## *Factors affecting flower abscission in field beans (Vicia Faba L. Minor)*

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# FACTORS AFFECTING FLOWER ABSCISSION IN FIELD BEANS (*VICIA FABA* L. *MINOR*)

A thesis submitted in accordance with the requirements of the University of Durham for the degree of Doctor of Philosophy

by

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CONTENTS

## ABSTRACT

A study of flower abscission in commercial varieties of faba beans grown under field conditions showed that least flower drop occurred to flowers situated on proximal flower positions and most on distal positions within every raceme. Application of stresses to plants increased flower drop, most of which occurred to flowers situated on middle and upper flower positions. Many fertilised flowers abscised and manual tripping of all flowers did not ensure a high level of pod set. Decreasing within plant competition and the application of growth regulators all reduced flower drop.

An ultrastructural study showed that either abscission or pod set occurred after a series of clearly defined cellular and enzymatic changes at the pedicel/peduncle junction.

Flower removal experiments demonstrated that all flowers were capable of setting a pod and that much flower drop was initiated by the presence of small proximally situated pods.

Observations on plants with different floral and plant morphologies revealed two inbred lines which displayed minimal flower drop. Experiments showed that there had been no change in the gross morphology of the stem vasculature. In commercial varieties the first formed flower was, in many cases, independent of other flowers, while the second and third flowers were connected to other flowers via the vascular strands. The inbred lines possessed an independent vascular supply to all flowers within every raceme. This arrangement circumvented any communication between proximal and distal flowers, allowed for an even distribution of assimilates, so a high level of pod set was achieved. Initial experiments showed that independent vascular supply lines were more tolerant to stress.

The results obtained are discussed in the thesis.

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# CHAPTER 1

# INTRODUCTION

## Biology, origin and classification

The faba bean, Vicia faba L. is an annual, the dominant phenotype having an indeterminate growth habit with inflorescences developing after between five and ten vegetative nodes have formed. The flowers are commonly off-white with dark spotted wing petals, the standard petal possessing dark stripes. They are borne on axillary racemes which develop acropetally. The species is diploid (2n = 12), although a tetraploid (2n = 4x = 24), has now been described (Poulson and Martin, 1977). The wild progenitors are unknown and its evolutionary origins obscure (Ladizinsky, 1975). The closest wild relative is considered to be Vicia galilaea. Plitm. et. Zoh., however Vicia narbonensis L. is also a close wild relative (Smartt, 1980), but no successful hybridisation of these two species with V. faba has yet been achieved. Such hybrids would be useful for increasing the genetic variability of the crop, which is considered somewhat limited (Bond, 1976), although not as yet fully exploited (Lawes, 1980).

The intraspecific classification of Muratova (1931) is still used, based on the criterion of seed size. The opinion of Cubero (1974) is that there are four subspecies faba (broad), equina (horse), minor (field) and paucijuga (tick), faba being commonly known as the variety major.

## History and food value of the crop

Vicia faba has been an important seed protein crop in Great Britain from at least the bronze age (Hyams, 1971).

Further archaeological investigation has enabled the area of domestication to be pinpointed as the Eastern Mediterranean area (Schultz-motel, 1972; Zohary, 1977; Smartt, 1980). It has now spread in cultivation to cover the area between central Asia, Western Europe, Northern Africa and South America. At present over 70% of the crop is produced in China (F.A.O., 1976), but it is also an important legume in most northern temperate areas, and is grown at higher altitudes and in cool seasons in the sub tropics (Lawes, 1980).

The value of the crop lies in the high protein level of the seeds ranging from 22% to 36% crude protein, which although characteristically low in the sulphur amino acids cysteine and methionine, is high in lysine (Griffiths and Lawes, 1977, 1978). The crop provides, in some African and Mediterranean countries, a substantial part of the protein in human diets. In Western Europe its use as a human food is, at present, confined to fresh, frozen or canned major types (Lawes, 1980). The equina and minor varieties are used mainly for animal feed, and the smaller seeded types are locally valuable for racing pigeons. The faba bean is also a potentially valuable source of novel protein food. The first United Kingdom spun meat analogue ("Kesp") was produced from faba bean isolates by the Courtaulds company. In addition faba bean flours have been found to be acceptable as a protein additive in bread, biscuits and pasta up to levels of 10% in the U.S.A. (Jonas, 1981).

The field bean crop

The acreage of field beans grown in England has declined steadily from 200,000 hectares in the late nineteenth century

to 42,000 hectares in 1979 (Jonas, 1981) ). The national average yield of the crop is three tonnes per hectare (Smith and Altrich, 1967), however, yields can be as high as nine tonnes per hectare or as low as one tonne per hectare (Sprent, Bradford and Norton, 1977). The annual fluctuation in yield is the chief reason for the unpopularity of the crop amongst farmers and research has shown that several factors contribute to this instability.

Weeds

Before the advent of modern selective herbicides, the traditional method of weed control was manual hoeing. Although the problem of annual weeds has since been reduced, there are as yet no selective herbicides that will control broad leaved weeds such as thistles (Circium sp.) and field bindweed (Convolvulus arvensis L.) In addition perennial grasses, such as couch (Agropyron repens.) reduces yield by competition (Hewson, Roberts and Bond, 1973). Couch grass in particular is a carrier of Take-all (Gauanomyces gramminis) and other diseases of cereals. Its presence in field beans can nullify their value as a break crop in intensive cereal production (Anon, 1970).

Pests

The most serious insect pest of faba beans is Aphis fabae, the black bean aphid. The degree of damage caused by A. fabae depends upon the size of the pest population and the stage of crop development at which the attack occurs. Infection can now be controlled by systemic insecticides, so preventing killing bees, necessary for cross-pollination. However work is also underway to seek resistance to this pest (Bond and Pope, 1975) and also to predict infestation from the

levels of overwintering aphid egg populations (Cammell and Way, 1977). In North-West France, a phycomycete, Entomopthora, can sometimes provide control (Lawes, 1980).

Fungal Diseases

The major fungal pathogens of the crop are chocolate spot (Botrytis fabae) and leaf spot (Ascochyta fabae). The chocolate spot fungus occurs in non-aggressive and aggressive forms. The non-aggressive form nearly always occurs as small chocolate spots on most field bean crops, and causes little damage to plants. It is the aggressive form which can devastate winter sown beans, and has been a contributory factor for the poor yields of recent years (Hebblethwaite and Davis, 1971). Leaf spot is predominantly a seed-borne disease, although crops may also become infected during growth by spore transmission. Other fungal diseases that are known to infect faba beans are Sclerotinia bifoliorium (stem rot), Perononspora viciae, Rhizotonia solani, Stemphylium botryosum, Uromyces fabae, Fusarium avenaceum, F. fabae, F. oxysporium, F. solani.

In comparison with many legumes, there is relatively little genetic variation in the resistance to disease, however partial resistance to Uromyces fabae, Ascochyta fabae and Botrytis fabae have recently been reported (Chapman, 1981). Chemicals can be used for control in some instances (Lizenberger, 1974) but applying them may cause mechanical damage. The best form of control for leaf spot is the use of only healthy seed, checked for the absence of Ascochyta.

Virus diseases

Virus diseases include bean leaf roll virus, bean yellow mosaic virus, broad bean stain virus and broad bean true

mosaic virus. In Great Britain, bean leaf roll virus can be serious in spring field beans. It is transmitted by aphids especially Acyrthosiphon pisum. Some resistance has been reported to this virus in commercial variety Maris Bead (Chapman, 1981), but the best form of control is by use of clean seed, and control of the aphid vector.

