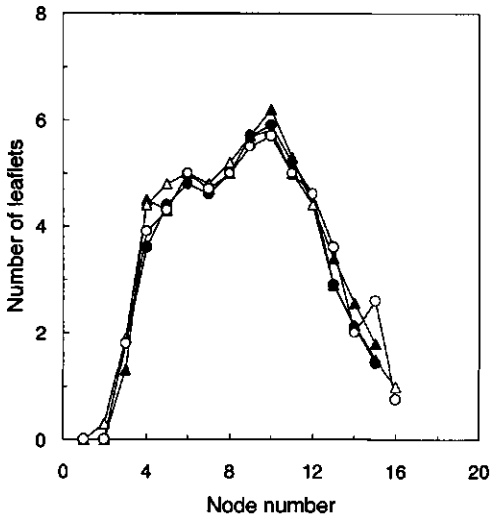


FIG. 3. Effect of number of leaves on the parent shoot on number of leaflets per leaf along the shoot of rose 'Motrea'. Nodes are numbered acropetally. Data are means of 10 replicate plants (Experiment 1).
(▲) 2/5 leaf: three of the five leaflets removed; (△) 1 leaf; (●) 5 leaves; (○) 9 leaves.

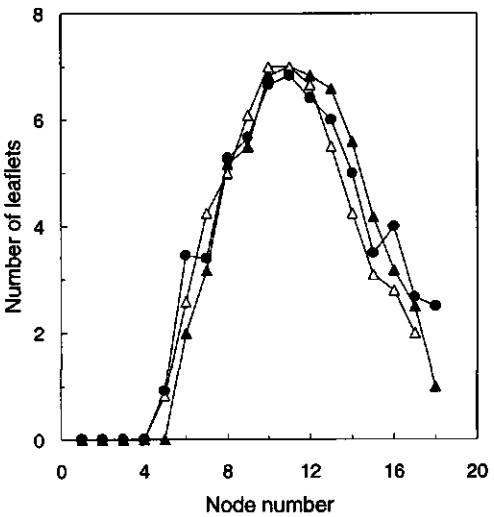


FIG. 4. Effect of number of leaves on the parent shoot on number of leaflets per leaf along the shoot of rose 'Sweet Promise'. Nodes are numbered acropetally. Data are means of 12 replicate plants (Experiment 2).
(▲) 1 leaf; (△) 3 leaves; (●) 5 leaves.

on the same plant, the effects were similar to the effect of reducing the number of leaves. Although the sprouting uppermost bud was found to exert apical control over lower shoots on the same branch by diversion of assimilates away from them (Mor *et al.* 1981), our data show that assimilate supply for the upper shoot was reduced by the competing lower shoots.

Although the leaf area and the shoot length were clearly affected by the assimilate supply during shoot growth, the number of leaves and the leaf shape (number of leaflets per leaf) were not influenced. Rylski & Halevy (1972) also found for pepper and McDaniel (1980) for tobacco no effect of leaf removal on the number of leaves preceding the flower. The number of leaves formed by the axillary bud meristem before flower formation was not affected by lower sprouting buds, which is in accordance with data of McDaniel *et al.* (1989) for tobacco. When we varied the assimilate supply by applying different light intensities (10-70 W m^{-2}), the number of leaves preceding the flower was also not influenced (data not shown). Although at the start of the treatments only about 9 leaf primordia (Motrea) and 11 leaf primordia (Sweet Promise) were present in the axillary bud, the results suggest that the bud meristem had already been determined to produce a specific number of leaves and develop into a flower when the treatments were started, as was shown for tobacco (McDaniel 1978). The observation that the number of leaflets per leaf was independent on the assimilate supply may support this hypothesis.

An increase in assimilate supply resulted in thicker shoots, which was largely caused by an increase in pith tissue. Furthermore the po-

tential diameter of a shoot is to a large extent determined by the potential diameter of the pith, which in turn is determined soon after bud break (Chapter 2.1). It can be calculated that at harvest the fraction of pith in the total diameter was still approximately 60%. The area of both pith and remaining tissues (xylem, phloem and cortex) increased at increasing assimilate supply, indicating a greater capacity for transport and storage. Assimilate supply during shoot growth did not clearly affect the number of pith cells in transverse section, which might be explained by the observation that the number of pith cells has already been formed in the axillary bud; after bud break cell division in cross section ceases and only cell enlargement occurs (Chapter 2.1). Shoot elongation is the result of transverse divisions of pith cells, leading to vertical files of cells (Esau 1977; Mauseth 1988). The slight decrease in number of cells at decreasing assimilate supply (Table 1) might indicate that not all cells contribute to shoot elongation.

In a commercial rose crop, the number of buds that sprout after harvesting of the flower varies from one to four (Mor *et al.* 1981), while also the number of leaves remaining on the plant varies. As a result of these differences in assimilate supply, not only shoot length and diameter but also time elapsing between one harvest and the next will differ between shoots, resulting in variation in the crop.

5.2. Effect of temperature on bud break and shoot growth

Marcelis-van Acker C.A.M. 1994. Growth and morphology of the rose shoot as affected by temperature. (submitted).

Abstract. The effect of temperature on growth and morphology of shoots of rose cultivars Sweet Promise and Motrea was studied. Rate of development of the shoot, reflected in length of growth period and rates of leaf appearance and stem elongation, was accelerated with increasing temperatures. Appearance of the flower bud occurred at or shortly after the time the maximum rate of elongation was reached. Total number of leaves preceding the flower was not distinctly affected by temperature, indicating that the bud meristem was already at the start of the treatments determined to initiate a limited number of additional leaves before developing into a flower. The number of additional leaves was higher for 'Motrea' than for 'Sweet Promise'. The number of leaflets of the uppermost leaves increased with increasing temperature. Shoot size at harvest decreased with increasing temperature. 'Sweet Promise' formed larger shoots within a shorter time period than 'Motrea'. An increase in diameter at lower temperatures was the result of an increase in diameter of both pith tissue and remaining tissues. The increase in pith diameter was to a large extent due to an increase in cell size.

Introduction

Temperature has a pronounced effect on shoot development of greenhouse roses (Moe 1972; De Vries & Smeets 1979; Van den Berg 1987). Generally, higher temperature hastens development, resulting in a shorter growth period and smaller shoots. Effects of temperature on leaf appearance and shoot morphology in rose, however, are not well documented. Furthermore, most temperature-experiments described in the literature were carried out with large full-grown rose plants, wherein effects of temperature on growth of an individual shoot are difficult to interpret, in view of competition of other shoots and differences in the storage and remobilization of assimilates.

In order to study interrelationships between several independent environmental factors in their effect on growth and development of flowering shoots of rose, attempts have been made to develop a simulation model of shoot growth (Hopper & Hammer 1991; Lieth & Pasian 1991). Such a model can be useful to optimize rose crop production. However, quantitative data on shoot growth and morphology, necessary to develop such a model, are scarce (Lieth & Pasian 1991).

In the present study the effect of temperature on shoot growth and morphology of *Rosa hybrida* cultivar Sweet Promise (a large sized rose) and *Rosa hybrida* cultivar Motrea (a small sized rose) was studied. To prevent indirect effects of temperature on shoot growth by effects on storage and remobilization of assimilates, experiments were carried out with rooted cuttings in which only one shoot was allowed to grow. Effects of temperature on rates of leaf appearance and stem

elongation and on shoot size and morphology were recorded. Moreover, to get a better insight in the effect of temperature on the internal structure of the shoot, cell number and cell size of the pith were studied. The pith reflects the primary growth of the shoot. Shoot diameter, which is an important parameter for quality of a flower shoot, highly correlates with the diameter of the pith (Chapter 2.1). Furthermore, the pith was found to contribute to a large part of the shoot diameter (Chapter 2.1).

Materials and Methods

Rosa hybrida cv. Sweet Promise and *Rosa hybrida* cv. Motrea were grown in a greenhouse (temperature set at 21°C). In October double node cuttings, as described by Marcelis-van Acker & Leutscher (1993), were cut and rooted in the same greenhouse. When cuttings had rooted, of each cultivar four groups of 27 cuttings each were transferred to four growth chambers, that were maintained at a temperature of 13°C, 17°C, 21°C or 25°C (day and night) and a relative air humidity of about 70%. Light intensity was 40 W m^{-2} PAR provided by Philips high pressure sodium lamps (SON/T) and metal halide lamps (HPI/T). Day length was 16 h. As soon as the cuttings had been transferred to the growth chambers, they were pruned above the lower leaf to release the bud in its axil from inhibition. In this way bud break and shoot growth occurred at the imposed temperatures, while axillary bud formation and rooting of the cutting had occurred at similar conditions for all plants. At the day of transfer to the growth chambers, the number of leaves and leaf primordia in the axillary bud of the lower leaf of nine cuttings of each cultivar was recorded by use of a dissecting microscope (x50).

Shoot length, number of compound leaves visible without dissection (> 0.5 cm) and number of compound leaves with fully unfolded leaflets of nine randomly chosen plants were recorded three times a week. When the flower of the shoot was at the harvestable stage (sepals reflexing) its length, diameter (at 1 cm from the base of the shoot), fresh weight, number of leaves (including scales) and leaflets (per leaf) and total leaf area were determined. For each temperature treatment thin transverse hand cut sections were made at 1 cm from the shoot base of nine randomly chosen plants of each cultivar. Per transverse section the diameter of the pith was measured using an ocular micrometer and the number of pith cells on a diameter line was recorded. This was repeated for three diameter lines per shoot. Cell diameter was calculated as the ratio between pith diameter and cell number.

Data were analysed by fitting linear and quadratic regression functions. Significance of the regression parameters was tested at the 5% level.

Results

Cuttings of cv. Sweet Promise clearly formed larger shoots within a shorter time period than cuttings of cv. Motrea.

At the start of the treatments the axillary bud contained 12.1±0.3 ('Sweet Promise') or 11.0±0.3 ('Motrea') leaves including leaf primordia. The outer 6 to 7 leaves were reduced to scales. Time until bud break (start of shoot elongation) decreased with increasing temperature for both 'Sweet Promise' (Fig. 1A) and 'Motrea' (Fig. 1B). Maximum rate of elongation increased with increasing tempera-

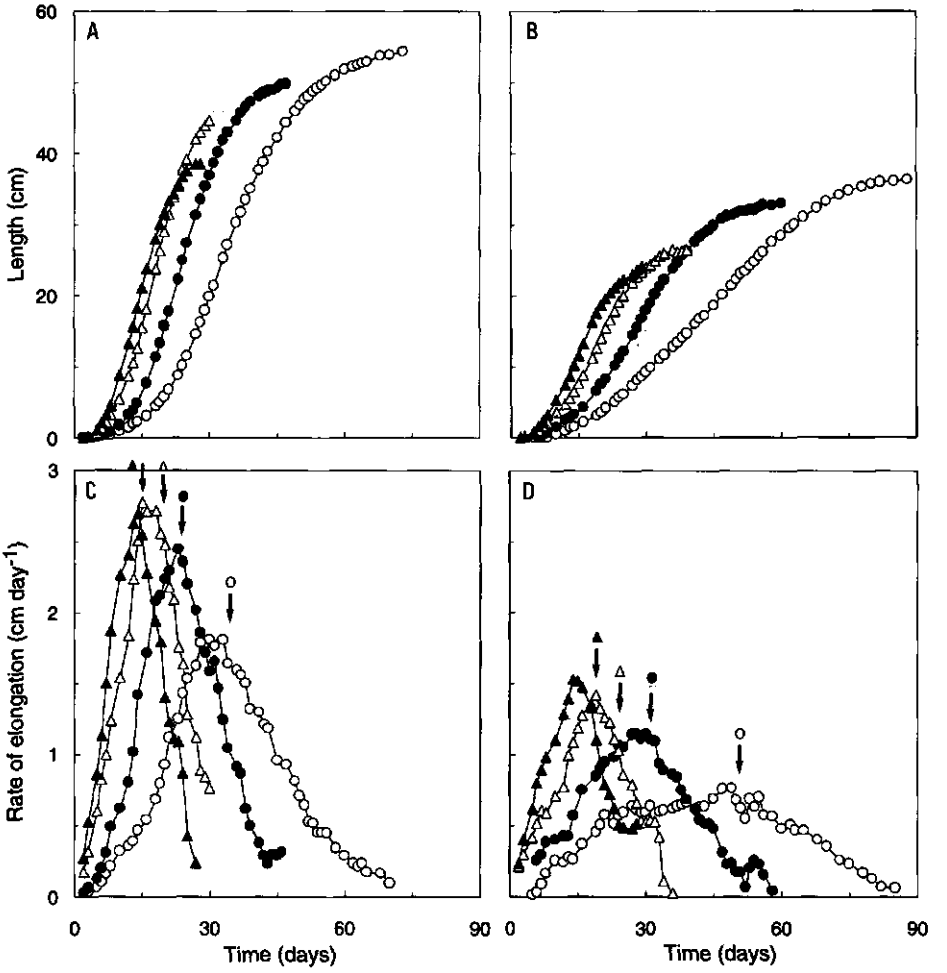


FIG. 1. Effect of temperature on shoot length (A,B) and rate of elongation (C,D) of rose 'Sweet Promise' (A,C) and 'Motrea' (B,D) in relation to time after release from correlative inhibition. Shoots were harvested when the flower was in the stage 'sepals reflexing'. Arrows indicate the time that the flower bud was visible without dissection.
○: 13°C; ●: 17°C; △: 21°C; ▲: 25°C.

ture, but for 'Sweet Promise' maximum rate of elongation at 25°C was the same as at 21°C (Fig. 1B,D). Final shoot length, however, decreased with increasing temperature. Appearance of the flower bud occurred at or shortly after the time the maximum rate of elongation was reached (Fig. 1C,D). The reciprocals of the time from pruning until bud break (buds 0.5 cm long) and from bud break until harvest increased linearly with temperature and were larger for 'Sweet Promise' than for 'Motrea' (Fig. 2).

