STUDIES TO ELUCIDATE SOME EFFECTS OF CYTOKININS ON THE REPRODUCTIVE POTENTIAL OF *VICIA FABA* L.

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Abstract

Abscission of flowers and loss of pods appear to be part of normal reproductive development in *Vicia faba* L. The biological processes leading to this loss of reproductive potential are yet to be elucidated, but may be mediated by a lack of an adequate cytokinin supply to the reproductive organs. External application of cytokinins was used in this study to help further understand the regulation of reproductive loss in this species.

The effect of six cytokinins on the reproductive potential of broad beans, grown under controlled conditions, was studied by local application of the chemicals on every inflorescence having flowers prior to and at full petal opening. 6-(benzylamino) purine (BA) and 6-[benzylamino-9-(2-tetrahydropyranyl)] purine ([9tP]BA) were the most active compounds in preventing flower abscission, regardless of the flower position on the plant. However, this early increase in reproductive potential was eliminated due to compensatory pod-drop during their development. It was suggested in the discussion that repeated applications of these compounds may prevent abortion of the pods.

In field experiments using various cultivars of *V. faba* L., the reproductive portions of plants were sprayed twice with cytokinins, at early flowering and at early pod-initiation. Both BA-compounds resulted in almost complete flower retention. It was the varieties for all subspecies that normally set fewer pods that showed the better response to cytokinin treatment. The response of subspecies of *Vicia faba* to cytokinins in descending order was *major* > *equina* > *minor*. At harvest, analysis of the cytokinin effects on yield components indicated a great variation in plants within each variety. Thus, recorded increases in the dry weight of harvestable organs were not statistically different from controls apart from cv. Toret, a uniform variety.

Depending on the variety, consistent enhancement in fruit-set in faba bean due to BA-application was associated with increased sucrose synthase activity and/or acid invertase activity at carpels before or at pollination, measured 24h after the application of the growth substance. Subsequently, an investigation of the inter-relationships between sucrose content of the carpel and successful pod-set after application of BA was performed. Each flower was at a known stage of development. The probability of each flower achieving pod-set was also known by virtue of its position on the inflorescence. Treatment with BA 24 hours before anthesis caused all flowers to set a pod, and this was accompanied by an increase in sugar content of the carpel one day after application. Decreases in glucose and fructose content were also found, a few days later, when flower-petals collapsed. These results are fully discussed in relation to sink development in *Vicia faba*.

In conclusion it is suggested that cytokinins strongly influence pod-set in *V. faba* L. The metabolism of sugar *in vivo* before or at pollination of the flower appears to be, at least part of the way cytokinin effects its action. To investigate this further, new methodologies need to be developed to elucidate the mode of action of cytokinin on sugar metabolism before or at anthesis of the flower and its relation to successful pod-set.

Dedication

This work is dedicated to my wife Eudokia,
precious companion and
groundwork of every happiness.

Declaration

I, the undersigned, hereby declare that this thesis has been composed entirely by myself and that all the work carried out herein is my own, except where specifically stated.

Efstathios I. Stavrianos
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Yea, the Lord is become my refuge,
and my God the helper of my hope.
(Psalm 93, 2)

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List of abbreviations

[9R]Z	zeatin riboside
[9tP]BA	6-[benzylamino-9-(2-tetrahydropyranyl)] purine
A_{520}	absorbance measured at 520 nm wave-length
ADP	adenosine-5-diphosphate
AI	acid invertase
ATP	adenosine-5-triphosphate
BA	benzyladenine
BSA	bovine serum albumine
ck	cytokinin
cv.	cultivar
D	margin of error of treatment mean
DW	dry weight
d.f.	degrees of freedom
DIECA	sodium diethyldithiocarbamate
DTT	DL-dithiothreitol
dw	dry weight
EC	European Community
EDTA	disodium ethylenediamine tetraacetate
ELISA	enzyme-linked immunosorbent assay
F-6-P	fructose 6-phosphate
fds	flower development stage
FW	fresh weight
G-6-P	glucose-6-phosphate
HPLC	high performance liquid chromatography
iP	isopentenyl adenine
KIN	kinetin
LSD	least significant difference
NADP	nicotinamide-adenine dinucleotide phosphate
NADPH	reduced nicotinamide-adenine dinucleotide phosphate
p	probability
PGR	plant growth retardant
PVPP	polyvinylpolypyrrolidone
SPS	sucrose phosphate synthase
SS	sucrose synthase
Suc	sucrose
TFW	*Vicia faba* L. *cv.* Three Fold White
UDP-G	uridine[5']diphospho[1]-α-D-glucopyranoside
UV	ultra-violet
Z	zeatin

Chapter 1

General introduction, background and rationale

1.1 Plants and man

Life here on Earth depends on the flow of solar energy. Only 1 percent of the sunlight energy reaching the earth becomes, through a series of operations performed by the cells of plants and other photosynthetic organisms, the energy that drives all the processes of life (Curtis & Barnes, 1989). The 5.3 billion people now on Earth are dependent on plants for their existence (Belopopsky & Oikonomou, 1996). There are at least 400,000 species of plants world wide. Of these, roughly 75,000 are known to be edible yet humans rely on only 20 species for 90% of their nutrition. Moreover, these 20 species are grown on only 10% of the entire Earth's land surface (Valaoras, 1992). Thus, crop production, i.e. the management of useful plants, can be regarded as the very basis of our civilisation (Forbes & Watson, 1991).

The first production of food by crop cultivation dates back to between 7,000 to 10,000 years ago, in the Neolithic era (Sauer, 1952). Today, even though over one million jobs world-wide depend either directly or indirectly on agriculture, the goal of proper nutrition for all people on earth remains an endeavour yet to be achieved (F.A.O., 1992).

During the history of man as a plant grower the increase of crop output per unit of land area has been achieved through the adoption of two broad strategies: (1) optimising the environment to suit the crop plants; (2) changing the crop plant to suit the environment in which it is grown (Janick *et al.*, 1974). Altering the environment principally involves the provision of more nearly optimal amounts of water (via irrigation and drainage) and of inorganic nutrients (e.g. fertilisers), and for horticultural crops the use of glass-houses (e.g. increased temperatures and sometimes CO_2-enrichment of the air), but also, and very importantly, the use of pesticides and herbicides to reduce competition in the environment. Changing crop plants to suit the environment has been historically achieved through plant breeding, but there is also a second approach based upon discoveries of various chemicals (e.g. plant growth substances) that can be applied to alter the rate of plant growth or the pattern of plant development in desired directions (Leopold *et al.*, 1975; Jeffcoat, 1981; Scott, 1984; Purohit, 1985; Grossman, 1990).

Some of these plant growth substances are related on the basis of either their chemical structure or their biological function to the five groups of the natural endogenous hormones of plants. Although, the mechanism of different plant hormone actions are not understood (Guern, 1987; Thomas, 1991; Naqvi, 1995) this has not prevented some of these substances becoming useful tools in agricultural and

horticultural practices (Luckwill, 1981; Bruisma, 1985; Schuch, 1990; Hoffmann, 1991). However, future progress in this field has to be linked with advances in whole-plant physiology, particularly of the endogenous mechanisms that control growth, fruiting and hence ultimately, the yield of crops.

1.2 Internal control of plant growth and development

All plant products are at the time of harvest or utilisation the result of changes in their structure and appearance as they grow. Development is the process where both quantitative differences in the numbers and arrangement of cells within different organs, and qualitative differences between cells, tissues and organs, occur in an organism (Wareing & Phillips, 1982). The term growth is applied to quantitative changes that occur during development and may be defined as an irreversible change in the size of a cell, organ or whole organism (Causton & Venus, 1981). The external form of an organ is primarily the result of differential growth along certain axes (Burgess, 1985). The term differentiation is applied on the qualitative changes that take place during development. Therefore, differentiation involves, in a broad sense, any situation in which meristematic cells give rise to two or more types of cell, tissue or organ which differ from each other in anatomical and physiological specialisation and organisation. Thus, growth and differentiation are considered as the two major developmental processes in plant life (Wareing & Phillips, 1982). Usually they take place concurrently during development, but under certain conditions growth can be obtained without differentiation, as in the growth of a mass of callus cells.

The growth of a plant is a dynamic, complex and strictly controlled process (Stitt & Schulze, 1994). This means that growth in different parts of the plant must be integrated and co-ordinated. The co-ordination of growth between different parts of the plant must clearly involve mechanisms of control . The development of organs, such as leaves or stems, involves an orderly sequence of phases of cell division and cell extension, so that there is also co-ordination of growth in time (Charles-Edwards *et al.*, 1986).

In multicellular organisms like higher plants, differentiation comes about as a result of divergent growth within and among cells. This is accomplished in an orderly and systematic way, with mitotic cell division ensuring genetically the continuity of all cells. The differentiation of genetically identical cells in plants is a process not as yet completely understood. The way in which differentiation is accomplished appears to

depend on the interaction between the cell's genetically controlled processes and the external environment. In plants particular cells take over the control of differentiation through the action of "chemical messengers". These compounds that are natural plant products are active in extremely small amounts, controlling presumably via concentration or influencing with control perhaps being via changes in cell sensitivity to the growth substance (Trewavas, 1981, 1982; Trewavas & Cleland, 1983) various plant processes, most of which are examples of growth and development. These chemicals, are known as plant hormones (from the meaning of the Greek origin of the word i.e. to set in motion or to stimulate), or more accurately, plant growth substances (Davies, 1995). Plant growth substances are organic substances, other than nutrients, which when present in small amounts can evoke specific biochemical, physiological or morphological responses (Moore, 1979). This category of substances can be divided into two groups: plant hormones and artificial plant growth regulators. Plant growth regulators include: growth inhibitors (defined at Section 1.5.5.2), growth retardants (defined at Section 1.5.5.3), hormone transport inhibitors and ethylene promoters or inhibitors.

As a result of studies extending over the last 60 years in the chemical control systems affecting physiological functions in plants, a number of important groups of hormone-like substances have been defined: auxins, gibberellins, cytokinins, ethylene and abscisic acid. Furthermore, a growing body of evidence shows that a variety of plant responses occurs with small oligosaccharides (York *et al.,* 1984; Tran *et al.,* 1985) and strongly suggests that there are also other natural groups of plant chemicals that can modify plant growth. The molecular basis, however, for the chemical control of many distinct growth patterns, such as flowering, remains unknown (Schell *et al.*, 1993).

Since growth is a co-ordinated activity, the various processes that proceed simultaneously in a plant are not independent, but are closely linked, one with another. Endogenous plant growth substances play important roles in the correlation of growth in different parts of the plant. Examples of growth correlation include the correlative inhibition of buds (apical dominance); the stimulation of fruit growth by hormones produced by the developing seeds etc. It is likely that all growth correlations are in one way or another affected by patterns of plant growth substances distribution within the plant which appears to influence accumulation of biomass (fresh matter) among plant organs (Weaver & Johnson, 1985). To some extent these growth correlations are explicable in terms of the availability of assimilates and the competition between growing regions for these substances (Farrar, 1992).

Assimilate partitioning is an important topic for both theoretical and applied plant physiology, because the incorporation of assimilates into the economically important components of a crop determines the final yield and thus the potential of economic reward (Gifford *et al.*, 1984; Daie, 1985; Gifford, 1986; Henrix, 1995). How organ growth and dry matter partitioning are regulated are the subject of scientific dispute hence no single unequivocal theory is acceptable at present (Gifford and Evans, 1981; Wolswinkel, 1985; Farrar, 1988; Patrick, 1988; Wardlaw, 1990). However, a very large amount of research in the field of plant growth substances has demonstrated that cytokinins, and indeed other plant growth substances, play a decisive role in regulating almost every aspect of plant development by influencing cell division and enlargement, cell differentiation and partitioning processes (Wilkins, 1984; Weaver and Johnson, 1985; Kuiper, 1993).

1.3 Nature and role of cytokinins in controlling developmental processes

1.3.1 Discovery and definition

The existence of specific substances that could control cell division were first postulated by scientists in the latter half of the nineteenth century (Wiesner, 1892). The first substance discovered that affected plant cell division and growth was called kinetin (Fig. 2.1.1) or more correctly 6-furfurylaminopurine (Miller *et al.*, 1955, 1956). Since then, a number of synthetic (e.g. BA) and naturally occurring (e.g. zeatin) 6-substituted aminopurine derivatives have been characterised (Horgan, 1984; McGaw & Burch, 1995). On the basis of their physiological capacity to promote *in vitro* callus cell division and growth (i.e. cytokinesis) in the same manner as kinetin, this class of substances were arbitrarily given the generic name cytokinin (Skoog *et al.*, 1965).

1.3.2 Occurrence in plants

Cytokinins, due to their predominant production in the roots, have been postulated as a chemical root signal transferred by the xylem sap to the shoot (Van Staden & Davey, 1979; Letham & Palni, 1983; Neumann *et al.*, 1990; McGaw & Burch, 1995). Recently, however, evidence for the translocation of cytokinin and some of its metabolites in the phloem have been reviewed by Hoad (1995). Irrespective of cytokinin production in other meristematic tissues e.g. corn kernels (Miller 1967) or seeds (Van Staden, 1983), the root-produced cytokinins have been interpreted as

master signals in the control of shoot growth (Sitton *et al.,* 1967; Komor, 1994). They occur in plants as free compounds (*de novo* biosynthesis) and as constituents of some tRNAs. According to the current theory on the biosynthesis and metabolism of cytokinins (Koshimizu & Iwamura, 1986), the nucleotides represent the first cytokinin-type compounds in the biosynthetic pathway from 5'-adenosine monophosphate to the great variety of species of this plant hormone group. However, the relative contributions of the various sources of these substances under different physiological conditions, and the pathway of cytokinin synthesis in normal plant tissues still needs to be elucidated by further research (Horgan, 1992).

1.3.3 Cytokinin metabolism in plants

Knowledge of cytokinin metabolism is incomplete (Letham & Palni, 1983; Koshimizu & Iwamura, 1986; McGaw, 1988), because until the 1980s these growth substances could only be measured with overall bioassays. Today, HPLC combined with ELISA techniques provide powerful analytical procedures to identify cytokinin patterns in plant tissues and to quantify the individual constituents (Fusseder *et al.,* 1988; Sayavedra-Soto *et al.,* 1988; Horgan, 1992b). Their metabolism consists of: (1) interconversion of bases, nucleosides and nucleotides, (2) N-glucosylation and alanine conjugation of the purine ring, (3) O-glucosylation and alanine conjugation of the purine ring, (4) side-chain reduction, and (5) side chain cleavage (Letham & Palni, 1983; McGaw & Burch, 1995).

Cytokinins are known for their capacity to inter-convert between a number of different forms e.g. BA metabolism (Letham & Gollnow, 1985). It should be noted that very little is known about the significance of these changes to the biological function of the cytokinins. There is indirect evidence that the free bases (i.e. unsubstituted cytokinin-molecules which are not incorporated into macromolecules as tRNA, such as kinetin, zeatin, benzyladenine etc.) are most likely to be the biologically active form of cytokinins (Horgan, 1992a). This indicates that the rate of conversion of cytokinin ribosides to bases may control the activity of cytokinin in plant cells (Kaminek, 1992).

1.3.4 Physiological effects on developing "sinks"

Cytokinins are generally associated with cell division and enlargement (Letham & Bollard, 1961; Miller, 1961; Fosket *et al.,* 1977; Nishinari & Syono, 1980; Horgan,

1984). They also apparently play an important role in nutrient mobilisation within plants (Mothes *et al.,* 1959, 1961; Mothes & Engelbrecht, 1961). Both these processes are of the utmost importance in plant growth and development (Ho, 1988). However, not only are the molecular mechanisms by which cytokinins mediate growth (Romanov, 1990; Trewavas, 1991) but also the cytokinin relations (biosynthesis, metabolism, translocation) of a plant are still not completely known.

There is circumstantial evidence from both correlative and pharmacological experiments that this class of growth substances establish strong sinks for assimilates and nutrients, often accumulating resources at the expense of other organs, even under stress conditions (Weaver & Johnson, 1985; Amzallag *et al.*, 1992; Ronzhina *et al.*, 1995). Replacement studies in which kinetin could restore assimilate movement to the point of application after excision of a sink organ provide further evidence that cytokinins influence sink strength, i.e. the competitive ability of a sink to attract assimilates (Wareing & Patrick, 1975; Patrick & Wareing, 1981; Wolswinkel, 1985; Kuiper, 1993).

The effect of cytokinins on sink strength could be indirect via the stimulation of cell growth and thus an increase caused in sink size. More likely, cytokinins, by stimulating the cell division activity of a sink (Bernier *et al.* 1977), may indirectly increase a tissue's demand for photosynthates and nutrients, thus causing enhanced sink strength and, in turn, greater phloem unloading. Rapid production of mRNAs from nuclear-encoded genes was observed after treatment of cotyledons with BA (Ohya & Suzuki, 1991), an observation which is in line with this interpretation of the mode of sink strength regulation by cytokinins.

However, a more direct effect through stimulation of sink activity (some aspect of metabolism per unit weight of sink tissue), although proposed by several authors, is still controversial. Patrick and Wareing (1981) suggested that the transfer of assimilate across the phloem-boundary membranes into the sink free space was a good candidate for hormonal control of sink activity. In addition, increased utilisation of assimilates in the sink, which requires a high metabolic rate, may cause more assimilates to be directed into this sink and an increased uptake of carbohydrates (in terms of sucrose) from the phloem (Ho, 1979; Morris, 1982; Giaquinta, 1983; Sung *et al.*, 1988). It is likely that cytokinins affect these processes because it is known that these plant growth substances enlarge both the capacity (by protein synthesis or activation of precursor proteins) and the activity of the proton

pumps to transport protons and possibly the energization of the transport of carbohydrates over the plasma membrane (Kuiper *et al.*, 1992), and thus in turn the activity of plant sinks.

1.3.5 Biological activities in plants and potential uses

Among other biological activities, the interaction of cytokinins with auxins, in the induction of organ formation in plant-tissue cultures is of major importance for plant biotechnology (Schuch, 1990; Tao & Verbelen, 1996). While a high auxin:cytokinin concentration ratio in the culture medium induces rooting, the reverse ratio favours bud and shoot formation (Skoog & Miller, 1957). This principle has been successfully exploited in the development of clonal and micropropagation techniques for a large number of plant species (Evans, 1989).

The application of cytokinins also promotes the growth of lateral buds by reducing the dominance of the apical bud (Sachs & Thimann, 1967; Cline, 1991 for literature) and , in this way, affects the shape of plants and the formation of fertile branches. Recently, results have been presented (Bangerth, 1994; Li *et al.*, 1995) suggesting as a possible regulatory mechanism of apical dominance the mutual interaction between the basipolar auxin transport system and the endogenous cytokinins transported via the xylem into the stem of leguminous plants.

Cytokinins also enhance the resistance of plants to various forms of stress (salinity, moisture, high temperature); they regulate plant growth under drought conditions, delay senescence of intact plants and excised plant parts (Brown *et al.* 1991; Grabau, 1995), reduce abscission of plant organs possibly by indirectly delaying senescence (Kuang *et al.*, 1992) and stimulate the development of chloroplasts (Halmann, 1990). At least some of these effects may be related to the ability of cytokinins in certain plant species to regulate the expression of specific genes (Chen, 1989; Chen *et al.*, 1993).

Recent evidence indicates that applying cytokinins to cereals just after anthesis enhanced grain setting and grain yields of wheat, barley, rye, triticale and oat (Hradecka & Petr, 1992). This effect is especially pronounced in plants grown with a reduced supply of nitrate, and is of both economical and ecological importance (Bouwman, 1996) because the crop may benefit from the application of cytokinins and thus a decrease in N-fertiliser intensity (tones fertiliser/tones biomass) may be achieved together with rising crop yield.

Although cytokinins exert spectacular effects on the growth and development of plants, it would therefore seem obvious to link these compounds with the control of these processes. A distinction, however, should be noted between the clear cut effects of externally applied cytokinins and the, as yet, very incomplete evidence for their endogenous roles (Horgan, 1984). Their low solubility and apparent poor transport within the plant and their pleiotropy of events, along with economic and sociological reasons (Rademacher, 1991; Gianfagna, 1995) have been further limitations in the adaptive use of cytokinins in agricultural practice. However a great diversity of laboratory experiments continues with this group of chemical plant growth substances. Regulation of the cytokinin content of specific plant parts at certain stages of plant development and/or under certain environmental conditions represents a promising approach for current research aimed at enhancing plant productivity and the yield stability of major crop plants (Kaminek, 1992).

The stability of yield appears to be a more important goal than simply boosting yield (Bond, 1987; Russell, *et al.*, 1989; Ney, 1992; Evans, 1993; Alexandratos, 1995). In subsistence farming, year-to-year variations can produce periods of feast and famine. The famine has disastrous effects on the survival and stability of the communities (i.e. the people inhabiting a particular area) involved, and indeed on the political stability of entire nations (Nwokolo, 1996). On the other hand, in highly developed economies with intensive systems of agriculture, market instability due to yield variation is still a central problem. Therefore, one challenge for plant scientists is to investigate the ability of cytokinins to reduce the gap between maximum potential yield and average yield in practice of some economically important species which suffer from yield instability, such as *Vicia faba* L. (Hawtin & Hebblethwaite, 1983; Stelling *et al.*, 1994). This in turn will enhance food security in developing countries and safeguard farm incomes in developed countries (Alexandratos, 1995).

1.4 The plant *Vicia faba* L.

1.4.1 Background and history

Vicia faba L. is one of the oldest food legumes and has been cultivated since the beginning of human history. *Vicia faba* L. is thought to have been first domesticated in west or central Asia about 6000 years ago but interestingly large seeded types (*Vicia faba* L. *major*) did not appear anywhere until recorded in the Mediterranean region in about 500 AD (Cubero, 1974; Ladizinski, 1975). By 1200 AD both *major* and *minor* types had been introduced into China. The crop was unknown in North

America until after the arrival of Columbus in the late 1400's. The Spanish and Portuguese were thought to have introduced the plant to Central and South America in the 16th century (Bond, 1976). As discussed by Hawtin & Hebblethwaite (1983) both the progenitor species and place of origin remain uncertain.

In Europe faba beans were the only bean known in the pre-Columbian era, before the introduction of *Phaseolus* beans from the New World. Their relative high yield potential, good storing ability and soil improving properties ensured them a prominent role in much of the European agriculture up to modern times (Hawtin & Hebblethwaite, 1983).

Faba bean are known botanically as *Vicia faba* L. Although there is some disagreement in the literature, the subspecific classification of Muratova (1931) is generally accepted based on the criterion of seed size, with species being divided into the two subspecies *paucijuga* and *eu-faba.* Within the *eu-fabae*, three varieties are commonly recognised: (1) the large seeded variety *major*, commonly known as broad beans with large flattened seeds, (2) the intermediate variety *equina*, Scotch or horse beans with medium sized seeds, and (3) the variety *minor* with small rounded seeds. *Paucijuga* is sometimes referred to as tick beans and those varieties have the smallest seeds (Hawtin & Hebblethwaite, 1983).

The common English name of faba beans has been adopted to refer to plants belonging to the species *Vicia faba* L. This was necessary because in the USA the term field bean is used to denote *Phaseolus vulgaris*.

The faba bean differs from other *Vicia* species in that it lacks tendrils, also the hilum is at right angles to the seed (Gunn, 1978). It is typically a tall annual, the dominant phenotype having an indeterminate growth habit with axillary racemes developing after between five and ten vegetative nodes have formed. The flowers are commonly off-white with dark stripes. The racemes develop acropetally. Typically between 6-12 racemes are formed, although the number is strongly influenced by environmental conditions prevalent at flowering. Evans (1959) has shown that faba beans exhibit a quantitative long day response, the critical photoperiod for flower initiation being 12-13 hours. More recent studies in controlled environmental conditions (Ellis *et al.*, 1988a,b) have confirmed these findings. Work by Summerfield and Roberts (1988) has shown that the progress towards flowering in *Vicia faba* L. is affected by vernalisation, post vernalisation average temperature and photoperiod.

In addition to indeterminate varieties which have many source-sink units on one shoot, there are physiologically semideterminate ones which have the complete source-sink relation as a whole shoot (Kogure, 1984). The semideterminate plants are early-maturing genotypes and are essential in places with cool summers such as in Northern Europe in order to bring harvest forward and thus enable land to be prepared for autumn sowing (Lawes *et al.*, 1983; Knott *et al.*, 1994).

Early maturing varieties with a determinate habit have also been bred, the most notable being Ticol (Bond *et al.*, 1983; Debely & Derbensky, 1992). These form flowers on four axillary inflorescences, the fifth and final inflorescence forms a terminal raceme ('topless' type). These types branch profusely. They yield less than the indeterminate forms (Pilbeam *et al.*, 1989; Pilbeam *et al.*, 1990). In addition to the limitation of grain yield, there is also variation for flower colour, seed characteristics, stem branching, inflorescence arrangement, pod orientation and characteristics, nutritional content of seeds, ploidy status as well as evidence of drought and salinity tolerance (Chapman, 1981). In experiments with various accessions of indeterminate field beans grown in Australia, Stoddard (1993) demonstrated that the field bean plant was morphologically determinate and produced a terminal inflorescence when flowers were stripped for all of the normal reproductive cycle. It was also shown in this study that the rate of production of flowering nodes was primarily controlled by environmental factors, particularly nocturnal temperatures, while their potential number was genetically determined.

The average seed weight is the most stable component of yield after number of ovules per pod, both having high heritability. These two characters and the growth habit of the plant have been used in variety classification (Higgins *et al.*, 1988).

1.4.2 World cropping areas

Faba bean production is widespread in temperate and subtropical regions of the world. It is the worlds seventh most important grain legume crop after soybean, phaseolus bean, groundnut, dry pea, chickpea and pigeon pea. The total world production of dry broad beans (term used by F.A.O for faba bean) was around 3.4 million tons in 1996 (F.A.O., 1996b). World-wide the area of cultivation is small (2.9 million ha), but nearly 2 millions ha are grown in China and over 5% of the arable land in Egypt is used for growing the crop (F.A.O., 1996b). In Morocco,

Tunisia and Algeria more than 300,000 ha are grown mainly for human consumption (Bamouh, 1995). However, only 14% of faba bean crop is grown in developed countries (F.A.O., 1996b).

Vicia faba L. is the second ranking seed legume in Europe after peas. Its total area was 172,000 ha in 1994 and its production basin progressively moved from the South to the North (F.A.O., 1994). The UK, Italy, Germany and Spain produce 95% of the European faba bean crop. The UK crop increased to 163,000 ha in 1993; an area not seen for 100 years and actually 72.8% of the maximum area ever recorded in the 1870s (Crofton, 1996). With a total area of 149,300 ha in 1994 the UK was the major producer of faba beans in the European Union (Bond & Pope, 1995). The area of field beans, grown mainly for stockfeed, fell to 120,000 ha in 1995, and may be attributed in part to a fail in yields experienced the year before and to strong competition from alternative break-crops. Overall production decreased from 453,900 tonnes in 1994 to 342,000 tonnes in 1995 (M.A.F.F., 1996). Since 1978 the European Commission has supported the price for dry pea and faba bean but not that of chickpea and lentil. This support allowed a rapid development during the early eighties. Its policy was to stimulate vegetable protein production for use in animal rations. Since 1993, supports have been given directly to farmers according to the area of cultivation (Carrouee, 1993).

In Mediterranean areas, faba beans are the major food legume despite a proportion of the population having an allergy to the crop known as "favism". Italy is the major producer of dry broad beans in these areas and produced 103,000 tonnes of dry broad beans and about 89,000 tonnes of green broad beans in 1995 (F.A.O., 1996a). They are autumn sown and harvested green in early summer. Elsewhere in Europe, small seeded types are grown predominantly for animal feed.

Spring sown types are successfully grown throughout all countries in the EU. However, their yields are notoriously variable, and the majority of the European hectareage tends to be sown with winter types because of their generally superior yield stability. Average yields have increased over the years with the genetic contribution being estimated as 1 t/ha between the mid 1970s and mid 1980s but with little increase since then (Fox & Milford, 1996). Breeders have worked to produce hybrid bean varieties with modified yield components and improved yield stability (Duc *et al.*, 1992) but progress, so far, has been slow.

1.4.3 Value and uses of the crop

The value of the crop lies in the high protein level of the seed ranging from 22% to 37% (Bressani & Elias, 1988). There is major underproduction of plant protein within the European Union with the consequence that 27% of the overall protein required for animal feed and over 63% of that used by the animal feed industry is imported (Anonymous, 1994). The protein is produced by the faba bean crop with less than half the support energy required by an equivalent cereal crop. In faba bean protein, the level of lysine is comparable with that in soybean protein but the levels of S-containing amino acids threonine and tryptophan are lower (Huisman, 1991). Consequently, a balanced blend of amino acids may be obtained by mixing faba bean flour with cereal flour. The resulting mixture may have a greater nutritional value than either ingredient alone (Newman *et al.,* 1988; Russell *et al.*, 1989). The small-seeded varieties are used mainly for pigeon and chicken feed. The importance of faba bean as a source of vegetable protein has been increased because of the progress in reducing the levels of specific anti-nutritional factors contained in the grain (Gatel, 1992). Tannins exist in the seed-coat of coloured-flowered genotypes; they bind to the proteins and lower their digestibility. Tannin-free varieties have been produced in faba beans, and this characteristic is strongly linked to white-flowers, the absence of black spots on the stipule and a lack of pigmentation in the stem (Crofts *et al.*, 1980; Cabrera, 1988; Helsper *et al.*, 1993). Also, accessions and induced mutants of *Vicia faba* with much reduced levels of vicine and convicine exist (Duc *et al.*, 1989; Ramsay *et al.*, 1991); these grains improve egg weight in poultry and "favism" in humans.

Moreover, in areas where cereals are predominant, faba beans can serve as a break crop. Their benefit to a subsequent wheat crop can be worth 50 kg N fertiliser per ha and yields of wheat after faba bean have been 5 or 10% larger than for continuous wheat (Dyke & Prew, 1983). Their ability to fix atmospheric nitrogen makes it likely that they will have a significant role in the future development of sustainable farming systems (Sprent & Mannetje, 1996; Attkinson, 1996). Furthermore, disease occurrence and weed populations are controlled and soil conditions are improved. In addition new methods of organic farming and "set aside" strategy may depend much more on a "traditional" arable rotation system which would include a legume like faba bean (Thomas, 1988; Karlen *et al.*, 1994; Knott *et al.*, 1994).

Apart from Europe where faba bean is fed to animals, its main use particularly in developing countries is as a protein rich food cooked in various ways (Adsule & Akpapunam, 1996). *Minor* and *equina* are harvested dry, whereas *major* is mainly

harvested as a green vegetable. In China it is used for human consumption and as a green fodder crop.

In conclusion, it can be said that *Vicia faba* L. appears to have a more promising future provided that some of the major problems of the crop, such as its reported yield instability, can be overcome. Many studies on this topic indicate that biological features of the plant itself (reproductive abscission, pollination and physiological factors) and the response of the crop to its environment are related to yield fluctuation in faba bean.

1.5 Reproductive abscission of *Vicia faba* L.

1.5.1 Bud abortion

Premature reproductive abscission in *Vicia faba* is one of several critical physiological traits causing both limitation and fluctuation in yield. The loss of the reproductive structures in faba beans can occur as bud abortion, flower abscission or as pod drop (Gates *et al.*, 1983; Clifford *et al.*, 1990).

The loss of visible flower buds is not normally of major concern with values around 9% - 15% of reproductive loss (Hodgeson & Blackman, 1956; Kambal, 1969). Bud abortion is likely to be greatest on the first formed nodes. An experiment where plants were shaded at bud initiation (Smith, 1982b) promoted bud abortion where initials had already been formed once the shading was removed, inflorescences formed normally but flowering was extended compared with control plants. It therefore appears to occur when photosynthetic assimilates are in limited supply for reproductive growth. Bud abortion at lower flowering nodes, is probably caused by constraints on assimilates to the first formed inflorescences, due to rapid vegetative growth at the apex, and at the apical nodes when competition with pods for assimilates occurs in the later stages of crop growth. Therefore there is little evidence that bud abortion significantly contributes to limitation of yield potential (Gates *et al.*, 1983; Clifford *et al.*, 1990; Knott *et al.*, 1994).

1.5.2 Flower abscission

Flower drop is the major cause of reproductive loss in faba beans, it is a phenomenon that varies between varieties and season and is sensitive to various environmental factors (Smith, 1982a). Estimates for flower loss range between 48% - 97% (Soper, 1952; Kambal, 1969; Jaquiery & Keller, 1978a, b; Rylott, 1991).

1.5.2.1 Description of abscission

Abscission is the process of detachment of plant organs from the rest of the plant. It occurs at the abscission zone, a region at the base of a leaf, fruit, flower, and floral parts or any region with a protective and abscission layer (Esau, 1960; Sexton, 1995). The abscission of a flower is an active process. The tissue in the genetically determined abscission zone shows distinct differences in metabolic condition. The distal side shows a condition characteristic of senescence (Carns, 1966). In contrast, the proximal side is in a state of high metabolic activity. The most notable features being increased presence of dictysome vesicles within the golgi apparatus, abundant rough endoplasmic reticulum, plasmodesmata and mitochondria indicative of active RNA and protein synthesis (Baird *et al.*, 1978). An early event during abscission is the enlargement of cortical cells that eventually swell to physically push the plant part away from the rest of the plant.

The first set fruits may cause abscission of flowers by release of abscission inducing factors (Tamas *et al.*, 1979; Huff & Dybing, 1980). Alternatively the high concentration of cytokinins in young fruit sets up an imbalance with the rest of the flowers on the raceme resulting in abscission (Rylott & Smith, 1990). Flower drop may also be promoted by competition of available assimilates (Baylis & Clifford, 1991). Assimilate partitioning to reproductive sinks may be regulated in part by plant growth substances (Thimann, 1992; Kuiper, 1993; Brenner & Cheikh, 1995) and hence are of paramount importance in influencing flower drop.

1.5.2.2 Pattern of flower abscission

Flower drop in *Vicia faba* L. follows two characteristic patterns. The first, within each raceme formed, is that most pods set on proximal positions, whereas flowers at distal positions usually abscise. The second pattern, the overall flower drop at each raceme formed, also progressively increases at each successive raceme. This seems to be a phenomenon common to most cultivars. Furthermore, for most varieties maximum flower retention was recorded in axillary racemes between flowering nodes two and eight (Gates *et al.*, 1983; Clifford *et al.*, 1990; Rylott, 1991).

Having described the severity and pattern of premature reproductive abscission in *Vicia faba*, it is appropriate to consider the recognition from many authors in the literature that these result from a complex interaction of genotype and environment.

1.5.3 Environmental effects

The majority of modern cultivars can give high yields (up to 7-8 t/ha) in favourable environments, but the same cultivars yield quite inadequately (1 t/ha) when conditions are in some way suboptimal (Pilbeam & Hebblethwaite, 1994). The number of flowers produced per node and on each plant depends on the cultivar and the growing conditions. Pod set, seed set and a reliable seed yield depend on the further retention and development of seeds until maturity and harvest. This must be achieved as a response of the crop to its environment (Picard, 1979; Gates *et al.*, 1983; Pilbeam & Hebblethwaite, 1990; Knott & Biddle, 1994).

1.5.3.1 Water supply

Vicia faba L. has comparatively shallow roots but it is often grown in semi-arid areas. Thus, good growth during the long growing season requires the availability of a large amount of water to the crop. Crop dry matter production is related to water use in two main ways: firstly, carbon dioxide uptake (CO_2) is accompanied by water loss; secondly the solar energy received by a plant correlates well with net radiated energy used for evapotranspiration (De Wit, 1958). Accordingly, water has an important effect in regulating dry matter yield and yield of beans.

Irrigation during flowering observed by Dantuma and Thompson (1983) resulted in enhanced vegetative growth at the expense of reproductive growth. The majority of investigations carried out on irrigation have shown that faba bean plants do not respond to irrigation at flowering time (Day & Legg, 1983). Generally leaf area is increased if plants are well supplied with water (Karamanos, 1978; Dennett *et al.*, 1993). Irrigation at flowering resulted in an average 20% more flower abscission in the four varieties examined compared with control unirrigated plants (Smith, 1982c). Plants are induced to become more vegetative, with an increase in shading of lowest flowering nodes. The number of flowering nodes increased, pod set was delayed and final seed yield of irrigated beans was only 52 % of the controls. The pattern of flower drop, whereby most pods set on proximal positions was retained, but more dropped at each and every flower position due to irrigation. Increased abscission due to irrigation has also been reported by El-Rhaman *et al.* (1980), Rowland *et al.* (1982) and Grasshoff (1990).

Farah *et al.*, (1988) found that faba beans are less sensitive to moisture stress before rather than after flowering. They found that the best treatment for irrigating faba beans in the Sudan, was every 14 days until flowering and then 7 days after

flowering had finished. This treatment produced the best yield. Grasshoff (1990) also found that mild water shortage during flowering followed by plenty of water after fruit set resulted in high seed yields at lower stem nodes in cultivar Alfred. The inverse treatment, plenty of water during flowering followed by increased water shortage at flowering showed 20-60% lower seed yields at those nodes; the effect was mainly due to a lower number of pods per node. Rowland *et al.* (1982) have suggested that increased flower shedding induced by irrigation may be due to a decrease in flower fertilisation through inhibition of pollen germination or by a change in chemical composition of the style or micropyle. What has been observed by many workers, is that irrigation at flowering reduces the ability of the plant to partition dry matter to reproductive organs (Smith, 1982c; Gates *et al.*, 1983; Dantuma & Thompson, 1983; Grasshoff, 1990). This may in part be due to hormonal imbalance within the plant that may either inhibit fertilisation or promote abscission (Rylott & Smith, 1990; Smith & Rylott, 1992).

1.5.3.2 Population density of planting and shading

As reviewed by Gates *et al.* (1983), many studies have shown that as planting density increased, branching, number of nodes per stem, number of flowering bearing nodes and the number of pods reaching maturity, all decreased but, within limits (11-67 plant/m^2), yield per unit area increased. Similar findings have been reported more recently by Pilbeam *et al.* (1990). In addition, results have been presented showing that the interaction between uncontrolled environmental conditions in the field (i.e. year of cultivation) and plant density have a great influence on pod set in faba beans (Amato *et al.*, 1992)

Smith (1982a) has demonstrated that growing plants of *Vicia faba* at increased density also increased flower abscission especially in the middle and distal position on each raceme. Basal pod set was a stable characteristic. Aufhammer and Gotz-Lee (1991) found that at high population density (60 plants/m^2) numbers of pods and seed weight from about the third node upwards were even more reduced than those at lower nodes. Hodgeson and Blackman (1957) showed that shading basal nodes led to more pod production at the top of the stem whereas defoliation of the lower half of the plant depressed pod development in the upper plant. They concluded that the effects of increased plant density were more likely to be attributable to altered hormonal balance within plants than to mutual shading. Dantuma and Thompson (1983) reported that increased density caused greater competition in the later stages

of the life cycle that resulted in reduced harvest index. This may be because most assimilation is taking place in densely populated plants in the upper portion of the plant. Most of this assimilate accumulates in the stem and needs to be redistributed to the seeds. At lower density, most assimilates are found in mid-stem leaves proportional to leaf area. Two thirds of this assimilate appears in beans after 6 hours (Crompton *et al.* 1984).

Shading for a two week period during the middle of flowering increased flower drop and induced increased vegetative growth (Smith, 1982b), similar to that observed when irrigation was applied during flowering. Shading imposed during flowering reduced the yield of plants progressively according to the duration of time shading was imposed (Aufhammer & Gotz-lee, 1989, 1991).

1.5.3.3 Summary

Although the proportion of dry matter devoted to seed production is all important, a high allocation of assimilate to reproductive growth may be obstructed by highly fertile growing conditions and frequently water supply is implicated. However, shading at mid flowering resulted in greater vegetative growth (Smith, 1982b) and irrigation at flowering also resulted in enhanced vegetative growth at the expense of the reproductive growth, especially in terms of pod abortion at the lower nodes (Smith, 1982c). There is a critical stage for about two weeks after mid-flowering when retention of young pods at the lower nodes is most important. Therefore production of biomass *per se* without a mechanism to adequately switch production from vegetative biomass in faba beans would appear to have very little benefit (Thompson, 1983).

From observations made by many workers on the effects of various environmental and agronomic factors on pod set of faba beans, a linking factor is apparent, that may explain the sensitivity of the crop to extrinsic factors experienced during the flowering period. This factor is the inability of *Vicia faba* L. in some environmental conditions to co-ordinate source and sink activities to optimal effect to exploit assimilation from the leaves and the potential storage capacity of assimilates into to pods (Aufhammer & Gotz-Lee, 1989, 1991). In addition, the balance of various intrinsic plant growth substances may play a key role in the establishment of sinks and hence help to optimise the use of available assimilates by the sinks.

1.5.4 Intrinsic factors

1.5.4.1 Pollination of faba bean flowers

Factors affecting the pollination of faba bean flowers were extensively reviewed by Bond and Poulsen (1983). The sepals of the flower are combined into a single five toothed calyx. The corolla is made up of five petals, the standard, two wings and two lower petals fused to form the keel petal. The flower has ten stamens, the upper one is physically free whereas the filaments of the other nine are united to form a sheath that encloses the ovary. The unilocular ovary may possess between two and nine ovules. The style is situated at right angles to the ovary and possesses hairs at the upper end, just below the stigma (Kambal *et al.*, 1976). The surface of the stigma is covered with papillae which when broken form an exudate which induces pollen germination (Bond and Poulsen, 1983).

As in other legumes, the petals of *Vicia faba* are joined and hinged. Tripping occurs when an insect depresses the wing and keel petals, releasing the stigma and pollen. During tripping the hairs on the style may brush pollen out of the style so helping collection by a visiting "pollen collecting" bee. The hairs also rub against the hairs of a "nectar collecting" bee allowing transport of pollen to other flowers (Bond & Poulsen, 1983). As reviewed by Crofton (1996), only bees can pollinate bean flowers in the UK.

Variation in flower structure exists in *V. faba* . This according to Bond and Poulsen (1983) determines whether the flowers are self or cross pollinated. The stigma becomes receptive to pollen before anthers dehisce and up to 6 days after the flower opens. Time to complete fertilisation in faba bean can range from 24 h to 72 h after pollination (Stoddard, 1985; Johansson & Walles, 1994; Lazaridou, personal communication). The degree of self-fertilisation is dependent on the emergence of exudate from the stigma tips, that occurs earlier in undisturbed flowers of autofertile plants, the latter having no exudate until after flower opening (Paul *et al.*, 1978). It is possible to rupture the papillae by hand pollination so leading to early release of stigmatic exudate. In some plants, the stigma is not receptive to pollen until after the flower has opened. So although anthers dehisce before the flower opens, there are mechanisms to delay pollination until after flower opening (Bond & Poulsen, 1983). However, in addition to differences in flower structure, the organisation and mechanical strength of the stigma cuticle also affect the incidence of self pollination. It appears that the stigmatic cuticle may be weaker in predominantly autogamous

lines of faba beans (Lord & Heslop-Harrison, 1984) leading to production of stigmatic exudate.

The floral biology of faba beans leads to a breeding system intermediate between totally self pollination and obligate cross-pollination. The amount of natural cross-fertilisation is around 35% with a range from between 4% and 84% (Bond & Poulsen, 1983). This is influenced by the genotype and the environment (Link *et al.*, 1994). Most varieties of faba beans produce more flowers than pods. In some conditions, lack of insect pollination may constrain yield. Rowland *et al.* (1982) found fewer pollinated flowers and even fewer fertilised ovules in irrigated compared with non irrigated faba beans. However under normal field conditions, fertilisation failure is unlikely to be a factor constraining yield. It has been demonstrated repeatedly that many abscised flowers are fertilised (Kambal, 1969; Chapman *et al.* 1979; Smith, 1982a).

Large differences have been found between crops of beans in the proportion of flowers that have been fertilised, depending on location and size of field (Stoddard & Bond, 1987). In some crops this figure was as low as 17%. While crops can sometimes produce an adequate yield in the absence of bees, the presence of bees results in more pods being set on lower nodes earlier in the season (Hebblethwaite *et al.*, 1984; Stoddard, 1986).

In some circumstances, poor pollination can no doubt result in a reduced pod-set, but pollination is increasingly discounted as a primary limiting factor especially with the newer varieties (Knott *et al.*, 1994; Crofton, 1996).

1.5.4.2 Assimilate partitioning and intra-plant competition

In the faba bean cultivars with indeterminate growth, during the relatively long period of flowering and pod development, different reproductive organs develop at the same time, competing among themselves and with the vegetative organs still growing. Such competition leads to flower and pod loss (Peat, 1983; Gates *et al.*, 1983). The losses of sink units due to reproductive abscission as occurs in *V. faba* leads to the thought that source output is insufficient, at least at critical times, to match overall sink demand. Removal of lower racemes reduced flower shedding on successive nodes (Smith, 1982a), demonstrating the competitive interaction between lower nodes with developing pods and those at higher positions which had yet to reach anthesis. Moreover, Aufhammer and Gotz-Lee (1989) reported that following the removal of the basal three racemes of *V. faba*, over-compensation (i.e. when the

yield of a treated plant is greater than the yield of an untreated control plant) occurred, whereby the overall number of pods and yield per plant could be substantially increased. It has also been shown (Aufhammer *et al.*, 1989) that the concentration of pod set at the upper nodes, which was established by removal of the inflorescences from the nodes 1-4, delayed the establishment of generative sinks in favour of leaves, internodes and roots.

The apex imports assimilates from the leaves until the end of the flowering period and competition between apex and fruits is responsible for pod shedding (Jacquiery & Keller, 1978,1980; Gehriger & Keller, 1980). Experiments (MacEwen, 1972; Gehriger *et al.*, 1979; Chapman *et al.*, 1979) in which the balance between vegetative and reproductive growth has been artificially manipulated have not led to any generalised conclusions, and although top removal sometimes improved pod set, it is by no means clear that this was through additional assimilate being made directly available. Apex removal initially increased pod set, although these young pods subsequently shed in a pattern similar to that of flower drop on control plants (Chapman *et al.*, 1978). However, removal of the apex, a major hormone source and simultaneously an active sink, evidently stimulates the early stages of pod development (Clifford *et al.*, 1992). It has been reported (Chapman & Sadjadi, 1981), that the pods which set first compete for assimilates with the growing roots and stem apex, as well as with other developing pods. There is a lag phase during early pod growth when fruits are incapable of competing with alternative vegetative sinks (Peat, 1983). During this period vascular differentiation in the pedicel and peduncle occurs, and it has been demonstrated that pod abortion is a result of failure of vascular differentiation in these organs (Gates *et al.*, 1982), and is therefore limited by the capacity of transport tissues rather than assimilate availability.

The genetic prevention of the indeterminate stem growth (e.g. terminal inflorescence genotype Ti) was expected to reduce the number of competing sinks within the plant and to result in an enhanced pod development. As has been reported by Thompson (1983), the determinate cultivar was not more effective in dry matter partitioning into reproductive structures than the indeterminate ones. Stutzel and Aufhammer (1991) experimentally tested their model of dry matter partitioning in *V. faba* by using two contrasting genotypes, an indeterminate and an determinate one. They concluded that the smaller proportions of dry matter found in pods and seeds of the determinate plant than the indeterminate variety was due to the limited number of pods formed and not due to the intrinsic ability of these organs to attract assimilates for their growth. Although the role of the branches in topless cultivars may be the same as

that of the apex of indeterminate plants with respect to assimilate attraction (Baker *et al.*, 1983), the internal regulation of partitioning is different. Late formed tillers of determinate plants are certainly not equivalent to the apex of an indeterminate one in terms of productivity per unit of dry matter invested due to the production of small leaves (Aufhammer *et al.*, 1991).

It has been demonstrated that the vegetative organs act as a temporary sink for assimilates, which are then demobilised and distributed to active sinks in all parts of the plant (Ismail & Sagar, 1981; Kogure *et al.*, 1992). However, Gates *et al.* (1981) have suggested that inadequate vascular development to the young pod may be a major factor determining the rapid transport of assimilates and that raceme morphology, and the influence of earlier set pods determines this.

The evidence which has already been mentioned, indicates that manipulation of source-sink relationships of the plant in favour of reproductive sinks may alter internal competition as well as the hormonal balance within the plant. Although it is possible that direct competition between vegetative and reproductive development for available assimilate may on occasion be the cause of poor pod-set, the evidence is far from conclusive. The assimilate transport capacity may be an important limiting factor in pod development. It seems likely that some other factor(s) are often involved in the determination of generative organ retention, amongst which may be the hormonal balance of the plant.

1.5.4.3 Interaction between pods and flowers

The interaction between pods and flowers has been reviewed by Gates and his co-workers (1983). It has been shown that the pattern of flower abscission within racemes can be related directly to the acropetal flower anthesis within them and to the architecture at the vascular system interconnecting flowers within a raceme (Gates *et al.*, 1981; Smith, 1982a)

The typical vascular system of a raceme in current commercial varieties is such that the basal flower has a separated vascular supply, whereas the flowers above are linked to successive apical flowers by a dichotomously branched vascular system. It has been shown by Smith (1982a) that removal of the basal independent flower has no effect on abscission of higher flowers, whereas removal of the basal two or three flowers drastically reduces flower shedding at higher raceme positions. That leads to the conclusion that an abscission-promoting signal is translocated from young basal pods to distal buds and flowers. The results, however, of the determination of some

of the endogenous substances in abscising and not-abscising flowers and pods were not conclusive (Diethelm *et al.*, 1988).

Based on the observed autoinhibitory effect of indolacetic acid (IAA) export from a dominant organ on IAA transported out of an inhibited organ, Bangerth (1989) suggested that the polar IAA transport system is sufficient to explain the exerted growth inhibition effect. In this hypothesis called 'primigenic dominance', second messengers which have been proposed by Cline (1994) are not essential. In addition to the inhibitory factor, an antagonising system, established on the interaction between cytokinins and IAA, may be involved (Bangerth, 1994) in inducing more distal bud and flower growth when cytokinin level rises in these organs and the dominance of basal pods ceases.

1.5.5 Plant growth substances

1.5.5.1 Effects of plant growth substances

As discussed in Section 1.2, plant growth substances have been used by man as a tool to manipulate the developmental physiology of plants. In *Vicia faba,* the effects of exogenous application of plant growth substances on reproductive loss were comprehensively reviewed by Keller and Bellucci (1983). In a more recent investigation performed by Rylott and Smith (1990), 4-chloroindole, 6-benzylamino purine and gibberellic acid (GA_3) were applied to each and every flower, 24 hours before or 24 hours after pollination in glasshouse grown plants. Controls, sprayed with distilled water shed most of their flowers. Chloroindole and gibberellic acid applied before pollination had no effect on pod set pattern. Gibberellic acid applied after pollination increased pod set at the basal position by 21%. Chloroindole also had a similar effect on proximally set pods when applied after pollination. Application of the cytokinin resulted in almost complete pod set on all racemes, whether applied before or after pollination. The effect of cytokinin was not due to any changes in flower synchrony. In addition, parthenocarpic pod set was not enhanced by this treatment (Smith & Rylott, 1992). The change in pattern of flower drop and pod set exhibited by plants supplied with auxin and gibberellin after pollination, suggests that a "hormonal switch" may operate to make tissues sensitive to these plant growth substances. Investigations into plant growth substance levels in fruits have been performed. Shortly after fertilisation, coincident with high rates of cell division in developing legume seeds, high concentration of cytokinins and

gibberellins are measured (Carlson *et al.*, 1987; Diethelm *et al.*, 1988; Baylis & Clifford, 1991). In addition, cytokinins have been shown to be present in high concentrations in the xylem sap, suggesting that cytokinins produced in other plant parts, for example the root nodules (Henson & Wheeler, 1976), are translocated into developing seeds (Lorenzi *et al.*, 1978; Carlson *et al.*, 1987). The suspensor of runner beans (*Phaseolus coccineus*) contains high concentrations of cytokinins and gibberellins presumably supporting early development of the seed (Lorenzi *et al.*, 1978). In the light of this evidence it is supposed that cytokinins play an important role in establishing sinks in *Vicia faba*. It is possible, however, that the more developmentally advanced, proximally situated pods monopolise the supply of cytokinins and hence assimilates early in pod development. Applying exogenous cytokinin temporally restores the supply of cytokinin to the other flowers on the inflorescence. Hence assimilates are directed to these potential sinks, vascular tissue differentiation proceeds at the pedicel/peduncle junction (Gates *et al.*, 1981) and pod set is accomplished. However, many of these young pods subsequently drop (Rylott, 1991) as the temporary effects of cytokinins are reversed by the plant.

Jaquiery and Keller (1978) stated that pods do not become active sinks until they are 4-6 cm in length. In experiments carried out by Rylott (1991) much of the increased yield potential, due to application of 6-benzylamino purine was lost by pod drop during the critical stage between pod set (1-2 cm in length) and pods becoming active sinks. Application of a combination of chloroindole and cytokinin to field grown faba beans when flowers were opening at flowering node four, increased the number of harvestable pods by 36% compared to controls (Rylott, 1991). Therefore, this combination of plant growth substances could increase the reproductive sink strength. However, the pods were only retained on flowering raceme four. It is known that cytokinins are not mobile, and their effects short lived. To retain more flowers further application would be need to be made latter in the flowering period. Besides auxin and cytokinin, gibberellins are probably required to induce assimilate flow into pods (Rylott, 1991). It is possible that the applications of gibberellic acid during flowering and pod set in *Vicia faba* may also be necessary. Comprehensive experiments on the role of plant growth substances to improve sink strength in faba beans need to be conducted.

In summary, although plant growth substances can overcome the limitations in the number of reproductive sinks in *Vicia faba* caused by premature flower and pod

drop, the effects are transient and temporary. The plant growth substances, their relative concentrations and combinations needed to enhance sink strength and hence promote reliable dry matter seed production is poorly understood (Aufhammer, 1990; Baylis & Clifford, 1991). It would appear that different cultivars and seasonal effects cause differential responses to plant growth substances (Peat, 1983). A co-ordinated and multi-disciplinary approach to these problems appears to be required if we are to better understand the significance of plant growth substances in supplying assimilates to developing sinks.

1.5.5.2 Effects of plant growth inhibitors

Plant growth inhibitors are artificial chemicals that block movement of endogenous plant hormones. They can in turn lead to a local accumulation of these hormones and hence induce physiological changes. The substance TIBA (2,3,5-tri, iodo benzoic acid) is thought to act in this way by blocking the downward movement of IAA from the stem apex (Luckwill, 1981). In trials it has been shown that TIBA caused reduction in plant height and increased the number of *V. faba* tillers and pods (El-Zawily *et al.,* 1985). Yield increases of 10% were also described (Attiya *et al.,* 1983) after application to faba beans at the six leaf stage and the commencement of flowering. Newaz and Lawes (1980) demonstrated that the application of TIBA could lead to 75% greater faba bean yields. Smith (1982a) found that TIBA reduced flower abscission in cultivar Maris Bead by 11%. Chapman and Sadjadi (1981) could improve pod set in *Vicia faba* with TIBA but effects on yield were variable, suggesting problems in assimilate supply to a greater fruit load.

Morphatins, derivatives of florene-9-carboxylic acid were reported by Schneider *et al.* (1965) to induce a gradual inhibition of growth often accompanied by stimulation of branching. As with TIBA, this effect is due to an interruption of downward movement of auxins from the plant apex (Garrod, 1982). Application of the morphatin CME 73170P (2-chloro-9-hydroxyfluoren-9-carboxylic acid) at between 1-10 mg/l increased yield of *V. faba* by up to 50% (El Zawily *et al.,* 1985). Yield increases were ascribed to increase in tillers and pod retention.

1.5.5.3 Effects of plant growth retardants (PGRs)

Growth retardants are synthetic organic chemicals which when applied to responsive plants reduce the rate of stem elongation by inhibiting sub-apical meristem activity,

normally without affecting leaf production and development or inducing growth malformations (Cathey, 1964). These synthetic substances inhibit gibberellin biosynthesis, often at the same point in the metabolic pathway, i.e. between kaurene-kaurenol and kaurenal to kaurenoic acid (Grossman, 1990). Chlormequat, 2-chloroethyl trimethyl ammonium chloride (Hassan & El-Mousi, 1982); Daminozide (Abou-Elleil and El Wazeri, 1978; Chapman & Sadjadi, 1981; Dekker & Neuvel, 1983; Rylott, 1991); Paclobutrazol and Chlormequat as PP333 or JF10405 (Attiya *et al.*, 1983; Field *et al.*, 1989; Rylott, 1991; Batts & Hebblethwaite, 1992); Flurprimidol (Rylott, 1991) have all produced decreases in flower drop and increased yield with their application to faba beans. Rylott (1991) found in field trials over three years with Daminozide, PP333, Flurprimidol that the best time to apply was at mid-flowering (first raceme, first flower fully open). Increases in pod set were evident on all racemes. Attiya *et al.* (1983) thought that the reduction in stem length was the major reason why pod set increased. The application of a PGR was thought to have increased the ability of pods to compete for assimilates because of a reduction in apical dominance. Pod set, however, is not purely dependent on assimilate availability. The presence or lack of intrinsic plant growth substances can affect the development of sinks. For example, more assimilates are known to be diverted in the reproductive organs of faba beans which are treated with cytokinins (Chapman & Sadjadi, 1981). It may be possible therefore that the reduction in available gibberellins by the application of these PGRs resulted in a relative increase in the proportion of intrinsic plant growth substances, especially the cytokinins and this pre-disposed the inflorescences to set more pods (Rylott, 1990). Although plant growth retardants may cause an increase in pod set in *Vicia faba*, often the pods are not retained until harvest. This may be due to assimilates becoming limited because of the greater number of developing sinks. Alternatively, the better intrinsic balance of plant growth substances within the plant, that encouraged sink development is gradually lost. The balance prevailing before PGR application is re-established. It has been observed that the proximal pods gradually resume their naturally dominant status after plant growth regulator application (Rylott, 1991). Much of this may be due to better vascular development within the raceme (White *et al.*, 1984) which favours assimilate supply to proximal, more developmentally-advanced pods.

1.6 Aim and objectives of the current study

With increasing pressure for environmentally conscious farming and the need to change land use strategies due to overproduction of cereals the cultivation of grain

legumes, such as faba beans, has been proposed (Thomas, 1988; Attkinson, 1996). Breeding of new varieties, in a long term, and the application of plant growth substances, in a shorter term, may secure yield of *Vicia faba* by achieving higher pod load, regardless of the environmental changes in a particular area of faba bean cultivation. On the basis of the improvement of *V. faba* productivity, either by plant breeding or by using plant growth substances, lies the knowledge of the physiological processes of the plant (Russell *et al.*, 1993) which result in a profitable harvest for the farmer. Externally applied cytokinins on faba bean plants appear to play a leading role in the processes which control the capacity of the reproductive organs on the plant and thus an important component of potential yield. This physiological response, however, has yet to be elucidated.

The aim of this study is to obtain a better understanding of the regulation of pod set and pod development by applying cytokinin compounds in *V. faba* plants during the reproductive phase of their development. This study attempts to explain reproductive load of *V. faba* as (the result of) a dynamic process in terms of an internal competition among organs for cytokinins and/or assimilates in relation to their position on the plant, their stage of development and the intrinsic ability of the plant to retain them to maturity.

To this end, studies dealing with the screening of various cytokinin analogs and the assessment of their biological activity in terms of pod load were performed in an attempt to identify active compounds.

The most active substances found above were tested under field conditions using commercial varieties with differences in their ability to retain their reproductive organs. Determination of dry matter distribution to harvestable organs was also carried out to assess varietal response to treatments and whether this approach may help to increase yield and stability of yield in the field.

Correlative studies focused on changes in sucrose metabolism in the reproductive sinks at crucial developmental stages during the period when pod set occurred with enhancement of pod set due to cytokinin treatment, were also performed. Using these findings a more physiological approach to the flower developmental-stage response to applied cytokinins could be made.

Experimentation was also carried out to examine the effects of exogenous application of active cytokinin on each flower on levels of soluble carbohydrates in developing carpels and this was related to successful pod set.

♦ ♦ ♦

Chapter 2

Materials and methods

2.1 Materials

2.1.1 Plant material

These are listed in Table 2.1.1.

Table 2.1.1: *Vicia faba L. varieties used in the various experiments.*

Variety name	Plant and Floral Characteristics	Supplier
Three Fold White	*Major* variety, white flowered, indeterminate growth habit, used for human consumption.	Elsoms seeds, Spalding, Lincs
Maris Bead	*Minor* variety with coloured flowers, indeterminate growth habit, long relatively stiff straw, recommended for the production of small round beans which are used for pigeon feed.	Dalgety pulses, King's Lynn, Norfolk
Troy	*Minor* variety with coloured flowers, short strawed, semideterminate, preferred in Scotland because of the earliness	Dalgety pulses, King's Lynn, Norfolk
Cresta	*Equina* variety, white flowered, short stiff and moderately early to ripen. Cresta is included in the 'preferred' group of varieties in Scotland because of the good yield and earliness.	PBI, Cambridge
Toret	*Equina* variety, white flowered, moderately early to mature and has very short, stiff straw, is the only exception among short strawed varieties for resistance to rust (*Uromyces fabae*).	Nickerson Seeds, Lincs

2.1.2 Cytokinins

Six cytokinin analogs were used in this study; all were obtained from Sigma Chemical Co., Dorset, UK (Table 2.1.2).

