AN ABSTRACT OF THE THESIS

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Title: PHOTOSYNTHATE DISTRIBUTION, CARBOHYDRATE LEVEL IN YOUNG PODS, AND VIABILITY OF DEVELOPING SEEDS IN RELATION TO ABSCISSION OF REPRODUCTIVE STRUCTURES IN PHASEOLUS VULGARIS L.

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The fifth trifoliate leaf of field-grown bush snap beans (Phaseolus vulgaris L. cv Oregon 1604) was pulsed with $^{14}CO_2$ during anthesis and early pod development at the first (RN1) and fourth (RN4) terminal mainstem raceme nodes. Pod abscission at RN1 and RN4 was 3% and 29%, respectively. Gain in pod wall dry weight at RN1 and RN4 was similar following anthesis, but at 4 days after anthesis (DAA) pod weight at RN1 was 3 fold greater than at RN4. The level of ^{14}C-activity (dpm) recovered in the pods reflected their dry weight. The fraction of total raceme ^{14}C-activity recovered in the pods at RN1 increased from 2% to 16% during 1 to 5 DAA, whereas less than 1% was found in pods at RN4 Seed received much less ^{14}C-photosynthate but the pattern of its aquisition was similar to pods. Debudding the three most proximal raceme nodes improved pod at the remaining fourth node (RN4-d), so that at 5 DAA pod wall weight was 6 fold greater than in intact controls. This treatment reduced abscission at fourth raceme node to 56%. The ^{14}C-activity recovered in pod walls at RN4-d increased from zero at 1 DAA to 14%, but less than 1% was found at the same

raceme node of the control. No significant amount of radioactivity was recovered in the developing seeds from distal pods of either treatment. Marked callose deposition, as determined by aniline blue fluoresence, was observed in the phloem of the vascular strand entering presumably aborted seeds of recently abscised flowers. Apparent viability at 4 DAA (i.e, lacking fluoresence) was 87% and 37% for pods at RN1 and RN4, respectively. Debudding improved the seed viability at the fourth raceme node to 81%. Greater callose deposition in the phloem and low recovery of ^{14}C-activity in seeds at RN4 suggest metabolite flow is restricted at distal raceme positions. This could cause starvation, abortion, and ultimately pod abscission. Starch and glucose concentrations in petals decreased dramatically after anthesis, whereas the decline in sucrose was less marked. Carbohydrate levels in petals were highest at RN4-d. The concentration of starch in pods steadily increased for 3 days following anthesis, then rapidly decreased. During the rise, the concentration at RN4-d was about double that at RN1 or RN4. The amount of starch per pod also increased for 3 days following anthesis, changed little between 3 and 4 DAA, and then fell (RN4 and RN4-d) or increased sharply (RN1). Concentrations of glucose and sucrose were similar in pods from all three nodes; values were highest at anthesis and declined to low levels by 2-3 DAA. The similarity in carbohydrate levels in organs at normally setting (RN1) and abscising (RN4) nodes does not support the concept that an assimilate deficiency is a major cause of pod abscission at distal nodes in intact racemes. Although debudding improved the carbohydrate status and reduced

abscission at the distal node, it did not lower abscission to the level observed at RN1. Preliminary results with detached flowers suggest that petals may be a source of assimilate for young pods.

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CHAPTER 1

INTRODUCTION

Dry beans, Phaseolus vulgaris L., are a popular legume crop in developed and developing nations in part because of their nutritional value. They are considered an important source of protein and calories in the human diet (Scrimshaw and Young, 1976). Snap beans, also known as common, string, garden and fresh and French beans, refer to bean pods produced for consumption as a fresh or processed vegetable. The pods, which are harvested immature, are an important vegetable component in the diet and widely consumed in developed nations as a supplementary source of minerals and vitamins (Silbernagel, 1988). In 1980, about 370,000 acres of snap bean were harvested commercially in the United States with an approximate farm value of $192 million (Silbernagel, 1988). Nationally, Oregon is the second leading state in production of snap beans for processing (Crabtree et al., 1989).

As much as 50% of the world's population suffers from malnutrition as a result of inadequate calorie intake or deficiencies in protein, vitamin or minerals (Litzenberger, 1973). To increase the supply of dietary protein, higher yields of high protein crops such cereal and legumes are needed. However, excessive premature abscission of flowers and young pods in beans

and other legumes is considered one of the barriers to increased yield. Often an excess of 80% of their total reproductive organs abscise (Shibles, 1979). Understanding factors and mechanisms regulating abscission processes can play a key role in improving legume yield.

Many environmental factors such as drought (Gableman and Williams, 1962; Shibles et al., 1975), extreme temperature (Mann and Jaworski, 1970), low irradiance level (Schou et al., 1978) and photoperiod (Van Shaick and Probst, 1958) have been shown to increase the abscission of reproductive structures. These environmental conditions also reduce photosynthesis (Salisbury and Ross, 1978). Since environmental stress can increase abscission and lower photosynthesis, it has been hypothesized that available photosynthate during flowering and early pod fill in legumes lead to competition among reproductive organs and ultimately in flower/pod abscission (Adams, 1967).

The rate of flower/pod abscission varies among node positions within a raceme. Usually it is the late forming more distal flowers which abscise most frequently, while early and proximal organs abscise less and contribute heavily to the final yield (Brun and Betts, 1984; Mauk et al., 1984; Tamas et al., 1979; Huff and Dybing, 1980; Pechan and Webster, 1986; Pate and Farrington, 1981; Ojehomon, 1972). The difference in abscission rate among floral positions has been attributed to the acropetal succession of flower development within an inflorescence (Huff and Dybing, 1980; Van Steveninck, 1958; Ojehomon, 1972; Mauk and Breen, 1986). Newly opened flowers and young pods at distal raceme locations are at a disadvantage, since they compete for photosynthate and

nutrients with older, larger pods at proximal positions. Removal of early flowers reduced the rate of abscission of distal flowers and young pods (Brun and Betts, 1984; Huff and Dybing, 1980; Ojehomon, 1971). These studies suggest that older fruits at basal positions either so dominate a limited supply of photosynthate that insufficient amounts reach distal flowers, or they produce inhibitors which, directly or indirectly, reduce the ability of distal organs to accumulate photosynthate.

The objective of this research is to determine whether at the same developmental stages, proximal young bean pods are more competitive for photosynthate, have higher carbohydrate levels, and higher seed viability than pods at distal positions. An additional objective is to determine whether absence of proximal pods would alter these atributes in pods at distal raceme nodes.

CHAPTER 2

LITERATURE REVIEW

The genus Phaseolus comprises more than 100 species, but P. vulgaris L. (common bean), P. coccineus L. (runner bean), P. lunatus L. (lima bean), and P. acutifolius Gray var. lutifolius (tepary bean) are the only cultivated species, all of which originated in the Americas. The most widely grown of the four cultivated beans is the common bean, which by itself accounts for 95 percent of the total world phaseolus bean production (Laing et al., 1984).

For the last decade, yield of common beans and other legumes have stagnated in comparison to a marked increase in cereal crops (Summerfield, 1980). A major obstacle to increased yield of beans is premature and excessive abscission of flowers and young pods. Unfortunately the mechanism controlling abscission is not well understood.

The following literature review gives an overview of the problem of abscission of reproductive structures in beans and other legumes and identifies some of the factors associated with the shedding of these organs.

Abscission of flowers and young pods

It has been reported that as much as 64% to 82% of all bean reproductive structures abscise under field conditions (Izquierdo and Hosfield, 1981). Several environmental factors have been shown to cause abscission, such as drought (Gableman and Williams, 1962; Shibles et al., 1975), extreme temperature (Mann and Jaworski, 1970), low irradiance level (Schou et al., 1978) and

long photoperiod (Van Shaick and Probst, 1958). Because these environmental conditions influence both abscission and photosynthesis, it has been hypothesized that insufficient photosynthate during flowering and "pod fill" may induce abscission (Adams, 1967).

The role of available photosynthate in limiting pod set is also supported by experimental manipulation of the source/sink ratio. Procedures which decrease the ratio, such as shading (Mann and Jaworski, 1970) and severe defoliation in soybean (McAlister and Krober, 1958; Tanaka and Fujita, 1979) and bean (Binnie and Clifford, 1980) during anthesis and early pod growth, markedly enhance the abscission of reproductive structures. Conversely, increasing the source/sink ratio reduces the abscission rate. For example, improving the light environment within a soybean canopy with reflectors during flowering and early pod growth nearly doubled the final number of pods per plant as compared to controls (Schou et al., 1978). Continous CO_2 enrichment of snap bean after flowering increased the number of pods per plant and increased pod dry weight at harvest (Gustafson, 1983). Furthermore, depodding soybeans (Huff and Dybing, 1980; McAlister and Krober, 1958), lupin (Steveninck, 1957), and beans (Gage, 1978; Subhadrabandhu et al., 1978; Tanaka and Fujita, 1979) significantly reduced abscission of pods not removed. This strongly supports the hypothesis that available photosynthate is a factor limiting pod set.

Bean flowers are borne on racemes, each arising at a node with a trifoliate leaf. Flowers are self-pollinated with pollination

occurring in the late bud stage, usually at night, with pollen tube growth commencing immediately (Bliss, 1980). A pollen tube reaches an ovule micropyle and penetrates the embryo sac within 8-9 hours after pollination, fertilization soon follows (Weinstein, 1926). Flowers open in the early morning in response to light and temperature (Bliss, 1980). Pod growth in beans begins immediately after anthesis and is completed in 16 to 17 days (Carr and Skene, 1960).

The sequence of flowering within a bean cultivar depends on whether it is determinate or indeterminate. In determinate types, first flowers to open are those at the base of the inflorescence arising from the upper most leaf axil of the mainstem (Wivutvongvana and Mack, 1974; Ojehomon, 1966). Indeterminate types produce only axillary inflorescences and the node number of the first lateral inflorescence differentiated is variable and considered a cultivar characteristics (Ojehomon, 1969).

Determinate bean cultivars produce anywhere from 1 to 5 flowering nodes per raceme (Wivutvongvana and Mack, 1974), whereas soybeans inflorescence generally have 2 to 3 nodes (Kato et al., 1955). Flowers within a raceme open in an acropetal fashion, i.e., flowering succession is from proximal to distal nodes. In the snap bean cultivar Oregon 1604, basal flowers of the mainstem raceme bloom and set pods 1-2 days prior to those at the next higher raceme node. When flowers open at raceme node four, about 7 days after anthesis at the proximal node, pods at the basal node already weigh 0.6 to 0.12 g (Mauk and Breen, 1986).

In beans, and other legume crops, early flowers which develop at the base of an inflorescence experience lower abscission than

those developing later at distal positions. Frequent abscission of distal flowers is attributed to the acropetal pattern of flowering within a raceme. This pattern puts distally located flowers at a disadvatage, since they compete for photoassimilate and nutrients with rapidly growing pods at proximal positions. Removing early formed flowers reduces the abscission rate of later flowers. For example, an isoline line of Clark soybean produces long axillary racemes in which abscission rates at nodes 1-4, 5-8 and 9 and above are 17%, 47% and 75%, respectively (Brun and Betts, 1984). When flowers are removed from positions 1 to 4, abscission is reduced to 20% at nodes 5-8 and 70% at 9 and above. Similar results were found in lupin (Van Steveninck, 1957), beans (Gage, 1978), and cowpeas (Ojehomon, 1972). Thus older fruits at basal raceme nodes have a negative effect on sink development at distal nodes, possibly because they monopolize photosynthate and other nutrients or produce inhibitors that reduce the ability of distal organs to accumulate photosynthate. The amount of floral abscission in depodded bean plants depends upon the development stage at which pods are removed. Removal of older slow growing pods (low rates of cell expansion) had no effect on subsequent flower abscission, whereas excision of younger pods undergoing rapid cell expansion drastically reduced abscission (Gage, 1978).

Mauk et al. (1987) examined the proportion of abscised flowers/pods relative to developmental stages in 'Oregon 1604' beans. They found 2.5% were shed prior to anthesis, 15% at anthesis, 40% one day past anthesis and the remaining 43% from 2 days after anthesis to harvest. The developmental stage at which

flowers or pods abscise varies with their position within a raceme. Sage and Webster (1987) reported that bean flowers at raceme distal nodes are shed one day after anthesis when their seeds contain a proembryo of 5 to 8 cells, whereas those at proximal positions abscise at 3 to 4 days after anthesis, when the embryo is at late globular stage.

Abscission of reproductive structures is also related to carbohydrate content. Horticulturalists have known for many years that plants with an abundance of carbohydrates as less likely to drop fruit than those with a low carbohydrate status. Retention of fruits by deciduous tree crops depends upon the accumulation of carbohydrate reserves in the previous season. Greater abscission of young fruits is observed when reserves are low (Addicott, 1982). Similarly, cotton plants growing under favorable light conditions were shown to shed only 20% percent of their young fruits, but this increased to 90% when plants were placed in total darkness for 48 hr. The rise in abscission rate was correlated with greatly reduced translocation of carbohydrates into young fruits (Raby and Bate, 1978). Supplying bean explants with sucrose considerably enhanced starch deposition and retarded abscission of leaflets (Addicott, 1982).

Setting and abscising legume pods differ in their carbohydrate content. Dybing et al. (1986) reported that normally setting soybean pods accumulated higher levels of soluble carbohydrate and starch than distal pods which usually abscise. They suggested that pods at the base of the raceme can better compete for photosynthate than those at distal positions and, therefore, have higher carbohydrate levels. On the other hand, a depletion

of stored reserves in distal pods would be expected to accompany a lower photosynthate supply. When Tanaka and Fujita (1979) measured the sugar and starch content in bean pod walls at 20 days after bloom, they found that normally growing pods had much higher sugar and starch contents than those senecsing and devoid of seeds. A shortage of photosynthate during pod wall growth was proposed as one of the causes of abortion.

It is still not clear whether unavailability of photosynthate is a cause of abscission. There is evidence that older fruits of soybean (Huff and Dybing, 1980; Shibles et al., 1975) and bean (Tamas et al., 1979) inhibit development of younger, distal fruits via chemical substances. Huff and Dybing (1980) extracted an unidentified substance from 6 day old soybeans pods, which when applied on pedicel scars remaining after removal of proximal flowers, promoted abscission of distal flowers.