Leaf appearance was accelerated by increasing the temperature for both cultivars (Fig. 3). The effect of temperature on leaf unfolding was similar to the effect on leaf ap-

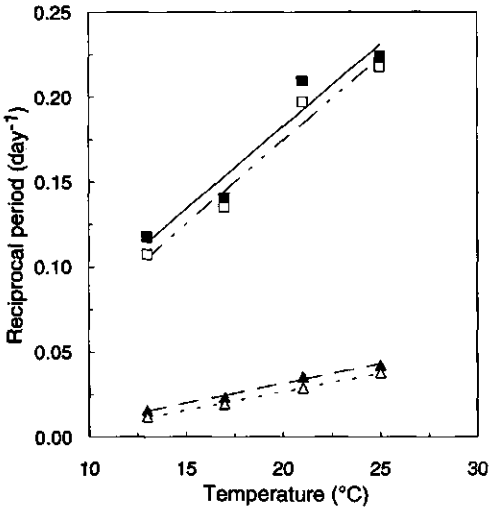


FIG. 2. Effect of temperature on reciprocals of time from pruning until bud break (▲△) and from bud break until 'sepals reflexing' (■□) of shoots of rose 'Sweet Promise' (closed symbols) and 'Motrea' (open symbols). The curves were fitted as a linear function of temperature.
——: Y = -0.0119+0.00972T; P=0.03
– – –: Y = -0.0226+0.00984T; P=0.02
— —: Y = -0.0149+0.00232T; P=0.005
- - - -: Y = -0.0168+0.00217T; P<0.001

pearance (data not shown). For 'Sweet Promise' total number of leaves was not affected by temperature, whereas for 'Motrea' it might be slightly higher at the lowest temperature, but this was not statistically significant. The number of additional leaves, i.e. leaves initiated after release from correlative inhibition, and the total number of compound leaves were larger for 'Motrea' than for 'Sweet Promise' (Fig. 3). Higher temperature resulted in more leaflets per leaf of the upper leaves, whereas the number of leaflets of lower positioned leaves was not affected (Fig. 4). The numbers of non-elongated and elongated internodes were not affected by temperature, but the average length of the elongated internodes decreased with increasing temperature (Fig. 5). Internodes of 'Sweet Promise' were almost twice as long as those of 'Motrea'.

At harvest, leaf area of the shoot showed an optimum at a temperature of 17 to 21°C for both cultivars (Table 1). Weight and diameter of the shoot increased with decreasing temperature, although no difference in weight between 17°C and 13°C shoots was found. The increase in shoot diameter was the result of an increase in diameter of both pith tissue and remaining tissues (xylem, phloem and cortex). The increase in pith diameter was to a large extent the result of an increase in cell size. Cell number was not affected by temperature for 'Sweet Promise' and slightly decreased with increasing temperature for 'Motrea' (Table 1).

Discussion

Most of the observed effects of temperature were similar for both cultivars, although their shoot size differed largely. Rate of development of the shoot, reflected in growth period and rates of leaf appearance and stem elongation, was accelerated at higher temperature, which is in accordance with the general response of plant organs to increasing temperature (Moe & Heins 1991; Hopper & Hammer 1991; Marcelis & Baan Hofman-Eijer 1993). The reciprocal of the time between two events is a measure of the rate at which a process is completed (Dennet *et al.* 1979). Rates of development are linear with increasing temperature (Porter & Delecolle 1988), which is in accordance with our results. The increase in rate of leaf appearance with temperature fits in with results of Pieters (1974) on poplar, Hay & Tunnicliffe Wilson (1982) on wheat and Marcelis (1993b) on cucumber. It should be mentioned that leaf appearance (macro-

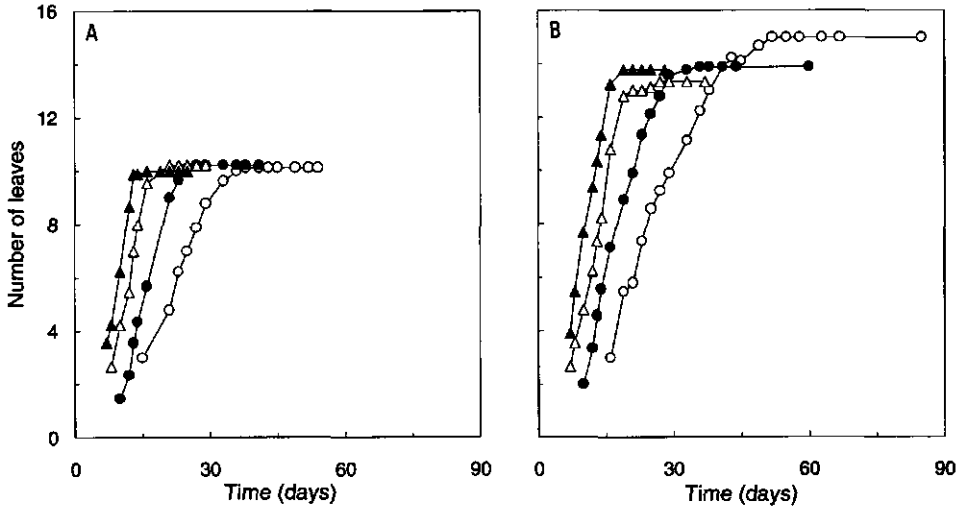


FIG. 3. Effect of temperature on appearance of compound leaves per shoot of rose 'Sweet Promise' (A) and 'Motrea' (B) in relation to time after release from correlative inhibition.
○: 13°C; ●: 17°C; △: 21°C; ▲: 25°C.

scopically visible leaves) may not be only regulated by the environment but may also depend on rate of leaf initiation as well, as reported by Hay & Kemp (1990) for wheat. In rose, a clear effect of temperature on leaf initiation rate during axillary bud formation was found (Chapter 4.4). Although the rate of leaf appearance increased, final number of

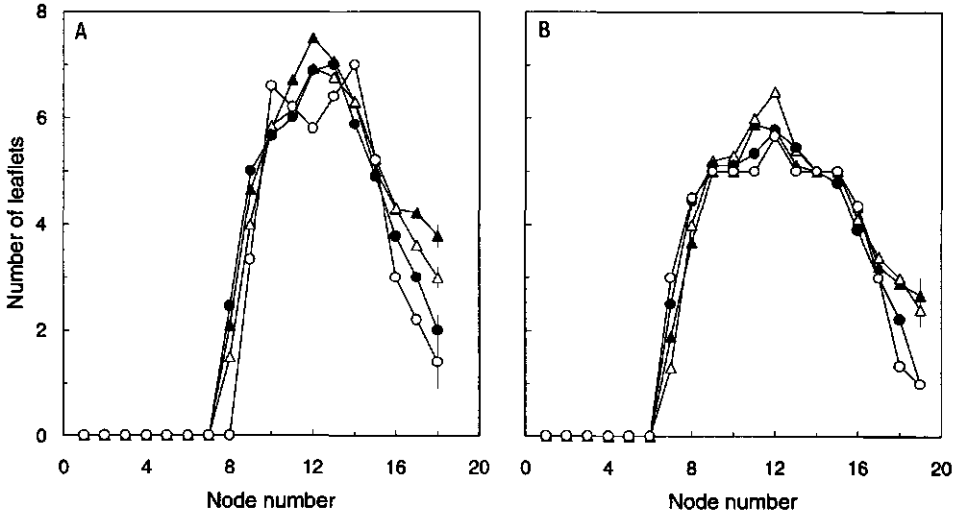


FIG. 4. Effect of temperature on number of leaflets per leaf along the shoot of rose 'Sweet Promise' (A) and 'Motrea' (B). Nodes are numbered acropetally. Vertical bars at the highest node indicate SE_{mean}, when larger than symbols. ○: 13°C; ●: 17°C; △: 21°C; ▲: 25°C.

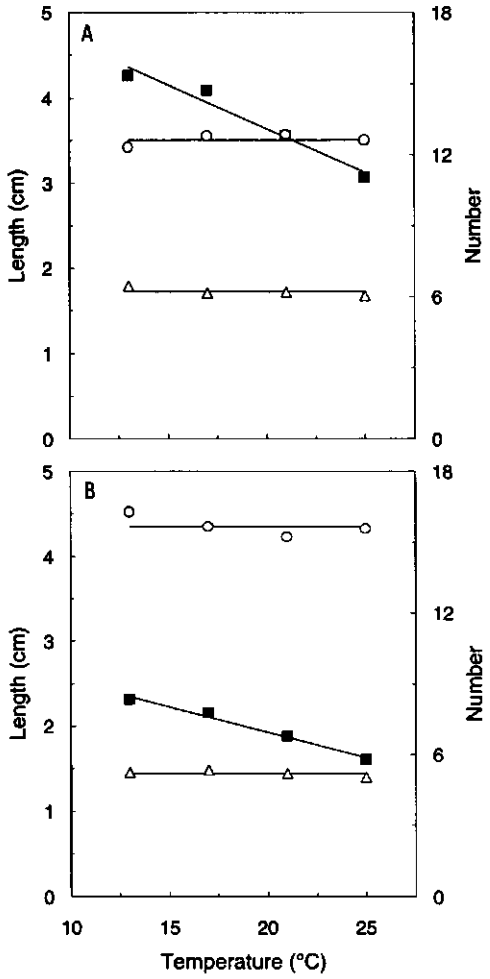


FIG. 5. Effect of temperature on numbers of non-elongated (△) and elongated (○) internodes and average length of elongated internodes (■) of shoots of rose 'Sweet Promise' (A) and 'Motrea' (B).
A. Length: Y = 5.706-0.1032T; P=0.018
B. Length: Y = 3.123-0.0598T; P=0.007

leaves, including scale-like leaves, was not affected by temperature. These results confirm results of Moe (1972), Van den Berg (1987) and Hopper & Hammer (1991), although they obviously did not consider the scale-like leaves at the base of the shoot. The number of additional leaves, i.e. leaves initiated after release from correlative inhibition of the axillary bud, was larger for 'Motrea' than for 'Sweet Promise', indicating that in an axillary bud of 'Sweet Promise' a (relatively) larger part of the future shoot is already initiated than in buds of 'Motrea'. As a result, the apical meristem of 'Motrea' might be affected by environmental influences during shoot growth to a larger extent than the apical meristem of 'Sweet Promise'.

Low temperature had a pronounced positive effect on final shoot size. Although lower temperatures had a negative effect on growth rate, the increased duration of growth resulted in larger shoots. Temperature may also affect photosynthesis and cell division and expansion. Net photosynthesis of individual rose leaves has a wide optimum temperature range from 15 to 37°C at saturating irradiance and ambient CO_2 concentration (Bozarth *et al.* 1982; Lieth & Pasian 1990). However, temperature affected leaf area of the individual leaves, which may result in an effect on net photosynthesis of the shoot. For 'Samantha' roses, the optimum temperature range for whole plant net photosynthesis at saturating irradiance and ambient CO_2 level was reported to be between 20 and 25°C at all stages of shoot development (Jiao *et al.* 1991), whereas at low irradiance a reduction in air temperature increased whole plant net photosynthesis by reducing respiration. At low temperatures, carbohydrate content will increase since growth falls more than the rate of photosynthesis (Farrar 1988).

As the air humidity was kept constant in the experiment, the vapour pressure deficit differed between the treatments. It cannot be ruled out that this may have affected the results, as reported by Hoffman (1979). However, Grange & Hand (1987) reported that humidities between 1.0 and 0.2 kPa va-

TABLE 1. Effect of temperature on some shoot characteristics of rose 'Sweet Promise' and 'Motrea'. Data are means ± SE_{mean}. Shoots were harvested when the flower was at the stage 'sepals reflexing'. Shoot characteristics were fitted as a linear and a quadratic function of temperature.

Cultivar	Temperature (°C)	Fresh weight (g)	Leaf area (cm^2)	Diameter shoot (mm)	Diameter pith (mm)	No. of cells	Cell diameter (µm)
Sonia	13	22.5±0.5	373±9	6.6±0.2	3.3±0.1	31.5±0.6	103±2
	17	25.2±0.5	528±10	6.3±0.1	3.0±0.1	31.1±0.5	97±2
	21	19.8±0.5	553±14	5.2±0.1	2.9±0.1	31.0±0.6	93±1
	25	15.0±0.4	471±12	4.7±0.1	2.5±0.1	30.1±0.4	85±2
	Linear	ns	ns	P=0.04[b]	P=0.01[c]	ns	P=0.01[d]
	Quadratic	ns	P=0.03[a]	ns	ns	ns	ns
Motrea	13	16.4±0.4	299±7	5.2±0.1	2.3±0.1	30.8±0.4	74±1
	17	16.4±0.5	412±10	4.7±0.1	2.2±0.1	30.0±0.7	74±2
	21	12.4±0.3	397±10	4.2±0.1	2.1±0.1	29.6±0.7	71±1
	25	9.3±0.2	317±7	3.9±0.1	1.9±0.1	28.6±0.6	67±2
	Linear	P=0.05[e]	ns	P=0.01[f]	P=0.03[g]	P=0.01[h]	ns
	Quadratic	ns	ns	ns	ns	ns	ns

[a] $Y=-935+148.91T-3.709T^2$
[b] $Y=8.371-0.1628T$
[c] $Y=4.01-0.05665T$
[d] $Y=122.84-1.488T$
[e] $Y=25.67-0.633T$
[f] $Y=6.716-0.11244T$
[g] $Y=2.6829-0.02889T$
[h] $Y=33.012-0.1713T$
ns = not significant (P=0.05)

pour pressure deficit have little effect on the physiology and development of horticultural crops. The vapour pressure deficits at the temperatures in our experiments fell within this range.

Shoot length decreased with increasing temperature. Internode length similarly decreased, as was also found by Moe & Kristoffersen (1969) and Van den Berg (1987). Moe & Heins (1991), however, reported that in a wide range of pot and bedding plants internode length was little affected by average daily temperature, while in *Lilium longiflorum* Erwin *et al.* (1989) found an increased internode length with increasing temperature. The shorter internodes at higher temperatures may be caused by the increased rate of leaf initiation. In poplar, the length of growth periods of leaves and subtending internodes were related (Pieters 1974). Furthermore, the carbohydrate availability in the elongation region of the stem may have been altered by the temperature. The differences in elongation may also be mediated through differences in hormone synthesis or action, probably gibberellin (Erwin *et al.* 1989). Variation in internode length is due to differences in cell number and/or cell length. In *Helianthus* cell enlargement appeared to be the dominant factor in internodal development (Wetmore & Garrison 1966), but Brown & Sommer (1992) reported a dominant role of cell division and increases in cell number to final internode length in several woody plants. It would be interesting to study the cellular basis of internodal elongation in rose.

High temperature increased the number of leaflets per leaf for the upper leaves only. Since the flower bud in rose may act as a strong sink (Mor & Halevy 1979), the number of leaflets of the upper leaves might be affected by competition for assimilates between the leaf primordia and the flower bud. The effect of temperature on the number of leaflets of the upper leaves might be due to an effect on sink strength of these leaf primordia relative to that of the flower bud. The number of leaflets was not affected in the lower leaves, which were already initiated in the axillary bud before start of the temperature treatment. Obviously, the specific number of leaflets of a leaf was already determined at that early stage. Only final leaf size could be affected by temperature yet. In contrast to our results on rose, Humphries & Wheeler (1963) reported that in general lower temperature results in more leaflets per leaf.