Table 2.1.2: *Cytokinin analogs used in experiments.*

Abbreviation	Trivial Name	Systematic Name	Empirical Formula
BA	Benzyladenine	6-(benzylamino) purine	$C_{12}H_{11}N_5$
KIN	Kinetin	6-(furfurylamino) purine	$C_{10}H_9N_5O$
Z	Zeatin	6-(4-hydroxy-3-methylbut-2-enylamino) purine	$C_{10}H_{13}N_5O$
iP	Isopentenyl adenine	6-(methylbut-2-enylamino) purine	$C_{10}H_{13}N_5$
[9R]Z	Zeatin riboside	6-(4-hydroxy-3-methylbut-trans-2-enylamino-9-b-D-ribofuranosyl) purine	$C_{15}H_{21}N_5O_5$
[9tP]BA	BPA	6-[benzylamino-9-(2-tetrahydropyranyl)] purine	$C_{17}H_{19}N_5O$

In naming cytokinins, which are substituted 6-aminopurines, the numbering of the purine nucleus is used to define the positions of substitution (Figure 2.1.1). However, in the literature there are a number of different ways to name these compounds. At least three (IUPAC-IUB, 1970; Letham, 1978; Crouch *et al.* 1993) abbreviation systems have been used for these substances. For simplicity, the system adopted in this study is based on the system proposed by Crouch *et al.* (1993) which is a modification of a previous system proposed by Letham (1978). According to the rules of this system substitution in the purine ring and in the side chain are noted in square brackets and round brackets respectively. Benzyladenine (BA), kinetin (KIN), zeatin (Z), dihydrozeatin (DHZ) and isopentenyladenine (iP) are regarded as basic compounds and their derivatives are denoted in an analogous manner. For instance, *trans*-zeatin is indicated as Z and *trans*-zeatin riboside as [9R]Z. Since the adopted system is only semi-systematic and consequently some, but not all, structural information is conveyed, the full systematic name and the empirical chemical formula of each compound is also given (Table 2.1.2).

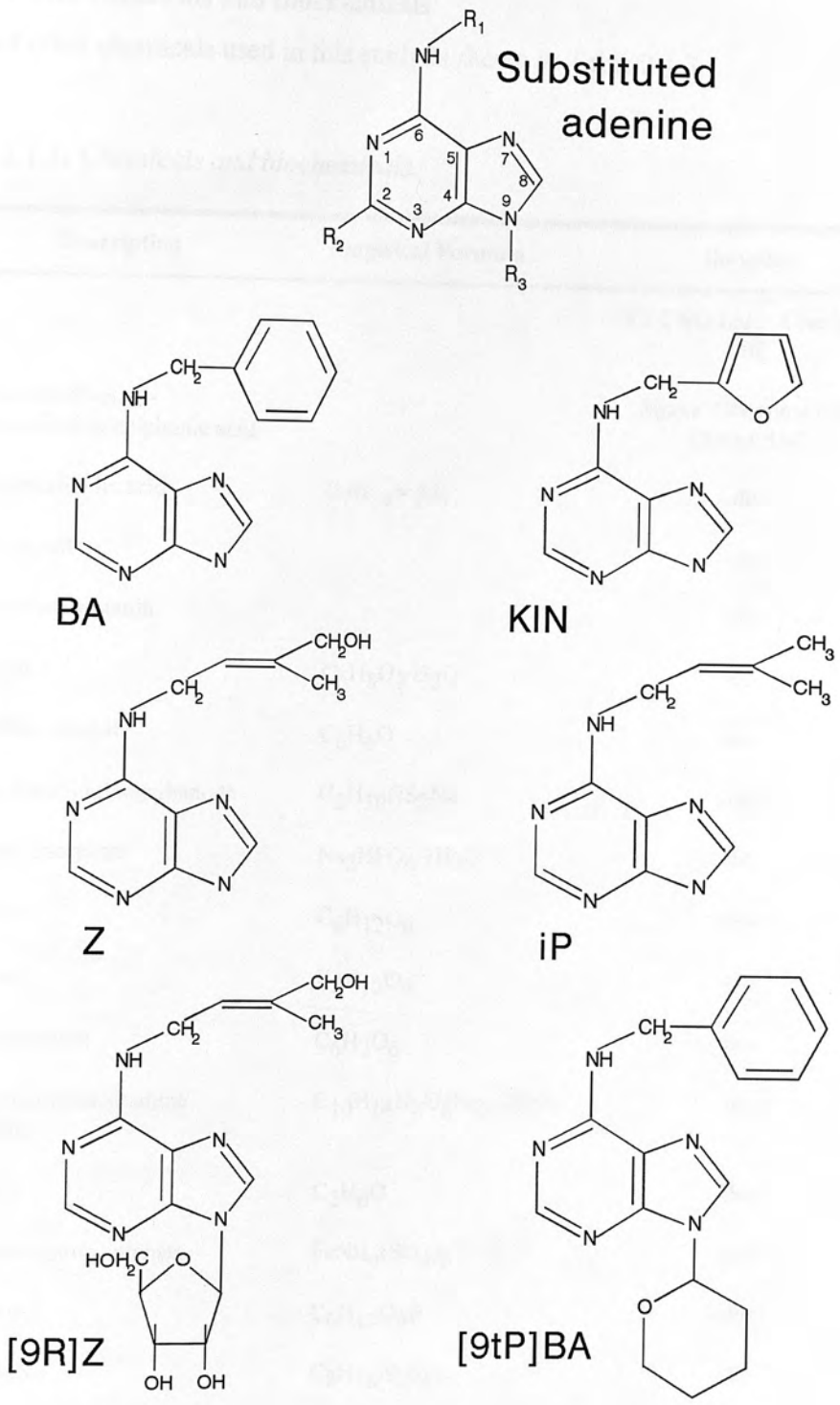

Figure 2.1.1: Structures of all cytokinin analogs used in this study. The structure of a substituted adenine is also depicted.

2.1.3 Other chemicals and biochemicals

A list of other chemicals used in this study is shown in Table 2.1.3.

Table 2.1.3: *Chemicals and biochemicals.*

Description	Empirical Formula	Supplier
Agral		ICI CND Ltd., Cheshire, UK
2,2-azino-bis-(3-ethyl-benzthiazoline)-6-sulphonic acid		Sigma Chemical Co., Dorset, UK
3,5-dinitrosalicylic acid	$C_7H_{14}N_2O_7$	-do-
ADTS reagent		-do-
Bovine serum albumin		-do-
Citric acid	$C_6H_8O_7 \cdot H_2O$	-do-
Crystallised phenol	C_6H_6O	-do-
Sodium diethyldithiocarbamate	$C_5H_{10}NS_2Na$	-do-
Disodium phosphate	$Na_2HPO_4 \cdot 7H_2O$	-do-
D-Fructose	$C_6H_{12}O_6$	-do-
D-Glucose	$C_6H_{12}O_6$	-do-
DL-Dithiothreitol	$C_6H_2O_6$	-do-
Disodium ethylenediamine tetraacetate	$C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$	-do-
Ethanol	C_2H_6O	-do-
Ferric ammonium sulphate	$FeNH_4(SO_4)_2 \cdot 12H_2O$	-do-
Fructose 6-P	$C_6H_{11}O_9P$	-do-
Hepes buffer	$C_8H_{18}N_2O_4S$	-do-
Hydrochloric acid	HCl	-do-
Magnesium chloride hexahydrate	$MgCl_2 \cdot 6H_2O$	-do-

Continued

Description	Empirical Formula	Supplier
Magnesium chloride hexahydrate	$MgCl_2 \cdot 6H_2O$	Sigma Chemical Co.
Nitrogen (liquid)	N_2	-do-
Perchloric acid	$HClO_4$	-do-
Potassium ferricyanide	$K_3Fe(CN)_6$	-do-
Polyvinylpolypyrryolidone		-do-
Potasium hydroxide	KOH	-do-
Resorcinol (1,3-Benzenediol)	$C_6H_6O_2$	-do-
Sodium potasium tartrate	$NaKC_6H_6O_4.4H_2O$	-do-
Sodium bisulfite	$NaHSO_3$	-do-
Sodium carbonate	Na_2CO_3	-do-
Sodium fluoride	NaF	-do-
Sodium hydroxide	NaOH	-do-
Sodium molybdate	$Na_2MoO_4 \cdot 2H_2O$	-do-
Sucrose	$C_{12}H_{22}O_{11}$	-do-
Test-combination sucrose/D-glucose/ D-fructose		Boehringer Mannheim Ltd., East Sussex, UK
Uridine[5']diphospho[1]-α-D-glucopyranoside	$C_{15}H_{22}N_2O_{17}P_2$	-do-

2.2 General Methods

2.2.1 Enumeration of racemes and flowers

Each flower position within a raceme was assigned a number, 1 being the most proximal flower, 2 the next and so on (Figure 2.2.1). Raceme position was also enumerated. The first raceme recorded in this study was the one that formed buds which survived until full flower development. The next raceme, was numbered 2 and so on until all reproductive nodes had been accounted for (Figure 2.2.1). In this way each and every flower (on the main stem) had its own number. This allowed observations to be recorded accurately, as well as a detailed analysis of flower and pod drop to be performed.

3.2.3 Overall percentage pod set

Spraying BA on to racemes increased ($p<0.001$) overall percentage pod set by 39% compared to control plants. This increase was related to the number of setting pods per plant, while the number of flowers formed was unaffected (Table 3.2.1).

The application of KIN or Z on the racemes had little effect on overall percentage pod set compared to control plants (Table 3.2.1).

Table 3.2.1: *Effect of external application of BA, KIN and Z on flowering racemes, on overall characteristics.*

Treatment	No. of flowers per plant	No. of pods set	% pod set	No. of mature pods	% mature pods	No. of seeds per mature pod
Control	67	8	13	4	5	4
BA	69^{ns}	36***	52***	6*	8*	4^{ns}
KIN	70^{ns}	11^{ns}	15^{ns}	5^{ns}	6^{ns}	4^{ns}
Z	60^{ns}	8^{ns}	13^{ns}	3^{ns}	5^{ns}	4^{ns}

ns : no statistically significant difference from the control
*, *** : different from the control at 0.05, 0.001 probability level

3.2.4 Intra-raceme percentage of mature pods

In control plants, the highest proportion of flowers which formed mature pods was recorded on the first (proximal) flower position of the raceme, at 18%. Very few pods (6% or less) set on the flower positions 2 to 8 (Table 3.2.2).

Table 3.2.2: *Effect of external application of BA, KIN and Z on intra-raceme percentage mature pods in cv. Three Fold White.*

Treatment	Flower position							
	1	2	3	4	5	6	7	8
	Percentage of mature pods							
Control	18	6	1	4	1	5	4	3
BA	12^{ns}	8^{ns}	8*	1^{ns}	7^{ns}	8^{ns}	7^{ns}	17*
KIN	21^{ns}	7^{ns}	4^{ns}	4^{ns}	6^{ns}	3^{ns}	2^{ns}	5^{ns}
Z	25^{ns}	6^{ns}	0^{ns}	0^{ns}	0^{ns}	3^{ns}	3^{ns}	0^{ns}

ns: no statistically significant difference from the control
* : different from the control at 0.05 probability level

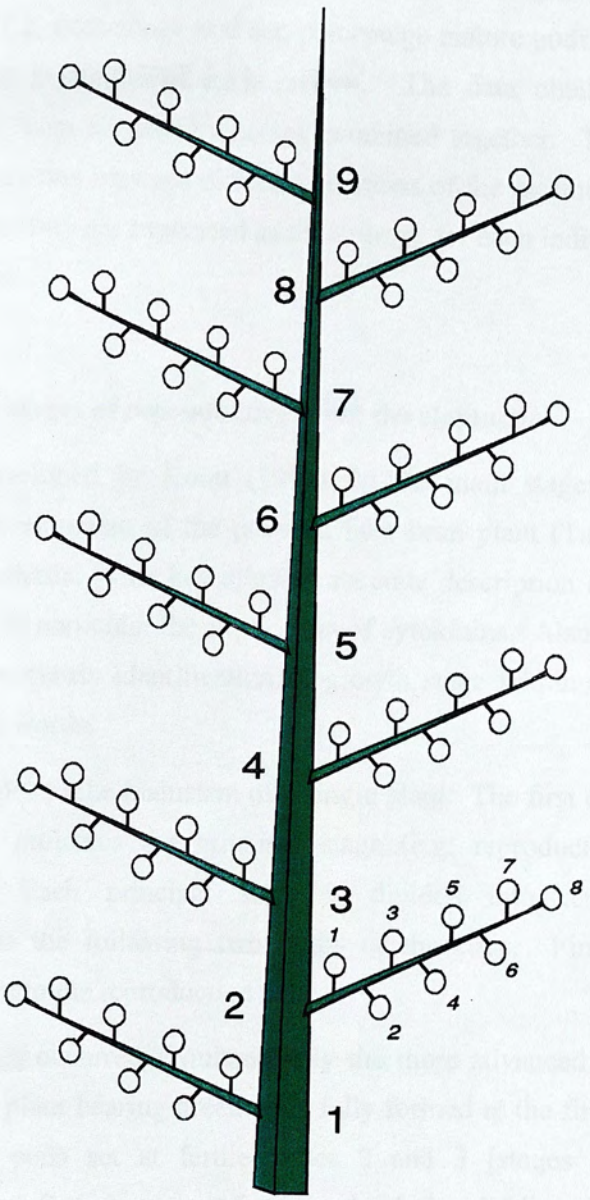

Figure 2.2.1: Schematic layout of *Vicia faba* L. mainstem flowering nodes showing enumeration of raceme positions (numbers along the mainstem) and flower positions (numbers along the peduncle of the 2nd inflorescence).

2.2.2 Definition of intra- and inter-raceme interactions

The term intra-raceme is used in order to describe differences of a recorded characteristic (e.g. percentage pod set, percentage mature pods), from one to another position on the peduncle of each raceme. The data obtained for intra-raceme comparisons is from all tested racemes combined together. The term inter-raceme refers to comparisons between different positions of the racemes on the mainstem of the plant. These data are expressed as the average for each individual flower position on every raceme.

2.2.3 Growth stages of reproductive plant development

The system developed by Knott (1990) for the main stages that occur during reproductive development of the pods on faba bean plant (Table 2.2.1) have been adopted in this thesis. This key allowed accurate description of the timing of field operations, and in particular the application of cytokinins. Also the use of the digital codes allowed accurate identification of growth stage within the text, without the excessive use of words.

Descriptions apply to the mainstem of a single plant. The first digit of the code (e.g. the number 2) indicates the principal stage (e.g. reproductive phase) of plant development. Each principal stage is divided into ten secondary stages corresponding to the following two digits of the code. Finally, the number in parenthesis refers to the reproductive node.

Where two stages occurred simultaneously the more advanced stage was recorded. Thus for a bean plant bearing green pods fully formed at the first fertile node [stage 205(1)], young pods set at fertile nodes 2 and 3 [stages 204(2) and 204(3)] respectively], open flowers open at fertile nodes 4, 5 and 6 [stages 203(4), 203(5) and 203(6) respectively], and buds visible at fertile nodes 7, 8, 9 and 10 [stages 201(7), 201(8), 201(9) and 201(10) respectively], only the stage 205(1) was used for identification.

Table 2.2.1: *Growth stages of reproductive development of faba bean plant (Knott, 1990 and personal communication).*

Code	Definition	Description
201(1)	Flower buds visible	First buds visible and still green
202(1)	Coloured buds visible	First buds showing colour
203(1)	First open flowers	First open flowers on first racemes
204(1)	First pod set	First pods visible at first fertile node
205(1)	Pods fully formed, pods green	Pods fully formed but with small immature seeds within
206(1)	Green harvest stage	Green harvest stage of a broad bean
207(1)	Pod fill, pods green	Seeds at maximum size fill the pod cavity
208(1)	Seed dehydration	Pod and seeds begin to lose moisture
209(1)	Seed rubbery, pods still pliable, turning black	
210(1)	"Dry seed"	Seed dry and hard, pods dry and black

2.2.4 Growth stages of flower development

The growth stages of flower development (fds) chosen in this study (Figure 2.5.1), were defined according to a scale developed by Smith (1982), which described morphologically the stages of flower development from bud initiation (fds-1) to petal collapse (fds-10). Anthesis was regarded to have occurred at the time of anther dehiscence which usually took place before the full opening of the petals (fds-9) (Figure 2.5.1).

2.2.5 Hand -tripping

Each flower of the plants grown under controlled environmental conditions was hand-tripped (Plate 2.2.1) when it was fully opened (fds-9) in order to ensure that each flower was pollinated. The technique is described elsewhere (see section 1.5.4.1).

Plate 2.2.1: *Hand-tripping of faba bean flower.*

2.2.6 Pod-set

The process of pod-set was judged complete, when a young green pod of 1-2 cm in length was clearly visible after petal abscission. The percentage of pod-set refers to the number of pods set at any one position, expressed as a percentage of the number of flowers at that position. Overall percentage pod-set is the total number of pods set on the mainstem, expressed as a percentage of the total number of flowers formed on the mainstem.

2.2.7 Mature pods

This definition refers to all pods, regardless of size, present at the time of harvest with full, partial or no seed development. At that time, the deposition of major food reserves in cotyledons of all varieties had already finished; thus the seeds were at the last stage of their physiological development, the stage of dehydration and ripening. The percentage of mature pods therefore is the number of mature pods expressed as a percentage of the number of flowers.

For KIN and Z treated inflorescences, the intra-raceme pattern of percentage mature pods was not altered compared to controls. BA application increased 7% and 13% ($p<0.05$) the percentage of mature pods at flower positions 3 and 8 respectively, over controls, (Table 3.2.2).

3.2.5 Inter-raceme percentage of mature pods

Control plants on average retained 5% of the original numbers of flowers as mature pods on each of the lower six racemes, whereas the figure was 7% on the upper three racemes (Table 3.2.3). The application of cytokinins to each flowering inflorescence did not affect the inter-raceme percentage of mature pods apart from on the lower racemes 1 and 2. The application of BA increased ($p<0.05$) the percentage of mature pods at raceme 1 by 6% and raceme 2 by 12% compared to control plants (Table 3.2.3).

Table 3.2.3: *Effect of external application of BA, KIN and Z on inter-raceme percentage mature pods in cv. Three Fold White.*

Treatment	Raceme position								
	1	2	3	4	5	6	7	8	9
	Percentage of mature pods								
Control	3	4	6	9	5	3	9	6	5
BA	9*	16*	11^{ns}	8^{ns}	5^{ns}	8^{ns}	5^{ns}	8^{ns}	4^{ns}
KIN	0^{ns}	4^{ns}	5^{ns}	2^{ns}	11^{ns}	11^{ns}	8^{ns}	9^{ns}	9^{ns}
Z	2^{ns}	0^{ns}	8^{ns}	8^{ns}	9^{ns}	5^{ns}	11^{ns}	2^{ns}	5^{ns}

ns: no statistically significant difference from the control
* : different from the control at 0.05 probability level

3.2.6 Overall percentage of mature pods

Control plants produced on average four mature pods per plant (Table 3.2.1). This represented a mature pod figure from original flowers of 5%. Application of KIN and Z had no effect on the number of mature pods per plant. Plants treated by BA, however, retained 2 more ($p<0.05$) pods compared to control plants (Table 3.2.1). This was equivalent to a 3% greater ($p<0.05$) mature pods on BA-treated plants over controls (Table 3.2.1).

2.2.8 Harvestable Pods

This term defines those pods at harvest which have at least one seed developed at a harvestable size. The number and the weight of these seeds determine the yield of the crop. The percentage of harvestable pods refers to the number of harvestable pods expressed as a percentage of the number of flowers.

2.3 Experimental procedures Chapter 3: Assessment of the biological activity of six cytokinin analogs

Two experiments (Experiment 3.1 and Experiment 3.2) were carried out to assess the effects of six cytokinin analogs on reproductive load of faba bean plants. The following description applies to both experiments.

2.3.1 Plant material

Two seeds of the variety Three Fold White (*Vicia faba* L. *sp. major*) were sown on 1 October 1992 (Experiment 3.1) and 30 October 1992 (Experiment 3.2) into 18 cm pots containing Levingtons compost at the Scottish Agricultural College, Edinburgh.

Within the bee-proof glass-house the plants were subject to a day-time minimum temperature of 21^0 C and a night-time temperature of 16^0 C. 400W sodium lamps suspended at a constant distance 1m above the top of the plant and spaced at 1 lamp per m^{-2}, provided a 16h photoperiod. Plants were thinned to one per pot when the third leaf had unfolded .

Each experiment was arranged as a completely randomised design, each treatment (Table 2.3.1) comprised 8 replicated plants.

2.3.2 Treatments

Because of the low solubility of cytokinins in water, BA, KIN, Z, iP and [9R]Z powder were first dissolved directly into a few drops of 1M NaOH while [9tP]BA were first dissolved in 95% (v/v) ethanol. Each solution was diluted to strength in distilled water containing 1 ml l^{-1} of the non-ionic wetter "Agral". Control plants were treated with water containing the wetter (Table 2.3.1). The concentration selected was based on previous studies (Kellerhals, 1984; Rylott & Smith, 1990; Smith & Rylott, 1992) where cytokinin was found to give maximum response in *V. faba* L.

3.2.7 Number of seeds per mature pod

Each pod that reached maturity on control plants contained on average 4 seeds (Table 3.2.1). No cytokinin treatment used in experiment 3.1 altered the number of seeds per mature pod.

3.2.8 Synchrony of flowering

Synchrony of flower opening was not generally affected by the application of BA, KIN and Z. In only two cases was the synchrony ratio altered and in each of these cases flowers became less synchronous (Table 3.2.4). At raceme 7, with KIN and Z the ratio was increased ($p<0.001$) hence flowers were less synchronous in their development compared to controls. In all cases the correlation between the synchrony ratio and the percentage pod set achieved on each raceme ranged between $\pm$ 0.17 (Table 3.2.4).

In addition, the number of days from the start of flowering until all flowers on raceme 9 had reached the stage at which pollination could take place, was not significantly altered by the external application of cytokinins. The length of time in all cases ranged between 18 and 21 days.

Table 3.2.4: *Effect of cytokinins on the intra-raceme synchrony of flowering; lower values indicate greater synchrony than higher values. The correlation coefficient between synchrony of flowering and mean percentage pod set on all racemes is also given.*

Treatment	Raceme position									Correlation coefficient
	1	2	3	4	5	6	7	8	9	
	Days									
Control	0.77	0.81	0.68	0.72	0.64	0.64	0.52	0.51	0.55	-0.012
BA	0.65^{ns}	0.76^{ns}	0.74^{ns}	0.67^{ns}	0.71^{ns}	0.68^{ns}	0.63^{ns}	0.63^{ns}	0.60^{ns}	-0.076
KIN	0.75^{ns}	0.72^{ns}	0.71^{ns}	0.62^{ns}	0.74^{ns}	0.74^{ns}	0.67**	0.62^{ns}	0.59^{ns}	-0.167
Z	0.71^{ns}	0.75^{ns}	0.68^{ns}	0.67^{ns}	0.72^{ns}	0.66^{ns}	0.68**	0.62^{ns}	0.53^{ns}	-0.032

ns: no statistically significant difference from the control
** : different from the control at 0.01 probability level

Table 2.3.1: *Treatments applied during experiments 3.1 and 3.2.*

	Treatment	Active ingredient	Concentration
Experiment 3.1	Control	water + wetter	
	BA	benzyladenine + wetter	$1x10^{-4}$ M
	KIN	kinetin + wetter	$1x10^{-4}$ M
	Z	zeatin + wetter	$1x10^{-4}$ M
Experiment 3.2	Control	water + wetter	
	iP	isopentenyl adenine + wetter	$1x10^{-4}$ M
	[9R]Z	zeatin riboside + wetter	$1x10^{-4}$ M
	[9tP]BA	BPA + wetter	$1x10^{-4}$ M

2.3.3 Application of treatment solution to flowers

In both experiments, all the flowers and flower buds on an inflorescence were treated at a single time. At this time the inflorescence had three basal flowers open (fds 9) in which anthesis had occurred. The majority of other flowers on this raceme were ranged over all pre-anthesis developmental stages (which were 5-8). In these experiments, treatment solutions were applied to all tissues of the flowers or buds on the raceme with a spray-gun (Humbrol Ltd., Hull, England). Spraying of mutual racemes was avoided by enclosing the sprayed inflorescence within a plastic shield.

2.3.4 Recorded characteristics

Plants were scored for pod-set, number of mature pods and number of seeds per pod. In addition, in order to investigate the possible effects of hormone treatment on intra-raceme flower development a detailed analysis of synchrony of flowering was performed. This was calculated by measurement of the time difference, in days, between the proximal flower having fully opened (fds 9) and the distal flower on the same raceme reaching the same stage of development. This figure was divided by the number of flowers on that raceme. The correlation coefficient was calculated between this intra-raceme flowering synchrony figure and the percentage pod set

3.3 Experiment 3.2: Assessment of iP, [9R]Z and [9tP]BA

3.3.1 Intra-raceme percentage pod set

Typical pod set distribution was displayed by control plants, with average percentage pod set on the proximal three flower positions being 20%. Average percentage pod set on the distal five positions was 4% (Figure 3.3.1.a).

Percentage pod set on the plants treated with iP or [9R]Z was not altered at any individual flower position compared to control plants.

The application of [9tP]BA increased the average pod set on the three proximal flowers to 62%. At flower position 1 this increase was 33% greater ($p<0.05$) compared to control plants, and at positions 2 and 3 it was 48% and 46% greater ($p<0.001$) respectively. On average, at the five more distal flower positions on the inflorescence, percentage of pod initiation was increased ($p<0.001$) to 79% (Figure 3.3.1.a).

3.3.2 Inter-raceme percentage pod set

Mean pod set on the lower four racemes was 14% in control plants (Figure 3.3.b). [9tP]BA application on each raceme resulted in higher ($p<0.001$) pod set at each individual raceme position on the lower four reproductive nodes, giving on average a 70% increase compared to the same raceme positions on control plants. A similar pattern of increase was observed on the five upper inflorescences measured. At racemes 5-9, the proportion of flowers that had developed pods was greater ($p<0.001$) by 54%, 55%, 55%, 58% and 61% respectively compared to that on control plants. Slight changes in pod set due to treatments of iP and [9R]Z were recorded at all raceme positions in comparison to control plants (Figure 3.3.b).

3.3.3 Overall percentage pod set

Average overall pod set on control plants was 10%. Pod set increased ($p<0.001$) to 73% due to [9tP]BA application on each flowering node. This change was attributed to the increased number of developing pods, since the number of flowers was not affected (Table 3.3.1).

Overall pod set measured on the plants treated by iP and [9R]Z was 11 and 12% respectively (Table 3.3.1), thus no effect in overall pod set due to the application of these two cytokinins was recorded.

achieved on the same raceme. This procedure was repeated for every raceme formed for each plant in all treatments. It was usual for plants to produce reproductive axillary shoots but data collection for all plants was restricted to the first nine racemes produced on the main stem.

2.4 Experimental procedures Chapter 4: Response of faba bean cultivars to BA-type cytokinins

2.4.1 Plant material and treatments

Seeds of 5 commercial varieties of *Vicia faba* L. -Three Fold White, Maris Bead, Troy, Cresta and Toret- were sown at Bush Estate, Edinburgh School of Agriculture on 2 April 1993. Each plot measured 1.0 x 1.2 m and seeds were sown at regular intervals along 20 cm rows in order to attain a plant density equal to 36 plants m^{-2}. Reserve plants were transplanted in order to fill the gaps of ungerminated seeds. A distance of 1m was left between each plot.

Soil samples were measured for the indices of P and K and according to the results compound fertiliser was placed in the seed-bed at the rate 60 kg/ha P and 90 kg/ha K. No herbicides were used on the plots, and during the early stages of growth the plots were kept weed free by hoeing.

Two cytokinin analogs were used: BA and [9tP]BA each at the concentration $1x10^{-4}$ M. Solutions were prepared as described in section 2.3.2.

Two applications of the chemicals were made during the flowering period of the plants because it has been shown that cytokinin effects are transient and are effective for only 3-4 raceme positions (Rylott, 1991). All applications of chemicals at growth stages, 203(1) (first open flowers) and 204(1) (first pod set) were made with a Cooper Peglar knapsack sprayer fitted with fan-jet nozzles and were applied to "run off". The vegetative apex of the stem was not sprayed and the chemicals were directed mainly to the reproductive organs of the plant. Control plants were sprayed with a mixture of water and wetter at the same growth stages. This method was found by Rylott (1991) to produce the best results in enhancing pod set of the first eight racemes on the mainstem of cv. Three Fold White, a broad bean variety tested for three years under field conditions in Scotland.

The experiment was designed as a Randomised Block. Each treatment contained 4 replicated plots. In order to prevent edge effects, 5 plants were chosen at random from within the centre of the plots and tagged to allow accurate identification.

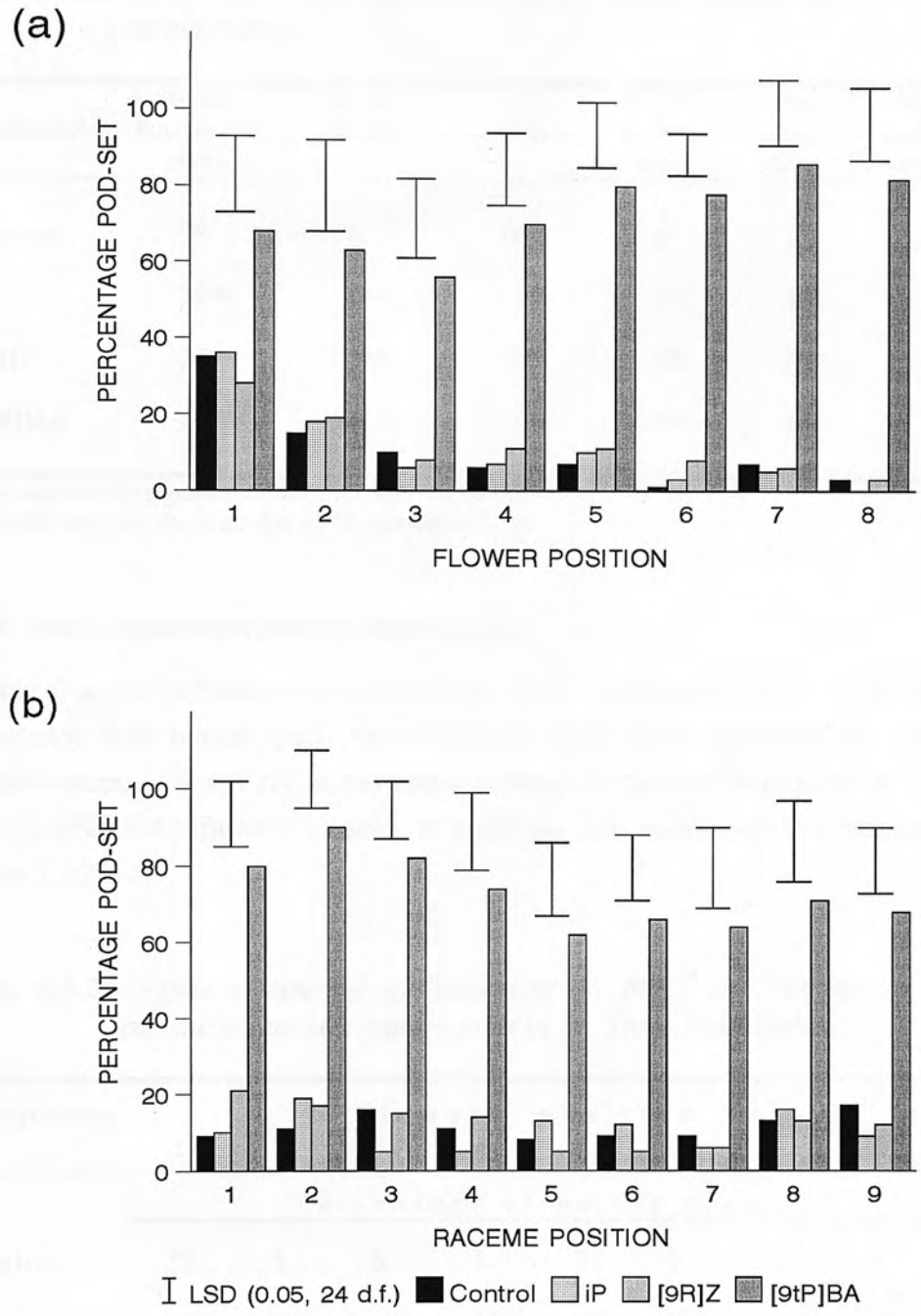

Figure 3.3.1: The effect of iP, [9R]Z and [9tP]BA on (a) intra-raceme and (b) inter-raceme percentage pod set. (Actual figures are shown in Appendix 3.2).

2.4.2 Recorded characteristics

The number of flowers formed and pods set on the mainstems of these plants were scored. At the harvest (12-13 September) when the plants were at the stage 209(1) (seed rubbery, pods still pliable, turning black), a record was made of the number of mature pods, dry weight of hulls (mature pods without seeds) and dry weight of seeds on the mainstem, number of seeds per harvestable pod and number of seeds per mainstem. Dry weights were determined by placing the sample into an oven set at 90^0C until the weights reached equilibrium. The distribution of dry matter between hulls and seeds of harvestable pods was also calculated. It was usual for plants to produce axillary reproductive shoots, but the data presented here were restricted to racemes produced on the mainstem only.

Standard analysis of variance has been applied to the average of all five plants selected from a plot. Also, the variability between single-plant sampling units was measured in order to extract information about the uniformity of each variety and the precision of each character of interest in this trial. This additional source of variation between the individual plants of the same variety was estimated by the margin of error of treatment mean (D) expressed as a percentage of the mean value of the same variety (Gomez & Gomez, 1984).

2.5 Experimental procedures Chapter 5: Effects of BA application on the activity of developing reproductive sinks

2.5.1 Experiment 5.1: Field trial 1993 - Effects of BA on the activity of sucrose metabolising enzymes in carpels

2.5.1.1 Plant material

Plants from the field trial described in section 2.4 were used for this study. However, results for only three of the varieties i.e. Maris Bead, Troy and Toret, are presented here, and only for two of the treatments (i) Control and (ii) BA because technical restrictions made it impossible to collect flowers for biochemical studies from the whole field trial. With regard to the characteristic of pod set, based on the observations after the first application of BA, Toret appeared to have a good positive response to cytokinin and a low intrinsic ability to set pods while Maris Bead and Troy were less responsive to BA but had good ability to retain their pods. Therefore the varieties were chosen for (a) contrasting levels of pod set and (b) different

Table 3.3.1: *Effect of external application of iP, [9R]Z and [9tP]BA on overall characteristics.*

Treatment	No. of flowers per plant	No. of pods set	% pod set	No. of mature pods	% mature pods	No. of seeds per mature pod
Control	75	8	10	4	5	4
iP	75^{ns}	8^{ns}	11^{ns}	3^{ns}	4^{ns}	4^{ns}
[9R]Z	74^{ns}	9^{ns}	12^{ns}	5^{ns}	6^{ns}	3^{ns}
[9tP]BA	74^{ns}	54***	73***	5^{ns}	6^{ns}	4^{ns}

ns : no statistically significant difference from the control
*** : different from the control at 0.001 probability level

3.3.4 Intra-raceme percentage mature pods

As was observed in Experiment 3.1 (Section 3.2.4), the intrinsic ability of the flowers to develop into mature pods was lower at more distal positions of untreated inflorescences. Thus, 22% of the initial number of flowers at position 1, and on average 3% of the flowers formed at positions 2-8, developed into mature pods (Table 3.3.2).

Table 3.3.2: *Effect of external application of iP, [9R]Z and [9tP]BA on intra-raceme percentage mature pods in cv. Three Fold White.*

Treatment	Flower position							
	1	2	3	4	5	6	7	8
	Percentage of mature pods							
Control	22	3	6	1	3	1	4	1
iP	25^{ns}	1^{ns}	1^{ns}	1^{ns}	3^{ns}	0^{ns}	0^{ns}	2^{ns}
[9R]Z	22^{ns}	8^{ns}	4^{ns}	6^{ns}	6^{ns}	0^{ns}	3^{ns}	0^{ns}
[9tP]BA	14^{ns}	6^{ns}	1^{ns}	7^{ns}	10^{ns}	7*	2^{ns}	8*

ns: no statistically significant difference from the control
* : different from the control at 0.05 probability level

responsiveness to cytokinins and hence were suitable for comparative studies between the varieties on the effects of external application of cytokinin on sucrose metabolism in relation to pod set.

2.5.1.2 Sample collection

Flowers at three different growth-development stages (Figure 2.5.1) were collected from randomly selected plants from the middle of each plot. The collection of flowers took place 24h after the second application of control and BA treatments. All the flowers were collected from the lower 4-5 racemes of the plant. Flower collection took place on the same day for control and BA-treated plants of each variety. Troy and Toret were sampled simultaneously and Maris Bead was sampled a few hours later. In both cases during the sampling the weather was bright and cool with high clouds. Flower cutting took place in the afternoon when the flowers were fully opened and the growth stages were easily recognised visually. The carpels of the flowers were extracted and immediately afterwards the fresh weight was determined. Each sample consisted of 30-50 carpels which gave 0.6-1.0 g fresh weight depending on the number of carpels and the growth stage. The samples were placed in containers to avoid evaporation of water from the tissue. Before storing, at -80^{0}C, samples were dipped in liquid N_2. A few weeks later enzyme extraction was performed, and the analyses for enzyme activity followed. Each measurement of enzyme activity which is presented in this study was taken from three separate samples which came from three corresponding replicated plots of each treatment in the field. Furthermore, each sample was assayed in triplicate under laboratory conditions and the results averaged.

2.5.1.3 Enzyme extraction

Tissue-soluble proteins were extracted by grinding 0.6-1.0g tissue in a precooled porcelain mortar using liquid N_2. The powder was transferred into a test tube and diluted in a ratio of 1:5 (w/v) tissue to buffer in the extraction medium (Khayat & Zieslin, 1987) containing: 50 mM Hepes-NaOH buffer (pH 7.5), 0.5 mM $MgCl_2$, 1mM $Na_2EDTA{\cdot}2H_2O$, 2 mM DIECA, 2.5 mM DTT, 1%(w/v) BSA, and 2%(w/v) PVPP. The mixture of tissue powder and extraction medium was homogenised by an electric homogeniser, Ultra-Turrax T25 for 30 seconds (8000 No load/min).

In general, the application of cytokinins locally on each raceme at anthesis did not affect the percentage of mature pods. A slight reduction in pod drop during pod development until maturity was caused by [9tP]BA at flowers 6 and 8; which, in turn, resulted 6% and 7% respectively, greater ($p<0.05$) percentage pod set compared to control plants (Table 3.3.2).

3.3.5 Inter-raceme percentage mature pods

The number of mature pods on control plants were quite uniform over all racemes (Table 3.3.3). External application of iP, [9R]Z and [9tP]BA on each raceme generally did not alter the number of mature pods retained on each reproductive node apart from on racemes 1 and 9. At raceme 1, [9tP]BA caused 12% more ($p<0.05$) pods to mature while at reproductive node 9, iP resulted 8% higher percentage of mature pods compared to control plants (Table 3.3.3).

Table 3.3.3: *Effect of external application of cytokinins on inter-raceme percentage mature pods in cv. Three Fold White.*

Treatment	Raceme position								
	1	2	3	4	5	6	7	8	9
	Percentage of mature pods								
Control	3	7	8	6	1	6	3	10	1
iP	7^{ns}	7^{ns}	1^{ns}	0^{ns}	1^{ns}	1^{ns}	3^{ns}	7^{ns}	9^{*}
[9R]Z	12^{ns}	11^{ns}	9^{ns}	5^{ns}	1^{ns}	4^{ns}	5^{ns}	6^{ns}	8^{ns}
[9tP]BA	15^{*}	10^{ns}	5^{ns}	4^{ns}	6^{ns}	4^{ns}	4^{ns}	4^{ns}	6^{ns}

ns: no statistically significant difference from the control
$*$: different from the control at 0.05 probability level

3.3.6 Overall percentage mature pods

Only 5% of the flowers formed on control plants reached the stage of mature pod (Table 3.3.1). None of the three cytokinins used in this study altered this figure.

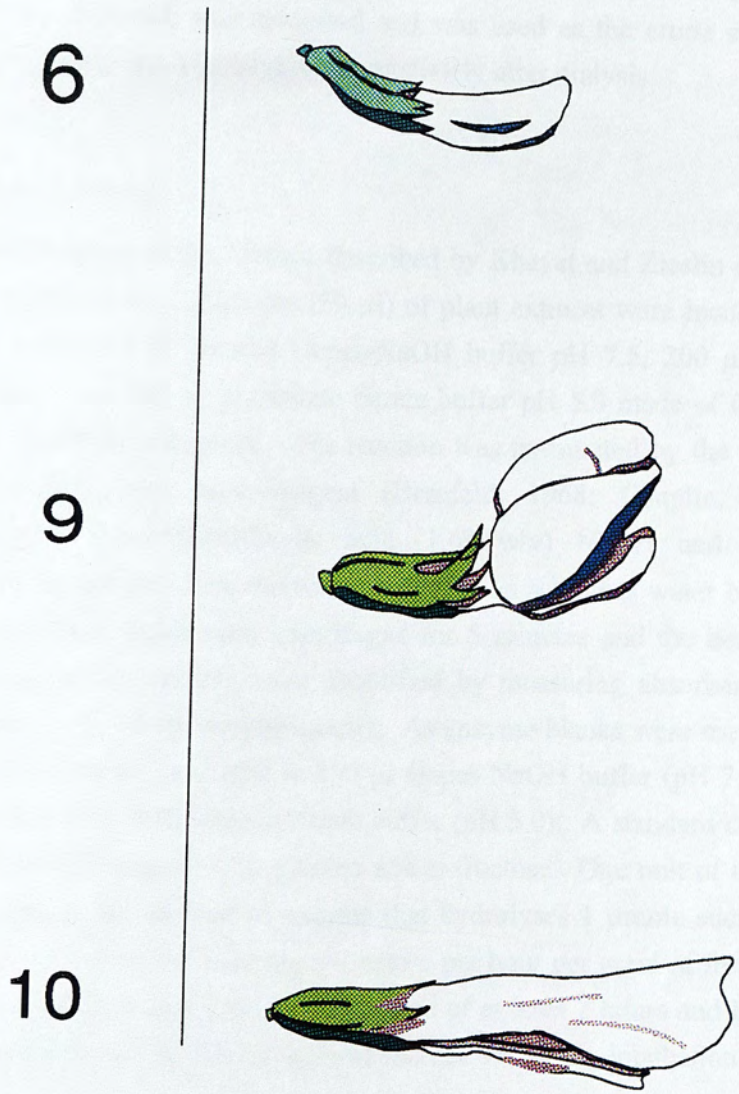

Figure 2.5.1: Three stages of flower development in *Vicia faba* L. (scale proposed by Smith, 1982a).

3.3.7 Number of seeds per mature pod

Mean number of seeds measured per matured pod of control plants was 4 (Table 3.3.1). This characteristic was not affected by the application of any of the three cytokinin analogs assessed in Experiment 3.2.

3.3.8 Synchrony of flowering

The application of iP and [9tP]BA did not affect the synchrony ratio of flower opening at any raceme position (Table 3.3.4). A slightly negative correlation was found (not more than 0.3) between the ratio of flower opening of each raceme and the percentage pod set achieved on that raceme. Spraying the racemes with cytokinins did not alter the total period of flowering of the first 9 reproductive nodes of each plant. In all cases the number of days of this period ranged between 17 and 19.

Table 3.3.4: *Effect of cytokinins on the intra-raceme synchrony of flowering; lower values indicate greater synchrony than higher values. The correlation coefficient between synchrony of flowering and mean percentage pod set on all racemes is also given.*

Treatment	Raceme position									Correlation coefficient
	1	2	3	4	5	6	7	8	9	
	Days									
Control	0.61	0.61	0.65	0.69	0.62	0.62	0.66	0.63	0.64	0.324
iP	0.61^{ns}	0.64^{ns}	0.73^{ns}	0.66^{ns}	0.73^{ns}	0.68^{ns}	0.61^{ns}	0.64^{ns}	0.66^{ns}	-0.275
[9R]Z	0.65^{ns}	0.62^{ns}	0.67^{ns}	0.59^{ns}	0.64^{ns}	0.64^{ns}	0.60^{ns}	0.63^{ns}	0.67^{ns}	-0.167
[9tP]BA	0.65^{ns}	0.65^{ns}	0.64^{ns}	0.59^{ns}	0.67^{ns}	0.66^{ns}	0.61^{ns}	0.63^{ns}	0.61^{ns}	-0.032

ns: no statistically significant difference from the control

3.4 Discussion

The reproductive potential in *V. faba* is directly related to the number of flowers formed on the plant and their ability to set pods. The analysis of intra-raceme pattern of pod set in untreated plants in the preceding experiments demonstrated that first opened flowers on the raceme are more likely to initiate a pod than those which open latter. This is a common characteristic in most grain legumes including *V. faba*

Immediately afterwards, the extract was collected carefully with a Pasteur pipette and centrifuged for 10 min by a Hawkslay MBC centrifuge at 16,000 rpm. Then, the supernatant was dialysed against 100fold of the same buffer without BSA and PVPP. The dialysis took place in a cold room (4° C) for 24 h. Finally, the volume of the resulting dialysate was measured and was used as the crude enzyme extract. All enzyme activities were assayed immediately after dialysis.

2.5.1.4 AI assay

A modification of the method described by Khayat and Zieslin (1987) was used for faba bean carpels. Aliquots (50 µl) of plant extracts were incubated for 30 min at 37°C with 150 µl 50 mM Hepes-NaOH buffer pH 7.5, 200 µl of 1M sucrose as substrate, and 600 µl phosphate citrate buffer pH 5.0 made of 0.1M citric acid and 0.2M disodium phosphate. The reaction was terminated by the addition of 1 ml of 3,5-dinitrosalicylic acid reagent (Bernfeld, 1968; Chaplin, 1986) containing: 0.1%(w/v) 3,5-dinitrosalicylic acid, 1.6%(w/v) NaOH and 30%(w/v) sodium potassium tartrate. The mixture was heated in a boiling water bath for 10 minutes. After cooling, tubes were centrifuged for 5 minutes and the hexoses produced (D-glucose and D-fructose) were quantified by measuring absorbance at A_{570} with a Beckman DU-65 spectrophotometer. As enzyme blanks were used: 50 µl of enzyme extract that were incubated in 150 µl Hepes-NaOH buffer (pH 7.5), 200 µl distilled water and 600 µl phosphate citrate buffer (pH 5.0). A standard curve was set up for an equimolar mixture of D-glucose and D-fructose. One unit of invertase activity in this study is the amount of enzyme that hydrolyses 1 µmole sucrose by releasing 1 µmole D-glucose and 1 µmole D-fructose per hour per gram of fresh weight of tissue. Enzyme activity was linear over a period of at least 2 hours and linear with quantity of tissue extract. Acid hydrolysis of sucrose during the incubation was negligible.

2.5.1.5 SS and SPS assay

Aliquots (10 µl) of tissue extract were incubated for 30 min at 37°C with 60 µl extraction solution and 70 µl of reaction mixture according to the method of Khayat and Zieslin (1987) for measurement of SS activity. The reaction mixture contained: 15 mM UDP-G, 15 mM fructose, 5 mM $MgCl_2 \cdot 6H_2O$, 5 mM NaF, 5 mM $Na_2MoO_4 \cdot 2H_2O$ and 50 mM Hepes-NaOH buffer pH 7.5. The same method slightly modified was used for measurement of SPS activity. Thus, 70 µl of tissue extract were incubated for 30 min at 37°C with an equal volume of reaction mixture (Khayat

(Baylis & Clifford, 1991). Competition effects are supposedly the cause of this phenomenon in reproductive developmental hierarchy of faba bean plants (Jaquiery & Keller, 1978; Chapman *et al.*, 1979; Peat, 1984). In addition, work with soybean (Huff & Dybing, 1979, 1980) indicated the existence of an abscission-inducing signal for the control of abscission of the distal flowers of an inflorescence as suggested by Gates *et al.* (1981) and Smith (1982a) for *V. faba.*

Usually the earlier developed organ dominates over later developed ones. Synchronisation of flower development by breeding or other manipulations (e.g. increased temperature and photoperiod) is expected to eliminate dominance almost completely. This has been reported in a number of examples of other species (Fisher, 1973; Bangerth & Ho, 1984; Bohner & Bangerth, 1988). In *V. faba* it has been suggested by Gates and his co-workers (1981) that inbred lines which form few flowers on each inflorescence, and all opening within 1-2 days, are more likely to set pods than those with many flowers per inflorescence. In the present study, control plants showed no correlation between synchrony of flowering and resulting pod set. Also, spraying of the racemes with BA and [9tP]BA resulted in increased pod set and was followed by a flowering pattern less synchronous than that of control plants. Consequently, since the application of cytokinins resulted in no significant change in the length of the flowering period, it is suggested that the increased pod initiation in this experimentation is a consequence of cytokinin action which is irrelevant to any effects on the synchrony of flowering. These findings are consistent with those presented by Rylott and Smith (1990) working with the same variety and applying BA on each flower.

It was clearly demonstrated here that exogenous application of BA and [9tP]BA locally on flowering inflorescences, dramatically increased pod set; and the supposition can be made that cytokinin substances intrinsically produced in the plant may lie on the physiological basis of pod set. In the literature, unfortunately, there is a lack of studies that relate specifically to endogenous cytokinins and the process of pod set in *Vicia faba.* In *Glysine max*, however, a leguminous species that also suffers from excessive flower drop, studies have shown a strong correlation to exist between the probability of a flower setting a fruit and the total cytokinin flux in the xylem exudate at the time of flower opening (Carlson *et al.*, 1987). Ovules of abscising flowers ceased development at the four- to eight-cell stage, which may be due to constraints on cell division imposed by a reduction in availability of

& Zieslin, 1987). The reaction mixture contained: 15 mM UDP-G, 15 mM fructose 6-P, 5 mM $MgCl_2 \cdot 6H_2O$, 5 mM NaF, 5 mM $Na_2MoO_4 \cdot 2H_2O$ and 50 mM Hepes-NaOH buffer pH 7.5. In both cases, the reaction was terminated by the addition of 70 ml of 1M NaOH and unreacted fructose was destroyed by heating in a boiling water bath for 10 minutes. Sucrose formation was determined by the resorcinol colorimetric method (Roe, 1934; Rufty & Huber, 1983). It should be noted that the basis of the colorimetric method is a reaction between the chromogenic reagents and fructose which is formed from sucrose. Thus, after cooling, 250 µl of 0.1%(w/v) resorcinol in 95% ethanol and 750 µl of 30% HCl were added and the tubes were incubated at 80^0C for 8 minutes. Then, the tubes were allowed to cool before centrifugation for 5 minutes with a Hawkslay MBC centrifuge at 16,000 rpm. Enzymatic activity was determined by reference to a blank reaction mixture in which the UDP-glucose solution was replaced by water (15 µl), and absorbance was measured at A_{520} with the same spectrophotometer. A standard curve was prepared with sucrose. The amount of enzyme solution and reaction time had previously been shown to be in the linear range of the reaction. One unit of SS or SPS activity in this study is the amount of the enzyme that synthesises 1 µmole sucrose per hour per gram of fresh weight of tissue. SS activity and SPS activity were found to be linear for at least 1.5 hours.

2.5.2 Experiment 5.2: Field trial 1994 - Effects of BA on the activity of sucrose metabolising enzymes in pedicel and peduncle.

2.5.2.1 Plant material and treatments

Three cultivars of *Vicia faba* L. were used in this study. Seeds of cvs Three Fold White, Troy and Toret were sown in late April 1994, later than usual because of the wet weather in March (Appendix 5.1), on a field at Bush Estate, Edinburgh Centre for Rural Economy. The experiment was a complete block design. Each treatment consisted of three replicated plots; each plot measured 1.4 x 2 m and seeds were sown at regular spacing along 20 cm rows in order to achieve plant population of 36 plants m^{-1}. A distance of 1m was left between plots. Reserved plants were transplanted in order to fill the gaps of ungerminated seeds.

The previous crop was faba bean. Soil samples indicated that the indices for P and K were normal for faba beans; thus no compound fertiliser was placed in the seed bed. No herbicides or pesticides were applied to the growing crop. Plots were kept weed-free during the growing period of the crop by hoeing.

cytokinins. A second line of evidence coming from studies on the roots of field beans have shown that root nodules generate compounds with cytokinin activity and their levels were twelve to thirteen times those detected in the roots and stems (Henson & Wheeler, 1976). Also, it has been reported that application of antigibberellins to faba bean plants increases root nodules (Hassan & El-Mousi, 1982). The same growth substances when applied to field beans enhanced pod set (Richards & Smith, 1987). Therefore, it can be postulated that the ontogenetic variation in the amount of cytokinins exported from the roots and carried through the xylem into the flowers with the transpiration stream is involved in the physiological functions which lead to successful pod initiation in *V. faba.* A deficiency of root-produced cytokinins during flowering and early pod development due to reduced root growth and nodulation during this period may determine floral abscission of the plants.

The increased pod set achieved in this study by BA-type cytokinin treatments was an indication that the faba bean plant is capable of supporting initially a greater pod load. However, the small proportion of flowers that developed into mature pods, due to high levels of pod drop before their maturity, implies that the increased reproductive load achieved immediately after setting of pods placed a demand on the plant that it was not entirely able to supply with cytokinins and/or photoassimilates and mineral nutrients for further growth and development. This view is also supported by evidence obtained by many studies in other legumes (Clifford, 1981; Crosby *et al.*, 1981; Peterson *et al.*, 1984; Carlson *et al.*, 1987) and *Vicia faba* (Rylott, 1991), where BA was applied externally on the flowers. It has also been reported that endogenous cytokinin levels of Z, [9R]Z, DHZ (dihydrozeatin) and [9R]DHZ (dihydrozeatin ribonucleoside) dropped sharply before the start of pod fill (Nooden *et al.*, 1989) in soybean, and that the pods of this species suppress cytokinin production in the roots as they inhibit root growth (Nooden, 1988; Nooden & Guiamet, 1989) quite early in their development. These observations on pod development and cytokinin status of the plant lead to the conclusion that failure of young pods to reach maturity and thus to contribute to the final yield may be attributed to limited cytokinin supply in these organs. Therefore, it could be proposed that multiple applications of cytokinins to the young pods may be required to attain increased pod number at maturity. Kinet *et al.*, (1985) supported this proposal and suggested that a continuous supply of these growth substances is an

The synthetic cytokinin BA was used at the concentration $1x10^{-4}$ M and solution was prepared as described in Section 2.3.2. As in the previous field experiment, two applications of the chemical had been initially planned for early flowering and early pod set. However, only one cytokinin spraying was performed at stage 203(5) (i.e. first flowers open on the fifth raceme), due to rainy and windy weather at early flowering (see Appendix 5.1b). The application of the chemical was made as described in Section 2.4.1. Control plants were sprayed with a mixture of water and wetter at the same growth stage.

Reproductive development was observed on the first five reproductive nodes of the mainstem of each plant. The total number of flowers formed on these racemes and the number of pods set were recorded. Percentage pod set on these racemes was calculated based on 5 plants randomly selected in each experimental plot.

2.5.2.2 Sample collection

On the day following BA-application, samples were taken between 1400 and 1800 h: thus the various stages of flower development could be accurately recognised since new flowers open in the afternoon (Bond & Poulsen, 1983). Five plants (different from those tagged for pod set measurements) were selected within the centre of each plot and the five lower racemes of the mainstem were removed. The racemes were refrigerated and were brought into the laboratory near the field. Each raceme bore flowers at various developmental stages; mostly at stages from 6 to 10 (Figure 2.5.1). Stages of flower development were observed visually as in Experiment 5.1, and the flowers at stages 6, 9 and 10, were excised below the flower receptacle, thus removing all floral organs but leaving the pedicel intact and attached on the peduncle. Immediately after flower removal, the peduncle was cut just above and just below the pedicel-peduncle junction, and the excised pedicel and rachis part (intact pedicel and part of peduncle) were placed in a container placed in ice, according to the developmental stage of the cut flower. When all segments of the sampled racemes in the plot had been collected, the fresh weight of the tissue was recorded before placing it in the liquid N_2. Storage of the tissue followed at -80^0C for a few days, until the enzyme analyses were performed as described in Sections 2.5.1.3 - 2.5.1.5. Each measurement of enzyme activity that is presented in this study was taken from three separate samples which came from three corresponding replicated plots of each treatment in the field. Furthermore, each sample was assayed in triplicate under laboratory conditions and the results averaged.

important aspect of their action: this was based on evidence from many different species. However, in addition to cytokinins, gibberellins and auxins are likely to function in directing the development of pods in broad beans (Diethelm *et al.*, 1988; Rylott, 1991).

Of the six cytokinin analogs tested here BA and its derivative [9tP]BA were the only effective substances, particularly in enhancing pod set. KIN, Z, iP and [9R]Z did not alter any of the reproductive characteristics under investigation in these experiments. The lack of activity observed in non-BA compounds may be due partly to the immobility of these substances and hence they do not reach the active site in the plant. It is well documented that the cuticle is the first limiting barrier in foliar uptake of non-volatile agrochemicals (Kirkwood, 1991) like those employed here. The non-ionic surfactant Agral used in the present study enhance the rate of efficiency of cuticle penetration and absorption of the cytokinin. Surfactants can enhance the biological effectiveness of active ingredients, (Foy, 1992; Bukovak & Petracek, 1993; Kirkwood, 1993; Stock & Holloway, 1993) but it is usually not clear how this is accomplished. The sites and mechanisms of surfactant action have been considered by Stock and Holloway (1993). They may act at four possible sites in treated plant organs, namely, on the surface, cuticle, and epidermis or within the internal tissues. Albeit recent information suggests that in the case of lipophilic formulations (like cytokinins) non-ionic surfactants may be preferable (Kirkwood, 1991) there are no studies in the literature investigating the interactive effect of cytokinin compounds and surface active agents with regard to the rate of uptake of the growth substance. Therefore, it is unknown how much of the plant growth substance entered the plant tissue.

Following ease of uptake, differences in translocation within the plant probably account for some cytokinin effects on the reproductive development of pulse crops. In recent work (Atkins & Pigeaire, 1993) with *Lupinus angustifolius* L., it has been demonstrated that when [^{14}C]-BA was applied to the petals, sepals and outside surface of stamens and ovaries, most of the label which migrated was recorded in the pedicel of the fed flower. However, on the basis of radioactivity per unit weight of tissue, the ovules had attracted more ^{14}C than the ovary or the pedicel. These findings imply that site of action may be different from site of application, with the latter being also of decisive importance. It is also important to note here that although the use of non-ionic surfactants increases the uptake of chemical into the

2.5.2.3 Enzyme extraction and analysis

The methods are described in Sections 2.5.1.3 - 2.5.1.5.

2.6 Experimental procedures Chapter 6: Effects of BA application on water-soluble sugar content of developing carpels

2.6.1 Plant material

All plants used in this study were grown in plastic pots under controlled conditions in a climate chamber at the Scottish Agricultural College, Edinburgh. Two seeds of *Vicia faba* L. cv. Toret were sown in 18 cm plastic pots containing Levington peat-based potting compost on 16th January 1995. Climate chamber day-time temperature was 21^0C and night temperature was 16^0C. Photoperiod was 16 h with lighting provided by 400W High Pressure Mercury lamps at the number of 4 lamps/3 m^2 suspended at a constant distance of 1 m above the plants. Plants were thinned to one per pot at the stage when the third leaf was unfolding and staked with canes to prevent lodging. All branches were removed to ensure uniform plant material. Plants were watered daily with tap water. All flowers were hand-pollinated once petals had fully opened (fds-9).

2.6.2 BA application

Cytokinin and control treatments were formulated according to a modified method of Rylott and Smith (1990). Thus, every flower on each plant was treated by gently applying the solution on to the standard petal and the calyx, with a fine artists paintbrush. Control plants were treated with distilled water containing 1 ml Agral surfactant/litre and cytokinin treated plants with $1x10^{-4}$ M BA. The preparation of solutions was performed as in section 2.3.2. Each flower was treated once with the growth substance at fds-6 (Figure 2.5.1), immediately prior to the standard petal folding back on itself, in order to avoid self fertilisation since at this stage anther dehiscence begins. Treatments were related to the flower development stage, as the time to complete fertilisation is difficult to determine and can range from 24 to 72 h after pollination (Stoddard, 1986; Lazaridou personal communication). All treatments are described in Table 2.6.1.

plant organ it does not otherwise influence its transport patterns within the plant tissue (Bromilow & Chamberlain, 1991).