Abscisic acid (ABA) has been given wide attention for its role in controlling reproductive organ abscission. It was identified by Van Steveninck (1957, 1958) as the compound in lupin tissue which induced abscission in leaf explants. Abscisic acid has been linked with cotton (Davis and Addicot, 1972) and peach fruit abscission (Martin and Nishijima, 1972). However, the role of ABA in regulating abscission in bean and soybean is conflicting. Tamas et al. (1979) found in two bean cultivars, Redkloud and Redkote, that removing older fruits growing at the base of a raceme reduced abortion of younger, distal pods and also lowered their ABA concentration in comparison to intact controls. The authors suggested that older fruit promote abscission of young

fruit at upper positions by increasing their level of ABA. In another study, Subhadrabandhu et al., (1979) found contradictory results regarding an association between ABA and pod abscission in bean. In the bean cultivar Seafarer, ABA content of late and distally positioned pods was directly correlated with pod abscission, whereas in 'Black Turtle Soup' it was inversely related to abscission. However, removal of early flowers from both cultivars reduced ABA content and abscission of subsequently formed fruits. These authors concluded that ABA does not regulate fruit abscission in beans. Moreover, Spollen et al. (1986) found removal of proximal soybean pods had no effect on the amount or concentration of ABA in seeds of distal pods but it did increase the ABA content in pod walls, which agrees with the results of Subhadrabandhu et al. More recently, Diethelm et al. (1988) reported that the ABA content in abscising and non-abscising Vicia faba flowers was similar, and although removal of older pods resulted in less abscission among distal flowers, it did not affect their ABA level. Huff and Dybing (1980) also found that ABA did not mimic the effect of the unknown abscission-inducing substance extracted from proximal pods. Similarly, when ABA was injected into flower bearing nodes of lupin, no increase in the abscission rate was obtained at any position in the inflorescence (Porter, 1977).

Ethylene is also acknowledged for its role in promoting abscission of reproductive organs. Webster et al. (1976) found that exogenous application of an ethylene-generating compound, ethephon, increased abscission of reproductive structures in beans when applied at concentration of 1000-2000 ppm. They also reported that bud and flower abscission were more sensitive to ethephon

treatment than pods. Additionally, high internal ethylene levels were associated with high levels of flower/pod abscission in beans (Izquierdo and Hosfield, 1979) and faba beans (El-Beltagy and Hall, 1975). The flower abscission zone in legumes is fully differentiated at the base of the pedicel very early in flower bud development. According to Osborne (1984), the zone remains quiescent until appropriate levels of ethelene stimulate activity of hydrolytic enzymes, eventually resulting in ovary-pedicel separation.

Floral Abscission Zone

As a plant organ approaches the end of its functional life due to senescence or injury, it passes through series of chemical and physiologial changes. Chemicals products of these changes reach the abscission zone and initiate the separation process (Addicott, 1982).

Webster and Chiu (1975) compared ultrastructural changes of the floral abscission zone in Red Kidney beans to those of the leaf abscission zone. The abscission zone between the pedicel and peduncle was found to consist of four to five rows of relatively small and compactly arranged parenchyma cells. Disintegration of the cell wall zone was observed within 24 hr after anthesis and abscission occurred within 48 hr. Separation of the bean flower pedicel proceeds after the loss of pectic substances and breakdown of both the middle lamella and primary wall of abscission zone cells (Kupaya and Hoza, 1983; Webster and Chiu, 1975). Changes occur at a much slower pace in the leaf abscission zone of bean, here cell wall degeneration begins about 10 days after the leaf

reaches maximum expansion and abscission occurs only after an additional 8 days (Webster and Chiu, 1975).

Photosynthate partitioning to reproductive structures

The distribution of photosynthate to metabolic sinks is as important as its production. Recent cereal crop varieties out yield older varieties and wild types because of alterations in photosynthate distribution, as expressed in a greater harvest index (ratio of grain yield to grain + straw yield) (Austin et al., 1980). Improvement in the productivity in beans, and legumes in general, may well be accelerated by understanding the mechanisms which regulate photosynthate distribution to reproductive structures.

Photosynthate transport occurs in the phloem of the vascular system, the conducting elements are sieve tubes which are composed of specialized seive cell members (Esau, 1960). The vascular system forms a highly reticulate network interconnecting plant tissues from root to shoot tips. (Esau, 1960; Canny, 1973). Doutt (1932) studied the anatomy of the vascular system of 'Black Valentine' bean in detail and characterized developmental changes in vascular bundles at each node throughout growth. She also traced the branching of vascular bundles from the pedicel of the flower to bracts, petals, pod walls and ovules. Vascularization studies of the fruit of soybean (Thorne, 1981), beans (Doutt, 1932; Reeve and Brown, 1986), and peas (Hardham, 1976) show that two ventral bundles of the ovary alternately provide a single trace to each ovule. Thus adjacent ovules are supplied by different bundles. The ovules are attached to the placenta by the funiculus, through which the vascular tissue

passes to the integuments where it branches into two bundles, each reticulating to an extent that is species dependent.

Thorne (1985) reviewed the mechanism of assimilate transport into developing seeds of several crops. He concluded that incoming assimilate reaching the reticulate venation of the seed coat is unloaded prior to its delivery to embryonic storage tissue. Unloading occurs entirely within maternal tissues since there is lack of symplastic connections between generations. The actual unloading sites of photosynthate from sieve tubes of the reticulate venation may be via companion cells which are linked to the sieve tube lumen symplastically via plasmodesmata.

Using ^{14}C, researchers are able to conveniently study the distribution of photosynthate to many sinks in a plant at different developmental stages. This is done by exposing a leaf to $^{14}CO_2$ and assying for radioactivity sometime later at initially unlabeled sites.

Translocation studies show that the onset of reproductive development in plants is characterized by an abrupt shift in photosynthate distribution patterns which favor growth of reproductive organs. In mung beans, Kuo et al. (1978) found that photosynthate fixed at the time of anthesis largely remained in vegetative tissue. However, a high proportion migrated to reproductive organs at later times. Waters et al. (1980) traced the movement of ^{14}C-photosynthate in bean and reported that during pod fill over 85% of the labeled assimilate exported by the eighth trifoliate was recovered in pods with only 1% in the root.

The relation between photoassimilate distribution and abscis-

sion of reproductive organs has been studied in soybean (Brun and Betts 1984; Heithold et al., 1987; Spollen et al., 1987), snap bean (Mauk and Breen, 1986), cowpea (Ojehmon, 1972), and lupin (Pate and Farrington, 1981) by examining the partitioning of translocated ^{14}C-photosynthate to proximal and distal flowers, which are normally setting and abscising, respectively. Proximal flowers were found to accumulate much more labeled photosynthate than distal flowers. When older pods were removed, distal flowers accumulated greater amounts of ^{14}C-photosynthate and their abscission was reduced (Brun and Betts, 1984; Heitholt et al., 1987; Spollen et al., 1987; Pate and Farrington, 1981). These results further support the nutritional hypothesis that older pods compete with newly opened flowers for photosynthate, and abscission of distal flowers is a result of inadequate photoassimilate.

Pate and Farrington (1981) collected phloem sap from lupin flower stalks of normally setting (raceme node 2) and abscising (node 10) flowers. Sap was gathered from the stalk at two sites, just below the abscission zone and above it, at the base of the gynoecium. Bleeding occurred at both sites in both setting and abscising flowers until the corolla senesced. Bleeding was less pronounced at the upper site of the flower stalk when petal senescence began. Sap flow at the upper site quickly returned to full intensity at node 2 as pod formation continued but no bleeding was evident distal to the abscission zone at node 10. The authors suggested that phloem becomes obstructed or non-functional in flowers likely to be shed and that this occurs several days before their abscission is observed.

The amount of photosynthate translocated to given sink

reflects its strength and ability to compete with neighboring sinks. Sink strength is determined by the size of the sink, its growth rate, and proximity to the source (Evans, 1975).

Adams (1967) reported that sink strength parallels the developmental succession of growth from the base to apex of the bean inflorescence. Freshly pollinated flowers and developing embryos of young pods are considered the weakest sinks, whereas rapidly maturing pods the strongest.

Brun and Betts (1984) defined sink intensity in soybean as the competative ability of a sink to accumulate photoassimilate per unit mass. They found that sink intensity was very high in both setting and abscising flowers prior to anthesis, but then it rapidly dropped to a low level where it remained for several days. Sink intensity recovered in normally setting but not in abscising flowers. They speculated the decline in sink intensity after anthesis is due to utilization of carbohydrate reserves stored in soybean ovules.

Ovule abortion

Achievement of high seed yield in dry beans depends upon the number of seeds produced per pod and individual seed weight (De Mora and Foster, 1986). Because of the assumption that pod abscission is a result of embryo abortion, Shibles et al. (1975) suggested that major factors limiting legume yield occur prior to pod fill.

Seed set can be limited by deficincies in pre-fertilization events such as pollen production and viability and pollen tube growth (Pechan and Webster, 1986). Evidence shows, however, that

ovule abortion in legumes is generally not the result of a lack of fertilization. Histological observations showed that ovules of abscised soybean flowers contained proembryos of 4 to 8 cells each (Abernethy et al., 1977; Kato et al., 1955). Linck (1961) noticed that ovules at stylar and peduncle ends of a pea pod abort more frequently than central ones. The aborted ovules contained an embryo which had ceased to grow at 4 to 7 days following anthesis. Abortion of ovules at the peduncle end was not attributed to the greater distance required for pollen tube growth, since ovules at the stylar end also frequently aborted. In an examination of normally abscising bean flowers, Pechan and Webster (1986) found that 93% of the pollen grains appeared healthy and all ovules within an ovary were penetrated by pollen tubes at the micropyle. These studies indicate that pod and seed abortion in legumes is not related to lack of fertilization but rather due to post-fertilization events.

The ultimate fate of a pod appears to be determined by the presence and health of developing seeds. In peas, Eewuens and Schwabe (1975) found that growth of the pod wall depends upon continued development of fertilized ovules. They showed when developing seeds were killed 2 days after bloom, fruits grew very little *in vitro* in comparison to controls. However, growth of fruits with killed seeds were restored to normal by application of gibberellins and naphthaleneacetic acid. They speculated that developing pea seeds supply some of the hormones required for growth of the pod wall.

Cytological analysis showed that the ontogenetic stage of

embryos in dependent upon raceme positions (Sage and Webster, 1987). Distally positioned pods aborted at one day after anthesis and their seeds contained a proembryo of 5-8 cells. Pods at basal positions aborted at 3-4 days after anthesis and contained embryos at the late globular stage (Sage and Webster, 1987). Kato et al. (1955) examined abscised buds, flowers, and pods of soybean and classified them according to stage of ovule and seed development. They found approximately 5% abscise at flower differentiation, 16% at reproductive cell division (megasporogenesis), 4% at flowering and fertilization, 43% at an initial proembryo stage, 13% at a later proembryo stage, and 18% at the cotyledon stage. Abernethy et al., (1977) suggested that cessation of soybean proembryo development resulted from reduced levels of unkown cell division mediating factors or increased levels of inhibitory hormones or both.

Depletion of carbohydrate reserves in developing seeds is an early indication of seed abortion. Almonds ovaries usually contain two ovules, one develops normally and becomes fertilized and the other aborts. At pollination, starch grains are evident in integument and nucellus tissues of both ovules. However, 4 days later starch is nearly depleted from one ovule but accumulates in the one judged viable (Pimienta and Polito, 1980) Sage and Webster (1988) recently reported that early symptoms of abortion-related changes in bean ovules occur in the maternal tissue, including rapid depletion of starch in integuments and nucellar cells, increased vacuolation, and collapse of some cells. This is followed by termination of embryo growth and vascular development.

Disfunctioning of sieve tubes has been linked to deposition

of callose, a polysaccaride composed of 1,3 ß-glucans, at the sieve plates. Callose can be detected histologically by staining with resorcin blue or aniline blue (Escherich, 1975; Dumas and Knox, 1983). The role of callose in phloem sieve tubes and its function as a sealing system were reviewed by Escherich (1975). Sieve plates accumulate callose as sieve elements age, and in annual plants the plates become occluded with callose before the sieve elements are obliterated. In perennial plants approching the cold season, callose is deposited permanently or temprorily in sieve elements depending on the age of the phloem. Permanent callose is deposited in early formed sieve elements which become permanently non-functional. Temporary callose, which is also termed dormancy callose, is deposited in more recently formed seive elements but then dissolved during reactivation in early spring. Callose is not detected in trees during active growth in summer. Based on anatomical observations it has been assumed that callose deposits in the sieve plates slows or stops transport by narrowing or sealing seive pores (Eschrich, 1975). Additionally, heat treatments which induce callose deposition in phloem tissue also decrease lateral movement of ^{14}C-assimilate in bean petioles (Webster and Currier, 1967).

Callose deposition is also associated with ovule abortion in many fruits such as almond (Pimienta and Polito, 1982), cherry (Stosser and Anwari, 1982), and pea (Briggs et al., 1987). Aborted almond ovules show callose deposition in the inner integuments which extends as a ring around the nucellus. Movement of a fluorescent dye (uranin) into the ovules

terminated where callose was deposited (Pimienta and Polito, 1982). It was suggested that almond ovule abortion was related to blockage of transported metabolites by callose. Similarly, Briggs et al. (1986) observed lignin and callose between the maternal vascular trace and embryo sac in aborted pea seed, and they speculated that this reduced the permeability of the nucellar region to translocatable nutrients, causing embryo starvation.

CHAPTER 3

PARTITIONING OF ^{14}C-PHOTOSYNTHATE INTO YOUNG PODS AND DEVELOPING SEEDS AT SETTING AND ABSCISING FLOWERING NODES OF SNAP BEANS

Abstract. The fifth trifoliate leaf of field-grown bush snap beans (Phaseolus vulgaris L. cv Oregon 1604) was pulsed with $^{14}CO_2$ during anthesis and early pod development at the first (RN1) and fourth (RN4) terminal mainstem raceme nodes, which are normally setting and abscising, respectively. Gain in pod wall dry weight at RN1 and RN4 was similar following anthesis, but at 4 days after anthesis (DAA) pod weight at RN1 was 3 fold greater than at RN4. The level of ^{14}C-activity (dpm) recovered in the pods reflected their dry weight. Seeds received much less radioactivity but the pattern of aquisition was similar to pods. The fraction of total raceme ^{14}C-activity recovered in the pods at RN1 increased from 2% to 16% during 1 to 5 DAA, whereas little more than 1% was found in pods at RN4. Debudding the three most proximal raceme nodes improved the growth rate of pods at RN4, so that at 5 DAA pod wall weight was about 6 fold greater than in intact controls. The ^{14}C-activity recovered in pod walls at RN4 increased from zero at 1 DAA to 14% at 5 DAA in the debudded raceme, but less than 1% was found at the same raceme node of the control. No significant amount of radioactivity was recovered in the developing seeds from distal pods of either treatment. Marked callose deposition, as determined by aniline blue fluorescence, was observed in the phloem of the vascular strand entering presumably aborted seeds of recently abscised flowers. Apparent viability at 4 DAA, as determined from callose deposition, was 87% and 37% for pods at

RN1 and RN4, respectively. Debudding improved the seed viability at RN4 to 81%. Greater callose deposition in the phloem and low recovery of ^{14}C-activity in seeds at RN4 suggest metabolite flow is restricted at distal raceme positions. This could cause seed starvation, abortion and ultimately pod abscission.