Plant parasites

The parasitic plant Orobanche crenata (Broomrape), can in the dry climates of Spain and Italy completely destroy the crop. Two lines are known to be resistant to this plant (Chapman, 1981). However in humid climates Broomrape is seldom a major problem.

Flower abscission

Adverse weather conditions can have a detrimental effect, especially in winter sown beans, on the final yield of the crop (Hebblethwaite and Davis, 1971). In addition failure of all ovules to set seed and parthenocarpic pod formation have also been recognized as factors influencing the overall yield of faba beans (Chapman, Fagg and Peat, 1979). However high levels of flower and pod drop have been identified as one of the major contributory factors limiting the yield of field beans (Kambal, 1969; Sekurali, Frauen and Chr. Paul, 1978; Chapman and Peat, 1978; Gates and Boulter, unpublished).

Abscission of flowers in Legumes

Abscission of reproductive structures is a common phenomenon in many wild and cultivated plants. It occurs in many crops of economic importance to man including cotton, Gossypium hirsutum (Dunlup, 1943), tobacco, Nicotiana tabacum (Valdovinos and Jensen, 1968) and tomato, Lycopersion

esculentum (Levy, Rabinowitch and Kedar, 1978). The problem seems to be of particular significance in the legumes, where flower drop has been recognized as an important factor limiting yields in many species, for example lima beans, Phaseolus lunatus (Cordner, 1953); cowpea, vigna unguiculata (Adesomoju et al, 1979), peanuts, Arachis hypogaea (Smith, 1954), soya beans, Glycine max (Huff and Dybing, 1980), Phaseolus vulgaris (Subhadrabandhu, Adams and Reicosky, 1978), pigeon peas, Cajanus cajan (Sheldrake, Narayanan and Venkataratnam, 1979) and lupin, Lupinus sp. (Porter, 1977).

Abscission of flowers in V. faba appears to be particularly acute, various estimates of which have been reported, ranging from 70% - 97% (Soper, 1952; Hodgeson and Blackman, 1956; Rowlands, 1961; Kambal, 1969, Jaquiery and Keller, 1978).

The physiological basis of Abscission

The process of detachment of plant organs, abscission, has been defined by Esau (1960) as occurring in the "abscission zone" which is the zone at the base of leaf, fruit or flower, or other plant part, that contains the "abscission layer" and the "protective layer", both of which play a role in the separation of the plant part from the plant. The abscission layer is a layer of cells, the disruption or breakdown of which separates a plant part from the plant (Simons, 1973). The protective layer safeguards the rest of the plant from subsequent invasion by pests or disease.

The cellular changes leading towards separation, have been described by a number of authors as involving the dissolution of the middle lamella and subsequent expansion of parenchyma cells which causes the fracture of the xylem

vessels. These cells subsequently collapse, and a layer of meristematic cells then grow over the wound to prevent possible infection (Weisner, 1885; Tison, 1899; MacDaniels, 1936; Valdovinos and Jensen, 1968; Sexton and Redshaw, 1981).

Some descriptions have been at variance to the general outline of abscission given above for example Bornman, Spurr and Addicott (1967) described cellular separation occurring through a layer of newly formed meristematic cells. In addition Webster (1968) noted that dissolution of both middle lamella and the primary wall could result in the abscission of P. vulgaris. In dwarf mistletoe Arceuthobium sp. seed dispersal, the fruit explodes, along an abscission zone in which the cells were ruptured, rather than intact, with little evidence of dissolution of the middle lamella (Toth and Kuijt, 1978).

In general it appears that the distal side of the abscission zone tissue shows a condition characteristic of senescence (Carns, 1966). In contrast, the proximal side seems to be in a state of high metabolic activity. The most notable ultrastructural features being a rise in dictysome vesicles within the Golgi apparatus, an increase in rough endoplasmic reticulum, abundant plasmodesmata, abundant mitochondria and a rise in RNA and protein synthesis (Baird, Reid and Webster, 1978).

Regulators of abscission

There is much evidence to support the hypothesis that ethylene is the natural regulator of leaf abscission (Morgan and Durham, 1980). In V. faba increased ethylene appears to be correlated with abscission, and marked abscission has been shown to occur when ethylene levels are rising

(El Beltagy and Hall, 1975).

The role of indole acetic acid (IAA) remains unclear (Huff and Dybing, 1980) although two theories for the role of auxin have been proposed. The first, the auxin gradient theory, states that auxin is the principal endogenous regulator of abscission, its gradient across the abscission zone regulates the onset and rate of abscission. Abscission does not occur with auxin gradients characteristic of healthy, mature tissue: with high auxin distal to the abscission zone and low auxin proximal to the abscission zone. Abscission occurs after a fall in the ratio of distal to proximal auxin and is accelerated when the gradient is reversed (Addicott, Lynch and Carns, 1955). The second states that the primary action of auxin is directly on the abscission zone and is of a two phase type, with low concentration accelerating abscission and high concentration inhibiting. The differences between responses to proximal and distal application of auxin, it was claimed, could be explained on the basis of difference of transport, without the necessity of an auxin gradient involvement (Biggs and Leopold, 1955).

## Environmental factors affecting flower abscission

### Temperature

It has been demonstrated that high temperature in tomato flowers affected gametogenesis, hence pollen production and also changed the rate of germination and pollen tube growth into the style. These factors contributed to the failure of fertilization (to occur) which was the prime cause of flower drop in tomato (Levy, Rabinowitch and Kedar, 1978). High and rising temperatures also appeared to increase flower

shedding in a variety of legumes (Cordner, 1933; Lambeth, 1950; Davis, 1945).

With V. faba flowering and flower drop were reduced during the course of a cold stress period. When plants were transferred to warmer conditions the flowers initiated during the cold stress period stayed on longer, but fell finally in a pattern similar to the control plants (Gehriger, 1980). In addition faba beans seem to require a soil temperature above 5°C for 1000 degree days for satisfactory dry matter production. It has been suggested that low soil temperature in initial vegetative growth may be responsible for the failure to achieve full potential crop growth.

Water

Water supply appears to be the most critical environmental factor affecting yield in most crops. Water stress applied to legumes during the pod fill stage rather than at flowering has the most severe effect on subsequent yield (Doss, Pearson and Rogers, 1974). Little work, however, has been performed on the specific effects of water stress on flower abscission in faba beans. In addition the effects of irrigation on flower drop in V. faba has been little studied, although Stolp (1955) has reported that faba beans gave their maximum yield response when irrigation was applied during the flowering period.

Density

The density of the crop canopy can be an important factor in determining pod set and hence overall yield. Most studies have compared the differences in pods per plant and overall yield, rather than of flower drop. In lupin,

investigations have shown that at high density pod set was reduced and so consequently was seed yield (McGibbon and Williams, 1980). Similar results have been shown to occur with faba beans (Sprent, Bradford and Norton, 1977). Overall yield in faba beans, however, increases with increasing density. It therefore appears that seed yield is a direct function of the number of mature pods per unit area, and not mature pods per plant (Keller and Berkhard, 1981).

Light

The length of the photoperiod is an important contributory factor for bud abscission and flower formation. Phaseolus vulgaris, if subjected to a daylength in excess of 11 hours underwent considerable abscission of flower buds (Zehni and Morgan, 1976). In addition with soybean, long photoperiods of over 14 hours resulted in plants that did not flower (Fisher, 1955). Little work has been performed on V. faba, but Evans (1959) has shown that the plant exhibited a quantitative long day response, the critical photoperiod for flower expansion being 12-13 hours.