Relevant to the cytokinin translocation within the plant tissue is its metabolism. Following supply of ^{14}C-BA to *Phaseolus vulgaris* plant roots, only [9R]BA was detected in xylem sap collected from the stem (Ramina, 1979). Such evidence strengthens the view that the cytokinin riboside is the form that is translocated in plants. *In vitro* culture of tobacco reproductive organs showed that internal levels of free, non-metabolised base of BA appeared important in the initiation of physiological responses (van der Krieken *et al.*, 1988). There is also evidence that plant tissues convert exogenous BA into a great diversity of metabolites which include products of ring substitution (e.g. ribosides, nucleotides, N-glucosides), and products of sidechain cleavage (e.g. adenine, adenosine etc.) (Letham & Palni, 1983; van Staden & Crouch, 1995). Cytokinin metabolites possess functional, though somewhat obscure roles in plants (Letham & Palni, 1983; Wagner & Beck, 1993), contributing either to an active or to an inactive pool. The identity of the active form(s) of cytokinin, namely the molecule(s) which bind possibly to a receptor to evoke a growth or physiological response, is an unresolved issue (Venis, 1985; van Staden & Crouch, 1995). Matsubara (1990) considered BA to be the most active cytokinin in the class of ring-substituted aminopurines. Generally a second substituent in the 9-position of N^6-substituted purines lowers, but does not eliminate cytokinin activity (Skoog *et al.*, 1967; Matsubara, 1990). In the present study BA and its 9-substituted analog [9tP]BA were both the most active compounds in successful pod initiation. The limited success of KIN, Z, iP and [9R]Z to reduce flower drop in faba bean plants may also be explained, in addition to their uptake, by changes in metabolism of the externally applied cytokinins after entering the plant tissue which in turn reflect changes in their biological activity.

This diversity of action leaves plenty of work for future research. Further investigations into all these aspects using *Vicia faba* plants as biological material are extremely important in order to explain structure-activity relationships and response of faba bean plants to different forms of cytokinin growth substances. However, the analysis of experimental data from individual nodes and flower positions showed large experimental errors (Figures 3.2.1 and 3.3.1). The ability of faba bean plant to set pods has also been noted as a variable character by other workers (e.g. Aufhammer & Gotz-Lee, 1991; Rylott, 1991). The explanation for this variation

Table 2.6.1: *Treatments and number of plants per treatment.*

Treatment	Solution	Flower Development Stage 6	9	10	Pod-set measurement
		Number of plants			
Control	Water + Wetter	4	4	4	6
BA	BA + Wetter	4	4	4	6

2.6.3 Pod-set measurements

Twelve plants were selected of which 6 plants were used as control and 6 plants were treated with BA (Table 2.6.1). Each plant was recorded as one replication. Pod-set was monitored on all 3 to 8 midcanopy mainstem racemes.

2.6.4 Sampling technique

For sugar analysis, sets of 4 plants for each treatment were selected, each plant being one replication. Since removal of a flower during sampling makes it impossible to determine whether that particular carpel would really have developed into a pod, if left on the raceme, the sampling procedure must maximise the probability for pod set or abscission, whichever is desired for a given treatment. The following procedure was used. Sampling was restricted to racemes 3 to 8 on the mainstem and flower positions 4 to 7 on each raceme (Figure 2.6.1). Preliminary studies had shown that on untreated plants of cv. Toret pod set was almost zero at those flower positions.

Flowers treated with BA had high probability for pod-set. Untreated flowers had high probability of pod-drop. The advantages of this procedure were that control and BA-treated samples were inserted at the same position on the raceme and can be collected on the same day for a particular physiological age. A disadvantage is that actual percent abscission for the control sample is likely to be less than 100% since at least a few pods usually are set at these insertion positions on the raceme. Additionally, BA treatment is not 100% reliable.

Flowers from predetermined flower positions on the mainstem were sampled at three successive developmental stages -6, 9 and 10- throughout the sampling period. At flower development stage 6, a sample flower was treated and the number of its

must be mainly due to genetic control. In the present study, each selected plant was treated as one replication. This approach may have introduced greater error because plant to plant variation is large in commercial varieties of faba beans. A better approach to minimise this error would have been to select a group of plants as a single replication. This technique was worth applying in the following field experiment (Chapter 4) where space and labour limitations could have been overcome.

Although various problems outlined above are associated with the exogenous application of cytokinins to plant systems, the effects of BA and [9tP]BA documented in this study seem to have potential for further investigation. It is interesting to check the consistency of these effects in various cultivars of *Vicia faba* with contrasting ability to keep their reproductive structures. This research would lead to more conclusive evidence concerning the control of yield instability of faba beans with cytokinin compounds.

♦ ♦ ♦

position on the raceme was recorded on a tag attached at this node. This tagging procedure ensured that all harvested carpels for a particular sample were of a similar age i.e. at the same stage of development, and that they were treated once 24 hours ago.

Flowers were removed by cutting below the calyx and the cut flowers were taken to the laboratory for carpel removal. The time from cutting to initial dissection was 10-20 minutes. The carpel of each flower was quickly removed, weighed, and stored at -80^{0}C.

2.6.5 Soluble sugar extraction

This was a modified method used by Cochrane (personal communication) in cereal grains. Twenty-four carpels were dissected and homogenised on ice in 0.25 M ice cold perchloric acid (1 ml) for 5 min using an Ultra-Turax T25 (Janke & Kunkel, IKA-Laboratechnick, Germany) electric homogeniser. The same number of carpels were used for each stage of development. Chilled distilled water (3 ml) was added and the mixture further homogenised on ice for 5 min. Immediately afterwards the homogenate was centrifuged for 10 min at 5000 g and 0^{0}C in a Sigma 202MK refrigerated centrifuge (Sigma laboratory centrifuges Gmbh., Germany). The resulting supernatant was retained on ice and the pellet resuspended in 0.25M perchloric acid (0.5 ml) and homogenised on ice for 5 minutes. Chilled distilled water (1.5 ml) was added and, following an additional period of homogenising on ice (5 min) the resuspended pellet was centrifuged as before. Following centrifugation the second supernatant was added to the first and the final pellet was discarded. The combined supernatants were then neutralised by adding 1 M KOH and 0.1 M KOH to pH 7.0. The neutralised supernatant was then recentrifuged as previously described and the supernatant measured for volume and stored at -20^{0}C. The pellet was discarded.

2.6.6 Determination of sucrose, D-glucose and D-fructose

Sucrose, D-glucose and D-fructose were determined by enzymatic analysis using a test combination kit supplied by Boehringer Mannheim (see Appendix 2.6.1).

Chapter 4

Field study on the response of faba bean cultivars to externally applied BA-type cytokinins

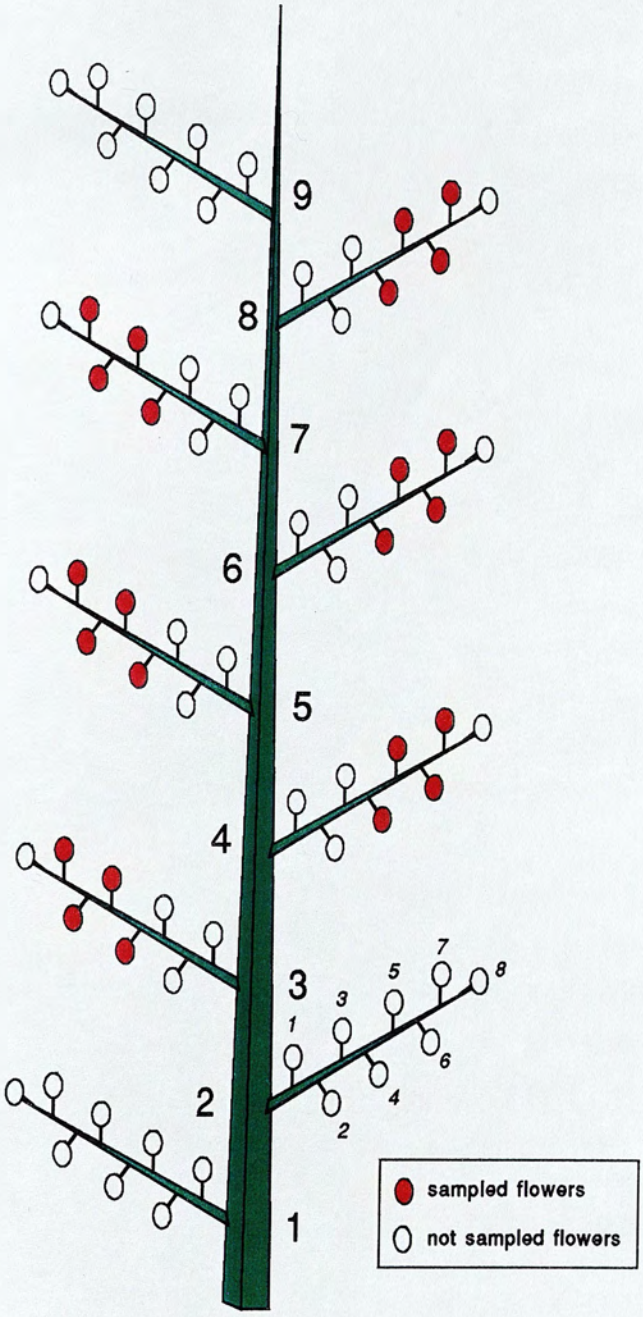

Figure 2.6.1: Schematic layout of faba bean mainstem flowering nodes showing sites at which flowers were sampled.

4.1 Introduction

It was found in Chapter 3, that application of BA or [9tP]BA, locally on each flowering raceme, increased pod set and markedly modified the pattern of flower loss of broad bean plants cv Three Fold White. However, the profile of pods retained until maturity did not differ from that in control plants. It was suggested that repeated applications of cytokinins to young pods, possibly during the lag period of their development starting after fertilisation (Peat, 1983), may increase pod retention with possible advantages to final reproductive load.

It is well known that yield stability, and thus the number of seed-bearing pods per plant is a characteristic with low heritability in *Vicia faba* L. (Lawes *et al.*, 1983; Stelling *et al.*, 1994). Keller (1974) and Gates *et al.* (1983) observed losses of reproductive organs during reproductive development in faba beans which ranged from 36% to 87%, depending on variety and season. Wide variation has also been observed among faba bean genotypes, as regards the proportion of potential pods attaining final harvest maturity (Duc & Picard, 1981; Suso *et al.*, 1996).

Many studies (as reviewed in Sections 1.5.3 to 1.5.5) suggest that the indeterminate growth habit of the plant results in high levels of internal competition between reproductive (seeds and pods) and vegetative (apex, stem and developing leaves) sinks, particularly at the phase from the beginning to the end of flowering, to such an extent that reproductive organs fail to grow and are rejected. External application of cytokinins alone or in combination with other growth substances on the reproductive structures of faba bean is able in some but not all instances to increase the final yield (Chapman & Sadjadi, 1981; Kellerhals, 1983; El-Abd *et al.*, 1989; Rylott, 1990), possibly by diminishing the competitive relationships within the plant.

Therefore, the main objective of this experiment was to determine the effects of the two active cytokinin-compounds of the previous experiments (Chapter 3), BA and [9tP]BA, applied at specific growth stages during flowering (Section 2.4), on five varieties with diverse reproductive loss, and to evaluate which parameter of the reproductive potential was affected. Also, the reliability of the treatments and the consistency of the results over these cultivars were tested. The experiment was performed under field conditions, as it is clearly not possible because of technical limitations to do this study in a controlled environment. Application timing was based upon flower opening (Section 2.2.3). Exact time of application could therefore be determined and so compared with future experiments. The variability within the plants of each variety was also measured for each recorded characteristic, so that the validity of the hormonal effect could be assessed.

4.2 Effect of cytokinin on intra-raceme percentage pod set

4.2.1 Effect of cytokinin on each variety

4.2.1.1 Three Fold White (TFW)

Control plants of the broad bean variety TFW normally had the ability to set most of the pods at the four proximal flower positions, resulting in average pod set 55%; while the figure for the upper five positions was only 2% (Table 4.2.1).

The application of BA resulted in greater ($p<0.001$) percentage pod set at flower positions 4, 6, 7 and 8 by 53, 57, and 64% respectively (Table 4.2.1). At the positions 3 and 5, percentage pod set was 38% and 62% higher ($p<0.01$) than the control plants, while at positions 1, 2 and 9 no differences were recorded. These increases gave an average of 82% pod set at the proximal four flowers, and 56% at the five distal flowers.

The application of [9tP]BA did not change the level of percentage of the pods set at flowers 1 and 9; but it caused more ($p<0.01$) flowers to set pods at positions 2, 3 and 5, as well as at positions 4, 6, 7 and 8 ($p<0.001$, Table 4.2.1).

4.2.1.2 Maris Bead

The average percentage pod set at the four proximal positions of control plants of Maris Bead was 92%, and at the upper 5 flowers on the raceme 56% (Table 4.2.1).

The application of BA caused more flowers to set pods at positions 4, 5 ($p<0.05$), and 6,7,8 ($p<0.01$), 23% more from controls on the upper five flower positions, while the increase on the four proximal flowers was only 5% (Table 4.2.1).

The effect of [9tP]BA was similar to that observed for BA. Thus, the figure for the four proximal positions was 98%, and for the upper five it was 86% (Table 4.2.1).

4.2.1.3 Troy

In this study, the racemes of Troy were found to form no more than seven flowers. Of those, almost complete pod set (95%) was achieved at the lower four flowers in control plants (Table 4.2.1).

The application of BA increased the percentage of pod set at positions 4 ($p<0.05$) and 6 ($p<0.001$, Table 4.2.1).

Chapter 3

Effects of various cytokinin analogs on the reproductive load of broad bean cv. Three Fold White

4.2 Effect of cytokinin on intra-raceme percentage pod set

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The application of BA increased the percentage of pod set at positions 4 ($p<0.05$) and 6 ($p<0.001$, Table 4.2.1).

3.1 Introduction

As outlined in Section 1.5, half or more of the reproductive structures borne by a faba bean plant may be shed without contributing to seed yield. This is a characteristic common to pulse crops, and the pod number has been proposed as the most important component of the variation of their yield (Dantuma & Thompson, 1983; Hardwick, 1988). Experiments with decapitated faba bean plants (Chapman *et al.*, 1979; Gates *et al.*, 1983; Diethelm *et al.*, 1988) led to the conclusion that an indeterminate growth habit would favour excessive growth of stems and leaves instead of generative organs and hence be of no advantage. Furthermore, faba beans exhibit dominance effects from older fruits, causing inhibition of the development of younger fruits (Crompton *et al.*, 1981; Smith, 1982a). Application of plant growth substances have been shown to modify such competitive effects and enhance reproductive growth at the expense of vegetative growth by influencing plant development and growth habit (Section 1.5.5). Studies on hormonal factors that may be causally involved have indicated that cytokinins seem to play a leading role (Baylis & Clifford, 1991), possibly in the distribution of source output to developing reproductive sinks (Aufhammer, 1990). It was even possible, under both controlled (Chapman & Sadjadi, 1981; Rylott & Smith, 1990; Smith & Rylott, 1992; Clifford *et al.*, 1992) and field conditions (Kellerhals, 1983; Rylott, 1991) to reduce abscission along the main stem by application of cytokinins.

The ability of external application of BA during flowering to enhance reproductive capacity in faba bean (Rylott & Smith, 1990; Smith & Rylott, 1992) and other legumes such as soybean (*Glysine max*) (Crosby *et al.*, 1978; Dyer *et al.*, 1987; Dybing & Westgate, 1989; Kuang *et al.*, 1991; Reese *et al.*, 1995), peas (*Pisum sativum*) (Garcia-Martinez & Carbonell, 1980), cowpea (*Vigna unguiculata)* (Adedipe *et al.*, 1976; Argall & Stewart, 1984) and narrow-leafed lupin (*Lupinus angustifolius*) (Atkins & Pigeaire, 1993), makes this system especially useful for studying the natural regulation of pod set and further pod development.

Experimentation with KIN sprayed on broad beans resulted in increased number of flowers and pods per plant, and seeds per pod, implying that a specificity may exist between the typed cytokinin applied and characteristic affected (El-Abd *et al.*, 1989). In *Vicia faba* L., however, most commonly the synthetic compound BA has been evaluated for premature abscission reduction activity; hence little is known about the response of this plant to various cytokinin analogs and the optimum chemical structure for the best response.

Increases ($p<0.001$) at positions 4 and 6 were also recorded as a result of the application of [9tP]BA (Table 4.2.1).

Table 4.2.1: *Effect of BA and [9tP]BA on intra-raceme percentage of pod set in each variety.*

Variety	Treatment	Flower position								
		1	2	3	4	5	6	7	8	9
		Percentage of pod set								
Three Fold White	Control	87	72	38	21	8	5	0	0	0
	BA	91^{ns}	86^{ns}	76**	74***	64**	67***	57***	64***	30^{ns}
	[9tP]BA	96^{ns}	92**	78**	72***	61**	56***	60***	45***	19^{ns}
Maris Bead	Control	95	95	91	87	72	59	52	44	42
	BA	97^{ns}	99^{ns}	96^{ns}	95*	92*	95**	87**	74**	50^{ns}
	[9tP]BA	98^{ns}	98^{ns}	98^{ns}	96*	88^{ns}	84**	90**	94**	75^{ns}
Troy	Control	100	95	95	91	85	65	52	n f	n f
	BA	99^{ns}	96^{ns}	98^{ns}	96*	96^{ns}	100**	88^{ns}	n f	n f
	[9tP]BA	99^{ns}	98^{ns}	98^{ns}	97*	94^{ns}	89*	63^{ns}	n f	n f
Cresta	Control	97	97	92	77	66	45	24	18	19
	BA	99^{ns}	98^{ns}	94^{ns}	93*	84*	85**	85**	100**	100**
	[9tP]BA	98^{ns}	95^{ns}	99^{ns}	95*	92**	85**	80**	89**	81**
Toret	Control	98	93	68	43	24	15	4	0	n f
	BA	99^{ns}	99**	96**	90***	84***	84***	79***	78*	n f
	[9tP]BA	99^{ns}	95^{ns}	93**	94***	85***	84***	72***	84*	n f

ns : no statistically significant difference from the control
*, **, *** : different from the control at 0.05, 0.01, 0.001 probability level
nf : flowers were not formed

4.2.1.4 Cresta

In control plants, very high levels of percentage pod set were recorded on the three proximal flowers giving on average 95%, while average percentage pod set at the six distal flowers averaged 42% (Table 4.2.1).

The application of BA resulted in higher percentage pod set at the six distal flower positions, resulting in the average pod set for these positions being 91% (Table 4.2.1).

Therefore, the aim of this experimentation was to determine which out of six of the most biologically-active cytokinins, either synthetic or naturally occurring, applied externally to developing flowers affected the reproductive retention of an indeterminate broad bean variety (Section 2.3). These results were considered in relation to the developmental physiology of the plant. Thus positional effects on the plant were also analysed.

3.2 Experiment 3.1: Assessment of BA, KIN and Z.

3.2.1 Intra-raceme percentage pod set

In all control plants the pattern of pod set was the same with most pods set at proximal flower positions on the raceme, especially positions 1 and 2 (Figure 3.2.1.a). Few pods set at positions more distal than flower position 3.

The application of 6-benzylaminopurine (BA) to the whole raceme resulted in a greater ($p<0.001$) pod set at all flower positions except flower position 1 (Figure 3.2.1.a), compared to controls and compared to other treatments.

However, the application of kinetin (KIN) and zeatin (Z) resulted in an intra-raceme pod set pattern similar to control plants without changes in the percentage of pod set at all flower positions (Figure 3.2.1.a) compared to control plants.

3.2.2 Inter-raceme percentage pod set.

Control plants set most pods on the upper three reproductive nodes formed (Figure 3.2.1.b). Average pod set for each of these nodes was 20%, while for the racemes 1-6 it was on average 9%.

The application of BA to each inflorescence resulted in greater pod set on all racemes, with little difference between the lower part of the reproductive region (51%) and the upper portion (53%, Figure 3.2.1.b). The lower four racemes and racemes 7 and 8 had a greater ($p<0.001$) pod set percentage than on control plants and other treated plants. In addition, more ($p<0.05$) pods set at the racemes 6 and 9 compared to control plants.

None of the other treatments, KIN and Z, caused any changes in the percentage of pod set at all treated racemes compared to control plants (Figure 3.2.1.b).

Increases compared with control plants, were also evident at flowers 4 ($p<0.05$) and 5, 6, 7, 8, 9 ($p<0.01$) due to the application of [9tP]BA, giving an average for the distal 6 flowers of 87% (Table 4.2.1). As in the case of BA the application of [9tP]BA caused no difference between control and treated plants at the three proximal positions.

4.2.1.5 Toret

Control plants of Toret followed a pattern of intra-raceme percentage pod set very similar to that observed in TFW (Section 4.2.1.1). Most of the pods set on the proximal three flower positions gave an average of 86%, while the proportion of the flowers which formed at the five distal positions and set pods was only 22% (Table 4.2.1).

BA treatment caused an increase over control plants in pod initiation at all flower positions apart from position 1 where almost all the flowers formed on both control and treated plants set pods (Table 4.2.1). Thus, at the positions 2 and 3 the percentage pod set was 6% and 28% higher ($p<0.01$) than in controls, average pod set of the proximal three positions of flowers being 98%. Increases were also evident at positions 4, 5, 6, 7 ($p<0.001$) and 8 ($p<0.05$), with average percentage pod set being 83% (Table 4.2.1).

The application of [9tP]BA resulted in percentage pod set figures that were similar to those with BA treatment, at all flowers. Percentage pod set increased at flowers 3($p<0.05$), 4, 5, 6, 7 ($p<0.001$) and 8 ($p<0.05$) as compared with controls. Average pod set was 96% on the proximal three flowers and 84% on the five upper flowers (Table 4.2.1).

4.2.2 The main effect of cytokinin on intra-raceme pod set

As in previous experiments, control plants set the majority of pods at proximal flower positions on the raceme, the average for the first four flower positions being 82% while average pod set at the distal five flowers was 30% (Table 4.2.2).

The application of BA at growth stages 203(1) and 204(1) resulted in increased percentage of pod set at all flower positions apart from position 1 (Table 4.2.2). At flower position 2 percentage pod set was 6% higher ($p<0.01$) than in controls; at positions 3, 4, 5, 6, 7, 8, it was 15%, 26%, 33%, 48%, 53% and 63% higher ($p<0.001$), and at the most distal flower it was 40% higher ($p<0.05$) than in controls.

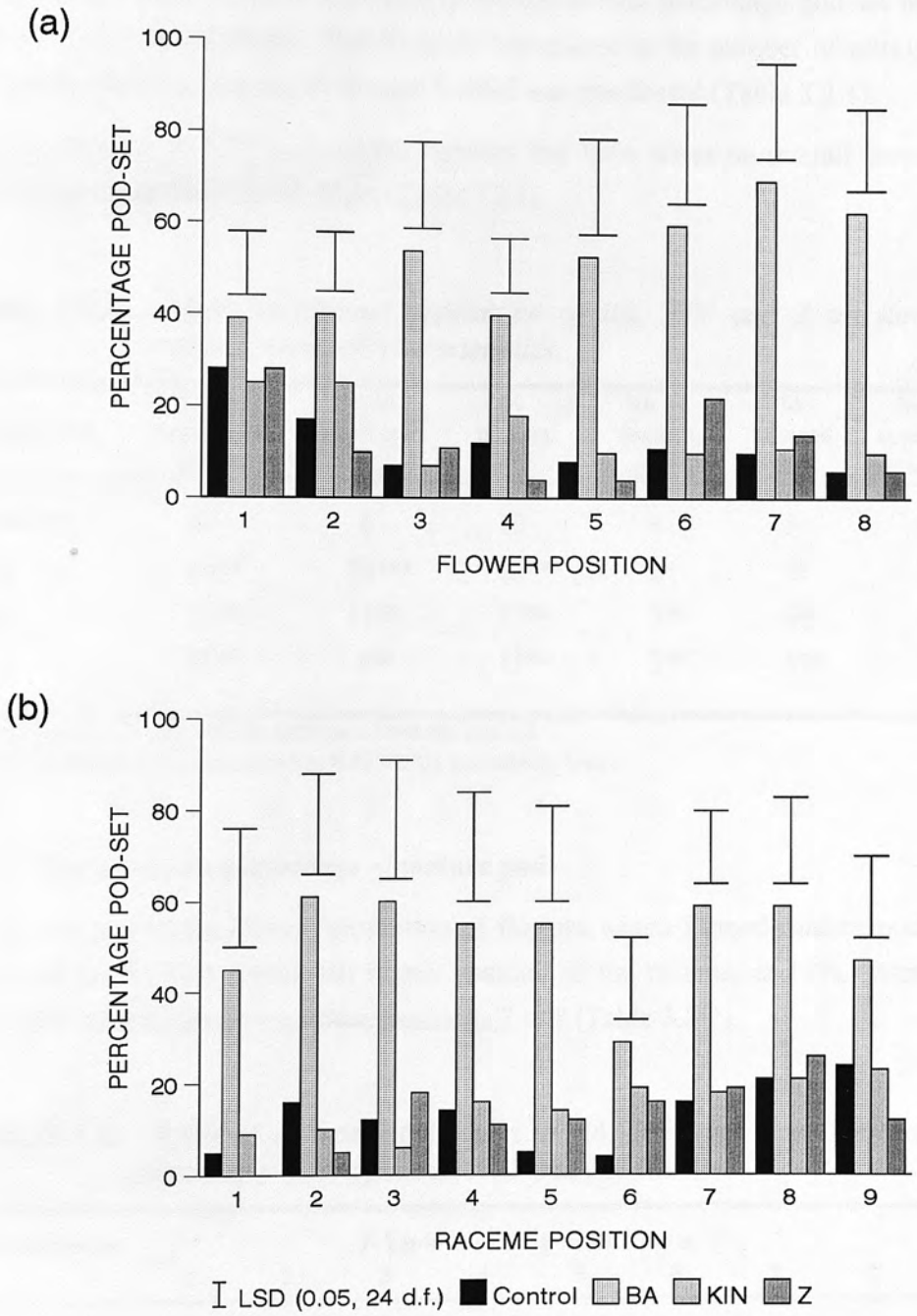

Figure 3.2.1: The effect of BA, KIN and Z on (a) intra-raceme and (b) inter-raceme percentage pod set. (Actual figures are contained in Appendix 3.1).

These changes gave an average percentage pod set at the four proximal flower positions of 94%, while at the three distal flowers it was 77.6%.

Table 4.2.2: *Overall effect of BA and [9tP]BA on intra-raceme percentage of pod set of faba bean plants.*

Treatment	Flower position								
	1	2	3	4	5	6	7	8	9
	Percentage of pod set								
Control	95	90	77	64	51	38	26	16	20
BA	97^{ns}	96**	92***	90***	84***	86***	79***	79***	60*
[9tP]BA	98^{ns}	96**	93***	91***	84***	79***	73***	78***	58*

ns : no statistically significant difference from the control
*, **, *** : different from the control at 0.05, 0.01, 0.001 probability level

The application of [9tP]BA resulted in similar percentage pod set figures to those in plants treated by BA; on the proximal four flowers it was 95% and on the distal five flowers 74% (Table 4.2.2).

4.2.3 The main effect of variety on intra-raceme pod set

The *major* variety Three Fold White set in general less pods at all flower positions than did *minor* and *equina* varieties (Table 4.2.3). However, at flower position 1 percentage pod set figures of TFW were similar to those of Maris Bead, while at positions 7 and 8 they were similar to those of Toret.

Between the two *minor* varieties, Maris Bead and Troy, no differences in the percentage of pods set at any flower position were recorded, apart from position 1 where Troy set 3% more ($p<0.001$) pods than Maris Bead plants (Table 4.2.3).

Between the *equina* varieties used in this study, Cresta and Toret, the figures of percentage pod set at flower positions 1, 2, 3, 6, 7, and 8 were similar (Table 4.2.3).

At the positions 4 and 5 Toret set less ($p<0.001$) pods than Cresta, which reached the levels of percentage pod set of the two *minor* varieties, Maris Bead and Troy. In addition, no difference was recorded between *minor* and *equina* varieties at flower positions 2, 3, 7 and 8 (Table 4.2.3).

Table 4.2.3: *Overall varietal effect on intra-raceme percentage of pod set. Probability values of interaction between variety and cytokinin are also presented.*

Variety	Flower position								
	1	2	3	4	5	6	7	8	9
	Percentage of pod set								
TFW	91[a]	83[a]	64[a]	56[a]	45[a]	42[a]	39[a]	36[a]	16[a]
Maris Bead	96[ab]	98[b]	95[b]	93[c]	84[c]	79[c]	76[b]	71[b]	56[b]
Troy	99[b]	96[b]	97[b]	95[c]	92[c]	84[c]	67[b]	n f	n f
Cresta	98[b]	97[b]	95[b]	88[c]	81[c]	72[bc]	63[b]	69[b]	67[b]
Toret	99[b]	96[b]	86[b]	76[b]	64[b]	61[b]	52[ab]	54[ab]	n f
LSD (42 d.f.)	*5.7*(3)	*7.8*(3)	*11.0*(3)	*10.5*(3)	*13.6*(3)	*14.6*(3)	*26.2*(3)	*23.6*(2)	*33.4*(2)
Var. x Treat.	0.500	0.022	<0.001	<0.001	<0.001	0.001	0.080	0.126	0.230

nf : flowers were not formed
a, b, c : each letter indicates a group of means which do not differ statistically
(2), (3) : LSD calculated at 0.01, 0.001 probability level

4.2.4 Interactive effect of variety and of cytokinin treatment

No interactive effect of variety and cytokinin treatment was found at flower positions 1, 7, 8 and 9 (Table 4.2.3). However, at flower positions 2-6 interaction was present between variety and cytokinin. At those flower positions the varieties TFW and Toret responded better than Maris Bead, Troy and Cresta to the external application of cytokinins in terms of increase in percentage pod set (Table 4.2.1).

4.3 Effect of cytokinin on inter-raceme percentage pod set

4.3.1 Effect of cytokinin on each variety

4.3.1.1 Three Fold White

Control plants set most of their pods on the upper five racemes. Average percentage pod set on the upper five racemes was 53% and on the lower four racemes 32% (Table 4.3.1).

The application of BA at plant growth stages 203(1) and 204(1), resulted in higher percentage pod set over control plants at all racemes (Table 4.3.1). Thus percentage pod set at the lower four racemes averaged 73%, and at the upper five 84%.

Increase in the proportion of flowers which set pods was also recorded at all racemes apart from raceme 7 due to the application of [9tP]BA (Table 4.3.1). The figures were similar to those observed in the plants treated by BA, leading to an average percentage pod set at the lower four racemes of 75% and at the five upper racemes of 84%.

4.3.1.2 Maris Bead

As in the variety TFW, control plants of Maris Bead set slightly more pods at the upper reproductive nodes on the mainstem than at the lower nodes. The average for the lower four nodes was 74%, while for the upper five it was 84% (Table 4.3.1).

More flowers than in the control initiated a pod at reproductive nodes 1, 2, 5, 6 ($p<0.05$) and 4 ($p<0.001$) due to BA treatment, resulting in average pod set for the first four reproductive nodes being 94% and for the upper five nodes 95% (Table 4.3.1).

The application of [9tP]BA resulted in higher percentage pod set at racemes 1, 2, 3, 5, 6 ($p<0.05$) and 4 ($p<0.001$), giving an average for the lower four racemes of 96% and for the upper five of 93% (Table 4.3.1).

4.3.1.3 Troy

Control plants of Troy showed a very high percentage pod set at all reproductive nodes on the mainstem (Table 4.3.1). Average percentage pod set at the lower four racemes was 87% and at the upper five racemes 91%.

External application of BA resulted in almost complete (99%) pod set at the lower four racemes. In the upper five racemes, an increase was evident only at raceme 7 as compared with control plants, and the pod set at these racemes in average was 97% (Table 4.3.1).

The application of [9tP]BA resulted in increases only at the four lower racemes over control plants, resulting in an average pod set at these racemes of 97% (Table 4.3.1). No differences were recorded on the upper five racemes.

Table 4.3.1: *Effect of BA and [9tP]BA on inter-raceme percentage of pod set in each variety.*

Variety	Treatment	Raceme position								
		1	2	3	4	5	6	7	8	9
		Percentage of pod set								
Three Fold White	Control	28	28	37	35	45	58	66	58	40
	BA	74***	85***	64*	70**	90***	87**	88*	83**	72**
	[9tP]BA	83***	81***	66*	68**	90***	90**	70^{ns}	86**	86**
Maris Bead	Control	74	67	76	79	81	85	88	83	84
	BA	91*	96*	94^{ns}	99***	99*	98*	95^{ns}	92^{ns}	89^{ns}
	[9tP]BA	98*	88*	97*	100**	100*	97*	90^{ns}	91^{ns}	91^{ns}
Troy	Control	92	77	83	97	99	99	89	86	82
	BA	98*	99**	97**	100*	97^{ns}	99^{ns}	99*	95^{ns}	94^{ns}
	[9tP]BA	100*	100**	95**	100*	100^{ns}	99^{ns}	98^{ns}	92^{ns}	92^{ns}
Cresta	Control	79	74	74	73	76	77	84	84	93
	BA	93**	94*	91**	94**	96^{ns}	94*	91^{ns}	94^{ns}	93^{ns}
	[9tP]BA	94**	92*	94**	96**	93^{ns}	98*	87^{ns}	89^{ns}	87^{ns}
Toret	Control	53	69	54	47	56	62	65	57	50
	BA	92**	99**	99***	100***	99***	97**	92**	78**	63^{ns}
	[9tP]BA	89**	95**	91***	96***	97***	98**	91**	84**	78*

ns : no statistically significant difference from the control
*, **, *** : different from the control at 0.05, 0.01, 0.001 probability level

4.3.1.4 Cresta

Control plants developed pods in 75% of the flowers formed on the four lower racemes, and in 83% of those formed on the upper five racemes (Table 4.3.1).

The application of BA caused more ($p<0.01$) flowers to set pods than in control plants at the racemes 1, 3 and 4. Increase ($p<0.05$) was also observed at racemes 2 and 6. Average pod set for the lower four racemes was 93% and for the upper five racemes it was 94% (Table 4.3.1).

The application of [9tP]BA increased the percentage pod set at racemes 1, 3, 4 ($p<0.01$) and 2, 6 ($p<0.05$), giving figures very similar to those measured in BA-treated plants. Thus, average of pods set on the basal four racemes was 94% and on the upper five racemes 91% (Table 4.3.1).

4.3.1.5 Toret

The pattern followed by control plants of Toret was similar to the pattern observed in all other varieties, with greater pod set at the upper five racemes than at the lower four racemes (Table 4.3.1). Average percentage pod set for the first four racemes was 56% and for the upper five 58%.

The application of BA increased ($p<0.001$) the percentage pod set at racemes 3, 4 and 5 (Table 4.3.1). Higher ($p<0.01$) percentage pod set than in the control plants was also recorded at racemes 1, 2, 6, 7 and 8. Thus the figures of average percentage pod set for the four lower and for the five upper racemes were 98% and 86% respectively.

The application of [9tP]BA caused more flowers to set a pod than in controls at all raceme positions (Table 4.3.1) resulting in average pod set for the four lower reproductive nodes of 93% and for the upper five nodes 90%.

4.3.2 The main effect of cytokinin on inter-raceme pod set

In general, the percentage pod set figures which were recorded on the mainstem of control plants were similar for the various racemes (Table 4.3.2). On average, 65% of the flowers formed on the lower four racemes set pods and 74% of those on the upper five racemes.

Table 4.3.2: *Overall effect of BA and [9tP]BA on inter-raceme percentage of pod set in faba bean plants.*

Treatment	Raceme position								
	1	2	3	4	5	6	7	8	9
	Percentage of pod set								
Control	65	63	65	66	71	76	78	74	70
BA	90***	94***	89***	93***	96***	95***	93***	88***	83***
[9tP]BA	93***	91***	89***	92***	96***	96***	88***	88***	87***

*** : different from the control at 0.001 probability level

External application of BA at stages 203(1) and 204(1) resulted in increases ($p<0.001$), at all raceme positions over control plants (Table 4.3.2): average

percentage pod set at the lower four racemes was 92% and at the upper five racemes 91%.

External application of [9tP]BA at the same growth stages resulted in percentage pod set figures that were very similar to those recorded for BA treated plants (Table 4.3.2). Percentage pod set was increased ($p<0,001$) at all reproductive nodes over control plants. Average pod set on the four lower racemes was 91% and on the upper five racemes 90%.

4.3.3 The main effect of variety on inter-raceme pod set

The broad bean variety TFW exhibited lower ($p<0.001$) levels of percentage pod set on the first four racemes than all other varieties examined (Table 4.3.3). On the upper five nodes the figures of percentage pod set in TFW were lower ($p<0.001$) than in the varieties Maris Bead, Troy and Cresta, but they were similar to those recorded at the same nodes of Toret plants (Table 4.3.3).

Table 4.3.3: *Overall varietal effect on inter-raceme percentage of pod set. Probability values of interaction between variety and cytokinin are also presented.*

Variety	Raceme position								
	1	2	3	4	5	6	7	8	9
	Percentage of pod set								
TFW	62^{a}	65^{a}	56^{a}	57^{a}	75^{a}	79^{a}	75^{a}	76^{a}	67^{a}
Maris Bead	88^{b}	84^{b}	89^{b}	93^{bc}	93^{bc}	93^{b}	91^{bc}	89^{b}	88^{b}
Troy	97^{c}	92^{b}	92^{b}	99^{c}	99^{c}	99^{c}	96^{c}	92^{b}	89^{b}
Cresta	89^{c}	87^{b}	87^{b}	88^{bc}	89^{bc}	90^{b}	88^{bc}	89^{b}	91^{b}
Toret	78^{b}	87^{b}	82^{b}	82^{b}	84^{ab}	86^{ab}	83^{ab}	73^{a}	64^{a}
LSD (42 d.f.)	$10.3^{(3)}$	$12.3^{(3)}$	$13.9^{(3)}$	$13.7^{(3)}$	$11.0^{(3)}$	$9.8^{(3)}$	$11.7^{(3)}$	$11.1^{(3)}$	$16.6^{(3)}$
Var. x Treat.	<0.001	0.001	0.076	0.059	<0.001	<0.001	0.026	0.038	0.004

a, b, c : each letter indicates a group of means which do not differ statistically
(3) : LSD calculated at 0.001 probability level

No differences in the levels of intra-raceme percentage pod set were observed between the two *minor* faba bean varieties at any raceme position, apart from the first formed where Troy set 11% more ($p<0.001$) pods than Maris Bead (Table 4.3.3).

Similar levels of percentage pod set were also recorded between the *equina* varieties, Cresta and Toret, at the raceme positions 2, 3, 4, 5, 6 and 7 (Table 4.3.3). However, at the positions 1, 8 and 9 Cresta set more ($p<0.001$) pods than Toret. Also, at the racemes 2, 3, 4, 5, 7, 8 and 9 the *equina* variety Cresta exhibited as high a pod set as the two *minor* varieties Maris Bead and Troy.

4.3.4 Interactive effect of variety and cytokinin treatment

The varieties TFW and Toret, in general, responded better than others to the external application of BA and [9tP]BA, showing in some racemes (e.g. 1, 5, and 6) strong ($p<0.001$) interaction between variety and cytokinin analogue (Table 4.3.3). Next to those two varieties, Troy also showed good response to cytokinin treatments at racemes 2 and 7.

4.4 Effect of cytokinin on overall percentage pod set

4.4.1 Effect of cytokinins on each variety

4.4.1.1 Three Fold White

Control plants set 19 pods on the mainstem while those treated with either BA or [9tP]BA set 15 pods more ($p<0.01$) than controls (Table 4.4.1). The number of flowers was not affected. Thus, the increase in the number of pods due to the application of cytokinins was 39% ($p<0.001$) of the overall pod set on the mainstem of treated plants.

4.4.1.2 Maris Bead

In control plants of Maris Bead, the number of flowers was 59 and it was reduced ($p<0.05$) to 56 by [9tP]BA application. The average number of pods set was increased from 48 in control plants to 56 ($p<0.01$) in BA treated plants and to 53 ($p<0.05$) in [9tP]BA treated plants (Table 4.4.1). The lower number of flowers and concomitant higher number of pods per mainstem caused an overall increase in average percentage pod set on the mainstem, from 80% in control plants to 95% in

plants treated by [9tP]BA (Table 4.4.1). BA application gave the same level of increase ($p<0.01$, Table 4.3).

Table 4.4.1: *Effect of BA and [9tP]BA on the formation of reproductive organs (flowers, setting pods, mature pods and harvestable pods) on the mainstem of each variety.*

Variety	Treatment	No. flowers	No. pods set	% pod set	No. mature pods	% mature pods	No. harvest. pods	% harvest. pods
Three Fold White	Control	47	19	40	8	17	7	16
	BA	44^{ns}	34**	79***	9^{ns}	21^{ns}	9^{ns}	21^{ns}
	[9tP]BA	43^{ns}	34**	79***	8^{ns}	18^{ns}	7^{ns}	17^{ns}
Maris Bead	Control	59	48	80	31	51	24	40
	BA	60	56**	95**	39^{ns}	65*	25^{ns}	43^{ns}
	[9tP]BA	56*	53*	95**	38^{ns}	68*	29^{ns}	52^{ns}
Troy	Control	45	40	89	31	70	25	57
	BA	43^{ns}	42^{ns}	98*	32^{ns}	75^{ns}	25^{ns}	58^{ns}
	[9tP]BA	45^{ns}	43^{ns}	96*	32^{ns}	72^{ns}	25^{ns}	57^{ns}
Cresta	Control	53	40	76	20	38	18	34
	BA	47^{ns}	44^{ns}	93**	18^{ns}	39^{ns}	16^{ns}	34^{ns}
	[9tP]BA	49^{ns}	45^{ns}	94**	20^{ns}	40^{ns}	16^{ns}	34^{ns}
Toret	Control	52	30	57	15	28	14	27
	BA	54^{ns}	49***	92***	25***	46***	16^{ns}	29^{ns}
	[9tP]BA	56**	51***	91***	19**	35***	13^{ns}	23^{ns}

ns : no statistically significant difference from the control
*, **, *** : different from the control at 0.05, 0.01, 0.001 probability level

4.4.1.3 Troy

In general, the external application of cytokinins did not affect the number of flowers and the number of pods formed on the mainstem compared to control plants (Table 4.4.1). The average percentage pod set was increased from 89% in control plants to 98 ($p<0.01$) and 96% ($p<0.01$) in BA and [9tP]BA treated plants respectively.

4.4.1.4 Cresta

The overall average percentage pod set in control plants was 76% and this increased to 93% ($p<0.01$) and to 94% ($p<0.01$) due to the application of BA and [9tP]BA respectively.

4.4.1.5 Toret

The application of [9tP]BA increased the number of flowers established on the mainstem of Toret plants compared to control pants (Table 4.4.1). Each mainstem formed an average of 52 flowers on control plants, 54 in BA-treated and 56 ($p<0.01$) flowers in [9tP]BA-treated plants. Both treatments caused more pods to set on each mainstem compared to control plants (Table 4.4.1). The application of BA increased ($p<0.001$) the number of setting pods from 30 on controls to 49, and the application of [9tP]BA increased ($p<0.001$) the average number of pods to 51. These increases observed in the number of pods represented increases in the percentage pod set, too. On control plants, 57% of the flowers formed on the mainstem set pods while on either BA or [9tP]BA sprayed plants set pods at 92% ($p>0.001$) and 91% ($p<0.001$) of the flowers, respectively (Table 4.4.1).

4.4.2 Main effect of cytokinins

In general, the external application of cytokinins to faba bean plants had no effect on the number of flowers. However, the average number of pods per mainstem was increased by all treatments compared to control plants (Table 4.4.2).

Table 4.4.2: *Overall effect of BA and [9tP]BA on the formation of reproductive organs (flowers, setting pods, mature pods and harvestable pods) on the mainstem of faba bean plants.*

Treatment	No. flowers	No. pods set	% pod set	No. mature pods	% mature pods	No. harvest. pods	% harvest. pods
Control	51	35	68	21	41	18	35
BA	49^{ns}	45***	91***	25***	49***	18^{ns}	37^{ns}
[9tP]BA	50^{ns}	45***	91***	23**	47***	18^{ns}	37^{ns}

ns : no statistically significant difference from the control
, * : different from the control at 0.01, 0.001 probability level

BA treatment increased the number of pods from 30 to 49 ($p<0.001$) and [9tP]BA treatment to 51 ($p<0.001$). The increased number of pods per mainstem caused an overall increase ($p<0.001$) in percentage pod set from 57% in control plants to 91% in those treated by BA or [9tP]BA over control plants (Table 4.4.2).

4.4.3 Main effect of variety

The plants of the *major* variety TFW and the *minor* variety Troy formed less ($p<0.001$) flowers per mainstem compared to plants of Maris Bead, Cresta and Toret (Table 4.4.3). Also, the plants of TFW exhibited lower ability to set pods ($p<0.001$) compared to all other varieties, which in turn resulted in the lowest ($p<0.001$) average percentage pod set recorded in this study (Table 4.4.3).

In contrast to TFW, most of the flowers which were established on the mainstem of Troy plants set pods. Thus, greater percentage pod set over all other varieties apart Maris Bead was achieved by Troy (Table 4.4.3).

Table 4.4.3: *Overall varietal effect on the formation of reproductive organs (flowers, setting pods, mature pods and harvestable pods) on the mainstem.*

Variety	No. flowers	No. pods set	% pod set	No. mature pods	% mature pods	No. harvest. pods	% harvest. pods
TFW	44^{a}	29^{a}	66^{a}	8^{a}	19^{a}	8^{a}	18^{a}
Maris Bead	58^{c}	52^{c}	90^{cd}	36^{d}	61^{c}	26^{c}	28^{b}
Troy	44^{a}	42^{b}	95^{d}	32^{c}	72^{d}	25^{c}	57^{c}
Cresta	49^{b}	43^{b}	88^{c}	19^{b}	39^{b}	17^{b}	23^{ab}
Toret	54^{c}	43^{b}	80^{b}	20^{b}	36^{b}	14^{b}	19^{ab}
LSD (42 d.f.)	$4.5^{(3)}$	$5.8^{(3)}$	$6.4^{(3)}$	$3.4^{(3)}$	$6.06^{(3)}$	$4.8^{(3)}$	$9.0^{(3)}$
Var. x Treat.	0.128	<0.001	<0.001	<0.001	<0.001	0.354	0.231

a, b, c, d : each letter indicates a group of means which do not differ statistically
(1), (2), (3) : LSD calculated at 0.05, 0.01, 0.001 probability level

The two *equina* varieties set equal number of pods on the mainstem (Table 4.4.3). However, because Toret formed more ($p<0.001$) flowers on the mainstem compared to Cresta, the overall percentage pod set on the mainstem of Toret plants was lower ($p<0.001$) than the percentage pod set measured in Cresta.

4.4.4 Interactive effect of variety and of cytokinin treatment

There was no interactive effect between variety and cytokinin treatment with respect to the number of flowers (Table 4.4.3). In contrast, there was a strong interaction ($p<0.001$) between variety and cytokinin treatments for the number of pods set and percentage pod set per plant (Table 4.4.3). The varieties TFW and Toret responded better to the application of either BA or [9tP]BA compared to the other three cultivars used in this study, when they were scored for number of pods or percentage pod set on the mainstem of each plant (Table 4.4.1).

4.5 Effect of cytokinin on intra-raceme percentage of mature pods

4.5.1 Effect of cytokinin on each variety

4.5.1.1 TFW

Control plants matured all their pods on the five proximal flower positions, with the greatest portion being formed at positions 1 and 2. The average proportion of flowers which developed a mature pod at positions 1 to 5 was 16% (Table 4.5.1).

The application of BA increased the percentage of mature pods at flower sites 2 ($p<0.01$) and 3 ($p<0.05$), compared to control plants, resulting in an average 21% at the five proximal positions (Table 4.5.1).

In contrast, the application of [9tP]BA resulted in less ($p<0.05$) mature pods at the positions 2 and 3, compared to control plants and those treated by BA. Thus, the average percentage of mature pods at the proximal five positions was 16% (Table 4.5.1).

4.5.1.2 Maris Bead

The majority of pods mature on the proximal 4 positions on the inflorescence. In control plants, the average percentage of flowers that produced mature pods at the proximal four positions was 75%, while the average at the distal five positions was 14% (Table 4.5.1).

The application of cytokinins increased the percentage of mature pods over control plants at the positions 5, 6 ($p<0.05$) and 7 ($p<0.01$). The average percentage of mature pods for the five upper flowers were 25% and 23% for BA and [9tP]BA, respectively (Table 4.5.1).

Table 4.5.1: *Effect of BA and [9tP]BA on intra-raceme percentage of mature pods in each variety.*

Variety	Treatment	Flower position								
		1	2	3	4	5	6	7	8	9
		Percentage of mature pods								
Three Fold White	Control	57	24	8	1	1	0	0	0	0
	BA	57^{ns}	29**	12*	3^{ns}	4^{ns}	0^{ns}	0^{ns}	0^{ns}	0^{ns}
	[9tP]BA	59^{ns}	18*	4*	1^{ns}	0^{ns}	0^{ns}	0^{ns}	0^{ns}	0^{ns}
Maris Bead	Control	93	84	72	50	29	13	6	4	17
	BA	95^{ns}	92^{ns}	81^{ns}	69^{ns}	49*	37*	27**	10^{ns}	0^{ns}
	[9tP]BA	97^{ns}	95^{ns}	87^{ns}	73^{ns}	54*	35*	26**	0^{ns}	0^{ns}
Troy	Control	99	91	84	64	35	11	0	n f	n f
	BA	98^{ns}	87^{ns}	77^{ns}	65^{ns}	54^{ns}	24^{ns}	50^{ns}	n f	n f
	[9tP]BA	97^{ns}	85^{ns}	80^{ns}	59^{ns}	46^{ns}	29^{ns}	13^{ns}	n f	n f
Cresta	Control	96	66	47	17	3	0	0	0	0
	BA	84^{ns}	65^{ns}	48^{ns}	26^{ns}	11^{ns}	9*	0^{ns}	0^{ns}	0^{ns}
	[9tP]BA	88^{ns}	67^{ns}	46^{ns}	26^{ns}	7^{ns}	5^{ns}	0^{ns}	0^{ns}	0^{ns}
Toret	Control	89	57	16	2	1	0	0	0	n f
	BA	78**	70^{ns}	63***	40***	29***	10**	0^{ns}	0^{ns}	n f
	[9tP]BA	74**	58^{ns}	45***	27***	15*	2^{ns}	0^{ns}	0^{ns}	n f

ns : no statistically significant difference from the control
*, **, *** : different from the control at 0.05, 0.01, 0.001 probability level
nf : flowers were not formed

4.5.1.3 Troy

The application of cytokinins had no effect on the proportion of flowers that developed into mature pods at all flower positions (Table 4.5.1).

4.5.1.4 Cresta

Only the application of BA caused more ($p<0.05$) flowers to develop a mature pod at position 6, compared to control plants. The percentage of mature pods at flower 6 from 0% in controls increased to 9% in BA-treated plants. None of the treatments caused any effect at any of all the other flower positions compared to controls (Table 4.5.1).

4.5.1.5 Toret

All plants of Toret did not produce mature pods above flower position 6. In control plants, almost all the mature pods were recorded on the proximal four flower positions, on average 41% of the flowers formed pods at these positions. On the upper five positions of the inflorescence it was only 0.2% (Table 4.5.1).

The application of BA decreased the percentage of mature pods at the position 1 ($p<0.01$), while the same treatment caused more flowers to mature pods at sites 3, 4, 5 ($p<0.01$) and 6 ($p<0.01$) resulting in an average of 63% for the four proximal positions and 8% for the distal five positions (Table 4.5.1).

Similarly, the application of [9tP]BA caused more pods to mature at flowers 1 ($p<0.01$), 3, 4 ($p<0.001$) and 5 ($p<0.05$) compared to control plants, resulting in an average of 51% for the proximal four flowers and 3% for the upper five flowers (Table 4.5.1).

4.5.2 The main effect of cytokinin

The majority of mature pods on control plants were situated on the four proximal flower positions. The average percentage of flowers that produced mature pods at the four proximal positions was 55%, while at the five distal positions was 6% (Table 4.5.2).

The applications of BA at early and mid-flowering increased the percentage of mature pods at flower positions 3, 4, 5, 6 ($p<0.001$), 7($p<0.01$), and decreased this parameter at position 1 ($p<0.05$) compared to control plants, resulting in an average of 62% mature pods on the four proximal flowers and 13% on the five distal flowers (Table 4.5.2).

Increased percentage of mature pods was also evident due to the application of [9tP]BA, at the positions 3 ($p<0.01$), 4, 5 and 6 ($p<0.001$). At flower site 1, 3% decrease ($p<0.05$) was observed compared to control plants. The average was 59% and 9% for the four proximal and for the five distal positions respectively (Table 4.5.2).

Table 4.5.2: *Overall effect of BA and [9tP]BA on intra-raceme percentage of mature pods.*

Treatment	Flower position								
	1	2	3	4	5	6	7	8	9
	Percentage of mature pods								
Control	86	64	45	26	14	5	2	1	6
BA	83*	68^{ns}	56***	40***	29***	16***	15*	3^{ns}	0^{ns}
[9tP]BA	83*	65^{ns}	52**	37***	24***	14***	8^{ns}	0^{ns}	0^{ns}

ns : no statistically significant difference from the control
*, **, *** : different from the control at 0.05, 0.01, 0.001 probability level

4.5.3 Main effect of variety

Plants of TFW, the only *major* variety used in this field study, formed all their mature pods on the five proximal flower positions. The figures of percentage mature pods at the first four flower positions of TFW plants were the lowest ($p<0.001$) recorded in this study (Table 4.5.3).

Between the two *minor* varieties, Maris Bead and Troy, no difference was observed with respect to the percentage of mature pods at all flower positions. Both varieties at the positions 2, 3, 4, 5 and 6 matured more ($p<0.001$) pods over all the other varieties (Table 4.5.3).

Levels of mature pods were similar between Cresta and Toret at all flower positions apart from position 1, where Cresta matured more ($p<0.001$) pods compared to Toret. Also, at the same position no difference was observed between Maris Bead and Cresta. In general, at the four distal positions of TFW, Cresta and Toret almost all the pods dropped before reaching maturity (Table 4.5.3).

Table 4.5.3: *Overall varietal effect on intra-raceme percentage of mature pods. Probability values of interaction between variety and cytokinin are also presented.*

Variety	Flower position								
	1	2	3	4	5	6	7	8	9
	Percentage of mature pods								
TFW	58^{a}	23^{a}	8^{a}	2^{a}	2^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Maris Bead	95^{cd}	90^{c}	80^{c}	64^{c}	44^{c}	28^{b}	20^{a}	5^{b}	6^{a}
Troy	98^{d}	88^{c}	80^{c}	62^{c}	45^{c}	21^{b}	21^{a}	nf	nf
Cresta	89^{c}	66^{b}	47^{b}	23^{b}	7^{ab}	5^{a}	0^{a}	0^{a}	0^{a}
Toret	80^{b}	61^{b}	41^{b}	23^{b}	15^{b}	4^{a}	0^{a}	0^{a}	nf
LSD (42 d.f.)	$7.3^{(3)}$	$11.1^{(3)}$	$10.5^{(3)}$	$10.3^{(3)}$	$10.7^{(3)}$	$9.9^{(3)}$	$22.0^{(3)}$	$3.8^{(3)}$	$6.4^{(1)}$
Var. x Treat.	0.015	0.128	<0.001	<0.001	0.010	0.014	0.028	0.051	0.059

nf : flowers were not formed
a, b, c, d : each letter indicates a group of means which do not differ statistically
(1), (3) : LSD calculated at 0.05, 0.001 probability level

4.5.4 Interactive effect of variety and of cytokinin treatment

Differences between the varieties in their responsiveness to cytokinin treatments were observed at the positions 1, 3, 4, 5 and 6 (Table 4.5.3). Toret plants responded better to the externally applied cytokinins as regarded the percentage of mature pods at the positions 1, 3, 4, 5 and 6 (see Section 4.5.1.5), and Maris Bead plants at the flower positions 5, 6 and 7 (see Section 4.5.1.2).

4.6 Effect of cytokinin on inter-raceme percentage mature pods

4.6.1 Effect of cytokinins on each variety

4.6.1.1 Three Fold White

Control plants of TFW matured the majority of their pods on the lower four racemes, with the average percentage of mature pods being 21%. On the upper five racemes the average was 12% at each position (Table 4.6.1).

The application of BA resulted in a higher ($p<0.05$) proportion of flowers producing mature pods compared to control plants, at the racemes 2 and 8. The average for the lower four racemes was 31% and for the upper five it was 11% (Table 4.6.1).

The application of [9tP]BA did not affect the percentage of mature pods at all racemes apart from raceme 8 where more pods matured compared to controls. The average percentage of mature pods was 21% for the lower four and 15% for the upper five racemes, respectively (Table 4.6.1).

Table 4.6.1: *Effect of BA and [9tP]BA on inter-raceme percentage of mature pods of each variety.*

Variety	Treatment	Raceme position								
		1	2	3	4	5	6	7	8	9
		Percentage of mature pods								
Three Fold White	Control	22	20	16	26	20	21	13	2	4
	BA	38^{ns}	37*	30^{ns}	20^{ns}	15^{ns}	17^{ns}	8^{ns}	7*	6^{ns}
	[9tP]BA	27^{ns}	20^{ns}	17^{ns}	19^{ns}	14^{ns}	13^{ns}	22^{ns}	13*	14^{ns}
Maris Bead	Control	53	50	49	60	56	50	48	50	40
	BA	71^{ns}	75*	72**	75*	69^{ns}	61^{ns}	62*	50^{ns}	42^{ns}
	[9tP]BA	77^{ns}	74*	79**	87*	71^{ns}	71^{ns}	55^{ns}	50^{ns}	52^{ns}
Troy	Control	76	62	68	80	84	75	72	61	64
	BA	84^{ns}	80**	78^{ns}	86^{ns}	86^{ns}	82^{ns}	65^{ns}	53^{ns}	58^{ns}
	[9tP]BA	83^{ns}	88**	85^{ns}	89^{ns}	82^{ns}	74^{ns}	58^{ns}	50^{ns}	44^{ns}
Cresta	Control	45	43	41	38	39	36	34	39	47
	BA	57*	60***	32^{ns}	38^{ns}	38^{ns}	40^{ns}	33^{ns}	13*	20*
	[9tP]BA	64*	49**	44^{ns}	50*	35^{ns}	42^{ns}	27^{ns}	24^{ns}	24*
Toret	Control	40	40	33	35	31	27	23	23	10
	BA	87***	87**	79**	64**	44^{ns}	33^{ns}	23^{ns}	9**	4*
	[9tP]BA	74***	67**	63**	45*	43^{ns}	25^{ns}	12^{ns}	2**	1*

ns : no statistically significant difference from the control
*, **, *** : different from the control at 0.05, 0.01, 0.001 probability level
nf : flowers were not formed

4.6.1.2 Maris Bead

In general, all racemes of control plants produced similar levels of mature pods. The average for the lower four racemes was 53% and for the upper five it was 43% (Table 4.6.1).

The application of BA resulted in a higher percentage of mature pods at the racemes 2 ($p<0.05$), 3 ($p<0.01$), 4 ($p<0.05$) and 7 ($p<0.05$). On average, 73% of the flowers formed on the lower four racemes produced mature pods and 71% of the flowers formed on the upper five racemes (Table 4.6.1).

The application of [9tP]BA resulted in more mature pods on reproductive nodes 2 ($p<0.05$), 3 ($p<0.01$) and 4 ($p<0.05$). The average for the lower four racemes was 76% and for the upper five racemes it was 60% (Table 4.6.1).

4.6.1.3 Troy

In control plants, the average percentage of mature pods for the lower four racemes of control plants was 72% and 71% was for the upper five racemes (Table 4.6.1).

The application of BA resulted in more ($p<0.01$) pods, compared to controls, but only on the second reproductive node, that resulted in the average percentage mature pods on the lower four racemes being 82% and on the upper five 69% respectively (Table 4.6.1).

An increase ($p<0.01$) in the number of mature pods was also observed on raceme 2 due to application of [9tP]BA. The average for the lower four racemes was 86% and for the upper five it was 62% (Table 4.6.1).

4.6.1.4 Cresta

For control plants of Cresta 42% of the flowers resulted in mature pods on the lower four racemes and 39% of flowers formed mature pods on the upper five racemes (Table 4.6.1).

The application of BA increased the percentage of mature pods at racemes 1 ($p<0.05$) and 2 ($p<0.001$) at the expense of racemes 8 and 9 where a decrease ($p<0.05$) was recorded. The average percentage mature pods for the lower four racemes was 47% while for the upper five racemes it was 29% (Table 4.6.1).