Low temperature increased the diameter of both pith tissue and remaining tissues (xylem, phloem and cortex), resulting in an increased capacity for storage and transport of assimilates. Pith diameter was measured as a parameter for primary growth. The effect of temperature on pith diameter was to a large extent due to an effect on cell expansion, although for 'Motrea' also the number of cells increased at low temperature. The increase in cell size might result from an increase in osmotic value at low temperature, as was suggested by Hoek *et al.* (1993). Temperature during shoot growth seemed not to have a large effect on cell number in cross section. Number of pith cells in a cross section was found to be fairly constant after bud break (Chapter 2.1) and might mainly be affected by growth conditions before bud break.

6. General discussion

Although axillary buds are responsible for building the frame of the rose plant and determine potential flower production, they have been no frequent subject of study. In the present study the development of axillary buds and shoots has been evaluated, in order to get a better insight in the development of a rose plant and in factors influencing its development. In this Chapter results from the preceding Chapters are integrated into a coherent view on the development of a bud and shoot as a flexible and plastic programme. The programme described holds for both cultivars studied. The differences between the cultivars are discussed in the preceding Chapters. Furthermore, consequences for practical rose growing will be raised.

Developmental programme

The development from a bud meristem into a flowering shoot can be regarded as a continuous developmental programme, comprising initiation of stem units and finally a flower. The buds in the axils of the 1-3 leaves immediately below the flower are different from the lower positioned buds. These buds are assumed to belong to the inflorescence (De Vries & Dubois, 1992) and are not taken into account in the following discussion. Several stages can be distinguished. First, axillary buds need to reach a certain developmental stage before they are able to break. When this requirement is met, release from correlative inhibition is prerequisite for bud break. Finally, initiation of the flower is controlled by apical dominance and does not occur before bud break. Thus, there are several check points along the sequence of the programme and the stage which is reached depends on the prevailing internal and external conditions (Fig. 1).

Axillary bud

Formation of axillary bud

A flowering shoot starts its developmental programme as an axillary bud meristem. In each leaf axil only one axillary bud is formed, unlike several other species where a number of equivalent buds are formed within a leaf axil (Gould *et al.* 1987; Tourn *et al.* 1992). Usually, axillary buds are inhibited by higher situated growth centres. Although axillary buds are sinks for assimilates (Maillette 1982; Kozlowski 1992), the sink strength of the shoot apex predominates. When the distance between the axillary bud and the apex increases, due to growth of the apex, the relative sink strength of an axillary bud meristem at a certain position on the shoot decreases, resulting in a slowing down of its growth and development. Although bud age increases towards the base of the shoot, permitting an enhanced development, development of lower buds is less compared to that of higher positioned buds (Chapter 4.2), indicating disadvantageous conditions prevailing in the lower buds. The correlative inhibition of the bud by upper plant parts increases from top towards

the base of the shoot, in accordance with results of Zieslin & Halevy (1976) for rose, Jennings (1987) for raspberry and Suzuki (1990) for mulberry. The effect of the shoot apex on the rest of the plant may depend on its rate of development, the faster its growth the larger its inhibiting effect, as suggested by Sachs (1991). In our study, however, it was shown that an increase in assimilate supply accelerated shoot development (Chapter 5.1), but no clear effect on development and growth potential of the bud was found (Chapter 4.3). Moreover, with increasing temperature during axillary bud formation, rate of development of the apex increased, but no increase in inhibiting effect on the axillary bud was found (Chapter 4.4).

The present study showed that the inhibition is restricted to outgrowth of the bud, since the bud meristem remains active as indicated by continued initiation of leaf primordia and cell divisions in the pith rib meristem (Chapter 4.1). Increase in number of leaves was also recorded in resting pear buds (Young *et al.* 1974). Although, as long as buds are inhibited from sprouting, bud and pith diameter and number of leaves increase, the meristem does not switch to the generative stage (Chapters 2.2 and 4.1), in contrast to buds of several tree species that may become reproductive in that situation (Fulford 1966; Maillette 1982). Development and size of the bud are affected by position along the shoot, age and growth conditions (Chapter 4),

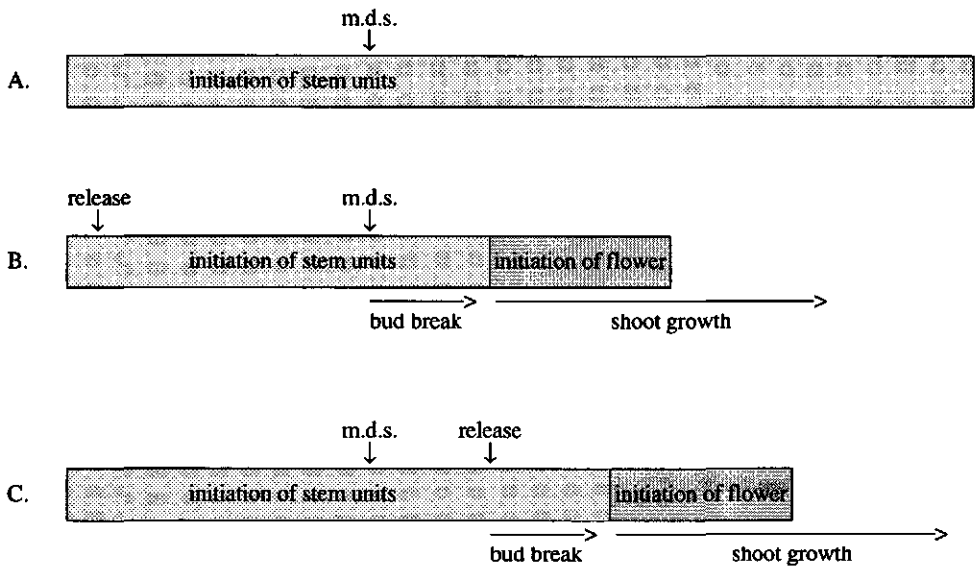


FIG. 1. Developmental programme of an axillary bud (excluding the 1-3 buds immediately below the flower).
A. No release from correlative inhibition. The bud does not break. No flower initiation;
B. Release from correlative inhibition before the bud has reached the minimum developmental stage for bud break. Bud break occurs as soon as this stage is reached.
C. Release form correlative inhibition when the minimum stage for bud break has already been reached.
m.d.s.: Minimum developmental stage for bud break.
release: Release from correlative inhibition.

as schematically represented in Table 1.

A bud contains the lower part of the future shoot, i.e. a number of leaves and leaf primordia, and the so-called secondary buds in the axils of the lowermost leaves (Chapter 2.1). Hence, in fact in each leaf axil several buds are present, of which the primary bud dominates the secondary buds.

Within a bud there may also exist a gradient in development due to correlative inhibition. In this reasoning the apical meristem, initiating new leaves, acts as a sink for assimilates and/or a source for auxin and in this way inhibits the development of lower bud parts. Phillips (1975), however, mentioned that in correlatively inhibited buds the normal capacity for auxin and gibberellin synthesis is absent, and that this synthesis does not start before release from inhibition. Following the theory of Sachs (1991) that the inhibition effect of an apex is dependent on its growth rate, we must conclude that if there is an inhibition gradient within the bud, it must be very small, compared to the gradient existing in the shoot.

The lowermost leaves of a bud are reduced to scales (Chapter 2.1). At initiation all leaves may have the same potential, but during the early development of a leaf differences occur. According to Romberger (1963) primordia are not predestined to become compound leaves or scales, but at an early stage in their development environmental conditions determine their morphogenetic determination. Accordingly, Steeves & Sussex (1989) reported that the developmental fate of a leaf primordium is fixed quite early in organogenesis. Furthermore, with increasing age the leaf primordium may loose the potential to develop leaflets. This latter suggestion is supported by the observation that the longer a bud is inhibited the more leaf primordia are reduced to scales (Chapter 4.1; Cockshull & Horridge 1977), whereas when soft pinching (Post 1950) is applied, resulting in release from inhibition at a very young stage, the subsequent shoot bears no or only few scales (Chapter 4.1). Also for *Eucalyptus* it was reported that shoots, formed by buds that had been inhibited from sprouting for a long time, carry many small leaves at their base (Carr 1984).

Fate of axillary bud

Several different fates of axillary buds can be distinguished (Maillette 1982; Bell 1991): In rose a bud may die, remain quiescent or develop into a shoot. The fate of a bud is determined by internal and external conditions and changes with position on the plant. Buds

TABLE 1. Effects of several factors on axillary bud development.

Factor	Rate of development	No of leaves and leaf primordia	Weight	No. of pith cells
Bud position	+	0+	+	+-
Bud age		+	+	+
Assimilate supply	+	0+	+	+
Temperature	+	0	?	0-

\+ : higher at increased level of factor.
\- : lower at increased level of factor.
0 : no effect.
+- : optimum response curve.
? : not investigated.

in the lower parts of the shoot or plant, which remain quiescent during the life time of a plant, serve as guarantee for survival of the plant after damage or severe pruning. Buds which form a shoot, may do so (1) immediately upon formation or (2) after a period of inhibition. In the first case, the bud grows into a sylleptic shoot, which is characterized by a hypopodium and absence of scales at its base. In the second case the bud forms a proleptic shoot, which is characterized by scales at its base (Hallé *et al.* 1978; Bell 1991). Whether a bud grows into a proleptic or a sylleptic shoot depends on its age at the time of release from inhibition and on its position along the shoot (Chapter 4). Buds in the axils immediately below the flower always form sylleptic shoots. These buds initiate only a few leaves preceding the flower bud (Chapter 4.2) and are suggested to be part of the inflorescence (De Vries & Dubois 1992). Branching in the inflorescence is exclusively sylleptic (Hallé *et al.* 1978). It is also possible that these buds are not correlatively inhibited by the shoot apex, since, as suggested by Zhou & Hara (1989) and Stafstrom (1993), the inhibition declines with transition to the reproductive stage. Furthermore, the shoot is assumed to undergo a gradual ageing process represented by the loss of apical dominance (Poethig 1990).

Bud break

The present study has demonstrated that buds appear to need a certain developmental stage before they are able to sprout, and that this stage associates with the number of leaves and the number of pith cells (Chapter 4.1). This stage might also depend on the bud position along the shoot, but further study is required. Our results indicate that the meristem has to mature to a certain extent before it is able to break. During the axillary bud stage, the meristem is organized and programmed to form leaves, axillary buds and stem tissues. This is a stable developmentally regulated state (McDaniel 1984), which may persist either for a long period or only briefly. Release from correlative inhibition, either spontaneously or induced by removal of the plant part above the bud, is the signal or condition necessary to induce bud break. This condition is supposed to be based on a hormonal balance between auxin produced in the shoot apex and cytokinins produced in the roots. Stafstrom (1993) proposed that apical dominance is controlled by opposite gradients of auxin and cytokinin. The gradients may vary during plant development due to root and shoot activity and the buds may vary in sensitivity to the hormones. Basal buds probably escape the inhibition imposed by the upper shoot parts, which may indicate that these buds are less sensitive to auxin or more sensitive to cytokinins. Furthermore, rootstocks are supposed to differ in cytokinin production (De Vries 1993), which might be the reason for differences in ability to induce the formation of basal shoots.

Axillary shoot

Flower formation

When released from inhibition axillary buds seem to develop autonomously (Chapter 4). Each sprouting bud has the potential to form a flower primordium. The present study validates the hypothesis of Cockshull & Horridge (1977) that transition of the meristem to the reproductive stage is blocked by apical dominance. As soon as the inhibition of a bud is released, its meristem becomes determined to form a flower. The apex rapidly initiates a number of leaves and switches from the

vegetative to the reproductive stage. Similar to tobacco (McDaniel 1984), the transition from vegetative to reproductive development seems to be precisely regulated and endogenously controlled. Removal of full grown leaves has no effect on the number of nodes (leaves) preceding the flower (Chapters 2.2 and 5.1), as also found for pepper (Rylski & Halevy 1972) and tobacco (McDaniel 1980). Removal of leaf primordia may delay flowering, although a direct effect of developing leaves on the maturation of apices is not likely (Sachs 1991). The transition may be influenced by the physiological distance of the apices from the roots, for which the number of nodes is a rough measure, and a stable change within the apices themselves, both factors internal to the plant (Sachs 1991).

Buds need no specific information from the parent plant for completing their developmental programme, as indicated by *in vitro* (Chapter 3) and grafting (Chapter 4) experiments. The *in vitro* experiments showed that cytokinin is necessary for the bud meristem to complete the developmental programme including initiation of a flower bud (Chapter 3); in the *in vivo* situation cytokinins will be provided by the roots. Without cytokinin in the culture medium, buds were able to sprout, but usually only the leaves that were already formed in the axillary bud at the time of excision unfolded; thereafter the meristem died. The stage of development that will be reached may depend on the cytokinin content of the bud itself, which was reported to be dependent on the position of the bud along the shoot (Van Staden *et al.* 1981).

In black current, close proximity of roots to the lateral buds was inhibitory to flowering; gibberellins produced in the roots were assumed to be the inhibitory factor (Schwabe & Al-Doori 1973). In rose, the number of nodes (leaves) preceding the flower was higher for lower positioned axillary buds, which might also be related to the "distance" from the roots. Thus, in basal shoots which arise close to the root system, the number of leaves preceding the flower is often high. Sachs (1991) suggested that the distance to the roots may be a function of the contacts between vascular channels, through which signals from the root are transported. This would account for "distance" not being related to stem length in any simple way. The present study has shown that buds which sprout in the presence of the apex of the parent shoot, initiate a new vascular system alongside that serving the parent shoot (Chapter 2.3). The vascular channels serving the sprouting bud do not contact those serving the parent shoot, whereas they may do so when the parent shoot has been decapitated, as has been shown for pea by Sachs (1970). The new vascular system might be "physiologically shorter" than any previous system, inducing a higher number of nodes before floral transition. Basal shoots arise from relatively old buds, in proximity of the roots and in the presence of the parent shoot, all factors that induce a high number of nodes preceding the flower. Furthermore, the number of additional leaves, formed after release from inhibition, appeared to depend on the assimilate supply during axillary bud formation (Chapters 4.3 and 4.4). Similarly, Sachs (1991) reported that this number may be small in poor conditions and considerably larger when plants grow vigorously. In contrast, in tomato increase in assimilate supply decreased the number of leaves preceding the first inflorescence (Dieleman & Heuvelink 1992).

Whether the flower primordium grows into a flower or aborts, depends on conditions after bud break, especially conditions affecting

the assimilate supply, although hormonal control might also be involved (Moe 1971).