The effects of shading on yield has been investigated in a number of crops including wheat, Triticum aestivum (Pendleton and Weibel, 1965); rice, Oryza sativa (Stansel et al, 1965); chickpea, Cicer arietinium (Pandey, Singh and Singh, 1980); pea, Pisum sativum (Hole and Scott, 1981); oil seed rape, Brassica napus subsp. oleifera (Tayo and Morgan, 1969) and faba beans (Hodgeson and Blackman, 1956). In all cases it was found that shading had an adverse effect on yield when applied during reproductive rather than vegetative growth. There have been few investigations, however, on the

relationship between shading and flower drop in faba beans.

## Physiological factors affecting abscission

### Breeding system, pollination and fertilization

Faba beans have a breeding system intermediate between total autogamy and total allogamy, with outbreeding averaging 30% although a range of values have been reported (Holden and Bond, 1960; Hanna and Lawes, 1967; Voluzneva, 1971; Bond and Pope, 1974; Poulsen, 1975). The flowers normally require bee visits, predominantly Bombus hortorum and B. agrorum, to trip the floral mechanism and to effect cross-pollination. A field population of faba beans, therefore, contains both hybrid and inbred plants. Flowers of hybrid plants are able to set seed from autopollination, and their progeny are produced mainly by self-fertilization. Flowers of inbred plants appear to have stigmas that are more difficult to rupture (Toynbee-Clarke, 1971; 1974) and although self-fertile, do not set seed unless visited by insects, therefore about half their progeny result from cross-fertilization (Free and Williams, 1976; Drayner, 1959).

The floral morphology of self or autofertile flowers has been described (Kambal, Bond and Toynbee-Clarke, 1976), but the physiological basis for autofertility appears to be due to the timing of stigma receptivity and production of a stigmatic exudate, relative to the time of anther dehiscence. In autosterile lines, requiring bee tripping of the flower, no exudate is observed until after anthesis, thus allowing effective cross-pollination before self-pollen can germinate on the stigma (Paul et al, 1978).

Therefore, because a population of faba beans consists of approximately one third hybrid plants and two thirds inbred

plants, it might be expected that insect pollination or mechanical tripping would increase yield. This was found to be the case by a number of authors (Kambal, 1969; Kendall and Smith, 1975; Poulsen, 1975; Free and Williams, 1976). In contrast a few authors have shown that there is no advantage in ensuring that all flowers are tripped (Free, 1966; Williams, 1972). Bond and Pope (1974) have shown that pod set at the centre of fields was lower than that at the border in one year, but observed no difference the next. They suggested that some of the difference was due to problems with post-fertilization differentiation of embryos, but no evidence could be found that bees failed to penetrate to the centre of large fields.

The view that bee visitation, because of local variations in population and adverse weather conditions could be a source of yield instability, has prompted the investigation of the possibility of converting the crop to total allogamy (Lawes, 1974; Hanna and Lawes, 1976; Adcock and Lawes, 1976; Chapman and Peat, 1976; Poulsen, 1977). Sources of auto-fertility are known to exist in some Middle Eastern, Indian and African populations, especially in subspecies paucijuga. These types have fewer flowers per node, with short, weak stems as compared to European minor types (Lawes, 1980).

Plant competition and source-sink relationships

As V. faba is an indeterminate species, the first set pods compete for assimilates with growing roots and stem apex, as well as with developing pods further up the stem (Crompton, Lloyd-Jones, Hill-Cottingham, 1981). The competition within the plant is such that Chapman, Fagg and Peat (1979) proposed that increasing within plant competition will

predispose a plant to flower drop and premature pod drop, decreased competition having a reverse effect. Competition between apex and fruits, as well as between single fruits was shown to be responsible for premature pod drop (Jaquiery and Keller, 1980).

Little work has been performed on the effects of competition of the first set pods within a raceme on abscission of flowers on racemes higher up the plant in V. faba, although investigations on the effect of flower and pod removal have been performed on other legumes including yellow lupin, Lupinus luteus (Van Steveninick,1959), snap bean, P. vulgaris (Tamas et al, 1979), pigeon pea, C. cajan (Sheldrake, Narayanan and Venkataratnam, 1979), soya bean, G. max (Huff and Dybing, 1980) and cowpea, V. unguiculata (Ojehomon, 1972). They have variously reached two hypotheses for the abscission of younger distal fruits. The first, involved the production of growth promoters or inhibitors by older basal fruit, which travels up the peduncle and promotes abscission of younger distal fruit. The second is that the oldest fruit at the lowest raceme position monopolises the majority of nutrients available to the whole inflorescences, so distal young fruits and flowers starve, abort and are shed.

Evidence of the distribution of carbon assimilates within the stem leaves and fruit of V. faba, indicates that the majority of fixed carbon comes from the leaf subtending the pod, with other leaves supplying carbon in smaller quantities (Kipps and Boulter, 1973). It also appears that the stem acts as a temporary sink for assimilates, especially if there is no demand for them elsewhere (Ismail and Sagar, 1981(a) ). However the distribution of carbon assimilates within racemes,

and the exact contribution made by other leaves within *V. faba* has been poorly studied and could help to explain source/sink relationships within the plant and may be a determining factor in flower abscission and hence overall crop yield.

## Genetic variability, breeding methods and current breeding objectives

The genetic variability available to plant breeding is considered somewhat restricted, although there has been under-exploitation of what is present, in current breeding programmes. A terminal genotype (Sjodin, 1971), a closed flower mutant (Poulsen, 1977), large variability in flower and seed colour as well as evidence for differences in peduncle morphology, and flower synchrony have now been described (Chapman, 1981).

The breeding system has been manipulated from partial allogamy to complete allogamy or complete autogamy to allow exploitation of standard plant breeding methods, mainly mass and recurrent selection. Recently, synthetic varieties have become available (Bond and Fyfe, 1962; Bond, 1974; Bond, 1981) and attempts have been made in Britain and France to breed $F_1$ hybrids using male sterility (Bond, 1968, 1972; Bond, Fyfe and Toynbee-Clarke, 1964, 1966). $F_1$ hybrids have proved unsuccessful due to incomplete male sterility, and difficulties of large scale production of seed (Plant Breeding Institute annual reports 1965-75; Bond, Fyfe and Toynbee-Clarke, 1966), although yield advantages conferred by heterosis and independence from bee pollinators have been shown to be considerable (Bond, 1970).

A recent programme, now terminated, at the Welsh Plant Breeding Station, Aberystwyth has been to increase auto-fertility, using the inherent autogamous nature of exotic

material, especially paucijuga types. The programme produced two spring bean varieties, Dacre and Deiniol, for which there was claimed to be increased self-fertility (Lawes, 1981).

Attempts have also been made to breed earlier maturing varieties. Present indeterminate varieties are late maturing, ripening between mid-September and mid-October, when conditions can be inclement to harvest (Aylmer and Walsh, 1979). However there is evidence that earlier varieties tend to have lower yields (Poulsen, 1977), so a balance must be achieved between lower yield and earliness.

The primary objectives of most faba bean breeding programmes are for improved yield stability and increased dry or green seed yield. Current opinion seems to be that a greater chance of success for the indirect selection for yield, might come from selection of phenological or morphological attributes (Hawtin, 1981). Characters such as onset and duration of flowering and grain filling, number of tillers, plant height, determinate growth habit, exploitation of heterosis, increased resistance to pests and diseases and reduced flower drop are all recognized as factors that could stabilize and possibly improve yield (Picard, 1974; Chapman, 1977; De Vries, 1979; Hawtin, 1981). Most of the above improvements would involve the production of inbred lines for subsequent use in crossing programmes.