The application of [9tP]BA caused more pods to reach maturity, compared to control plants, at the racemes 1 ($p<0.05$), 2 ($p<0.01$) and 4 ($p<0.05$), while less ($p<0.05$) pods matured on raceme 9. On average, 52% of possible mature pods was recorded on the lower four racemes and for the upper five this figure was 30% (Table 4.6.1).

4.6.1.5 Toret

Control plants of Toret had more mature pods on the lower four reproductive nodes compared to the upper five reproductive nodes. The figures were 37% and 23% respectively (Table 4.6.1).

The application of BA resulted in more mature pods at the racemes 1 ($p<0.001$), 2 ($p<0.01$), 3 ($p<0.01$) and 4 ($p<0.01$) but fewer at racemes 8 ($p<0.01$) and 9 ($p<0.05$). The average possible mature pods for the lower four racemes was 79% while for the upper five it was 23% (Table 4.6.1).

The application of [9tP]BA increased the percentage of mature pods at the racemes 1 ($p<0.001$), 2 ($p<0.01$), 3 ($p<0.01$) and 4 ($p<0.05$) but fewer pods formed on racemes 8 ($p<0.01$) and 9 ($p<0.05$). The average percentage of mature pods for the lower four racemes was 62% but for the upper five racemes it was only 17% (Table 4.6.1).

4.6.2 Main effect of cytokinins

On control plants, the average percentage of mature pods on the lower four racemes was 45% while on the upper five racemes this figure was 39% (Table 4.6.2).

Table 4.6.2: *Overall effect of BA and [9tP]BA on inter-raceme percentage of mature pods.*

Treatment	Raceme position								
	1	2	3	4	5	6	7	8	9
	Percentage of mature pods								
Control	47	43	42	48	46	42	38	35	33
BA	67***	68***	58***	57*	51^{ns}	47^{ns}	38^{ns}	26*	26*
[9tP]BA	65***	59***	58***	58**	49^{ns}	45^{ns}	35^{ns}	28*	27^{ns}

ns : no statistically significant difference from the control
*, **, *** : different from the control at 0.05, 0.01, 0.001 probability level

The application of BA increased the percentage of mature pods at racemes 1, 2, 3, ($p<0.001$) and 4 ($p<0.05$) resulting in the average for the lower four racemes being 63%. On the upper five racemes, no effect of cytokinins was observed on racemes 5, 6 and 7, although a decrease ($p<0.05$) in mature pods was evident at racemes 8 and 9 (Table 4.6.2).

The application of [9tP]BA increased the percentage of mature pods, compared to control plants, at the racemes 1, 2, 3 ($p<0.001$) and 4 ($p<0.01$) but decreased ($p<0.05$) the number of pods at raceme 8. The average for the lower four racemes was 60% and for the upper five racemes it was 37% (Table 4.6.2).

4.6.3 The main effect of variety

The plants of the *major* variety TFW exhibited the lowest percentage of mature pods on the lower five reproductive nodes, compared to all other varieties used in this study. On the upper four racemes, similar levels of percentage mature pods were recorded between the varieties TFW and Toret (Table 4.6.3).

Table 4.6.3: *Overall varietal effect on intra-raceme percentage of mature pods. Probability values of variety x cytokinin interaction are also presented.*

Variety	Raceme position								
	1	2	3	4	5	6	7	8	9
	Percentage of mature pods								
TFW	29^{a}	26^{a}	21^{a}	22^{a}	17^{a}	17^{a}	14^{a}	7^{a}	8^{a}
Maris Bead	67^{bc}	66^{bc}	67^{cd}	74^{c}	65^{c}	61^{c}	55^{c}	50^{c}	45^{c}
Troy	81^{c}	77^{c}	77^{d}	85^{c}	84^{d}	77^{d}	65^{c}	55^{c}	55^{c}
Cresta	55^{b}	51^{b}	39^{b}	42^{b}	37^{b}	39^{b}	31^{b}	25^{b}	30^{b}
Toret	67^{bc}	65^{bc}	58^{c}	48^{b}	39^{b}	28^{ab}	19^{ab}	11^{ab}	5^{a}
LSD (42 d.f.)	$21.1^{(3)}$	$18.3^{(3)}$	$17.4^{(3)}$	$15.9^{(3)}$	$11.7^{(3)}$	$15.9^{(3)}$	$13.1^{(3)}$	$14.7^{(3)}$	$14.3^{(3)}$
Var. x Treat.	0.187	0.079	0.003	0.036	0.132	0.274	0.042	0.042	0.004

a, b, c, d : each letter indicates a group of means which do not differ statistically
(1), (2), (3) : LSD calculated at 0.05, 0.01, 0.001 probability level

Between the two *minor* varieties, similar levels of percentage mature pods were measured at all raceme positions apart from racemes 5 and 6 where Troy matured more ($p<0.05$) pods compared to Maris Bead (Table 4.6.3).

Between *equina* varieties differences as regards the percentage of mature pods were observed only at racemes 3 and 9. At raceme 3, Toret plants matured more ($p<0.001$) pods compared to Cresta plants, while at raceme 9 they matured less ($p<0.001$) compared to Cresta (Table 4.6.3). In addition, the figures of the percentage of mature pods measured on the two lower raceme positions of *equina* plants were as high as those figures observed on the same racemes of Maris Bead plants (Table 4.6.3).

4.6.4 Interactions of variety and cytokinin treatment

Strong interactions ($p<0.003$) between variety and cytokinin application were present at the raceme positions 3 and 9. At the raceme position 3, plants of Maris Bead and Toret responded better to the application of BA and [9tP]BA compared to the other three faba bean varieties, and at the position 9 a better response was shown only by Toret (see Section 4.6.1). Interaction was also evident at the positions 4, 7 and 8 (Table 4.6.3).

4.7 Effect of cytokinin on overall percentage of mature pods

4.7.1 Effect of cytokinins on each variety

4.7.1.1 Three Fold White

Control plants produced on average 8 pods on the first 9 racemes of their mainstem, or a proportion of 17% of the flowers formed on each mainstem. External application of cytokinins did not affect the number and the percentage of mature pods (Table 4.4.1).

4.7.1.2 Maris Bead

Control plants possessed an average of 31 pods on the mainstem or as proportion of flowers, 51% produced mature pods. Application of BA and [9tP]BA caused more ($p<0.05$) mature pods to be produced at 65% and 68% of the flowers, respectively (Table 4.4.1).

4.7.1.3 Troy

On the mainstem of control plants 31 pods were recorded at maturity. This number represented 70% of the flowers originally measured on the plant. No treatment improved this figure (Table 4.4.1).

4.7.1.4 Cresta

The number of mature pods on control plants was 20; this represented 38% of the original flowers. No effect was observed due to the application of BA-type cytokinins (Table 4.4.1).

4.7.1.5 Toret

Control plants retained 15 pods on the mainstem. The application of BA resulted in 10 pods more ($p<0.001$) to mature over control plants, which in turn meant an increase in percentage of mature pods from 28% to 46%. The application of [9tP]BA, also, increased the number of mature pods to 19 and the percentage of mature pods to 35% (Table 4.4.1).

4.7.2 Main effect of cytokinins

The number of mature pods retained on control plants was 21. The application of BA increased ($p<0.001$) this number to 25, or an increase of possible mature pod production from 41% to 49%. The application of [9tP]BA increased ($p<0.01$) the number of mature pods on the mainstem to 23. The percentage of mature pods was therefore increased ($p<0.001$) by 6%, over control plants (Table 4.4.2).

4.7.3 Main effect of variety

The fewest ($p<0.001$) mature pods on the mainstem were observed on the broad bean plants. The number of mature pods on TFW plants was 8 and the percentage of mature pods was 19% of original flowers (Table 4.4.3).

Field bean varieties retained the greatest number of mature pods on their mainstem over *major* and *equina* varieties. Maris Bead plants matured 4 pods more ($p<0.001$) compared to Troy, but the percentage of mature pods was 9% less ($p<0.001$) than the percentage measured in Troy plants because Maris Bead formed more flowers on the mainstem than Troy (Table 4.4.3).

Similar numbers and percentages of mature pods were observed between the two equina varieties employed in this study. Both varieties, Cresta and Toret, retained more ($p<0.001$) mature pods compared to TFW, but less ($p<0.001$) mature pods compared to either Maris Bead or Troy (Table 4.4.3).

4.7.4 Interactive effect of variety and of cytokinin treatment

A strong interaction ($p<0.001$) between variety and cytokinin application was observed in both the number of pods and percentage of mature pods (Table 4.4.3). Toret plants matured more pods (Sec. 4.7.1.5) when they were sprayed either with BA or [9tP]BA, compared to controls, while none of the other four varieties responded to the external application of cytokinins concerning the same characteristic.

4.8 Effect of cytokinin on intra-raceme percentage of harvestable pods

4.8.1 Effect of cytokinin on each variety

4.8.1.1 Three Fold White

No flower formed a harvestable pod above flower position 5 in both control and cytokinin treated plants. Control plants retained the majority of harvestable pods at the two proximal flower positions. The average percentage of harvestable pods at the lower four flower positions was 21% while at the upper 5 positions it was 0.2% (Table 4.8.1).

The application of BA did not affect the percentage of harvestable pods at any flower position on the raceme (Table 4.8.1).

The application of [9tP]BA resulted in a lower ($p<0.05$) percentage of harvestable pods at flower position 3, while no effect was observed at any other flower site as compared with control plants. The average number of harvestable pods was 20% for the four proximal flowers and 0% for the upper five flowers (Table 4.8.1).

4.8.1.2 Maris Bead

Control plants followed the usual pattern of intra-raceme percentage of harvestable pods where more pods reach a harvestable size at the lower flower positions on the raceme. The figures were 60% for the four proximal positions and 5% for the five upper positions (Table 4.8.1). External application of cytokinin did not alter these figures (Table 4.8.1).

4.8.1.3 Troy

A similar pattern to that in Maris Bead, was also observed in control plants of Troy. The proportion of flowers which developed to harvestable pods on the four lower racemes was on average 69%; for the next three racemes - because flowers were not formed at positions 8 and 9 - it was 9% (Table 4.8.1).

Table 4.8.1: *Effect of BA and [9tP]BA on intra-raceme percentage of harvestable pods for each variety.*

Variety	Treatment	Flower position								
		1	2	3	4	5	6	7	8	9
		Percentage of harvestable pods								
Three Fold White	Control	53	23	8	1	1	0	0	0	0
	BA	55^{ns}	26^{ns}	12^{ns}	3^{ns}	3^{ns}	0^{ns}	0^{ns}	0^{ns}	0^{ns}
	[9tP]BA	61^{ns}	16^{ns}	3*	1^{ns}	0^{ns}	0^{ns}	0^{ns}	0^{ns}	0^{ns}
Maris Bead	Control	79	72	54	33	16	6	2	0	0
	BA	86^{ns}	70^{ns}	53^{ns}	30^{ns}	22^{ns}	20^{ns}	8^{ns}	4^{ns}	0^{ns}
	[9tP]BA	89^{ns}	75^{ns}	63^{ns}	50^{ns}	24^{ns}	13^{ns}	6^{ns}	0^{ns}	0^{ns}
Troy	Control	95	65	71	43	19	7	0	n f	n f
	BA	94^{ns}	79^{ns}	60^{ns}	39^{ns}	26^{ns}	11^{ns}	13^{ns}	n f	n f
	[9tP]BA	91^{ns}	69^{ns}	65^{ns}	42^{ns}	26^{ns}	13^{ns}	0^{ns}	n f	n f
Cresta	Control	92	48	38	11	3	0	0	0	0
	BA	75^{ns}	51^{ns}	32^{ns}	20^{ns}	5^{ns}	2^{ns}	0^{ns}	0^{ns}	0^{ns}
	[9tP]BA	80^{ns}	54^{ns}	35^{ns}	20^{ns}	4^{ns}	4^{ns}	0^{ns}	0^{ns}	0^{ns}
Toret	Control	79	42	11	0	0	0	0	0	n f
	BA	62*	43^{ns}	41**	15*	10**	3^{ns}	0^{ns}	0^{ns}	n f
	[9tP]BA	61*	40^{ns}	28**	13*	6*	0^{ns}	0^{ns}	0^{ns}	n f

ns : no statistically significant difference from the control
*, **, *** : different from the control at 0.05, 0.01, 0.001 probability level
nf : flowers were not formed

The applications of either BA or [9tP]BA at early and mid flowering did not cause any changes in the level of percentage of harvestable pods at any flower position compared to untreated plants (Table 4.8.1).

4.8.1.4 Cresta

The average percentage of harvestable pods at the four proximal flowers was 47% while for the upper five it was 0.6%. External application of cytokinins did not affect this characteristic (Table 4.8.1).

4.8.1.5 Toret

All the harvestable pods in control plants were retained on the three lower flower positions, giving an average of 33% at the four proximal flower sites (Table 4.8.1).

The application of BA increased the percentage of harvestable pods at the positions 3 ($p<0.01$), 4 ($p<0.05$) and 5 ($p<0.05$) while a decrease ($p<0.05$) was recorded at position 1; the average for the four proximal positions was 40% and for the four upper positions 3% (Table 4.8.1).

The application of [9tP]BA resulted in a higher percentage of harvestable pods at flower sites 3 ($p<0.01$), 4 ($p<0.05$) and 5 ($p<0.05$), but lower at position 1 ($p<0.05$). At the four proximal positions, 36% of the flowers developed harvestable pods, and 2% at the four upper positions (Table 4.8.1).

4.8.2 The main effect of cytokinin

In control plants, the proximal flowers formed the majority of harvestable pods. The average percentage of harvestable pods at the four lower flower positions was 46% while at the five upper it was 2% (Table 4.8.2).

Table 4.8.2: *Overall effect of BA and [9tP]BA on intra-raceme percentage of harvestable pods.*

Treatment	Flower position								
	1	2	3	4	5	6	7	8	9
	Percentage of harvestable pods								
Control	80	50	37	17	8	3	0	0	0
BA	74^{ns}	54^{ns}	40^{ns}	21^{ns}	13^{ns}	7*	4*	1^{ns}	0^{ns}
[9tP]BA	76^{ns}	51^{ns}	39^{ns}	25*	12^{ns}	6*	1^{ns}	0^{ns}	0^{ns}

ns : no statistically significant difference from the control
*: different from the control at 0.05 probability level

The application of BA increased ($p<0.05$) the percentage of harvestable pods at the flowers 6 and 7 resulting in an average of 47% at the four proximal flower sites and of 5% at the five distal flower sites (Table 4.8.2).

In [9tP]BA-treated plants, a higher percentage of harvestable pods was recorded than in control plants, at the flower sites 4 and 6; the average at the four proximal flowers was 48% while at the five distal positions it was 4% (Table 4.8.2).

4.8.3 Main effect of variety

In general, the lowest percentage of harvestable pods was recorded on the plants of *major* and *equina* varieties. The large-seeded variety TFW at the positions 1, 5, 6, 7, 8 and 9 gave similar levels of harvestable pods to those levels measured in the *equina* varieties Cresta and Toret. In addition, both Toret and TFW plants retained less ($p<0.05$) harvestable pods than Maris Bead, Troy and Cresta plants at flower position 1 (Table 4.8.3).

Table 4.8.3: *Overall varietal effect on intra-raceme percentage of harvestable pods. Probability values of variety x cytokinin interaction are also presented.*

Variety	Flower position								
	1	2	3	4	5	6	7	8	9
	Percentage of harvestable pods								
TFW	56^{a}	22^{a}	8^{a}	2^{a}	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Maris Bead	85^{b}	73^{c}	57^{c}	38^{bc}	21^{b}	13^{b}	5^{b}	1^{a}	0^{a}
Troy	93^{b}	71^{c}	65^{c}	41^{c}	24^{b}	10^{b}	4^{ab}	nf	nf
Cresta	82^{b}	51^{b}	35^{b}	17^{b}	4^{a}	2^{a}	0^{a}	0^{a}	0^{a}
Toret	67^{a}	42^{b}	27^{b}	9^{ab}	5^{a}	1^{a}	0^{a}	0^{a}	nf
LSD (42 d.f.)	*11.0*(3)	*14.0*(3)	*14.6*(3)	*12.2*(3)	*10.0*(3)	*7.0*(3)	*4.6*(2)	*1.1*(1)	-
Var. x Treat.	0.006	0.617	0.010	0.070	0.058	0.203	0.059	0.050	-

nf : flowers were not formed
- : denominator of F-test is zero
a, b, c, d : each letter indicates a group of means which do not differ statistically
(1), (2), (3) : LSD calculated at 0.05, 0.01, 0.001 probability level

Between the two *minor* varieties similar figures of intra-raceme percentage of harvestable pods were observed at the lower seven flower positions. No differences

were also evident between the two *equina* varieties at any flower position apart from position 1 where Toret retained less ($p<0.001$) harvestable pods than Cresta (Table 4.8.3).

4.8.4 Interactions of variety and cytokinin treatment

Interactions between variety and cytokinin treatment were found at the flower positions 1 and 3 at the inflorescence, with regard to the percentage of harvestable pods (Table 4.8.3). At flower 1, Toret showed a negative response to the external application of cytokinins, while at position 3 the same variety responded positively to treatments (see Section 4.8.1.5).

4.9 Effects on inter-raceme percentage of harvestable pods

4.9.1 Effect of cytokinins on each variety

4.9.1.1 Three Fold White

On control plants the majority of harvestable pods was retained on the lower racemes. The average percentage of harvestable pods for the lower four racemes was 21% and for the upper five racemes it was 12% (Table 4.9.1).

The application of BA resulted in higher ($p<0.05$) percentage of harvestable pods, compared with control plants, only on raceme position 2. On average, for the lower four racemes the percentage of harvestable pods was 31% and for the upper five racemes it was 10% (Table 4.9.1).

The application of [9tP]BA did not affect the percentage of harvestable pods at any raceme position, resulting in an average of 20% for the lower four racemes and 13% for the upper five raceme positions (Table 4.9.1).

4.9.1.2 Maris Bead

On control plants the average percentage of harvestable pods on the lower four racemes was 44% and on the upper five racemes it was 36%. BA treatment did not alter these figures at any reproductive node. The application of [9tP]BA increased ($p<0.05$) the percentage of harvestable pods at racemes 3 and 4, resulting in an average of 70% for the lower four racemes (Table 4.9.1).

Table 4.9.1: *Effect of BA and [9tP]BA on inter-raceme percentage of harvestable pods of each variety.*

Variety	Treatment	Raceme position								
		1	2	3	4	5	6	7	8	9
		Percentage of harvestable pods								
Three Fold White	Control	22	20	15	26	18	20	9	0	4
	BA	42^{ns}	37*	28^{ns}	18^{ns}	14^{ns}	16^{ns}	8^{ns}	7^{ns}	6^{ns}
	[9tP]BA	25^{ns}	20^{ns}	17^{ns}	18^{ns}	14^{ns}	12^{ns}	17^{ns}	10^{ns}	14^{ns}
Maris Bead	Control	47	43	40	46	46	40	39	31	25
	BA	61^{ns}	55^{ns}	45^{ns}	56^{ns}	45^{ns}	38^{ns}	35^{ns}	26^{ns}	33^{ns}
	[9tP]BA	74^{ns}	64^{ns}	66*	74*	55^{ns}	49^{ns}	38^{ns}	32^{ns}	28^{ns}
Troy	Control	68	58	58	72	71	53	51	48	43
	BA	73**	66^{ns}	65^{ns}	72^{ns}	66^{ns}	70*	48^{ns}	28^{ns}	43^{ns}
	[9tP]BA	77**	74*	67^{ns}	81^{ns}	72^{ns}	47^{ns}	43^{ns}	35^{ns}	27^{ns}
Cresta	Control	43	39	36	32	34	30	30	33	39
	BA	55^{ns}	54**	30^{ns}	29^{ns}	31^{ns}	36^{ns}	31^{ns}	11*	17*
	[9tP]BA	59^{ns}	48*	38^{ns}	41*	25^{ns}	37^{ns}	23^{ns}	21^{ns}	17*
Toret	Control	40	40	33	70	19	18	17	10	5
	BA	71**	72***	55*	38^{ns}	21^{ns}	14*	7*	2*	1*
	[9tP]BA	68**	55***	46*	28^{ns}	20^{ns}	6*	4*	0*	0*

ns : no statistically significant difference from the control
*, **, *** : different from the control at 0.05, 0.01, 0.001 probability level

4.9.1.3 Troy

On the mainstem of control plants, an average of 64% of the flowers formed on the lower four racemes developed to pods of a harvestable size. The average for the upper five racemes was 53% (Table 4.9.1).

The application of BA produced more harvestable pods at racemes 1 ($p<0.01$) and 6 ($p<0.05$); for the lower four racemes 69% and for the upper five racemes 51% (Table 4.9.1) of pods were harvested.

The application of [9tP]BA caused more harvestable pods at racemes 1 ($p<0.01$) and 2 ($p<0.05$) resulting in an average for the lower four racemes of 75%. The average number of harvestable pods for the upper five racemes was 45% (Table 4.9.1).

4.9.1.4 Cresta

On control plants, the average percentage of harvestable pods was 38% and 33% for the lower four and for the upper five racemes, respectively (Table 4.9.1).

The application of BA caused more ($p<0.01$) flowers to develop into harvestable pods at raceme position 2 , at the expense of racemes 8 and 9 where the percentage of harvestable pods was lower ($p<0.05$), compared to control plants. On average, 42% of the flower positions on the lower four racemes formed a harvestable pod. The average for the upper five racemes was 25% (Table 4.9.1).

The application of [9tP]BA resulted in more ($p<0.05$) harvestable pods at racemes 2 and 4, and less at raceme 9, compared to control plants. The average for the lower four racemes was 47% and for the upper five racemes it was 25% (Table 4.9.1).

4.9.1.5 Toret

Control plants produced more harvestable pods at lower racemes, with an average of 46%. The average percentage of harvestable pods on the upper five racemes was 14% (Table 4.9.1).

BA increased the percentage of harvestable pods at racemes 1 ($p<0.01$), 2 ($p<0.01$) and 3 ($p<0.05$), this increased the average on the lower four racemes to 59%. However, fewer (9%; $p<0.05$) harvestable pods were observed on the upper five racemes, compared to control plants (Table 4.9.1).

Application of [9tP]BA increased harvestable pods at racemes 1 ($p<0.01$), 2 ($p<0.001$) and 3 ($p<0.05$), but decreased ($p<0.05$) the pods at racemes 6, 7, 8 and 9. The average percentage of harvestable pods on the lower four racemes was 49% and 6% on the upper five racemes (Table 4.9.1).

4.9.2 Main effect of cytokinins

In general, the lower racemes on the mainstem retained the majority of harvestable pods. For control plants 42% of possible harvestable pods were located on the lower four racemes but 29% were located on the upper five racemes (Table 4.9.2).

Table 4.9.2: *Overall effect of BA and [9tP]BA on inter-raceme percentage of harvestable pods.*

Treatment	Raceme position								
	1	2	3	4	5	6	7	8	9
	Percentage of harvestable pods								
Control	44	40	36	49	38	32	29	24	23
BA	60**	57**	45*	43^{ns}	35^{ns}	35^{ns}	26^{ns}	15**	20^{ns}
[9tP]BA	61**	52**	47*	48^{ns}	37^{ns}	30^{ns}	25^{ns}	20^{ns}	17^{ns}

ns : no statistically significant difference from the control
*, ** : different from the control at 0.05, 0.01 probability level

BA applied at growth stages 203(1) and 204(1) caused more harvestable pods at racemes 1 ($p<0.01$), 2 ($p<0.01$), 3 ($p<0.05$) but less at raceme 8 ($p<0.05$), compared to control plants. Thus, the average percentage of harvestable pods for the lower four racemes increased to 51% while on the upper five racemes the number of pods decreased to 26% (Table 4.9.2).

The application of [9tP]BA resulted in more harvestable pods at positions 1 ($p<0.01$), 2 ($p<0.01$) and 3 ($p<0.05$), but had no effect on the upper five raceme positions. Average figures were 52% for the first four reproductive nodes and 26% for the five apical racemes (Table 4.9.2).

4.9.3 Main effect of variety

On the lower five raceme positions of the plants of the broad bean variety TFW, the lowest numbers of harvestable pods were recorded of any of the varieties tested. For racemes 1, 3 and 4, plants of cv. Cresta, showed similar figures for harvestable pods to those observed in TFW, while for racemes 5, 6, 7, 8 and 9 the number of pods produced by Toret were similar to that recorded in TFW (Table 4.9.3).

Similar proportions of harvestable pods were produced by the two *minor* varieties at all racemes apart from racemes 4 and 5 where Troy plants retained more harvestable pods compared to Maris Bead. No differences in the percentage of harvestable pods were observed between equina varieties at the lower five racemes, while on the upper four racemes Cresta produced more ($p<0.001$) harvestable pods compared to Toret (Table 4.9.3).

Table 4.9.3: *Overall varietal effect on inter-raceme percentage of harvestable pods. Probability values of variety x cytokinin interaction are also presented.*

Variety	Raceme position								
	1	2	3	4	5	6	7	8	9
	Percentage of harvestable pods								
TFW	30^{a}	26^{a}	20^{a}	21^{a}	15^{a}	16^{ab}	12^{a}	6^{a}	8^{a}
Maris Bead	61^{b}	54^{b}	50^{bc}	59^{b}	49^{c}	42^{cd}	37^{bc}	30^{bc}	29^{b}
Troy	73^{b}	66^{b}	63^{c}	75^{c}	70^{d}	57^{d}	47^{c}	37^{c}	38^{b}
Cresta	52^{ab}	47^{b}	35^{ab}	34^{ab}	30^{b}	34^{bc}	28^{b}	22^{b}	25^{b}
Toret	60^{b}	55^{b}	44^{b}	46^{b}	20^{ab}	13^{a}	9^{a}	4^{a}	2^{a}
LSD (42 d.f.)	*26.0*(3)	*19.6*(3)	*18.1*(3)	*15.9*(3)	*13.2*(3)	*19.1*(3)	*15.1*(3)	*11.6*(3)	*14.2*(3)
Var. x Treat.	0.713	0.333	0.144	0.098	0.711	0.312	0.615	0.018	0.021

a, b, c, d : each letter indicates a group of means which do not differ statistically
(3) : LSD calculated at 0.001 probability level

4.9.4 Effect of variety and cytokinin treatment

No interactive effect due to variety and cytokinin treatment was present at the lower seven flower positions for the percentage of harvestable pods. However, slight interaction ($p<0.05$) was evident at the upper two raceme positions (Table 4.9.3). At those positions the application of either BA or [9tP]BA to *equina* varieties (see Section 4.9.1) resulted in fewer harvestable pods, compared to control plants.

4.10 Effect of cytokinin on overall percentage of harvestable pods

Synthetic BA-type cytokinin applications at stages 203(1) and 204(1) had no effect on both the overall number and the overall percentage of harvestable pods of faba bean plants (Table 4.4.1 and 4.4.2).

Differences ($p<0.001$) were observed between different varieties. The number of harvestable pods retained on the mainstem of *major* plants was lower ($p<0.001$) compared to *minor* and to *equina* plants. The percentage of harvestable pods of *major* plants was lower ($p<0.001$) only compared to *minor* and not to *equina* varieties because the plants of the latter formed more flowers on the mainstem. In addition, the plants of *minor* varieties retained more pods until the harvest, compared

to *equina* varieties. However, only plants of Troy exhibited higher ($p<0.001$) percentage of harvestable pods compared to Cresta and Toret (Table 4.4.3).

4.11 Effect of cytokinin on number of seeds

4.11.1 Effect of cytokinin on each variety

The application of cytokinins to the varieties TFW, Maris Bead, Troy and Cresta had no effect on the number of seeds contained on the mainstem of each plant.

In cv. Toret, control plants produced 43 seeds per mainstem; it increased ($p<0.05$) to 58 seeds for those treated with BA. Treatment with [9tP]BA did not alter the number of seeds (Table 4.11.1).

Table 4.11.1: *Effect of BA and [9tP]BA on number of seeds and dry weight of harvestable reproductive organs on the mainstem.*

Variety	Treatment	No. seeds per plant	No. seeds per harvest. pod	Dry weight hulls (g)	Dry weight seeds (g)	Dry weight pods (g)
Three Fold White	Control	30	4.0	14.2	17.8	32.0
	BA	34^{ns}	3.7^{ns}	17.6^{ns}	20.9^{ns}	38.5*
	[9tP]BA	29^{ns}	4.0^{ns}	14.8^{ns}	17.8^{ns}	32.6^{ns}
Maris Bead	Control	83	3.6	13.7	22.0	35.7
	BA	91^{ns}	3.5^{ns}	14.5^{ns}	25.7^{ns}	40.2^{ns}
	[9tP]BA	109^{ns}	3.7^{ns}	17.3^{ns}	26.8^{ns}	44.1^{ns}
Troy	Control	90	3.6	18.5	30.8	49.3
	BA	90^{ns}	3.7^{ns}	16.2^{ns}	32.5^{ns}	48.8^{ns}
	[9tP]BA	88^{ns}	3.5^{ns}	19.4^{ns}	34.8^{ns}	54.2^{ns}
Cresta	Control	66	3.8	20.3	26.9	47.1
	BA	55^{ns}	3.4**	15.5^{ns}	27.5^{ns}	43.1^{ns}
	[9tP]BA	58^{ns}	3.6*	15.8^{ns}	26.2^{ns}	42.0^{ns}
Toret	Control	43	3.2	11.0	20.1	31.2
	BA	58*	3.7^{ns}	12.8^{ns}	27.2**	40.0**
	[9tP]BA	47^{ns}	3.7^{ns}	10.9^{ns}	21.7^{ns}	32.6^{ns}

ns : no statistically significant difference from the control
*, **, *** : different from the control at 0.05, 0.01, 0.001 probability level

The number of seeds per harvestable pod was not affected by the cytokinin analogs used in this field study for all varieties apart from cv. Cresta. Each harvestable pod of control plants of Cresta contained on average 3.8 seeds. The application of BA and [9tP]BA decreased this number to 3.4 ($p<0.01$) and 3.6 ($p<0.05$) respectively (Table 4.11.1).

4.11.2 Main effect of cytokinins

Control faba bean plants formed, on average, 62 seeds on the mainstem, having 3.6 seeds per harvestable pod. The application of either BA or [9tP]BA at stages 203(1) and 204(1) did not alter these figures (Table 4.11.2).

Table 4.11.2: *Overall effect of BA and [9tP]BA on number of seeds and dry weight of harvestable reproductive organs on the mainstem.*

Treatment	No. seeds per plant	No. seeds per harvest. pod	Dry weight hulls (g)	Dry weight seeds (g)	Dry weight pods (g)
Control	62	3.6	15.5	23.5	39.0
BA	65^{ns}	3.6^{ns}	15.3^{ns}	26.8*	42.1^{ns}
[9tP]BA	66^{ns}	3.6^{ns}	15.7^{ns}	25.5^{ns}	41.1^{ns}

ns : no statistically significant difference from the control
* : different from the control at 0.05 probability level

4.11.3 Main effect of variety

The lowest number of seeds per mainstem was recorded on the plants of the *major* variety TFW (31 seeds). The *equina* varieties Cresta and Toret had 60 and 49 seeds per mainstem, respectively. The number of seeds per mainstem of *equina* plants was higher ($p<0.001$) compared to *major* plants, but it was lower ($p<0.001$) compared to *minor* plants. Plants of Maris Bead and Troy, formed 94 and 90 seeds per mainstem, respectively (Table 4.11.3).

Each harvestable pod of the *major* variety TFW contained more ($p<0.01$) seeds compared to all *minor* and *equina* varieties assessed in this field trial. In addition, no difference in the number of seeds per harvestable pod was recorded between *minor* and *equina* faba bean plants (Table 4.11.3).

Table 4.11.3: *Overall varietal effect on number of seeds and dry weight of harvestable reproductive organs of the mainstem. Probability values of interaction between variety and cytokinin are also presented.*

Variety	No. seeds per plant	No. seeds per harvest. pod	Dry weight hulls (g)	Dry weight seeds (g)	Dry weight pods (g)
TFW	31^{a}	3.9^{b}	15.5^{a}	18.8^{a}	34.4^{a}
Maris Bead	94^{c}	3.6^{a}	15.2^{a}	24.8^{ab}	40.0^{ab}
Troy	90^{c}	3.6^{a}	18.0^{b}	32.7^{c}	50.7^{c}
Cresta	60^{b}	3.6^{a}	17.2^{b}	26.9^{bc}	44.1^{bc}
Toret	49^{b}	3.5^{a}	11.6^{a}	23.0^{ab}	34.6^{a}
LSD (42 d.f.)	*17.3*(3)	*0.29*(2)	*4.21*(3)	*5.85*(3)	*9.36*(3)
Var. x Treat. p val	0.107	0.026	0.057	0.539	0.237

a, b, c : each letter indicates a group of means which do not differ statistically
(1), (2), (3) : LSD calculated at 0.05, 0.01, 0.001 probability level

4.11.4 Effect of variety and cytokinin treatment

No interactive effect of variety and cytokinin treatment was observed on the number of seeds per mainstem (Table 4.11.3).

Slight interaction ($p<0.05$) between faba bean variety and external application of cytokinin was evident with respect to the number of seeds per harvestable pod (Table 4.11.3). Both treatments caused less seeds to be formed on each harvestable pod of Cresta plants (see Section 4.11.1).

4.12 Effects on dry weight of harvestable pods

4.12.1 Effect of cytokinins on each variety

None of the cytokinins used in this study affected the dry weight of the harvestable pods on the mainstem of cv. Maris Bead, Troy and Cresta plants. In contrast, the application of BA on plants of TFW and Toret increased the dry weight of harvestable pods. On TFW plants, it increased ($p<0.01$) from 32.0 g on controls to 38.5 g on BA treated plants, and on Toret plants from 31,2 g to 40.0 g ($p<0.05$), respectively (Table 4.11.1).

4.12.2 Main effect of cytokinin

On average, the dry weight of harvestable pods formed on the mainstem of control plants was 39.0 g. This figure was not affected by any of the cytokinin treatments (Table 4.11.2).

4.12.3 Main effect of variety

The lowest dry weight of harvestable pods was recorded on broad bean plants of cv. TFW. Similar figures were observed for varieties Maris Bead and Toret. Troy exhibited a greater ($p<0.001$) dry weight of pods compared to the other *minor* variety Maris Bead, and cvs TFW and Toret (Table 4.11.3).

Between *equina* varieties the dry weight of harvestable pods on the mainstem of Cresta plants was higher ($p<0.001$) than those of cv. Toret (Table 4.11.3).

No interaction was found between variety and cytokinin treatment (Table 4.11.3).

4.13 Estimation of sampling error within each variety

4.13.1 Three Fold White

In broad bean plants of cv. TFW the variation between individual plants was estimated to be slightly less than 10% of the mean value for the observations of number of flowers, number of seeds per plant, dry weight of harvestable pods and percentage of dry matter distribution within harvestable pods. The highest margin error was observed for the percentage of harvestable pods, at 19% (Table 4.13.1).

4.13.2 Maris Bead

In Maris Bead plants the margin of error of the means of the studied characteristics ranged from 2 to 32%. This variety was quite uniform with regard to the observations of number of flowers, number of pods set, percentage of pod set, number of seeds per harvestable pod and dry matter distribution within harvestable pods. However, the margin of error for the number of harvestable pods, percentage of harvestable pods, number of seeds per plant, and dry weight of pods were 27, 26, 32 and 26 respectively (Table 4.13.1).

4.13.3 Troy

In all the cases, the variation among cv. Troy plants did not exceed 10% of the mean of each characteristic under investigation. The lowest margin of error was estimated for the percentage of pod set and the highest for the number of seeds per plant (Table 4.13.1).

Table 4.13.1: *Margin of error for each characteristic recorded within each variety (data were collected only from the mainstem).*

Characteristic	Variety				
	Three Fold White	Maris Bead	Troy	Cresta	Toret
	Margin of error (%)				
No. Flowers	9	2	5	10	2
No. Pods set	17	6	5	13	4
% Pod-set	10	6	1	4	2
No. Mat. Pods	14	11	7	11	6
% Mat. Pods	17	11	5	9	4
No. Harv. Pods	12	27	5	14	18
% Harv. Pods	19	26	3	15	15
No. Seeds/plant	9	32	8	16	15
No. Seeds/Harv. Pod	11	5	3	2	6
DW Hulls	13	27	11	15	8
DW Seeds	8	8	8	13	7
DW Pods	9	26	6	13	7

4.13.4 Cresta

Plants of cv. Cresta exhibited a margin of error less than 10% of the mean value when they were scored for percentage of pod set, percentage of mature pods, number of seeds per harvestable pod and dry matter distribution within harvestable pods. The highest margin of error was 16% and it was recorded on the number of seeds per plant (Table 4.13.1).

4.13.5 Toret

The variation among plants of cv. Toret, in all the cases of the characters studied, was found to be less than 10% of the mean value for each characteristic, apart from number of harvestable pods, percentage of harvestable pods and number of seeds per plant where it was 18%, 15% and 15%, respectively (Table 4.13.1).

4.14 Discussion

The difference in flowering date among the varieties probably had little effect on the yield potential, as the climatic conditions were quite stable during the flowering period (see Appendix 4.1b). It can also be assumed that pollination was not a determinant of flower drop: the small size of the field, the absence of competing bee-forage crops in the area, and the stable weather during flowering support this assumption. In addition, it has been reported that high cross-fertilisation levels occur more often in cooler environments such as Scotland (Link *et al.*, 1994). Therefore, the application of BA-compounds may be regarded as the main factor that affected the reproductive loss of faba bean plants in this field experiment.

Keller and Bellucci (1983), while reviewing the effects of plant growth substances on the development and yield of *Vicia faba* plants, questioned the consistency of results and the reliability of the treatments under controlled and field conditions. In the present study, it was shown that overall pod set was increased by BA-type cytokinins applied externally to *V. faba* plants at growth stages 203(1) and 204(1), i.e. early flowering and early pod set. These findings confirmed previous results (Chapter 3) where the same substances were tested under controlled conditions and only in one broad bean variety. In the literature, similar results have been reported from field studies when faba beans were sprayed with BA during flowering at the same concentration (10^{-4}M) (Kellerhals, 1983; Rylott, 1991).

Both cytokinins BA and [9tP]BA exhibited similar levels of activity in enhancing pod set on each individual variety. The rating of the varieties in descending order of response to pod set was Three Fold White (increase 39%), Toret (increase 35%), Cresta (increase 28%), Maris Bead (increase 15%) and Troy (increase 8%). This order of varieties coincides with the ascending order of the normal ability of each cultivar to set pods. Thus, the effects of cytokinins on pod initiation were more profound in the varieties which suffered more from flower abscission. However, it is interesting to notice that even the *minor* varieties, Maris Bead and Toret, which

naturally shed a very small fraction of their flowers, benefited from BA-type cytokinins and achieved almost complete pod set. In these small-seeded cultivars, the limited effect of BA-compounds on their overall pod set could be interpreted as a reflection of adequate balance of endogenous cytokinins and/or increased sensitivity to these compounds in the reproductive organs (buds and flowers) during flowering. On the other hand, increases in pod initiation, due to external cytokinins, in the other varieties may be an indication of relative deficiency of endogenous cytokinins and/or limited responsiveness at the hormone-responsive active site of the reproductive organ to these substances; hence the exogenous application of BA-compounds resulted in increased pod set.

The data of pod initiation presented here suggest that the physiological limitations in pod set, and thus in yield potential, are different in each variety and thus reflect differences at the genetic level. This is in agreement with previous findings reported by Gates and his co-workers (1983).

The present experiment showed that an increased supply of BA-type cytokinins exogenously to faba bean plants at flowering overcame this early loss of reproductive load independently of the normal intrinsic ability of each variety to control this. It can be seen also that, in general, increases in pod set were evident both within each raceme and between racemes, excluding the positions where almost complete pod set was recorded without treatment or where inflated variation resulted in non-significant increases (e.g. flower positions more distal than 6). This indicated that the application of the chemical suppressed the plant's inherent dominance towards the lower racemes and proximal flowers. The physiological processes which were affected by the cytokinins are unknown; however, it could be suggested that both competition and dominance may be involved.

In this species, apart from cytokinin application, pod set can be increased by various treatments at flowering which alter the endogenous hormonal balance of the plant and reduce the dominance of the vegetative apex. It has been shown that chemical retardation of vegetative growth (Keller & Bellucci, 1980; Smith 1982a; Kellerhals & Keller, 1984; Hack *et al.*, 1985; Field *et al.*, 1989; Batts *et al.*, 1991; Child & Wenyu, 1991; Rylott, 1991; Batts & Hebblethwaite, 1992), topping (Chapman *et al.*, 1978; Duc & Picard, 1981; Smith 1982; Diethelm *et al.*, 1988), antiauxin application (Chapman & Sadjadi, 1981; Smith, 1982a), and inhibition of auxin translocation (Smith, 1982a; Clifford *et al.*, 1992) diminish flower drop and favour reproductive rather than vegetative growth. Since auxin relations in the plant are responsible for dominance effects between apex and reproductive organs, between lower and upper

nodes and between proximal and distal positions within inflorescence (Tamas *et al.*, 1985; Diethelm *et al.*, 198; Peter *et al.*, 1989; Bangerth, 1989; Li *et al.*, 1995), the role of cytokinins in pod set must be viewed in the context of their interaction with endogenous auxin gradients.

The results of the present study suggest that external supply of BA-compounds applied at flowering and early pod initiation are one class of chemicals which enhance pod set. Carlson *et al.*, (1987) observed a strong correlation between the probability of a *G. max* flower setting a fruit and the total cytokinin flux in xylem exudate at the time of flower opening. It is possible, therefore, that the application of the cytokinins resulted in an increase in certain intrinsic hormones, e.g. by augmenting endogenous cytokinin levels, so that the cytokinin:auxin ratio was increased and thus reduced correlative inhibitory effects of auxins produced from the apex and from earlier formed sinks, which dominate development at early flowering and early pod set (Bangerth, 1994). This change in the endogenous hormonal balance pre-disposed the plant to set more pods. Data presented here for cv. Troy, support this suggestion. The stem growth of this semideterminate field bean variety is clearly terminated, i.e. the terminal node regularly develops flowers, but there is no pod initiation at this node. This variety, therefore, with reduced dominance of the vegetative apex, normally exhibits a relatively low degree of flower abscission.

Furthermore, changes that may be stimulated in the vascular tissue at the pedicel and peduncle of the raceme may also be involved in cytokinin action in controlling the competitive ability of generative sinks and hence the pod set (Gates *et al.*, 1981).

In addition, in most cases described in the literature (see reviews: Gates *et al.*, 1983; Bangerth, 1989; Aufhammer, 1990; Baylis & Clifford, 1991), it is difficult to make a clear distinction between dominance and competition for a limited assimilate supply with respect to early reproductive loss in grain legumes. Competition for assimilates may have been part of the explanation for the early decrease in reproductive potential. In this field experiment, the contribution of assimilates produced from leaves on the percentage pod set it is not known. However, it may be assumed that photoassimilate production at the whole plant level did not limit the process of pod set since conditions for growth (temperature, irradiance, water, fertilisation, weeding, pests and diseases control) were available in sufficient amounts. Furthermore, possible changes in the ability of the sources to produce photoassimilates due to cytokinin applications may be excluded as the main factor controlling pod set. This assumption is also supported by evidence from experiments in which, under conditions of low competition for light during flowering, an indeterminate faba bean

plant was unable to co-ordinate source and generative sink activities optimally to exploit the functional assimilation and storage capacity. Here it was suggested that available assimilate in the main did not limit the early establishment of pod load (Aufhammer & Gotz-Lee, 1989). Consequently, the role of exogenous cytokinins on enhancing pod set should be focused on the partitioning rather than on the production of the photoassimilates among developing sinks.

Many theories have been put forward to explain the mechanism by which assimilates are distributed among plant organs, but no unequivocal conclusion is available at present (Gifford & Evans, 1981; Wolswinkel, 1985; Farrar, 1988; Patrick, 1988; Wardlaw, 1990). However, it is suggested that the distribution of assimilates among sinks is primarily regulated by the sinks themselves (Evans, 1975; Gifford & Evans, 1981; Farrar, 1988; Ho, 1988; Marcelis 1994). It is well known that the presence or lack of certain hormones can affect assimilate partitioning to developing sinks (Aufhammer, 1990; Ronzhina & Mokronosov, 1994). Cytokinins have been demonstrated to increase sink strength (i.e. the potential capacity of a sink to accumulate and metabolise assimilates) of plant organs (Kuiper, 1993) and are particularly noted for the mobilisation of a wide variety of substances (Mothes & Engelbrecht, 1961; Letham, 1967; Ronzhina *et al.*, 1995). Therefore, the increase in pod set due to external supply of BA-compounds appears to be caused by increased potential of the flower as a sink (i.e. net assimilate importer) and thus by its relative ability to compete for assimilates and nutrients with other sinks on the plant.

Depending on the variety, cytokinin application can result in increased percentage of mature pods (Table 4.4.1). However, the percentage of the harvestable pods did not match this increase in pod load (i.e. number of pods) at maturity. The reason was that a proportion of the pods which were retained on the plant until harvest did not attain a harvestable size because of depressed growth during their development. This may be either because the assimilates needed for pod and hence seed growth were insufficient, or because the increased competitive effect on the sinks (pods) produced by the application of cytokinins gradually diminished. It can be seen from the mature pod distribution within the raceme (Tables 4.6.1 & 4.6.2), that after exogenous cytokinin application the naturally dominant pods gradually resume their status. Much of the reason for this may be the development of the vascular tissue within the raceme that favours assimilate supply to proximal more developmentally advanced pods (White *et al.*, 1984).

However, an increased percentage of mature pods at flower positions above 2 on the raceme was accompanied by a decrease in the percentage of mature pods at the

proximal flower position 1 (Table 4.5.2). This observation indicated that cytokinin application just before flower opening affected the interrelationship between developing pods on the same inflorescence. Presumably, the stimulation of secondary vascular differentiation on the pedicel and peduncle of the young pods (Aloni, 1993; Kuang *et al.*, 1991, 1992) reduced the inhibitory effect of the proximal pods on those pods developed at more distal positions. The exact mechanism for this phenomenon has not been elucidated, but it has been suggested that it is the adequacy of the supply of endogenous cytokinins and nutrients which controls the growth of pods in soybean (Nooden & Letham, 1993).

In addition, the majority of mature pods were retained on the lower reproductive nodes, and at these racemes the positive effect of cytokinins was observed (Table 4.6.1 & 4.6.2). This observation indicates that the lower four racemes benefited from the repeated application of cytokinins, presumably because of the diversion of assimilates and nutrients to very young pods formed on them for a longer period at the early stages of their development. Also, this suggests that by late pod set, for the racemes above reproductive node 4, the effects of BA-analogs may have been much less. This suggestion confirms the suggestion made in Chapter 3 that repeated applications of these growth substances may promote pod retention. However, it would appear that even if the yield potential of the plant could further be increased by more applications of growth substances, the plant would suffer from source limitations (Rylott, 1991). The decrease in the percentage of mature and harvestable pods observed on the more apical racemes lends credence to this speculation.

In Northern Europe, although there is wide variation among cultivars, the estimated proportion of faba bean flowers that produce mature pods to harvest is about 10% (Knott *et al.*, 1994). This study found a much higher percentage, of about 35%, but it took into consideration only fruits on the main stem and at the reproductive nodes 1 to 9, and these nodes exhibit lower reproductive loss compared to more apical nodes (Gates *et al.*, 1983; Aufhammer *et al.*, 1989; Clifford *et al.*, 1990; Rylott, 1991). In general, the number of pods growing on a plant changed considerably during the growing season, and depended primarily on how many fruits did not abort shortly after flowering.

The yields achieved in this experiment were higher than those normally obtained for field-grown plants. With a plant density of 36 plants per m^2 in each experimental plot the calculated forecast yield for control plants was 8.5 tonnes/ha whereas for BA-treated plants it was 9.6 tonnes/ha (based on DW of seeds per plant in Table 4.11.2). The small size of the plot relative to the area of a faba bean field in practice

and the favourable conditions of cultivation (agronomic, weather, soil etc.) were likely to influence the high levels of productivity observed here. Mainstem yield, represented by the DW of seeds, generally was increased ($p<0.05$) by 14% in BA-treated plants relative to control plants and by 9% (statistically insignificant) in those sprayed by [9tP]BA (Table 4.11.2). Further analysis, however, of the effects of cytokinins on the yield components within each variety showed varietal differences in the contribution of each yield component to final yield.

In cv. Toret, for example, BA application resulted in 35% relative increase of the DW of seeds, due in the main to the greater number of seeds formed on the mainstem, which was the result of the increased (statistically insignificant) number of seeds within each harvestable pod. The contrasting evidence concerning the number of seeds per pod of cv. Toret and cv. Cresta (see below) indicates that BA may affect flower fertilisation and/or ovule and seed abortion. Nevertheless, a lack of information about the breeding habits of these varieties does not allow further conclusions on the way cytokinin affected any possible action on flower physiology.

For cv. Cresta, on the other hand, in contrast to cv. Toret, application of BA-compounds reduced the number of seeds formed on each harvestable pod. However, total DW of seeds on the mainstem was not affected being compensated by the average seed size, since the number of harvestable pods was not altered. The response of this variety supports previous findings (Aufhammer *et al.*, 1989): whereas the number of seeds decreased, the weight of individual seeds generally increased.

Mainstem yield analysis of the broad bean variety Three Fold White, showed that BA-treated plants exhibited 20% increase in the DW of the harvestable pods above control plants. This effect was due to the combined contribution of the increases (statistically insignificant) in the DW of seeds and DW of hulls on the mainstem.

In the two small-seeded varieties used in this study, Maris Bead and Troy, yield increases were observed on the mainstem, but they were not significant. The main reason for this was the high degree of variability between plants within each variety, particularly Maris Bead, which is reflected in the analysis of the sampling error.

The plant material employed here came from commercial varieties (i.e. synthetic varieties), and high variability among individual plants of each variety was expected to be present in the experiment. For some of the characteristics studied, the variation in the measurements made because of non-uniform plants, resulted in an increased experimental error and thus in statistically insignificant differences due to cytokinin

applications. Therefore, an estimation of the uniformity in each cultivar was of primary importance, because it could be used as a criterion to choose more uniform biological material and more appropriate sampling techniques for future research. In this experiment, since observations within the sample were performed on individual plants of each cultivar, the measurement of the size of the sampling variance is an estimate of the level of uniformity of the cultivar for the characteristic studied.

Each cultivar was sown in 4 replicated plots in the field, and only 5 sampling units (i.e. plants on which actual measurements were made) within each plot were selected to be measured and used for estimating each plot value. An appropriate sampling technique is one that provides an estimate, or a sample value, that is as close as possible to the mean that would have been obtained had all plants in the population (i.e. plot in terms of statistics) been measured. In this study, as a population value is considered the average over all plots of the same cultivar and is called overall cultivar-mean. The difference between the sample value and the population value (overall cultivar-mean) constitutes the sampling error. It is evident that an increased number of sampling units (i.e. individual plants for this field trial) and/or replicated plots, and low levels of variability among these units (i.e. a more uniform plant population), would give a smaller sampling error and consequently a higher degree of precision (i.e. the reciprocal of the variance of the sample estimate) for the characteristic studied. The degree of precision is specified here in terms of the margin of error (D) of the overall cultivar-mean. The margin of error is presented in Table 4.13.1 as a percentage of the overall cultivar-mean for each recorded characteristic and gives the range within which the sample estimate deviates from the true value in 95% of the cases. Thus, for each variety, with 95% probability level, the mean of each particular characteristic can be expected to be within the range of the mean value plus or minus the margin of error (± D). If a level of the margin of error is satisfactory, then future research can accept the sampling procedure adopted for these experiments for flower and pod count, i.e. 4 replications and 5 plants per plot. In contrast, if the margin of error is higher than the desired level, then either or both of the following approaches could be taken into consideration: (a) increase the number of replications, (b) use a different sampling procedure with a change in sampling design, type of sampling unit or sample size. In practice, the desired level of precision (i.e. margin of error) for this study was prescribed to be 5%, based on experimental objective and previous experience (Chapter 3).

In *V. faba,* high intra-cultivar variation has been demonstrated by Goodrich *et al.* (1985) with the aid of electrophoretic techniques. In the present field experiment,

marked variability occurred in several characters within the plants of each variety and was reflected in the margin of error.

For example, in cv. Maris Bead variation between plants (sampling units) for the number of harvestable pods is much larger than that for the number of flowers (Table 4.13.1). Hence, although a sample size of 5 plants may be appropriate for number of flowers, it may not be so for harvestable pods count. With regard to the latter character, an increase in the number of sampled plants per plot from 5 to 130 would be needed to achieve an improvement in the degree of precision which will reduce the margin of error found in the present study from 27% to 5% which is an acceptable level. An alternative would be to increase the number of replications from 4 to 100. It is apparent that in either case the time spent in making measurements of the samples and the cost are beyond the means of this project.

For percentage of pod set, a very low (D<5%) inter-plant variation was observed in the varieties Toret and Troy, while the highest variation was measured in cv. Three Fold White. For the broad bean variety TFW, D could be reduced to 5% if, while keeping the sampling unit at 5 plants per plot, the number of replications was increased to 15; or, alternatively, by sampling 20 plants per plot of 4 replicated plots. In this case, it is better to increase the sample size, because adding more sampling units (i.e. plants per plot on which actual measurement is made), is less costly than adding more replications.

Among the varieties tested, Troy appeared to be very uniform in most of the characteristics studied here, probably because it is more inbred than the other cultivars (Lawes *et al.*, 1983).

It is evident that future experiments on the investigation of cytokinin effects on the reproductive potential of faba bean should include more replications in the experimental layout and/or more than five plants for each sample, depending on the variety and the characteristic studied. An alternative, in order to improve uniformity of plant material and precision of results, was to do the experiments on inbred lines. However, the cost involved and the fact that such lines are not commercially available, coupled with possible problems arising from inbreeding depression of the plants, would mean that this was not applicable for this thesis.

In summary, it has been demonstrated here that pod set, which markedly reduces the reproductive capacity of the faba bean plant at a very early stage of its establishment, was strongly influenced by external application of BA-compounds. Therefore, further investigation of the role of cytokinins in the hormonal control of this process

may be worthwhile. Whether a reduction in flower abortion due to cytokinins will increase seed yield is difficult to determine. The effects of these growth substances on parameters other than pod set, which limit the final yield of *V. faba,* was found to be very obscure because of high variability of measurements within the plants of each variety with regards to these parameters. Therefore, the case for employing in future experiments varieties such as Troy and Toret rests on: (a) their contrast in levels of normal setting of pods, which enables comparative studies on physiological processes that may be affected by cytokinins to be carried out in relation to pod initiation; (b) their very low variation within the plants, which gives precision to the results; and (c) their agronomic characters such as the fact that these two varieties flower at the same time (Appendix 4.1), which contributes to convenience of handling in the field and parallel investigation of the process of pod set.

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Chapter 5

Effect of BA on the activity of sucrose-metabolising enzymes in developing reproductive sinks

5.1 Introduction

It has been established in Chapters 3 and 4 that application of the synthetic cytokinin, benzyladenine (BA), to flowers of *Vicia faba* L. increased pod set. It was suggested in the discussion of the Section 4.14, that BA-cytokinins may effect their action in successful pod initiation by increasing the potential capacity of a reproductive sink to accumulate and metabolise assimilates i.e. its sink strength.

The concept of sink strength implies that utilisation of assimilate in the sink draws more nutrients into the sink from the source; therefore it is expected that by stimulating metabolic activity in the sink more assimilate will be diverted into this sink organ (Gifford & Evans, 1981; Wyse, 1986). Despite much disagreement in the literature concerning the definition and quantification of sink strength, in general it is agreed that sink activity is the most important determinant of sink strength (Farrar, 1993). Sink age (Chamont, 1993; Marcelis, 1993) or sink size (Ho, 1988; 1992; Jenner & Hawker, 1993) may also be concerned. Ho (1988; 1992) considered the physiological processes for the uptake and accumulation of imported assimilate in the sink cells (i.e. the regulation of both the phloem transport toward the fruit and the unloading and compartmentation of imported assimilates inside the fruit) to be a meaningful measure of sink activity.

It has been suggested that uptake and accumulation of assimilates from the phloem by the sink tissues is a factor controlling fluxes of phloem transport (Singh & Jenner, 1982). In most of the higher plants including *V. faba*, sucrose is the form in which carbohydrate is transported from the sites of synthesis (sources) to the sites of utilisation or storage (sinks) (Zimmerman & Brown, 1971; Cronshaw *et al.* 1986; Sung *et al.* 1988). It accounts for over 95% of translocated carbohydrate and 90% of all solutes (Thorne & Rainbird, 1983) in the plant. At sinks, this disaccharide is the major fuel for the biosynthesis of storage, structural, and respiratory products providing carbon and energy for sink growth, development and maintenance (Duffus & Duffus, 1984). There is strong experimental support for the view that the rate of import of sucrose into sinks is dependent on tissue utilisation of sucrose (Ho, 1979; Morris, 1982; Giaquinta, 1983; Sung *et al.*, 1988), and thus on their sink activity as it is defined above.

In most higher plants examined, sucrose *per se* is not utilised for metabolic processes; instead, it is hydrolysed prior to subsequent metabolism (Avigad, 1982).

Cleavage of sucrose arriving in a sink is the initial step in sucrose metabolism. Inside the sink cell, sucrose is metabolised into hexoses either by sucrose synthase or by invertase (Zimmerman & Brown, 1971; Avigad, 1982; Cronshaw *et al.*, 1986; Sung *et al.*, 1988, 1994).

The ability of the sink tissue to import assimilates is considered to be correlated with the activity of sucrose synthase (SS) in the cytosol (Claussen *et al.* 1985; Sung *et al.* 1989a,b; Xu *et al.* 1989; Sun *et al.* 1991; Ross & Davies, 1992; Wang *et al.* 1993; Ho, 1996) or of soluble acid invertase (AI) in the vacuole (Morris, 1982; Morris & Arthur, 1984; Sung *et al.* 1994). The predominance of each enzyme in the pathway of sucrose conversion may depend on the processes occurring in the tissue at that time (Sung *et al.*, 1994). Thus in many active and growing sink tissues, SS activity may predominate, and in others, particularly those undergoing expansion, acid invertase may predominate.

Sink activity can be modified by the molecular approach of altering biosynthetic pathways (Sonnewald & Willmitzer, 1992; Ho, 1991), and by the use of growth substances, in particular cytokinins (Kinet *et al.*, 1986; Fetene & Beck, 1993; Kuiper *et al.*, 1993; Ronzhina & Mokronosov, 1994). For the tomato raceme, a very weak sink (Kinet, 1977; Russell & Morris, 1983), the application of cytokinin and gibberellic acid increased import of carbohydrates in the inflorescence 24 h after the treatment (Kinet *et al.*, 1986). A similar chronicle of events has also been confirmed with BA application on leaves of *V. faba,* and the hormonal effects were linked to the stage of sink development (Ronzhina *et al.*, 1995).

In view of this evidence, it is possible that the promotion of pod set after external application of BA may be associated with an increased SS and/or AI activity in the reproductive sinks, which in turn enhances the ability of flowers (sink tissues) to accept assimilates necessary for ontogenetic processes of pod set. Thus, the objective of the present study was to obtain more information about the regulation of pod initiation by external application of BA in commercial varieties of *Vicia faba* L. by examining its relevance to AI and SS activity in developing reproductive sinks during the critical period of flower development when physiological changes that lead to pod set occur. Developing carpels, and pedicel and peduncle tissue, were evaluated with regard to their sucrose metabolism and the ability to set pods (Section 2.5).

5.2 Experiment 5.1: Effects of benzyladenine on sucrose-metabolising enzymes in developing carpels in relation to pod set in three varieties of *V. faba*.

5.2.1 Effect of BA on pod set

In control plants of cv. Maris Bead, the average percentage of pods that set on the lower five racemes was 75%. The application of BA at plant growth stages 203(1) and 204(1) caused more ($p<0.05$) flowers to set pods, resulting in an average of 96% (Table 5.2.1).

Table 5.2.1: *Effect of BA on the overall percentage of pod set at the lower five racemes in three varieties of faba bean plants grown in the field in 1993.*

Treatment	Variety		
	Maris Bead	**Troy**	**Toret**
	Percentage of pod set		
Control	75	90	56
BA	96*	98*	98*
LSD (0.05, 15 d.f.)	*15.9*	*6.6*	*22.9*

* : different from the control at 0.05 probability level

In the cultivar Troy, the proportion of flowers that initiated pods at the first five racemes of the mainstem of control plants was 90%. After BA application this increased ($p<0.05$) to 98% (Table 5.2.1).

For control plants of cv. Toret 56% of flowers formed pods on the lower five racemes. The application of BA increased ($p<0.05$) this to 98%, giving figures similar to those observed in treated plants of Maris Bead and Toret (Table 5.2.1).

5.2.2 The effects of benzyladenine on the fresh weight of carpel

5.2.2.1 Maris Bead

External application of BA to cv. Maris Bead plants had no effect on carpel fresh weight at any of the three stages of flower development studied in this experiment (Table 5.2.2).