Stem units

The shoot is composed of a succession of reiterated units; each unit consists of a leaf, an axillary bud, a node and an internode (Sussex 1989; Bell 1991). The lower units of a shoot are preformed, i.e. formed within the axillary bud, whereas the upper units are neoformed, i.e. formed after release from inhibition. The number of preformed nodes depends on the position (Chapter 4.2) and the physiological age (Chapter 4.1) of the bud at sprouting, and depends on conditions during axillary bud formation. The number of neoformed nodes appeared to be exclusively controlled by internal factors of the bud independent of environmental conditions after bud break (Chapter 5). The two cultivars included in the present study differ in the amount of pre- and neoformed units (Chapters 4 and 5). In 'Motrea' a larger part of the shoot is neoformed, indicating that environmental conditions during shoot growth may have a larger effect on shoot development than in 'Sweet Promise'.

Number of leaflets and leaf area per leaf, axillary bud size and internode length show an optimum halfway along the flowering shoot. The lower leaves, which are formed in the axillary bud stage, are already determined for their final form (number of leaflets) at the moment of bud break. The decrease in internode length and leaf size in the upper part of the shoot might be due to the limited growth potential of the stem apex, as suggested by Berrill (1961). This potential is assumed to be progressively exhausted. Furthermore, Berrill (1961) reported a direct relation between the size of the apex and the size of the leaves and internodes. Size of the axillary bud in its dormant state was reported to be proportionate to the size of the leaf at whose base it forms. It is also possible that when the apical meristem switches to the reproductive stage, the inhibition imposed by the apical meristem declines (Zhou & Hara 1989; Stafstrom 1993). However, in a later stage of shoot growth the developing flower bud may become a strong sink, as suggested by Mor & Halevy (1979), depriving assimilates from the upper part of the shoot. As a result the nodes and internodes of the shoot may have been exposed to different extents of inhibition, which may be reflected in the appearance of the nodes and internodes along the shoot. The more inhibition a node experiences and consequently the smaller its relative sink strength, the smaller its leaf and axillary bud and the shorter its subtending internode. Furthermore, the level of available assimilates, which affects the internode length to a large extent (Chapter 5; Brown & Sommer 1992), will vary for the various stem parts. Thus, in *Hydrangea*, internode elongation seemed to influence the degree of development of axillary buds (Zhou & Hara 1989). In rose axillary bud development also correlates positively with internode length. The degree of inhibition an axillary bud has experienced during its development, can be detected on the shoot which grows from the bud: the more inhibition, the higher the numbers of non-elongated internodes and scale-like leaves.

Shoot length

Shoot length is determined by the number and length of the stem units. The number of nodes preceding the flower is already determined in the axillary bud (Chapter 4), but length is primarily the result of extension of the internodes (Dickison 1992). The nodal regions do elongate but only slightly (Maksymowych & Orkwiszewski 1993). Differences in internode

length are due to differences in cell number and cell length. In a number of woody perennials, final internode length appeared to be highly correlated with cell number rather than cell length (Brown & Sommer 1992). Shoot elongation can be affected by conditions during shoot growth (Chapter 5), since it is dependent on growth rate, length of growth period, available assimilates and cell plasticity (Sinnott 1960). Moreover, endogenous levels of growth regulators might be involved.

Shoot diameter

Shoot diameter, an important parameter for flower shoot quality, appeared to correlate highly with the diameter of the pith (Chapter 2.1), an observation that has also been made in several other woody plant species (Sinnott 1936; Bostrack 1993). Furthermore, pith diameter correlated positively with the area of vascular tissue and as a result with the transport capacity of the shoot (Chapter 2.1).

Although usually little attention is paid to the pith, its importance in the development of a shoot cannot be denied, since the pith reflects the primary growth of the shoot. It persists in nearly all plant species and is present in old stems in size, shape and structure exactly as it was in the young twig (Eames & MacDaniels 1947) and therefore it is a reliable witness of primary shoot diameter. Pith cells are able to store reserves and remain alive for a long time in many tree species, which indicates their importance for storage purposes (Glerum 1980; Chapter 2.1). In Poplar, pith cells were even found to contain photosynthetically active chloroplasts (Van Cleve *et al.* 1993).

The final size of the pith is dependent on cell number and cell size. Cell number in cross section is largely set during the period before bud sprouting (Chapter 2.1) and was found to correlate positively with final pith diameter (Chapter 4.1) and, consequently, with shoot diameter. Internal factors, like age and position along the shoot appeared to determine the number of pith cells in cross section in the bud. Although the bud age decreases towards the shoot apex, the effect of position cannot be explained by the slight difference in age between the buds (Chapter 4).

Rootstocks were found to affect the diameter of basal shoots (De Vries 1993; Fuchs 194). Rootstocks may differ in cytokinin production, which might affect cell divisions in the basal buds and subsequently the potential diameter of the basal shoots. Since diameter of the basal shoots is an important parameter determining flower production (Kool & Van de Pol 1993; Fuchs 1994), further research is needed on this topic.

Pith diameter also positively correlated with the size of the pith cells which can be affected by conditions during shoot growth (Chapter 5). Although in an axillary bud all pith cells were similar, in the subsequent shoot two types of cells were found to differentiate (Chapter 2.1). Since not all pith cells in the bud expand during shoot growth, it would be interesting to study whether cells determined to expand and die or to stay small and alive can be recognized as early as in the axillary bud.

For the part of the shoot which remains after the flower is cut, i.e. the structural shoots of a rose plant, the contribution of the pith to the total shoot diameter decreases, due to an increase of vascular tissue by secondary growth. However, in shoots having a large pith diameter (i.e. a large initial shoot diameter) the area of vascular tissue is also large, which may result in a more vigorous shoot growth.

Growth period

The total growth period of a shoot is defined as the time between pruning the stem above a bud and harvest of the flowering shoot grown from that bud. It is an important determinant of flower production of a rose crop. Total growth period can be divided in two parts: the time from pruning until bud break and the time from bud break until harvest. Time until bud break depends on the temperature during bud break (Chapter 5.2; Van de Berg 1987), the position of the bud (Chapter, 4.2; Zieslin & Halevy 1976), rootstock (De Vries 1993), leaf subtending the bud and stem and leaves above the bud (Zieslin & Halevy 1976). Bud age and temperature during bud formation slightly influenced time until bud break (Chapters 4.1 and 4.4). Availability of assimilates when the inhibition of the bud is released did not affect bud break (Chapter 5.1). However, in our experiments the number of buds allowed to sprout was restricted to one. When all buds are allowed to sprout, it may be expected that assimilate supply will positively affect the number of sprouting buds. Time from bud break until harvest increases with decreasing temperature or assimilate supply during shoot growth (Chapter 5), with decreasing distance from the base of the plant and, slightly, with decreasing temperature during bud formation (Chapter 4).

Basal shoots

After propagation, strong growing shoots (basal shoots) arise at the base of the plant. Basal shoots were shown to develop from the axillary buds in the axils of the scale-like leaves at the base of the shoot (Chapter 2.2). The number of buds present at the base of the plant depends on the number of non-elongated internodes, which is affected by age and position of the bud which was used for propagation (Chapter 4). Except for very young buds or buds from the uppermost part of the shoot, the number of basal buds is non-limiting for the formation of basal shoots. The lowermost two buds, which are the oldest ones, dominate the other buds, since in a normal situation these buds grow into basal shoots (Chapter 2.2). The first basal shoots are formed from buds which were initiated on the parent plant. The number of basal buds growing at the same time depends on the vigour of the plant. Later formed shoots usually develop from basal buds of the basal shoots and may restrict the growth of the older basal shoots (Chapter 2.3; Kool *et al.* 1991). The size (length, diameter, weight) of the basal shoot is dependent on the age of the bud at sprouting and the growth conditions during subsequent growth.

Practical consequences

When harvesting a flower shoot, the most distal axillary buds will sprout and each grow into a shoot. In case of propagation, the axillary bud will form the above ground part of the future plant. This study showed that some characteristics of the shoot, which develop from the bud, are already determined in the axillary bud, whereas several other characteristics can yet be influenced during shoot growth. The major results of this study, as far as effects of influencing factors are concerned, are summarized in Table 2. Length, diameter and weight of the shoot are important parameters for shoot quality, whereas the growth period from pruning until harvest determines the flower yield per plant. Furthermore, flower yield depends on the number of sprouting buds forming a flower shoot. However, in this study the number of sprouting buds was usually restricted to one in order

to avoid interactions between imposed treatments and number of competing shoots, which disturb the effects of the treatments.

Shoot characteristics determined during axillary bud formation

The total number of leaves preceding the flower is determined during axillary bud formation and cannot be influenced after bud break. The more assimilates are available during axillary bud formation or the older the axillary bud, the more leaves are formed. It should be noticed that when bud age increases, the increase in number of leaves mainly concerns the number of scale-like leaves at the base of the shoot.

As the number of pith cells on the diameter line is determined within the axillary bud, the potential diameter of the subsequent shoot is affected during axillary bud formation. The more assimilates available and the older the bud, the higher the number of cells. Final size of the shoot can be influenced during shoot growth. The total growth period of the shoot can be affected during axillary bud formation to a certain extent, but the effects of conditions after bud break are larger.

It should be noted that in commercial practice, factors influencing axillary bud formation, also affect the growth of the parent shoot. Since axillary buds develop into shoots attached to the parent shoot, the effects of the imposed factor on subsequent shoot growth concern direct effects on the growth potential of the axillary bud as well as indirect effects via the parent plant.

TABLE 2. Effects of several factors on axillary shoot development.

Factor	Rate of bud break	Growth period	No. of leaves (incl. scales)	Shoot length	Shoot diameter	Shoot weight	Leaf area
Factors during axillary bud formation							
Bud age							
in situ	0+	0	+	0	+	0	0
in isolation	0+	0	+	0	0+	0	0
Bud position							
in situ	+	-	-	-	0+	+	±
in isolation	+	-	-	-	?	0	?
Assimilate supply							
in situ	0	-	+	+	+	+	+
in isolation	0	0	+	0	0	0	0
Temperature							
in situ	+	-	-	±	±	±	0
in isolation	+	-	-	0-	0	-	-
Factors during bud break and shoot growth							
Assimilate supply	0	-	0	+	+	+	+
Temperature	+	-	0	-	-	+-	+-

\+ : higher at increased level of factor.
\- : lower at increased level of factor.
0 : no effect.
+- : optimum response curve.
± : variable effect.
? : not investigated.

Position of the bud along the shoot clearly affects subsequent growth. At a higher position, bud break is enhanced and the growth period shortened. However, higher positioned buds may grow into smaller shoots compared to lower positioned buds. When higher positioned buds are used for propagation, basal shoots will be formed faster. Using cuttings from all positions of the shoot will lead to a large variability in subsequent shoot growth. Size of the axillary bud correlated positively with the size of the shoot bearing the axillary bud. Furthermore, differences in shoot diameter and leaf area of the parent shoot will lead to variation in subsequent growth of the cutting. This variability can be reduced by selecting cuttings according to the size of the shoot and position along the shoot and by using double node cuttings, as described elsewhere (Marcelis-van Acker & Leutscher 1993).

Shoot characteristics affected by conditions after release from inhibition

As discussed in Chapter 5, length, diameter, weight and leaf area of the shoot can be affected to a large extent during and after bud break. In general the more assimilates available, the bigger the shoot. Time to bud break decreases with increasing temperature; the total growth period can be reduced by an increase in temperature or assimilate supply.

Conclusion

Axillary buds remain in the vegetative state as long as they are correlatively inhibited. They are not dormant, but continue to grow although at a low rate. When released from inhibition, their developmental programme (bud break, leaf initiation and flower initiation) is already set to a large extent. However, they display a high degree of plasticity in their development into a shoot, in response to ambient conditions in which they are growing.

References

Altus D.P. & Canny M.J. 1985. Water pathways in wheat leaves. I. The division of fluxes between different vein types. Aust. J. Plant Physiol. 12: 173-181.

Anonymous 1993. CBS Landbouwtelling 1993. Misset, Doetinchem, 127pp.

Anonymous 1994. Top-25 snijbloemen en kamerplanten. Vakblad voor de Bloemisterij 3: 36.

Baldini E. & Mosse B. 1956. Observations on the origin and development of sphaeroblasts in the apple. J. Hortic. Sci. 31: 156-162.

Barlow H.W.B. 1970. Some aspects of morphogenesis in fruit trees. In: Physiology of trees crops (L.C. Luckwill & C.V. Cutting, eds). Acad. Press, London. pp. 25-45.

Bell A.D. 1991. Plant Form. An illustrated guide to flowering plant morphology. Oxford Univ. Press, Oxford. 341pp.

Bernier G., Kinet J.-M. & Sachs R.M. 1981. The physiology of flowering. Vol.II. CRC press, Boca Raton, 231pp.

Berrill N.J. 1961. Growth, development and pattern. W.H. Freeman and Comp., San Francisco, 555pp.

Bohner J. & Bangerth F. 1988. Cell number, cell size and hormone levels in semi-isogenic mutants of *Lycopersicon pimpinellifolium* differing in fruit size. Physiol. Plant. 72: 316-320.

Bornman, C.H. & Vogelmann T.C. 1984. Effect of rigidity of gel medium on benzyladenine-induced adventitious bud formation and vitrification *in vitro* in *Picea abies*. Physiol. Plant. 61: 505-512.

Bostrack J.M. 1993. The relationship between size of shoot apices and morphological features of mature leaves and stems of four species of angiosperm trees. Ann. Bot. 72: 341-347.

Bozarth C.S., Kennedy R.A. & Schekel K.A. 1982. The effects of leaf age on photosynthesis in rose. J. Am. Soc. Hortic. Sci. 107: 707-712.

Bressan P.H., Kim Y.-J., Hyndman S.E., Hasegawa P.M. & Bressan R.A. 1982. Factors affecting *in vitro* propagation of rose. J. Am. Soc. Hortic. Sci. 107: 979-990.

Brown C.L. & Sommer H.E. 1992. Shoot growth and histogenesis of trees possessing diverse patterns of shoot development. Am. J. Bot. 79: 335-346.

Burrows G.E. 1986. Axillary meristem ontogeny in *Araucaria cunninghamii* Aiton ex D.Don. Aust. J. Bot. 34: 357-375.

Byrne T.G. & Doss R.P. 1981. Development time of 'Cara Mia' rose shoots as influenced by pruning position and parent shoot diameter. J. Am. Soc. Hortic. Sci. 106: 98-100.

Canny M.J. 1990. What becomes of the transpiration stream? New Phytol. 114: 341-368.