Aims

The aim of the work presented here was to provide an accurate description of the phenomenon of flower drop in Vicia faba L. To this end observations of flower abscission in commercial and inbred lines, under normal and stress conditions in the field and glasshouse were made. In addition

a morphological and biochemical study of the cellular and enzymatic changes occurring during abscission and pod set was undertaken. Observations were also made on material obtained from exotic and crossed material, lines exhibiting low flower drop were selected and by using radiocarbon and eosin feeding experiments together with fluorescence and light microscopy, a comparative study of the vascular anatomy of commercial varieties and lines exhibiting low flower drop was made. These data were used to define a plant ideotype which exhibits minimal flower drop. Following this, preliminary data, comparing these new ideotypes with present commercial varieties was obtained.

# CHAPTER 2

# MATERIALS AND METHODS

## Biological material

Commercial and inbred lines which were used in glasshouse and field experiments are shown in Table 2.1. Inbred material scored for flower drop from the crossing programme is shown in Table 2.2 and Figure 2.1 indicates the origin of the crossed material. Table 2.3 lists other legumes employed in the ultrastructural examination of abscission zones.

## Chemicals, biochemicals and histological stains

A list of the chemical materials used in this study are shown in Table 2.4

## Growth conditions and experimental design

### Greenhouse

Seeds were sown in Levington's potting compost in 12 or 14 cm diameter plastic pots. To ensure nodulation benches were inoculated with *Rhizobia* obtained from field grown material and satisfactory nodulation of plants was observed. Nodules under these conditions were well formed by the time of first flower bud formation.

Seeds shown in short days were placed under high pressure 400 W sodium lamps, type SON/T (Anon, 1973), which were suspended 1 m above the pods and maintained at that height. This supplementary lighting was used to give the plants a 16 h day until natural daylength exceeded this.

Plants were watered weekly with a commercial nutrient feed, Maxicrop (Maxicrop Ltd.) containing all the necessary elements, including nitrogen, and at all other times with

Table 2.1 Varieties and inbred lines of Vicia faba

| Variety name or identifier | Source | Identity |
|---|---|---|
| Maris Bead | PBI | Commercial variety ex EEC trial seed. |
| Deiniol | PBI | -do- |
| Herz Freya | PBI | -do- |
| Kristall | PBI | -do- |
| Strubes | PBI | -do- |
| Minica | PBI | Commercial equina variety ex EEC trial seed. |
| Weirboon | PBI | Commercial major variety ex EEC trial seed. |
| Montica | PBI | -do- |
| Cockfield | PBI | Commercial equina variety. |
| TI Col | PBI | Topless mutant, coloured flowers. |
| line 3 | PBI | 67, inbred component of synthetic variety "Bulldog". |
| line 4 | PBI | 224, equina winter bean. |
| line 5 | PBI | 51/3. |
| line 8 | PBI | Inbred autofertile line 224. |
| line 21 | WPBS | Ch 467 Topless mutant. |
| line 22 | PBI | Sudanese triple white (STW) |
| T51 | PBI | Autofertile inbred line. |
| T2 | PBI | Autosterile inbred line. |

22 x 21 = original cross

56 = line number of $F_1$ generation

56/143 = 143rd seed of $F_2$ derived from line 56.

56/143/7 = 7th seed of 56/143 seed selected in $F_2$

Inbred for further two generations.

Figure 2.1 Crossing programme and explanation of identifiers

Table 2.2 Inbred lines derived from crossing programme

| Identifier | Generation Scored | Plant and Floral Architecture |
|---|---|---|
| 56/98/10 | F4 | Segregating for TI and drawfism, white flowered. |
| 56/107/1 | " | TI basal but no stem branching, white flowered, rapid senescence, sheds leaves. |
| 56/107/4 | " | TI stem no basal branching, white flowered, rapid senescence, sheds leaves. |
| 56/109/7 | " | TI, no stem or basal branching, white flowered. |
| 56/109/15 | " | Segregating for TI, white flowered, little stem branching. |
| 56/117/1 | " | TI, black spotted flowers, no stem or basal branching. |
| 56/118/8 | " | TI, white flowers, basal branching. |
| 56/118/20 | " | Semideterminate, sparsely tillering, synchronous white flowers. |
| 56/130/1 | " | Semideterminate, no tillers, synchronous white flowers. |
| 56/134/7 | " | TI, basal no stem branching, white flowered terminal flowers in clusters. |
| 56/134/12 | " | TI, no branching, white flowered, terminal flowers in clusters. |
| 56/134/14 | " | TI, basal branching, white flowers, clustered terminal flowers. |
| 56/143/7 | " | TI, side branches, synchronous flowering, flowers with yellow spot. |
| 56/143/13 | " | TI, no basal but stem branches synchronous flowering, flowers with yellow spot. |
| 56/143/18 | " | TI, basal and side branches, synchronous flowering, flowers with yellow spot. |
| 56/14/F | F6 | Black spotted flowers, indeterminate, little branching. F - field grown material. |
| 56/62/F | " | Segregating for yellow spot, black spot, white flowers, indeterminate, field grown material. |
| 56/107/1 - 4 | " | Segregating for yellow spot, black spot, white flowers, TI and non TI, monoculm, leaf shedder, rapid senescence. |

Table 2.2 (cont..)

| Identifier | Generation Scored | Plant and Floral Architecture |
|---|---|---|
| 56/118/20 | F6 | Semideterminate, synchronous white flowers, little branching. |
| 56/134/12 + 14 | " | White flowered TI with stem and basal branching. |
| 56/143/9(G) | " | Semideterminate, synchronous white flowers, little branching. |
| 56/143/13 - 18 | " | TI, yellow spot flowers, rapid senescing with stem and side branches. |

Table 2.3 Legume varieties used for scanning electron microscope examination of abscission layers

| Species | Source |
|---|---|
| *Vicia atropupurea* Dest. | Estacao Agronomica Nacional, Portugal. |
| *V. pannonica* Cr. cv Magledi | Research Centre for Agrobotany (NIAVT), Hungary. |
| *V. villosa* Roth. cv Kartali | -do- |
| *Lupinus* sp. Russel strain | Suttons Seeds Ltd., Torquay, U.K. |
| *Pisum sativum* L. cv. Feltham First | -do- |
| *Canavalia ensiformis* D.C. | Sigma Chemicals Ltd., Poole, Dorset, U.K. |
| *Phaseolus vulgaris* L. cv. 519N | Institute de Nutricion de Centra America Y Panama. |
| *Vigna unquiculata* L. Walp. | International Institute of Tropical Agriculture, Ibadan, Nigeria. |
| *Phaseolus lunatus* L. | Tyneside Seed Stores, Gateshead, U.K. |
| *P. aureus* L. | -do- |
| *Cicer arietinum* L. | -do- |
| *Dolichos lablab* Lam. | -do- |
| *Glycine max* L. merr. | -do- |

Table 2.4 Chemical materials used and their commercial sources

| Chemical | Source |
|---|---|
| Acetic acid | B.D.H. Biochemicals Ltd., Poole, Dorset, U.K. |
| Aniline blue | |
| Carmine | |
| Citric acid | |
| Disodium hydrogen orthophosphate | |
| Dipotassium hydrogen orthophosphate | |
| Ethanol | |
| Ferric chloride | |
| Hydrochloric acid | |
| Hydrogen peroxide | |
| Iodine | |
| Lactic acid | |
| Periodic acid | |
| Phloroglucinol | |
| Potassium dihydrogen phosphate | |
| Potassium iodide | |
| Ruthenium red | |
| Silver nitrate | |
| Sodium hydroxide | |
| Sulphuric acid | |
| Toludine blue | |
| Toluene | |
| Xylene | |
| Agarose | Bethsada Chemicals Inc., Rockville, Maryland, U.S.A. |

Table 2.4 (cont...)