In snap bean, and other legumes, abscission of flowers and young pods can reach 45%-80% (Subhadrabandu et al., 1978a; Binnie and Clifford, 1981), resulting in substantial losses of potential yield. It has been hypothsized that insufficient photosynthate during flowering and early pod fill intensifies competition among reproductive structures, causing some to abscise (Adams, 1967). Experimental manipulations of the source/sink ratio support the hypothesis of a photosynthate limitation. Pod removal in soybean (Brun and Betts, 1984; Huff and Dybing, 1980; McAlister and Krober, 1958), lupin (Van Stevenink, 1957), bean (Gage, 1978; Subhadrabandhu et al., 1978; Tamas et al., 1979), and cowpea (Ojehomon, 1972) reduces abscission of pods not removed. Increased source activity of legumes during flowering and early pod growth, such as through CO_2 enrichment (Gustafson, 1983; Hardman and Brun, 1971) or improved light environment (Schou et al., 1977), was shown to improve pod set. Conversely, shading (Mann and Jaworski, 1970) and severe defoliation (Heitholt et al., 1986) during this development period greatly enhances abscission of reproductive structures.

Flowers of many legumes develop acropetally along a raceme, and both flowers and young pods at proximal (basal) raceme nodes abscise less frequently than those at more distal locations (Brun and Betts, 1984; Heitholt et al., 1986; Huff and Dybing, 1980; Mauk et al. 1984; Pechan and Webster, 1986; Spollen et al., 1986). The higher abscission rate at distal raceme nodes is not attributed to greater failure of fertilization (Abernethy et al., 1977; Kato et al., 1955; Pechan and Webster, 1986).

Distal flowers/pods may be at a disadvatage since they develop concurrently with rapid pod growth at more proximal nodes, whereas young proximal pods grow in the absence of competing older pods. Studies with soybean (Brun and Betts, 1986; Heitholt et al., 1986; Spollen et al., 1986), lupin (Pate and Farrington, 1981), and beans (Mauk and Breen, 1986) show that distal flower nodes receive less recently translocated ^{14}C-photosynthate than proximal ones. Furthermore, removal of soybean pods from proximal nodes increases the accumulation of photosynthate by distal pods and reduces their abscission (Brun and Betts, 1984). Monopoly of assimilates by rapidly growing proximal pods may limit the supply to distal organs, causing abortion of ovules or developing seeds and ultimately flower/pod abscission.

The terminal mainstem raceme of the snap bean 'Oregon 1604' frequently produces four or five nodes, flowers at the proximal raceme node (RN1) bloom and set pods at least 7 days before anthesis occurs at the fourth node (RN4). Mauk and Breen (1986) showed that in this cultivar flowers and pods at distal raceme nodes had a higher abscission rate and received less ^{14}C-photosynthate than those at proximal nodes. No information,

however, is available on the effect of raceme node position on photoassimilate supply to ovules and developing seeds, seed viability, or whether the absence of proximal pods alters these properties at distal nodes.

The objectives of this work are, 1) to determine whether raceme node position (RN1 vs. RN4) influences translocation of recently fixed photosynthate to pods, developing seeds and/or affects seed viability, and 2) to determine if a reduction in competition within a raceme, by preventing the formation of proximal pods, affects photosynthate partitioning and viability of developing seeds of distal pods.

Materials and Methods

The determinate cultivar Oregon 1604 was field-grown on a Chehalis silty loam soil. Prior to planting, fertilizer was applied at 8, 24, and 8 kg/ha of N-P-K, respectively. Seeds were planted 6 cm apart in rows separated by 90 cm and alternate rows were planted on 12 or 23 June, 1987. The 11 day separation between plantings permitted flower development at RN1 and RN4 of the mainstem raceme of separate plants to occur under the same environmental conditions. Thus the early (12 June) and late (23 June) plantings provided plants with flowers at the same stages of development (i.e., days after anthesis, DAA) at RN4 and RN1, respectively. Plants were further selected so that the pair of flowers at each raceme node under comparison were at the same DAA.

Two experiments were conducted. In experiment 1 the distribution of ^{14}C-photosynthate to proximal and distal nodes of the mainstem was compared (Appendix Fig. 1A). Raceme nodes were marked with colored tags and the date of anthesis of each pair of flowers recorded. On 26 Aug., plants with flowers at RN4 or RN1 at 0, 1 and 2 DAA and were dosed with $^{14}CO_2$. Plants with flowers at 3 and 4 DAA were dosed on 28 Aug. Each raceme position and stage of flower development consisted of 4-6 replicates and each raceme replicate was the average of the two flowers of each node under comparison.

In the second experiment, selected plants from the late planting were treated by removal of flower buds from raceme node positions 1, 2, and 3 at about 5 days before anthesis at RN1. Organs at raceme node four of debudded plants, designated RN4-d, were compared with those at RN4 of intact controls. On 5

Sept., selected plants with RN4 or RN4-d flowers at 1, 2, 3 or 4 DAA and were dosed with $^{14}CO_2$ (Appendix Fig. 1B). Each flowering stage had 3 replicates.

Application of $^{14}CO_2$. The terminal mainstem raceme of 'Oregon 1604' arises at the sixth node, counting from the node bearing primary leaves. When flowers at RN1 reach anthesis, the subtending trifoliate leaf is usually small, rapidly expanding, and presumably a strong sink. Therefore, in all treatments the fully expanded fifth trifoliate was pulsed with $^{14}CO_2$. At approximately 10 AM, this leaf was inserted into a polyethelene bag (Ziplock) along with a small beaker containing $Na_2H^{14}CO_3$ (462 kBq, 36.2 mCi/mmol). The bag was sealed, with the aid of modeling clay around the petiole, and 10% phosphoric acid injected through the bag into the beaker, and the hole sealed with tape. The bag was removed after 1 hr and the terminal raceme harvested after 24 hr. Flowers/pods were removed from the raceme and further separated into pods, petals, and bracts, which were placed on ice and transferred to the laboratory. The developing seeds were dissected out and stored in a freezer. They were too small to accurately weigh. Pod walls and the remainder of the raceme were oven-dried at 70°C for 24 hr and weighed. Samples were combusted in a Packard Sample Oxidizer and radioactivity determined with a liquid scintillation counter. Radioactivity per sample was expressed in absolute counts (dpm) after correcting for quenching. Distribution within the raceme was expressed as percentage radioactivity in a sample compared to total activity recovered in the entire raceme (%dpm). Sink intensity was calculated by

dividing percentage radioactivity by sample dry weight (%dpm/dry wt). Each data point represents the mean value of the replicates. Analysis of variance was conducted to evaluate treatment effects with Tukey's honestly significant difference test used for mean seperation. All statistical analyses were conducted using SIGSTAT software.

Viability of developing seeds. Increased callose deposits are observed in aborted ovules of many fruit, including cherries (Stosser and Anwari, 1982), almonds (Pimienta and Polito, 1982) and peas (Briggs et al., 1987). In this study we tested the apparent viability of developing bean seeds by detecting callose deposition using the aniline blue fluorescence method.

When the early planting reached flowering on 12 Aug., lower nodes of the mainstem raceme of several plants were debudded as described above. Flowers at similar DAA were collected from RN1, RN4 and RN4-d at 10 AM between 20 and 28 Aug. Flowers were immediately fixed in formaldehyde:propionic acid:95% ethanol (5:5:90,v/v/v). Tissues were softened with 8N NaOH and developing seeds stained with a 0.1% solution of aniline blue for callose detection (Martin, 1958). Seeds were observed with a fluorescence microscope (Leitz-Wetzlar).

For comparison purposes, flowers 1 to 2 DAA, and which abscised when touched, were collected randomly from different racemes. Upon staining, intense fluorescence was observed in the phloem of the vascular strand entering the developing seeds isolated from these flowers (Fig. 4A). Developing seeds of flowers collected from RN1, RN4, and RN4-d were classified as "non-viable"

when the location and intensity of fluoresence was similar to that of seeds from abscised flowers. Seeds were categorized as to whether they showed intense, intermediate and no fluorescence. Each collection per raceme node consisted of 3 to 6 samples with 40-46 seeds were examined per sample.

Results

The size and growth pattern of the mainstem raceme differed during development of proximal and distal flowers (Fig. 1A). The raceme was relatively small and slow growing when pods at RN1 were developing and its dry weight increased only 74 mg between 1 and 5 DAA. The two pods growing at this node accounted for 34% of this increase. Because of rapid pod growth of pods at lower raceme nodes, raceme dry weight was over 3 fold higher when anthesis occurred at RN4 than at RN1. Total raceme dry weight more than doubled during the 4 days following anthesis at RN4, but pods developing at this node only contributed about 3% to this weight gain.

Average dry weight per "pod", (i.e., pod wall, pod less developing seeds) increased after anthesis at both RN1 and RN4 (Fig. 1B). The increase was similar at both nodes for several days following anthesis, but at 4 and 5 DAA pod weight at RN1 was 3 and 1.6 folds higher than that at RN4 respectively.

Raceme node position did not affect petal and bract dry weight. Petal dry weight per flower was 10.2 mg at 1 DAA, declining to 8.3 mg by the next day. Petals frequently abscised at 2 DAA. Bracts weighed an average of 2.3 mg and showed no weight change with pod development. However total flower ^{14}C-activity was significantly higher at RN1 than RN4 on 1 DAA (Appendix Table 1) much of this difference contributed by the petals.

Ovules were less than a millimeter in length at anthesis, but developing seeds from proximal pods had probably grown to 3 mm by 4 DAA (Walbot et al., 1972). Seeds from pods at RN4 always appeared smaller than those from RN1, this was especially evident

at 3 and 4 DAA. Pods contained 6-8 developing seeds, with raceme position having no effect on number per pod.

The level of ^{14}C-activity (dpm) recovered in pods at proximal and distal raceme nodes was similar for the first 3 days following anthesis, however, at 4 and 5 DAA pods at RN1 acquired significantly higher radioactivity than those at RN4 (Fig. 2A). Although they received much less ^{14}C-photosynthate, the pattern of acquisition by developing seeds was similar to that of pods (Fig. 2B). The correlation between the level of radioactivity in a pod and corresponding seeds was significant (R^2 = 0.72, n = 52). During early development, RN4 seeds received less ^{14}C-activity than RN1 but the difference was not significant.

Pods at both RN1 or RN4 acquired only a small fraction of the ^{14}C-photosynthate translocated to the raceme at 1 and 2 DAA (Table 1). Thereafter, RN1 pods received an increasing proportion, and at 5 DAA each pod at this node contained nearly 16% of the recovered ^{14}C-activity. In contrast, pods at RN4 acquired little more than 1% of the ^{14}C-photosynthate translocated to the raceme. The relative distribution of ^{14}C-activity (%dpm) to proximal and distal pods was similar to their proportional contribution to raceme dry weight. Seeds from pods borne at RN1 and RN4 accumulated, respectively, less than 0.4% and 0.03% of the radioactivity recovered in the raceme (data not shown).

Sink intensity (%dpm/dry wt.) is an expression of the relative competitive ability to accumulate photoassimilate per unit mass (Brun and Betts, 1984). Although sink intensity of pods at RN1 was generally much higher than that at RN4, its change with

DAA was similar at both raceme nodes (Fig. 2C). Sink intensity declined sharply to a minimum at 2 DAA followed by a full (RN1) or partial (RN4) recovery the next day.

In experiment 2, distal RN4 pods of intact raceme nodes showed little growth, but removal of flower buds from raceme nodes 1, 2, and 3 resulted in a 5 fold increase in pod weight at RN4-d between 2 and 5 DAA (Fig. 3A). Even so, the weight gain of RN4-d pods was about 60% of the gain in RN1 pods in experiment 1, in which RN4 pods also showed considerable growth (Fig. 1B). The discrepancy in pod growth between the two experiments may be related to temperature differences. Starting 6 days before dosing with $^{14}CO_2$, plants in experiment 2 experienced four consecutive days in which maximum temperature ranged from 33-38°C. Such temperature can cause excessive flower/pod abscission in 'Oregon 1604' (Mauk et al., 1987) as well misshapen pods lacking fully developed ovules (Stobbe et al., 1966).

The intact raceme in experiment 2 gained over 850 mg of dry matter between 2 and 4 DAA and, in comparison to the slowly growing debudded raceme, was a strong sink for ^{14}C-photosynthate from the fifth trifoliate leaf (Table 2). No ^{14}C-activity, however, was detected in distal pods of the intact raceme except at 3 DAA (Fig. 3B). The fraction of radioactivity recovered in each RN4-d pod of the debudded raceme increased from zero at 2 DAA to over 14% per pod at 5 DAA. Little or no ^{14}C-activity was detected in developing seeds from pods at RN4 or RN4-d (data not shown).

Viability of Developing Seeds. Figure 4B shows one developing

seed each from pods borne at RN1 and RN4. The fluorescence intensity of the vascular strand of the RN4 seed is simialr to that of the aborted seed (Fig. 4A), an indication that these seeds contained similar amounts of callose and suggesting that the RN4 seed was also non-viable. The RN1 seed showed much less fluorescence (intermediate level) than aborted seeds. At early development stages (i.e., 0-2 DAA) vascular strands of a majority of seeds from RN1 and RN4-d failed to show any fluorescence (Fig 5). At each stage, the highest proportion of non-viable seeds exhibiting intense fluoresence were from RN4 reaching 65% on 4 DAA. Debudding tended to increase apparent viability of seeds from distal pods as shown by lower callose deposition to 81% at 4 DAA.

Discussion

Greater raceme growth during development of distal than proximal pods of 'Oregon 1604' and relatively small size of distal pods in comparison to the entire raceme, demonstrate the intense competition distal young pods might experience in obtaining translocated assimilates. Indeed, Mauk and Breen (1986) showed that flowers/pods at increasing higher nodes within a raceme receive a decreasing proportion of the photosynthate translocated from a nearby leaf. In the present study, comparisons were made between pods at proximal and distal raceme nodes at the same developmental stages (DAA). At each stage, pods at RN4 received a lower fraction of ^{14}C-photosynthate transported to the raceme then those at RN1 and differences increased with pod development (Table 1). However, for several days following anthesis, young distal pods in experiment 1 apparently were not at a disadvantage in obtaining photoassimilate since their dry weight at comparable developmental stages was similar to that of proximal pods. Moreover, like amounts of photosynthate were probably transported to proximal and distal pods during their early development since they acquired about the same amount of ^{14}C-activity when the fifth trifoliate leaf was dosed with $^{14}CO_2$.

Debudding the three lowest raceme nodes allowed distal organs (RN4-d) to develop without competition or other influences from nearby older pods, improving both their growth and accumulation of ^{14}C-photosynthate in comparison to distal pod of intact racemes.

The much poorer growth and low sink activity of distal pods (RN4) of the intact racemes in experiment 2 than 1 suggest that

distal organs experienced some stress in the second study. The effect of the stress however was mitigated by preventing pods from forming at more proximal raceme nodes. The relatively poor pod growth in the debudding experiment may have resulted from damage due to high temperature (33-38°C). This would explain the low ^{14}C-activity recovered in seeds and pods from RN4 and RN4-d. Day/night temperature of 35/26°C were shown to cause a high proportion of embryo sacs in beans to degenerate (Ormord et al., 1967) and continued growth of the pod wall may depend upon development of fertilized ovules, as it does in pea (Eeuwens and Schwabe, 1975). Debudding may have improved the assimilate and hormonal supply to flowers and pods at RN4-d and reduced the dependency of pod wall growth on developing seeds. Very small pods are often present on 'Oregon 1604' and Gustafson (1983) reported that at commercial harvest half the pods of this cultivar are small and lack a developing seed.