Carpenter W.J. & Rodriguez D.C. 1971. The effect of plant growth regulating chemicals on rose shoot development from basal and axillary buds. J. Am. Soc. Hortic. Sci. 96: 389-391.

Carr D.J. 1984. Positional information and plant morphology. In: Positional control in plant development (P.W. Barlow & D.J. Carr, eds). Cambridge Univ. Press, Cambridge, pp. 349-374.

Cassels A.C. & Minas G. 1983. Plant and *in vitro* factors influencing the micropropagation of Pelargonium cultivars by bud-tip culture. Scientia Hortic. 21: 53-65.

Caulfield F. & Bunce J.A. 1991. Influence of the environment during seed development on the morphology and growth rate of soybean seedlings. Bot. Gaz. 152: 59-63.

Church T.W. & Godman R.M. 1966. The formation and development of dormant buds in sugar maple. For. Sci. 12: 301-306.

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

Cline M.G. 1994. The role of hormones in apical dominance. New approaches to an old problem in plant development. Physiol. Plant. 90: 230-237.

Cockshull K.E. & Horridge J.S. 1977. Apical dominance and flower initiation in the rose. J. Hortic. Sci. 52: 421-427.

Darlington A.B. & Dixon M.A. 1991. The hydraulic architecture of roses (*Rosa hybrida*). Can. J. Bot. 69: 702-710.

De Vries D.P. 1993. The vigour of glasshouse roses. Dissertation Agric. Univ. Wageningen, 169pp.

De Vries D.P. & Dubois L.A.M. 1983. Relations between basal bottom-breaks and harvested shoots in own-rooted hybrid tea-rose seedlings and their clones. Gartenbauwiss. 48: 189-192.

De Vries D.P. & Dubois L.A.M. 1992. Variation in the shoot production of 'Sonia'; cut rose plants, originating from grafting scions of different nodal positions. Gartenbauwiss. 57: 64-68.

De Vries D.P. & Smeets L. 1979. Effects of temperature on growth and development of hybrid tea-rose seedlings. Scientia Hortic. 11: 261-268.

Debergh P.C. 1983. Effects of agar brand and concentration on the tissue culture medium. Physiol. Plant. 59: 270-276.

Dennet M.D., Elston J. & Milford J.R. 1979. The effect of temperature on the growth of individual leaves of *Vicia faba* L. in the field. Ann. Bot. 43: 197-20.

Dickison W.C. 1992. Comparative morphology and anatomy of the stem of the flowering plants. Progr. Bot. 53: 44-62.

Dieleman J.A. & Heuvelink E. 1992. Factors affecting the number of leaves preceding the first inflorescence in the tomato. J. Hortic. Sci. 67: 1-10.

Dixon M.A., Butt J.A., Murr D.P. & Tsujita M.J. 1988. Water relations of cut greenhouse roses: the relationship between stem water potential, hydraulic conductance and cavitation. Scientia Hortic. 36: 109-118.

Dubois L.A.M., Roggemans J., Soyeurt G. & De Vries D.P. 1988. Comparison of the growth and development of dwarf rose cultivars propagated *in vitro* and *in vivo* by softwood cuttings. Scientia Hortic. 35: 293-299.

Dutcher R.D. & Powell L.E. 1972. Culture of apple shoots from buds *in vitro*. J. Am. Soc. Hortic. Sci. 97: 511-514.

Eames A.J. & MacDaniels L.H. 1947. An introduction to plant anatomy. McGraw-Hill Book Comp., New York, 427pp.

Erwin J.E., Heins R.D. & Karlsson M.G. 1989. Thermomorphogenesis in *Lilium longiflorum*. Am. J. Bot. 76: 47-52.

Esau K. 1965. Plant Anatomy. John Wiley, New York, 767pp.

Esau K. 1977. Anatomy of Seed Plants. John Wiley, New York, 550pp.

Farrar J.F. 1988. Temperature and the partitioning and translocation of carbon. In: Plants and temperature (S.P. Long & F.I. Woodward, eds). Symp. Soc. Exp. Biol. 42. Company of Biologists, Cambridge, pp. 203-235.

Fink S. 1983. The occurrence of adventitious and preventitious buds within the bark of some temperate and tropical trees. Am. J. Bot. 70: 532-542.

Fisher J.B. & Ewers F.W. 1992. Xylem pathways in liana stems with variant secondary growth. Bot. J. Linn. Soc. 108: 181-202.

Fisher O.W. & Kofranek A.M. 1949. Bottom break production of rose plants as influenced by plot location in the greenhouse. Proc. Am. Soc. Hortic. Sci. 53: 501-502.

Francis D. & Barlow P.W. 1988. Temperature and the cell cycle. In: Plants and temperature (S.P. Long & F.I. Woodward, eds) . Symp. Soc. Exp. Biol. 42. Company of Biologists, Cambridge, pp. 181-201.

Fuchs H.W.M. 1994. Scion-rootstock relationships and root behaviour in glasshouse roses. Dissertation Agric. Univ. Wageningen, 127pp.

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

Furr J.R., Cooper W.C. & Reece P.C. 1947. An investigation of flower formation in adult and juvenile citrus trees. Am. J. Bot. 34: 1-18.

Garrison R. 1949. Origin and development of axillary buds: *Syringa vulgaris* L. Am. J. Bot. 36: 205-213.

Ghashghaie J., Brenckmann F. & Saugier B. 1991. Effects of agar concentration on water status and growth of rose plants cultured *in vitro*. Physiol. Plant. 82: 73-78.

Ghashghaie J., Brenckmann F. & Saugier B. 1992. Water relations and growth of rose plants cultured *in vitro* under various relative humidities. Plant Cell Tiss. Org. Cult. 30: 51-57.

Glerum C. 1980. Food sinks and food reserves of trees in temperate climates. N. Z. J. For. Sci. 10: 176-185.

Goodwin P.B. 1967. The control of branch growth on potato tubers. I. Anatomy of buds in relation to dormancy and correlative inhibition. J. Exp. Bot. 18: 78-99.

Gould K.S., Cutter E.G., Young J.P.W. & Charlton W.A. 1987. Positional differences in size, morphology, and *in vitro* performance of pea axillary buds. Can. J. Bot. 65: 406-411.

Grange R.I. & Hand D.W. 1987. A review of the effects of atmospheric humidity on the growth of horticultural crops. J. Hortic. Sci. 62: 125-134.

Gregory F.C. & Veale J.A. 1957. A reassesment of the problem of apical dominance. Symp. Soc. Exp. Biol. 11: 1-20.

Gris M.A. 1872. Sur la moelle des plantes ligneuses. Ann. Sci. Nat. Serie 5: 34-79.

Halevy A.H. 1972. Phytohormones in flowering regulation of self-inductive plants. Proc. 18th Int. Hortic. Congr. 5: 187-198.

Halevy A.H. & Zieslin N. 1969. The development and causes of petal blackening and malformation of Baccara rose flowers. Acta Hortic. 14: 149-156.

Halim H., Edwards G.R. Coombe B.G. & Aspinall D. 1988. The dormancy of buds of *Citrus sinensis* (L.) Osbeck inserted into rootstock stems: Factors intrinsic to the inserted bud. Ann. Bot. 61: 525-529.

Hallé F., Oldeman R.A.A. & Tomlinson P.B. 1978. Tropical trees and forests. An architectural analysis. Springer-Verlag, Berlin, 441pp.

Hansen J. 1989. Influence of cutting position and temperature during rooting on adventitious root formation and axillary bud break of *Stephanotis floribunda*. Scientia Hortic. 40: 345-354.

Hasegawa P.M. 1979. *In vitro* propagation of rose. HortSci. 14: 610-612.

Hay R.K.M. & Kemp D.R. 1990. Primordium initiation at the stem apex as the primary event controlling plant development: preliminary evidence from wheat for the regulation of leaf development. Plant, Cell Environ. 13: 1005-1008.

Hay R.K.M. & Tunnicliffe Wilson G. 1982. Leaf appearance and extension in field-grown winter wheat plants: the importance of soil temperature during vegetative growth. J. Agr. Sci. 99: 403-410.

Herr J.M.(Jr) 1971. A new clearing-squash technique for the study of ovule development in angiosperms. Am. J. Bot. 58: 785-790.

Hillman J.R. 1984. Apical dominance. In: Advanced plant physiology (M.B. Wilkins, ed.). Longman, New York, pp. 127-148.

Ho L.C. 1992. Fruit growth and sink strength. In: Fruit and seed production: aspects of development, environmental physiology and ecology. (C. Marshall & J. Grace, eds). Soc. Exp. Biol., Seminar series 47. Cambridge Univ. Press, Cambridge, pp. 101-124.

Hoek I.H.S., Hänisch ten Cate C.H., Keijzer C.J., Schel J.H. & Dons H.J.M. 1993. Development of the fifth leaf is indicative for whole plant performance at low temperature in tomato. Ann. Bot. 72: 367-374.

Hoffman G.J. 1979. Humidity. In: Controlled environment guidelines for plant research (T.W. Tibbits & T.T. Kozlowski, eds). Acad. Press, New York, pp. 141-172.

Hopper D.A. & Hammer P.A. 1991. Regression models describing *Rosa hybrida* response to day/night temperature and photosynthetic photon flux. J. Am. Soc. Hortic. Sci. 116: 609-617.

Horridge J.S. & Cockshull K.E. 1974. Flower initiation and development in the glasshouse rose. Scientia Hortic. 2: 273-284.

Humphries E.C. & Wheeler A.W. 1963. The physiology of leaf growth. Annu. Rev. Plant Physiol. 14: 385-410.

Hveslof-Eide K. 1991. The effect of temperature, daylength and irradiance on the growth of mother plants of *Nephrolepis exaltata* (L.) Schott and on the subsequent growth *in vitro* of runner tip explants. Scientia Hortic. 47: 137-147.

Jacobs G., Allan P. & Bornman C.H. 1969. Tissue culture studies on rose: use of shoot tip explants. I. Auxin: cytokinin effects. Agroplant. 1: 179-188.

Jacobs G., Zieslin N. & Halevy A.H. 1980. Factors affecting ^{14}C-sucrose uptake by single-node explants of rose flower stems. Physiol. Plant. 50: 251-254.

Jennings D.L. 1987. Some effects of secondary dormancy and correlative inhibition on the development of lateral buds of raspberry canes (*Rubus idaeus* L.). Crop Res. 27: 119-129.

Jiao J., Tsujita M.J. & Grodzinski B. 1991. Influence of temperature on net CO2 exchange in roses. Can. J. Plant Sci. 71: 235-243.

Kassner G. 1884. Mark einiger Holzpflanzen. Univ. Basel. 38pp.

Kauppi A., Rinne P. & Ferm A. 1987. Initiation, structure and sprouting of dormant basal buds in *Betula pubescens*. Flora 179: 55-83.

Khayat E. & Zieslin N. 1982. Environmental factors involved in the regulation of sprouting of basal buds in rose plants. J. Exp. Bot. 33: 1286-1292.

Kool M.T.N. & Van de Pol P.A. 1993. Controlling the plant development of *R. hybrida* 'Motrea'. Scientia Hortic. 53: 239-248.

Kool M.T.N., Van de Pol P.A. & Berentzen W.T.J. 1991. Formation and early development of bottom breaks in 'Motrea' roses. Scientia Hortic. 48: 293-298.

Kozlowski T.T. 1992. Carbohydrate sources and sinks in woody plants. Bot. Rev. 58: 107-222.

Kozlowski T.T. & Winget C.H. 1963. Patterns of water movement in forest trees. Bot. Gaz. 124: 301-311.

Kozlowski T.T. Torrie J.H. & Marshall P.E. 1973. Predictability of shoot length from bud size in *Pinus resinosa* Ait. Can. J. For. Res. 3: 34-38.

Kozlowski T.T. Kramer P.J. & Pallardy S.G. 1991. The physiological ecology of woody plants. Acad. Press, San Diego, 657pp.

Ladell J.L. 1963. Needle density, pith size and tracheid length in Pine. Commonwealth forestry Inst. Univ. Oxford. Inst. paper no. 36, 76pp

Lieth J.H. & Pasian C.C. 1990. A model for net photosynthesis of rose leaves as a function of photosynthetically active radiation, lea temperature, and leaf age. J. Am. Soc. Hortic. Sci. 115: 486-491.

Lieth J.H. & Pasian C.C. 1991. A simulation model for the growth and development of flowering rose shoots. Scientia Hortic. 46: 109-128.

Litz R.E. & Conover R.A. 1981. Effect of sex type, season, and other factors on *in vitro* establishment and culture of *Carica papaya* L. explants. J. Am. Soc. Hortic. Sci. 106: 792-794.

Maene L. & Debergh P. 1985. Liquid medium additions to established tissue cultures to improve elongation and rooting *in vivo*. Plant Cell Tiss. Org. Cult. 5: 23-33.

Mailette L. 1982. Structural dynamics of silver birch. I. The fates of buds. J. Appl. Ecol. 19: 203-218.

Maksymowych R. & Orkwiszewski A.J. 1993. The role of nodal regions in growth of *Xanthium* (Compositae) stem. Am. J. Bot. 80: 1318-1322.

Marcelis L.F.M. 1993a. Effect of assimilate supply on the growth of individual cucumber fruits. Physiol. Plant. 87: 313-320.

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

Marcelis L.F.M. & Baan Hofman-Eijer L.R. 1993. Effect of temperature on the growth of individual cucumber fruits. Physiol. Plant. 87: 321-328.

Marcelis-van Acker C.A.M. & Leutscher K.J. 1993. Effect of type of cutting on heterogeneity and growth of *Rosa hybrida* cv. Motrea and *Schefflera arboricola* cv. Compacta. Scientia Hortic. 54: 59-67.

Marks T.R. & Myers P.E. 1992. Effect of explant location upon early culture development *in vitro*. J. Hortic. Sci. 67: 583-591.

Mauseth J.D. 1988. Plant Anatomy. Benjamin/Cummings Publ. Comp., California, 560 pp.

Mayak S., Halevy A.H., Sagie S., Bar-Yoseph A. & Bravdo B. 1974. The water balance of cut rose flowers. Physiol. Plant. 31: 15-22.

McDaniel C.N. 1978. Determination for growth pattern in axillary buds of *Nicotiana tabacum* L. Develop. Biol. 66: 250-255.

McDaniel C.N. 1980. Influence of leaves and roots on meristem development in *Nicotiana tabacum* L. cv. Wisconsin 38. Planta 148: 462-467.