Carpels collected from fully opened flowers (fds-9) had 10.2% ($p<0.05$) higher fresh weight compared to those collected at fds-6 and fds-10 (Table 5.2.2).

A slight ($p<0.05$) interaction between BA application and flower development stage for fresh weight of the carpels was found (Table 5.2.2).

5.2.2.2. Troy

Fresh weight of carpels at any of the three stages of flower development was not affected by BA-application on cv. Troy plants (Table 5.2.2).

Among flower development stages, at fds-6, the lowest fresh weight per carpel recorded was 19.7 mg. Carpels of flowers at stages 9 and 10 were heavier than carpels at fds-6 by 12.7% ($p<0.001$) and 20% ($p<0.001$), respectively (Table 5.2.2).

Table 5.2.2: *Effects of flower development stage and of cytokinin application on fresh weight of developing faba bean carpels.*

Flower Development Stage	Treatment	Fresh weight (mg / carpel) cv. Maris Bead	Fresh weight (mg / carpel) cv. Troy	Fresh weight (mg / carpel) cv. Toret
fds-6	Control	20.3	19.5	18.4
	BA	18.6^{ns}	19.9^{ns}	19.6^{ns}
fds-9	Control	21.6	22.3	20.9
	BA	21.4^{ns}	22.0^{ns}	22.8^{ns}
fds-10	Control	18.5	24.7	23.6
	BA	20.4^{ns}	22.5^{ns}	23.5^{ns}
Main effect of cytokinin	Control	20.1	22.2	21.0
	BA	20.1^{ns}	21.5^{ns}	22.0^{ns}
cytokinin x fds	p value	0.043	0.043	0.682

ns : no statistically significant difference from the control

An interaction ($p<0.05$) was found between fds and cytokinin treatment with respect to fresh weight per carpel (Table 5.2.2).

5.2.2.3 Toret

As in previous findings, BA-treatment did not affect the fresh weight of carpels at any of the three stages of flower development studied here (Table 5.2.2).

No interaction was present between fds and cytokinin application (Table 5.2.2).

5.2.3 Effects of benzyladenine on acid invertase activity in carpels of cv. Maris Bead

In general, AI activity of untreated carpels decreased progressively as the stage of flower development was more advanced. The highest AI activity measured was at stage 6 (Figures 5.2.1a & 5.2.2a).

Application of BA to cv. Maris Bead plants increased the activity of AI in carpels of fds-6 and fds-9, by 52% ($p<0.001$) and by 167% ($p<0.001$) over the controls, when the activity was expressed per unit of carpel fresh weight (Figure 5.2.1a), or 39% ($p<0.001$) and 163% ($p<0.001$) when the activity was measured on per carpel basis (Figure 5.2.2a). At flower stage 10, AI activity per g fresh weight was not altered by BA treatment (Figure 5.2.1a & 5.2.2a).

Table 5.2.3: *Overall effect of BA application on AI, SS and SPS activity of developing carpels collected from plants of cv. Maris Bead.*

Treatment	AI (I) activity	AI (II) activity	SS (III) activity	SS (IV) activity	SPS (III) activity	SPS (IV) activity
Control	76	1.56	71	1.42	7.06	0.14
BA	138***	2.75***	84**	1.69**	7.19^{ns}	0.14^{ns}

(I) : µmoles sucrose hydrolysed g^{-1} fresh weight h^{-1}
(II) : µmoles sucrose hydrolysed $carpel^{-1}$ h^{-1}
(III): µmoles sucrose synthesised g^{-1} fresh weight h^{-1}
(IV): µmoles sucrose synthesised $carpel^{-1}$ h^{-1}
ns : no statistically significant difference from the control
, * : different from the control at 0.01, 0.001 probability level

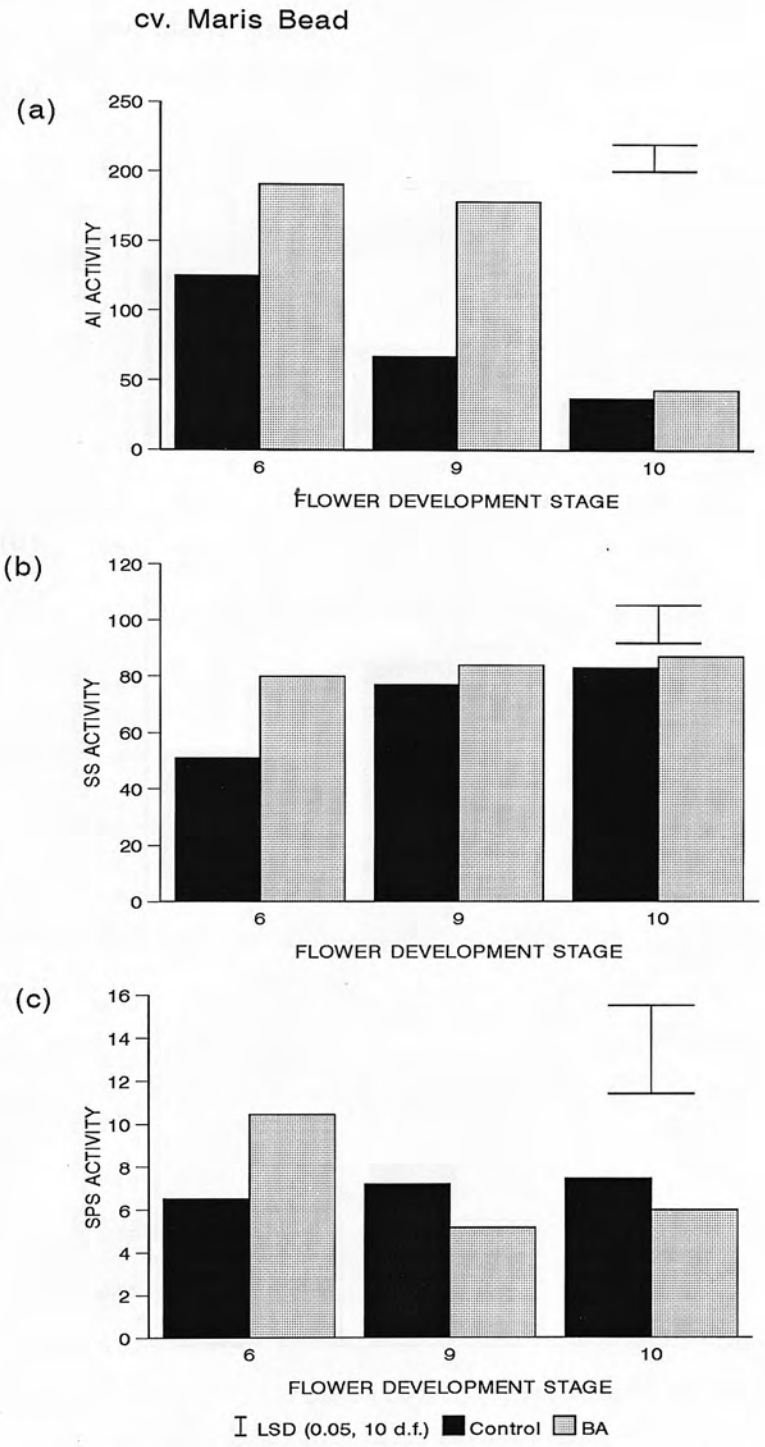

Figure 5.2.1: The effect of BA on (a) acid invertase activity (μmoles sucrose hydrolysed g^{-1} FW h^{-1}), (b) sucrose synthase activity (μmoles sucrose synthesised g^{-1} FW h^{-1}), and (c) sucrose phosphate synthase activity (μmoles sucrose synthesised g^{-1} FW h^{-1}) in developing carpels of cv. Maris Bead. (Actual figures are contained in Appendix 5.2.1).

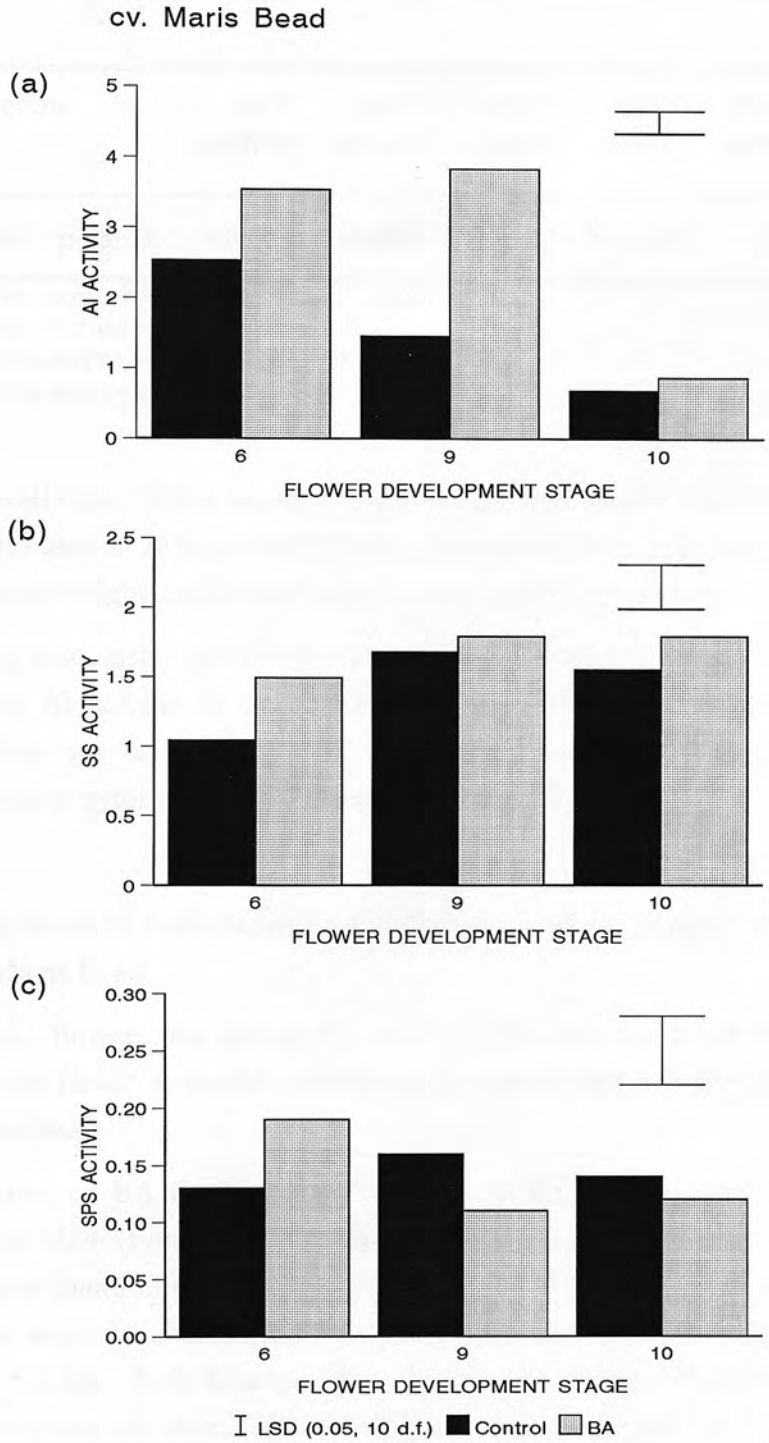

Figure 5.2.2: The effect of BA on (a) acid invertase activity (μmoles sucrose hydrolysed carpel^{-1} h^{-1}), (b) sucrose synthase activity (μmoles sucrose synthesised carpel^{-1} h^{-1}), and (c) sucrose phosphate synthase activity (μmoles sucrose synthesised carpel^{-1} h^{-1}) in developing carpels of cv. Maris Bead. (Actual figures are contained in Appendix 5.2.2).

Table 5.2.4: *Interaction of cytokinin and flower development stage on AI, SS and SPS activity in developing carpels collected from plants of cv. Maris Bead.*

Interaction		AI (I) activity	AI (II) activity	SS (III) activity	SS (IV) activity	SPS (III) activity	SPS (IV) activity
ck x fds	p value	<0.001	<0.001	0.030	0.306	0.057	0.057

(I) : µmoles sucrose hydrolysed g^{-1} fresh weight h^{-1}
(II) : µmoles sucrose hydrolysed $carpel^{-1}$ h^{-1}
(III): µmoles sucrose synthesised g^{-1} fresh weight h^{-1}
(IV): µmoles sucrose synthesised $carpel^{-1}$ h^{-1}

The overall effect of BA on AI activity of faba bean carpels was an increase of 82% ($p<0.001$) and of 76% ($p<0.001$) when the activity was calculated on the basis of carpel fresh weight and on each carpel respectively (Table 5.2.3).

A strong relationship ($p<0.001$) was present between BA application and fds, with regard to AI activity of cv. Maris Bead developing carpels (Table 5.2.4). This interaction was due to increased AI activity in carpels of flowers before or at pollination in cytokinin-treated plants (Figure 5.2.2a).

5.2.4 Effects of benzyladenine on sucrose synthase activity in carpels of cv. Maris Bead

In control flowers, the lowest SS activity expressed on carpel fresh weight was recorded at fds-6. A similar pattern was observed when activity was measured on a per-carpel basis.

Application of BA resulted in 57% higher ($p<0.01$) SS activity per carpel fresh weight at fds-6 (Figure 5.2.1b). In addition, each carpel at stage 6 of BA-treated plants was found to have 43.3% ($p<0.05$) higher SS activity over untreated carpels, while at stage 9 no difference between treated and untreated plants was recorded (Figure 5.2.2b). With faba bean carpels collected after pollination and fertilisation, SS activity was not altered after cytokinin application (Figure 5.2.2b)

On average, BA treatment increased SS activity of carpels by 18.3% ($p<0.01$) and 19% ($p<0.01$) when enzyme activity was expressed per unit of organ fresh weight and per individual organ respectively (Table 5.2.3).

An interaction between fds and BA application was present only when activity was expressed on per carpel fresh weight (Table 5.2.4). This interactive effect was due to better response of flowers at fds-6 and at fds-9 to externally applied cytokinin with regard to SS activity (Figure 5.2.1b).

5.2.5 Effects of benzyladenine on sucrose phosphate synthase activity in carpels of cv. Maris Bead

In general, all the stages of flower development tested in this study exhibited very low activity of SPS, whether expressed on carpel fresh weight or on each organ. This activity ranged between 6.71-8.49 μmoles sucrose synthesised g^{-1} FW h^{-1} and 0.13-0.16 μmoles sucrose synthesised carpel^{-1} h^{-1}, and the levels of activity were similar between the different stages of flower development.

SPS activity was not altered by BA treatment at any of the three stages of flower development studied here.

5.2.6 Effects of benzyladenine on acid invertase activity in carpels of cv. Troy

AI activity in developing carpels was not altered with BA application at any of the three stages of flower development studied (Figures 5.2.3a & 5.2.4a).

Table 5.2.5: *Overall effect of cytokinin application on AI, SS and SPS activity in developing carpels collected from plants of cv. Troy.*

Treatment	AI (I) activity	AI (II) activity	SS (III) activity	SS (IV) activity	SPS (III) activity	SPS (IV) activity
Control	65	1.37	83	1.84	6.43	0.15
BA	59^{ns}	1.23^{ns}	97^{ns}	2.08^{ns}	7.35*	0.16^{ns}

(I) : μmoles sucrose hydrolysed g^{-1} fresh weight h^{-1}
(II) : μmoles sucrose hydrolysed carpel^{-1} h^{-1}
(III): μmoles sucrose synthesised g^{-1} fresh weight h^{-1}
(IV): μmoles sucrose synthesised carpel^{-1} h^{-1}
ns : no statistically significant difference from the control
* : different from the control at 0.05 probability level

Table 5.2.6: *Interactive effect of flower development stage and BA on AI, SS and SPS activity of developing carpels collected from plants of cv. Troy.*

Interaction		AI (I) activity	AI (II) activity	SS (III) activity	SS (IV) activity	SPS (III) activity	SPS (IV) activity
ck x fds	p value	0.287	0.445	0.417	0.269	0.061	0.054

(I) : μmoles sucrose hydrolysed g^{-1} fresh weight h^{-1}
(II) : μmoles sucrose hydrolysed $carpel^{-1}$ h^{-1}
(III): μmoles sucrose synthesised g^{-1} fresh weight h^{-1}
(IV): μmoles sucrose synthesised $carpel^{-1}$ h^{-1}

On average, AI activity was 65 μmoles sucrose hydrolysed g^{-1} FW h^{-1} or 1.37 μmoles sucrose hydrolysed per carpel h^{-1} in control plants. In neither case was the activity affected by BA-treatment applied on cv. Troy plants (Table 5.2.5).

No interaction between BA-application and stage of flower development with respect to sucrose hydrolysis by AI was observed (Table 5.2.6).

5.2.7 Effects of benzyladenine on sucrose synthase activity in carpels of cv. Troy

SS activity in cv. Troy carpels at any of the three flower developmental stages tested was not affected by BA-treatment (Figures 5.2.3b & 5.2.4b).

Average SS activity of carpels in control plants was 83 μmoles sucrose synthesised g^{-1} FW h^{-1} or 1.84 μmoles sucrose synthesised $carpel^{-1}$ h^{-1}. Application of BA had no effect on SS activity (Table 5.2.5).

There was no interaction between developmental stage and BA-treatment with regard to SS activity in developing faba bean carpels (Table 5.2.6).

5.2.8 Effects of benzyladenine on sucrose phosphate synthase activity of carpels in cv. Troy

The SPS activity of cv. Troy untreated carpels ranged from 5.04 to 7.41 μmoles sucrose synthesised g^{-1} FW h^{-1} or from 0.10 to 0.18 μmoles sucrose synthesised $carpel^{-1}$ h^{-1}.

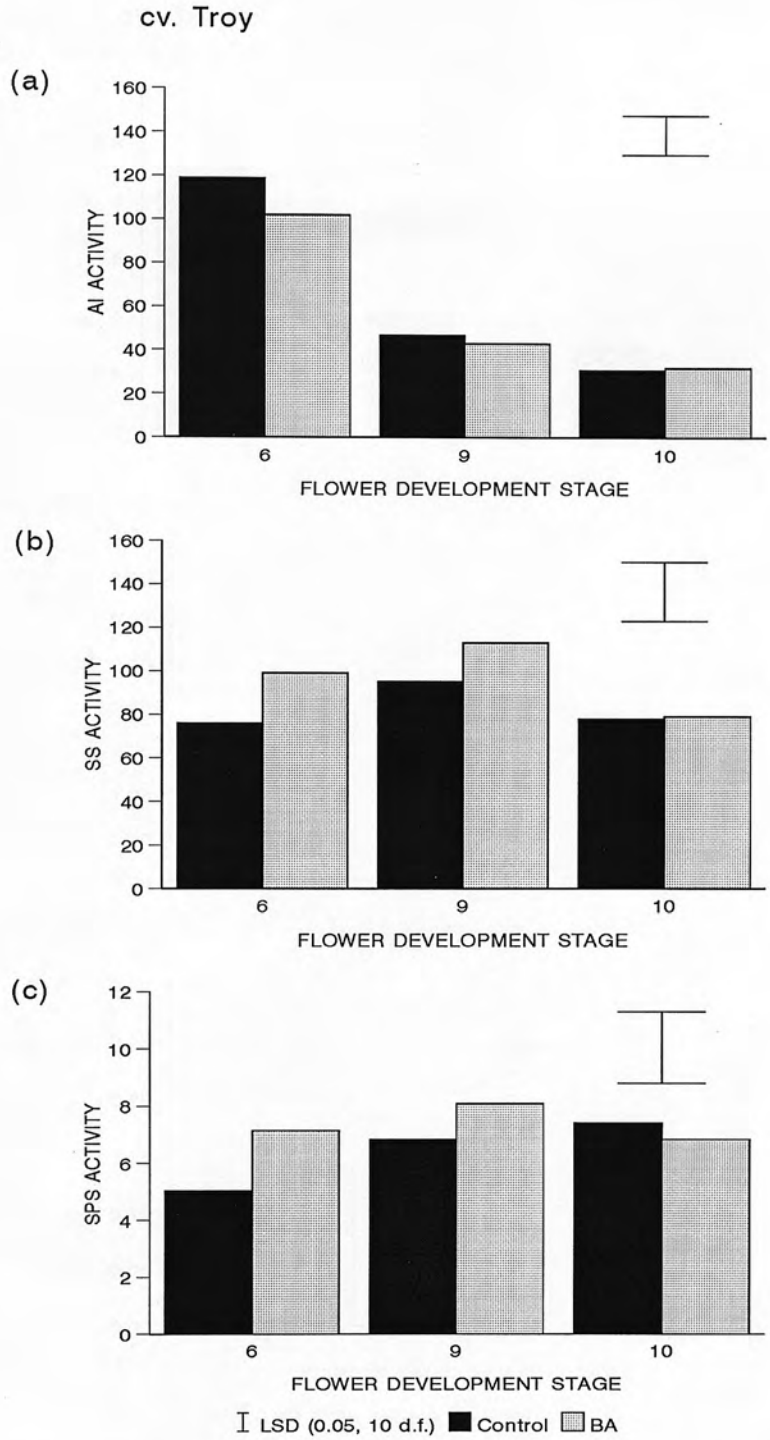

Figure 5.2.3: The effect of BA on (a) acid invertase activity (μmoles sucrose hydrolysed g^{-1} FW h^{-1}), (b) sucrose synthase activity (μmoles sucrose synthesised g^{-1} FW h^{-1}), and (c) sucrose phosphate synthase activity (μmoles sucrose synthesised g^{-1} FW h^{-1}) in developing carpels of cv. Troy. (Actual figures are contained in Appendix 5.3.1).

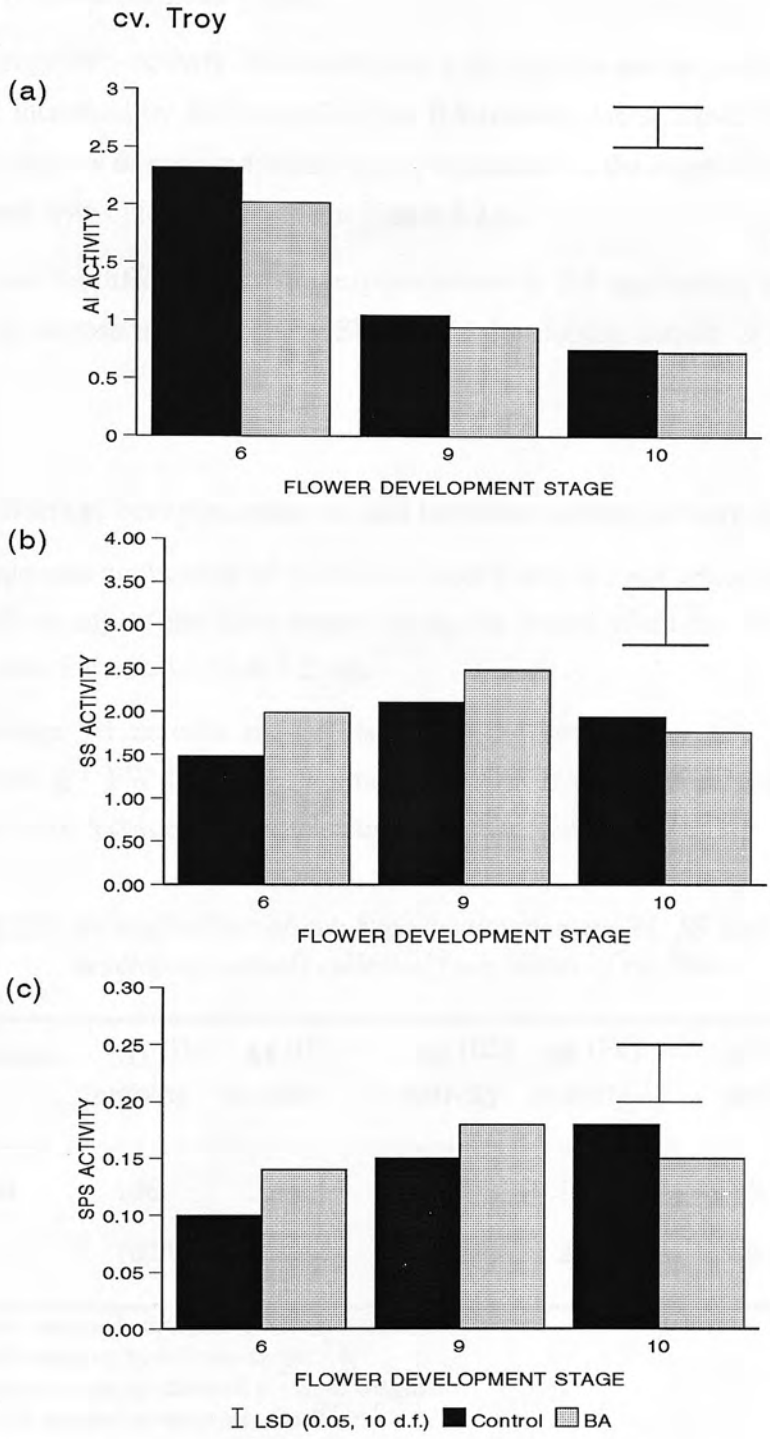

Figure 5.2.4: The effect of BA on (a) acid invertase activity (μmoles sucrose hydrolysed carpel^{-1} h^{-1}), (b) sucrose synthase activity (μmoles sucrose synthesised carpel^{-1} h^{-1}), and (c) sucrose phosphate synthase activity (μmoles sucrose synthesised carpel^{-1} h^{-1}) in developing carpels of cv. Troy. (Actual figures are contained in Appendix 5.3.2).

At neither stage of flower development had BA application any effect on SPS activity (Figures 5.2.3c & 5.2.4c).

The average SPS activity of controls was 6.43 μmoles sucrose synthesised g^{-1} FW h^{-1}, and increased by 14.3% ($p<0.05$) in BA-treated plants (Table 5.2.5). However, the SPS activity of each individual carpel regardless of the stage of development was not altered with cytokinin treatment (Table 5.2.5).

There were no differences in the responsiveness to BA application between fds with regard to sucrose metabolism by SPS in the developing carpels of cv. Troy (Table 5.2.6).

5.2.9 Effects of benzyladenine on acid invertase activity of carpels in cv. Toret

The exogenous application of BA to cv. Toret plants did not affect the activity of AI in carpels at any of the three stages during the period when the process of pod set takes place (Figures 5.2.5a & 5.2.6a).

The average AI activity in carpels of control plants was 106 μmoles sucrose hydrolysed g^{-1} FW h^{-1} or 2.19 μmoles sucrose hydrolysed $carpel^{-1}$ h^{-1}. Similar amounts were recorded after application with BA (Table 5.2.7).

Table 5.2.7: *Overall effect of cytokinin application on AI, SS and SPS activity of developing carpels collected from plants of cv. Toret.*

Treatment	AI (I) activity	AI (II) activity	SS (III) activity	SS (IV) activity	SPS (III) activity	SPS (IV) activity
Control	106	2.19	87	1.83	6.18	0.13
BA	102^{ns}	2.16^{ns}	93^{ns}	2.04**	6.15^{ns}	0.13^{ns}

(I) : μmoles sucrose hydrolysed g^{-1} fresh weight h^{-1}
(II) : μmoles sucrose hydrolysed $carpel^{-1}$ h^{-1}
(III): μmoles sucrose synthesised g^{-1} fresh weight h^{-1}
(IV): μmoles sucrose synthesised $carpel^{-1}$ h^{-1}
ns : no statistically significant difference from the control
** : different from the control at 0.01 probability level

Interactive effect of cytokinin treatment coupled with developmental stage on AI activity of the carpels was not found in cv. Toret plants (Table 5.2.8).

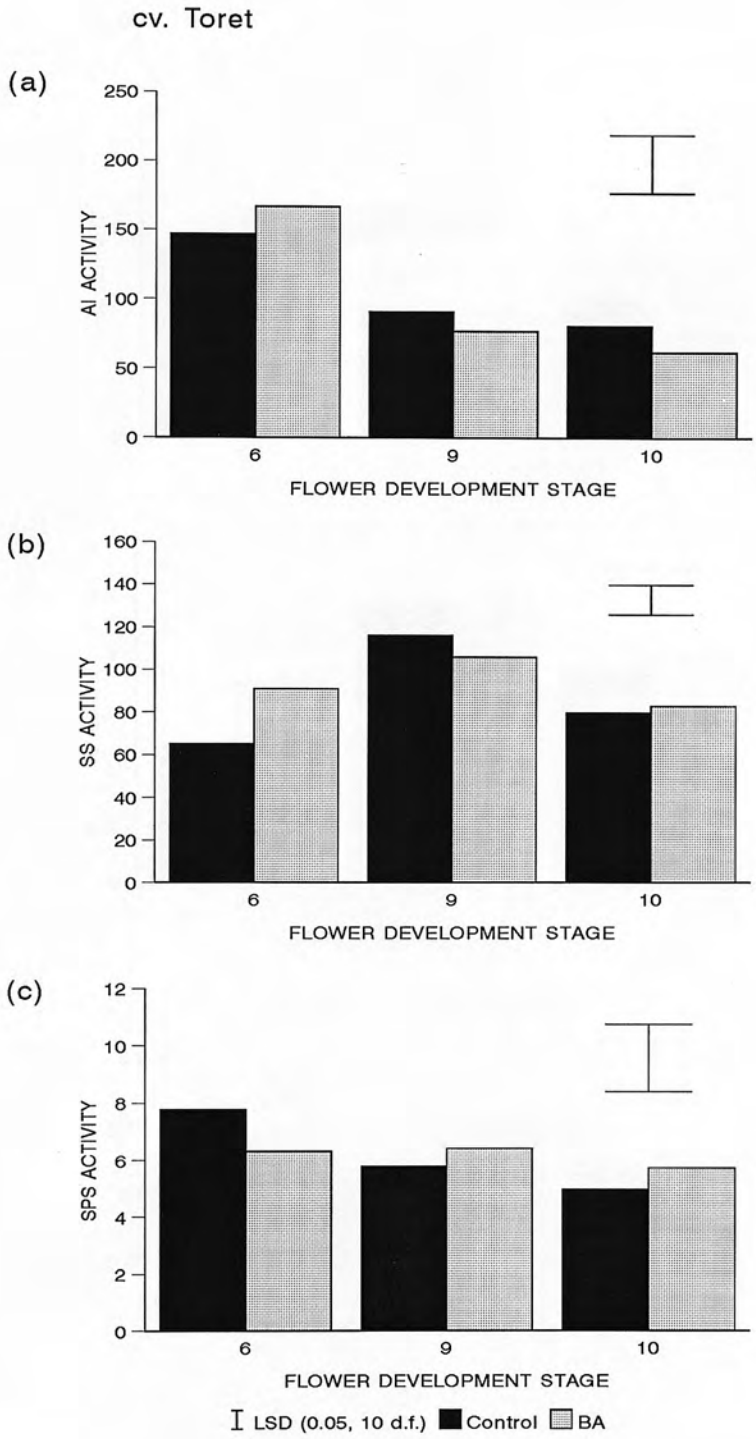

Figure 5.2.5: The effect of BA on (a) acid invertase activity (μmoles sucrose hydrolysed g^{-1} FW h^{-1}), (b) sucrose synthase activity (μmoles sucrose synthesised g^{-1} FW h^{-1}), and (c) sucrose phosphate synthase activity (μmoles sucrose synthesised g^{-1} FW h^{-1}) in developing carpels of cv. Toret. (Actual figures are contained in Appendix 5.4.1).

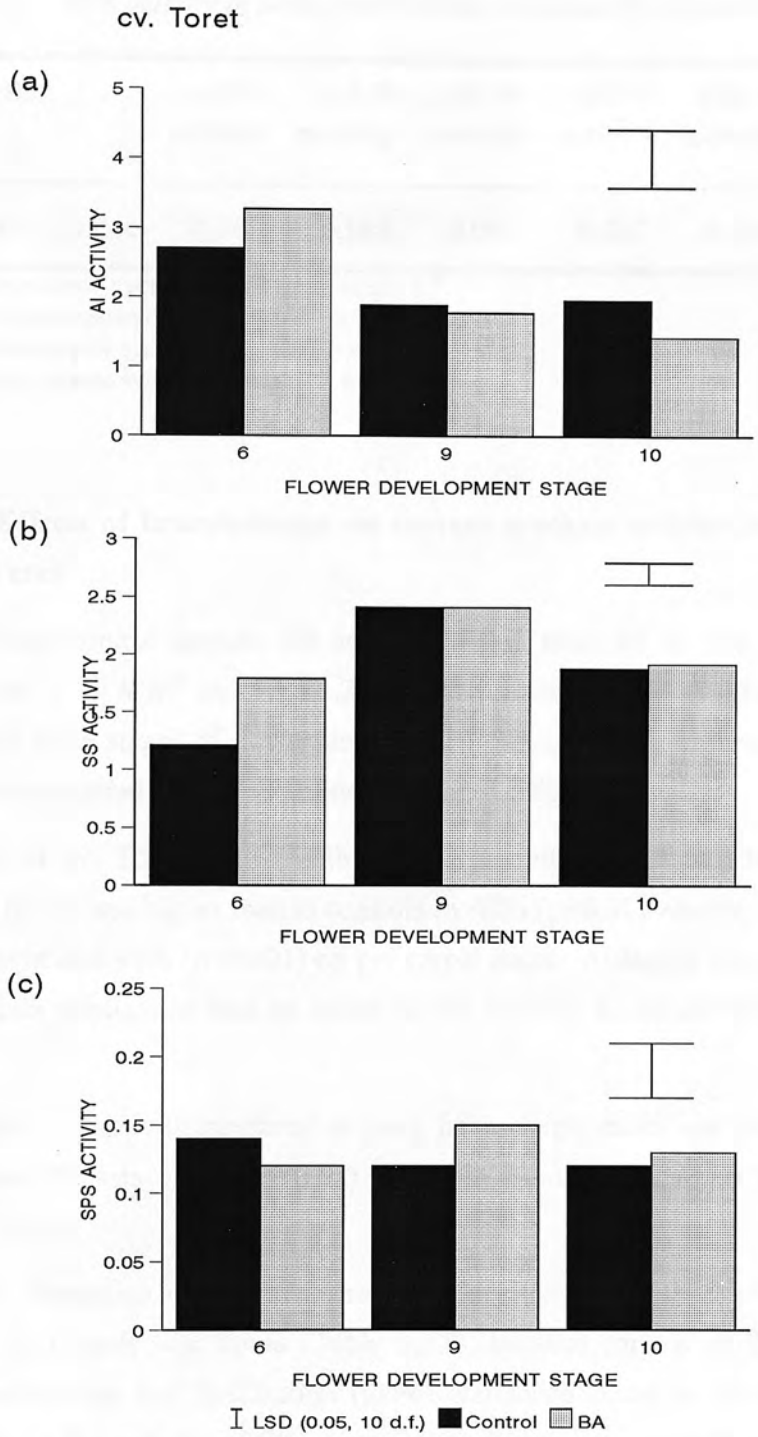

Figure 5.2.6: The effect of BA on (a) acid invertase activity (μmoles sucrose hydrolysed carpel^{-1} h^{-1}), (b) sucrose synthase activity (μmoles sucrose synthesised carpel^{-1} h^{-1}), and (c) sucrose phosphate synthase activity (μmoles sucrose synthesised carpel^{-1} h^{-1}) in developing carpels of cv. Toret. (Actual figures are contained in Appendix 5.4.2).

Table 5.2.8: *Interactive effect of flower development stage and BA on AI, SS and SPS activity in developing carpels collected from plants of cv. Toret.*

Interaction		AI (I) activity	AI (II) activity	SS (III) activity	SS (IV) activity	SPS (III) activity	SPS (IV) activity
ck x fds	p value	0.343	0.164	0.006	0.001	0.289	0.268

(I) : µmoles sucrose hydrolysed g^{-1} fresh weight h^{-1}
(II) : µmoles sucrose hydrolysed $carpel^{-1}$ h^{-1}
(III): µmoles sucrose synthesised g^{-1} fresh weight h^{-1}
(IV): µmoles sucrose synthesised $carpel^{-1}$ h^{-1}

5.2.10 Effects of benzyladenine on sucrose synthase activity in carpels of cv. Toret

In cv. Toret control carpels, SS activity ranged from 65 to 116 µmoles sucrose synthesised g^{-1} FW h^{-1} and from 1.21 to 2.41 µmoles sucrose synthesised $carpel^{-1}$ h^{-1} over the three stages of flower development studied. The highest figures in both cases were recorded at fds-9 (Figures 5.2.5b & 5.2.6b).

In plants of cv. Toret treated with BA, SS activity in the carpels of unfertilised flowers (fds-6) was higher than in controls by 40% ($p<0.01$) when expressed on per g fresh weight and 48% ($p<0.001$) on per carpel basis. At stages more advanced than 6, cytokinin application had no effect on SS activity in carpels (Figures 5.2.5b & 5.2.6b).

On average, SS activity measured on per g fresh weight basis was 6.8% ($p<0.01$) and on per carpel basis 11.5% ($p<0.01$) higher in BA-treated carpels than in controls (Table 5.2.7).

A strong interaction of BA treatment and developmental stage with regard to SS activity in carpels was found (Table 5.2.8), because carpels of flowers collected before pollination and fertilisation (fds-6) responded better to cytokinin treatment which raised the activity of SS, compared to flowers at stages 9 and 10 where SS activity was not altered (Figures 5.2.5b & 5.2.6b).

5.2.11 Effects of benzyladenine on sucrose phosphate synthase activity in carpels of cv. Toret

SPS activity ranged from 4.97 to 7.79 μmoles sucrose synthesised g^{-1} FW h^{-1} or from 0.12 to 0.14 μmoles sucrose synthesised $carpel^{-1}$ h^{-1} over the three developmental stages studied here in control plants (Figures 5.2.5c & 5.2.6c).

The SPS activity of cv. Toret carpels was not affected by BA application at any of the three stages of flower development studied in this experiment (Figures 5.2.5c & 5.2.6c).

The average SPS activity in carpels of control plants was 6.18 μmoles sucrose synthesised g^{-1} FW h^{-1} or 0.13 μmoles sucrose synthesised $carpel^{-1}$ h^{-1}. The application of BA did not affect SPS activity (Table 5.2.7).

5.3 Experiment 5.2: Effects of benzyladenine on sucrose-metabolising enzymes in the pedicel and peduncle in relation to pod set in three varieties of *V. faba*.

5.3.1 Effect of benzyladenine on pod set

In control plants of the broad bean variety TFW the proportion of flowers formed on the lower five racemes of the mainstem that initiated pods was 27%. Application of BA at stage 203(5) caused more ($p<0.05$) flowers to set pods compared with controls, resulting in an average 64% on the lower five racemes (Table 5.3.1).

Table 5.3.1: *Effect of BA on the overall percentage of pod set at the lower five racemes of faba bean plants grown in the field in 1994.*

Treatment	Variety		
	TFW	Troy	Toret
	Percentage of pod set		
Control	27	73	39
BA	64*	86	72*
LSD (0.05, 15 d.f.)	*30.4*	*13.9*	*28.7*

* : different from the control at 0.05 probability level

In cv. Troy plants, at the same reproductive nodes the application of cytokinin did not affect pod set. Pod set was 73% in controls and 86% in BA-treated plants (Table 5.3.1).

In cv. Toret, pod initiation in untreated plants was 39%, and in BA treated plants this increased by 33% ($p<0.05$, Table 5.3.1).

5.3.2 Effects of benzyladenine on acid invertase activity in pedicel and peduncle tissue of cv. Three Fold White

Among the three stages of flower development studied here, the highest AI activity was recorded in unfertilised flowers (fds-6), this activity decreased ($p<0.05$) when flowers were fully opened (fds-9) and further decreased at petal collapse (Table 5.3.3).

An interactive effect between flower development stage and cytokinin application was not found (Table 5.3.3).

The rate of sucrose hydrolysis by AI in the pedicel and peduncle of unfertilised flowers of cv. Three Fold White control plants was 50 μmoles sucrose hydrolysed g^{-1} FW h^{-1}. The application of BA resulted in a 12% decrease ($p<0.05$) of AI activity relative to controls (Figure 5.3.1a).

With flowers at fds-9 and fds-10 the external supply of BA to plants did not affect AI activity at the pedicel and peduncle of the flowers (Figure 5.3.1a).

Table 5.3.2: *Overall effect of cytokinin application on AI, SS and SPS activity in the pedicel and peduncle tissue collected from plants of cv. Three Fold White.*

Treatment	AI (I) activity	SS (II) activity	SPS (II) activity
Control	41	45	12.0
BA	38*	51ns	12.8ns
LSD (0.05, 10 d.f.)	*2.9*	*8.9*	*2.27*

(I) : μmoles sucrose hydrolysed g^{-1} fresh weight h^{-1}
(II) : μmoles sucrose synthesised g^{-1} fresh weight h^{-1}
ns : no statistically significant difference from the control
* : different from the control at 0.05 probability level

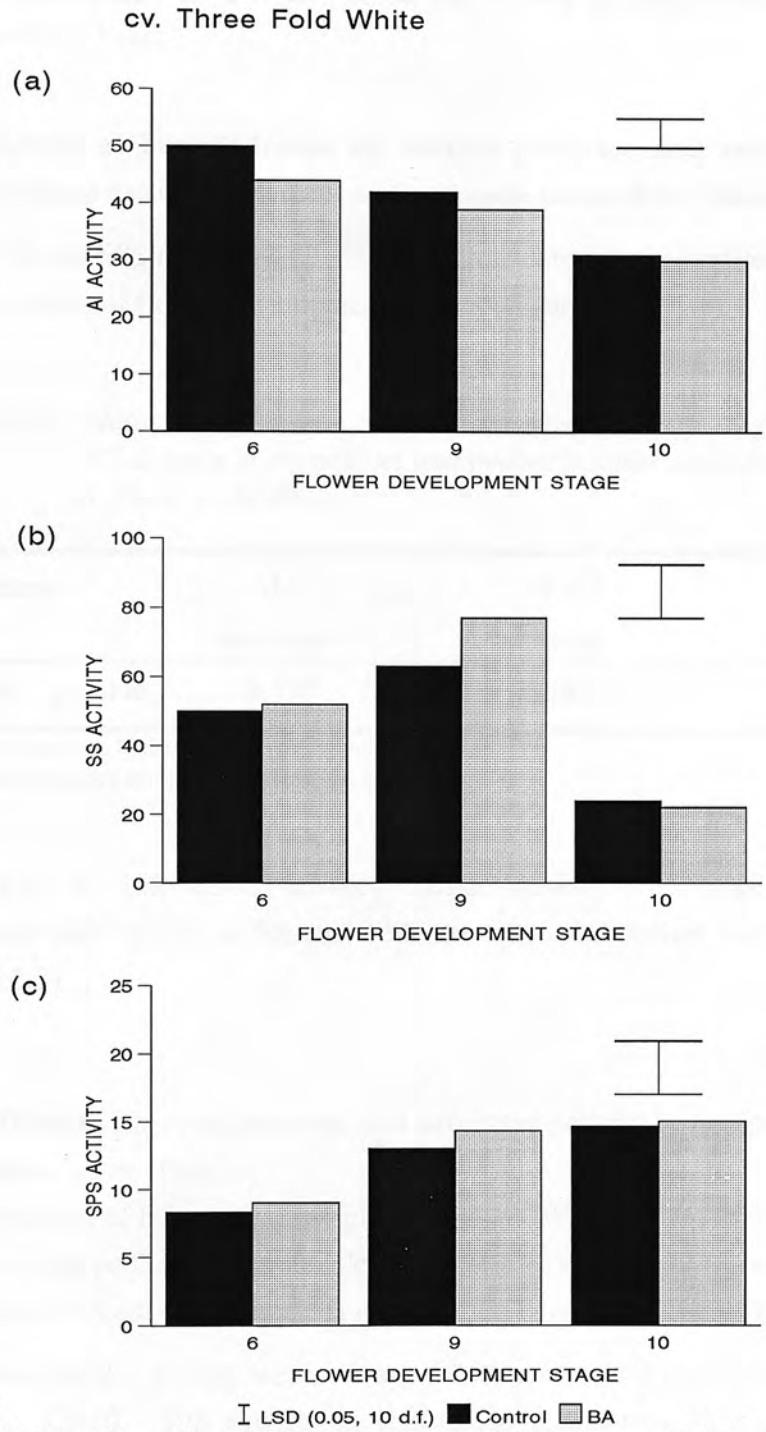

Figure 5.3.1: The effect of BA on (a) acid invertase activity (μmoles sucrose hydrolysed g^{-1} FW h^{-1}), (b) sucrose synthase activity (μmoles sucrose synthesised g^{-1} FW h^{-1}), and (c) sucrose phosphate synthase activity (μmoles sucrose synthesised g^{-1} FW h^{-1}) in pedicel and peduncle tissue collected from plants of cv. Three Fold White. (Actual figures are contained in Appendix 5.5).

In control plants, the average AI activity over all three stages studied was 41 μmoles sucrose hydrolysed g^{-1} FW h^{-1}, while the activity in BA-treated plants was 7% lower ($p<0.05$, Table 5.3.2).

5.3.3 Effect of benzyladenine on sucrose synthase and sucrose phosphate synthase activity in pedicel and peduncle tissue of cv. Three Fold White

Neither SS nor SPS activity were affected by the external application of BA at any of the three stages of flower development studied (Figures 5.3.1b, c).

Table 5.3.3: *Interactive effect of cytokinin and developmental stage on AI, SS and SPS activity in the pedicel and peduncle tissue collected from plants of cv. Three Fold White.*

Interaction		AI (I) activity	SS (II) activity	SPS (II) activity
ck x fds	p value	0.338	0.287	0.931

(I) : μmoles sucrose hydrolysed g fresh $weight^{-1}$ h^{-1}
(II) : μmoles sucrose synthesised g fresh $weight^{-1}$ h^{-1}

There was no interaction between flower development stage and cytokinin application with respect to SS and SPS activity in the pedicel and peduncle tissue (Table 5.3.3).

5.3.4 Effects of benzyladenine on acid invertase activity in pedicel and peduncle tissue of cv. Troy

The application of BA on cv. Troy plants at stage 203(5) did not affect the AI and SS activity at the pedicel and peduncle of flowers at any of the three developmental stages examined either separately (Figure 5.3.2a, b) or collectively (Table 5.3.4).

Differences in SPS activity were measured between control and BA-treated plants at fds-6 and fds-10. SPS activity in BA-treated plants was 33% and 22% higher ($p<0.05$) at fds-6 and fds-10 respectively than equivalent flowers on control plants (Figure 5.3.2c). The differences found at fds-6 and fds-10 reflected a general increase in the average SPS activity over all three stages from 4.4 μmoles sucrose synthesised g^{-1} FW h^{-1} in control plants to 5.0 μmoles sucrose synthesised g^{-1} FW h^{-1} in plants treated with BA (Table 5.3.4).

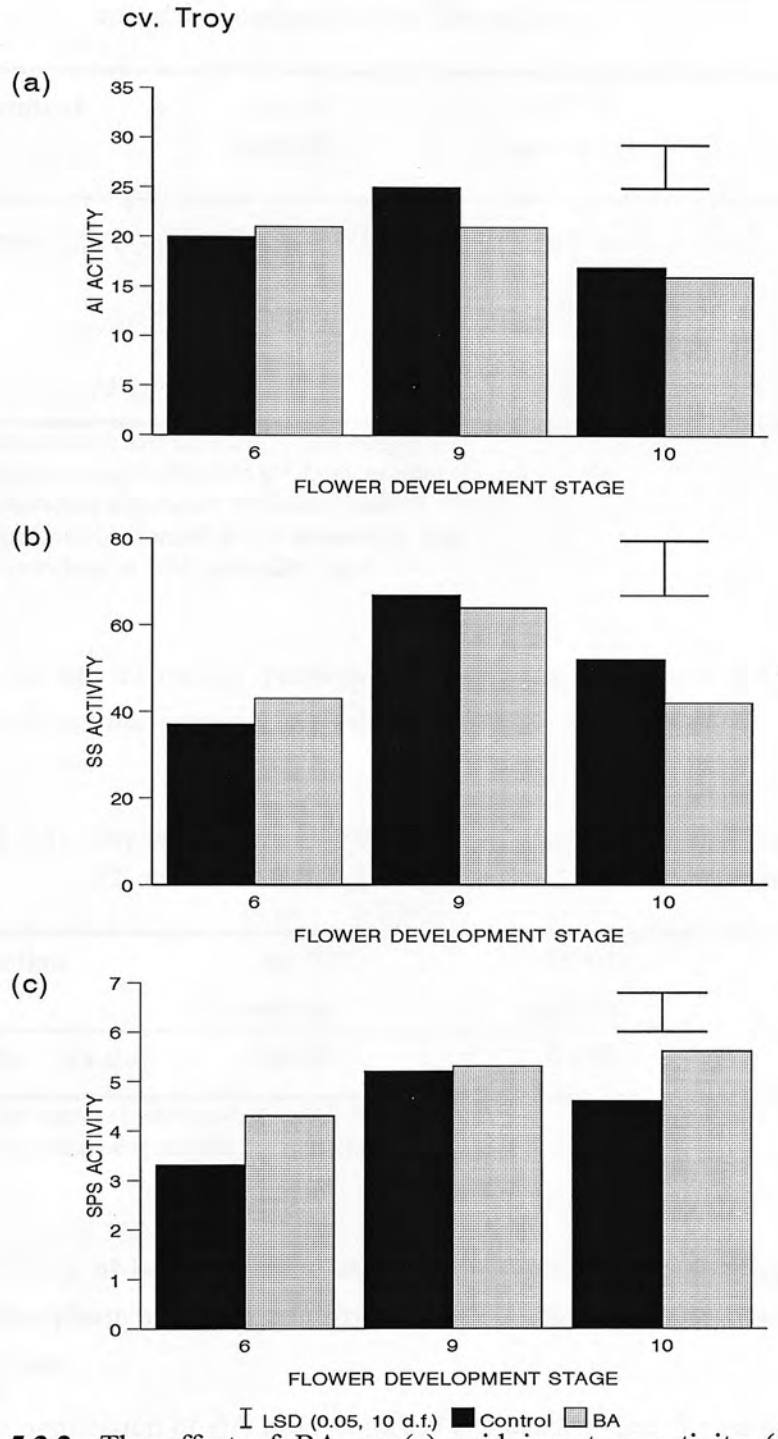

Figure 5.3.2: The effect of BA on (a) acid invertase activity (μmoles sucrose hydrolysed g^{-1} FW h^{-1}), (b) sucrose synthase activity (μmoles sucrose synthesised g^{-1} FW h^{-1}), and (c) sucrose phosphate synthase activity (μmoles sucrose synthesised g^{-1} FW h^{-1}) in pedicel and peduncle tissue collected from plants of cv. Troy. (Actual figures are contained in Appendix 5.6).

Table 5.3.4: *Overall effect of cytokinin application on AI, SS and SPS activity in the pedicel and peduncle of cv. Troy plants.*

Treatment	AI (I) activity	SS (II) activity	SPS (II) activity
Control	21	52	4.4
BA	19^{ns}	50^{ns}	5.0^{*}
LSD (0.05, 10 d.f.)	*2.5*	*7.3*	*0.46*

(I) : µmoles sucrose hydrolysed g^{-1} fresh weight h^{-1}
(II) : µmoles sucrose synthesised g^{-1} fresh weight h^{-1}
ns : no statistically significant difference from the control
$*$: different from the control at 0.05 probability level
(1): LSD calculated at 0.05 probability level

There was no interaction between developmental stage and BA application for activity of the three enzymes studied (Table 5.3.5).

Table 5.3.5: *Interactive effect of flower development stage and BA on AI, SS and SPS activity in the pedicel and peduncle of cv. Troy plants.*

Interaction		AI (I) activity	SS (II) activity	SPS (II) activity
ck x **fds**	p value	0.420	0.198	0.247

(I) : µmoles sucrose hydrolysed g^{-1} fresh weight h^{-1}
(II) : µmoles sucrose synthesised g^{-1} fresh weight h^{-1}

5.3.5 Effects of benzyladenine on acid invertase, sucrose synthase and sucrose phosphate synthase activity in pedicel and peduncle tissue of cv. Toret plants

External application of BA to cv. Toret plants did not alter the AI activity at any of the three stages of flower development studied in this experiment (Figure 5.3.3a).

SS activity at stage 6 was 58 µmoles sucrose synthesised g^{-1} FW h^{-1}. BA application increased SS activity by 26% ($p<0.05$). No effect of cytokinin on the activity of SS was found at stages 9 and 10 (Figure 5.3.3b).

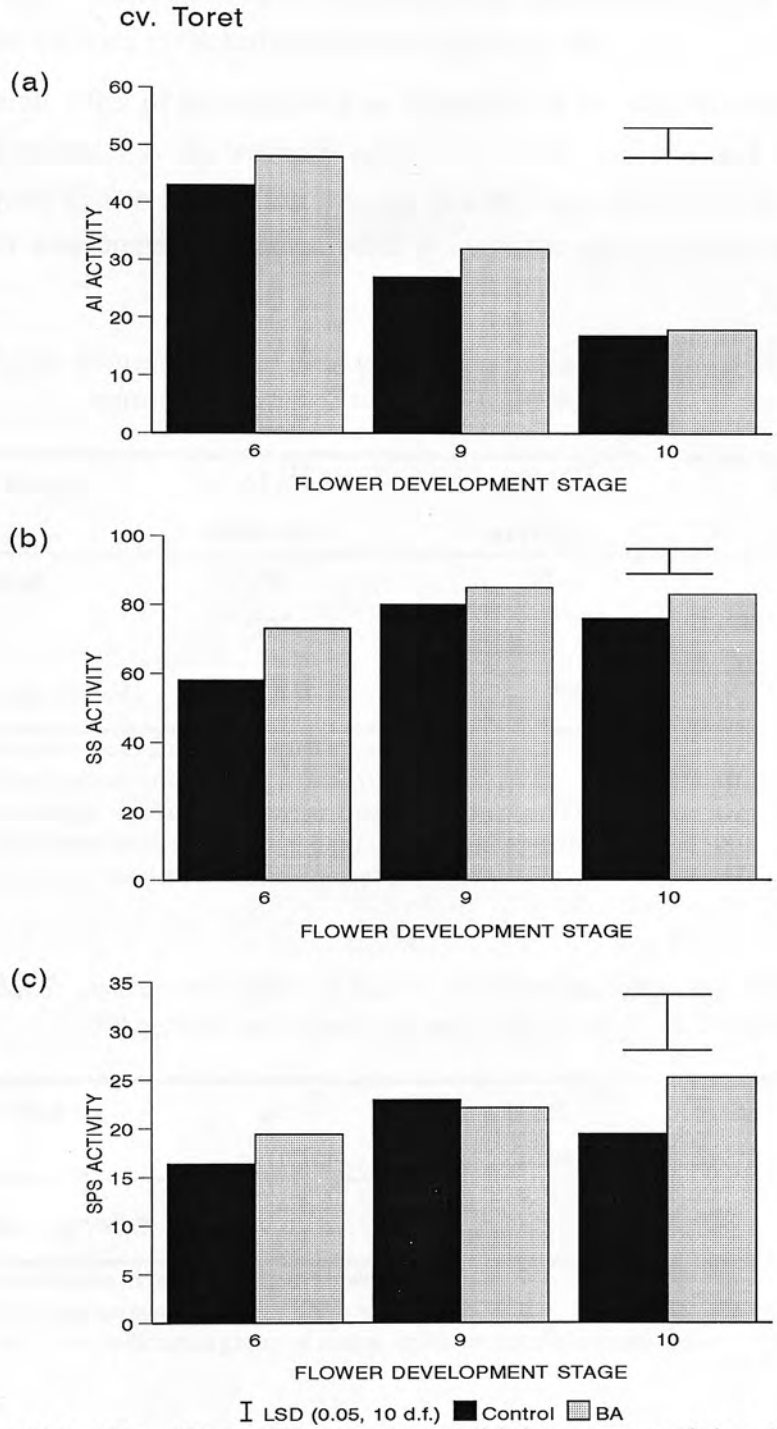

Figure 5.3.3: The effect of BA on (a) acid invertase activity (μmoles sucrose hydrolysed g^{-1} FW h^{-1}), (b) sucrose synthase activity (μmoles sucrose synthesised g^{-1} FW h^{-1}), and (c) sucrose phosphate synthase activity (μmoles sucrose synthesised g^{-1} FW h^{-1}) in pedicel and peduncle tissue collected from plants of cv. Toret. (Actual figures are contained in Appendix 5.7).

In BA-treated plants of cv. Toret, at stage 10, SPS activity was 23% higher ($p<0.05$) than in equivalent flowers on control plants. At stages 6 and 9, SPS activity was not altered by external cytokinin application (Figure 5.3.3c).

The overall effect of BA treatment on AI and SS at the pedicel and peduncle of cv. Toret flowers, was to increase activity by 10% ($p<0.05$) and 13% ($p<0.001$) respectively (Table 5.3.6). The average SPS activity measured over all three stages of flower development was not affected by cytokinin application (Table 5.3.6).

Table 5.3.6: *Overall effect of cytokinin application on AI, SS and SPS activity in the pedicel and peduncle tissue collected from plants of cv. Toret.*

Treatment	AI (I) activity	SS (II) activity	SPS (II) activity
Control	29	71	19.6
BA	32*	80***	22.3ns
LSD (10 d.f.)	*2.4*(1)	*8.7*(3)	*3.3*(1)

(I) : µmoles sucrose hydrolysed g^{-1} fresh weight h^{-1}
(II) : µmoles sucrose synthesised g^{-1} fresh weight h^{-1}
ns : no statistically significant difference from the control
*, *** : different from the control at 0.05, 0.001 probability level
(1), (3): LSD calculated at 0.05, 0.001 probability level

Table 5.3.7: *Interactivel effect of flower development stage and BA on AI, SS and SPS activity in the pedicel and peduncle of plants of cv. Toret.*

Interaction		AI (I) activity	SS (II) activity	SPS (II) activity
ck x **fds**	p value	0.304	0.111	0.233

(I) : µmoles sucrose hydrolysed g^{-1} fresh weight h^{-1}
(II) : µmoles sucrose synthesised g^{-1} fresh weight h^{-1}
a, b, c : each letter indicates a group of means which do not differ statistically

An interactive effect of growth substance application to stage of flower development was not recorded at any of the three enzymes studied in this experiment (Table 5.3.7).

5.4 Discussion

In both these two field studies (Experiments 5.1 & 5.2), pod set was increased with BA treatment. This cytokinin resulted in lower flower drop and hence greater overall pod set at the first five racemes of all varieties tested in 1993. A similar trend was apparent in 1994.

In general it was observed that in the plants sown in early April 1993, there was a greater tendency towards increased pod set than in the plants sown in late April of the following year. In addition, the relative increases in pod initiation with cytokinin treatment appeared to be more profound in 1993 than in 1994 over all tested faba bean cultivars. It is well known that pod set of faba beans is notoriously variable from season to season, particularly on higher racemes (Lawes *et al.*, 1983; Gates *et al.*, 1983; Knott *et al.*, 1994). Therefore, in this study, the differences observed in the proportion of flowers that developed pods in *V. faba* plants in different years may be an effect of the environmental conditions (Appendices 4.1, 5.1) of each year. The environment at Bush, which in general tends to be wet, in combination with the rainy weather in March 1994, resulted in a very late sowing (21 April) that year. In addition, the unusually dry period for Scotland in May and early June 1994 may have further reduced the reproductive potential of the plants. There is enough evidence in the literature to show that yield in *Vicia faba* is responsive to early sowing (Hebblethwaite *et al.*, 1983; Pilbeam *et al.*, 1989; Stutzel *et al.*, 1994); but it is not yet clear which environmental factors are responsible for higher seed yield in earlier sown crops, and how these factors affect the establishment of the reproductive potential of the plant. A possible explanation which has been noted (Hebblethwaite *et al.*, 1991) and which can also be applied in the present study, is that early sown plants have time to develop their root systems to maximum depth by the time the crop reaches the critical stages of pod set; this often means that the early, unlike the late-sown, crop has completed a critical development of roots before water stress occurs. However, it is important to note that, in both years, BA application caused a greater number of pods to set.

Baylis and Clifford (1991) suggested in their review that supplies of assimilates to premature reproductive sinks throughout their development is an important factor that limits the pattern and severity of reproductive loss in grain legumes. It has also been reported that reproductive organs of faba beans (before, at and after anthesis) depend for their development on the supply of assimilates from the reserve in the plant stem as well as from the assimilates concurrently produced in the leaves (Peat,

1983). This observation was indirectly confirmed here by the activity of SPS, which can be regarded as an indicator of sucrose formation in the cytosol of photosynthesising organs (Stitt & Quick, 1989; Cheikh & Brenner, 1992; Galtier *et al.*, 1996). In both experiments (5.1 and 5.2), the activity of SPS was very low relative to the activities of AI and SS. Increases of SPS activity with BA application, as for example in cv. Troy, appeared to be less important for pod set than the changes in the activity of AI and SS in the same sinks. Therefore, sucrose synthesis from photosynthesis in the sampled reproductive tissues may be excluded as a leading factor controlling pod set in this study.