Since early development of pods at both RN1 and RN4-d occurs in the abscence of competition from organs at other nodes within a raceme, pods at these raceme nodes might be expected to show similar sink activity and growth. Although growth of pods at RN4-d was similar to that of RN4 pods of experiment 1, it was substantially less than that at the proximal nodes (i.e., RN1). At 2 DAA in experiment 1 mean pod dry weight at RN1 and RN4 was 2.8 and 2.1 mg respectivily, whereas in experiment 2 pod dry weight was 1.1 and 1.6 mg at RN4 and RN4-d, respectiviely. Damaging temperatures may have prevented pods at RN4-d from equalling the growth of those at RN1, however competition or inhibitory effects from pods developing on other racemes could also have been

factors. Also, distal flowers/pods of bean may be inherently weaker than those developing at proximal nodes. Proximal, but not distal, soybean pods enlarge significantly when grown in vitro (Huff and Dybing, 1980). Similar comparisons in beans between proximal and distal pods or distal pods from intact and debudded racemes have not been made.

Flower petals of 'Oregon 1604' are white at anthesis, usually creamy the next day, and frequently abscise by 2 DAA. The decline in sink activity of pods between 1 and 2 DAA (Fig. 2C) coincided with the period of petal senescence and fall. Pate and Farrington (1981) observed similar results in basal, setting flowers of lupin, in which sink activity showed a sharp drop near anthesis but a rapid resurgence when the corolla senesced. Brun and Betts (1984) also observed a temporary decline in sink intensity of soybean flowers/pods shortly after anthesis, which they thought was related to carbohydrate needs being met by degenerating starch granules in the embryo sac. The embryo sac of Phaseolus species probably does not store starch, since George et al. (1979) were unable to detect starch granules in the embryo sac of Phaseolus aureus. Starch granules are visible in the integuments of developing seeds of 'Oregon 1604' (Appendix Fig. 3A and B), and Sage and Webster (1987) observed that starch depletion in integuments and nucellar tissue is gradual in nonaborting bean embryos. Although carbohydrates stored in ovules could met the metabolic needs of embryos and associated tissues, it is unlikely they would be available for growth of the more massive pod wall. An alternate carbohydrate source could be senescing petals, which

rapidly lose starch (Chapter 4) and show a dry weight loss between 1 and 2 DAA of about 2 mg, which is comparable to the ovary weight at this development stage. Doutt (1932) described vascular connections between bean petals and the ovary, and translocation of sucrose from petals to the ovary has been demonstrated in senescing flowers of carnation (Nichols, 1975; Halaba and Rudnicki, 1985) and orchids (Hsiag, 1951).

The poor accumulation of ^{14}C-photoassimilate and increased callose deposition in the phloem of developing seeds at distal nodes may indicate that they received insufficient nutrients to support their continued development. A reduction in the supply of photoassimilate to developing seeds would be expected to enhance utilization of their reserve materials. In a preliminary examination of developing seeds of 'Oregon 1604', integuments from RN4 seeds at 4 DAA were nearly depleted of starch, whereas comparable tissue from proximal RN1 seeds contained numerous large starch grains (Appendix Fig. 3A and B). This is in agreement with a breif report by Sage and Webster (1988) indicating that starch is rapidly lost from integuments and nucellar tissue of aborting bean seeds between anthesis and 5 DAA. Thereafter, embryo development and vascular differentiation ceased. The higher incidence of callose deposition in seeds of RN4 compared to those at RN1 and RN4-d may be associated with a reduction in assimilate supply, since callose has been shown to interfere with photosynthate movement (Webster and Currier, 1968). Pimienta and Polito (1982) observed movement of the dye, uranin, through the vascular system of almonds ovules and found that it terminated at sites of callose deposition in aborted ovules, leading them to

conclude that blockage of metabolite transport is associated with ovule abortion. Similarly, Briggs et al. (1986) found callose and lignin accumulated between the vascular trace and embryo sac of aborted pea seeds and speculated that this reduced the permeability of the nucellar region to translocated nutrients, causing embryo starvation. Since callose is deposited in response to phloem injury and disfunction (Eschrich, 1975), the higher amounts observed in developing seeds at RN4 may result from phloem failure as a consequence of low sink activity and embryo death. The lower occurrence of callose deposition (intense plus intermediate fluorescence) in seeds at the distal raceme node of debudded plants is consisitent with studies in which this treatment reduced abscission and increased partitioning of ^{14}C-photosynthate to distal pods (Chapter 4; Brun and Betts, 1984; Huff and Dybing, 1980; Pate and Farrington, 1981).

A sampling problem encounterd in studying translocation to organs at abscising raceme nodes is that over time the attached, harvested organs may represent a population increasingly different from that of flowers/pods that are about to or have just abscised. In this study, if a plant had shed one or both flowers/pods at a particular raceme node prior to pulsing with $^{14}CO_2$, it was substituted with one having both pods intact. Since over half the organs which abscised from the mainstem raceme of 'Oregon 1604' do so before or on 1 DAA (Mauk et al., 1987), this selection biased sampling toward decidedly "healthier" organs. It did or not, however, insure that both ovaries at a node or their seeds were fully or equally functional. Thus the 6-8 seeds of some pods were

devoid of measurable ^{14}C-activity, suggesting that they aborted before or early in the 24 hr translocation period. Degenerative changes may not have been limited to ovules and seeds, since Webster and Chiu (1975) observed alterations in cell walls of the floral abscission zone in bean within 24 hr of anthesis and that abscission followed within 48 hr. The abscission zone may actually regulate assimialte flow to the developing flower or pod, since Pate and Farrington (1981) reported that although bleeding sap was observed proximal to the abscission zone of a lupin flower at a generally abscising raceme node location, sap was not evident distal to the zone. They concluded that phloem tissue was blocked in flowers destined to abscise in a few days.

Table 1. Percent dry weight and radioactivity (dpm) of pod walls at raceme node one (RN1) and raceme node four (RN4) from whole mainstem raceme relative to days after anthesis.

Raceme node	Pod wall Days after athesis 1	2	3	4	5
	% dry wt. of raceme				
RN1	1.38a[z]	2.59a	3.56a	6.24a	6.30a
RN4	0.59a	0.71b	1.11b	0.61b	1.16b
	% dpm				
RN1	1.93a	0.71a	9.06a	11.93a	15.62a
RN4	0.70a	0.02a	1.13b	0.47b	0.95b

[z]Mean values followed by the same letter in a coloumn are not significantly different (Tukey's test, 5% level).

Table 2. Total raceme dry weight and ^{14}C-activity in intact and debudded mainstem racemes relative to days after anthesis of flowers at raceme node four.

Treatment	Total raceme			
	Days after anthesis			
	2	3	4	5
	Dry weight (mg)			
Intact	374a[z]	641a	1232a	863a
Debudded	80b	108b	114b	123b
	^{14}C-activity (dpm x 10^{-3})			
Intact	1,328a	2,276a	7,381a	4,901a
Debudded	8b	25b	1b	9b

[z]Mean followed by different letter in a coloumn are significantly different (Tukey's test, 5% level).

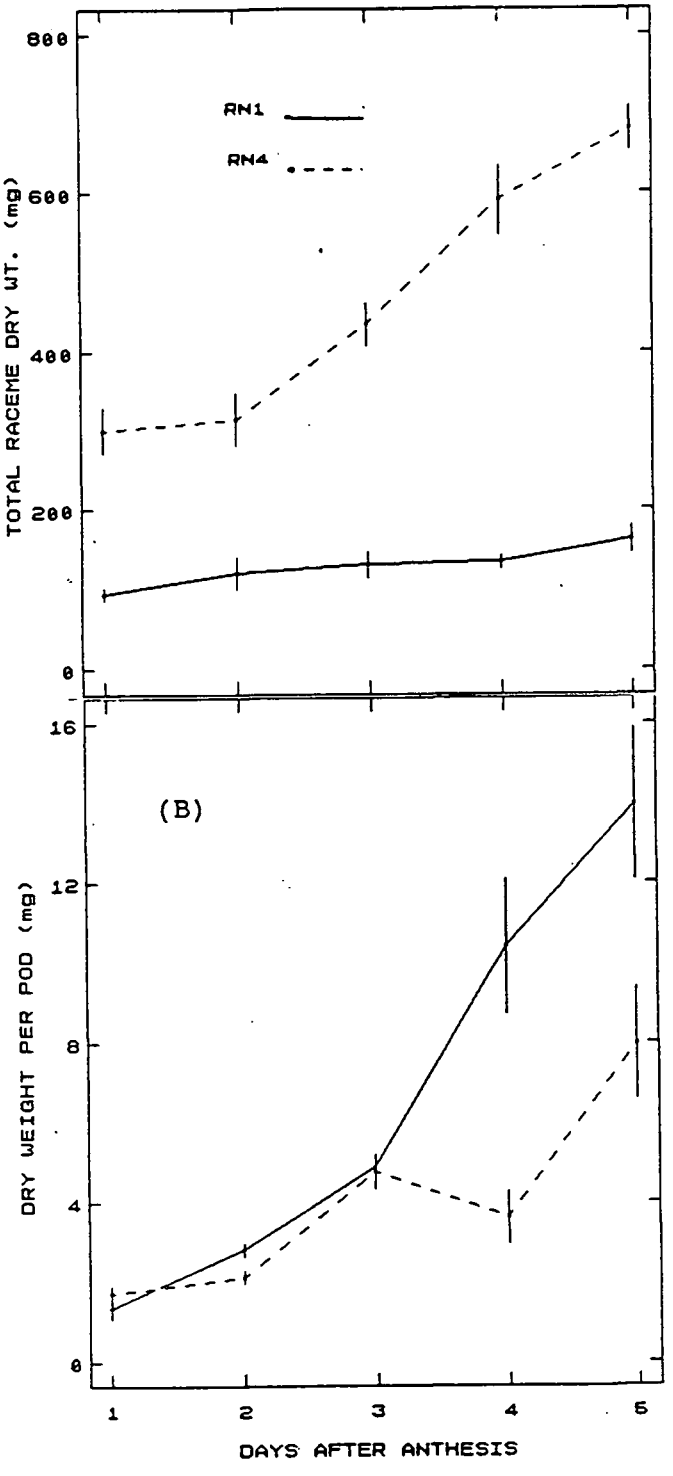


Fig. 1 Changes in dry weight of (A) total raceme during pod development at raceme node one (RN1) and raceme node four (RN4) and (B) pod wall at RN1 and RN4, relative to days after anthesis at that raceme node. Vertical bars are SDs of the means.

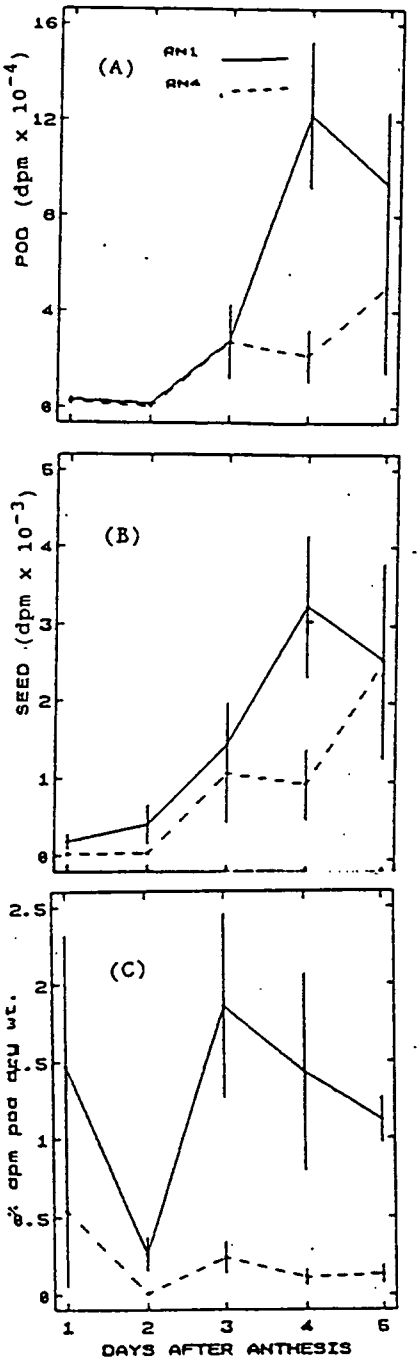


Fig. 2 Changes in (A) ^{14}C-activity per pod, (B) seed and (C) sink intensity per pod at raceme node one (RN1) and four (RN4). Vertical bars are SDs of the means.