McDaniel C.N. 1984. Shoot meristem development. In: Positional control in plant development (P.W. Barlow & D.J. Carr, eds). Cambridge Univ. Press, Cambridge, pp. 319-347.

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

McIntyre G.I. & Hsiao A.I. 1990. The role of expanded leaves in the correlative inhibition of axillary buds in milkweed (*Asclepias syriaca*). Can. J. Bot. 68: 1280-1285.

Medford J.I. 1992. Vegetative apical meristems. Plant Cell 4: 1029-1039.

Michno-Zatorska Z., Szczesniak T. & Ciesielska S. 1986. Localization of axillary meristems during different stages of radish ontogenesis. Biol. Plant. 28: 241-248.

Milburn J.A. 1979. Water flow in plants. Longman, London, 225pp.

Moe R. 1971. Factors affecting flower abortion and malformation in roses. Physiol. Plant. 24: 291-300.

Moe R. 1972. Effect of daylength, light intensity, and temperature on growth and flowering in roses. J. Am. Soc. Hort. Sci. 97: 796-800.

Moe R. & Andersen A.S. 1988. Stock plant environment and subsequent adventitious rooting. In: Adventitious root formation in cuttings (T.D. Davies, B.E. Haissig & N. Sankhala, eds) Dios-Condes Press, Portland, pp.214-234.

Moe R. & Heins R. 1991. Control of plant morphogenesis and flowering by light quality and temperature. Acta Hortic. 272: 81-89.

Moe R. & Kristoffersen T. 1969. The effet of temperature and light on growth and flowering of *Rosa* 'Baccara' in greenhouses. Acta Hortic. 14: 157-163.

Mor Y. & Halevy A.H. 1979. Translocation of ^{14}C-assimilates in roses. I. The effect of the age of the shoot and the location of the source leaf. Physiol. Plant. 45: 177-182.

Mor Y. & Zieslin N. 1987. Plant growth regulators in rose plants. Hortic. Rev. 9: 53-73.

Mor Y., Spiegelstein H. & Halevy A.H. 1981. Translocation of ^{14}C-assimilates in roses. II. The effect of shoot darkening and cytokinin application. Physiol. Plant. 52: 197-200.

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

Murashige T. & Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Nadel B.L., Altman A., Pleban S. & Hüttermann A. 1991. *In vitro* development of mature *Fagus sylvatica* L. buds. I. The effect of medium and plant growth regulators on bud growth and protein profiles. J. Plant Physiol. 138: 596-601.

Neeff F. 1914. Uber Zellumlagerung. Ein Beitrag zur experimentellen Anatomie. Zeitschr. Bot. 6: 465-547.

Nell T.A. & Rasmussen H.P. 1979. Floral development and blindness in roses: an SEM study. J. Am. Soc. Hortic. Sci. 104: 18-20.

Nelson N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153: 375-380.

Norton M.E. & Norton C.R. 1986. Change in shoot proliferation with repeated *in vitro* subculture of shoots of woody species of *Rosaceae*. Plant Cell Tiss. Org. Cult. 5: 187-197.

Okhawa K. 1979. Promotion of renewal canes in greenhouse roses by 6-benzylaminopurine without cutback. HortSci. 14: 612-613.

Parups E.V. 1971. Use of 6-benzylaminopurine and adenine to induce bottom-breaks in greenhouse roses. HortSci. 6: 456-457.

Patrick J.W. 1988. Assimilate partitioning in relation to crop productivity. HortSci. 23: 33-40.

Paul E.M.M., Hardwick R.C. & Parker P.E. 1984. Genotypic variation in the response to sub-optimal temperatures of growth on tomato (*Lycopersicon esculentum* Mill.). New Phytol. 98: 221-230.

Peterson C.A. & Fletcher R.A. 1973. Apical dominance is not due to a lack of functional xylem and phloem in inhibited buds. J. Exp. Bot. 24: 97-103.

Peterson C.A. & Fletcher R.A. 1975. Lateral bud outgrowth on excised stem segments: Effect of the stem. Can. J. Bot. 53: 243-248.

Phillips I.D.J. 1975. Apical dominance. Annu. Rev. Plant Physiol. 26: 341-367.

Pieters G.A. 1974. The growth of sun and shade leaves of *Populus euramericana* 'Robusta' in relation to age, light intensity and temperature. Meded. Landbouwhogesch. Wageningen 74(11): 1-107.

Poethig R.S. 1990. Phase change and the regulation of shoot morphogenesis in plants. Science 250: 923-930.

Popham R.A. & Chan A.P. 1950. Zonation in the vegetative stem tip of *Chrysanthemum morifolium* Bailey. Am. J. Bot. 37: 476-484.

Porter J.R. & Delecolle R. 1988. Interaction of temperature with other environmental factors controlling the development of plants. In: Plants and temperature (S.P. Long & F.I. Woodward, eds). Symp. Soc. Exp. Biol. 42. Company of Biologists, Cambridge, pp. 133-156.

Post K. 1950. Florist crop production and marketing. Orange Judd Publ. Comp., New York, 891pp.

Pukacki P., Giertych M. & Chalupka W. 1980. Light filtering function of bud scales in woody plants. Planta 150: 132-133.

Rastogi R. & Sawhney V.K. 1989. *In vitro* development of angiosperm floral buds and organs. Plant Cell Tiss. Org. Cult. 16: 145-174.

Richards F.J. 1959. A flexible growth function for empirical use. J. Exp. Bot. 10: 290-300.

Richards J.H. & Larson P.R. 1981. Morphology and development of *Populus deltoides* branches in different environments. Bot. Gaz. 142: 382-393.

Roberts G.L., Tsujita M.J. & Dansereau B. 1993. Supplemental light quality affects budbreak, yield and vase life of cut roses. HortSci. 28: 621-622.

Romberger J.A. 1963. Meristems, growth and development in woody plants. U.S.D.A. Tech. Bull. No. 1293, 214pp.

Rouffa A.S. & Gunckel J.E. 1951a. A comparative study of vegetative shoot apices in the *Rosaceae*. Am. J. Bot. 38: 290-300.

Rouffa A.S. & Gunckel J.E. 1951b. Leaf initiation, origin, and pattern of pith development in the *Rosaceae*. Am. J. Bot. 38: 301-307.

Rubinstein B. & Nagao M.A. 1976. Lateral bud outgrowth and its control by the apex. Bot. Rev. 42: 83-113.

Rudinsky J.A. & Vité J.P. 1959. Certain ecological and phylogenetic aspects of the pattern of water conduction in conifers. For. Sci. 5: 259-266.

Rylski I. & Halevy A.H. 1972. Factors controlling the readiness to flower of buds along the main axis of pepper (*Capsicum annuum* L.). J. Am. Soc. Hortic. Sci. 97: 309-312.

Sachs R.M. 1965. Stem elongation. Annu. Rev. Plant Physiol. 16: 73-96.

Sachs T. 1970. A control of bud growth by vascular tissue differentiation. Isr. J. Bot. 19: 484-498.

Sachs T. 1991. Pattern formation in plant tissues. Cambridge Univ. Press, Cambridge, 234pp.

Sachs R.M. & Hackett W.P. 1969. Control of vegetative and reproductive development in seed plants. HortSci. 4: 103-107.

Scherer P.A., Muller E., Lippert H. & Wolff G. 1988. Multi-element analysis of agar and gelrite impurities investigated by inductively coupled plasma emission spectrometry as well as physical properties of tissue culture media prepared with agar or the gellan gum gelrite. Acta Hortic. 226: 655-658.

Schwabe W.W. & Al-Doori A.H. 1973. Analysis of a juvenile-like condition affecting flowering in the black currant (*Ribes nigrum*). J. Exp. Bot 24: 969-981.

Shigo A.L. 1985. How tree branches are attached to trunks. Can. J. Bot. 63: 1391-1401.

Short K.C. & Roberts A.V. 1991. *Rosa spp.* (roses): *In vitro* culture, micropropagation, and the production of secondary products. In: Biotechnology in agriculture and forestry. Vol 5. Medicinal and Aromatic Plants III (Y.P.S. Bajaj, ed.). Springer Verlag, Berlin, pp. 376-397.

Singer S.R., Hannon C.H. & Huber S.C. 1992. Acquisition of competence for floral development in *Nicotiana* buds. Planta 188: 546-550.

Sinnott E.W. 1921. The relation between body size and organ size in plants. Am. Nat. LV: 385-403.

Sinnott E.W. 1936. The relation of organ size to tissue development in the stem. Am. J. Bot. 23: 418-421.

Sinnott E.W. 1960. Plant morphogenesis. McGraw-Hill Book Comp., New York. 550pp.

Sorokin H.P. & Thimann K.V. 1964. The histological basis for inhibition of axillary buds in *Pisum sativum* and the effects of auxins and kinetin on xylem development. Protopl. 59: 326-350.

Stafstrom J.P. 1993. Axillary bud development in pea: apical dominance, growth cycles, hormonal regulation and plant architecture. In: Cellular communication in plants (R.M. Amasino, ed.). Plenum Press, New York, pp. 75-86.

Stafstrom J.P. & Sussex I.M. 1988. Patterns of protein synthesis in dormant and growing vegetative buds of pea. Planta 176: 497-505.

Steeves T.A. & Sussex I.M. 1989. Patterns in plant development. Cambridge Univ. Press, Cambridge. 388pp.

Sussex I.M. 1989. Developmental programming of the shoot meristem. Cell 56: 225-229.

Suzuki T. 1990. Apical dominance in mulberry (*Morus alba*): Effects of position of lateral and accessory buds and leaves. Physiol. Plant. 78: 468-474.

Swartz H.J., Geyer A.S., Powell L.E. & Lin S.-H.C. 1984. The role of bud scales in the dormancy of apples. J. Am. Soc. Hortic. Sci. 109: 745-749.

Tamas I.A. 1987. Hormonal regulation of apical dominance. In: Plant hormones and their role in plant growth and development (P.J. Davies, ed.). Martinus Nijhoff Publ., Dordrecht, pp. 393-410.

Tepper H.B. 1993. Developmental features accompanying the imposition and release of apical dominance in pea. J. Plant. Physiol. 142: 722-729.

Terry N., Waldron L.J. & Taylor S.E. 1983. Environmental influences on leaf expansion. In: The growth and functioning of leaves (J.E. Dale & F.L. Milthorpe, eds). Cambridge Univ. Press, 179-207.

Tourn G.M., Tortosa R.D. & Medan D. 1992. *Rhamnaceae* with multiple lateral buds: an architectural analysis. Bot. J. Linn. Soc. 108: 275-286.

Trewavas A. 1981. How do plant growth substances work? Plant, Cell Environ. 4: 203-228.

Trewavas A. 1991. How do plant growth substances work? II. Plant, Cell Environ. 14: 1-12.

Tromp J., Jonkers H. & Wertheim S.J. 1976. Grondslagen van de fruitteelt. Staatsuitgeverij, 's Gravenhage, 356pp.

Tweddle D., Roberts A.V. & Short K.C. 1984. *In vitro* culture of roses. In: Plant tissue and cell culture application to crop improvement (F.J. Novak, L. Havel & J. Dolezel, eds). Czechoslovak Acad. Sci., Prague, pp 529-530.

Van Cleve B., Forreiter C., Sauter J.J. & Apel K. 1993. Pith cells of poplar contain photosynthetically active chloroplasts. Planta 189: 70-73.

Van de Pol P.A. & Breukelaar A. 1982. Stenting of roses; A method for quick propagation by simultaneously cutting and grafting. Scientia Hortic. 17: 187-196.

Van de Pol P.A. & Marcelis L.F.M. 1988. Apical application of aqueous solutions to roses via flower tubes - a technique with possibilities. Scientia Hortic. 34: 123-129.

Van den Berg G.A. 1987. Influence of temperature on bud-break, shoot growth, flower bud atrophy and winter production of glasshouse roses. Dissertation Agric. Univ. Wageningen, 170pp.

Van Harten A.M. 1978. Mutation breeding techniques and behaviour of irradiated shoot apices of potato. Agricultural Research Reports 873. Pudoc, Wageningen, 132pp.

Van Staden J., Spiegelstein H., Zieslin N. & Halevy A.H. 1981. Endogenous cytokinins and lateral bud growth in roses. Bot. Gaz. 142: 177-182.

Vijaya N. & Satyanarayana G. 1991. Effect of culture media and growth regulators on *in vitro* propagation of rose. In: Horticulture - New technologies and applications (J. Prakash & R.L.M. Pierik, eds). Kluwer Acad. Publ., Dordrecht, pp. 209-214.

Vité J.P. & Rudinsky J.A. 1959. The water-conducting systems in conifers and their importance to the distribution of trunk injected chemicals. Contr. Boyce Thompson Inst. 20: 27-38.

Wang Y.-T. & Boogher C.A. 1988. Effect of nodal position, cutting length and root retention on the propagation of golden pothos. HortSci. 23: 347-349.

Wetmore R.H. & Garrison R. 1966. The morphological ontogeny of the leafy shoot. In: Trends in plant morphogenesis (E.G. Cutter, ed.). Longmans, London. pp. 187-199.

Wilkins H.F. 1988. Techniques to maximize cutting production. Acta Hortic. 226: 137-143.

Yemm E.W. & Willis A.J. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57: 508-514.

Young L.C.T., Winneberger J.T. & Bennett J.P. 1974. Growth of resting buds. J. Am. Soc. Hortic. Sci. 99: 146-149.

Zamski E., Oshri S. & Zieslin N. 1985. Comparative morphology and anatomy of axillary buds along a rose shoot. Bot. Gaz. 146: 208-212.

Zhou T.S. & Hara N. 1989. Development of shoot in *Hydrangea macrophylla.* II. Sequence and timing. Bot. Mag. Tokyo 102: 193-206.

Zieslin N. 1981. Plant management of greenhouse roses. Flower cutting procedure. Scientia Hortic. 15: 179-186.

Zieslin N. 1992. Regulation of flower formation in rose plants: a reappraisal. Scientia Hortic. 49: 305-310.

Zieslin N. & Halevy A.H. 1976. Components of axillary bud inhibition in rose plants. I. Effect of different plant parts (correlative inhibition). Bot. Gaz. 137: 291-296.

Zieslin N. & Halevy A.H. 1978. Components of axillary bud inhibition in rose plants. III. Effect of stem orientation and changes of bud position on the stem by budding. Bot. Gaz. 139: 60-63.

Zieslin N. & Moe R. 1985. *Rosa.* In: Handbook of flowering (A.H. Halevy, ed.). Vol. 4, CRC Press, Boca Raton, pp 214-225.

Zieslin N. & Mor Y. 1981. Plant management of greenhouse roses. Formation of renewal canes. Scientia Hortic. 15: 67-75.