In addition, the existing knowledge of the behaviour of growth substances in the plant (Mothes & Engelbrecht, 1961; Horgan, 1984; Kaminek, 1992; Kuiper, 1993; Ronzhina *et al.*, 1995) suggests that nutrients (sugars and amino acids) can be transported preferentially to regions of high cytokinin activity. On the basis of this evidence, it can be suggested that the application of exogenous cytokinin such as BA to the faba bean plants at flowering overrides the natural plant hormonal system by reducing intra-plant competition and hence giving more flowers the opportunity to set a pod probably by directing assimilates to them. However, the limiting physiological processes for the uptake and accumulation of imported assimilate in the reproductive sinks as well as the way they are affected by cytokinins have not yet been elucidated (Brenner & Cheikh, 1995).

Regulation of the net flow of photoassimilates is an integrated process in higher plants (Stitt & Schulze, 1994). Cytokinins have been reported to be indispensable signals from the root of the plant for assimilate distribution because of their capability of controlling the activity of sinks i.e. the physiological and biochemical processes within the sink that determine its ability to attract assimilates (Beck, 1994). Most of the recent research in sink metabolism has attempted to describe sink activity in biochemical terms (Wyse, 1986; Jenner & Hawker, 1993; Black, 1993; Riffkin *et al.*, 1995). The cleavage of sucrose has been proposed to be an important metabolic step in regulating import of this disaccharide to reproductive organs (see Section 5.1 & below).

Huber and Akazawa (1986) presented the concept of two distinct pathways for sucrose degradation, one mediated by SS and the other by invertase; but their functional significance was not clear. Invertase is responsible for the hydrolysis of sucrose to glucose and fructose (Avigad, 1982). Soluble acid invertase functions in tissues undergoing rapid growth and development, where hexoses are rapidly utilised and sucrose rapidly hydrolysed (ap Rees, 1974; Giaquinta, 1978; Schaffer, 1986).

On the other hand, the main physiological importance of SS appears to lie in its ability to cleave sucrose in the presence of UDP into UDP-glucose and fructose. The reaction is reversible but, as suggested elsewhere (Turner & Turner, 1980; Avigad, 1982; Hawker, 1985), SS is generally considered to act only in the breakdown of sucrose *in vivo*. This reaction requires half the net energy of the sucrose metabolic pathway catalysed by invertase (Black *et al.* 1987). The UDP-glucose released by this cleavage, and its derivatives, are substrates for synthesis of structural and storage polysaccharides (Feingold & Avigad, 1980).

Given the importance of sinks in controlling assimilate distribution (Gifford & Evans, 1981) it seems likely that the diversity in the cytokinin content of the sinks and/or their sensitivity to this substance may affect sucrose hydrolysis which in turn may be an important factor in the relative ability of each sink to import assimilates. Morris (1982) reviewed the correlated changes observed where hormones, including cytokinins, influenced AI activity and assimilate import in a number of plant systems. The role of exogenous cytokinins was inhibitory or without effect.

In the present study, the application of the synthetic cytokinin BA on cv. Maris Bead resulted in higher AI activity in the carpels of flowers, as well as in an increase in the percentage of pod set. However, the same treatment in cv. Troy and cv. Toret enhanced pod set but did not cause any change in AI activity of the carpels. In the pedicel and peduncle tissue studied during the following year's experimentation (field trial 1994), similar results were obtained for Troy and Toret, but in TFW, an increase in pod set with BA application was accompanied by a decrease in AI activity at fds-6. These results, may be regarded as an indication of a different varietal response to cytokinins with respect to sucrose inversion by AI in the vacuole of sink cells and its relationship to pod initiation. However, this interpretation may not be conclusive since the varieties Troy and Toret, and Maris Bead and TFW flowered at different dates. Thus, it is possible that apart from genetic factors, an environmental effect may also be involved in the diversity of the rate of sucrose hydrolysis by AI as affected by exogenous cytokinin (Geiger *et al.*, 1996).

It has been shown that the relationship between soluble acid invertase activity and import rate of ^{14}C was particularly clear during the first week after pollination in bean (*Phaseolus vulgaris* L.) pod enlargement in which this enzyme predominates (Sung *et al.*, 1988). In the literature there is lack of information about the role of soluble AI in developing reproductive sinks before pollination and anthesis, the period which according to our results, seems to be crucial for pod set. This lack of information in reproductive physiology may partly be due to technical difficulties in

collecting enough and uniform (i.e. at the same physiological age and with the same probability of abscission) plant tissue/organs for enzyme analysis and measurement of growth or of developmental parameters. In addition the co-existence in developing fruits of different processes involving assimilate transport, such as pod growth, seed development and transport from pod to seeds, makes it very difficult to interpret the experimental results. Furthermore, the use of growth substances makes this task still more complicated. However, if sucrose hydrolysis before anthesis is a limiting step in the control of pod set, this hypothesis can be indirectly supported by investigations on the ratios of hexoses to sucrose in the reproductive organs with known degrees of abortion.

The second enzyme assayed in this study which is involved in sucrose cleavage was that of sucrose synthase. Application of BA to the plants was accompanied by a rise in SS activity in extracts of developing carpels when the flowers were at fds-6 in cv. Toret and in cv. Maris Bead. Since it is known that cytokinin stimulates cell division at sinks (Bernier *et al.*, 1977; Section 1.3), elevated SS activity at the period before pollination probably resulted from stimulation of active cell-division in the BA-treated carpel, and would therefore be followed by an increased demand for structural carbohydrates. Thus an increase in the capacity of sucrose cleavage by SS might be required to provide hexoses and sugar nucleotides for both cell wall synthesis and respiration at the carpels. These metabolic changes in turn, cause more assimilates to be mobilised to the carpels, giving them the opportunity to continue their development and the flowers to set pods. This explanation is also in line with findings showing that near and at the opening of the petals, the flowers of *V. faba* are in a period of decreased growth (Peat, 1983). These findings are also confirmed here with the data showing carpel growth among developmental stages in terms of fresh weight (Table 5.2.2). At this stage (i.e. near fds-9) the destiny of a flower to develop into a pod or to abscise is determined. Flowers competing better for assimilates (measured in disintegration of ^{14}C per minute) were those which set a pod (Peat, 1983).

The role of SS may also be, apart from the supply of hexoses for maintenance or for structural products, to supply substrates for starch synthesis (Robinson *et al.*, 1988; Riffkin *et al.*, 1995). Recent work on the immunolocalisation of SS in the seed-coat of peas shows that the enzyme was closely associated with the starch parenchyma, and SS activity was closely associated with starch synthesis within the pea embryo (Rochat *et al.*, 1996). These observations are paralleled by studies in histochemical localisation of SS in developing kernels of maize (Wittich, 1996). The SS was found

to be particularly active in the top of the endosperm where starch was synthesised, confirming the results of enzyme extraction in the same tissue (Doehlert, 1990).

The importance of this role of SS, i.e. its involvement in starch synthesis, may be crucial for pod set at the pedicel and peduncle area where vascular differentiation takes place. Gates and his co-workers (1981), in their cytological studies on the pedicel:peduncle junction observed the presence of starch in the cells of a sheath and parenchyma around the vascular bundles near the opening of the flower of *V. faba*. This amount of storage carbohydrate subsequently disappeared as the vascular differentiation in this area proceeded which in turn led to successful pod set. In the present field experiment in 1994, the application of BA on cv. Toret caused 33% higher percentage of pod set in treated than in untreated plants. This increase in pod set was accompanied by an analogous increase (26%) in SS activity in the pedicel and peduncle at fds-6, i.e. before pollination and fertilisation. It can be suggested therefore that BA increased the ability of a flower to set a pod by stimulating SS activity, which consecutively resulted in a greater amount of starch being stored transiently for later support of vascular differentiation, which in turn resulted in great mechanical strength at the pedicel and peduncle junction and thus led to successful pod initiation. This suggestion is indirectly supported by recent evidence which shows the presence of a positive relationship between exogenously applied cytokinins in cereals and starch content in the plants (Kaminek *et al.*, 1996). However, the lack of direct evidence to support this hypothesis, and the observation in the present study that in the broad bean variety TFW, a 37% increase in pod set with BA application was not parallel with a concomitant increase in SS activity, do not allow further generalisations. Nevertheless, the role of cytokinin in starch accumulation in the reproductive organs preceding abscission remains an interesting area for future research, because it has also been claimed (Oberholster *et al.*, 1991) that this starch is a source of energy supply for the differentiation of separation layers and protective layers in the abscission zone. However, as in the case of AI, quantitative studies on this aspect involve the difficulties mentioned above for invertase.

Most transfer processes contributing to assimilate translocation through the source-path-sink transport system would appear to be susceptible to hormonal control (Patrick, 1982; 1991). The interaction of sink metabolism with source metabolism and phloem transportation of photoassimilates, and the way in which this interaction controls pod set in *Vicia faba* and other grain legumes has not yet been elucidated.

The present preliminary work attempted to connect the exogenous supply of cytokinin, pod set and reproductive sink activity in terms of sucrose cleavage in the reproductive organs of faba beans. The activity of single enzymes was used as a suitable measure of sink activity. This approach is based on the assumption that these enzymes are the limiting and thus controlling determinants in the metabolic pathway, and that their activity measured *in vitro* corresponds with that *in vivo*. It should be noted, however, that sucrose hydrolysis by soluble AI and by SS forms only two of many possible biochemical controlling steps in regulating the import of sucrose into sink organs. Recent findings have revealed the existence of various isoforms (bound and free SS, and cell-wall-bound, vacuolar and cytosolic invertase) of these two sucrose-cleaving enzymes in plant cells (Krausgrill *et al.*, 1996; Sturm, 1996). These findings combined with the unknown regulatory mechanisms of each metabolic pathway in which each one of these isoforms is involved, at each part of the cell, at each tissue of the sink organ, at each developmental stage and at each cultivar, makes the system too complex to be investigated within the limits of this thesis, because it involves a very great commitment of time, resources and labour effort. An alternative approach might be the measurement of the concentrations of the main metabolites in the pathway of sucrose cleavage, and the way they are affected by BA.

The immediate period of flower development before anthesis appears to be a time of crucial physiological changes controlling pod set, with the cytokinins playing a leading role. Thus, further investigation should be focused on local application of cytokinins on each flower at fds-6.

Furthermore, the difficulties which arose because of the unfavourable weather, in applying the growth substances, particularly in 1994, and the variation in the flowering dates among the varieties testified to the need to continue experimental work under controlled environmental conditions.

♦ ♦ ♦

Chapter 6

Effects of BA on sucrose, glucose and fructose contents of developing carpels in relation to pod set

6.1 Introduction

External application of the synthetic cytokinin 6-benzylaminopurine (BA) has been found to give consistent enhancement of fruit set in faba beans both under controlled (Chapter 3) and field (Chapters 4 & 5) conditions. Its physiological effect on reproductive sinks, however, has yet to be clearly elucidated.

As discussed in Sections 4.14 and 5.4, flower retention and development in the faba bean plant appear to be regulated by a complex network of underlying physiological processes and biochemical mechanisms in which cytokinins seem to play an important role. The hypothesis was developed in Chapter 5 that sucrose cleavage in sinks before pollination may be a significant component of this interaction. The results of the experimentation, however, were not sufficient to show with certainty that stimulation of sucrose metabolism either by AI or SS in sink tissues of *V. faba* with the use of BA is the leading factor that enhances pod set (Section 5.4).

The sucrose content of a sink is the net result of the rates of its supply and use. Therefore, the measurement of the levels of sucrose in relation to other sugars produced at the initial stages of sucrose metabolism can provide valuable information on the balance between supply and demand for assimilated carbon at a given sink tissue or organ (Farrar & Williams, 1991a, b; Koch *et al.*, 1992, 1995; Koch & Nolte, 1995; Pollock & Farrar, 1996).

It was suggested in Section 5.4 that the investigation of the various factors involved in successful pod initiation could be simplified provided that localised applications of BA could be used on each flower, and both the positional effects and the stage of flower development could be taken into account. In cv. Toret, the observations that: (a) BA treatment steadily enhanced pod initiation (Chapters 4 & 5), (b) its plants exhibit the typical intra- and inter-raceme pattern of pod set, with pod initiation at proximal flower positions and lower racemes, (c) there is low inter-plant variation for pod set (Chapter 4), and (d) fds-6 appeared to be more responsive to cytokinin application in terms of sucrose cleavage (Chapter 5) provided adequate grounds for using this cultivar in the present experiment.

The purpose of this study therefore was to examine the effect of exogenous application of BA to flowers of *Vicia faba* cv. Toret on the content of key soluble carbohydrates (Section 2.6) involved in the initial steps of sucrose metabolism in developing carpels, and relate these data to successful pod set.

6.2 The effect of benzyladenine on percentage pod set

In control plants, none of the flowers formed at the first three flower positions under investigation set any pods (Plate 6.2.1). However, at position 4 20% of the flowers developed into pods (Table 6.2.1). Local application of BA on each flower before pollination and fertilisation (fds-6) dramatically increased percentage pod set at all flower positions (Plate 6.2.2). Complete pod set was obtained at the positions 4,5,6 ($p<0.001$), and 95% ($p<0.001$) at position 7 (Table 6.2.1).

Overall percentage of pod set at the sites where sampling took place was also increased from only 5% in controls to 99% ($p<0.001$) in BA-treated flowers (Table 6.2.1).

Table 6.2.1: *Effect of BA on percentage pod set at those positions where sampling took place.*

Treatment	Flower position on the raceme				
	4	5	6	7	*mean*
	Percentage of pod set				
Control	20	0	0	0	*5*
BA	100***	100***	100***	95***	*99****

*** : different from the control at 0.001 probability level

6.3 The effects of benzyladenine and of flower development stage on fresh weight of carpels

Brushing of BA solution on faba bean flowers before pollination and fertilisation did not affect the fresh weight of carpels collected at any of the three developmental stages after the application of the chemical (Table 6.3.1).

Each carpel of the untreated flowers had an average fresh weight of 20.5 mg. This did not change markedly in carpels of BA-treated flowers (Table 6.3.1).

The stage of carpel development had no effect on the average fresh weight of individual carpels, which varied between 21.0 mg at fds-6 and 22.6 mg at fds-10 (Table 6.3.1).

Plate 6.2.1: *Pattern of pod set on control plants.*

Plate 6.2.2: *Pattern of pod set on plants treated with BA.*

Table 6.3.1: *Effect of BA on the fresh weight of faba bean developing carpels.*

Treatment	fds-6	fds-9	fds-10	mean
	Fresh weight (mg carpel^{-1})			
Control	19.5	21.8	20.0	20.5
BA	22.2^{ns}	22.5^{ns}	25.3^{ns}	23.3^{ns}
mean	21.0^{a}	22.1^{a}	22.6^{a}	*1.8$^{(1)}$*

ns : no statistically significant difference from the control
(1) : LSD (15 d.f., 0.05 probability level) for fds means
a : this letter indicates a group of means which do not differ statistically

6.4 The effects of benzyladenine on the content of water-soluble sugars

6.4.1 Sucrose content

The application of the synthetic cytokinin BA on the flowers at stage 6, induced 63% ($p<0.05$) more sucrose accumulation per g of carpel fresh weight than in the controls, measured 24h after the treatment (Figure 6.4.1a).

When sucrose content was measured on the basis of each carpel, it was 88.9% higher ($p<0.05$) in BA-treated flowers than in controls at fds-6 (Figure 6.4.2a). At none of the stages more advanced than fds-6, were any changes in sucrose content due to BA-application recorded in the reproductive organs measured either on a per g of carpel fresh weight or per carpel basis (Figure 6.4.1a & 6.4.2a).

Table 6.4.1: *Overall effect of cytokinin and interactive effect of flower development stage and cytokinin on sucrose content in developing carpels.*

Factor	Treatment	Sucrose mg/g FW	Sucrose mg/carpel
Cytokinin	Control	11.1	0.23
	BA	11.9^{ns}	0.27^{ns}
ck x fds	p value	0.035	0.036

ns: no statistically significant difference from the control

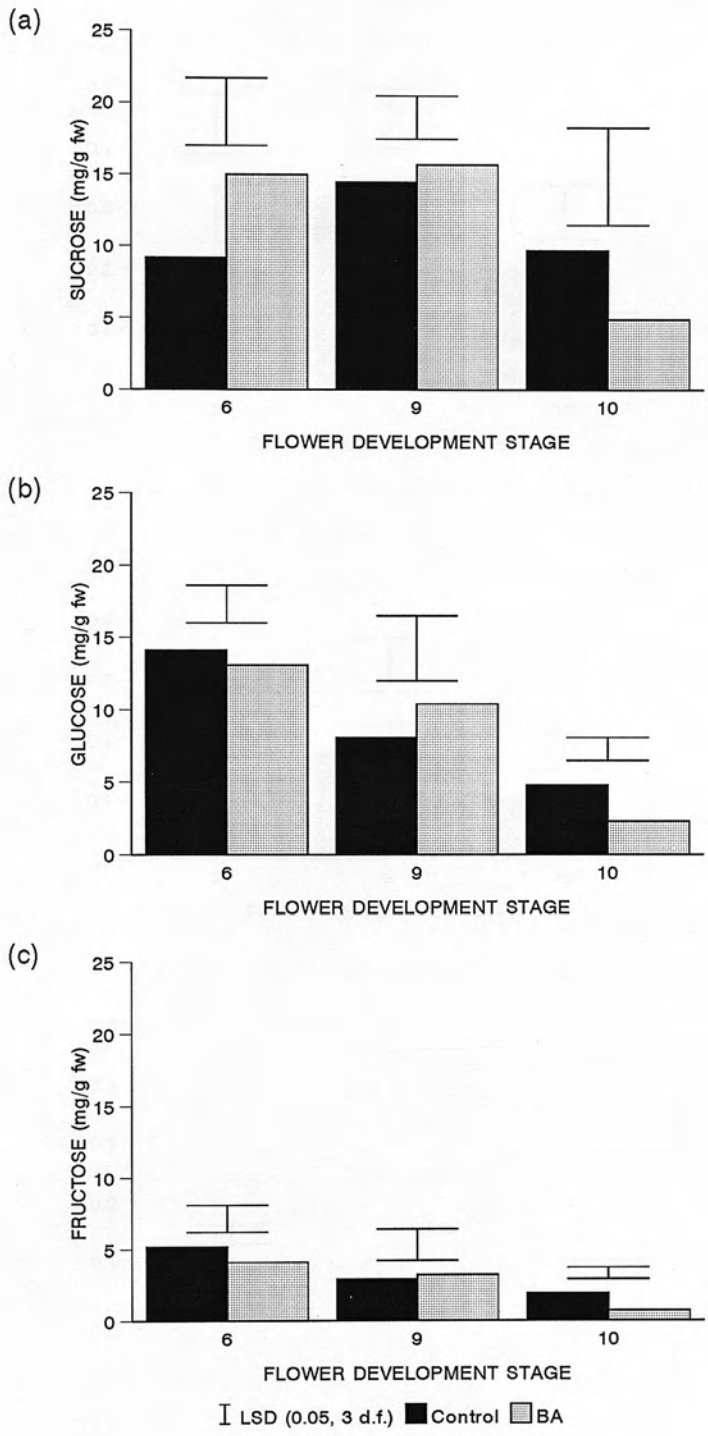

Figure 6.4.1: Effects of BA on (a) sucrose, (b) glucose and (c) fructose contents (mg g^{-1} FW) of carpels, measured at three stages of flower development.

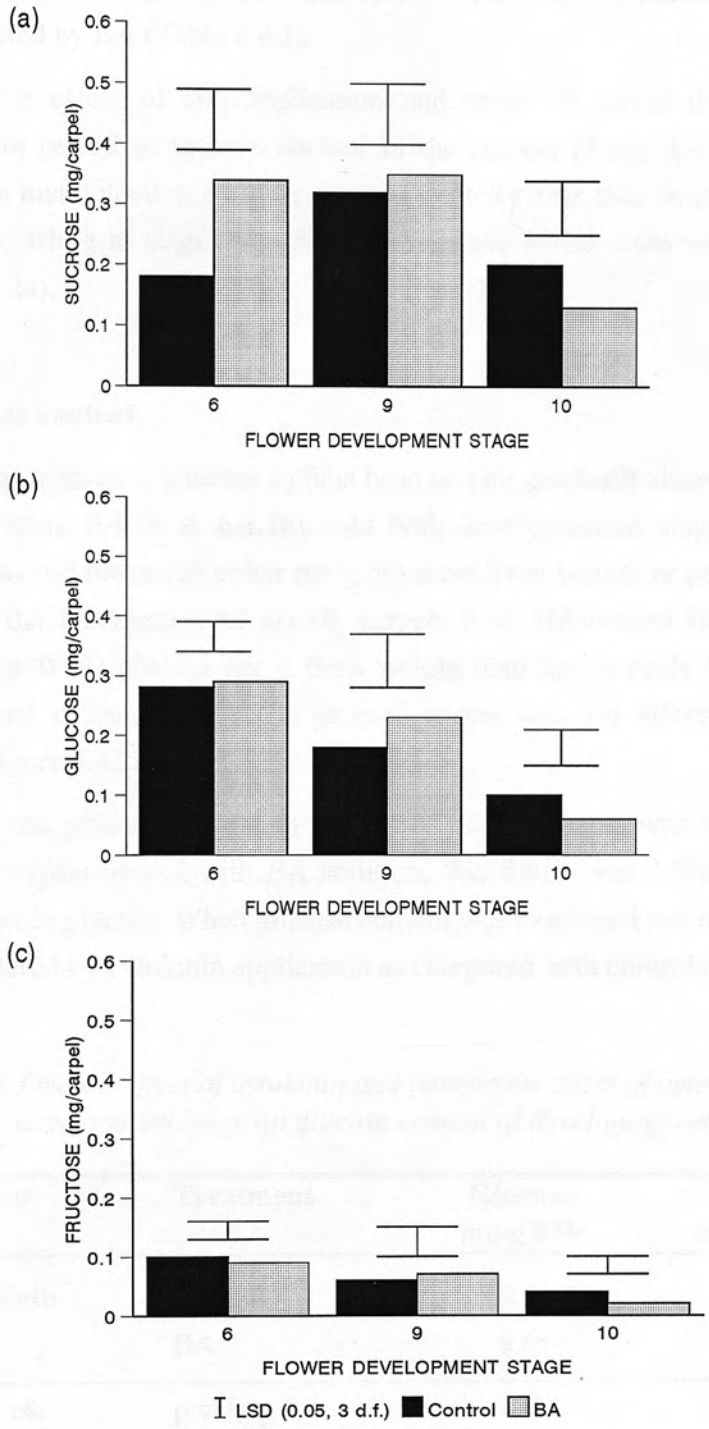

Figure 6.4.2: Effects of BA on (a) sucrose, (b) glucose and (c) fructose contents (mg carpel^{-1}) of carpels, measured at three stages of flower development.

The average amount of sucrose found in carpels collected from control plants was 11.1 mg g^{-1} fresh weight or 0.23 mg $carpel^{-1}$ sucrose. In neither case was this amount affected by BA (Table 6.4.1).

An interactive effect of BA application and stage of carpel development was observed with regard to sucrose content in the carpels (Table 6.4.1). BA caused sucrose to be maintained at a higher level in treated rather than in control flowers at stage 6 only, while at stages 9 and 10 the sucrose levels were unaltered (Figures 6.4.1a & 6.4.2a).

6.4.2 Glucose content

In general, the amount of glucose in faba bean carpels gradually decreased from fds-6 to fds-10 (Figure 6.4.1b & 6.4.2b). At both developmental stages 6 and 9, the amount of glucose measured either per g of carpel fresh weight or per carpel was not affected by BA treatment. At fds-10, carpels from BA-treated flowers contained 52.1% less ($p<0.05$) glucose per g fresh weight than the controls (Figures 6.4.1b), while the total amount of glucose in each carpel was not affected by the same treatment (Figure 6.4.2b).

On average, the glucose content in carpels of control plants was 9.0 mg g^{-1} fresh weight. In carpels treated with BA solution, this figure was 4.5% lower ($p<0.05$) than in untreated plants. When glucose content was expressed per individual carpel, it was not altered by cytokinin application as compared with controls (Table 6.4).

Table 6.4.2: *Overall effect of cytokinin and interactive effect of cytokinin and flower development stage on glucose content of developing carpels.*

Factor	Treatment	Glucose mg/g FW	Glucose mg/carpel
Cytokinin	Control	9.0	0.18
	BA	8.6*	0.19^{ns}
fds x ck	p value	0.024	0.038

ns: no statistically significant difference from the control
* : different from the control at 0.05 probability level

Interaction between stage of carpel development and BA treatment was observed in the glucose content of the carpels (Tables 6.4.2). This interactive effect was a

reflection of the decreased glucose content at fds-10 because of BA treatment (Figures 6.4.1b & 6.4.2b).

6.4.3 Fructose content

As with glucose content, fructose content of carpels collected either at fds-6 or fds-9 was not affected by the application of BA-solution locally on each flower before its pollination and fertilisation (Figures 6.4.1c & 6.4.2c). At fds-10, the amount of fructose per g fresh weight of BA-treated carpels was found to be 63.2% lower ($p<0.05$) than in controls (Figure 6.4.1c), whereas the total amount of fructose in each carpel was not affected by this treatment (Figure 6.4.2c).

In control carpels, the average content of fructose g^{-1} fresh weight over all three flower development stages studied was 3.3 mg. In cytokinin treated plants, this figure was 18.2% lower ($p<0.05$) than in the controls (Table 6.4.3). On per organ basis, the average amount of fructose in controls was 0.07 mg, and it was not affected by the external application of BA (Table 6.4.3).

Table 6.4.3: *Overall effect of cytokinin, and interactive effect of cytokinin and flower development stage on fructose content of developing carpels.*

Factor	Treatment	Fructose mg/g FW	Fructose mg/carpel
Cytokinin	Control	3.3	0.07
	BA	2.7*	0.06^{ns}
fds x ck	p value	0.506	0.601

ns: no statistically significant difference from the control
* : different from the control at 0.05 probability level

There was no interaction between stage of carpel development and cytokinin treatment with regard to fructose content in the carpels (Tables 6.4.3).

6.4.4 Total content of reducing sugars

The greatest effect of BA on reducing-sugar content (i.e. glucose plus fructose) expressed per g of carpel fresh weight, was obtained at fds-10. BA application caused a 54% ($p<0.05$) reduction compared to controls (Table 6.4.4).

The amount of glucose plus fructose in each carpel was not affected by cytokinin treatment, at any stage of carpel development (Table 6.4.4).

Table 6.4.4: *Effect of BA on the content of reducing-sugars in carpels at three stages of flower development.*

Flower development stage	Treatment	Reducing Sugars mg/g FW	Reducing Sugars mg/carpel
fds-6	Control	19.5	0.38
	BA	17.3^{ns}	0.38^{ns}
fds-9	Control	11.3	0.24
	BA	13.8^{ns}	0.30^{ns}
fds-10	Control	6.5	0.14
	BA	3.0*	0.08^{ns}

ns: no statistically significant difference from the control
* : different from the control at 0.05 probability level

The average amount of reducing sugars over all three stages of carpel development was not altered by BA application to flowers as compared with controls (Table 6.4.5).

Table 6.4.5: *Overall effect of cytokinin and interactive effect of cytokinin and flower development stage on the content of reducing sugars in developing carpels.*

Factor	Treatment	Reducing sugars mg/g FW	Reducing sugars mg/carpel
Cytokinin	Control	12.4	0.25
	BA	11.3^{ns}	0.25^{ns}
fds x ck	p value	0.041	0.038

ns: no statistically significant difference from the control

An interaction of developmental stage and BA treatment was measured with regard to the content of reducing sugars in carpels (Table 6.4.5). Flowers collected after petal collapse responded to BA application by reducing the amount of glucose and fructose in the carpels (Table 6.4.4).

6.4.5 Total content of water-soluble sugars

The accumulation of all three water-soluble sugars measured in this study, i.e. sucrose plus glucose plus fructose, in carpels of flowers treated with BA at a stage before pollination and fertilisation did not differ markedly compared with the controls at any of the three stages of flower development (Table 6.4.6).

Table 6.4.6: *Effect of BA on the content of water-soluble sugars in carpels at three developmental stages.*

Flower development stage	Treatment	Soluble sugars mg/g FW	Soluble sugars mg/carpel
fds-6	Control	28.5	0.56
	BA	32.0^{ns}	0.72^{ns}
fds-9	Control	25.5	0.55
	BA	29.3^{ns}	0.66^{ns}
fds-10	Control	16.5	0.34
	BA	8.3^{ns}	0.20^{ns}

ns: no statistically significant difference from the control
$*$: different from the control at 0.05 probability level

On average, the content of water-soluble sugars in control carpels was 23.5 mg g^{-1} fresh weight or 0.48 mg $carpel^{-1}$ (Table 6.4.7). In neither case was any effect observed due to BA treatment (Tables 6.4.7).

Table 6.4.7: *Overall effect of cytokinin and interactive effect of cytokinin and flower development stage on the content of water-soluble sugars in developing carpels.*

Factor	Treatment	Soluble Sugars mg/g FW	Soluble sugars mg/carpel
Cytokinin	Control	23.5	0.48
	BA	23.2^{ns}	0.53^{ns}
fds x ck	p value	0.028	0.026

ns: no statistically significant difference from the control

An interactive effect between BA application and flower development stage was found when sugar content was measured both per g of carpel fresh weight and per carpel (Table 6.4.7).

6.5 Discussion

Flower and pod development on individual faba bean racemes in cv. Toret plants proceeds sequentially from proximal to distal flowers with a greater proportion of abscission occurring at floral sites more distal than site 3 (Section 4.5.1.5). For the purposes of this study, plants were grown in a growth chamber and the treatments were limited to flowers at positions 4 to 7 and at stages 6 to 10, in order to ensure that both treated and control flowers were at comparable stages of development and subject to identical environmental conditions during the specific treatment periods (Section 2.6). Furthermore, working with potentially abscising flowers located at distal nodes on racemes ensured proper controls to investigate the possible changes in soluble-sugar content caused by BA application that may allow reproductive organs to set during the critical period of flower development when the retention or abscission of the flower is determined. The sampling system relied on probability of pod set based on the location on the raceme and the effect of cytokinin. The results showed that this procedure had the ability to determine the desired levels of percentage pod set. On average, the samples from BA treated plants, had almost all set pods and the abscising (i.e. control) samples only 5% (Table 6.2.1).

This experiment also demonstrates the important role of cytokinin at fds-6 in initiating pod set in *Vicia faba*, thus supporting the findings of Chapter 5 (Section

5.4.2). In this experiment, through carpel excision of flowers at fds-6, the start of anther opening and the release of pollen grains, i.e. the beginning of anthesis was observed. This is in agreement with previous reports that anther dehiscence usually occurs at the hooded stage of bud development in *V. faba* (Lawes *et al.*, 1983) i.e. fds-6. Brushing of BA on the sepals of the calyx 24 h before anthesis (i.e. before anther dehiscence) completely eliminated abscission of flowers in cv. Toret plants. This result was due to the external application of the cytokinin before pollination (i.e. hand -tripping) and before any possible self-fertilisation of the flower in the absence of tripping (auto-fertility), since the time-interval between pollination and fertilisation in *Vicia faba* has been found to be less than a day (Johansson & Walles, 1993a; Lazaridou, personal communication). A similar effect of BA has been reported by Rylott & Smith (1990) in the broad bean variety Three Fold White. These workers selected two stages of flower development, 24 h before and 24 h after hand-tripping and studied the timing of cytokinin treatment on broad bean flowers. They found that all treated inflorescences showed enhanced pod initiation as compared with their respective controls whatever the timing of application. However, the percentage of pod set in those flowers treated with BA 24 h before pollination was higher than in those treated 24 h after pollination. These observations have also been confirmed by more recent findings in localised BA application on flowers at various developmental stages in the main inflorescence of *Lupinus angustifolius* L. (Atkins & Pigeaire, 1993).

The induction of pod initiation with BA application coincided with 63% or 89% more sucrose in carpels at fds-6 as compared with untreated flowers, expressed on concentration or on carpel basis respectively (Section 6.4.1). Sucrose content in BA-treated flowers was kept at a high level until the petals of the flower were fully opened (fds-9); at this stage it was not significantly different from that in control plants. Afterwards, from fds-9 until fds-10, sucrose content of carpels fell with no difference between treated and untreated flowers. The data for pod set come from plants of the same experiment (grown at the same time under the same conditions) as those in which carbohydrates were determined; so this change in the sucrose status of the reproductive sinks in response to external BA application suggests that sucrose accumulation in carpels of *Vicia faba* at the beginning of anthesis (stage 6) and possibly its maintenance at a high level until full flower opening (stage 9) is important for successful pod initiation.

Furthermore, the net increase in sucrose level 24 h after cytokinin application indicates that, in the carpels destined to set pods, the rate of sucrose import and/or

accumulation is higher than the rate of its metabolism; i.e. sucrose supply exceeds demand in these organs. It can be assumed that there were no differences in the supply of sucrose either from current photosynthesis or from mobilising reserves in the stem (Peat, 1983) between control and cytokinin-treated plants, since all were cultivated under the same precisely-controlled conditions. Thus, given this assumption of equal supply, it can be supposed that demand for carbohydrates differs between cytokinin-treated and untreated carpels. This in turn means that in carpels destined to set a pod as compared with those destined to drop either sucrose consumption by the carpel was lower, and/or import of sucrose from the sources was higher, since the carpels at fds-6 were regarded as non-photosynthesising organs and thus they could not produce sucrose by photosynthesis (Section 5.4). It can be suggested, therefore, that the higher level of sucrose at the beginning of flower anthesis may be a critical carbon source for maintaining the growth and development of the carpels during the lag phase which follows after fertilisation when assimilates are also being used for vascular differentiation of the pedicel-peduncle junction (Gates *et al.* 1981). The presence of this lag phase on post-anthesis flower development was indirectly revealed in this study by the fall in the contents of all three carbohydrates measured at fds-10 in untreated as well as treated flowers (Tables 6.4.2, 6.4.3 & 6.4.5).

An important point is that in the present study the measurement of sucrose and the measurements of glucose and fructose at the same tissues were done at the same time. These data show that the content of sucrose detected at fds-6 was higher in BA-treated carpels than in untreated, and was concomitant with unchanged levels of glucose or fructose contents as compared with the controls. Thus it would appear that sucrose depletion in both treated and untreated carpels was at similar levels. This was so, because if sucrose was metabolised in the treated carpel, and its initial stages of metabolism are cleavage by either SS or invertase (Section 5.1), as a consequence, sucrose breakdown would result in higher levels of glucose and/or fructose, something which was not found in this study. It seems therefore that either sucrose import into or sucrose production within the carpel possibly from the degradation of storage products such as starch (Hill-Cottingham, 1983) was induced during the first 24 h after the application of the growth substance. Rapid starch turnover has been reported to occur in many sinks, but it does not appear to act as a buffer between import and metabolism of sucrose (Farrar, 1991 and references therein). By comparison, sucrose status of a tissue as it is affected by growth substances has been shown to have a controlling role in sink metabolism, and thus in

its development, probably at the level of gene expression (Kauffman *et al.*, 1973; Claussen, *et al.*, 1986). In addition, it has been reported that sucrose as a substrate affects the biochemical control of carbon metabolism which, in turn, regulates carbon partitioning in source and sink regions (Geiger & Servaites, 1994). In the data presented here, however, not all the carbohydrate resources which are physiologically significant for carpel development have been measured. If there is such a pool of carbohydrates, it can be identified by measuring simultaneously starch and soluble sugars and by including radio-active carbon data reflecting contributions from current photosynthesis.

Differences were observed among the contents of sucrose, glucose and fructose expressed on per carpel basis, at fds-6 and fds-9; but no difference was found when all three sugars were measured together. The data displayed at Table 6.4.6 show that the content of total soluble sugars (i.e. sucrose, fructose and glucose together) was not affected by BA application at any stage of flower development. These results combined with the clear effect of BA on pod set, indicate the importance of measuring the contents of each individual carbohydrate compound, since measuring only their pooled content seems to be of little value because it remains constant and thus does not shed much light on the physiological processes under investigation.

The results presented in Table 6.4.7 concerning total soluble sugars appear to contradict observations of previous workers on soybean plants (Dybing *et al.*, 1986). These workers presented a timecourse of biochemical events covering the period from anthesis to abscission of developing carpels. It was shown in their studies that the content of ethanol-soluble carbohydrates was higher in setting than in abscising ovaries when it was calculated on per organ basis, while it was unchanged on per mg of ovary fresh weight basis. Part of the reason for the disagreement with the results of this study may be the differences in plant species and thus in metabolism, experimental conditions, and sugar analyses employed in each case; these make further discussion in terms of soluble sugars and pod set unprofitable. Nevertheless, it should be noted that the measurements of the fresh weight in this soybean study revealed that carpels that set grew more rapidly than abscising carpels. This finding is partly in agreement with the results presented here in Table 6.4.2, which show that BA-treated, i.e. setting, carpels at fds-10 were 26.5% heavier (statistically insignificant) than the corresponding controls. Because of this increase in carpel fresh weight of treated flowers at fds-10, the contents of glucose and fructose showed a decrease of 52.1% and 63.2% respectively when expressed on per g fresh weight, while they remained unchanged when measured on per carpel basis (Figure 6.4.2).

This evidence of fresh-weight increases may be regarded as an indication that growth parameters of the reproductive sinks such as growth rate and relative growth rate, and their inter-relationships with hormonal factors like cytokinin and with successful pod initiation need to be examined in future studies.

In conclusion, the results of the present study seem not to confirm the results of Chapter 5 concerning the hormonal control of the levels of activity of sucrose-cleaving enzymes (i.e. the activity of reproductive sinks) which is probably related to pod set. It is more likely that the response of the faba bean flowers to external application of cytokinins in terms of pod set and sugar status of the reproductive sinks is a complex and integrated sequence of biochemical reactions in which the increase in sucrose cleavage activity may be secondary rather than primary. However, these observations come from two different experiments, i.e. (1) from the field trial of 1993 and (2) from the present experiment. In both experiments an increase in pod set was achieved with external application of cytokinin, but different methodologies were employed in biochemical analysis and thus their results are not comparable. However, all these findings point to the need for more conclusive studies when flowers are at the beginning of anthesis (fds-6) i.e. before pollination. Soluble sugar contents and starch quantity, respiration and sucrose import rates, and the activity of certain enzymes (e.g. SS) in crude extracts need to be comparatively studied during anthesis in both cytokinin-treated and untreated flowers under the same experimental conditions. These investigations are important for establishing a timetable of biochemical events that might be useful in identifying processes that initiate flower retention and pod set, but may be difficult because of the problems outlined in Sections 4.14 and 5.4.

♦ ♦ ♦

Chapter 7

General discussion, conclusions and future prospects

7.1 General discussion

Among crop plants there are two distinct developmental pathways for the storage organs, depending on whether the growth of the harvestable parts is predominant and terminal (as in the cereals) or continues over a long period in equilibrium with the growth of other organs (as in sugar cane and beet). Pulse crops like *V. faba* are in an intermediate position having for a while vegetative and reproductive growth simultaneously (Peat, 1983; Evans, 1993). A characteristic of the reproductive development of the faba bean plant is that it suffers from excessive loss of its generative organs at various stages of their development (Sections 1.5). This loss is very variable and has a great influence on the final yield of the crop (Dantuma & Thompson, 1983; Rylott, 1991; Flores *et al.*, 1996). Genetic factors (Gates *et al.*, 1983) and environmental conditions (El-Rhaman *et al.*, 1980; Husain *et al.*, 1988), as well as their interaction (Smith, 1982c; Pilbeam *et al.*, 1989; Pilbeam *et al.*, 1990; Flores *et al.*, 1996) have been shown to determine the levels of the drop of reproductive organs in faba bean plants (Section 1.5). There is lack of understanding about the controlling mechanisms in many crucial reproductive loss- or retention-determining processes (Baylis & Clifford, 1991; Marshall & Grace, 1992) and hence in the year to year variation in pod set ability of faba bean plants.

It is generally accepted that the environment (considered as weather, soil and biological environment), apart from indirect effects on crop yield through disease spread and lodging, influences also the rate of the physiological processes (e.g. photosynthesis, respiration, partitioning) of crop plants (Thompson , 1983; Geiger *et al.*, 1996) and thus, in turn, the production of reproductive organs. Russell and his colleagues (1993) discussed in their review the difficulties in identifying and quantifying the critical environmental factors that have the potential to limit (or control) crop yield in particular circumstances. Environmental physiologists, however, has focus there interest in the view that developmental responses to environmental stimuli such as light or temperature are mediated by a hormonal factor (Biddington, 1985; Pharis & Reid, 1985; Binns, 1994).

Particularly for cytokinins, external factors such as root temperature (Skene, 1975; Udomprasert *et al.*, 1995), water stress (Itai & Vaadia, 1971; Reid & Wample, 1985; Davies *et al.*, 1986; Bano *et al.*, 1994), hypoxia (Neumann *et al.*, 1990), light conditions (Horgan, 1984; Borisova *et al.*, 1990) and nitrogen supply (Sattelmacher & Marschner, 1978; Salama & Wareing, 1979; Kuiper, 1988; Kuiper *et al.*, 1989; Wagner & Beck, 1993) exert an influence on the endogenous content of the plant. In addition to the effects of environmental stress on endogenous levels of plant

hormones, exogenous application of growth substances may be used successfully to protect plants from stress conditions (Biddington, 1985; Trewavas, 1991; Gianfanga *et al.*, 1991) suggesting that, within the plant, endogenous mechanisms for stress resistance are often hormonally regulated. Palta and Ludwig (1996) in their recent work with narrow-leafed lupin (*Lupinus angustifolius*) showed that pod set was increased with exogenous application of BA on the flowers, even though plants were under water stress indicating that the well-known effect of water deficit on flower abscission in lupines (Biddiscombe, 1975; Farrington & Pate, 1981; French & Turner, 1991) was negated by the cytokinin treatment. In the present study, of the various cytokinin analogs used, BA-compounds were found to cause the most consistent increases in pod set for faba bean plants grown under both controlled and field conditions. This group of chemicals was found, also, to be most effective when applied to different commercial varieties which varied in their intrinsic ability to retain their pods. In control plants the average pod set at the first nine reproductive nodes on the mainstem in the different experiments (Experiments 3.1, 3.2 & field trial 1993) varied between 10% - 89%. None of the externally applied cytokinins reduced pod set. In the cases where increases (either statistically significant or not significant) were recorded these ranged from 1% - 63% as compared with the control plants. When pod-set measurements were performed at specific sites on the mainstem, as for example in Chapter 6 (Figure, 2.6.1), the average percentage of pod set was 5% in control plants and 99% in treated plants (Table 6.1). On the basis of this evidence it is concluded that externally applied BA-cytokinin may reduce some of the variation between years in pod set ability in *Vicia faba* regardless of the environmental conditions experienced during development and thus it can increase the potential reproductive capacity of the plant.

Two developmental scales were adopted in this thesis: one which describes the reproductive growth of the whole plant (Section 2.2.3) and the other which was applied in the development of buds and flowers (Section 2.2.4). The use of these scales allowed treatments to be made at specific developmental growth stages of the plant in order to try to reduce some of the variability caused by other factors (environmental changes, cultivation practice etc.) and to relate the changes in the reproductive potential to the developmental physiology of the plant. Increased pod set after cytokinin treatment(s) was found when the chemicals were applied at flowering, the period of greatest potential abscission (Smith, 1982c; Gates *et al.*, 1983; Rylott, 1991). In the 1993 field trial, spraying the whole plant at early- to mid-flowering resulted in higher pod initiation than in untreated plants. This was a

general observation over five faba bean cultivars. The same effects were observed when cytokinins were applied locally on each raceme at the beginning of the opening of its flowers (Chapter 3) and on each flower immediately before its anthesis (Chapter 6). These results are in agreement with previous studies with BA in *Vicia faba* (Chapman & Sadjadi, 1981; Kellerhals, 1983; Rylott & Smith, 1990; Smith & Rylott, 1992) as well as in other grain legumes (Crosby *et al.* 1981; Dybing & Westgate, 1989; Atkins & Pigeaire, 1993; Palta & Ludwig, 1996). Relevant to this discussion are also the findings of Smith and Rylott (1992) which show that in broad beans the enhanced pod set caused by BA application to flowers was not due to parthenocarpy and was more pronounced when hand-tripping of the flowers followed cytokinin application. During the period from the beginning of anthesis until shortly after ovule fertilisation vast physiological changes take place in the flower as for example anther maturation and dehiscence, nectary development, formation of stigma exudate, pollen growth in the style, hormonal production, fertilisation (Bond & Poulsen, 1983; Section 1.5.4.1), and in these physiological changes the function of cytokinins has not yet been examined.

This evidence, in combination with the general view that the effects of cytokinins on plant developmental processes are short-lived (Kinet, *et al.*, 1985), strongly indicate the need for further investigation of the role of cytokinins in the determination of the proportion of flowers that form pods during anthesis because the plant at this developmental stage was found to be responsive to BA-compounds. Recent studies on cytokinin signal perception in leaves of barley plants (*Hordeum vulgare*) showed that their high responsiveness to exogenous cytokinins depended on a dramatic decrease in endogenous cytokinin content during leaf growth (Kulaeva *et al.*, 1996). Moreover, the pool of biologically active cytokinins may be increased by application of exogenous cytokinin and this may affect plant development, for example, by causing leaf or flower senescence (Hockart *et al.*, 1991; Lukaszewska *et al.*, 1994). Spraying faba bean flowers, therefore, with BA-compounds might compensate for the reduction in supply of the endogenous cytokinins. This increase, in turn, in endogenous cytokinins at flowering may overcome competition and dominance between reproductive organs and between vegetative and reproductive sinks (Bangerth, 1994), and thus result in an increase of the potential reproductive capacity. It is uncertain, however, whether lack of endogenous cytokinins is a natural regulator of flower drop and to what extent these substances control pod set in *Vicia faba* L..

It appears that the quantitative analysis of endogenous cytokinin in relation to pod set could provide some evidence to answer these questions. Recently, the methods of HPLC-ELISA and HPLC together with fluorimetric detection was employed to measure endogenous cytokinins and auxins in developing grains of wheat (Kaminek *et al.*, 1996). In this experiment however, only the total content of endogenous hormones was measured. It is well known from studies on cytokinin metabolism and function that the biological activity of this group of hormones is dependent on the chemical structure of the compound (Brzobohaty *et al.,* 1994) and not on the total concentration of such chemicals present. The ineffectiveness of externally applied KIN, Z, iP and [4R]Z to increase pod set in experiments 3.1 and 3.2 may be due partly for this reason and partly to their immobility in the plant after application (Luckwill, 1978; Kirkwood, 1991). With regard to their physiological importance, cytokinin metabolites have been classified (Horgan, 1987; Van Staden & Crouch, 1996) into three categories: (1) 'oxidation products' with irreversible loss of their biological activity through oxidative degradation of the N^6 sidechain; (2) 'N-conjugates' with loss of, or reduction in activity through irreversible conjugation with alanine or glucose; (3) 'active pool' of inter-convertible compounds which are themselves active, or serve as storage forms which may be converted into active cytokinins. Conversion of these growth substances from active to inactive forms may be one of the most important facts affecting their physiological role in plant development. Consequently, one prerequisite for progress in the understanding of cytokinin action in pod set seems to be a detailed knowledge of their endogenous levels gained from both qualitative and quantitative studies simultaneously in the reproductive organs of faba beans.

However, apart from the problems reviewed by Davies (1995) in quantifying endogenous hormone concentrations at their active site, extraction of these compounds provides only a static picture of substances which are in a dynamic state; at any time their concentration is the outcome of their biosynthesis, accumulation, and metabolism (Canny, 1985). Hence variations of growth substance level in extracts may be very misleading if a timecourse pattern is not presented. Immunological technology described by Morris and his co-workers (1993) was applied to the analysis of endogenous cytokinins in cereal grains, starting immediately after pollination. From such analyses, the timecourse of changes in the major cytokinins present in the developing spikelets was determined. High endogenous levels of zeatin and zeatin riboside coincided with the period of maximum cell division in the endosperm. As the cell division ended all cytokinins

reduced to low levels. On the basis of these studies and the foregoing discussion, it would be worth applying these immunological techniques to developing reproductive organs of *V. faba* during anthesis in order to gain a better view of the relationship of the changes in endogenous cytokinins to the process of pod set.

Changes in the concentration of hormones, however, cannot always account for the differences in growth and development of plants. There is an ever-increasing literature showing that tissue sensitivity to a growth substance may also be a physiologically significant variable as well as the concentration of the growth substance (Trewavas 1981, 1991). As Firm (1986) has pointed out, a change in sensitivity is shown by the observation that the response to a given amount of hormone has changed. This could be caused by a change in the number of receptors, a change in receptor affinity, or a change in the subsequent chain of events (Davies, 1995). A receptor is a specific cellular recognition site that binds the ligand and in consequence instructs the cell to respond in the appropriate manner to the particular chemical signal (Venis, 1985). Many cytokinin-binding systems have been described, but in no case is there compelling evidence that the binding being studied is to a physiological receptor (Venis, 1985; Guern, 1987; Gan & Amasino, 1996). This seems to be the reason why in the literature, there is lack of studies on flower development that deal with these complex issues. Trewavas (1991) following the discussion on sensitivity (Trewavas, 1981), pointed out the need for unambiguous measurement of sensitivity only at the endogenous growth substance concentrations in order to clarify growth regulator function in the intact plant. A specific sensitivity measurement termed 'control strength' was proposed by him and the employment of fluorescence ratio imaging technology, which is under development in Edinburgh, appears to promise a solution to this problem. The advantage in measuring control strength is that it represents the portion of the total control of the physiological response which is exerted by the contributing molecule at its endogenous concentration. In other words, the contribution of the growth substance to the control of the endogenous process is measured. This approach of tissue sensitivity to endogenous plant growth substances is still in its infancy but progress in this domain is likely to be linked with the role of cytokinins in pod set of *Vicia faba*. The problems outlined above in measuring endogenous cytokinin concentrations at their active site simultaneously with changes in the sensitivity parameters make the elucidation of mode of action of these compounds extremely complex and difficult. Such a task would need to combine the technical experience and knowledge of various disciplines and could be attempted only by a research team.

The investigation of the natural control of reproductive potential by endogenous cytokinins, is likely to be far more complicated if attempted during the period of pod development. Although pod set increased after external application of BA-compounds in the vast majority of cases, there was no final increase in the retention of pods at harvest. It was shown in the 1993 field trial, that retention of mature and harvestable pod depended on variety. As discussed in sections 3.4 and 4.14, a continuous supply of cytokinins to the young pods shortly after set may be necessary to overcome the lag phase of development (Gates *et al.*, 1983; Rylott, 1991) so that they may become active sinks. Jaquiery and Keller (1978), stated that at the end of flowering the apex passed on its role as a predominant sink to the developing pods. Thus pods do not become active sinks until they are 4 - 6 cm in length. Much of the increased reproductive potential (due to increased pod set, achieved by BA-compound application), was lost during this critical stage between pod set (1 - 2 cm in length) and the pods attaining active sink status, because of increased levels of pod drop. Mauk and his co-workers (1990) studied pod development in soybean explants. They reported that it is influenced by a synergistic effect of cytokinin and minerals; which suggests that dynamic processes within the plant (such as growth, senescence, translocation of assimilates etc.) are becoming very integrated (i.e. incorporate the functions of several components) during the period of pod development.

Also, high inter-plant variation was found to be present (Section 4.14) with regard to the characteristics recorded during pod development. Increases in the number of replications or in the number of plants per sampling unit or both were suggested as appropriate means to tackle this problem (Section 4.14). Obviously, this in turn leads to greater problems of workload and resources, so that fewer varieties can be screened at any one time. None of the cultivars used in this study were pure lines, but were commercial varieties. Most faba bean cultivars are synthetics. They are consisted either from populations (i.e. non-inbred components) with narrower variation than the synthetic or from inbred lines, with tested combining ability (Lawes *et al.*, 1983; Bond, 1995). These blends of genotypes increase the frequency of desirable genes and gene combinations while avoiding inbreeding depression and yield shrinkage (Lawes *et al.*, 1983; Stelling *et al.*, 1994; Link, 1995). Much of the inter-plant variation, therefore, in the reproductive potential can be ascribed to the genetic variability present within the variety. This variation might be due to the various levels of crossbreeding during seed multiplication. Differing levels of cross breeding result in differing degrees of heterogeneity (i.e. genetic diversity between

plants) and heterozygosity (i.e. genetic diversity within the plant), while inbreeding leads to yield depression and instability (Stelling *et al.*, 1994). Consequently, inbred lines would lead to reduced levels of variability (and hence higher uniformity) and may be better model plants for physiological experiments but the results obtained would not relate to commercial practice.

It is evident, therefore, from the difficulties outlined above that any attempt to interpret the effects of cytokinins on pod retention by determining the relative amounts of a number of endogenous hormones and other factors and their inter-relations during this period is extremely difficult, if not presently impossible.

Throughout all the experimental work in this thesis, detailed positional analysis of the reproductive potential on the mainstem of faba bean plants was carried out. This analysis demonstrated that for any reproductive structure in control plants, likelihood of abscission or retention was dependent both on position on the raceme and raceme position on the stem. These patterns have been previously recognised by other workers (e.g. Gates *et al.*, 1983; Clifford *et al.*, 1990). The pods which set first could cause abscission of younger reproductive structures by the release of abscission-inducing factors (Tamas *et al.*, 1979; Huff and Dybing, 1980; Bangerth, 1989) or by competing for available assimilates, mineral nutrients or growth regulators (Clifford 1979; Chapman & Sadjadi, 1981). In the present study it was shown that the application of BA-cytokinins altered the normal pattern of pod set, and gave the flowers almost equal opportunity to set pods thus increasing their reproductive potential regardless of their position on the plant. However, interpreting the role of cytokinins as potential internal signals for the determination of the ability of each flower to compete with other sinks at that time points to a major gap in knowledge: the mode of stimulation of sink strength by externally applied cytokinin.

At present much discussion focuses on the question of whether the concept of sink strength is a useful one (Farrar, 1993a, 1996). Sink strength is considered either an attractive or a vague and confusing concept (Farrar, 1993a). Much confusion is due to the lack of a clear definition of sink strength. In accordance with many authors (e.g. Wareing & Patrick, 1975; Wolswinkel, 1985; Farrar, 1993b; Marcelis 1996), in this thesis the term sink strength is defined as the competitive ability of an organ to attract assimilates. As a measure of sink strength the actual rate of assimilate import or of growth has often been used (e.g. Warren Wilson, 1972). When measured in this way, sink strength in fact represents the net result of assimilate flow, and this may depend on the competitive ability of all sinks on a plant and on the assimilate supply (source strength). This does not seem to be a useful measure of sink strength, and it

is the prime cause why some authors reject the use of this concept. Minchin & Thorpe (1993) dismissed sink strength (as measured by the actual import rate) as a misnomer. On the other hand some authors (e.g. Patrick, 1993) have stated that it should be identified as a set of parameters to describe a sink's ability to influence assimilate import. These parameters should be independent of the rest of the plant. This description is what has been employed in this thesis by using the metabolic activity of sucrose as a measurement of sink strength (Chapter 5). The rate of sucrose cleavage by sucrose synthase (SS) within the reproductive structure at fds-6 appeared to correlate with the increase of the reproductive capacity at pod set in cv. Toret. In this variety, the application of BA resulted in higher SS activity both in carpels and pedicel and peduncle tissue at fds-6 (Section 5.4). These findings were confirmed in cv. Maris Bead but not in cv. TFW (Section 5.4). A correlative study like that, however, cannot distinguish between cause and effect but it can indicate interesting areas for further research. It remains uncertain, therefore, whether sucrose breakdown by SS before pollination and fertilisation is a key factor that quantitatively reflects the competitive ability of a flower and thus its possibility for retention or abscission, and whether this factor is under the control of cytokinins. Nevertheless, this work revealed the need for further investigation of the role of cytokinin in pod set in relation to sucrose metabolism before pollination, i.e. at fds-6.

In cv. Toret, locally applied BA on each flower before pollination and fertilisation resulted in complete pod set at sites where normally almost negligible flower retention was recorded. This increase in the reproductive potential at these flower positions was parallel with higher sucrose contents in treated carpels at fds-6 as compared with the controls (Section 6.5.3). These observations were not expected, based on previous findings of SS activity, but they strengthen the supposition that the potential of a flower to develop a pod may have been determined by earlier (before pollination & fertilisation) cytokinin action possibly on sucrose accumulation.

As discussed in Section 6.5, when partitioning of carbon assimilates are studied in reproductive organs, the carbon lost by respiration and the carbon used for dry matter production should be taken into account. In addition, a distinction might be needed between assimilates produced in the sink organ itself and those produced in other parts of the plant. In faba bean carpels the activity of sucrose phosphate synthase (SPS), and thus the photosynthetic activity of these organs, was found to be rather low relatively to sucrose cleavage by acid invertase (AI) or sucrose synthase (SS) (Section 5.4.2). However, at later stages of pod development photosynthesis does take place in the hulls (Koscielniak *et al.*, 1990) and this carbon source should also

be measured. Furthermore, if cytokinins induce the reproductive structures to attract more assimilates, a distinction should be made between the processes of storage and growth. It should be investigated whether when storage occurs, it is competitive with growth or it just involves a surplus which is not needed or cannot be used for growth at the time of formation for example of vascular bundles (Gates *et al.*, 1981). In addition, although a quantitative effect of cytokinins on the fresh weight of carpels of the treated flowers at fds-10 was not detected (Section 6.3), the regulation of the dry matter percentage and the relationship between growth in fresh and dry matter is still only poorly understood. Ehret and Ho (1986) and Ho *et al.*(1987) found indications that to some extent the accumulation of water might be independent of the accumulation of dry matter. As stated by Farrar (1993c) more research in this subject is needed in order to relate growth parameters of the sink with its ability to develop.

The sink strength of a plant's organ is not static but changes during the development of the plant. The total sink strength of all organs together is determined by the number of organs on a plant and the sink strength of each individual organ (Ho, 1996). Wareing & Patrick (1975) and Patrick (1988) have emphasised the importance of identifying whether organ growth is limited (a) by assimilate supply (source limited) or if the assimilate supply is more than a given sink needs, by the sinks ability to use it (sink limited). The elucidation of these factors might have implications for the control of organ growth. Externally applied cytokinins increased the proportion of young fruits retained on the plant by limiting their abscission rather than by limiting their formation i.e. decrease in the total number of flowers (Chapter 3, 4, 5, 6). This effect altered the sink/source balance in the whole plant, and may have caused a too great fluctuation in assimilate partitioning during the early stages of pod development. This means that the available amount of source-derived organic compounds will be divided according to a new hierarchy (Minchin & Thorpe, 1996). Because of an increase in the number of sinks at pod set the supply of assimilates may have been inadequate for further growth of those sinks. In view of this limitation during pod development a great number of pods was lost before they reached maturity and the reproductive potential was reduced (Chapters 3 and 4). Often the term "source-limitation" is interpreted to mean that organ growth is determined only by the source, while "sink limitation" is interpreted to mean that organ growth is determined only by the sinks. But Marcelis (1996) has suggested that the term "source limitation" should cover both source strength and organ's potential capacity and affinity to accumulate assimilates i.e. its sink strength. Farrar (1993c) suggested abandoning the traditional attempts to speak of sink- or source-

limitation, because control of fluxes (growth) will be shared by both source and sink rather than centred only in one of them. However, the messengers for the co-ordination of supply and assimilation are not yet known.

Fruit abscission seems not to be due solely to a shortage of assimilate supply, but also to other factors which are probably hormonally mediated, such as assimilate utilisation and dominance of competing fruits (Tamas *et al.*, 1979; Gates *et al.*, 1983; Schapendonk & Brouwer, 1984; Bangerth, 1989; Ruiz & Guardiola, 1994). Ganeshaiah & Uma Shaanker (1994) proposed in their opinion-paper that the development of generative organs and hence their successful retention on the mother plant is a process of self-organisation, where any resource molecule moving into a sink autocatalytically increases the probability of receiving further resources. It is unknown, however, what determines the hierarchy in priorities of assimilate partitioning between multiple equivalent sinks. The abscission of reproductive structures is still poorly understood, but it has a strong influence on the reproductive load and assimilate partitioning of the whole plant. It could be worthwhile studying whether endogenous cytokinins or, as suggested by Farrar (1992), sucrose are involved in the communication between sources and sinks. Researchers engaged in recent studies of this process have suggested potential roles for hormones in regulating carbon partitioning and growth in sink organs. For example, Thomas and Rodriguez (1994) have proposed a model for explaining how activation of gene expression by carbon metabolites and gibberellic acid can affect starch mobilisation and source and sink metabolism in cereal seedlings. Their work shows that studies on the regulation at the whole-plant level need to address important functions such as mechanisms for communication and integration in response to internal factors such as sucrose and specific hormone levels. Therefore, it remains for researchers to show how these mechanisms affect each other in the regulation of carbon partitioning and growth. It was clear in the present study that by manipulating the cytokinin levels and sucrose partitioning for a short period (14 days during flowering) the plant of *Vicia faba* L. achieved a higher reproductive capacity and so it has the potential for higher yield. Plant growth substances appear to be a potential mechanism to manipulate the retention of reproductive structures. A better understanding of how assimilate partitioning is affected by plant hormones may make for more effective progress in plant breeding. Furthermore a greater insight is needed into factors which control partitioning in order that an improvement of harvest index can be achieved; the limits of which in *Vicia faba* are still unknown (Evans, 1993).