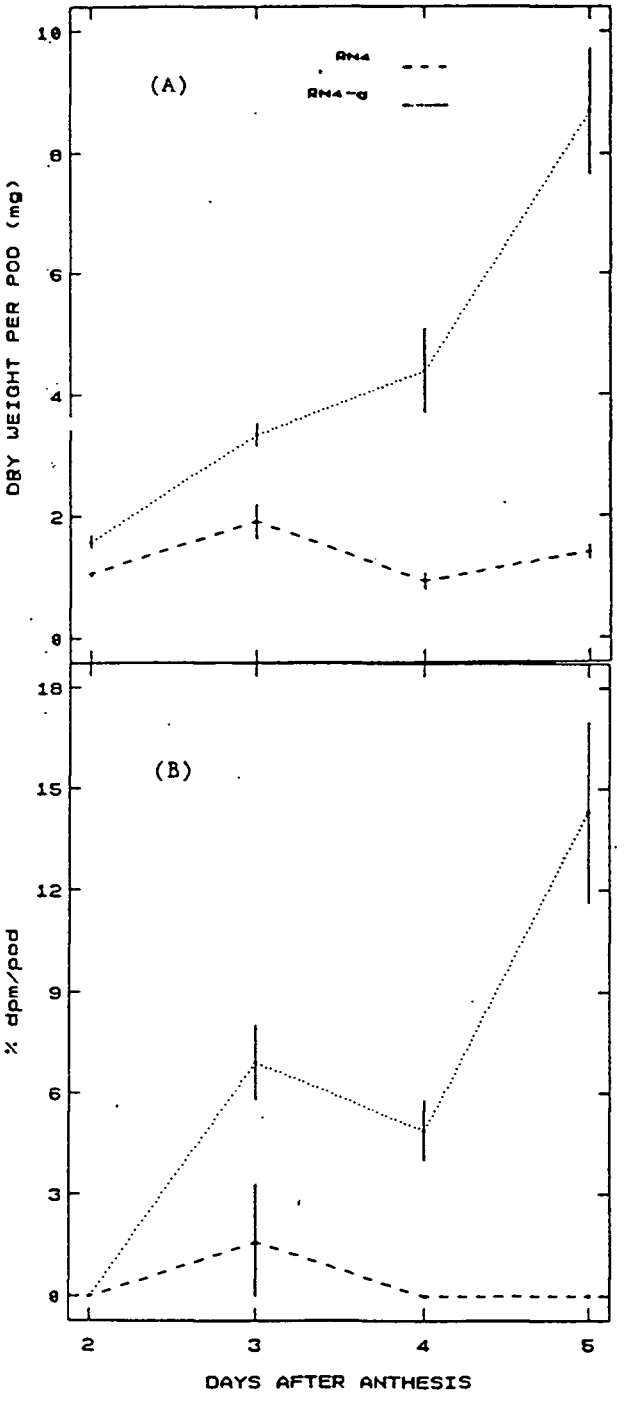
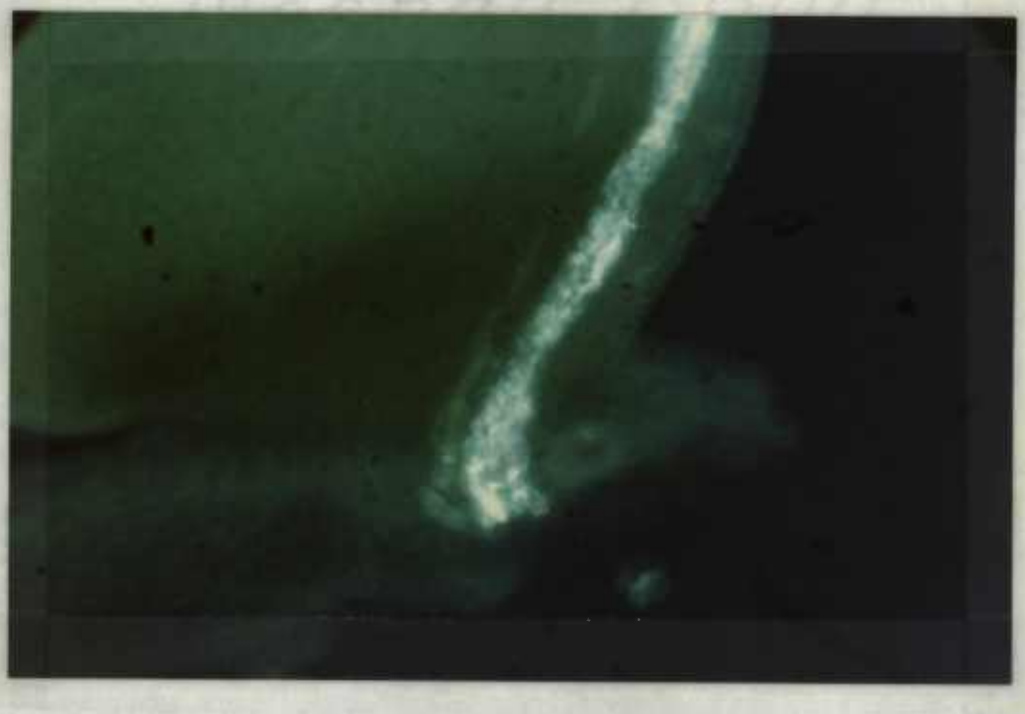
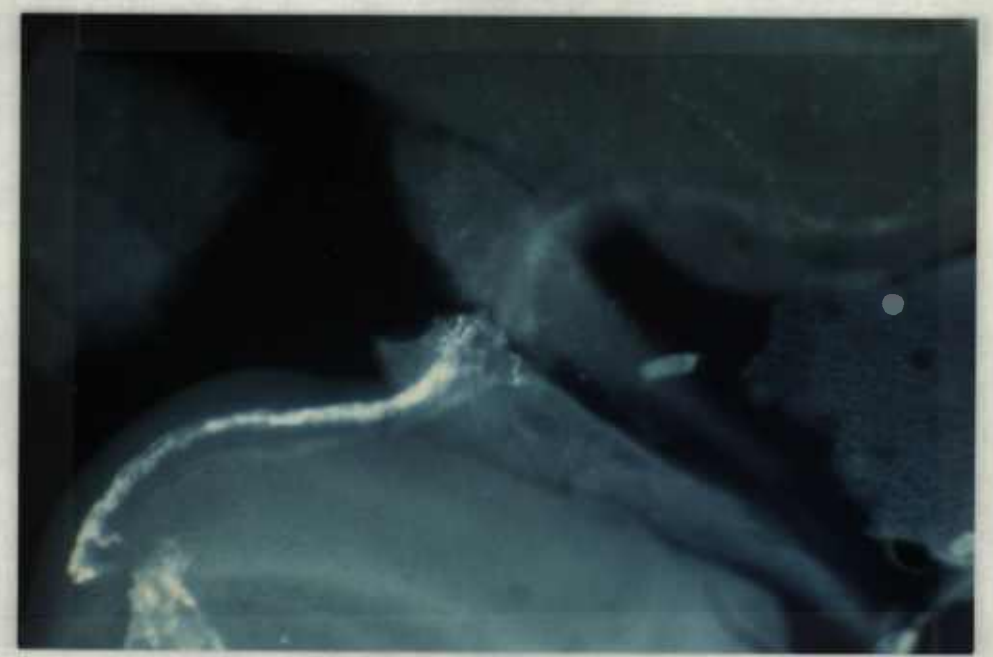


Fig. 3 Changes in pod wall (A) dry weight (B) percent ^{14}C-activity of raceme node four (RN4) and node four in a debudded raceme (RN4-d). Vertical bars are SDs of the means.



250 x

Fig. 4 (A) Photograph of a seed dissected from a pod of an abscised flower, showing intense fluorescence at the vascular strand entering the seed.



100 x

Fig. 4 (B) Photograph of two developing seeds at 4 DAA. Lower one is a non-viable seed from an RN4 pod showing intense fluorescence at phloem of the vascular strand entering the seed. Upper seed is viable from RN1, showing less fluorescence.

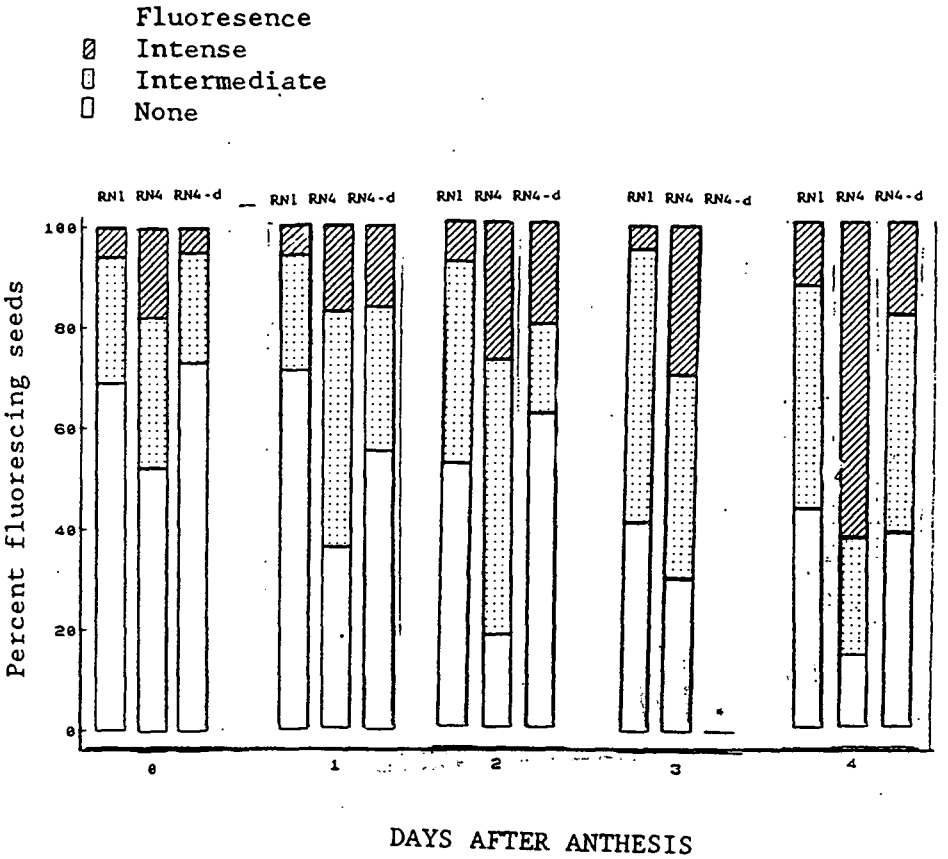


Fig. 5 Percent fluorescing seeds collected from young pods at raceme node one (RN1), four (RN4) or four in debudded raceme (RN4-d) relative to days after anthesis. * missing value. Number of seeds per sample 40-46.

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CHAPTER 4

CARBOHYDRATE LEVELS IN PETALS AND YOUNG PODS OF SNAP BEAN AT SETTING AND ABSCISING FLOWERING NODES

Abstract. Starch, glucose, and sucrose levels were determined in petals and pods from field-grown snap beans, Phaseolus vulgaris L. cv. Oregon 1604, at proximal node one (RN1) and distal node four (RN4) of intact, and node four (RN4-d) of debudded, mainstem racemes. Debudding consisted of removing flower buds from raceme nodes one through three. The rate of abscission at RN1, RN4, and RN4-d was 3%, 92%, and 56%, respectively. Pods retained at RN1 developed to normal size, whereas 80% of those at RN4-d and all at RN4 were small and devoid of seeds. Pods at all three nodes grew slowly for 3 days after anthesis (DAA), but between 3 and 4 DAA pods at RN1 and RN4-d doubled in dry weight while those at RN4 showed much less gain. By 5 DAA pod growth had ceased at RN4 and RN4-d but continued at RN1. Starch and glucose concentrations in petals decreased dramatically after anthesis, whereas the decline in sucrose was less marked. Carbohydrate levels in petals were highest in RN4-d. The concentration of starch in pods steadily increased for 3 days following anthesis, then rapidly decreased. During the rise, the concentration at RN4-d was about double that at RN1 and RN4. The amount of starch per pod increased for 3 days following anthesis, changed little between 3 and 4 DAA, and then fell (RN4 and RN4-d) or increased sharply (RN1). Concentration of glucose and sucrose were similar in pods from all three nodes; values were highest at anthesis and declined to low stable levels by 2-3 DAA. The similarity in carbohydrate levels in organs at

normally setting (RN1) and abscising (RN4) nodes does not support the concept that an assimilate deficiency is a major cause of pod abscission at distal nodes in intact racemes. Although debudding improved the carbohydrate status and reduced abscission at the distal node, it did not lower abscission to the level observed at RN1. Prelimiary results with detached flowers suggest that petals may be a source of assimilate for young pods.

Excessive premature shedding of flowers and developing pods is a major barrier to higher yield of legumes. As many as 80% of these reproductive organs may abscise in beans (Subhadrabandu et al., 1978; Binnie and Clifford, 1981). The rate of abscission, however, varies among floral positions within an inflorescence (raceme). Flowers and young pods at proximal raceme nodes abscise less frequently than those at more distal locations (Brun and Betts, 1984; Heitholt et al., 1986; Huff and Dybing, 1980; Mauk et al., 1984; Van Stevenick, 1957). This has been attributed to sequential acropetal development of flowers along a raceme which places distal flowers in competition with rapidly growing pods at proximal nodes (Mauk and Breen, 1986; Huff and Dybing, 1980). Studies with snap bean (Mauk and Breen, 1986), soybean (Brun and Betts, 1984; Spollen et al., 1986), and lupin (Pate and Farington, 1981) indicate that rapidly growing pods may so monopolize recently fixed photosynthate that insufficient amounts reach distal flowers/pods, and they abscise. A limitation in assimilate

supply to distal pods is also suggested in the work of Dybing et al., (1986) with soybean. They found that during the first week following anthesis the starch concentration declined more rapidly in normally abscising pods at distal nodes than those at proximal nodes, which usually are retained.

In our previous paper (Chapter 3), it was shown that dry weight increased in both proximal and distal pods between 1 and 2 days after anthesis (DAA), whereas petals lost an average of 2 mg dry weight. Node position (proximal vs distal) had no influence on pod dry weight and acquisition of ^{14}C-activity. After petal fall, distal pods accumulated dry matter and ^{14}C-activity at slower rate. It is not clear, however, whether raceme position also influences sugar and starch levels in bean flower parts and developing pods.

This study examined the carbohydrate status of flowers and young pods of bean in relation to their position within a raceme (proximal vs. distal) and whether debudding, to prevent the formation of competing proximal pods, influences carbohydrate levels at distal raceme nodes.

Materials and Methods

The experiment methodology used in this study was similar to that given in a previous paper (Chapter 3). Flowers of field-grown 'Oregon 1604' were collected from the mainstem terminal raceme at node one (RN1), four (RN4), and four of a debudded raceme (RN4-d) . Debudding consisted of removing flower buds from raceme nodes 1, 2, and 3 at about 5 days before anthesis at RN1.

Flowers/pods at 0 to 4 days after anthesis at RN1, RN4, and RN4-d were harvested between 16 and 20 Sept. and pods at 5 DAA on 25 Sept. Each flower/pod stage consisted of 3-6 replicates. Harvested flowers/pods were transferred to the laboratory on ice then heated in a microwave oven for 90 sec. Petals and pods, including seeds, were separated and weighed after drying at 70°C for 48 hr. Final abscission at RN1, RN4 and RN4-d was recorded when pods were dry and no longer green. Two flowers were assumued to have been produce per raceme node.

Starch and sugar analysis. Dried flower parts and pods were ground with a mortar and pestle and a 20 mg sample placed in 10 ml 80% ethanol (v/v), boiled for 10 min, centrifuged, and supernatant decanted. The pellet was dried at 50°C, suspended in 10 ml of 100 mM acetate buffer (pH 4.8), and boiled for 10 min. Starch was digested with 20 mg amyloglucosidase (Sigma, grade 2) at 55°C for 90 min. The digest was centrifuged at 12,000 x g for 5 min and a 50 ul aliquot of the supernatant analyzed for glucose by glucose oxidase-peroxidase procedure using o-dianisidine (Sigma, Technical Bulletin 510). Corn starch was used as a standard. The ethanol fraction, containing soluble sugars, was dried in a vacuum oven at

70°C then dissolved in 1.0 ml acetate buffer (100 mM; pH 4.8), and a 25 ul aliquot of assayed for glucose as above. The sucrose content was determined with glucose oxidase following digestion of 25 ul aliquot for 90 min at 55°C with 10 ul of a suspension of invertase (Sigma, grade 5) in acetate buffer (100 mM; pH 4.8). The sucrose concentration of the digest was calculated after correcting for the presence of glucose before digestion.

Analysis of variance was conducted to test for statistical differences among treatments and Tukey's Honest Test was used for mean seperation. All statistical analysis were conducted using SIGSTAT software.

Results

As anticipated, most pods at RN1 were retained whereas abscisson at RN4 was very high, but debudding the three lower raceme nodes reduced abscission at the distal node (Table 1). However, all pods retained at RN4 and 80% of those at RN4-d were small and devoid of developing seeds. In contrast, almost all the flowers at RN1 developed into seeded pods of normal size.