| Chemical | Source |
|---|---|
| Paraffin wax | Cheeseborough Ltd. |
| Flourescein | Edward Gurr Ltd., West London, U.K. |
| Glutaraldehyde | E.M. Scope Laboratories Ltd., Ashford, Kent, U.K. |
| Formaldehyde | -do- |
| Sodium cacodylate | |
| Spurrs resin | |
| Basic fushin | Kochlight Laboratories Ltd., Kolnbrook, Bucks., U.K. |
| 2,5,diphenyoxazole (PPO) | |
| Eosin | |
| Sodium chloride | |
| Triton X | |
| Calcofluor white | Polysciences Inc., Warrington, Pennsylvania, U.S.A. |
| 8-anilino-1-napthelene sulphonic acid (ANS) | Sigma Chemicals Ltd., Poole, Dorset, U.K. |
| Guaiacol | |
| Hydroxylamine | |
| Pectin | |
| Pectin methyl esterase | |
| Pectinase (polygalacturonase) | |
| Peroxidase | |
| Porcine pancreatic alpha-amylase | |
| Rice starch | |
| Sodium benzoate | |
| Tri-iodo-benzoic acid (TIBA) | |

tapwater. Vicia, Pisum and Lupinus were grown under cool conditions (maximum day temperature 15°C, minimum night temperature 10°C), whilst other legumes were grown with additional heating (maximum day temperature 25°C, minimum night temperature 15°C). All V. faba flowers were hand tripped at developmental stage 9 (Figure 2.2), unless stated otherwise.

For most experiments in the glasshouse plants were arranged either in a factorial or randomized block design depending on the number of treatments and varieties within the experiment.

Field

Durham

Seeds were sown directly in rows of 30 cm width with 8.5 cm spacing within rows, unless stated otherwise. No fertilizer treatment was applied. Most experiments were planned on a randomized block design with three replicate plots for each variety in the experiment. In all cases guard rows were included as part of the experimental design. Treatments were either arranged in blocks or within blocks, as available space allowed.

Plant Breeding Institute (PBI), Cambridge

Seeds were either drilled or hand sown, mostly in four row plots with a row width of 30 cm. The seeds were spaced 8.5 cm apart within rows. Most experiments were arranged as randomized blocks or a modification of this design as space allowed, with three replicate plots per treatment. All experiments included guard rows as part of the experimental design.

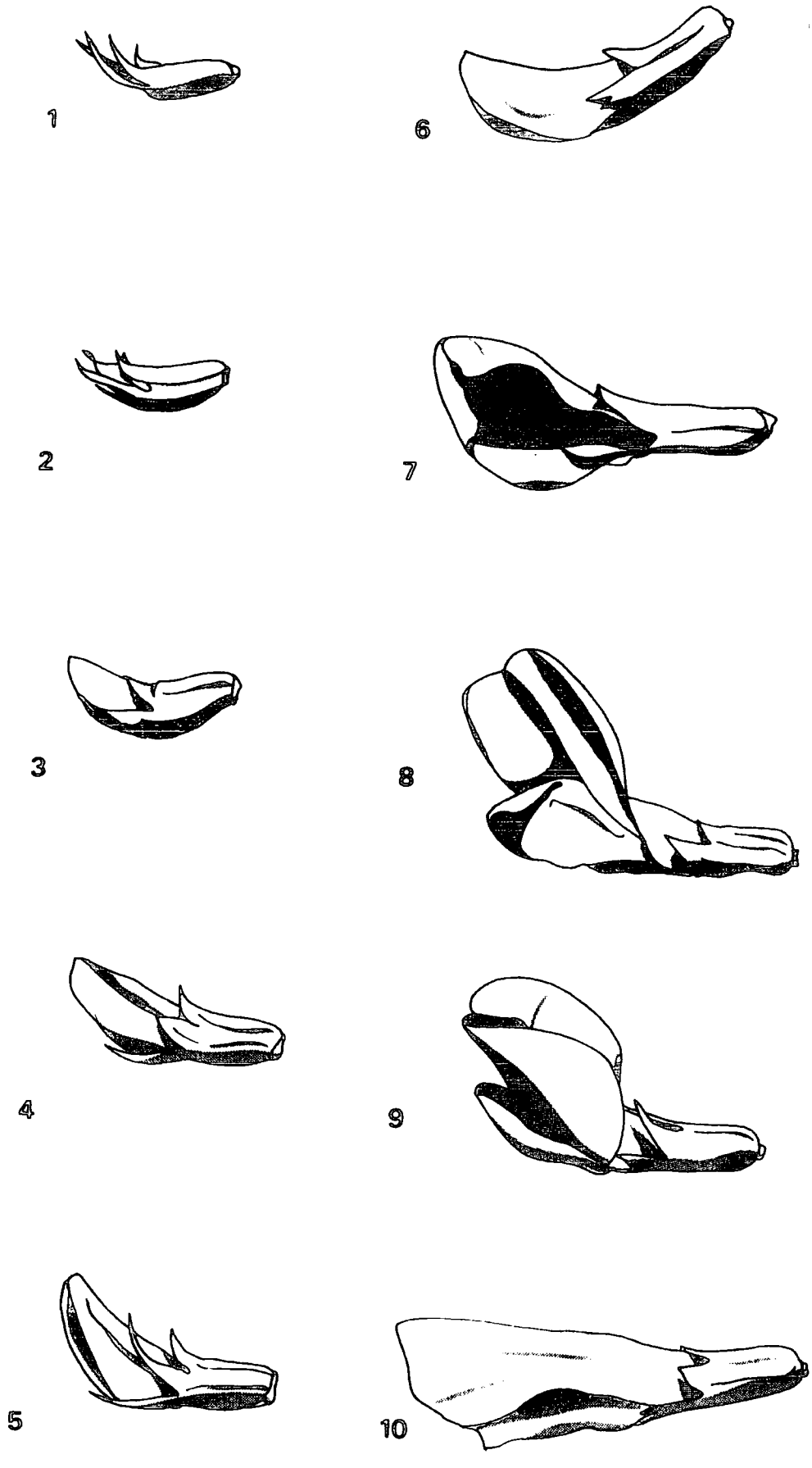



Figure 2.2: Stages of flower development in Vicia faba L.

Flower stages

In order to easily distinguish different stages of development, the differentiation of flowers was divided into ten stages (Figure 2.2) by a modification of the scheme proposed by Paul (1977).

Scoring for flower drop

Flower position within a raceme was assigned a number; one being the lowest, two the next lowest and so on (Figure 2.3). In this way, for each plant sampled the position of flower drop and pod set was quickly and accurately recorded for every flowering raceme.

Emasculation and crossing

Flowers were emasculated by the method of Erith (1930). At stage 3-4 the whole corolla was gripped with forceps and gently pulled. The petals separated from the calyx base, as they slide over the stigma and stamens, the anthers are removed, leaving behind an emasculated flower with an exposed stigma. This was immediately cross-pollinated. The cross 22 x 21 (56), released much variability of floral and plant architecture which was subsequently inbred (Table 2.2) and scored for flower drop.

Scoring of commercial varieties for flower drop

A detailed investigation of flower abscission within and on every raceme was performed during June and July, 1980, 1981 and 1982 at the PBI, Cambridge. Five plants were chosen and scored at random for each replicate plot of eight varieties comprising the EEC field bean trial.