Keller and Bellucci (1983) while reviewing the influence of plant growth substances on development and yield of *Vicia faba* L. noted the diversity between varieties in their reaction to the treatments of growth regulators. Even from the limited number (five) of varieties studied here, it was apparent that there were important differences among them in their reproductive potential and in their response to cytokinin treatments (Sections 4.14). It is because of this genetic variability in *Vicia faba* that plants with a contrasting ability to retain reproductive organs can be identified in cvs Troy and Toret in the present study. These varieties also had a very low inter-plant variation in terms of percentage pod set (Table 4.13.1). Such a system of contrasting plants allows comparative studies to be carried out to investigate to what extent differences in the levels of endogenous cytokinins are related to differences in the reproductive potential throughout plant development. This approach relies heavily on correlative interpretations which cannot prove or disprove the involvement of cytokinins in the development of the complex and highly interactive generative storage system of *Vicia faba* (Aufhammer *et al.*, 1986). As a useful tool to investigate how these interactions (between endogenous forms of cytokinins, cytokinins and other hormones, between cytokinins and developmental stages, between cytokinins and responsive sites etc.) may be regulated the use of genetically manipulated plants has been proposed (Klee & Romano, 1994; Morris, 1995), namely plants transformed with genes that regulate either a single process in question or a single hormone purported to influence a process. Here a selected and defined aspect can be altered, and the direct and indirect effects through the whole system can then be followed. This approach has the advantage of knowing what has been altered (provided that isogenic lines are used i.e. genetically identical but not necessarily homozygous plants) and any change will be a direct or indirect consequence of the initial intervention, so that it will often be possible to assign causality. Recent work in Scotland on *Vicia faba* cv. Toret (Ramsay & Middlefell-Williams, 1995) and elsewhere on other grain legumes (Nicolas, *et al.*, 1995; Saalbach *et al.*, 1995) has improved the prospects for reliable transformation in these species.

The genetic tools generated over the past decade from the study of the interaction between plant cells and pathogenic bacteria have provided a valuable mean in addressing the question of the action of plant hormones (Costacurta & Vanderleyden, 1995). The introduction of *Agrobacterium tumefaciens ipt* gene into plants has served to genetically engineer plants in which endogenous cytokinin can be elevated. The *ipt* gene is a gene encoding isopentenyl transferase which is a cytokinin

biosynthetic enzyme. Gan and Amasino (1996) summarised in their recent review the strategies that have been used to direct the expression of *ipt* gene in transgenic plants of various species. Based on studies in plant senescence, they proposed the development of a transgenic plant system that allows production of controlled levels of cytokinins in a specific tissue at a specific developmental stage. Although, to date, the autoregulatory production of cytokinin by the transgenic plant strategies has been assessed mainly in solanaceous species, a precisely controlled endogenous cytokinin level in a quantitative, spatial and temporal manner may have broad application in species like *V. faba* which is responsive to the externally applied cytokinin at a specific developmental stage (e.g. fds-6) with regard to the retention of reproductive organs. Such a transgenic approach will test the regulatory role of cytokinin in reproductive loss as it has been originated from external application studies. Also, it will provide a way to manipulate the reproductive retention in faba beans for potential agricultural benefit.

In conclusion, it is widely agreed that the development of plants is a very complex network of events interacting in time and space (Section 1.2). This is particularly apparent when considering the reproductive development of the faba bean plant, which coincides with parallel vegetative growth. In view of such complexity, the classical conception has been adopted in this thesis that cytokinin is one of the five types of hormonal messengers which are responsible for the control of plant development and the integration of the activities of the different plant organs (Section 1.3). It has long been accepted that the regulation of plant development by hormones is dependent on changes in endogenous hormone levels and the sensitivity of the cells to these substances. It was shown here that it is possible to control with BA-type cytokinins the reproductive potential of *Vicia faba* L. by increasing the number of pods that initially set. Often an increased level of endogenous cytokinins is assumed as a result of external application of synthetic cytokinins, though this has not been proved. Even with this assumption, however, the interpretation of results according to the classical hormone theory was difficult, because the observed responses are generally the consequence of complex interactions (a) at different levels (organ, tissue, cell etc.) of organisation and (b) at different stages of plant development. Perhaps our overall knowledge is improved by, and possibly practical applications can be developed from, the application of synthetic cytokinins externally; but from a physiologist's point of view, the "spray and weigh" strategy seems to be insufficient. Hypotheses on mode of action, and possible consequent generalisations, depend upon data about endogenous substances and about sequences

of responses at particular periods of plant development and at several levels of organisation. Some new techniques are applicable at this point which may provide information concerning the amount and form of the endogenous compounds, and the site of action of the biologically active form. Unfortunately there are no studies that relate specifically to the development of the reproductive structures of the faba bean plant. An attractive approach based on assimilate partitioning might be the investigation of the role of cytokinins as internal messengers controlling the ability of reproductive sinks to develop in conjunction with and in competition with other plant organs. However, a complex and multidiciplinary area like the hormonal control of the reproductive potential of *Vicia faba* in terms of the improvement of sink strength needs to be studied by a research team. Application of the tools of genetic manipulation of the endogenous cytokinins will hopefully lead to a better understanding of these processes in the future. For the performance of this task, the following ascertainment of Hobbie and his colleagues (1994) must always motivate the researchers interested in plant hormones when discourage and panic appear during their studies: "The plant hormones are the most interesting and challenging areas in plant biology. Ubiquitous in their occurrence and bewildering in the diversity of their responses, it is clear that a complete understanding of their biological role and mechanism of action will not come easily. However, there is plenty of cause for optimism."

7.2 Summary of major findings

- In the present study, of the various cytokinin analogs used, BA-compounds (BA and its derivative [9tP]BA) were the only cytokinins effective in enhancing the proportion of young fruits retained on the plant. This action was effected by limiting abscission rather than by altering, upwards or downwards the number of flowers formed (Chapter 3, 4, 5, 6).
- The application of BA-type cytokinins altered the normal pattern of flower drop, and gave the flowers almost an equal opportunity to set pods thus increasing their reproductive potential regardless of their position on the plant. This indicated that the application of the chemical suppressed the plant's inherent dominance towards the lower racemes and proximal flowers (Chapters 3, 4 & 6).
- Increased pod set after cytokinin treatment(s) was found when the chemicals were applied at flowering, the period of greatest potential abscission. The plant

at growth stages 203(1) and 204(1), i.e. early flowering and early pod set was found to be most responsive to BA-compounds. These findings strongly indicate the need for further investigation of the role of cytokinins in the determination of the proportion of flowers that form pods during this period (Chapter 4 & 5).

- The effects of BA-analogs on pod initiation were consistent when tested in various commercial varieties of *Vicia faba* with contrasting ability to keep their reproductive structures. However, the increase was more profound in the cultivars which suffered more from flower abscission (Chapters 4 & 5).
- It was clear in the present study that by manipulating the cytokinin levels for a short period (14 days during flowering) *Vicia faba* L. achieved a higher reproductive capacity and so it has the potential for higher yield. Mainstem yield, represented by the DW of seeds, generally was increased in cytokinin-treated plants as compared to controls (Table 4.11.2). Further analysis, however, of the effects of cytokinins on the yield components within each variety showed varietal differences in the contribution of each yield component to final yield (Chapter 4).
- Application of BA gave consistent enhancement of fruit set in faba beans both under controlled (Chapter 3) and field (Chapters 4 & 5) conditions. On the basis of this evidence it is concluded that externally applied BA-cytokinin may reduce some of the variation between years in pod set ability in *Vicia faba* regardless of the environmental conditions experienced during development and thus it is a potential mechanism to manipulate the reproductive capacity of the plant.
- BA resulted in lower flower drop and hence greater overall pod set at the first five racemes of all varieties tested under stress (field trial 1994) environmental conditions. These observations suggest that external applications of cytokinins preserve plants from stress conditions and appears that, within the plant, endogenous mechanisms for stress resistance may be hormonally regulated (Chapters 5).
- From the varieties tested, high inter-plant variation was measured (Section 4.14) in various characteristics recorded during pod development. Increases in the number of replications or in the number of plants per sampling unit or both were suggested as appropriate means to tackle this problem (Section 4.14). Obviously, this in turn leads to greater problems of workload and resources, so

that fewer varieties can be screened at any one time. This study reveals the need to choose more uniform biological material and more appropriate sampling techniques for future research.

- Genetic variability was found among *Vicia faba* cultivars so that plants with a contrasting ability to retain reproductive organs can be identified e.g. cvs Troy and Toret. These varieties also had a very low inter-plant variation in terms of percentage pod set (Chapter 4). Such a system of contrasting plants allows comparative studies to be carried out to investigate to what extent differences in the levels of endogenous cytokinins are related to differences in the reproductive potential throughout plant development.
- The activity of sucrose synthase (SS) within the reproductive sink at fds-6 appeared to correlate with the increase of the reproductive capacity at pod set in cv. Toret (Chapter 5). Investigations focused on local application of cytokinins on each flower at fds-6 strengthen the supposition that the potential of a flower to develop a pod may have been determined by earlier (i.e. prior to pollination & fertilisation i.e. at fds-6) cytokinin action possibly on sucrose accumulation (Chapter 6).
- Investigations for establishing a timecourse of biochemical events that might be useful in identifying processes that initiate flower retention and pod set is a challenging target, but may be complex to elucidate because of technical difficulties in collecting enough and uniform (i.e. at the same physiological age and with the same probability of abscission) plant tissue/organs for biochemical analysis and measurement of growth or of developmental parameters (Chapter 5 & 6).
- Hypotheses on the mode of action of cytokinins depend upon data about endogenous substances and about sequences of responses at particular periods of plant development and at several levels of organisation (Chapter 3 & 7). Some new techniques for quantitative and qualitative analysis of endogenous cytokinins, and fluorescence ratio imaging technology for measurement of the sensitivity only at the endogenous growth substance concentrations as well as the autoregulatory production of cytokinin by the transgenic plant strategies are applicable at this point (Chapter 7).

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Bibliography

Abou-Elleil, G. A. & El Wazeri, S. M. (1978). Significance of foliar application with certain growth substances for controlling shedding in field beans, *Vicia faba* L. *Agricultural Research Review* **56** (8), 59-63.

Adedipe, N. O., Fletcher, R. A. & Ormrod, D. P. (1976). Distribution of ^{14}C assimilates in the cowpea (*Vigna unguiculata* L.) in relation to fruit abscission and treatment with benzyladenine. *Annals of Botany* **40**, 731-737.

Adsule, R. N. & Akpapunam, M. (1996). Faba bean (*Vicia faba* L.). In *Legumes and Oilseeds in Nutrition* (E. Nwokolo & J. Smartt), pp. 197-202. London: Chapman & Hall.

Alexandratos, N. (Ed.) (1995). *World Agriculture: Towards 2010. An FAO study.* England: F.A.O. & J. Willey.

Aloni, R. (1993). The role of cytokinin in organised differentiation of vascular tissues. *Australian Journal of Plant Physiology* **20**, 601-608.

Amato, G., Cibella, R., Giambalvo, D. & Gristina, L. (1992). Observations of the reproductive development in faba bean (*Vicia faba var. equina*) in relation to plant density. *Proceedings of the 1st European conference in Grain Legumes* 1992, pp. 245-246. Angers: AEP.

Amzallag, G. N., Lerner, H. R. & Poljakoff-Mayber, A. (1992). Interaction between mineral nutrients, cytokinin and gibberellic acid during growth of sorghum at high NaCl salinity. *Journal of Experimental Botany* **43**, 81-87.

Anonymous (1994). *Ways to Improve the Quality of Domestically Produced Proteins for use in the Animal Feed Industry.* Brussels: European Commission.

ap Rees, T. (1974). Pathways of carbohydrate breakdown in higher plants. In *International Review of Science Plant Biochemistry, vol. 11,* (Ed. D. H. Northcote), pp. 89-127. London: Butterworth.

Argall, J. F. & Stewart, K. A. (1984). Effects of decapitation and benzyladenine on growth and yield of cowpea (*Vigna unguiculata* L. Walp.). *Annals of Botany* **54**, 439-444.

Atkins, C. A. & Pigeaire, A. (1993). Application of cytokinins to flowers to increase pod set in *Lupinus angustifolius* L. *Australian Journal of Agricultural Research* **44**, 1799-1819.

Atkinson, D. (1996). Legumes and sustainable farming: a vision for the future. In *Proceedings of the 30th Occasional Symposium of the British Grassland Society.* (Ed. D. Younie), pp. 326-331. SAC, Craibstone, Aberdeen, UK: British Grassland Society.

Attiya, H. J., Field, R. J. & Hill, G. D. (1983) Effect of PP333 and TIBA growth regulators on development and yield components of spring sown field beans (*Vicia faba* L.). *Proceedings of the Agronomy Society of New Zealand* **13,** 81-86.

Aufhammer, W. (1990). Hormones and sink development. *News Bulletin of the British Society for Plant Growth Regulation* **11,** 1-10.

Aufhammer, W. & Gotz-lee, I. (1989). Effects of inflorescence removal on seed yield performance of field beans (*Vicia faba*) under different shading treatments. *Journal of Agricultural Science, Cambridge* **113**, 317-323.

Aufhammer, W. & Gotz-Lee, I. (1991). Effects of inflorescence removal on interactions between reproductive storage sites of field beans (*Vicia faba*) under different shading treatments. *Journal of Agricultural Science, Cambridge* **116**, 409-415.

Aufhammer, W., Gotz, I. & Peter, M. (1986). Yield performance of field beans in relation to interactions between inflorescences at different nodes. *Journal of Agricultural Science, Cambridge* **108**, 479-486.

Aufhammer, W., Nalborczyk, E., Geyger, B., Gotz, I., Mack, C. & Paluch, S. (1989). Interactions between and within inflorescences in relation to the storage capacity of field beans (*Vicia faba*). *Journal of Agricultural Science, Cambridge* **112**, 419-424.

Avigad, G. (1982). Sucrose and other disaccharides. *In Plant Carbohydrates. Intracellular Carbohydrates. Encyclopaedia of Plant* Physiology; *New Series*, Vol. 13A (Eds F. A. Loewus & W. Tanner), pp. 217-347. Berlin-Heidelberg: Springer-Verlag.

Baird, L. A. M., Reid, P. D. & Webster, B. D. (1978). Ultrastructural modifications associated with the induction of abscission in *Coleus*. *Botanical Gazette* **139**, 165-170.

Baker, D. A., Chapman, G. P., Standish, M. & Bailey, M. (1983). Assimilate partitioning in a determinate variety of field bean. In *Temperate Legumes: Physiology, Genetics and Nodulation* (Eds G. D. Jones & D. R. Davis), pp. 191-198. London: Pitman.

Bamouh, A. (1995). Status of the grain legumes in the Maghreb. *Proceedings of the 2nd European conference in Grain Legumes* 1995, pp. 22-23. Copenhagen: AEP.

Bangerth, F. (1989). Dominance among fruits/sinks and the search for a correlative signal. *Physiologia Plantarum* **76**, 608-614.

Bangerth, F. (1994). Response of cytokinin concentration in the xylem exudate of bean (*Phaseolus vulgaris* L.) plants to decapitation and auxin treatment, and relationship to apical dominance. *Planta* **194**, 439-442.

Bangerth, F. & Ho, L. C. (1984). Fruit position and fruit set sequence in a truss as factors determining final size of tomato fruits. *Annals of Botany* **53**, 315-319.

Bano, A., Hansen, H., Dorffling, K. & Hahn, H. (1994). Changes in the contents of free and conjugated abscisic acid, phaseic acid and cytokinins in xylem sap of drought stressed sunflower plants. *Phytochemistry* **37**(2), 345-347.

Batts, G. R. & Hebblethwaite, P. D. (1992). Plant Growth Regulator use on *Vicia faba. Proceedings of the 1st European conference in Grain Legumes* 1992, pp. 227-228. Angers: AEP.

Batts, G. R., Hebblethwaite, P. D. & Baylis, A. D. (1991). Effects of the growth regulators JF10405 (PARLAY C) on the development, yield components and yield of winter sown field bean (*Vicia faba* L.). *In Manipulation of Canopy Structure in Arable Crops*, pp. 33-51. British Society for Plant Growth Regulation, Monograph 22.

Baylis, A. D. & Clifford, P. E. (1991). Control of reproductive abscission in grain legumes. *Annual Bulletin of the British Society for Plant Growth Regulation* **1**, 1-12.

Beck, E. (1994). The morphogenic response of plants to soil nitrogen: Adaptive regulation of biomass distribution and nitrogen metabolism by phytohormones. In *Flux Control in Biological Systems. From Enzymes to Populations and Ecosystems (*Ed. E. D. Schulze), pp. 119-152. San Diego, California, USA: Academic Press.

Belopopsky, A. & Oikonomou, D. (Eds) (1996). *Orthodoxy and Ecology. Resource Book.* Poland: Syndesmos.

Bernfeld, P. (1968). Amylases, α and β. In *Analytical Biochemistry.* (Ed. A. Dahlvist), vol. 22, No 99, pp 149-150.

Bernier, G., Kinet, J. M., Jacqmard, A., Havelange, A. & Bodson, M. (1977). Cytokinin as a possible component of the floral stimulus in *Sinapis alba. Plant Physiology* **60**, 282-285.

Biddington, N. L. (1985). The importance of mechanically induced stress and its relevance to growth regulator research. *News Bulletin of British Plant Growth Regulator Group* **7**, 1-9.

Biddiscombe, F. F. (1975). Effect of moisture stress on flower drop and seed yield of narrow-leafed lupin (*Lupinus angustifolius* L. cv. Unicrop). *Journal of the Australian Institute of Agricultural Science* **41**, 70-72.

Binns, A. N. (1994). Cytokinin accumulation and action: biochemical, genetic, and molecular approaches. *Annual Review of Plant Physiology and Plant Molecular Biology* **45**, 173-196.

Black, C. C. (1993). Sink strength: is it real, or measurable? *Plant, Cell and Environment* **16**, 1013-1046.

Black, C. C., Laszlo, M., Sung, S. S., Kormanik, P. P., Xu, D. P. & Paz, N. (1987). Regulation and roles for alternative pathways of hexose metabolism in plants. *Physiologia Plantarum* **69**, 387-394.

Bohner, J. & Bangerth, F. (1988). Effects of fruit set sequence and defoliation on cell number, cell size and hormone levels of tomato fruits (*Lycopersicon esculentum* Mill.) within a truss. *Plant Growth Regulation* **7**, 141-155.

Bond, D. A. (1976). *In vitro* digestibility of the testa in tannin-free field beans (*Vicia faba* L.). *Journal of Agricultural Science, Cambridge* **86**, 561-566.

Bond, D. A. (1987). Recent developments in breeding field beans (*Vicia faba* L.). *Plant Breeding* **99**, 1-26.

Bond, D. A. (1995). Faba bean: *Vicia faba* (Leguminosae-Papilionoideae). In *Evolution of Crop Plants, 2nd Edition.* (Eds J. Smartt & N. W. Simmonds), pp. 312-316. UK: Longman.

Bond, D. A. & Pope, M. (1995). Varietal improvement in faba beans in EU. *Grain Legume* **8**, 15.

Bond, D. A. & Poulsen, M. H. (1983). Pollination. In *The Faba bean (Vicia faba L.). A Basis for Improvement* (Ed. P. D. Hebblethwaite), pp. 77-102. London: Butterworths.

Bond, D. A., Lockwood, G., Jellis, G. J., Toynbee Clarke, G., Pope, M., Aubry, R. G. & Hall, J. A. (1983). Annual Report PBI, Cambridge, pp. 46-47.

Borisova, T., Makarova, R., Bonavanture, N., Machackova, I., Cermak, V. & Kefeli, V. (1990). Changes in auxin and cytokinin content in leaves and roots of *Triticum aestivum* due to light treatment. In *Physiology and Biochemistry of Cytokinins in Plants* (Eds M. Kaminek, D. W. S. Mok & E. Zazimalova), pp. 395-400. Hague, Netherlands: SPB Academic Publishing.

Bouwman, A. F. (1996). Agricultural statistics and environmental issues. FAO Quarterly Bulletin of Statistics **9**, iv-v.

Brenner, M. L. & Cheikh, N. (1995). The role of hormones in photosynthate partitioning and seed filling. *In Plant Hormones.* (Ed. P. J. Davies), pp. 649-670, The Netherlands: Kluer Academic Publishers.

Bressani, R. & Elias, L. G. (1988). Seed quality and nutritional goals in pea, lentil, faba bean and chickpea breeding. In *World Crops: Cool Season Food Legumes* (Ed. R. G. Summerfield), pp. 381-404. London: Kluwer Academic Publishers.

Bromilow, R. H. & Chamberlain, K. (1991). Pathways and mechanisms of transport of herbicides in plants. In *Target Sites for Herbicide Action* (Ed. R. C. Kirkwood), pp. 245-284. New York: Plenum Press.

Bruisma, J. (1985). Plant growth regulators, past and present. *Proceedings Growth Regulators in Horticulture 1985*, pp. 1-13. University of Reading: The British Plant Growth Regulator Group and the Horticultural Education Association.

Brzobohaty, B., Moore, I. & Palme, K. (1994). Cytokinin metabolism: implications for regulation of plant growth and development. *Plant Molecular Biology* **26**, 1483-1497.

Bukovak, M. J. & Petracek, P. D. (1993). Characterising pesticide and surfactant penetration with isolated plant cuticles. *Pesticide Science* **37**, 179-194.

Burgess, J. (Ed) (1985). In *An Introduction to Plant Cell Development.* Cambridge: Cambridge University Press.

Cabrera, A. (1988). Inheritance of flower colour in *Vicia faba* L. *FABIS Newsletter* **22**, 3-6.

Cannay, M. J. (1985). Ashby's law and the pursuit of plant hormones: a critique of accepted dogmas using the concept of variety. *Australian Journal of Plant Physiology* **12**, 1-7.

Carlson, D. R., Dyer, D. J., Cotterman, C. D. & Durley, R. (1987). The physiological basis for cytokinin induced increases in pod set in IX93-100 soybeans. *Plant Physiology* **84**, 233-239.

Carns, H. R. (1966). Abscission and it's control. *Annual Review of Plant Physiology* **17**, 295-314.

Carrouee, B. (1993). Pea and faba bean: new step in EEC production. *Grain Legumes* 1, 18-19.

Cathey, H. M. (1964). Physiology of growth retarding chemicals. *Annual Review of Plant Physiology* **15**, 271-302.

Causton, D. R. & Venus, J. C. (Eds) (1981). *The Biometry of Plant Growth.* London: Edward Arnold.

Chamont, S. (1993). Sink strength: The key for plant yield modelling. *Plant, Cell and Environment* **16**, 1013-1046.

Chaplin, M. F. (1986). Monosaccharides. In *Carbohydrate Analysis; A Practical Approach* (Eds M. F. Chaplin & J. F. Kennedy), pp. 1-36. Oxford: IRL Press.

Chapman, G. P. (1981). Genetic variation in *Vicia faba.* ICARDA, Alepo, Syria.

Chapman, G. P. & Sadjadi, A. S. (1981). Exogenous growth substances and internal competition in *Vicia faba* L. *Zeitschrift fur Pflanzenphysiologie* **104**, 265-273.

Chapman, G. P., Guest, H. L. & Peat, W. E. (1978). Top removal in single stem plants of *Vicia faba* L. *Zeitschrift fur Pflanzenphysiologie* **94**, 247-255.

Chapman, F. P., Fagg, C. W. & Peat, W. E. (1979a) Parthenocarpy and internal competition in *Vicia faba* L. *Zeitschrift fur Pflanzenphysiologie* **84**, 233-239.

Chapman, G. P., Fagg, C. W. & Peat, W. E. (1979b). Top removal on single stem plants of *Vicia faba* L. *Zeitschrift fur Pflanzenphysiologie* **89**, 118-127.

Charles-Edwards, D. A., Doley, D. & Rimmington, G. M. (Eds) (1986). *Modelling Plant Growth and Development.* North Ryde, Australia: Academic Press.

Cheikh, N. & Brenner, M. L. (1992). Regulation of key enzymes of sucrose biosynthesis in soybean leaves. Effect of dark and light conditions and role of gibberellins and abscisic acid. *Plant Physiology* **100**, 1230-1237.

Chen, C. M. (1989). Cytokinin-modulated macromolecular synthesis and gene expression. In *Plant Biotechnology* (Eds S. Kung & C. J. Arntzen), pp. 245-256. Stoneham, USA: Butterworths.

Chen, C., Jin, G., Andersen, B. R. & Ertl, R. (1993). Modulation of plant gene expression by cytokinins. *Australian Journal of Plant Physiology* **20**, 609-619.

Child, R. D. & Wenyu, Y. (1991). Improvement in shoot structure of faba bean using plant growth regulators. *Aspects of Applied Biology* **27**, 173-178.

Claussen, W., Loveys, B. R. & Hawker, J. S. (1985). Comparative investigations in the distribution of sucrose synthase activity and invertase activity within growing, mature and old leaves of some C3 and C4 plant species. *Physiologia Plantarum, Copenhagen* **65,** 275-280.

Claussen, W., Loveys, B. R. & Hawker, J. S. (1986). Influence of sucrose and hormones on the activity of sucrose synthase and invertase in detached leaves and leaf sections of eggplants (*Solanum melongena*). *Journal of Plant Physiology* **124,** 345-357.

Clifford, P. E. (1979). Source limitation of sink yield in mung beans. *Annals of Botany* **43**, 397-399.

Clifford, P. E. (1981). Control of reproductive sink yield in mung beans. *Zeitschrift fur Pflanzenphysiologie* **102**, 173-181.

Clifford, P. E., Pentland, B. S. & Baylis, A. D. (1990). Reproductive abscission patterns in faba bean (*Vicia faba* L. cv Troy). *FABIS Newsletter* **27**, 5-9.

Clifford, P. E., Pentland, B. S. & Baylis, A. D. (1992). Effects of growth regulators on reproductive abscission in faba bean (*Vicia faba* cv Troy). *Journal of Agricultural Science, Cambridge* **119**, 71-78.

Cline, G. M. (1991). Apical dominance. *Botanical Review* **57**, 318-358.

Cline, G. M. (1994). The role of hormones in apical dominance. New approaches to an old problem in plant development. *Physiologia Plantarum* **90**, 230- 237.

Costacurta, A. & Vanderleyden, J. (1995). Synthesis of phytohormones by plant-associated bacteria. *Critical Reviews in Microbiology* **21**, 1-18.

Crofton, G. R. A. (1996). A review of pollination and pod setting in faba beans (*Vicia faba* L.). *Plant Varieties and Seeds* **9**, 29-35.

Crofts, H. J., Evans, L. E. & WcVetty, P. B. E. (1980). Inheritance, characterisation and selection of tannin-free faba beans (*Vicia faba* L.). *Canadian Journal of Plant Science* **60**, 1135-1140.

Crompton, H. J., Hill-Cottingham, D. G., Hudd, G. A. & Lloyd-Jones, C. P. (1984). Effect of plant density on the uptake and distribution of ^{14}C in the field bean, *Vicia faba*. *Physiologia Plantarum* **60**, 543-546.

Crompton, H. J., Lloyd-Jones, C. P. & Hill-Cottingham, D. G. (1981). Translocation of labelled assimilates following photosynthesis of $^{14}CO_2$ by the field bean, *Vicia faba. Physiologia Plantarum* **51**, 189-194.

Cronshaw, J., Lucas, W. J. & Giaquinta, R. T. (Eds) (1986). *In Phloem transport. Plant Biology, vol. 1.* New York: Alan R. Liss.

Crosby, K. E., Aung, L. H. & Buss, G. R. (1978). Natural and synthetic cytokinins on soybean development and yield. In *Proceedings of Fifth Annual Meeting of Plant Growth Regulator Working Group, Blacksburg, Virginia, June 25-29*, pp. 86-93.

Crosby, K. E., Aung, L. H. & Buss, G. R. (1981). Influence of 6-benzylaminopurine on pod-set and seed development in two soybean, *Glysine max* (L.) Merr. genotypes. *Plant Physiology* **68**, 985-988.

Crouch, N. R., Smith, M. T. & van Staden, J. (1993). Purine cytokinin nomenclature: a working system of abbreviations. *Plant Growth Regulation* **13**, 13-20.

Cuberto, J. I. (1974). On the evolution of *Vicia faba* L. *Theoretical and Applied Genetics* **45**, 47-51.

Curtis, H. & Barnes, N. S. (Eds) (1989). *In Biology, 5th Edition.* New York: Worth Publishers.

Daie, J. (1985). Carbohydrate partitioning and metabolism in crops. *Horticultural Review* **7**, 69-108.

Dantuma, G. & Thompson, R. (1983). Whole crop physiology and yield components. In *The Faba bean (Vicia faba L.). A Basis for Improvement.* (Ed. P. D. Hebblethwaite), pp. 143-158, London: Butterworths.

Davies, P. J. (1995). The plant hormone concept: concentration, sensitivity and transport. In *Plant Hormones.* (Ed. P. J. Davies), pp. 13-38. The Netherlands: Kluer Academic Publishers.

Davies, P. J., Metcalfe, J., Lodge, T. A. & da Costa, A. R. (1986). Plant growth substances and the regulation of growth under drought. *Australian Journal of Plant Physiology* **13**, 105-125.

Day, W. & Legg, B. J. (1983). Water relation and irrigation response. In *The Faba bean (Vicia faba L.). A Basis for Improvement.* (Ed. P. D. Hebblethwaite), pp. 217-232, London: Butterworths.

De Wit, C. T. (1958). Transpiration and crop yields. Versl landbouwk Onderz Rijkslandb Proefstn **64** (6), 88.

Debely, G. & Derbensky, V. (1992). Determinate forms of pea (*Pisum sativum* L.) lupin (*Lupinus angustifolius* L.) and bean (*Vicia faba* L.) as the sources of early maturity and high seed yield for the breeding. *Proceedings of the 1st European conference in Grain Legumes* 1992, pp. 145-146. Angers: AEP.

Dekker, P. H. M. & Neuvel, J. J. (1983). The influence of daminozide (Alar-85) on the yield of broad beans , *Vicia faba* L. Abstract collection, 21st International Horticultural Congress, vol. 1.

Dennett, M. D., Nyalemegbe, K. & de Costa, W. A. J. M. (1993). Growth, water use and nitrogen fixation of determinate and indeterminate cultivars of *Vicia faba* L. under contrasting soil moisture regimes. *Aspects of Applied Biology* **34**, 269-278.

Diethelm, R., Keller, E. R. & Bangerth, F. (1988). Auxins, ABA and Gibberellin-like activity in abscising and non abscising flowers and pods of *Vicia faba* L. *Plant Growth Regulation* **7**, 75-90.

Doehlert, D. C. (1990). Distribution of enzyme-activities within the developing maize (*Zea mays*) kernel in relation to starch, oil and protein accumulation. *Physiologia Plantarum* **78**, 560-567.

Duc, G. & Picard, J. (1981). Variability among six different genotypes. Effect of top and flower removal. In *Vicia faba; Physiology and Breeding. World Crops: Production, Utilisation, Description, vol. 4,* (Ed. R. Thompson), pp. 283-298. Martinus Nijhoff Publishers.

Duc, G., Sixdenier, G., Lila, M. & Furstross, V. (1989). Search of genetic variability for vicine and convicine content in *Vicia faba* L. A first report of a gene which codes for nearly zero vicine and nearly zero convicine contents. *In Recent Advances of Research in Antinutritional Factors in Legume Seeds* (Eds J. Huisman, T. F. B. van der Poel & I. E. Liener), pp. 305-313.

Duc, G., Berthaut, N., Berthelem, P., Le Guen, J. & J. Picard. (1992). Hybrid breeding in faba bean (*Vicia faba*). *Proceedings of the 1st European conference in Grain Legumes* 1992, pp. 45-46. Angers: AEP.

Duffus, C. M. & Duffus, J. M. (Eds) (1984). *Carbohydrate metabolism in plants.* New York: Longman.

Dybing, C. D., Ghiasi, H. & Paech, C. (1986). Biochemical characterisation of soybean ovary growth from anthesis to abscission of aborting ovaries. *Plant Physiology* **81**, 1069-1074.

Dybing, C. D. & Westgate, M. E. (1989). Genotype, environment, and cytokinin effects on soybean flower abortion. *Proceedings of the Plant Growth Regulator Society of America, 16th Annual Meeting, August 1989*, pp. 29-30. Virginia.

Dyer, D. J., Carlson, D. R., Cotterman, C. D., Sikorski, J. A. & Ditson, S. L. (1987). Soybean pod set enhancement with synthetic cytokinin analogs. *Plant Physiology* **84**, 240-243.

Dyke, G. V. & Prew, R. D. (1983). Beans in crop rotations. In *The Faba bean (Vicia faba L.). A Basis for Improvement.* (Ed. P. D. Hebblethwaite), pp. 263-270, London: Butterworths.

Ehret, D. L. & Ho, L. C. (1986). The effects of salinity on dry matter partitioning and fruit growth in tomatoes grown in nutrient film culture. *Journal of Horticultural Science* **61**, 361-367.

El-Abd, S. O., Singer, S. M., El-Saeid, H. M. & Mahmoud, M. H. (1989). Effect of some levels of plant growth regulators and silver nitrate on the growth and yield of broad bean (*Vicia faba*) plants. *Egyptian Journal of Horticulture* **16**, 143-150.

El-Rhaman, K. A. A., El-Morshidy, M. A., Khalifa, M. A., Mussaballa, E. A. & El-Rahim, M. M. A. (1980). Flowering abscission, yield and quality of broad beans as affected by sowing date and irrigation frequency. *Research Bulletin, Faculty of Agriculture, Ain Sahams University, Cairo.*

El-Zawily, A. I., Zayed, E., Hassan, M. & Eissa, E. (1985). Growth, yield and endogenous phytohormone content of *Vicia faba* as affected by two plant growth regulators. *Angew Botanik* **59**,163-170.

Ellis, R. H., Roberts, E. H. & Summerfield, R. J. (1988a). Photothermal time for flowering in faba bean (*Vicia faba*) and the analysis of potential vernalisation responses. *Annals of Botany* **61**, 73-82.

Ellis, R. H., Summerfield, R. J. & Roberts, E. H. (1988b). Effects of temperature, photoperiod and seed vernalisation on flowering in faba bean *Vicia faba. Annals of Botany* **61**, 17-27.

Esau, K. (Ed.) (1960). *Anatomy of Seed Plants*. New York: Wiley.

Evans, D. A. (1989). Techniques in plant cell and tissue culture. *In Plant Biotechnology* (Eds S. Kung & C. J. Arntzen), pp. 53-76. Stoneham, USA: Butterworths.

Evans, L. T. (1959). Environmental control of flowering in *Vicia faba* L. *Annals of Botany* **23**, 521-546.

Evans, L. T. (1975). Beyond photosynthesis - the role of respiration , translocation and growth potential in determining productivity. *In Photosynthesis and Productivity in different Environments* (Ed. J. P. Cooper), pp. 501-507. Cambridge: Cambridge University Press.

Evans, L. T. (1993). The physiology of flower induction. Paradigms lost and paradigms regained. *Australian Journal of Plant Physiology* **20**, 655-660.

F.A.O. (1992). *The State of Food and Agriculture.* Rome: F.A.O.

F.A.O. (1994). F.A.O. *Production Yearbook.* Rome.

F.A.O. (1996a). FAOSTAT Database Results-1995 [On-line]. Revised July 1996. Available: http://apps.fao.org/lim500/nph-wrap.pl?Production Crops Primary&Domain=SUS.

F.A.O. (1996b). FAOSTAT Database Results-1996 [On-line]. Revised July 1996. Available: http://apps.fao.org/lim500/nph-wrap.pl?Production Crops Primary&Domain=SUS.

Farah, S. M., Arar, A., & Miller, D. E. (1988). Water requirements and the irrigation management of pea, lentil, faba bean and chickpea crops. In *World Crops: Cool Season Food Legumes* (Ed. R. G. Summerfield), pp. 271-278. London: Kluwer Academic Publishers.

Farrar, J. F. (1988). Temperature and the partitioning and translocation of carbon. In *Plants and Temperature* (Eds C. J. Pollock, J. F. Farrar & A. J. Gordon), pp. 163-179. Oxford, UK: BIOS Scientific Publishers.

Farrar, J. F. (1991) Starch turnover: its role in source-sink relations and a comparison with the role of sucrose. In *Recent Advances in Phloem Transport and Assimilate Compartmentation* (Eds J. L. Bonnemain, S. Delrot, W. J. Lucas & J. Dainty), pp. 213-223. Nantes, France: Quest Edition.

Farrar, J. F. (1992). The whole plant: Carbon partitioning during development. In *Carbon Partitioning within and between Organisms.* (Eds C. J. Pollock, J. F. Farrar, A. J. Gordon), pp. 163-179. Oxford, UK: BIOS Scientific Publishers.

Farrar, J. F. (1993a). Forum on sink strength: what is it and how do we measure it? *Plant, Cell and Environment* **16,** 1013-1046.

Farrar, J. F. (1993b). Sink strength: what is it and how do we measure it? Introduction. *Plant, Cell and Environment* **16**, 1013-1046.

Farrar, J. F. (1993c) Sink strength: what is it and how do we measure it? A summary. *Plant, Cell and Environment* **16**, 1013-1046.

Farrar, J. F. (1996). Sinks-integral parts of a whole plant. *Journal of Experimental Botany* **47**, 1273-1280.

Farrar, J. F. & Williams, M. L. (1991). The effects of increased atmospheric carbon dioxide and temperature on carbon partitioning, source-sink relations and respiration. *Plant Cell and Environment* **14**, 819-830.

Farrington, P. & Pate, J. S. (1981). Fruit set in *Lupinus angustifolius* L. cv. Unicrop. 1. Phenology and growth during flowering and early fruiting. *Australian Journal of Plant Physiology* **8**, 293-305.

Feingold, D. S. & Avigad, G. (1980). Sugar nucleotide transformations in plants. In *The Biochemistry of plants, vol. 3*, (Ed. J. Preiss), pp. 101-170, New York: Academic Press.

Fetene, M. & Beck, E. (1993). Reversal of the direction of photosynthate allocation in *Urtica dioica* plants by increasing cytokinin import into the shoot. *Botanica Acta* **106**, 235-240.

Field, R. J., Hill, G. D., Attiya, H. J. (1989). Improved yield and harvest index in field bean (*Vicia faba* L.) with paclobutrazol. *Proceedings of the Plant Growth Regulator Society of America* **16**, 23-28.

Fisher, J. E. (1973). Developmental morphology of the inflorescence in hexaploid wheat cultivars with and without the cultivar norin 10 in their ancestry. *Canadian Journal of Plant Science* **53**, 7-15.

Flores, F., Moreno, M. T., Martinez, A. & Cubero, J. I. (1996). Genotype-environment interaction in faba bean: comparison of AMMI and principal co-ordinate models. *Field Crops Research* **47**(2-3), 117-127.

Forbes, J. C. & Watson, R. D. (Eds) (1992). *Plants in Agriculture.* Cambridge, Cambridge University Press.

Fosket, D. E., Volk, M. J. & Goldsmith, M. R. (1977). Polyribosome formation in relation to cytokinin-induced cell division in suspension culture of *Glysine max* (L.) Merr. *Plant Physiology* **60**, 554-562.

Fox, K. & Milford, G. F. J. (1996). Improvements in the performance of peas, beans and lupins as grain legumes for British and European agriculture. *In Proceedings of the 30th Occasional Symposium of the British Grassland Society.* (Ed. D. Younie). Pp. 52-61, SAC, Craibstone, Aberdeen, UK: British Grassland Society.

Foy, C. L. (Ed.) (1992). *Adjuvants & Agrochemicals, Vol. 2,* pp. 17-35. Boca Raton, Florida, USA: CRC Press.

French, R. J. & Turner, N. C. (1991). Water deficits change dry matter partitioning and seed yield in narrow-leafed lupins (*Lupinus angustifolius* L.). *Australian Journal of Agricultural Research* **42**, 471-484.

Fusseder, A., Wagner, B. & Beck, E. (1988). Quantification by ELISA of cytokinins in root-pressure exudates of *Urtica dioica* plants grown under different nitrogen levels . *Botanica Acta* **101**, 214-219.

Galtier, N., Murchie, E., Betsche, T., Thepenier, C., Lasceve, G., Quick, P. & Foyer, C. H. (1996). Modification of carbon metabolism in tomato plants overexpressing SPS in the leaves. *Journal of Experimental Botany* **47**, 1306.

Gan, S. & Amasino, M. (1996). Cytokinins in plant senescence: from spray and pray to clone and play. *Bioessays* **18**, 557-565.

Ganeshaiah, K. N. & Uma Shaanker, R. (1994). Seed and fruit abortion as a process of self organisation among developing sinks. *Physiologia Plantarum* **91**, 81-89.

Garcia-Martinez, J. L. & Carbonell, J. (1980). Fruit set of unpollinated ovaries of *Pissum sativum* L. Influence of plant growth regulators. *Planta* **147**, 451-456.

Garrod, G. F. (1982). The discovery and developments of plant growth regulators. In *Plant Growth Regulator Potential and Practice* (Ed. T. H. Thomas), pp. 29-98. Wantage: British Crop Protection Council Publications.

Gatel, F. (1992). Protein quality of legume seeds for monogastrics animals. *Proceedings of the 1st European conference in Grain Legumes* 1992, pp. 461-473. Angers: AEP.

Gates, P., Yarwood, J. N., Harris, N., Smith, M. L. & Boulter, E. (1981). Cellular changes in the pedicel and peduncle during flower abscission in *Vicia faba*. In *Vicia faba, Physiology and Breeding; World Crops, vol. 4* (Ed. R. Thompson), pp. 299-316. The Hague: Martinus Nijhoff.

Gates, P., Smith, M. L., White, G. & Boulter, E. (1982). Reproductive physiology and yield stability in *Vicia faba* L. *In The Physiology, Genetics and Nodulation of Temperate Legumes* (Eds D. R. Davies & D. J. Jones) London: Pitman Books.

Gates, P., Smith, M. L., White, G. & Boulter, E. (1983). Reproductive physiology and yield stability in *Vicia faba* L. In *The Physiology, Genetics and Nodulation of Temperate legumes* (Eds D. R. Davies & D. J. Jones), pp. 43-54. London: Pitman.

Gates, P., Smith, M. L. & Boulter, E. (1983). Reproductive physiology of *Vicia faba*. In *The Faba bean (Vicia faba L.). A Basis for Improvement* (Ed. P. D. Hebblethwaite), pp. 133-142. London: Butterworths.

Gehriger, W., Bellucci, S. & Keller, E. R. (1979). Influence of decapitation and growth regulators on yield components and yield of *Vicia faba* L. In *Some Current Research of Vicia faba in Western Europe* (Ed. Bond, D. A., Scarazia-Mugnosa, G. T. & Poulsen, M. H.), pp. 421-434. Commission of the European Communities.

Gehriger, W. & Keller, E. R. (1980). Influence of topping of faba beans (*Vicia faba* L.) on their growth and on the supply of the flowers with ^{14}C. *FABIS Newsletter* **2**, 33.

Geiger, D. R. & Servaites, J. C. (1994). Dynamics of self-regulation of photosynthetic carbon metabolism. *Plant Physiology and Biochemistry* **32**, 173-183.

Geiger, D. R., Koch, K. E., Shieh, W-J. (1996). Effect on environmental factors on whole plant assimilate partitioning and associated gene expression. *Journal of Experimental Botany* **47**, 1229-1238.

Gianfanga, T. J., Durner, E. F. & Idriss, A. (1991). Mode of action and use of plant growth retardants in reducing the effects of environmental stress on horticultural crops. In *Progress in Plant Growth Regulation* (Eds L. C. Karssen, L. C. Van Loon & D., Vreugdenhil), pp. 21-26. Netherlands: Kluer Academic Publishers.

Gianfanga, T. (1995). Natural and synthetic growth regulators and their use in horticultural and agronomic crops. In *Plant Hormones.* (Ed. P. J. Davies), pp. 751-773, The Netherlands: Kluer Academic Publishers.

Giaquinta, R. T. (1978). Source and sink leaf metabolism in relation to phloem translocation. *Plant Physiology* **61**, 380-385.

Giaquinta, R. T. (1983). Phloem loading of sucrose. *Annual Review of Plant Physiology* **34**, 347-387.

Gifford, R. M. (1986). Partitioning of photoassimilate in the development of crop yield. In *Phloem transport. Plant Biology,* vol. 1, (Eds J. Cronshaw, W. J. Lucas & R. T. Giaquinta), pp. 535-549. New York: Alan R. Liss.

Gifford, R. M. & Evans, L. T. (1981). Photosynthesis, carbon partitioning and yield. *Annual Review of Plant Physiology* **32**, 485-509.

Gifford, R. M., Thorne, J. H., Hitz, W. D. & Giaquinta, R. T. (1984). Crop productivity and photoassimilate partitioning. *Science* **225**, 801-808.

Gomez, K. A. & Gomez, A. A. (Eds) (1984). *Statistical Procedures for Agricultural Research,* 2nd Edition. New York, Chichester: John Wiley.

Goodrich, W. J., Cooke, R. J. & Morgan, A. G. (1985). The application of electrophoresis to the characterisation of cultivars of *Vicia faba* L. *FABIS Newsletter* **13**, 8-11.

Grabau, L. J. (1995). Physiological mechanisms of plant senescence. In *Handbook of Plant and Crop Physiology, section II* (Ed. M. Pessarakli), pp. 483-496. New York: Marcel Dekker.

Grasshoff, C. (1990) Effect of pattern of water supply on *Vicia faba* L. 2. Pod retention and filling and dry matter partitioning, production and water use. *Journal of Agricultural Science, Netherlands* **38**, 131-143.

Grossman, K. (1990). Plant growth retardants as tools in physiological research. *Physiologia Plantarum* **78**, 640-648.

Guern, J. (1987). Regulation from within: the hormone dilemma. *Annals of Botany* **60** (supplement 4), 75-102.

Gunn, C. R. (1978). Genus *Vicia* with notes about Tribe *Vicieae* (Fabaceae) in Mexico and Central America. USDA Technical Bulletin No. 1601. Washington: USDA.

Hack, H., Lembrich, H. & Morris, D. B. (1985). The use of RSW 0411 as a growth regulator in different crops under different conditions. *Proceedings of the British Crop Protection Conference-Weeds*, vol. 1, pp. 113-120. Croydon: BCPC Publications.

Halmann, M. (1990). Synthetic plant growth regulators. *Advances in Agronomy* **43**, 47-105.

Hardwick, R. C. (1988). Critical physiological traits in pulse crops. In *World Crops: Cool Season Food Legumes* (Ed. R. J. Summerfield), pp. 885-895. Kluer Academic Publishers.

Hassan, E. A. & El-Mousi, A. (1982). Response on broad bean plants to low Cycosel concentration treatments in comparison to a commercial source of cytokinin and together. *Zeitschrift Acker und Pflanzenbau* **151**, 81-88.

Hawker, J. S. (1985). Sucrose. In *Biochemistry of Storage Carbohydrates in Green Plants*, (Ed. J. S. Hawker), pp. 1-54, London: Academic Press.

Hawtin, G. C. & Hebblethwaite, P. D. (1983). Background and history of faba bean production. In *The Faba bean (Vicia faba L.). A Basis for Improvement* (Ed. P. D. Hebblethwaite), pp. 3-22. London: Butterworths.

Hebblethwaite, P. D., Hawtin, G. C. & Lutman, P. J. W. (1983). The husbandry of establishment and maintenance. In *The Faba Bean: A Basis for Improvement* (Ed. P. D. Hebblethwaite), pp. 271-294. London: Butterworths.

Hebblethwaite, P. D., Scott, R. K. & Kogbe, J. O. S. (1984). The effect of irrigation and bees on the yield and yield components of *Vicia faba* L. In *Vicia faba: Agronomy, Physiology and Breeding. World Crops: Production, Utilisation, Description*, vol. 10 (Eds P. D. Hebblethwaite, T. C. K. Dawkins, M. C. Heath & G. Lockwood), pp. 71-94. The Hague: Martinus Nijhoff/W. Junk.

Hebblethwaite, P. D., Batts, G. R. & Kantar, F. R. (1991). Influence of sowing date, irrigation, plant growth regulators use and flower colour on yield of faba beans. *Annals of Applied Biology* **27**, 77-84.

Helsper, J. P. F. G., Hoogendijk, J. M., van Norel, A. & Burger-Meyer, K. (1993). Antinutritional factors in faba beans (*Vicia faba* L.) as affected by breeding towards the absence of condensed tannins. *Journal of Agricultural and Food Chemistry* **41**, 1058-1061.

Henrix, J. E. (1995). Assimilate transport and partitioning. In *Handbook of Plant and Crop Physiology, Section II* (Ed. M. Pessarakli), pp. 357-386. New York: Marcel Dekker.

Henson, I. E. & Wheeler, C. T. (1976). Hormones in plants bearing nitrogen-fixing root nodules: the distribution of cytokinins in *Vicia faba* L. *New Phytologist* **76**, 433-439.

Higgins, J., Evans, J. L. & Law, J. R. (1988). A revised classification and descriptions of faba bean cultivars (*Vicia faba* L.). *Plant Varieties and Seeds* **1**, 27-35.

Hill-Cottingham, D. G. (1983). Chemical constituents and biochemistry. In *The Faba bean (Vicia faba L.). A Basis for Improvement* (Ed. P. D. Hebblethwaite), pp. 159-180. London: Butterworths.

Ho, L.C. (1979). Regulation of assimilate translocation between leaves and fruits in the tomato. *Annals of Botany* **43**, 437-448.

Ho, L. C. (1988). Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. *Annual Review of Plant Physiology and Plant Molecular Biology* **39**, 355-378.

Ho, L. C. (1992). Fruit growth and sink strength. *In Fruit and Seed Production. Aspects of Environmental Physiology and Ecology.* (Eds, C. Marshall & J. Grace), pp. 101-124. Cambridge University Press, Cambridge, UK.

Ho, L. C. (1996). The mechanism of assimilate partitioning and carbohydrate compartmentation in fruit in relation to the quality and yield of tomato. *Journal of Experimental Botany* **47**, 1239-1244.

Ho, L. C., Grange, R. I. & Picken, A. J. (1987). An analysis of the accumulation of water and dry matter in tomato fruit. *Plant Cell and Environment* **10**, 157-162.

Ho, L. C., Lecharny, A. & Willenbrink, J. (1991). Sucrose cleavage in relation to import and metabolism of sugars in sink organs. In *Recent Advances in Phloem Transport and Assimilate Compartmentation* (Eds Bonnemain, J. L., Delrot, S., Lucas, W. J. & Dainty, J.), pp. 178-186. Nantes, France: Quest Edition.

Hoad, G. V. (1995). Transport of hormones in the phloem of higher plants. *Plant Growth Regulation* **16**, 173-182.

Hobbie, L., Timpte, C. & Estelle, M. (1994). Molecular genetics of auxin and cytokinin. *Plant Molecular Biology* **26**, 1499-1519.

Hockart, C. H., Letham, D. S., Wang, J., Cornish, E. & Parker, C. W. (1991). Control of cytokinin levels by inhibitors of metabolism, symbiosis and genetic manipulation. In *Progress in Plant Growth Regulation* (Eds L. C. Karssen, L. C. Van Loon & D. Vreugdenhil), pp. 605-616. Netherlands, Kluer Academic Publishers.

Hodgeson, G. L. & Blackman, G. E. (1956). An analysis of the influence of plant density on the growth of *Vicia faba* L. *New Phytologist* **76**, 433-439.

Hodgeson, G. L. & Blackman, G. E. (1957). An analysis of the influence of plant density on the growth of *Vicia faba.* II The significance of competition for light in relation to plant development at different densities. *Journal of Experimental Botany* **8**, 195-219.

Hoffmann, G. (1991). Use of plant growth regulators in arable crops: survey and outlook. In *Progress in Plant Growth Regulation* (Eds L. C. Karssen, L. C. Van Loon & D. Vreugdenhil), pp. 798-808. Netherlands, Kluer Academic Publishers.

Horgan, R. (1984). Cytokinins. In *Advanced Plant Physiology* (Ed. M. B. Wilkins), pp. 53-74. Bath, Great Britain: Pitman Press.

Horgan, R. (1987). Cytokinin genes. In *Hormone Action in Plant Development* (Eds G. V. Hoad, J. R. Lenton, M. B. Jackson & R. K. Atkin), pp. 119-128. London: Butterworths.

Horgan, R. (1992a). Present and future prospects for cytokinin research. In *Physiology and Biochemistry of Cytokinins in Plants* (Eds M., Kaminek, D.W.S. Mok & E., Zazimalova), pp. 3-13. Hague, Netherlands: SPB Academic Publishing.

Horgan, R. (1992b). Cytokinin analysis. In *Physiology and Biochemistry of Cytokinins in Plants* (Eds M., Kaminek, D.W.S. Mok & E., Zazimalova), pp. 429-435. Hague, Netherlands: SPB Academic Publishing.

Hradecka. D. & Petr, J. (1992). Spring barley grain weight after treatment with cytokinins. *Sbornik Vysoke Skoly Zemedelske v Praze, Fakulta Agronomicka* **54**, 153-162.

Huber, S. C. & Akazawa, T. (1986). A novel sucrose synthase pathway for sucrose degradation in cultured sycamore cells. *Plant Physiology* **81**, 1008-1013.

Huff, A. & Dybing, C. D. (1980). Factors affecting shedding of flowers in soybean (*Glysine max* L. Merrill). *Journal of Experimental Botany* **31**, 751-762.

Huisman, J. (1991). Quality and use of legumes seeds for animal nutrition. In *Aspects of Applied Biology 27: Production and Protection of Legumes* (Eds R. J. Froud-Williams, P. Gladders, M. C. Heath, J. F. Jenkyn, C. M. Knott, A. Lane & D. Pink), pp. 11-22. Association of Applied Biologists.

Husain, M. M., Hill, G. D. & Gallagher, J. N. (1988a). The response of field beans (*Vicia faba* L.) to irrigation and sowing date. 1. Yield and yield components. *Journal of Agricultural Science, Cambridge* **111**, 221-232.

Husain, M. M., Hill, G. D. & Gallagher, J. N. (1988b). The response of field beans (*Vicia faba* L.) to irrigation and sowing date. 2. Growth and development in relation to yield. *Journal of Agricultural Science, Cambridge* **111**, 233-254.

Ismail, A. M. A. & Sagar, G. R. (1981). The influence of leaf age, leaf position and sinks on the rate of export and partition of ^{14}C on different stages of development following assimilation of $^{14}CO_2$ by a single leaf of *Vicia faba* L. *Journal of Horticultural Science* **56**, 55-63.

Itai, C. & Vaadia, Y. (1971). Cytokinin activity in water-stressed shoots. *Plant Physiology* **47**, 87-90.

IUPAC-IUB Commission on Biochemical Nomenclature (CBN). (1970). Abbreviations and symbols for nucleic acids, polynucleotides and their constituents. *Journal of Biological Chemistry* **245**, 5171-5176.

Janick J., Schery R. W., Woods F. W. & Ruttan V. W. (Eds) (1974). *Plant Science. An Introduction to World Crops, 2nd Edition.* San Francisco: W. H. Freeman & Co.

Jaquiery, R. & Keller, E. R. (1978a). Influence of the distribution of assimilates on pod set in field beans, *Vicia faba* L. Part 1. *Angew Botanik* **52**, 261-276.

Jaquiery, R. & Keller, E. R. (1978b). Influence of the distribution of assimilates on pod set in field beans, *Vicia faba* L. Part 2. *Angew Botanik* **54**, 29-39.

Jeffcoat, B. (1981). *Aspects and Prospects of Plant Growth Regulators. Monograph 6.* British Plant Growth Regulator Group, Wantage.

Jenner, C. F. & Hawker, J. S. (1993). Sink strength: soluble starch synthase as a measure of sink strength in wheat endosperm. *Plant, Cell and Environment* **16**, 1013-1046.

Johansson, M. & Walles, B. (1993). Functional anatomy of the ovule in broad bean (*Vicia faba* L.). 1. Histogenesis prior to and after pollination. *International Journal of Plant Sciences* **154**(1), 80-89.

Johansson, M. & Walles, B. (1994). Functional anatomy of the ovule in broad bean (*Vicia faba* L.): Ultrastructural seed development and nutrient pathways. *Annals of Botany* **74,** 233-244.

Kambal, A. E. (1969). Flower drop and fruit set in field bean *Vicia faba* L. *Journal of Agricultural Science, Cambridge* **72**, 131-138.

Kambal, A. E., Bond, D. A. & Toynbee-Clarke, G. (1976). A study on the pollination mechanism in field beans (*Vicia faba* L.). *Journal of Agricultural Science, Cambridge* **87,** 519-526.

Kaminek, M. (1992). Progress in cytokinin research. *TIBTECH* **10**, 159-165.

Kaminek, M., Motyka, V. & Zazimalova, E. (1996). Hormonal control of wheat grain development and loading. *Journal of Experimental Botany* **47**, 1302.

Karamanos, A. J. (1978). Water stress and leaf growth of field beans (*Vicia faba* L.) in the field: leaf number and total leaf area. *Annals of Botany* **42**, 1393-1402.

Karlen, D. L., Varvel, G. E., Bullock, D. G. & Cruse, R. M. (1994). Crop rotations for the 21st century. *Advances in Agronomy* **53**, 2-37.

Kauffman, P. B., Ghosheh, N. S., Lacroix, J. D., Soni, S. L. & Ikuma, H. (1973). Regulation of invertase levels in *Avena* stem segments by gibberellic acid, sucrose, glucose and fructose. *Plant Physiology* **52**, 221-228.

Keller, E. R. (1974). Die ackerbohne (*Vicia faba* L.) eine vergessene kulturpflanze mit zukunftsaussichten? *Schweizerische Landwirtschafliche Forschung* **13**, 287-300.

Keller, E. R. & Bellucci, S. (1980). Effect of growth regulators on faba bean (*Vicia faba* L.) development. *FABIS Newsletter* **2**, 36.

Keller, E. R. & Bellucci, S. (1983). The influence of growth regulators on development and yield of *Vicia faba* L. In *The Faba bean (Vicia faba L.). A Basis for Improvement* (Ed. P. D. Hebblethwaite), pp. 181-196. London: Butterworths.

Kellerhals, M. & Keller, E. R. (1984). Effect of plant growth regulator combinations on faba bean development and yield components. In *Vicia faba, Agronomy, Physiology and Breeding. World Crops: Production, Utilisation, Description,* vol. 10 (Eds P. D. Hebblethwaite, T. C. K. Dawkins, M. C. Heath & G. Lockwood), pp. 37-46. The Hague: Martinus Nijhoff.

Khayat, E. & Zieslin, N. (1987). Effect of night temperature on the activity of sucrose phosphate synthase, acid invertase, and sucrose synthase in source and sink tissues of *Rosa hybrida* cv. Golden Times. *Plant Physiology* **84**, 447-449.

Kinet, J. M. (1977). Effect of light condition on the development of inflorescence in tomato. *Scientia Holticulturae* **6**, 15-26.

Kinet, J-M., Sachs, R. M. & Bernier, G. (Eds) (1985a). *The Physiology of Flowering. Volume III. The Development of Flowers.* Boca Raton, Florida, USA: CRC Press.

Kinet, J. M., Zime, V., Linotte, A., Jacqmond, A. & Bernier, G. (1986). Resumption of cellular activity induced by cytokinin and gibberellin in treatments in tomato flowers targeted for abortion in unfavourable light conditions. *Physiologia Plantarum* **64**, 67-73.

Kinet, J-M., Zune, V., Linotte, C., Jacqmard, A. & Bernier, G. (1985b). Resumption of cellular activity induced by cytokinin and gibberellin treatments in tomato flowers targeted for abortion in unfavourable light conditions. *Physiologia Plantarum* **64**, 67-73.

Kirkwood, R. C. (1991). Pathways and mechanisms of uptake of foliage-applied herbicides with particular reference to the role of surfactants. In *Target Sites for Herbicide Action* (Ed. R. C. Kirkwood), pp. 219-243. New York: Plenum Press.

Kirkwood, R. C. (1993). Use and mode of action of adjuvants for herbicides: a review of some current work. *Pesticide Science* **38**, 93-102.

Klee, H. J. & Romano, C. P. (1994). The roles of phytohormones in development as studied in transgenic plants. *Critical Reviews in Plant Sciences* **13**, 311-324.

Knott, C. M. (1990). A key for stages of development of the faba bean (*Vicia faba*). *Annals of Applied Biology* **116**, 391-404.