Pods grew slowly for 3 days following anthesis and during this period there were no significant differences among the three raceme nodes under comparison (Fig. 1). By the next day (4 DAA), however, basal pods of control plants (RN1) and distal pods of the debudded raceme (RN4-d) more than doubled in dry weight, with much less gain recorded at RN4. Pods at RN1 continued to grow between 4 and 5 DAA, whereas those at RN4 and RN4-d failed to gain additional dry matter. The apparent loss of pod dry weight at RN4-d between 4 and 5 DAA may have resulted in part from sampling errors because of the 5 day interval between harvest dates (i.e., 20 and 25 Sept.)

Pod dry weights in this experiment were generally less than that reported previously (Chapter 3). This may be related to low minimum temperature of 3-5°C which occurred on 5 nights just before and during the sampling period. Cool night temperatures can cause a severe reduction in photosynthesis in bean (Crookston et al., 1974) as well as reduced pod set and increase seed abortion (Dickson, 1981).

Low temperature may have also slowed senescence of flower petals of 'Oregon 1604'. One of the most obvious symptoms of flower senescence in bean is a change in the color of petals, from

white at anthesis, to creamy the next day, and yellow-orange on 2 DAA. The color changes are accompanied by a loss of freshness and shriveling, and petals are usually shed between 1 or 2 DAA. However, in this study petals were most often shed at 3 DAA.

Carbohydrate levels. During petal senescence, the concentration of starch and glucose decreased sharply at all raceme nodes (Fig. 2A and B), whereas that of sucrose changed little (Fig. 2C). Highest carbohydrate levels were found in petals of RN4-d. The petals of flowers at RN1 lost an average of 5.7 mg of dry matter before they abscised i.e., between anthesis and 2 DAA (data not shown).

The concentration of starch in pods at RN1 and RN4 of intact racemes was similar throughout the period studied, whereas debudding resulted in a 2 fold higher starch concentration in distal pods (RN4-d) between anthesis and 3 DAA (Fig. 3A). Starch concentration in pods at all three raceme nodes increased steadily for 3 days following anthesis and then rapidly declined. Expressing starch content on a per pod basis revealed greater differences between proximal and distal pods on intact racemes (Fig. 3B). Starch content began to decline after 3 DAA in RN4 pods, which were likely to abscise, but remained unchanged and then increased in setting pods at RN1. The amount of starch in RN4-d pods fell rapidly after 4 DAA to an intermediate level at 5 DAA. The pattern of change in sugar levels with pod development differed from that of starch (Fig. 3C and D). During the period when starch concentration was increasing in pods, glucose and sucrose concentrations dropped dramatically from maximum values at

anthesis to stable levels by 2-3 DAA. Sucrose concentrations were higher and the decline somewhat slower than glucose.

Discussion

Since dry weight of pods from different raceme nodes were similar for several days following anthesis, node position was not a factor determining initial size and early growth of retained pods even though it did affect their abscission and final size. It was not until rapid pod growth occurred that the weight of pods retained at RN4 or RN4-d showed the deletrious effect of a distal raceme node position. Rapid growth of the bean pod wall may depend upon continued development of enclosed seeds, as Eeuwans and Schwabe (1975) reported for pea. The high proportion of short flat pods at the distal nodes is evidence that seed development was incomplete. Sage and Webster (1987) found that seed abortion in distal bean fruit in intact racemes occurred at earlier stages of embryo development than in more proximal pods. The later cessation of pod growth at RN4-d than RN4 may have resulted from a delay in seed abortion due to debudding.

Pods still attached at the distal raceme nodes several days after anthesis represent a select sub-population of the ovaries present at anthesis. For Mauk et al. (1987) found that over 50% of the flowers/pods which abscise from the mainstem terminal raceme are shed before 2 DAA. Differences between retained pods at normally setting and abscising raceme nodes likely underestimates differences between the entire populations at these nodes. Flower petals and very young pods at proximal and distal nodes of intact racemes contained similar concentrations of sugars and starch, suggesting that raceme node did not influence their carbohydrate nutrition, at least until rapid pod growth began.

However, the higher starch levels in both petals and pods at RN4-d indicates that debudding probably improved the photosynthate supply to the distal node and allowed enhanced synthesis of carbohydrate reserves in flower parts prior to anthesis. If debudding did increase the assimilate supply to developing flowers at RN4-d, it did not continue during early pod development. At anthesis, a pod at RN4-d contained about 60 ug more starch than one at RN4, but this difference did not widen over the next few days (Fig. 3B). Although higher starch levels at RN4-d did not insure pod retention, improved carbohydrate nutrition may have been a factor in lowering abscission and improving pod growth at this raceme.

The steady rise in starch concentration in snap bean pods after anthesis differs from the changes in soybean reported by Dybing et al. (1987). They observed a continual decline in starch concentration in soybean pods for 4 days following anthesis, then a slight rise as the rate of pod growth increased. This loss in starch may be associated with results showing that even setting soybean pods are poor sinks for ^{14}C-photoassimilate for as many as 4 DAA (Brun and Betts, 1984). Young pods of 'Oregon 1604' also show a period of reduced photosynthate accumulation, however, this occurs for only about a day after anthesis (Chapter 3). The lack of an extended period of low sink activity in bean may permit continual synthesis of starch in the pod wall for several days following anthesis.

The rise in starch concentration in pod walls following anthesis may not have reflected changes occurring in developing seeds. For Sage and Webster (1987) observed that starch is

gradually depleted from cells of the integuments and nucellus of nonaborting bean embryos between anthesis and 5 DAA. The loss is greatly accelerated in aborting embryos. They concluded that, in addition to the loss of starch, increased vaculation and cell collapse in these tissues are the first sign of seed abortion and that these changes coincide with the decline in growth rate of abscising pods. Starch loss may have been more rapid in aborting than nonaborting seeds in the present study, since an electron microscopic examination of integuments at 4 DAA revealed fewer and smaller starch granules in seeds of RN4 than RN1 (Appendix Fig 3A).

Most of the decline in starch concentration in pods between 3 and 4 DAA resulted from rapid pod growth; changes in amount of starch per pod between these days were zero (RN1), plus 12% (RN4-d), or minus 12% (RN4). The marked increase in starch content in setting RN1 pods between 4 and 5 DAA accompanied continued rapid pod growth at this node and was in sharp contrast to the decrease in starch measured in pods at RN4 and RN4-d.

The loss of starch from pods at RN4 and RN4-d after 4 DAA accompanied the cessation of their growth and may have been associated with a loss of seed viability. The presence of viable seeds may be important to starch accumulation, for removal of viable seeds from tomato causes an increase in amylase activity in the ovary and a corresponding decrease in starch content (Marre and Murneek, 1952). In an earlier study (Chapter 3), 63% of the seeds in pods retained at RN4 showed excessive callose deposition in vascular strands by 4 DAA. This condition was thought to denote degeneration and loss of viability. However, only about

19% of the seeds from RN4-d pods showed excessive callose accumulation at 4 DAA, and this was similar to the percentage found at the setting RN1 node. The low percentage of RN4-d seeds showing degeneration at 4 DAA may have resulted from debudding treatment improving seed set and delaying seed abortion at the distal node.

In the previous chapter, we found that sink intensity of normally setting bean pods was high at 1 DAA and dropped to a low level at 2 DAA (Chapter 3). Similar observation have been reported in soybean (Brun and Betts, 1984) and lupin (Pate and Farrington, 1981). The decline in sink activity in soybean pods was attributed to utilization of starch grains in the embryo sac (Brun and Betts, 1984), which have been observed to disappear a few days after anthesis (Carlson, 1973). However, movement of carbohydrate from the embryo sac to maternal tissues of the seed or the pod wall has not been reported. Furthermore, Phaseolus species may not accumulate starch in the embryo sac since George et al. (1981) observed starch grains in the embryo sac of soybean but not in P. aureus.

The decline in sink activity in bean pods occurs coincident with senescence of petals. Similalrly, Pate and Farrington (1981) observed that sap flow from the flower stalk of lupin was lowest when petal senescence began and returned to full intensity when it was completed. Since the relatively large petals (15 mg) of the bean flower contained carbohydrate reserves which degrade during their senescence, they might function as an assimilate source for the pod and reduce its dependency on photosynthate from leaves. Doutt (1932) showed vascular connection between petals

and ovary of bean and sucrose is transported from petals to the ovary in flowers of some species (Nichols and Ho, 1975; Halaba and Rudinich, 1985; Hsiag, 1951).

The possibilty of bean petals acting as a carbohydrate source for a pod was examined in a prelimiary study in which seven groups of 70 flowers each were picked from the field at anthesis, other flowers were tagged but not removed. One group was used to determine initial dry weight and starch content. The remaining groups were placed in separate 250 ml flasks, three of which were then sealed and three left unsealed. It had previously been observed that placing bean flowers in sealed flasks delayed petal senescence. This effect may result from a build up of respiratory CO_2 which inhibits the action of ethylene (Maya and Halevy, 1980). One flask of each treatment was harvested at 1, 2, and 3 DAA. A control group of tagged flowers was also collected from the field at each of these times.

Petals abscised within 1 DAA in the unsealed flask and during this period their starch content rapidly declined, loosing an average of 2.5 mg dry wt./flower (Fig. 4A and B). Pod weight increased an average of 0.8 mg, an 80% increase, and then remained constant (Fig. 4C). Petals failed to abscise in sealed flasks and by 1 DAA lost only 0.5 mg dry weight and the gain per pod was 4 fold less than in unsealed flasks. Although the decline in starch content of petals during the first day after anthesis was similar in sealed and unsealed flasks, the changes in pods were very different (Fig. 4D). Pods in unsealed flasks rapidly accumulated starch, whereas those in sealed flasks showed a 76% loss in

starch by 1 DAA. In attached flowers, petal abscission occurred at 2 DAA and changes in dry weight and starch content in petals and pods most closely resembled those of the unsealed treatment.

These results show that early growth of the bean pod is not dependent upon a supply of photoassimilate from the leaves, since pod growth during the first day following anthesis was similar in attached and detached (unsealed) flowers. Presumably assimilates were translocated from petals to pod and petal senescence likely increased the availability of transportable substances. The source activity of bean petals may be responsible for the observed low sink activity of pods soon after anthesis (Chapter 3).

In summary, the similarity at proximal and distal nodes of carbohydrate levels in petals and pods and their changes for several days following anthesis does not support the concept that a carbohydrate deficiency is a major cause of abscission of organs at the distal node. The lower starch content and poor growth of retained distal pods at 4 and 5 DAA might well reflect degenerative changes leading finally to abscission. While the debudding treatment improved the carbohydrate status of flower/pods and reduced abscission at the distal node, a cause-effect relationship is unclear. Although the carbohydrate level at the proximal raceme node was lower than that at distal node of debudded plants, nearly all the proximal flowers developed into normal pods. Debudding may have eliminated some factor from proximal organs which interferes with seed and/or pod development at the distal node. Studies on the effect of debudding on processes associated with embryo development or degeneration may provide a clearer understanding on proximal pods enhance

flower/pod abscission of at more distal raceme nodes.

Table 3. Percent abscission and percent of retained pods that were unfilled at raceme nodes one (RM1), four (RN4), and four in debudded raceme (RN-4d).

Raceme node	Abscission (%)	Unfilled pods[z] (%)
RN1	3	2
RN4	92	100
RN4-d	56	80

[z]Short (< 6 cm), flat pod devoid of developing seeds.

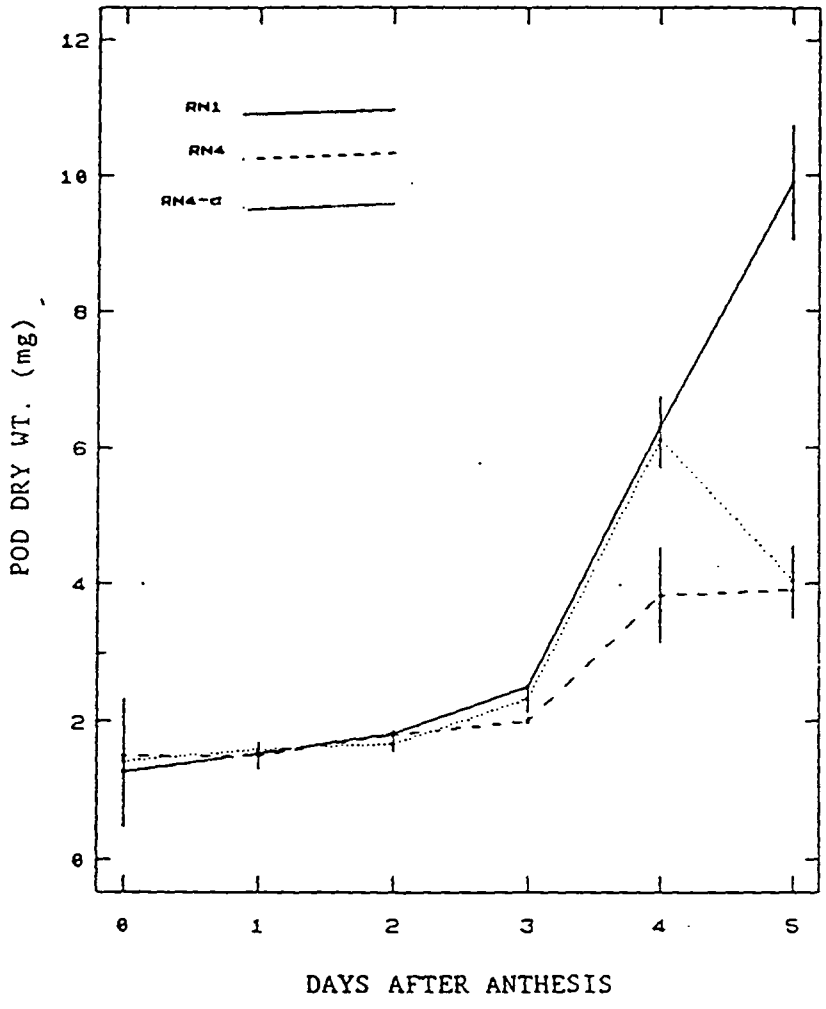

Fig. 6. Changes in dry weight per pod at raceme node one (RN1), raceme node four (RN4) or four in debudded raceme (RN4-d).