Zieslin N., Halevy A.H., Mor Y., Bachrach A. & Sapir I. 1972. Promotion of renewal canes in roses by ethephon. HortSci. 7: 75-76.

Zieslin N., Halevy A.H. & Biran I. 1973. Sources of variability in greenhouse rose flower production. J. Am. Soc. Hortic. Sci. 98: 321-324.

Zieslin N., Haaze H. & Halevy A.H. 1976a. Components of axillary bud inhibition in rose plants. II. The effect of bud position on degree of inhibition. Bot. Gaz. 137: 297-300.

Zieslin N., Mor Y., Bachrach A., Haaze H. & Kofranek A.M. 1976b. Controlling the growth and development of rose plants after planting. Scientia Hortic. 4: 63-72.

Zieslin N, Spiegelstein H, & Halevy A.H. 1978. Components of axillary bud inhibition in rose plants. IV. Inhibitory activity of plant extracts. Bot. Gaz. 139: 64-68.

Zimmermann M.H. 1978. Hydraulic architecture of some diffuse-porous trees. Can. J. Bot. 56: 2286-2295.

Zimmermann M.H. & Brown C.L. 1971. Trees; structure and function. Springer-Verlag, Berlin, 336pp.

Summary

The rose is the most important glasshouse cut flower in the Netherlands. Rose is a perennial woody shrub. The plants continuously form new flower shoots, which are the harvestable product. Within a rose crop there exists an undesired large variation in shoot number and size, which affects flower yield. Several developmental stages of shoot growth, varying from sprouting buds until harvestable shoots, occur within a crop at the same time, which makes manipulation of growth conditions complicated. Conditions which are optimal for shoots at a certain developmental stage may be suboptimal for shoots at a different developmental stage.

Axillary buds form the basis of production of a rose crop. They form the start of the upperground part of a new plant when propagated vegetatively and they determine the number of basal shoots, the degree of branching and eventually the number of flower shoots. However, little attention has been paid to axillary buds in rose research. The aim of the present study was to enlarge the knowledge and insight in the development of axillary rose buds. It was investigated to what extent the growth of an axillary bud into a shoot can be influenced during axillary bud formation and to what extent during actual outgrowth.

In Chapter 1 the organization of the shoot apical meristem is discussed. Furthermore, attention is paid to correlative inhibition.

In Chapter 2 the development of axillary buds and shoots is described. In each leaf axil one axillary bud is present. An axillary bud is an unextended partly developed shoot, containing the lower part of the future shoot. In the axils of the outermost scale-like leaves of this primary bud, a secondary bud is already present. The first secondary buds appear in the primary bud when the leaf subtending the primary bud unfolds. By that time the primary bud contains seven leaves and leaf primordia. During subsequent development of the shoot until it reaches the harvestable stage, the number of leaves and secondary buds in the primary bud increase to 11 and four, respectively. When the bud starts sprouting, additional leaves and a flower bud are formed within 10 days (Chapter 2.1).

In the axillary bud the cells of the pith are isodiametric and equal in size; they contain sugars and starch. The number of pith cells on a diameter line of the shoot does not change after bud break. The number of pith cells in an axillary bud reflects the potential diameter of the subsequent shoot. After bud break, pith diameter increases by cell expansion and final size of the pith is reached soon after start of shoot growth. Two types of cells have differentiated: small, vital cells and large dead cells. The small cells appear to form a network throughout the pith. The fraction of pith to shoot diameter decreases with time and is 50-60% in harvestable shoots ('sepals reflexing'). Final shoot diameter was found to be correlated with pith diameter (Chapter 2.1). The pith represents the primary growth of a shoot.

In Chapter 2.2 the origin and development of basal shoots was studied. Basal shoots are

vigorous shoots at the base of the plant. They form the frame of a rose plant and they determine the potential flower yield of a plant. Basal shoots appeared to be formed by buds in the axils of the scale-like leaves at the base of the shoot. Usually six to seven buds (potential basal shoots) were present at the base of the shoot. In Chapter 4 it was shown that this number depends on age and position of the bud used for propagation. The lowermost two buds were found most likely to sprout into basal shoots. These buds are already present as secondary buds in the axillary bud which is used for propagation. When a third basal shoot was formed, it arose from an axillary bud of one of the two basal shoots.

Chapter 2.3 focuses on the xylem (and water) pathways in a rose plant. By feeding dyes to roots or to shoots it was shown that each basal shoot is connected to only a part of the root xylem. When a new basal shoot starts growing, the existing root xylem becomes enveloped by a new xylem cylinder, serving the new shoot. As a result, the root xylem serving the former shoot may become fixed and its capacity may decrease with time. This may eventually lead to dying of the basal shoot.

Within the plant, axillary buds are subjected to correlative influences, i.e. influences of other plant parts. In order to study the growth potential of an axillary bud itself, an *in vitro* system has been developed (Chapter 3). Addition of cytokinin to the culture medium was necessary for the bud to complete its developmental programme up to formation of the flower bud. The shoots formed were morphologically comparable to shoots grown *in vivo*.

In the development of an axillary bud, two periods can be distinguished: (1) formation of axillary bud and its development as long as it is correlatively inhibited from sprouting, and (2) development after release from correlative inhibition. Chapter 4 focuses on the effects of several factors when applied before release from correlative inhibition, and Chapter 5 when applied after release from correlative inhibition. When factors were applied before release from correlative inhibition, shoot growth after release from inhibition ('growth potential' of the buds) was studied both when buds sprouted *in situ*, i.e. attached to the parent plant, and when buds sprouted in isolation. Growth potential in isolation was studied by culturing the buds *in vitro* as described in Chapter 3, or by grafting the buds in between the two leaves of a double-node cutting. After rooting, the cuttings were pruned above the grafted bud, which started to grow into a shoot. Shoot growth of buds *in situ* represents the situation in a crop, whereas shoot growth of buds in isolation enables to separate the effects on the bud itself from those on the parent plant.

In Chapter 4.1 the effect of bud age on development of axillary buds (from the middle part of a shoot) is described. Axillary buds need a certain developmental stage to be able to break. When this stage is reached, bud break requires release from correlative inhibition. However, axillary buds do not become dormant. Leaf initiation by the bud meristem continues, although at a low rate. As a result, the number of leaves in the bud and the weight of the bud increase. The bud stays in the vegetative stage, even for a period of more than one year. Transition of the meristem to the generative stage only occurs after release from correlative inhibition. The total number of leaves (including scales) preceding the flower of the subsequent shoot increased with increasing bud age and the

shoot carried more scale-like leaves and non-elongated internodes. Shoot length, weight and leaf area were not clearly affected by the age of the bud. The number of pith cells (on a diameter line) increased as buds got older, resulting in an increase in potential diameter of the subsequent shoot.

The anatomy and morphology of buds along the shoot was studied in Chapter 4.2. Along a shoot three groups of leaves can be distinguished: lower leaves (leaves with less than five leaflets), five-leaflet leaves (leaves with at least five leaflets) and upper leaves (leaves above the uppermost five-leaflet leaf, having less than five leaflets). The buds in the axils of the upper leaves often were generative and contained only a few leaves and a flower bud. These buds were suggested to be part of the inflorescence. Lower positioned buds were vegetative. Within the group five-leaflet leaves, weight of the bud increased towards the apex. Buds in the axils of the middle leaves contained most pith cells and the highest sugar and starch content. Pruning position on the shoot affected the growth of the buds into shoots. The contributions of assimilate supply, bud age and bud position to the effects of pruning position are discussed. Differences in final size of the shoot were largely the result of differences in assimilate supply created by the pruning height.

The effects of assimilate supply during axillary bud formation on development and growth potential of axillary buds are described in Chapter 4.3. Differences in assimilate supply were imposed by differential defoliation. At increasing assimilate supply weight of the axillary buds increased, whereas the number of leaves and leaf primordia in the bud only slightly increased. However, the total number of leaves preceding the flower of the subsequent shoot substantially increased. The number of pith cells (on a diameter line) increased with increasing assimilate supply. Size of the subsequent shoot at harvest ('sepals reflexing') was positively affected by an increased assimilate supply during axillary bud formation only when buds sprouted attached to the parent shoot.

Chapter 4.4 reports on the effect of a temperature pre-treatment on the development and growth potential of axillary buds. Rate of leaf initiation increased with increasing temperature, but the final number of leaves and leaf primordia in the bud was not clearly affected. The total number of leaves preceding the flower of the subsequent shoot, however, decreased with increasing temperature. Shoot size at harvest seemed to decrease with increasing pre-treatment temperature. Effects were more obvious for isolated buds than for *in situ* buds.

Assimilate supply and temperature after release from correlative inhibition largely affected the growth of the bud into a shoot (Chapter 5). In Chapter 5.1 assimilate supply was varied during shoot growth by retaining different numbers of leaves or different numbers of competing sprouting buds. Rate of bud break was not affected by an increased assimilate supply, whereas the subsequent growth period until harvest ('sepals reflexing') was shortened. Rate of elongation was stimulated until approximately three weeks after start of the treatments, indicating that at that time shoots became self-supporting for assimilates. Shoot size at harvest increased with increasing assimilate supply. The increase in shoot diameter was accompanied by an increase in pith diameter, which was due to more cell enlargement.

Temperature during shoot growth positively affected rate of development of the shoot (Chapter 5.2). Appearance of the flower

bud coincided with the maximum rate of elongation. Shoot size at harvest ('sepals reflexing') increased at lower temperatures as a result of the longer growth period. The effects of temperature on shoot size may have partly been mediated via an effect on assimilate supply.

In Chapter 4 it was shown that the total number of leaves preceding the flower was similar for buds sprouting *in situ* and in isolation. In Chapter 5 it appeared that the total number of leaves was not affected by temperature and/or assimilate supply during shoot growth. These results indicate that at the time of release from correlative inhibition the axillary bud is already determined to initiate a limited number of leaves before developing into a flower. Obviously, this number could only be influenced during axillary bud formation.

In Chapter 6 the results of the previous chapters are integrated into a coherent view on the developmental programme of an axillary bud. The programme can be regarded as a continuous process, comprising initiation of stem units and finally a flower. Axillary buds need to reach a certain developmental stage before they are able to break. When this requirement is met, release from correlative inhibition is prerequisite for bud break. Finally, initiation of the flower is controlled by apical dominance and does not occur before bud break (except for the upper 1-3 buds). The stage which is reached depends on prevailing internal and external conditions. Furthermore, it is discussed which parameters of shoot growth are already determined by the axillary bud and to what extent shoot growth can be affected after bud break. It can be concluded that the programme of an axillary bud is already set to a large extent at the time of release from inhibition. However, buds display a high degree of plasticity in their development into a shoot, in response to ambient conditions in which they are growing. Finally, some practical consequences of the results obtained are discussed.

Samenvatting

De kasroos is in Nederland de belangrijkste snijbloem. Het is een meerjarig gewas. De planten vormen continu nieuwe bloemscheuten. Binnen een rozegewas bestaat een ongewenst grote variatie in struikopbouw. Deze variatie betreft zowel het aantal, de dikte als de mate van vertakking van de stengels. De struikopbouw is sterk bepalend voor de potentiële produktie van de plant. Binnen het gewas komen op eenzelfde tijdstip verschillende ontwikkelingsstadia van scheutgroei voor, variërend van een uitlopende okselknop tot een oogstbare bloemstengel. Dit bemoeilijkt het sturen van de groeiomstandigheden. Immers, omstandigheden die optimaal zijn voor de groei van scheuten in een bepaald ontwikkelingsstadium hoeven dat niet te zijn voor scheuten in een ander stadium. Meer inzicht in de groei en ontwikkeling van een plant is nodig om de teelt beter te kunnen sturen.

Okselknoppen liggen aan de basis van de struikopbouw en van de bloemproduktie. In het geval van vegetatieve vermeerdering vormt een okselknop het beginpunt voor het bovengrondse deel van de nieuwe plant. Grondscheuten, de groeikrachtige scheuten aan de basis van de plant, ontstaan uit okselknoppen. Ook aan het optreden van vertakking ligt het al dan niet uitlopen van okselknoppen ten grondslag. Tenslotte begint iedere bloemscheut zijn ontwikkeling als knop in de oksel van een blad.

Ondanks het belang van okselknoppen is er bij de roos nog weinig inzicht in de ontwikkeling van een knop. Het doel van het hier beschreven onderzoek was om dit inzicht in de ontwikkeling van een knop te vergroten. Onderzocht is in hoeverre de ontwikkeling van een okselknop tot bloemscheut beïnvloed kan worden tijdens de aanleg van die knop en in hoeverre tijdens de werkelijke uitgroei ervan tot bloemscheut.

In hoofdstuk 1 wordt de opbouw van het apicale scheut meristeem (het groeipunt aan de top van een scheut) besproken. Verder wordt ingegaan op het optreden van correlatieve remming, dit is de remming die knoppen ondervinden die hen belet om uit te lopen.

In hoofdstuk 2 wordt de ontwikkeling van okselknoppen en bloemscheuten beschreven. In elke bladoksel is één knop aanwezig. De okselknop bevat in in-elkaar-geschoven vorm het onderste gedeelte van de toekomstige scheut. In de oksels van de buitenste knopschubben van deze zogenaamde primaire okselknop blijken al zogenaamde secundaire okselknoppen aanwezig te zijn. De eerste secundaire okselknoppen verschijnen in de primaire knop als de blaadjes van het blad, in de oksel waarvan de primaire knop staat, openvouwen. In de primaire knop zijn dan zeven bladprimordia aangelegd. Tijdens de ontwikkeling van de ouderscheut tot bloei neemt het aantal bladeren in de primaire knop toe tot ongeveer elf, terwijl het aantal bladeren met al een secundaire knop stijgt tot vier. Zodra de primaire knop uitloopt, worden binnen tien dagen nog een aantal bladeren met later weer okselknoppen en een bloemknop aangelegd (hoofdstuk 2.1).

In een okselknop zijn de mergcellen isodiametrisch en gelijk in grootte. De cellen

bevatten suiker en zetmeel. Het aantal mergcellen op een middellijn blijft onveranderd wanneer de knop uitloopt. Na knopuitloop neemt de diameter van het merg toe door celstrekking. De uiteindelijke diameter wordt vrij snel na knopuitloop bereikt. Twee typen cellen kunnen dan onderscheiden worden: Kleine levende cellen, waarin opslag van suiker en zetmeel plaatsvindt, en grote dode cellen, die gevuld zijn met lucht. De kleine cellen lijken een netwerk door het merg te vormen, dat in contact staat met de mergstralen. Het aandeel van het merg in de totale scheutdiameter neemt af in de tijd en bedraagt 50 à 60% bij oogstbare stengels. De scheutdiameter blijkt gecorreleerd met de mergdiameter (hoofdstuk 2.1). Het merg geeft de primaire groei van een scheut weer.