Knott, C. M., Biddle, A. J. & McKeown, B. M. (Eds) (1994). *The PGRO Field Bean Handbook.* England: Processors & Growers Research Organisation.

Koch, K. E. & Nolte, K. D. (1995). Sugar-modulated expression of genes for sucrose metabolism and their relationship for transport pathways. In *Carbon Partitioning and Source-Sink Interactions in Plants* (Eds M. Madore, W. J. Lucas), pp. 141-155. Rockville, MD: American Society of Plant Physiologists.

Koch, K. E., Nolte, K. D., Duke, E. D., McCarty, D. R. & Avigne, W. T. (1992). Sugar levels modulate differential expression of maize sucrose synthase genes. *The Plant Cell* **4**, 59-69.

Koch, K. E., Xu, J., Duke, E. D., McCarty, D. R., Yuan, C. X., Tan, B. C. & Avigne, W. T. (1995). Sucrose provides a long distance signal for coarse control of genes affecting its metabolism. In *Sucrose metabolism, Biochemistry and Molecular Biology* (Eds H. D. Pontis, G., Salerno & E., Echeverria), pp. 266-277. Rockville, MD: American Society of Plant Physiologists.

Kogure, K. (1984). Report of grant-in-aid from the Japanese ministry of Education, Science and Culture of 1982-1983, 111-120.

Kogure, K., Asanuma, K. & Kusutani, A. (1991). Mobility performance of chemical components on the indeterminate and determinate types of faba bean plant. *Proceedings of the 1st European conference in Grain Legumes* 1992, pp. 233-234. Angers: AEP.

Komor, E. (1994). Regulation by futile cycles: The transport of carbon and nitrogen in plants. In *Flux Control in Biological Systems. From Enzymes to Populations and Ecosystems* (Ed. E. D. Schulze), pp. 153-202. San Diego, California, USA: Academic Press.

Koscielniak, J., Filek, W. & Augustyniak, G. (1990). Photosynthetic activity in different organs of field beans (*Vicia faba* L. *minor*) with indeterminate and determinate growth habit. *Acta Physiologiae Plantarum* **12**(2), 95-103.

Koshimizu, K. & Iwamura, H. (1986). Cytokinins. In *Chemistry of Plant Hormones* (Ed. N. Takahashi), pp. 153-199. Boca Raton, Florida: CRC Press.

Krausgrill, S., Sander, A., Greiner, S., Weil, M. & Rausch, T. (1996). Regulation of cell wall invertase by a proteinaceous inhibitor. *Journal of Experimental Botany* **47**, 1193-1198.

Kuang, A., Peterson, C. M. & Dute, R. R. (1991). Changes in soybean raceme and petiole anatomy induced by 6-benzylaminopurine. *Annals of Botany* **67**, 23-28.

Kuang, A., Peterson, C. M. & Dute, R. R. (1991). Pedicel abscission and rachis morphology as influenced by BA. *Plant Growth Regulation* **10** (4), 291-303.

Kuang, A., Peterson, C. M. & Dute, R. R. (1992). Leaf abscission in soya bean: cytochemical and ultrastructural changes following benzylaminopurine treatment. *Journal of Experimental Botany* **43**(257), 1611-1619.

Kuiper, D. (1988). Growth response of *Plantago major* L. ssp. *pleiosperma* (Pilger) to changes in mineral supply. *Plant Physiology* **87**, 555-557.

Kuiper, D. (1993). Sink strength: established and regulated by plant growth regulators. *Plant, Cell and Environment* **16,** 1013-1046.

Kuiper, D., Kuiper, P. J. C., Lambers, H., Schuit, J. & Staal, M. (1989). Cytokinin concentration in relation to mineral nutrition and benzyladenine treatment in *Plantago major ssp pleiosperma. Physiologia Plantarum* **75**, 511-517.

Kuiper, D., Sommarin, M. & Kylin, A. (1992). The modulating effects of pH and benzyladenine on the Ca^{2+}- and Mg^{2+}- ATPases in the plasmalemma of wheat root cells. *Physiologia Plantarum* **84**, 367-373.

Kulaeva, O. N., Karavaiko, N. N., Selivankina, S. Y., Moshkov, I. E., Novikova, G. V., Zemlyachenko, Y. V., Shipilova, S. V. & Orudgev, E. M. (1996). Cytokinin signalling systems: From a whole plant to the molecular level. *Plant Growth Regulation* **18**(1-2), 29-37.

Ladizinski, G. (1975). On the origin of the broad bean *Vicia faba* L. *Israel Journal of Botany* **24**, 80-88.

Lawes, D. A., Bond, D. A. & Poulsen, M. H. (1983). Classification, origin, breeding methods and objectives. In *The Faba bean (Vicia faba L.). A Basis for Improvement* (Ed. P. D. Hebblethwaite), pp. 23-76, London: Butterworths.

Leopold, A. C. & Kriedemann, P. E. (Eds) (1975). In *Plant Growth and Development, 2nd Edition.* USA: McGraw-Hill.

Letham, D. S. (1967). Chemistry and physiology of kinetin-like compounds. *Annual Review of Plant Physiology* **18**, 349-364.

Letham, D. S. (1978). Cytokinins. In *Phytohormones and Related Compounds: A Comprehensive Treatise,* vol. 1 (Eds D. S. Letham, P. B. Goodwin & T. J. V. Higgins), pp. 205-264. Amsterdam: Elsevier.

Letham, D. S. & Bollard, E. G. (1961). Stimulants of cell division in developing fruits. *Nature* **191**, 119-1120.

Letham, D. S. & Gollnow, B. I. (1985). Regulators of cell division in plant tissues. XXX. Cytokinin metabolism in relation to radish cotyledon expansion and senescence. *Journal of Plant Growth Regulation* **4**, 129-145.

Letham, D. S. & Palni, L. M. S. (1983). The biosynthesis and metabolism of cytokinins. *Annual Review of Plant Physiology* **34**, 163-197.

Li, C. -J., Guevara, E., Herrera, J. & Bangerth, F. (1995). Effect of apex excision and replacement by 1-naphthylacetic acid on cytokinin concentration and apical dominance in pea plants. *Physiologia Plantarum* **94**, 465-469.

Link, W. (1995). Exploitation of hybrid vigour in faba bean (*Vicia faba* L.). *Proceedings of the 2nd European conference in Grain Legumes* 1995, pp. 218-219. Copenhagen: AEP.

Link, W., Ederer, W., Metz, P., Buiel, H. & Melchinger, A. E. (1994). Genotypic and environmental variation for degree of cross-fertilisation in faba bean. *Crop Science* **34**, 960-964.

Lord, E. M. & Heslop-Harrison, Y. (1984). Pollen-stigma interaction in the *Leguminosae*: Stigma organisation and the breeding system in *Vicia faba* L. *Annals of Botany* **54**, 827-836.

Lorenzi, R., Bennici, A., Cionini, P. G., Alpi, A. & D'Amato, F. (1978). Embryo-suspensor relations in *Phaseolus coccineus*: cytokinins during seed development. *Planta* **143**, 59-62.

Luckwill, L. C. (1978). The potential and limitations of growth regulators in crop production. In *Opportunities for Chemical Plant Growth Regulation.* Monograph 21. British Crop Protection Council.

Luckwill, L. C. (Ed.) (1981). *Growth Regulators in Crop Production. London: Edward Arnold.*

Lukaszewska, A. J., Bianco, J., Barthe, P. & Le Page-Degivry, M. T. (1994). Endogenous cytokinins in rose petals and the effect of exogenously applied cytokinins on flower senescence. *Plant Growth Regulation* **14**, 119-126.

M.A.F.F. (1996). *Agriculture in the United Kingdom 1995.* London: HMSO.

Marcelis, L. F. M. (1993). Effect of assimilate supply on the growth of individual cucumber fruits. *Physiologia Plantarum* **87**, 321-328.

Marcelis, L. F. M. (1994). Fruit shape in cucumber as influenced by fruit load, light and temperature. *Scientia Horticulture* **56**, 299-308.

Marcelis, L. F. M. (1996). Sink strength as a determinant of dry matter partitioning in the whole plant. *Journal of Experimental Botany* **47**, 1281-1292.

Marshall, C. & Grace, J. (1992). Preface. In *Fruit and Seed Production: Aspects of Environmental Physiology and Ecology* (Eds, C. Marshall & J. Grace), pp. xi, Cambridge, UK: Cambridge University Press.

Matsubara, S. (1990). Structure-activity relationships of cytokinins. *Critical Reviews in Plant Science* **9**, 17-57.

Mauk, C. S., Brinker, A. M. & Nooden, L. D. (1990). Probing monocarpic senescence and pod development through manipulation of cytokinin and mineral supplies in soybean explants. *Annals of Botany* **66**, 191-201.

McEwen, J. (1972). Effects of defoliating different zones on the plant of field beans (*Vicia faba* L.). *Journal of Agricultural Science, Cambridge* **72**, 131-138.

McGaw, B. A. (1988). Cytokinin biosynthesis and metabolism. In *Plant Hormones and Their Role in Plant Growth and Development* (Ed. P. D. Davies), pp. 76-93. Dordrecht, The Netherlands: Kluwer Academic Publisher.

McGaw, B. A. & Burch, L. R. (1995). Cytokinin biosynthesis and metabolism. In *Plant Hormones.* (Ed. P. J. Davies), pp. 98-117. The Netherlands: Kluer Academic Publishers.

Miller, C. O. (1961). A kinetin-like compound in maize. *Proceedings of the National Academy of Science, U.S.A.* **47**, 170-174.

Miller, C. O. (1967). Cytokinins in *Zea mays*. *Annals of New York Academy of Science* **144**, 251-257.

Miller, C. O., Skoog, F., Okomura, F. S., Saltza, M. H. & F. M. Strong. (1956). Isolation, structure and synthesis of kinetin, a substance promoting cell division. *Journal of American Chemical Society* **78**, 1345-1350.

Miller, C. O., Skoog, F., Saltza, M. H. & F. M. Strong. (1955). Kinetin, a cell division factor from deoxyribonucleic acid. *Journal of American Chemical Society* **77**, 1329-1334.

Minchin, P. E. H. & Thorpe, M. R. (1993). Sink strength: a misnomer, and best forgotten. *Plant, Cell and Environment* **16**, 1013-1046.

Minchin, P. E. H. & Thorpe, M. R. (1996). What determines carbon partitioning between competing sinks? *Journal of Experimental Botany* **47**, 1293-1296.

Moore, T. C. (Ed.) (1979). *Biochemistry and Physiology of Plant Hormones*. New York: Springer.

Morris, D. A. (1982). Hormonal regulation of sink invertase activity: Implications for the control of assimilate partitioning. In *Plant Growth Substances* (Ed. P. F. Wareing), pp. 659-668. Academic Press, UK.

Morris, D. A. & Arthur, E. D. (1984). Invertase activity in sinks undergoing cell expansion. *Plant Growth Regulation* **2**, 327-337.

Morris, D. A. & Arthur, E. D. (1985). Effects of gibberellic acid on patterns of carbohydrate distribution and acid invertase activity in *Phaseolus vulgaris*. *Physiologia Plantarum*, Copenhagen **65**, 257- 262.

Morris, O. R. (1995). The roles of phytohormones in development as studied in transgenic plants. *Critical Reviews in Plant Sciences* **13**, 311-324.

Morris, O. R., Blevins, G. D., Dietrich, T. J., Durley, C. R., Gelvin, B. S., Gray, J., Hommes, G. N., Kaminek, M., Mathews, J. L., Meilan, R., Reinbott, M. T. & Sayavedra-Soto, L. (1993). Cytokinins in plant pathogenic bacteria and developing cereal grains. *Australian Journal of Plant Physiology* **20**, 621-637.

Mothes, K. & Engelbrecht, L. (1961). Kinetin-induced directed transport of substances in excised leaves in the dark. *Phytochemistry* **1,** 58-62.

Mothes, K., Engelbrecht, L. & Kulajewa, O. (1959). Uber die wirkung des kinetins auf stickstoffverteilung und eiweiβsynthese in isolierten blatten. *Flora (Jena)* **147**, 445-465.

Mothes, K., Engelbrecht, L. & Schutte, H. R. (1961). Uber die akkumulation von a-aminoisobuttersaure im blattegewebe under dem einfluβ von kinetin. *Physiologia Plantarum* **14**, 72-75.

Muratova, V. (1931). Common beans, *Vicia faba* L. *Bulletin of Applied Botany Genetics and Plant Breeding, Leningrad* (Supplement), pp. 298.

Naqvi, S. S. M. (1995). Plant growth hormones: Growth promoters and inhibitors. In *Handbook of Plant and Crop Physiology, section III* (Ed. M. Pessarakli), pp. 527-556. New York: Marcel Dekker.

Neumann, D. S., Rood, S. B., & Smit, B. A. (1990). Does cytokinin transport from root-to-shoot in the xylem sap regulate leaf response to root hypoxia? *Journal of Experimental Botany* **41**, 1325-1333.

Newaz, M. A. & Lawes, D. A. (1980). Differential response of *Vicia faba* L. genotypes to 2,3,5-triiodobenzoic acid (TIBA). *Euphytica* **29**, 419-424.

Newman, C. W., Newman, R. K. & Lockerman, R. H.(1988). Utilisation of food legumes in human nutrition. In *World Crops: Cool Season Food Legumes* (Ed. R. J. Summerfield), pp. 405-411. Netherlands: Kluer Academic Publishers.

Ney, B. (1992). Some advances in crop physiology and possible utilisations for crop management and breeding. *Proceedings of the 1st European conference in Grain Legumes* 1992, pp. 187-193. Angers: AEP.

Nicolas, M., Gregoire, S. & Teoule, E. (1995). Gene transfer in pea (*P. sativum* L.) via somatic embryogenesis. *Proceedings of the 2nd European conference in Grain Legumes* 1995, pp. 424-425. Copenhagen: AEP.

Nishinari, N. & Syono, K. (1980). Changes in endogenous cytokinin levels in partially synchronized cultured tobacco cells. *Plant Physiology* **65**, 437-441.

Nooden, L. D. (1988). Whole plant senescence. In *Senescence and Ageing in Plants* (Eds L. D. Nooden & A. C. Leopold), pp. 391-439. San Diego: Academic Press.

Nooden, L. D. & Guiamet, J. J. (1989). Regulation of assimilation and senescence by the fruit in monocarpic plants. *Physiologia Plantarum* **77**, 267-274.

Nooden, L. D. & Letham, D. S. (1993). Cytokinin metabolism and signalling in the soybean plant. *Australian Journal of Plant Physiology* **20**, 639-653.

Nooden, L. D., Singh, S., Letham, D. S. (1989). Correlation of xylem sap cytokinin levels with monocarpic senescence in soybean. *Plant Physiology* **93**, 33-39.

Nwokolo, E. (1996). The need to increase consumption of pulses in the developing world. In *Legumes and Oilseeds in Nutrition* (Eds E. Nwokolo & J. Smartt), pp. 3-11. London: Chapman and Hall.

Oberholster, S. D., Peterson, C. M., Dute, R. R. (1991). Pedicel abscission of soybean: cytological and ultrastructural changes induced by auxin and ethephon. *Canadian Journal of Botany* **69**, 2177-2186.

Ohya, T. & Suzuki, H. (1991). The effect of BA on the accumulation of messenger RNAs that encode the large and small subunits of Rubisco and light-harvesting chlorophyll α/β protein in excised cucumber cotyledons. *Plant Cell Physiology* **32**, 577-580.

Palta, J. A. & Ludwig, C. (1996). Pod set and seed yield as affected by cytokinin application and terminal drought in narrow-leafed lupin. *Australian Journal of Agricultural Research* **48**(1), 81-90.

Patrick, J. W. (1982). Hormonal control of assimilate transport. In *Plant Growth Substances* (Ed. P. F. Wareing), pp. 659-668. Academic Press, UK.

Patrick, J W. (1988). Assimilate partitioning in relation to crop productivity. *Horticultural Science* **23**, 33-40.

Patrick, J. W. (1991). Control of phloem transport to and short-distance transfer in sink regions: an overview. In *Recent Advances in Phloem Transport and Assimilate Compartmentation.* (Eds J. L. Bonnemain, S. Delrot, W. J. Lucas & J. Dainty), pp. 167-177. France: Quest Editions.

Patrick, J. W. (1993). Sink strength: whole plant considerations. *Plant, Cell and Environment* **16**, 1013-1046.

Patrick, J. W. & Wareing, P. F. (1981). Hormonal control of assimilate movement and distribution. In *Aspects and Prospects of Plant Growth Regulators* (Ed. B. Jeffcoat), pp. 65-84. British Plant Growth Regulator Group Monograph 6.

Paul, C., Gates, P., Harris, N. & Boulter, D. (1978). Asynchronous sexual development determines the breeding system in field beans. *Nature, London* **275**, 54-55.

Peat, W. E. (1983). Developmental physiology. In *The Faba bean (Vicia faba L.). A Basis for Improvement.* (Ed. P. D. Hebblethwaite), pp. 103-132. London: Butterworth.

Peter, M., Aufhammer, W. & Bangerth, F. (1989). Dry matter accumulation at definite pod positions within field bean (*Vicia faba* L.) plants and possible relations with indolacetic acid and abscisic acid levels. *Plant Growth Regulation* **8**, 143-151.

Peterson, C. M., Folsom, M. W., Dalrymple, L. W. & Hoogenboom, G. (1984). Pod set on terminal racemes of soybean. Abstracts of the World Soybean Research Conference III, 12-17 August, pp. 40. Ames: Iowa State University.

Pharis, R. P. & Reid, D. M. (Eds) (1985). *Hormonal Regulation of Development; III. Role of Environmental Factors. Encyclopaedia of Plant Physiology; New Series,* vol. 11. Berlin-Heidelberg, Germany: Springer-Verlag,.

Picard, J. (1979). Some reflections on problems and prospects in *Vicia faba* breeding. In *Some Current Research on Vicia faba in Western Europe* (Eds D. A. Bond, G. T. Scarascia-Mugnozza & M. H. Poulsen), pp. 23-33. EEC publication EUR 6244.

Pilbeam, C. J. & Hebblethwaite, P. D. (1990). Effects of plant population density on spring-shown field beans (*Vicia faba*) with different growth habits. *Journal of Agricultural Science, Cambridge* **114**, 19-33.

Pillbeam, C. & Hebblethwaite, P. (1994). The faba bean. *Biologist*, **41**, 169-172.

Pillbeam, C. J., Hebblethwaite, P. D. & Richards, H. E. (1989). The response of determinate and indeterminate faba beans to different sowing dates in the spring. *Annals of Applied Biology* **114**, 377-390.

Pillbeam, C. J., Dug, G. & Hebblethwaite, P. D. (1990). Effects of plant population density on spring-sown field beans (*Vicia faba)* with different growth habits. *Journal of Agricultural Science, Cambridge* **114**, 19-33.

Pollock, C. & Farrar, J. (1996). Source-sink relations: the role of sucrose. In *Environmental Stress and Photosynthesis* (Ed. N. R. Baker), pp. 215-227. The Netherlands: Kluwer.

Purohit, S. S. (Ed.) (1985). *Hormonal regulation of plant growth and development, volume II.* Bikaner (India): Agro Botanical Publishers.

Rademacher, W. (1991). Actual and potential achievements with PGRs in arable crops. A critical assessment. In *Manipulation of Canopy Structure in Arable Crops*, pp. 97-101. British Society for Plant Growth Regulation, Monograph 22.

Ramina, A. (1979). Aspects of [$^{8-14}$C]benzylaminopurine metabolism in *Phaseolus vulgaris*. *Plant Physiology* **63**, 298-300.

Ramsay, G. & Middlefell-Williams, J. E. (1995). Towards efficient transformation of the grain legumes by optimising the plant-*Agrobacterium* interaction. *Proceedings of the 2nd European Conference in Grain Legumes* 1995, pp. 428-429. Copenhagen: AEP.

Ramsay, G., Griffiths, D. W. & Down, N. D. (1991). Spontaneous and induced variation in levels of vicine and convicine in faba beans. *Aspects of Applied Biology* **27**, 43-47.

Reese, R. N., Dybing, C. D., White, C. A., Page, S. M., Larson, J. E. (1995). Expression of vegetative storage protein (VSP-b) in soybean raceme tissues in response to flower set. *Journal of Experimental Botany* **46**, 957-964.

Reid, D. M. & Wample, R. L. (1985). Water relations and plant hormones. In *Hormonal Regulation of Development; III. Role of Environmental Factors. Encyclopaedia of Plant Physiology; New Series,* vol. 11 (Eds R. P. Pharis & D. M. Reid), pp. 513-578. Berlin-Heidelberg, Germany: Springer-Verlag,.

Richards, M. C. & Smith, M. L. (1987). Preliminary investigation into plant growth regulators on combining peas and field beans in Scotland. *Proceedings Crop Protection in Northern Britain* 1987, pp. 249-254. Invergrowrie: Scottish Crop Research Institute.

Riffkin, H. I., Duffus, C. M. & Bridges, I. C. (1995). Sucrose metabolism during endosperm development in wheat (*Triticum aestivum*). *Physiologia Plantarum* **93**, 123-131.

Robinson, N. L., Hewitt, ,J. D. & Bennett, A. B. (1988). Sink metabolism in tomato fruit. I. Developmental changes in carbohydrate metabolising enzymes. *Plant Physiology* **87**, 727-730.

Rochat, C., Dejardin, A., Maugenest, S., Lemontey, C., Hirel, B. & Boutin, J. -P. (1996). Is sucrose synthase a key parameter in sink strength determination of the developing pea seed? *Journal of Experimental Botany* **47**, 1329.

Roe, J. H. (1934). A colorimetric method for the determination of fructose in blood and urine. *Journal of Biological Chemistry* **106**, 15-22.

Romanov, G. A. (1990). Cytokinins and tRNAs: A hypothesis on their competitive interaction via specific receptor proteins. *Plant, Cell and Environment* **13**, 751-754.

Ronzhina, E. S. & Mokronosov, A. T. (1994). Source-sink relations and the role of cytokinins in the regulation of transport and partitioning of organic substances in plants. *Russian Journal of Plant Physiology* **41**, 396-406.

Ronzhina, E. S., Mokronosov, A. T., Sokolova, S. V. (1994). The action of cytokinins on the transport and partitioning of substances in detached leaves: 1. Sink components of cytokinin effects on plants with different types of terminal phloem. *Russian Journal of Plant Physiology* **41**, 623-628.

Ronzhina, E. S., Sokolova, S. V. & Mokronosov, A. T. (1995). Action of cytokinins on transport and partitioning of substances in detached leaves. 2. Conditions for developing the cytokinin effects. *Russian Journal of Plant Physiology* **42**, 52-57.

Ross, H. A. & Davies, H. V. (1992). Sucrose metabolism in tubers of potato (*Solanum tuberosum* L.). Effects of sink removal and sucrose flux on sucrose-degrading enzymes. *Plant Physiology* **98,** 287-293.

Rowland, G. C., Bond, D. A. & Parker, M. K. (1982). Estimates of the frequency of fertilisation in faba beans (*Vicia faba* L.). *Journal of Agricultural Science, Cambridge* **100**. 25-33.

Rufty, T. W. & Huber, S. C. (1983). Changes in starch formation and activities of sucrose phosphate synthase and cytoplasmic fructose-1,6-biphosphatase in response to source-sink alterations. *Plant Physiology* **72**, 474-480.

Ruiz, R. & Guardiola, J. L. (1994). Carbohydrate and mineral nutrition of orange fruitlets in relation to growth and abscission. *Physiologia Plantarum* **90**, 27-36.

Russell, C. R. & Morris, D. A. (1983). Patterns of assimilate distribution and source-sink relationships in the young reproductive tomato plant (*Lycopersicon esculentum* Mill.). *Annals of Botany* **52**, 357-363.

Russell, G., van Gardingen, P. & Wilson, G. W. (1993). Using physiological information about varieties: the way forward? *Aspects of Applied Biology (Physiology of Varieties)* **34**, 47-56.

Russell, J. S., Beech, D. F. & Jones, P. N. (1989). Grain legume productivity in subsistence agriculture. A global challenge. *Food Policy* **15**, 129-142.

Rylott, P. D. (1991). Some Effects of Plant Growth Substances on Broad Beans (*Vicia faba L. major*). Ph.D. Thesis, University of Edinburgh.

Rylott, P. D & Smith, M. L. (1990). Effects of applied plant growth substances on pod set in broad beans (*Vicia faba* L. *major*). *Journal of Agricultural Science, Cambridge* **114**, 41-47.

Saalbach, I., Waddell, D., Pickardt, T., Heimbeck, W., Fontaine, J., Horstmann, C. Schieder, O. & Muntz, K. (1995). Stable expression of the sulphur-rich 2S albumin gene in transgenic *Vicia narbonensis* increases the methionine content of seeds. *Proceedings of the 2nd European Conference in Grain Legumes* 1995, pp. 426-427. Copenhagen: AEP.

Sachs, T. & Thimann, K. V. (1967). The role of auxins and cytokinins in the release of buds dominance. *American Journal of Botany* **54**, 136-144.

Salama, A. M. S. E. D. A. & Wareing, P. F. (1979). Effects of mineral nutrition on endogenous cytokinins in plants of sunflower (*Helianthus annuus* L.). *Journal of Experimental Botany* **30**, 971-981.

Sattelmacher, B. & Marschner, H. (1978). Nitrogen nutrition and cytokinin activity in *Solanum tuberosum*. *Physiologia Plantarum* **42**, 185-189.

Sauer, C. O. (Ed.) (1952). *In Agricultural Origins and Dispersals*. New York: American Geographic Society.

Sayavedra-Soto, L. A., Durley, R. C., Trione, E. J. & Morris, R. O. (1988). Identification of cytokinins in young wheat spikes (*Triticum aestivum* cv. Chinese Spring). *Journal of Plant Growth Regulation* 7, 169-178.

Schaffer, A. A. (1986). Invertases in young and mature leaves of *Citrus cinensis*. *Phytochemistry* **25**(10), 2275-2277.

Schapendonk, A. H. C. M. & Brouwer, P. (1984). Fruit growth of cucumber in relation to assimilate supply and sink activity. *Scientia Horticulturae* **23**, 21-33.

Schell, J., Koncz, C., Spena, A., Palme, K. & Walden, R. (1993). Genes involved in the control of growth and differentiation in plants. *Gene* **135**, 245-249.

Schneider, G., Erdmann, D., Lust, S., Mohr, G. & Niethammer, K. (1965). Morphatins-a novel group of plant growth regulators. *Nature* **208**, 1013.

Schuch, W. (1990). Biotechnology and plant growth regulator research. In *Current and Future Methods in Plant Growth Regulator Research*, pp. 91-104. British Society for Plant Growth Regulation, Monograph 19.

Scott, T. K. (Ed.) (1984). *Hormonal Regulation of Development; II. The Functions of Hormones from the Level of the Cell to the Whole Plant.* (Encyclopaedia of Plant Physiology; New Series, volume 10). Berlin-Heidelberg, Springer-Verlag.

Sexton, R., Porter, A. E., Littlejohns, S., Thain, S. C. (1995). Effects of diazocyclopentadiene (DACP) and silver thiosulphate (STS) on ethylene regulated abscission of sweet pea flowers (*Lathyrus odoratus* L.). *Annals of Botany* **75**, 337-342.

Singh, B. K. & Jenner, C. F. (1982). Association between concentrations of organic nutrients in the grain, endosperm cell number and grain dry weight within the ear of wheat. *Australian Journal of Plant Physiology* **9**, 83-95.

Sitton, D., Itai, C. & Kende, H. (1967). Cytokinin production as a factor in shoot senescence. *Planta* **73**, 296-300.

Skene, K. G. M. (1975). Cytokinin production by roots as a factor in the control of plant growth. In *The development and Function of Roots* (Eds J. G. Torrey & Clarkson D. T.), pp. 365-396. London: Academic Press.

Skoog, F. & Miller, C. O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. *Symposium of the Society of Experimental Biology* **11**, 118-131.

Skoog, F., Strong, F. M. & Miller, C. O. (1965). Cytokinins. *Science* **148**, 532.

Skoog, F., Hamzi, H. Q., Szweykowska, A. M., Leonard, N. J., Carraway, K. C., Fujii, T., Helson, J. P. & Loeppky, R. N. (1967). Cytokinins: structure activity relationships. *Phytochemistry* **6**, 1169-1192.

Smith, M. L. (1982a). Factors affecting flower abscission in field beans (*Vicia faba* L. *minor*). PhD thesis, University of Durham.

Smith, M. L.(1982b) The effect of shading at different periods during flowering on flower abscission in *Vicia faba* L. *minor*. *FABIS* **4**, 32-34.

Smith, M. L.(1982c) Response of four genotypes of spring field beans (*Vicia faba* L. minor) to irrigation during the flowering period in the United Kingdom. *FABIS* **4**, 39-41.

Smith, M. L. & Rylott, P. D. (1992). The effects of cytokinin application on successful fruit development in faba beans (*Vicia faba* L.). *Proceedings of the 1st European conference in Grain Legumes* 1992, pp. 199-200. Angers: AEP.

Sonnewald, U. & Willmitzer, L. (1992). Molecular approaches to sink-source interactions. *Plant Physiology* **99**, 1267-1270.

Soper, M. H. R. (1952). A study of the principal factors affecting the establishment and development of the field bean (*Vicia faba* L.). *Journal of Agricultural Science, Cambridge* **42**, 335-346.

Sprent, J. I. & Mannetje, L. (1996). The role of legumes in sustainable farming systems: past, present and future. In *Proceedings of the 30th Occasional Symposium of the British Grassland Society.* (Ed. D. Younie), pp. 2-14, SAC, Craibstone, Aberdeen, UK: British Grassland Society.

Stelling, D., Ebmeyer, E. & Link, W. (1994). Yield stability in faba bean, *Vicia faba* L. 2. Effects of heterozygosity and heterogeneity. *Plant Breeding* **112**, 30-39.

Stitt, M. & Quick, W. P. (1989). Photosynthetic carbon partitioning: Its regulation and possibilities for manipulation. *Physiologia Plantarum* **77**, 633-641.

Stitt, M. & Schulze, E. D. (1994). Plant growth, storage, and resource allocation: From flux control in a metabolic chain to the whole-plant level. In *Flux Control in Biological Systems. From Enzymes to Populations and Ecosystems* (Ed. E. D. Schulze), pp. 57-118. San Diego, California, USA: Academic Press.

Stock, D. & Holloway, P. J. (1993). Possible mechanisms for surfactant-induced foliar uptake of agrochemicals. *Pesticide Science* **38**, 165-177.

Stoddard, F. L. (1985). Floral viability and pollen tube growth in *Vicia faba* L. *Journal of Plant Physiology* **123**, 249-262.

Stoddard, F. L. (1986a). Autofertility and bee visitation in winter and spring genotypes of faba beans (*Vicia faba* L.). *Journal of Agricultural Science, Cambridge* **97**, 171-182.

Stoddard, F. L. (1993). Termination of flowering in 'indeterminate' faba beans (*Vicia faba*). *Journal of Agricultural Science, Cambridge* **120,** 79-87.

Stoddard, F. L. & Bond, D. A. (1987). The pollination requirements of the faba bean. *Bee World* **68**, 144-152.

Sturm, A. (1996). Molecular characterisation and functional analysis of sucrose-cleaving enzymes in carrot (*Daucus carota* L.). *Journal of Experimental Botany* **47**, 1187-1192.

Stutzel, H. & Aufhammer, W. (1991). Canopy development of a determinate and an indeterminate cultivar of *Vicia faba* L. under contrasting plant distributions and densities. *Annals of Applied Biology* **118**, 185-199.

Stutzel, H. & Aufhammer, W. (1991b). Dry matter partitioning in a determinate and an indeterminate cultivar of *Vicia faba* L. under contrasting plant distributions and densities. *Annals of Botany* **67**, 487-495.

Stutzel, H., Aufhammer, W., Lober, A. (1994). Effects of sowing technique on yield formation of *Vicia faba* as affected by population density, sowing date and plant type. *Journal of Agricultural Science, Cambridge* **122,** 255-264.

Summerfield, R. G. and Roberts, E. H. (1988). Photo-thermal regulation of flowering in pea, lentil, faba bean and chickpea. In *World Crops: Cool Season Food Legumes* (Ed. R. G. Summerfield), pp. 911-922. London: Kluwer.

Sun, J., Loboda, T., Sung, S-J. S., Black, C. C. Jr. (1991). Sucrose synthase in wild tomato, Lycopersicon Chmielewskii, and tomato fruit sink strength. *Plant Physiology* **98**, 1163-1169.

Sung, S. S., Xu, D-P., Galloway, C. M. & Black, C. C. J. (1988). A reassessment of glycolisis and gluconeogenesis in higher plants. *Physiologia Plantarum* **72,** 650-654.

Sung, S. S., Shieh, W. J., Geiger, D. R. & Black, C. C. (1994). Growth, sucrose synthase, and invertase activities of developing *Phaseolus vulgaris* L. fruits. *Plant, Cell and Environment* **17**, 419-426.

Sung, S. S., Kormanik, P. P., Xu, D. P. & Black, C. C. (1989). Sucrose metabolic pathways in sweet gum and pecan seedlings. *Tree Physiology* **5**, 39-52.

Sung, S. S., Loboda, T. & Black, C. C. (1990). Sucrose, pyrophosphate, and plastid metabolism in relation to cellular communication. In *Perspective in Biochemical and Genetic Regulation of Photosynthesis,* vol. 10 (Ed. I. Zelitch), pp. 55-68. New York: Wiley-Liss.

Sung, S. S., Sheih, W. J., Geiger, D. R. and Black, C. C. (1994). Growth, sucrose synthase, and acid invertase activities of developing *Phaseolous vulgaris* L. fruits. *Plant, Cell and Environment* **17**, 419-426.

Sung S. S., Xu D., & Black C. C. (1988). Identification of actively filling sucrose sinks. *Plant Physiology* **89**, 1117-1121.

Suso, M. J., Moreno, M. T., Mondragao-Rodrigues, F. & Cubero, J. I. (1996). Reproductive biology of *Vicia faba*: role of pollination conditions. *Field Crops Research* **46**(1-3), 81-91.

Tamas, J. A., Davies, P. J., Mazur, B. K. & Cambell, L. B. (1985). Correlative effects of fruits on plant development. In *Proceedings of the World Soybean Conference III* (E. D. R. Shibles), pp. 858-865. Boulder and London: Westview Press.

Tamas, I. A., Wallace, D. A., Ludford, P. M. & Ozbun, J. L. (1979). Effect of older fruits on abortion and abscisic acid concentration of younger fruits in *Phaseolus vulgaris* L. *Plant Physiology* **64**, 620-622.

Tao, W. H. & Verbelen, J. P. (1996). Switching on and off cell-division and cell expansion in cultured mesophyll protoplasts of tobacco. *Plant Science* **116**, 107-115.

Thimann, K. V. (1992). Antagonisms and similarities between cytokinins, abscisic acid and auxin (mini review). In *Physiology and Biochemistry of Cytokinins in Plants* (Eds M. Kaminek, D.W.S. Mok & E. Zazimalova), pp. 395-400. Hague, Netherlands: SPB Academic Publishing.

Thomas, B. R. & Rodriguez, R. L. (1994). Metabolite signals regulate gene expression and source-sink relations in cereal seedlings. *Plant Physiology* **106**, 1235-1239.

Thomas, H. (1988). Plant growth regulator and herbicide use in relation to changing land used strategies. *British Plant Growth Regulator Group, News Bulletin,* February 1988, pp. 1-15.

Thomas, T. H. (1991). Some recent advances in plant hormone research in Europe. *Annual Bulletin of the British Society for Plant Growth Regulation* **1**, 1-12

Thompson, R. (1983). Changes in the partitioning of assimilates of *Vicia faba* in response to environment. In *Temperate Legumes: Physiology, Genetics and Nodulation.* (Eds G. D. Jones & D. R. Davis), pp. 175-190. London, GB: Pitman.

Thorne, J. H. & Rainbird, R. M. (1983). An *in vivo* technique for the study of phloem unloading in seed coats of developing soybean seeds. *Plant Physiology* **72**, 268-271.

Tran, T., Van, K., Toubart, P., Cousson, A., Darvil, A. G., Gollin, D. J., Chelf, P. & Albersheim, P. (1985). Manipulation of the morphogenetic pathways of tobacco explants by oligosaccharins. *Nature, London* **314**, 615-617.

Trewavas, A. (1981). How do plant growth substances work? *Plant, Cell and Environment* **4**, 203-228.

Trewavas, A. (1982). Growth substance sensitivity: The limiting factor in plant development. *Physiologia Plantarum* **55**, 60-72.

Trewavas, A. (1991). How do plant growth substances work? II *Plant, Cell and Environment* **14**, 1-12.

Trewavas, A. & Cleland, R. E. (1983). Is plant development regulated by changes in the concentration of growth substances or by changes in the sensitivity to growth substances? *Trends in Biochemical Science* **8**, 354-357.

Turner, J. F. & Turner, D. H. (1980). The regulation of glycolisis and the pentose phosphate pathway. In *The Biochemistry of plants, vol. 4* (Eds J. F. Stumpf & E. E. Conn), pp. 279-316. New York: Academic Press.

Udomprasert, N., Li, P. H., Davis, D. W., Markhart III, A. H. (1995). Root cytokinin level in relation to heat tolerance of *Phaseolus acutifolius* and *Phaseolus vulgaris*. *Crop Science* **35**, 486-490.

Valaoras, G. (1992). Agriculture and food supply. *Proceedings of the Inter-Orthodox Conference on Environmental Protection in November 1991*, pp. 95-96. Crete: Ecumenical Patriarchate of Constantinople.

Van der Krieken, W. M., Croes, A. F., Barendse, G. W. M. & Wullems, G. J. (1988). Uptake and metabolism of benzyladenine in the early stage of flower bud development in vitro in tobacco. *Physiologia Plantarum* **74**, 113-118.

Van Staden, J. (1983). Seeds and cytokinins. *Physiologia Plantarum* **58,** 340-346.

Van Staden, J. & Crouch, N. R. (1996). Benzyladenine & derivatives - their significance and interconversion in plants. *Plant Growth Regulation* **19**(2), 153-175.

Van Staden, J. & Davey, J. E. (1979). The synthesis, transport and metabolism of endogenous cytokinins. *Plant Cell and Environment* **2**, 93-106.

Venis, M. (1985). Receptor principles. In *Hormone Binding Sites in Plants*. (Ed. M., Venis), pp. 1-20, Longman, New York, USA.

Venis, M. (1985). Binding of cytokinins. Summary and future prospects. In *Hormone Binding Sites in Plants*. (Ed. M., Venis), pp. 154-189. New York, USA: Longman.

Venis, M. (Ed.) (1985). *Hormone Binding Sites in Plants*. New York, USA: Longman.

Wagner, B. M. Beck, E. (1993). Cytokinins in the perennial herb *Urtica dioica* L. as influenced by its nitrogen status. *Planta* **190,** 511-518.

Wang, F., Sanz, A., Brenner, M. L. & Smith, A. (1993). Sucrose synthase, starch accumulation, and tomato fruit sink strength. *Plant Physiology* **101**, 321-327.

Wardlaw, I. F. (1990). The control of carbon partitioning in plants. *New Phytologist* **116**, 341-381.

Wareing, P. F. & Patrick, J. (1975). Source sink relations and the partition of assimilates in the plant. In *Photosynthesis and Productivity in different Environments* (Ed. J. P. Cooper), pp. 481-499. Cambridge: Cambridge University Press.

Wareing, P. F. & Phillips, I. D. J. (Eds) (1982). *In Growth and Differentiation in plants*, 3d Edition. Great Britain: Pergamon Press.

Warren-Wilson, J. (1972). Control of crop processes. In *Crop Processes in Controlled Environments* (Eds A. R. Rees, K. E. Cockshull, D. W. Hand & R. G. Hurd), pp. 7-30. London: Academic Press.

Weaver, R. J. & Johnson, J. O. (1985). Relation of hormone to nutrient mobilisation and the internal environment of the plant: The supply of mineral nutrients and the photosynthate. In *Hormonal Regulation of Development; III. Role of Environmental Factors. Encyclopaedia of Plant Physiology; New Series,* vol. 11 (Eds R. P. Pharis & D. M. Reid), pp. 3-36. Berlin-Heidelberg, Germany: Springer-Verlag,.

White, G., Gates, P. & Boulter, D. (1984). Vascular development in the reproductive tissues of *Vicia faba* L. In *Vicia faba: Agronomy, Physiology and Breeding* (Eds P. D. Hebblethwaite, T. C. K. Dawkins, M. C. Heath & G. Lockwood), pp. 15-22. The Netherlands: Martinus Nijhoff.

Wiesner, J. (Ed.) (1892). *Die Elementarstruktur und das Wachstum der lebenden Substanz.* Vienna: A. Holder.

Wilkins, M. B. (Ed.) (1984). *Advanced Plant Physiology.* London: Pitman.

Wittich, P. E. (1996). Histochemical localisation of sucrose synthase and invertase activity in developing ovules. *Journal of Experimental Botany* **47**, 1311.

Wolswinkel, P. (1985). Phloem unloading and turgor-sensitivity transport: Factors involved in sink control of assimilate partitioning. *Physiologia Plantarum* **65**, 331-339.

Wyse, R. E. (1986). Sinks as determinants of assimilate partitioning: Possible sites for regulation. In *Phloem transport. Plant Biology,* vol. 1, (Eds J. Cronshaw, W. J. Lucas & R. T. Giaquinta), pp. 197-210. New York: Alan R. Liss.

Xu, D. P., Sung, S. S., Black, C. C. (1989). Sucrose metabolism in lima bean seeds. *Plant Physiology* **89**, 1106-1116.

York, W. S., Darvill, A. G. & Albersheim, P. (1984). Inhibition of 2,4-dichlorophenoxyacetic acid stimulated elongation of pea stem segments by a xyloglucan oligosaccharide. *Plant Physiology* **75**, 295-297.

Zimmerman, M. H. & Brown, C. L. (Eds). (1971). *Tree Structure and Function.* Pp. 241. Berlin: Springer-Verlag.

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Appendices for Chapter 2

Appendix 2.6.1: Principles and procedure of the enzymatic method for sucrose, D-glucose and D-fructose determination

The method is consisted of two assays, which can run in parallel. One is used for the determination of D-glucose and subsequently of D-fructose, before the inversion of sucrose. The other is used for the determination of D-glucose concentration after the enzymatic hydrolysis of sucrose.

a) Determination of D-glucose and D-fructose

At pH 7.6, the enzyme hexokinase catalyses the phosphorylation of D-glucose by adenosine-5-triphosphate (ATP) with the simultaneous formation of adenosine-5-diphosphate (ADP) (1).

$$(1)\quad \text{D}-\text{Glucose}+\text{ATP}\xrightarrow{\text{Hexokinase}}\text{G}-6-\text{P}+\text{ADP}$$

In the presence of glucose-6-phosphate dehydrogenase the glucose -6-phosphate formed is specifically oxidised by nicotinamide-adenine dinucleotide phosphate (NADP) to gluconate -6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) (2).

$$(2)\quad \text{G}-6-\text{P}+\text{NADP}^{+}\xrightarrow{\text{G}-6-\text{P dehydrogenase}}\text{gluconate}-6-\text{phosphate}+\text{NADPH}+\text{H}^{+}$$

The NADPH formed in this reaction is stoichiometric with the amount of D-glucose and is measured by means of its absorbance at 340 nm where is the absorption maximum of NADPH.

D-Fructose, also, in the presence of hexokinase and the aid of ATP is phopsphorylated to fructose-6-phosphate (F-6-P) (3).

$$(3)\quad \text{D}-\text{Fructose}+\text{ATP}\xrightarrow{\text{Hexokinase}}\text{F}-6-\text{P}+\text{ADP}$$

On completion of the reaction (3) F-6-P is converted by phosphoglucose isomerase to G-6-P (4).

$$(4)\quad \text{F}-6-\text{P}\xrightarrow{\text{Phosphoglucose isomerase}}\text{G}-6-\text{P}$$

G-6-P reacts again with NADP under formation of gluconate-6-phosphate and NADPH (2). The amount of NADPH formed now is stoichiometric with the amount of D-fructose.

b) Determination of Sucrose

At pH 4.6, sucrose is hydrolysed by the enzyme β-fructosidase to D-glucose and D-fructose (5).

(5) $\text{Sucrose} + H_2O \xrightarrow{\beta-\text{fructosidase}} \text{D-Glucose} + \text{D-Fructose}$

The determination of D-glucose after inversion (total D-glucose) is carried out according to the principle outlined above. The sucrose content is calculated by subtracting the D-glucose concentrations determined before the inversion (Reactions 1 & 2) from the D-glucose concentration measured after enzymatic hydrolysis of sucrose (Reactions 5, 1 & 2).

Appendices for Chapter 3

Appendix 3.1: Effects of external application of BA, KIN and Z on (a) intra-raceme and (b) inter-raceme percentage pod set.

(a)

Flower number	Treatment				*LSD*
	Control	**BA**	**KIN**	**Z**	*p=0.05, 24 d.f.*
1 (proximal)	28	39	25	28	*14.5*
2	17	40	25	10	*14.5*
3	7	54	7	11	*19.1*
4	12	40	18	4	*12.7*
5	8	53	10	4	*21.1*
6	11	60	10	22	*22.3*
7	10	70	11	14	*21.3*
8 (distal)	6	63	10	6	*18.8*

(b)

Raceme number	Treatment				*LSD*
	Control	**BA**	**KIN**	**Z**	*p=0.05, 24 d.f.*
1 (base)	5	45	9	0	*26.3*
2	16	61	10	5	*22.2*
3	12	60	6	18	*26.8*
4	14	55	16	11	*24.9*
5	5	55	14	12	*21.6*
6	4	29	19	16	*18.6*
7	16	59	18	19	*16.1*
8	21	59	21	26	*19.5*
9 (apex)	24	47	23	12	*20.8*

Appendix 3.2: Effects of external application of iP, [9R]Z and [9tP]BA on (a) intra-raceme and (b) inter-raceme percentage pod set.

(a)

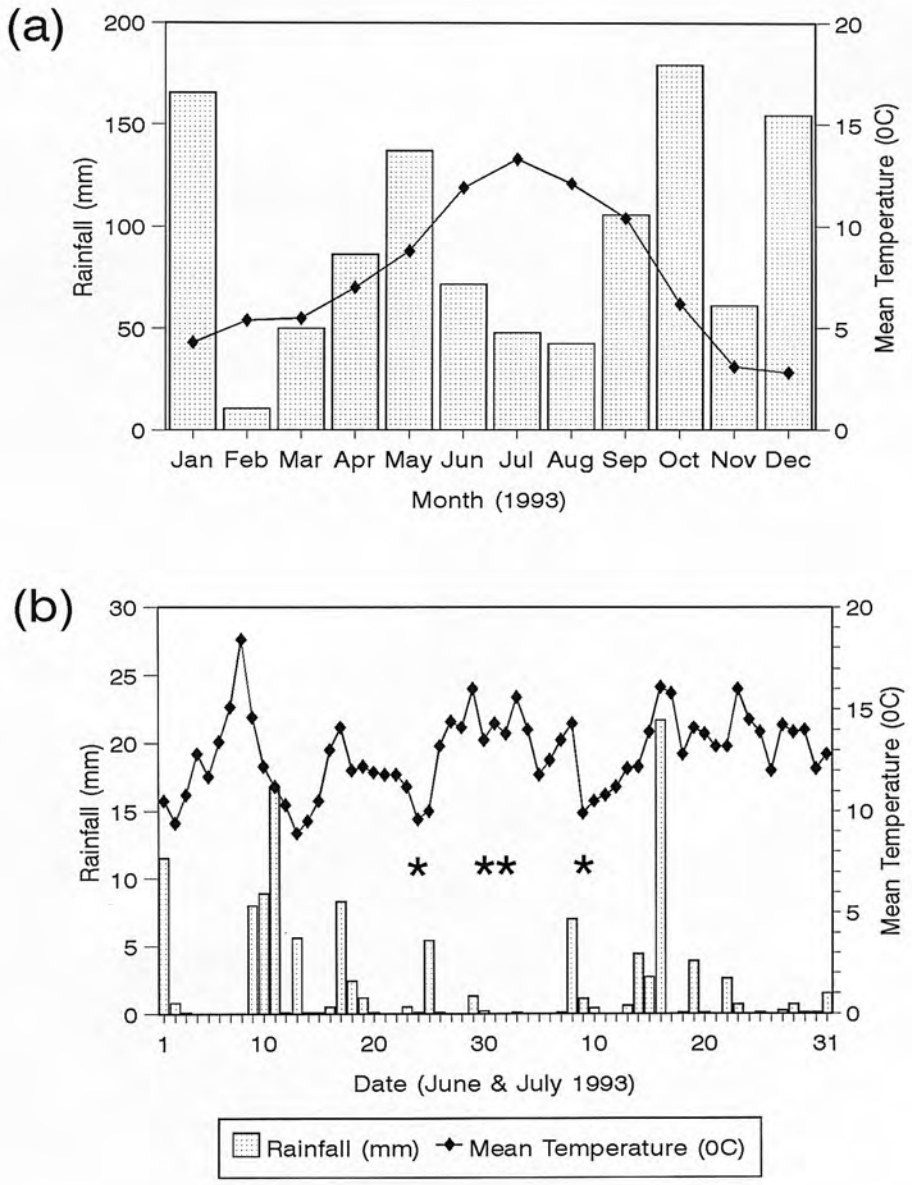
Flower number	Treatment				*LSD*
	Control	iP	[9R]Z	[9tP]BA	*p=0.05, 24 d.f.*
1 (proximal)	35	36	28	68	*21.1*
2	15	18	19	63	*24.3*
3	10	6	8	56	*21.5*
4	6	7	11	70	*19.0*
5	7	10	11	80	*17.8*
6	1	3	8	78	*11.3*
7	7	5	6	86	*17.0*
8 (distal)	3	0	3	82	*19.3*

(b)

Raceme number	Treatment				*LSD*
	Control	iP	[9R]Z	[9tP]BA	*p=0.05, 24 d.f.*
1 (base)	9	10	21	80	*17.7*
2	11	19	17	90	*15.9*
3	16	5	15	82	*15.6*
4	11	5	14	74	*20.9*
5	8	13	5	62	*19.2*
6	9	12	5	66	*17.7*
7	9	6	6	64	*21.3*
8	13	16	13	71	*21.6*
9 (apex)	7	9	12	68	*17.2*

Appendices for Chapter 4

Appendix 4.1: Climatological data of (a) monthly total rainfall and mean temperature for the year 1993, and (b) daily rainfall and mean temperature during the flowering period (June & July 1993). They were recorded at the Bush Estate meteorological station, approximately 0.3 km from the trial's site.

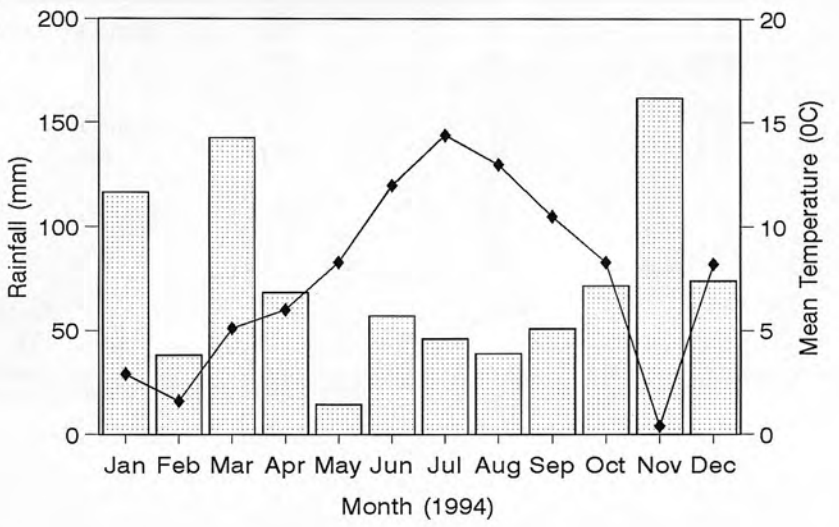


∗ : Application of chemicals. 24-6-1993 1st appl. cvs Three Fold White & Cresta
30-6-1993 1st appl. cvs Maris Bead, Troy & Toret
02-7-1993 2nd appl. cvs Three Fold White & Cresta
09-7-1993 2nd appl. cvs Maris Bead, Troy & Toret

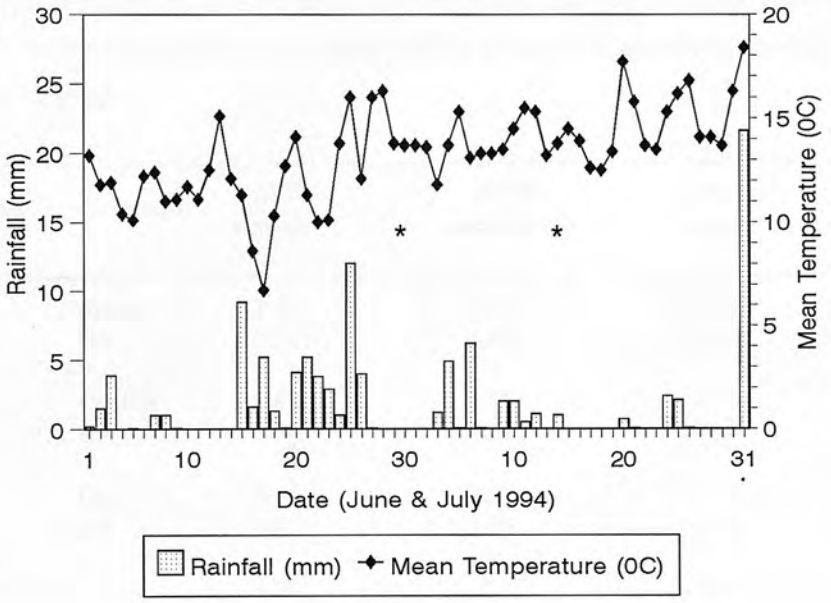
Appendices for Chapter 5

Appendix 5.1: Climatological data of (a) monthly total rainfall and mean temperature for the year 1994, and (b) daily rainfall and mean temperature during the flowering period (June & July 1994). They were recorded at the Bush Estate meteorological station, approximately 0.3 km from the trial's site.

(a)



(b)



∗ : Application of BA. 30-6-1994 spraying of cv. Three Fold White
14-7-1993 spraying of cvs Troy & Toret

Appendix 5.2.1: Effect of BA on acid invertase (AI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) activity of carpels of cv. Maris Bead.

Flower development stage	Treatment	AI (I) activity	SS (I) activity	SPS (I) activity
fds-6	Control	125	51	6.53
	BA	191	80	10.44
fds-9	Control	67	77	7.21
	BA	179	84	5.15
fds-10	Control	37	83	7.45
	BA	43	87	5.97
LSD (0.05, 10 d.f.)		*19.3*	*13.6*	*3.97*

(I): enzyme activity measured per g fresh weight

Appendix 5.2.2: Effect of BA on acid invertase (AI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) activity of carpels of cv. Maris Bead.

Flower development stage	Treatment	AI (II) activity	SS (II) activity	SPS (II) activity
fds-6	Control	2.54	1.04	0.13
	BA	3.54	1.49	0.19
fds-9	Control	1.46	1.68	0.16
	BA	3.84	1.79	0.11
fds-10	Control	0.68	1.55	0.14
	BA	0.87	1.79	0.12
LSD (0.05, 10 d.f.)		*0.32*	*0.32*	*0.07*

(II): enzyme activity measured per carpel

Appendix 5.3.1: Effect of BA on acid invertase (AI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) activity of carpels of cv. Troy.

Flower development stage	Treatment	AI (I) activity	SS (I) activity	SPS (I) activity
fds-6	Control	119	76	5.04
	BA	102	99	7.14
fds-9	Control	47	95	6.84
	BA	43	113	8.09
fds-10	Control	31	78	7.41
	BA	32	79	6.83
LSD (0.05, 10 d.f.)		*17.9*	*27.3*	*2.23*

(I): enzyme activity measured on per g fresh weight

Appendix 5.3.2: Effect of BA on acid invertase (AI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) activity of carpels of cv. Troy.

Flower development stage	Treatment	AI (II) activity	SS (II) activity	SPS (II) activity
fds-6	Control	2.32	1.48	0.10
	BA	2.02	1.99	0.14
fds-9	Control	1.04	2.11	0.15
	BA	0.94	2.48	0.18
fds-10	Control	0.75	1.94	0.18
	BA	0.73	1.77	0.15
LSD (0.05, 10 d.f.)		*0.35*	*0.65*	*0.05*

(II): enzyme activity measured on per carpel basis

Appendix 5.4.1: Effect of BA on acid invertase (AI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) activity of carpels of cv. Toret.

Flower development stage	Treatment	AI (I) activity	SS (I) activity	SPS (I) activity
fds-6	Control	147	65	7.79
	BA	167	91	6.31
fds-9	Control	91	116	5.78
	BA	77	106	6.41
fds-10	Control	81	80	4.97
	BA	62	83	5.72
LSD (0.05, 10 d.f.)		*42.5*	*13.9*	*2.35*

(II): enzyme activity measured in per carpel

Appendix 5.4.2: Effect of BA on acid invertase (AI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) activity of carpels of cv. Toret.

Flower development stage	Treatment	AI (II) activity	SS (II) activity	SPS (II) activity
fds-6	Control	2.71	1.21	0.14
	BA	3.27	1.79	0.12
fds-9	Control	1.89	2.41	0.12
	BA	1.78	2.41	0.15
fds-10	Control	1.97	1.88	0.12
	BA	1.43	1.92	0.13
LSD (0.05, 10 d.f.)		*0.84*	*0.19*	*0.04*

(II): enzyme activity measured in per carpel

Appendix 5.5: Effect of BA and of flower development stage on acid invertase (AI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) activity of pedicel: peduncle junction in cv. Three Fold White.

Flower development stage	Treatment	AI (I) activity	SS (I) activity	SPS (I) activity
fds-6	Control	50	50	8.3
	BA	44	52	9.0
fds-9	Control	42	63	13.0
	BA	39	77	14.3
fds-10	Control	31	24	14.6
	BA	30	22	15.0
LSD (0.05,10 d.f.)		*5.1*	*15.4*	*3.93*

(I): enzyme activity measured per g fresh weight

Appendix 5.6: Effect of BA and of flower development stage on acid invertase (AI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) activity of pedicel: peduncle junction in cv. Troy.

Flower development stage	Treatment	AI (I) activity	SS (I) activity	SPS (I) activity
fds-6	Control	20	37	3.3
	BA	21	43	4.3
fds-9	Control	25	67	5.2
	BA	21	64	5.3
fds-10	Control	17	52	4.6
	BA	16	42	5.6
LSD (0.05, 10 d.f.)		*4.4*	*12.6*	*0.79*

(I): enzyme activity measured per g fresh weight

Appendix 5.7: Effect of BA and of flower development stage on acid invertase (AI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) activity of pedicel: peduncle junction in cv. Toret.

Flower development stage	Treatment	AI (I) activity	SS (I) activity	SPS (I) activity
fds-6	Control	43	58	16.4
	BA	48	73	19.4
fds-9	Control	27	80	22.9
	BA	32	85	22.1
fds-10	Control	17	76	19.4
	BA	18	83	25.2
LSD (0.05, 10 d.f.)		*5.2*	*7.4*	*5.73*

(I): enzyme activity measured per g fresh weight
(1), (2): LSD calculated at p=0.05, p=0.01

Appendices for Chapter 6

Appendix 6.1: Effects of BA on sucrose, glucose and fructose contents (mg/g fw) in developing carpels.

Flower Development Stage	Treatment	Suc mg/g fw	Glu mg/g fw	Fru mg/g fw
fds-6	**Control**	9.2	14.1	5.2
	BA	15.0	13.1	4.1
	LSD (0.05, 3 d.f.)	*4.7*	*2.6*	*1.9*
fds-9	**Control**	14.5	8.1	2.9
	BA	15.7	10.4	3.2
	LSD (0.05, 3 d.f.)	*3.0*	*4.5*	*2.2*
fds-10	**Control**	9.7	4.8	1.9
	BA	4.9	2.3	0.7
	LSD (0.05, 3 d.f.)	*6.8*	*1.6*	*0.8*

Appendix 6.2: Effect of BA on sucrose, glucose and fructose contents (mg/carpel) in developing carpels.

Flower Development Stage	Treatment	Suc mg/carpel	Glu mg/carpel	Fru mg/carpel
fds-6	**Control**	0.18	0.28	0.10
	BA	0.34	0.29	0.09
	LSD (0.05, 3 d.f.	*0.10*	*0.05*	*0.03*
fds-9	**Control**	0.32	0.18	0.06
	BA	0.35	0.23	0.07
	LSD (0.05, 3 d.f.	*0.10*	*0.09*	*0.05*
fds-10	**Control**	0.20	0.10	0.04
	BA	0.13	0.06	0.02
	LSD (0.05, 3 d.f.	*0.09*	*0.06*	*0.03*