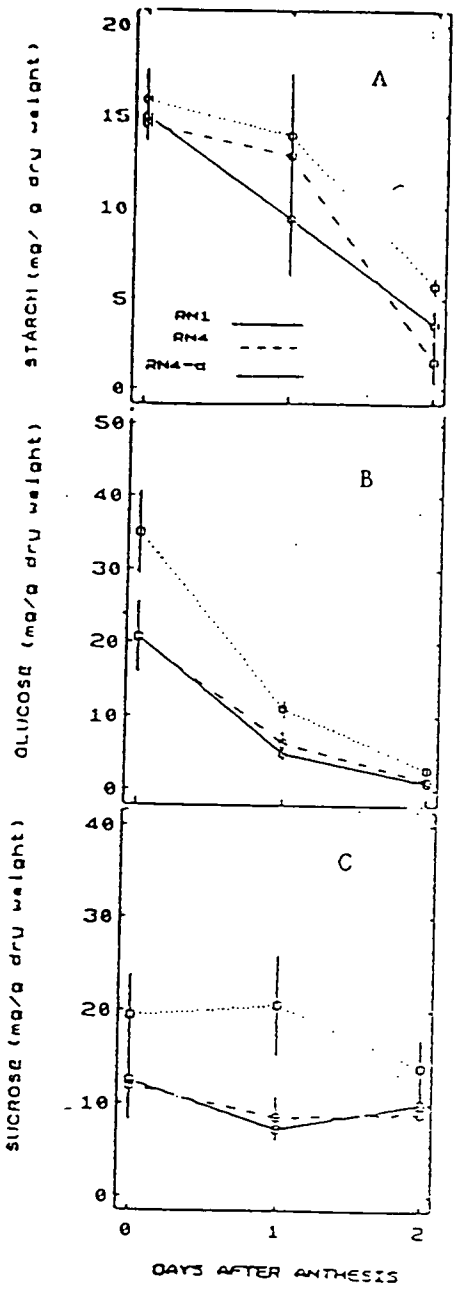

Fig. 7. Changes in petals of raceme node (RN1), raceme node four (RN4) or four in debudded raceme (RN4-d) relative to days after anthesis. (A) starch (B) glucose and (C) sucrose levels in mg/g dry weight. Vertical bars are SDs of the means

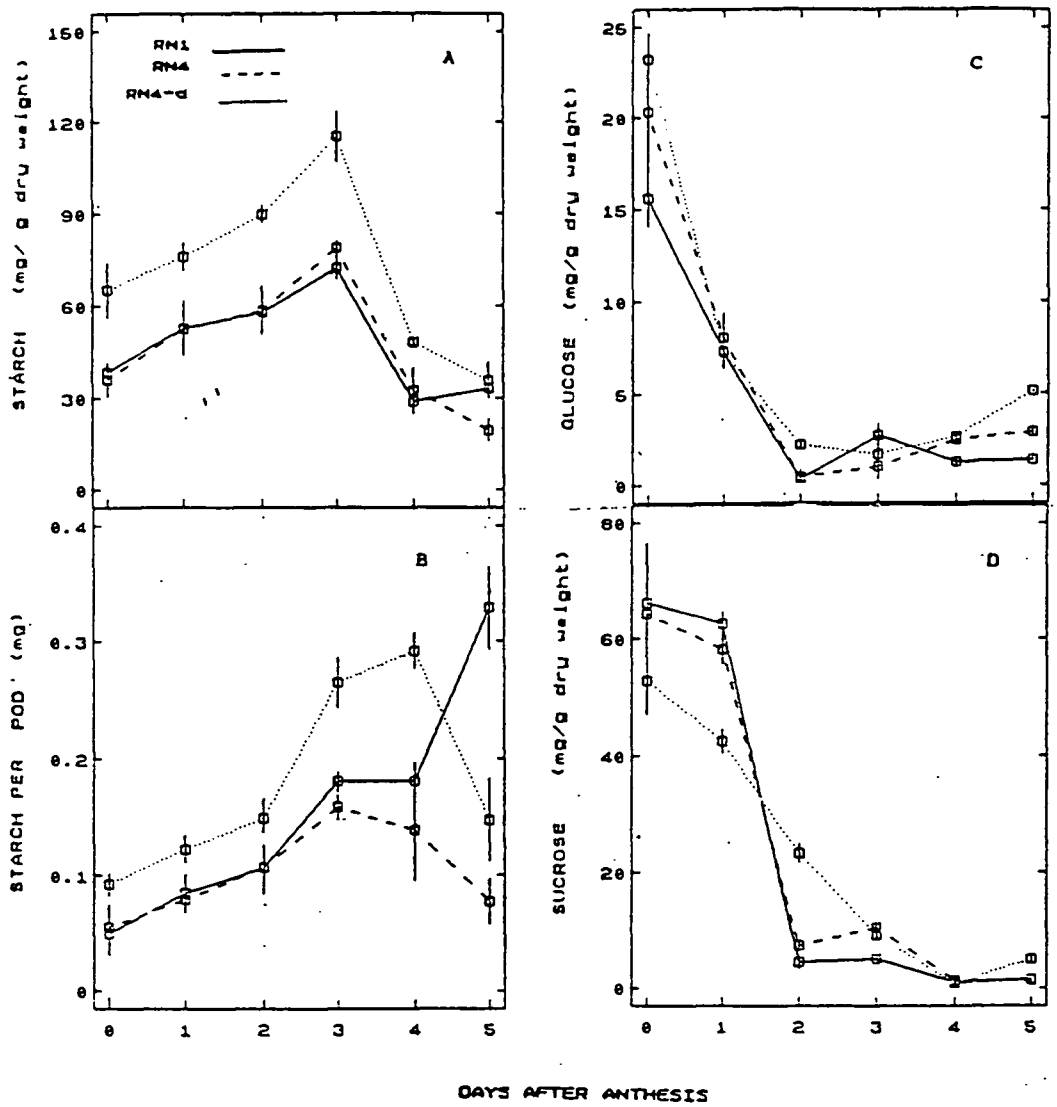

Fig. 8. Changes in raceme node one (RN1), raceme node four (RN4) or four in debudded raceme (RN4-d) relative to days after anthesis. (B) starch per pod (A) starch (C) glucose and (D) sucrose levels in mg/g dry weight. Vertical bars are SDs of the means.

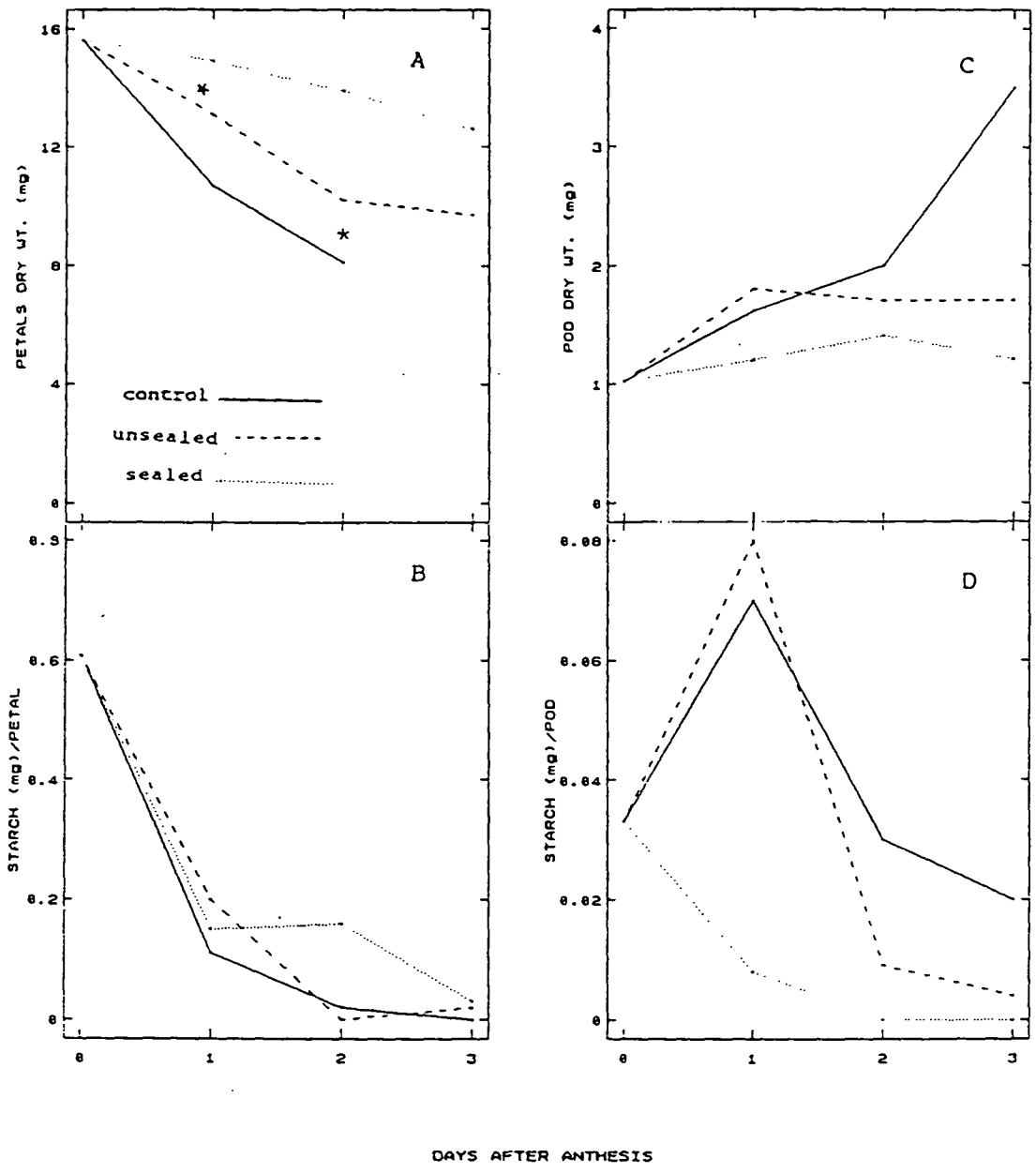

Fig. 9. Changes in dry weight per petal (A) and pod (B), starch per petal (C) and pod (D) of detached flowers in unsealed flasks, sealed flasks and intact control relative to days after anthesis. * Time when petals abscised.

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CHAPTER 5

EPILOGUE

Poor pod set at the distal position in bean racemes has been attributed to acropetal succession of flowering, which allows developmentally advanced proximal pods to interfere with the normal growth and development of distal organs. This interference could be in the form of diversion of nutrients, especially photoassimilates, away from distal organs and, thereby, reducing their growth and finally causing organ abortion and abscission. Another possibility is the release and transport of substances from proximal flower/pods which induce abortion and abscission at distal nodes. This study examined the nutrition hypothesis by determining, in proximal and distal organs, the distribution of ^{14}C-photoassimilate, carbohydrate status, and apparent viability of developing seeds. These properties were also determined at the distal node of racemes which were debudded to prevent pod formation at more proximal nodes.

In the presence of older pods, pod walls and seeds at distal raceme nodes were poor sinks after 3 DAA, as shown by a reduction in both dry matter accumulation and acquisition of ^{14}C-photosynthate. This was accompanied by poor seed viability, as indicated by a higher incidence of callose deposition. Greater callose deposition in the vascular strand entering seeds at RN4 and their low ^{14}C-activity suggest that metabolite flow to this distal position was restricted. Reduced assimilate flow could have caused starvation and abortion of developing seeds and ultimately pod abscission, or it could have been the result of seed failure

and slowing of pod growth.

It is difficult to invoke assimilate diversion as a negative influence during early stages of pod development at the distal node. For initial weight of organs at proximal and distal nodes, and subsequent pod growth and acquisition of ^{14}C-photosynthate, were similar for several days following anthesis, as were the carbohydrate levels in petals and pods. Furthermore, although sugar concentration decreased soon after anthesis, starch content steadily increased in both proximal and distal pods. It is unlikely that storage of carbohydrate reserves would continue in distal pods if the assimilate supply was insufficient to support normal seed and pod development. Differences between proximal and distal pods in growth, carbohydrate levels, and accumulation of ^{14}C-activity were observed by 4 and 5 DAA. Possibly by this time a large fraction of the pods still attached at the distal raceme node had few viable seeds and may have represented the population of small, flat pods observed at pod maturity.

Prevention of pods from forming at the three lower raceme nodes improved growth and fraction of ^{14}C-photosynthate, carbohydrate status, seed viability, and reduced abscission at the distal node. While these results indicate that proximal pods effectively reduce the transport of assimilate to distal pods, they provide little insight as to whether this is the result of successful direct competition for translocated assimilate or an indirect effect of abortion inducing substances which reduce the sink strenght of distal organs. Perhaps a more detailed analysis of the distribution of ^{14}C-photoassimilate within the raceme could

be used to separate these possibilties. If ^{14}C-activity in an intact raceme was found to be high just below node four and above node three it would be more likely that a hormonal influence and not domination of photosynthate by older pods is causing the abscission of distal pods.

Sink intensity, as measured by %dpm/mg, declined in normally setting bean pods between 1 and 2 DAA, then recovered after petal senescence. Similar changes were observed in soybean and lupin. Utilization of starch stored in the embryo sac was suggested to reduce the need for leaf-supplied assimilates in soybean, thus causing the observed decline in sink intensity. In the present study the temporary decline in sink intensity overlapped the period of petal senescence in which petals showed a marked loss of starch and total dry matter. Perhaps the petals were a source of assimilates for pods and this caused the drop in sink intensity. To test this hypothesis it may be necessary to quantify the assimilate contribution of petals of a given flower with that of a nearby source leaf. This could be determined by the following procedure:

a) Pulse a trifoliate subtending a raceme with $^{14}CO_2$.

b) Apply ^{3}H-sucrose to petals of a given flower on the raceme.

c) Assay the ^{14}C and ^{3}H activity in the pod.

If petals transport assimilate to the pod, ^{3}H activity would be detected in the pod. However, if ^{14}C is also detected in pod, this would suggest that both petals and the trifoliate are sources for the pod.

Even though prevention of pods formation reduced abscisson at the distal node, it is still not clear why retained

pods failed to develop to normal size. In peas it is known that pod wall growth ceases if the fertilized ovules fail to continue developing. Therefore, the flat immature pods of 'Oregon 1604' could be the result of late seed abortion. Determining the stage at which these seeds abort may help explain why pod wall growth ceased. Furthermore, it is also not known why young, distal pods, in the absence of older pods, did not show set and seed viability equal to that observed at proximal position. It may be necessary to determine if these distally located pods and/or their fertilized ovules are inherently weak, or if pods at distant racemes influence their development. This could be determined if comparisons in growth and seed viability between basal and distal pods were done when all other sinks (flowers and pods) are removed from the plants.