The effect of irrigation on flower drop

The relationship between supplementary watering and flower abscission was investigated at the PBI. Half the

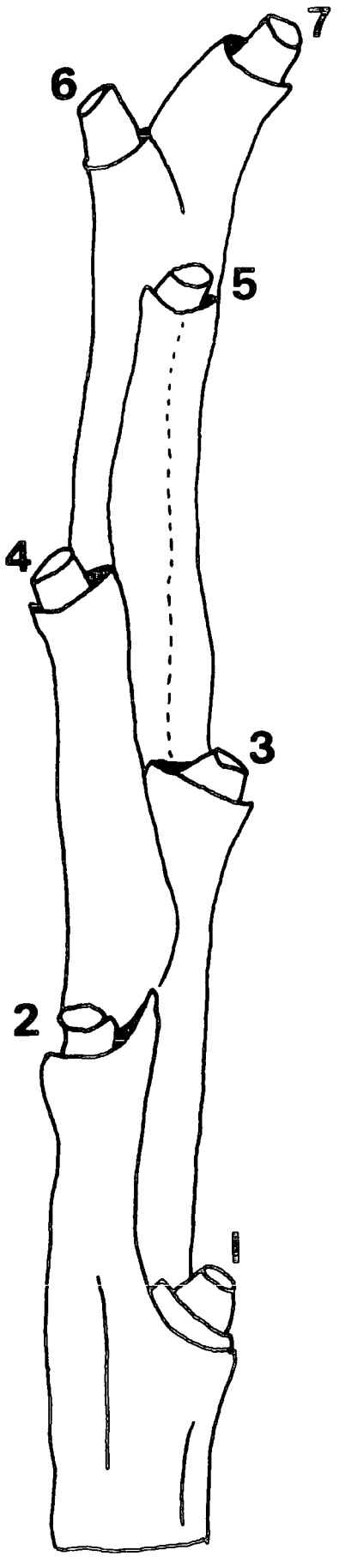



Figure 2.3: Enumeration of flowers on a raceme of Vicia faba L.

plot was irrigated for two hours on alternate days from bud initiation until pod set. The other half of the plot was left unirrigated. Five plants of each variety were picked at random from each subplot (ten plants per treatment) and were scored for flower drop.

The effect of overwatering on flower drop

In order to compliment the results obtained from the irrigation experiment, plants were subjected to an over-watering treatment at the glasshouses, Durham. When flowering had commenced plants from each variety were randomly assigned to the overwatering treatment, or to the control, where plants were watered so as to keep the compost moist, but not wet. Each treatment consisted of five plants per variety. The overwatered plants' compost was always kept very wet, achieved by placing plastic saucers underneath the pots which were always full of water. All plants were subsequently scored for flower drop.

Water stress and flower drop

The detailed effects of a drought stress on flower abscission within each raceme was studied on variety Maris Bead in the glasshouse at Durham. Watering was withheld at the following stages of flowering:- (a) first raceme, all flowers at stage 1; (b) first raceme all flowers at stage 5-6; (c) first raceme, all flowers at stage 8-9; (d) first two racemes, all flowers at stage 8-9 and (e) control. Under glasshouse conditions during June, 1980, the pots were dry within two-three days of water being withheld. The plants were left unwatered until one day after the leaves had gone flaccid, they were then watered. Watering was withheld in this manner until pods had set on the first three flowering

nodes. Watering was then commenced normally until harvest. Seed number, seed weight and yield per plant were established for all treatments.

Shading and flower drop

In order to study the effect of limiting the supply of photosynthetic assimilates on flower abscission at different stages of flowering plants were sown in randomized blocks with three replicates of each variety at the PBI. There was no replication of treatments because of limited space and shading frames. Three treatments and a control were employed, each treatment lasted two weeks. These began at the following stages of flowering, (a) bud initiation, (b) first raceme flowers at stage 9 and (c) at least five inflorescences flowering, the first inflorescence possessed pods. Three varieties in each subplot were shaded at one time by means of hessian screens placed over the appropriate plots which were selected at random. The north side of the frame was left open to allow access to bees. Each screen allowed approximately 60% of the ambient light to reach the canopy vegetation. Five plants of each variety were picked at random from each subplot (15 plants per treatment). Each plant was scored for position of flower drop and pod set within each raceme.

Cold shock and flower abscission

Application of a short low temperature shock on subsequent abscission was examined at the PBI. Plants were subjected to a temperature of $4^{o}C$ for five hours in a growth cabinet. The duration and temperature of the treatment was determined according to the meteorological records of the PBI for June 1969-1979. These show that the length of the

coldest period varied between 4 and 6 hours. The lowest minimum temperature recorded during this period was 1.5°C, and the highest minimum temperature recorded was 6°C, the average temperature being 4°C. The plants were acclimatised to the growth cabinet at 25°C and 14 h photoperiod a week before the cold shock was applied. Four plants of each variety were assigned to three treatments which were as follows: (a) flowers on racemes between stage 1 and stage 5-6, (b) flowers on racemes between stages 4-8 and stages 8-9 and (c) control. After application of the cold shock the plants were left overnight at 25°C and then transferred to an unheated glasshouse. They were then allowed to develop normally and subsequently scored for flower drop and pod set.

Frost damage and flower abscission

Frost during June 1980, damaged the apex of susceptible plants, rendering them topless. Three varieties comprising the EEC field bean trial were most affected; Weirboon, Strubes and Minica. Five plants so affected were picked at random from each subplot and scored for flower drop. These figures were subsequently compared to the equivalent number of flowering nodes on unaffected plants of the same varieties already scored for flower drop.

Planting density and flower abscission

An investigation of the effects of density on flower drop within each raceme and on each raceme was performed at Durham during July and August 1980. There were three density treatments, each seed being placed either at 45 cm, 30 cm or 15 cm apart within random plots. Four plants were labelled randomly from each subplot and scored for flower drop.

Tripping experiments

The effect on flower drop of differential tripping in both autosterile and autofertile lines was investigated in the glasshouse. Once flowering had commenced the following treatments were randomly applied to five replicate plants: (a) proximal two flowers tripped on each raceme, (b) distal two flowers tripped on each raceme, (c) all flowers tripped on each raceme, and (d) no flowers tripped. The plants were scored for flower drop during May 1982.

Presence of pollen tubes in ovaries of abscissed flowers

Detection of pollen tubes in ovaries of abscised flowers was performed by a modification of the method of Martin (1959). Aniline blue (2%) in 20% potassium phosphate stained the callose of pollen tubes in ovaries that had previously been softened with 1 M NaOH at 60$^{o}$C for 2 h. The samples were then examined under a Leitz orthoplan fluorescence microscope (Table 2.5).

Presence of pollen tubes in ovaries of tripped and untripped flowers

Plants were grown in the glasshouse and once flowering had commenced flowers on five plants of each line picked at random, were left untripped, a further five plants had all their flowers tripped. Flowers that abscised were assayed for the presence or absence of pollen tubes in ovaries, using the method described above.

The effect of growth regulators on flower abscission

The initial experiment with silver nitrate

Four plants of each line, which were flowering, were randomly selected and sprayed with 100 mg/l silver nitrate in distilled water together with two drops of a wetting agent

(Photoflo, Kodak, Ltd.) per 100 ml of silver nitrate solution. A further four plants of each line were sprayed with distilled water only. Each plant was subsequently scored for flower drop.

The initial experiment with sodium benzoate

Twenty plants were selected from variety Maris Bead, ten of which were sprayed twice with 100 mg/l sodium benzoate in distilled water with two drops of wetting agent, per 100 ml sodium benzoate solution. One spray was applied when the first inflorescence had reached stage 8, and one was applied when the first pods had set on the initial inflorescences. The other ten plants were sprayed with distilled water. The plants were scored for flower drop at all nodes.

Field experiment with sodium benzoate and silver nitrate

Silver nitrate (100 mg/l) or sodium benzoate (100 mg/l) were sprayed on separate subplots chosen at random. Spraying was performed twice, once at bud initiation and once at full flowering. Five plants were selected at random from each subplot and scored for flower drop.