In hoofdstuk 2.2 werd de herkomst en ontwikkeling van grondscheuten bestudeerd. Grondscheuten zijn de groeikrachtige scheuten die aan de basis van de plant ontstaan. Zij vormen het frame van een rozestruik en bepalen de potentiële bloemproduktie. Aangetoond werd dat grondscheuten afkomstig zijn van de knoppen in de oksels van de knopschubben aan de basis van de plant. In het algemeen waren er zes à zeven potentiële grondscheutknoppen aanwezig. In hoofdstuk 4 wordt aangetoond dat dit aantal afhankelijk is van de leeftijd en de positie van de knop die gebruikt wordt bij de vermeerdering. Van de potentiële grondscheutknoppen liepen over het algemeen alleen de onderste twee uit tot grondscheut. Zoals in hoofdstuk 2.1 beschreven, zijn deze knoppen al als secundaire knoppen aanwezig in de knop die gebruikt wordt voor de vermeerdering. Indien een derde grondscheut gevormd werd, ontstond deze uit een okselknop van een van de twee grondscheuten.

In hoofdstuk 2.3 werden xyleem (en water) transportbanen in de plant zichtbaar gemaakt door aan de scheut of aan de wortel met kleurstoffen gekleurd water aan te bieden. Iedere grondscheut bleek slechts met een gedeelte van het xyleem in de wortel in directe verbinding te staan. De kleuringspatronen leidden tot de volgende hypothese: Zodra een grondscheut uitloopt, wordt het xyleem van de wortel omgeven door nieuw xyleem, dat transport naar de nieuwe scheut verzorgt. Dit nieuwe xyleem kan als een ring het gehele oude xyleem omgeven. Hierdoor kan het xyleemgedeelte dat in directe verbinding staat met de primaire scheut of de eerste grondscheut beperkt worden in capaciteit. Uiteindelijk kan dit tot afsterven van deze scheut leiden.

De okselknoppen aan een plant ondervinden een correlatieve invloed, d.w.z. invloed uitgaande van andere delen van de plant. Teneinde in staat te zijn om de groeipotentie van de knop zelf te kunnen bestuderen, zonder de invloeden van de rest van de plant, is een *in vitro* systeem ontwikkeld, waarbij knoppen geïsoleerd van de plant uitgroeien tot scheuten die morfologisch vergelijkbaar zijn met in vivo gegroeide scheuten (hoofdstuk 3). Toevoeging van cytokinine aan de voedingsbodem bleek essentieel voor de knop om uit te groeien tot een complete scheut.

In de ontwikkeling van een okselknop kunnen twee perioden onderscheiden worden: (1) aanleg en ontwikkeling van de knop voordat de correlatieve remming opgeheven wordt, en (2) knopuitloop en ontwikkeling tot een oogstbare scheut. In hoofdstuk 4 worden voor een aantal beïnvloedende factoren de effecten op de eerste periode beschreven, en in hoofdstuk 5 de effecten op de tweede periode. Wanneer de beïnvloedende factor tijdens de eerste periode werd aangelegd, werd de

groeipotentie van de knoppen bestudeerd door de knoppen zowel aan de ouderplant als geïsoleerd van de plant te laten uitlopen. Bij de laatste methode werden de knoppen losgemaakt van de plant en op een voedingsmedium *in vitro* gezet (zoals beschreven in hoofdstuk 3) dan wel geoculeerd tussen de twee bladeren van een dubbelstek. Zodra de dubbelstek beworteld was, werd het stengeldeel boven de geoculeerde knop gesnoeid, zodat de geoculeerde knop uitliep. De groei van de knoppen aan de ouderplant geeft de situatie in een normaal gewas weer, de groei van de geïsoleerde knoppen maakt het mogelijk om de effecten van de beïnvloedende factor op de knop zelf te scheiden van die op de ouderplant.

In hoofdstuk 4.1 wordt het effect van knopleeftijd op de ontwikkeling van de knop beschreven. Okselknoppen dienen een bepaald ontwikkelingsstadium bereikt te hebben alvorens ze in staat zijn uit te lopen. Zodra dit stadium was bereikt, was opheffing van de correlatieve remming van de knop vereist om uit te kunnen lopen. Okselknoppen blijken niet in rust te gaan. De bladafsplitsing van het meristeem gaat door, zij het op een laag niveau. Als gevolg hiervan nemen zowel het aantal bladeren en bladprimordia in de knop als het gewicht van de knop toe naarmate de knop ouder wordt. De knop blijft echter vegetatief (vormt geen bloem), zelfs gedurende een periode van ruim een jaar. De overgang van het meristeem naar het generatieve stadium vond alleen plaats nadat de knop begon uit te lopen. Het totaal aantal bladeren (inclusief schubvormige bladeren) onder de bloem van de gevormde scheut nam toe naarmate de knop waaruit de scheut gevormd was, ouder was. Lengte, gewicht en bladoppervlak van de scheut werd niet duidelijk beïnvloed door de leeftijd van de knop. Het aantal mergcellen nam toe naarmate de knop ouder werd, zodat de potentiële dikte van de toekomstige scheut eveneens toenam.

De anatomie en morfologie van okselknoppen van verschillende posities aan de stengel werd bestudeerd in hoofdstuk 4.2. De bladeren aan een stengel konden in drie groepen ingedeeld worden: De onderste groep betreft de bladeren met minder dan vijf blaadjes, de middengroep zijn de vijfbladen, d.w.z. bladeren met minimaal vijf blaadjes, en bovenaan de stengel zit de groep topbladeren, welke weer minder dan vijf blaadjes hebben. De knoppen in de oksels van de bovenste bladeren aan een stengel bleken vaak generatief te zijn en slechts een paar bladeren en een bloemknop te bevatten. Er wordt verondersteld dat deze knoppen tot de bloeiwijze behoren. De knoppen lager aan de stengel waren vegetatief. Binnen de groep vijfbladen nam het gewicht van de knop toe naarmate de knop zich hoger aan de stengel bevond. De knoppen in de oksels van de middelste vijfbladen bevatten de meeste mergcellen en het hoogste suiker- en zetmeelgehalte. De hoogte waarop een stengel gesnoeid wordt, blijkt de groei van de scheut die vervolgens gevormd wordt te beïnvloeden. Wanneer de snoeihoogte wordt gevarieerd, zijn zowel de positie en de leeftijd van de knop die uit zal lopen, als de hoeveelheid blad die aan de stengel blijft verschillend. De bijdrage van elk van deze factoren aan het effect van snoeihoogte wordt bediscussieerd. Verschillen in uiteindelijke grootte van de nieuw gevormde scheut bleken grotendeels veroorzaakt te worden door verschillen in assimilatenvoorziening als gevolg van verschillen in hoeveelheid blad .

In hoofdstuk 4.3 wordt het effect van assimilatenaanbod geanalyseerd. Gedurende de periode van okselknopaanleg werd de hoeveelheid beschikbare assimilaten geva-

rieerd door een verschillend aantal bladeren aan de plant aan te houden. Naarmate er meer bladeren en daarmee meer assimilaten beschikbaar waren, was het gewicht van de okselknop hoger, terwijl het aantal bladeren in de knop slechts weinig toenam. Het totaal aantal bladeren aan de scheut die uit de knop groeide, nam echter duidelijk toe met de assimilatenbeschikbaarheid. Ook het aantal mergcellen nam toe. De lengte en het gewicht van de scheut die uit de knop groeide, werden alleen als deze aan de ouderplant uitgroeide beïnvloed door de hoeveelheid assimilaten tijdens okselknopaanleg.

In hoofdstuk 4.4 worden de effecten beschreven van een temperatuurvoorbehandeling op de daaropvolgende scheutgroei. Hiertoe werd de temperatuur tijdens okselknopaanleg gevarieerd. Met stijgende temperatuur nam de bladafsplitsingssnelheid weliswaar toe, maar het aantal bladeren in de knop aan het eind van de voorbehandeling was niet duidelijk beïnvloed. Daarentegen was het totaal aantal bladeren aan de scheut die vervolgens uit de knop groeide, lager naarmate de temperatuur tijdens de voorbehandeling hoger was. Lengte en gewicht van de bloemscheut bij de oogst leek af te nemen met een stijgende temperatuur. De effecten waren duidelijker wanneer de knoppen los van de ouderplant uitgroeiden.

De hoeveelheid beschikbare assimilaten en de temperatuur tijdens de uitgroei van een okselknop tot oogstbare bloemscheut bleken een duidelijke invloed te hebben op de groei en ontwikkeling van de scheuten (hoofdstuk 5). De hoeveelheid beschikbare assimilaten werd gevarieerd door het aantal bladeren of het aantal uitgroeiende zijscheuten te variëren (hoofdstuk 5.1). Hoewel de snelheid van knopuitloop niet afhankelijk bleek van de hoeveelheid assimilaten, was de groeiduur tot oogst duidelijk korter naarmate er meer assimilaten waren. De eerste drie weken van de groeiperiode was de snelheid van stengelstrekking hoger als er meer assimilaten beschikbaar waren. Waarschijnlijk was de uitgroeiende scheut na drie weken zelfvoorzienend, aangezien er vanaf dat moment geen verschil in snelheid van stengelstrekking tussen de behandelingen was. Lengte en gewicht van de bloemscheut bij oogst waren duidelijk afhankelijk van de hoeveelheid assimilaten tijdens de groei. De toename in scheutdikte bij een hoog assimilatenaanbod ging gepaard met een toename in de dikte van het merg, wat op zijn beurt een gevolg was van grotere cellen.

Temperatuur tijdens uitgroei van een okselknop tot oogstbare scheut had een duidelijk effect op de ontwikkelingssnelheid van de scheut (hoofdstuk 5.2). Het moment dat de bloemknop met het blote oog zichtbaar was, viel samen met het moment waarop de snelheid van stengelstrekking maximaal was. Lengte en gewicht van de bloemscheut bij de oogst nam toe naarmate de temperatuur lager was. De effecten van temperatuur waren mogelijk gedeeltelijk het gevolg van een bijeffect op de assimilatenvoorziening.

In hoofdstuk 4 werd aangetoond dat het aantal bladeren onder de bloem gelijk was indien knoppen aan de ouderplant dan wel los van de ouderplant uitgroeiden tot scheut. In hoofdstuk 5 bleek dat het aantal bladeren onder de bloem niet meer beïnvloed werd tijdens knopuitloop en scheutgroei. Dit wijst erop dat het totaal aantal bladeren al bepaald is in de okselknop op het moment dat die knop begint uit te lopen. Het aantal kan kennelijk alleen beïnvloed worden tijdens de aanleg van de okselknop.

In hoofdstuk 6 wordt ingegaan op het programma, dat een knop doorloopt vanaf aanleg

tot oogstbare bloemscheut. De resultaten van de voorgaande hoofdstukken zijn hierin geïntegreerd. Dit programma kan gezien worden als een continu proces, bestaande uit aanleg van stengeleenheden en uiteindelijk van een bloemknop. Okselknoppen hebben een bepaald ontwikkelingsstadium nodig alvorens ze uit kunnen lopen. Of ze vervolgens uitlopen is afhankelijk van de mate van correlatieve remming die ze ondervinden. Bloemknopaanleg tenslotte vindt alleen plaats als de okselknoppen uitlopen tot scheut, met uitzondering van de 1 à 3 bovenste okselknoppen (deze knoppen kunnen tot de bloeiwijze gerekend worden). Er zijn dus een aantal interne en externe condities die bepalen welk stadium van het programma een knop bereikt. In hoofdstuk 6 wordt tevens aangegeven welke aspecten van de groei van een knop tot een scheut al bepaald zijn in de okselknop en in hoeverre de scheutgroei nog beïnvloedbaar is na knopuitloop. Concluderend kan gezegd worden dat het programma van een knop grotendeels bepaald wordt tijdens knopaanleg, maar dat de grootte van de bloemscheut die uit de knop groeit nog aanzienlijk beïnvloed kan worden na knopaanleg. Tenslotte wordt een aantal consequenties van de in dit proefschrift beschreven resultaten voor de praktijk besproken.

Nawoord

Het in dit proefschrift beschreven onderzoek is tot stand gekomen in samenwerking tussen de vakgroep Plantencytologie en -morfologie (PCM) en de vakgroep Tuinbouwplantenteelt. Een multidisciplinaire aanpak geeft een duidelijke meerwaarde aan het onderzoek, hoewel het niet altijd even gemakkelijk is. Bovendien is het erg leerzaam om 'gedwongen' te worden zodanige formuleringen te gebruiken dat de tekst voor personen van verschillende vakgebieden begrijpelijk is. Deze pagina van mijn proefschrift wil ik graag gebruiken om de vele personen te bedanken, die op een of andere wijze eraan bijgedragen hebben dat mijn onderzoek over rozen ging.

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Christianne Marcelis-van Acker

Curriculum vitae

Christianne Augusta Maria van Acker werd geboren op 27 november 1963 in het Zeeuws-Vlaamse Sint Jansteen en groeide op in een boerengezin van zeven kinderen. In 1982 behaalde ze het Gymnasium-B diploma aan de Jansenius Scholengemeenschap in Hulst en begon ze de studie Tuinbouw aan de toenmalige Landbouwhogeschool in Wageningen. In 1985 liep ze stage bij de 'Bulb Physiology Group' van het toenmalige Glasshouse Crops Research Institute te Littlehampton in Engeland. Tijdens de doctoraalfase werden de afstudeervakken Bloementeelt, *In vitro* cultuur, Ontwikkeling van vegetatieve plantedelen en Plantenveredeling uitgevoerd. In 1987 breidde ze haar achternaam uit tot Marcelis-van Acker. In 1988 behaalde ze het doctoraalexamen met lof. In datzelfde jaar werd ze op grond van een zelf ingediend projectvoorstel aangesteld als AIO bij de vakgroepen Tuinbouwplantenteelt en Plantencytologie en -morfologie, waar ze onderzoek verrichtte naar anatomische, morfologische en fysiologische aspecten van de okselknopontwikkeling bij de roos. De resultaten van het onderzoek zijn beschreven in dit proefschrift. In januari 1993 werd ze aangesteld als transfercoördinator bij Agrotransfer, het transferpunt van de Christelijke Agrarische Hogeschool te Dronten. Vanaf september 1993 is ze werkzaam als secretaris bij de Stichting Nederlands Graan-Centrum.