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APPENDICES

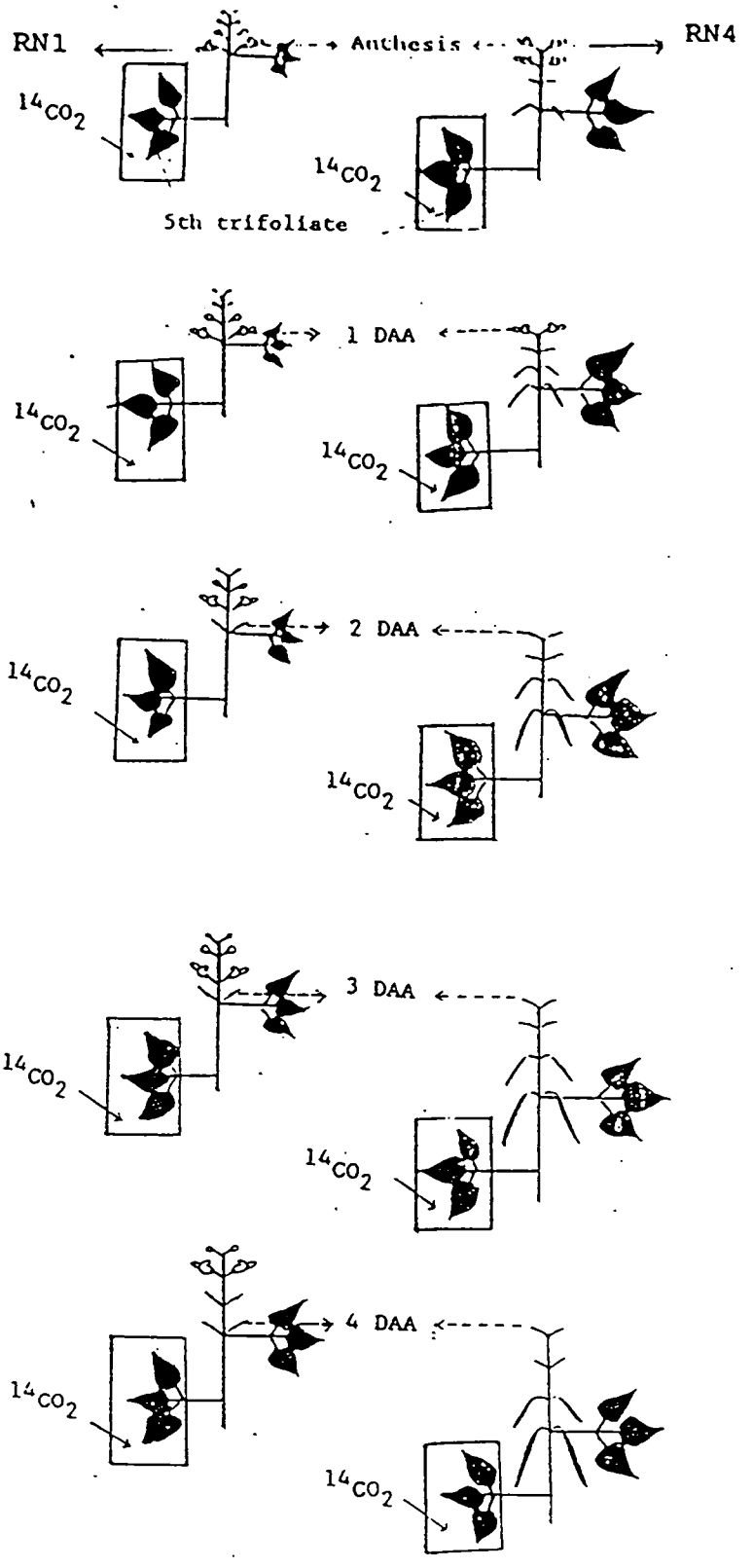


Fig. 1 A. Schematic diagram illustrating pulsing of the fifth trifoliate with $^{14}CO_2$ relative to days after anthesis of raceme node one (RN1) and four (RN4).

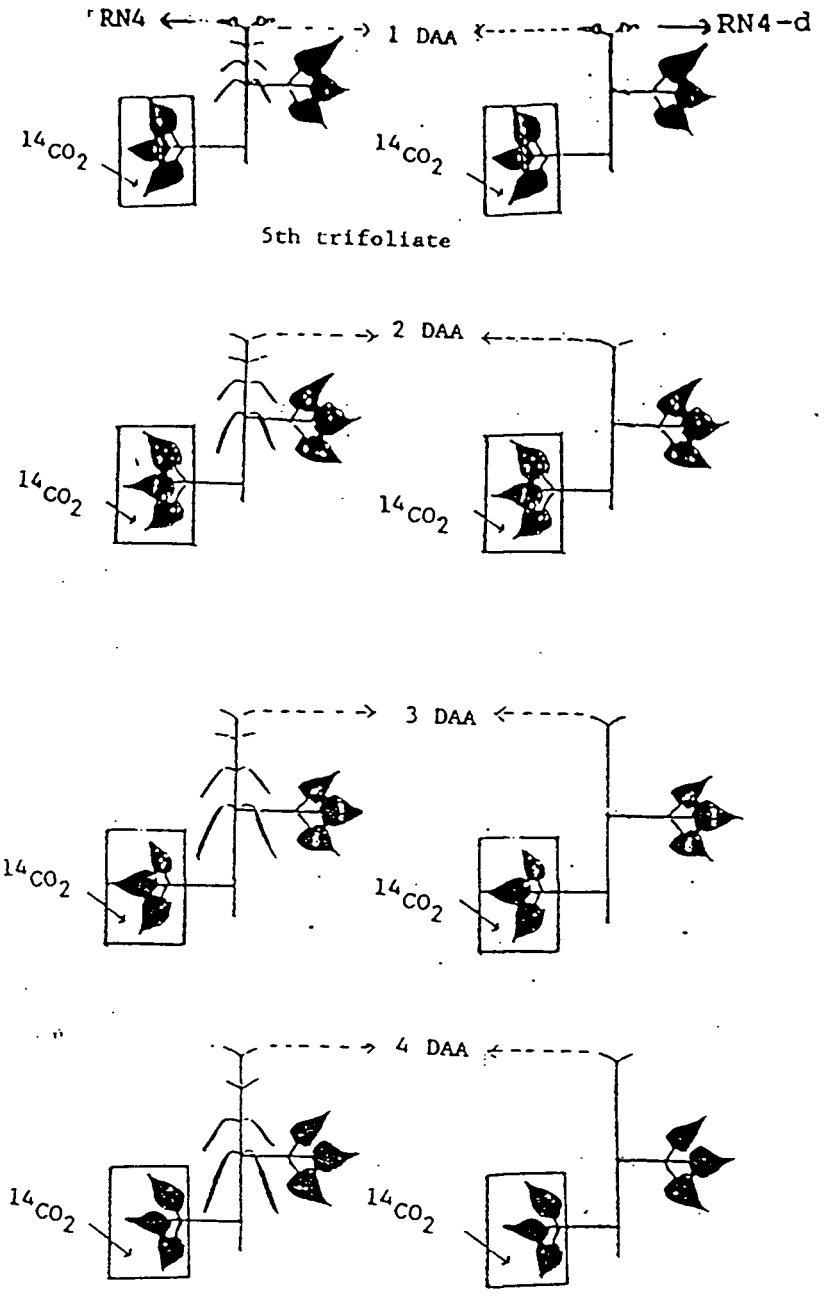
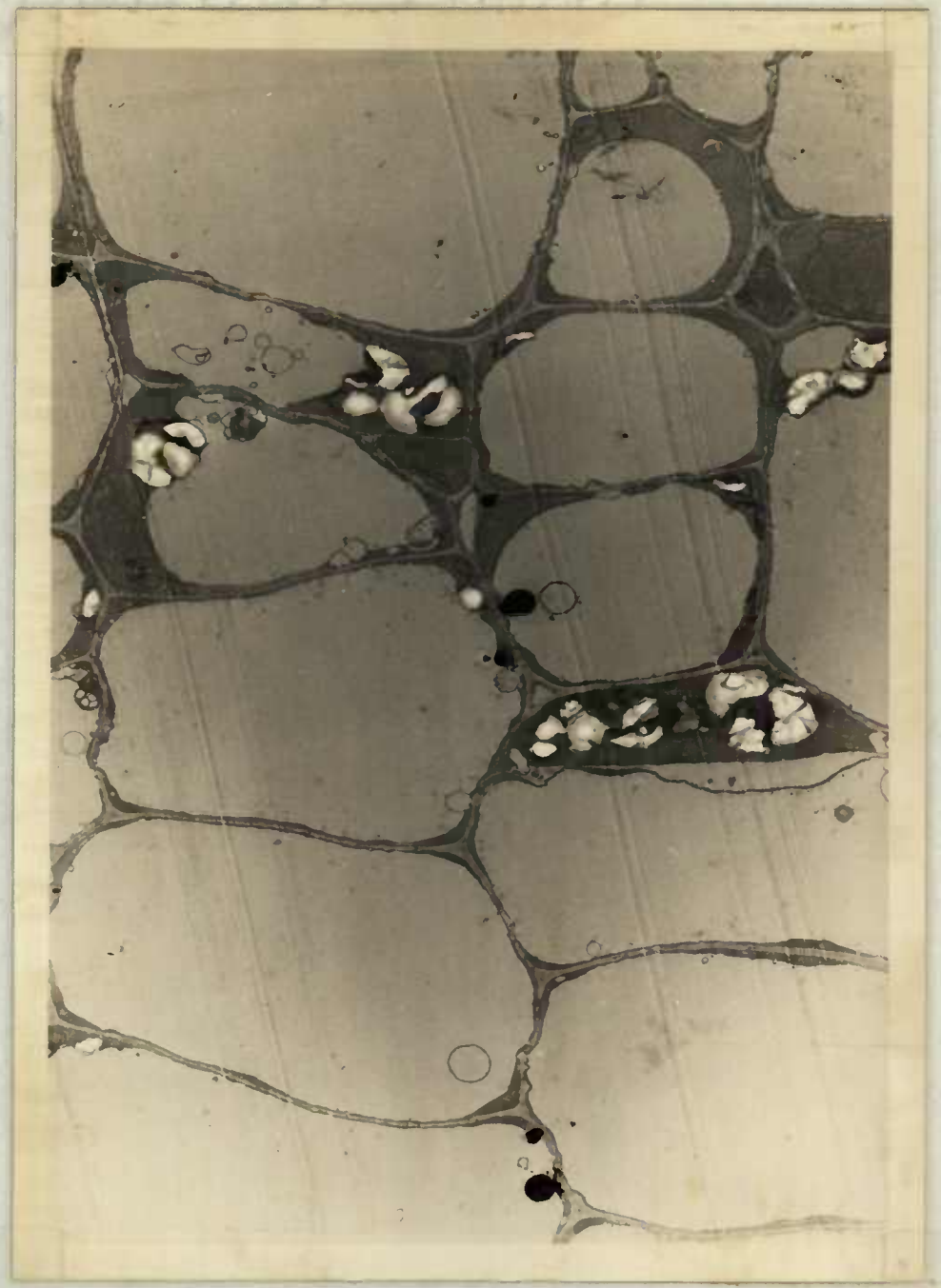


Fig. 1 B. Schematic diagram illustrating pulsing of the fifth trifoliate leaf with $^{14}CO_2$ relative to days after anthesis (DAA) at raceme node four in intact and debudded raceme.

Table 1. Radioactivity (dpm) in flower parts seeds, pod petals and bracts at raceme node one (RN1) and raceme node four (RN4) at one (1) and two (2) days after anthesis.

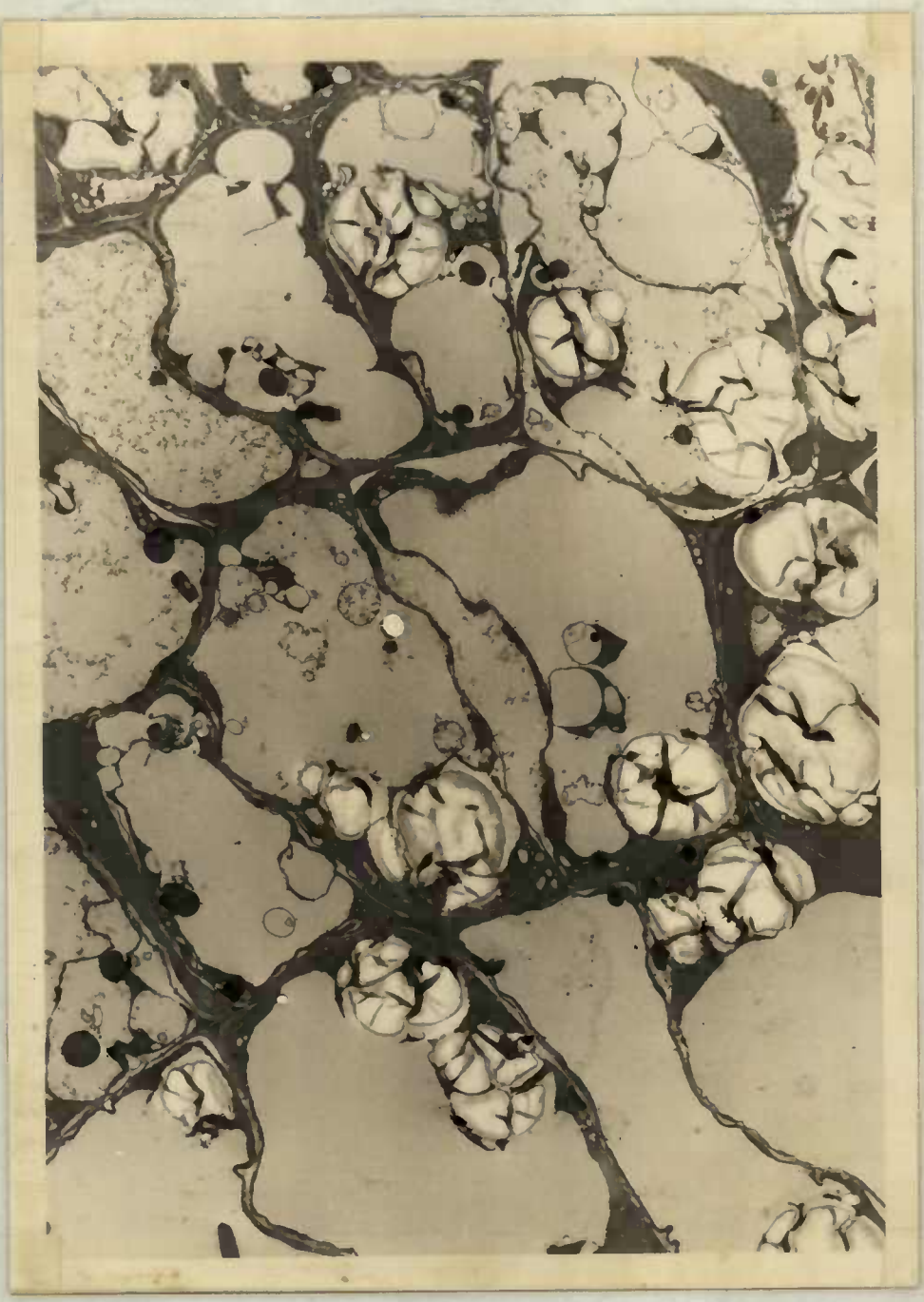
	Average absolute counts (dpm)									
	seeds		pod		petals		bracts		total	
Raceme node	1	2	1	2	1	2	1	2	1	2
RN1	194	424	3267	1794	3517*	570	334	133	7311*	2921
RN4	37	46	2216	716	905	194	166	21	3325	977

* significantly difference (Tukey's test 5%)



3900 x

Fig. 3 A. Electron micrograph of developing seed dissected from RN4 pods at 4 DAA, showing depletion of starch grains in some some cells of the integument.



3900 x

Fig. 3 B. Electron micrograph of a developing seed dissected from RN1 pods at 4 DAA showing cells of integument with many starch grains.