The effect of inflorescence or top removal on plants of line $T_2$ on flower drop

The following treatments were applied to four plants picked at random: (a) first raceme removed, (b) first two racemes removed, (c) first three racemes removed, (d) first four racemes removed, (e) first five racemes removed, (f) first six racemes removed.

The following decapitation treatments were also applied to four plants picked at random from the plot: (a) top removed after six flowering nodes had formed, (b) top removed after five flowering nodes had formed, (c) as (b) but after four flowering nodes had formed. Finally four plants were

selected to act as controls.

The racemes were removed once at least one flower on the inflorescence had reached developmental stage 9. The plants, where applicable, were scored up to and including the 20th flowering node.

The effect of surgical apex removal and decapitation on flower abscission

Three cultivars: Maris Bead, Cockfield and Deiniol, were grown at the PBI. Five plants of each variety were assigned to each treatment. These were: (a) surgical removal of the apex above the first five raceme initials, (b) decapitation of the apex after five flowering nodes had fully formed and (c) controls. Five plants of genotype TI Col. were grown at the same time as the above for comparison. All plants were scored during July, 1981.

The effect of leaf removal on flower abscission

Leaves were removed from plants of cv Cockfield, once they had fully formed from each successive node. Each treatment consisted of five replicates, these were: (a) controls, (b) all leaves on vegetative nodes removed, but no removal of leaves subtending inflorescences, (c) leaves on vegetative nodes not removed, but alternate leaves subtending racemes were removed from the first flowering node upwards, (d) as (c) but leaf removal initiated from the second flowering node upwards, (e) as (c) but leaves from vegetative nodes also removed, (f) all fully formed leaves removed, except for those leaves situated on vegetative nodes at the apical portion of the plant, (g) leaves only removed on vegetative nodes on the apical portion of the plant, (h) all leaves, subtending inflorescences were removed. The plants were scored for flower drop during May, 1981.

This experiment was repeated during September 1981, but only with treatments (a), (b), (c), (f) and (h) described above, and the leaves were covered with silver foil, instead of being removed. This was performed in order to eliminate any possibility that the physical removal of leaves would have an adverse effect on subsequent flower abscission.

Collection of material for microscopy

The appearance of a distinct brown line was used to predict flower shedding but in the absence of this marker incipient flower drop was tested by tapping flowers lightly with forceps: pedicels and peduncles of those which dropped were fixed.

Material for fluorescence microscopy was removed from the plant and observed directly in the laboratory.

Fixation and preparation of material for microscopy

Material was fixed for thick sectioning in formalin: ethanol:acetic acid (1:9:1) for 1 h, dehydrated in an alcohol series, transferred to xylene and embedded in wax. Sections (12 μm) were cut on a rotary microtome, dehydrated through an alcohol series and stained either in toludine blue, periodic acid Schiffs reagent, iodine/potassium iodide solution, acetocarmine, ruthenium red or hydroxylamine-ferric chloride according to Jensen (1962).

Alternatively sections were fixed in 0.05 M phosphate buffer pH 7.0 and 1% glutaraldehyde to avoid shrinkage of abscission zone cells. Tissue was fixed for thin sectioning for 2 h in 2.5% glutaraldehyde and 1% formaldehyde in pH 7.0 cacodylate buffer, washed in buffer for 2 h, post fixed in 1% aqueous osmium tetroxide for 3 h, washed in water, dehydrated through an alcohol series, embedded in Spurr's resin

and sectioned at 1 µm with an ultramicrotome (LKB ultratome).

Thin sections for transmission electron microscopy were stained in uranyl acetate and alkaline lead citrate and examined on a Phillips EM 400 electron microscope.

Specimens for scanning electron microscopy were prepared by fixation in 0.05 M phosphate buffer pH 7.0 plus 1% glutaraldehyde for 12 h, dehydrated through an acetone series, critical point dried with $CO_2$, coated with gold-palladium alloy and examined in a Cambridge stereoscan 600 scanning electron microscope (SEM).

Material for fluorescence microscopy was either sectioned by hand or on a tissue chopper (Mickle Engineering Co.). Either an aqueous solution (0.1%) of calcofluor white or 0.1% ANS, in 0.1 M citric acid buffer was applied to the fresh cut surface and examined under a Leitz orthoplan fluorescence microscope, for wavelengths and filters used see Table 2.5.

Table 2.5 Wavelength and Leitz filters used for various fluorescent stains

| Fluorescent stain | Suppression filter | Exciting filter | Wavelength range (nm) |
|---|---|---|---|
| ANS | K460 | UV-filter UG1 | 290 - 395 |
| Aniline blue | K460 | " | " |
| Calcofluor white | K460 | " | " |
| Fluorescein | K515 | Blue filter KP500 | 360 - 500 |

Gel diffusion enzyme assays

Estimates of the concentration of the enzymes, amylase, pectinmethyl esterase and pectinase were made for pedicel/peduncle junctions at different stages of flower development.

Peroxidase concentration was also estimated in pedicel/peduncle junctions at different developmental stages to monitor developmental differences between varieties with different floral architectures. The method adopted was a modification of the technique of radial diffusion in an agarose gel (Mestecky et al, 1969; Schill and Schumacher, 1979).

Buffers

Amylase

0.15 M Sørensen phosphate buffer, pH 7.0.

Pectin methyl esterase

The buffer was identical to that used in the amylase assay, except for the addition of 0.3 M NaCl, the pH was corrected to 7.0.

Pectinase

Citrate phosphate buffer pH 4.0 was used in this assay. Citric acid (0.1 M) and 0.2 M disodium hydrogen phosphate was made up with distilled water, separately as stock solutions.

Peroxidase

Potassium dihydrogen phosphate (20 mM) pH 6.1 buffer was used in this assay.

Amylase assay method

Agarose (1 g) was suspended in 100 ml phosphate buffer containing 0.1% purified rice starch. This was heated until all the starch and agarose had dissolved. Aliquots (9 ml) of hot agarose/starch mixture were poured onto each of a number of gel diffusion plates (Miles Biochemicals Ltd.) on a levelling table. After solidification and equilibration (at least 24 h in a moist atmosphere at $4^{\circ}C$), wells of 20 $\mu$l capacity were cut out of the plates.

Standard dilutions of alpha amylase, activity 1000 U/mg protein, were prepared fresh between 1000 and 0.1 µg protein/ml. Samples were crushed in a microhomogenisor and extracted with 100 µl buffer, 20 µl samples of which were applied into wells. At least five replicates of each sample and standard were used in each assay performed. Diffusion of the enzyme into the agarose starch gel was allowed to proceed for 16 h at room temperature (approximately 22°C). The agarose slabs were then covered for two minutes with 10% potassium iodide/1% iodine solution. The I/KI solution stains the highly polymerized starch a dark blue colour. The diffusion zones of the enzyme were visible as transparent discs. These were measured in two perpendicular diameters. A standard curve was constructed of $\log_{10}$ concentration of standard α-amylase versus the average diameter (mm) of the transparent discs. From this curve the concentration of amylase in each sample was evaluated.

Pectin methyl esterase assay method

Agarose (1%) and 0.1% purified pectin was dissolved in buffer and plates poured as previously described for the amylase assay. Standard pectin methyl esterase in a concentration range of 500 µg protein/ml to 0.1 µg/protein/ml, and samples were prepared, dispensed and allowed to diffuse into the gel as described for the amylase assay.

Diffusion zones were stained for two hours with an aqueous solution of ruthenium red (1:5000 w/v). Pectin that was unaffected by the enzyme appeared light pink, pectic substances (e.g. pectic acids) reacted with the stain to give a red colouration. Therefore the extent of the enzyme action

was noted by red circles in a pink gel. The diffusion zones were measured and a standard curve tabulated as previously described for the amylase assay.

Pectinase

Agarose/pectin plates were prepared, as for the pectin methyl esterase assay, but with citrate/phosphate buffer.

Standard pectinase (polygalacturonase), in a concentration range of 500 $\mu$g protein/ml to 0.1 $\mu$g protein/ml were prepared, dispensed and allowed to diffuse into the gel as previously described.

It was found that 0.05% I/5% KI solution, was the only available stain that would show up pectinase diffusion zones as light yellow circles in a dark yellow gel. The zones were measured and a standard curve constructed as described before.

Peroxidase

Agarose (1%) together with 5 mM guaiacol were suspended in phosphate buffer which were then prepared, poured and equilibrated as described for the amylase assay.

Standard horseradish peroxidase was used in a concentration range of 500 $\mu$g/protein/ml to 0.1 $\mu$g protein/ml together with samples which were prepared as previously described. Samples and standards were allowed to diffuse into the gel as previously described. Diffusion zones were stained with a 10 mM hydrogen peroxide solution, which showed up the zones, as dark brown rings in a colourless gel. The zones were measured and a standard curve constructed as described before.

## Experiments to estimate amylase concentrations in pedicel/peduncle junctions at different developmental stages

### Emasculation and polination effects on amylase concentration of the pedicel/peduncle junction

Flowers of line 22 plants were subjected to the following treatments: (a) stage 5 flowers emasculated, left unpollinated; (b) stage 5 flowers emasculated and pollinated; (c) stage 8-9 flowers pollinated; (d) stage 8-9 flowers left unpollinated.

All treatments were performed on at least five flowers. After 24 h the pedicel/peduncle junction of each flower was assayed for amylase as described previously. This experiment was repeated twice more.

### Pollination effects on amylase concentration of the pedicel/peduncle junction

Amylase concentration was estimated in five line 22 pedicel/peduncle junctions obtained from flowers at developmental stages 6, 7, 8, 8-9 and 9 respectively, 24 h after tripping had been performed. This experiment was repeated twice more.

## Estimation of pectinmethylesterase (PME) concentration in pedicel/peduncle junctions of flowers at different developmental stages

Concentrations of PME were estimated in pedicel/peduncle junctions obtained from flowers of Maris Bead. The pedicel/peduncles were removed from flowers which had reached the following developmental stages: stage 9; stage 9, petals beginning to die, no signs of ovary expansion; Stage 9, petals beginning to die, signs of ovary expansion; Stage 10 early pod set; Stage 10 Abscising. The experiment was repeated three times, with five replicates for each of the above treatments.

Estimation of pectinase in pedical/peduncle junctions

Concentrations of pectinase were estimated in pedicel/peduncle junctions obtained from flowers of variety Maris Bead. The pedicel/peduncle junctions were removed from flowers which had reached the following developmental stages: stage 7, stage 8, stage 9, stage 10, pod set, and abscising. The experiment was repeated three times, with five replicates for each of the above treatments.

Flower removal experiments

The effect of removal of the proximal flower within each raceme on flower drop

Once flowering had commenced, ten plants from each variety, picked at random, had the first flower from each raceme removed. The other ten plants were left untreated. The plants were scored during May, 1981.

The effect of removal of proximal flowers on flower drop in Deiniol plants

The following treatments were employed for all flowering nodes: (a) first two flowers of each raceme removed, (b) the first three flowers of each raceme were removed, (c) the first four flowers of each raceme were removed and (d) controls. Five plants were assigned to each treatment and scored in October 1980 for flower drop.

The above experiment was repeated using variety Cockfield and was scored for flower drop during January and February 1981.

The effect of removal of proximal flowers on flower drop of plants grown in the field

The following treatments were assigned to each raceme of each of five plants picked at random from each subplot: (a) control, (b) lowest flower removed, (c) first two flowers removed and (d) first three flowers removed. The plants were scored for flower drop during late August, 1981.

The effect of distal flower removal within each raceme on flower abscission

Plants of cv Cockfield were subjected during flowering, to the following treatments: (a) top two flowers removed, (b) top three flowers removed, (c) top four flowers removed and (d) control. Five replicates were randomly assigned to each treatment. The plants were scored for flower drop during April, 1981.

Estimation of peroxidase concentration in pedicel/peduncle junctions from variety Maris Bead and line 56/118/20

Racemes of plants of line 56/118/20 were selected for flowers, which at all positions, had reached developmental stage 10. For racemes on Maris Bead plants, these were selected for flowers that had reached stage 10 at proximal raceme positions and stage 9 at distal raceme positions. Every pedicel/peduncle junction from five racemes of each variety was excised and placed in Durham tubes in order of raceme position. These were then crushed and assayed for peroxidase, using the method already detailed.

Estimation of peroxidase concentration in pedicel/peduncle junctions of variety Deiniol and line 56/143/9 at different stages of development

Racemes of plants of variety Deiniol and line 56/143/9 were selected for flowers, which at all positions had reached developmental stage 10, and for racemes where pods had set. For both stages of development, every pedicel/peduncle junction from five racemes of each variety was excised and placed in Durham tubes in order of raceme position. These samples were then assayed for peroxidase as detailed previously.

## Observations of flower drop on plants with differing floral and plant architectures

### Initial observations

A range of plants were scored for flower drop that exhibited different floral and plant architectures. All plants were $F_4$ inbred lines derived from the crossing programme (Table 2.2). They were scored for flower drop and pod set in the glasshouse during December, 1980.

### Field observations

Seven $F_6$ inbred lines (Table 2.2) were grown in plots at the PBI. Five plants from each subplot were picked at random and scored for flower drop and pod set during July, 1981.

### Observations on the transport of the dye eosin within the vascular tissue of plant lines exhibiting low flower drop and of commercial varieties

Racemes from plants of variety Deiniol and line 56/143/9 were surrounded by parafilm (Gallenkamp Ltd) which was made water-tight with paraffin wax, to form a well. Eosin was poured into the well so as to cover either the first or second flower position on a raceme. The first or second flower was then removed, depending on the experiment, underneath the eosin solution, with a scalpel blade. In this way eosin travelled up the vascular tissue of the peduncle, via the cut tissue.

At least five racemes of both lines, had their proximal flower removed, to allow eosin transport up the vascular tissue of the peduncle. The same number of racemes, of both lines, had the flower situated on the second raceme position removed to allow eosin to transport up the vascular system of the peduncle.

## Investigation of the distribution of assimilates within the bean plant by using $^{14}CO_2$

### Demonstration of communication of flowers within a raceme

The proximal flower of each raceme tested was enclosed in a perspex box, containing 10 $\mu Ci$ of H $^{14}CO_3$ (Amersham, International, PLC) in a small well in the bottom of the box. The box was sealed with paraffin wax. A syringe was inserted through a small hole in the box, which had previously been sealed with plasticine, and 0.5 ml of 50% lactic acid was injected into the well containing the radio labelled carbon. After one hour the reaction was quenched with the injection of 1 M NaOH. The fed flower and other flowers within the raceme were removed, extracted for 2 h with 80% at 70°C. Aliquots (1 ml) of extractant were removed and placed in 5 ml of scintillant. The scintillant used was toluene and triton x (2:1 v/c), to which was added 5 g PPO per litre of toluene. The samples were counted for 1 minute on a Packard Prias $^{TM}$PL/PLD automatic scintillation counter.

### The incorporation of $^{14}CO_2$ into racemes

Individual leaves from plants of line 22 and from commercial variety Maris Bead were fed with 20 $\mu Ci$ $^{14}CO_2$ for 2 h according to the methods previously described. The fed leaf and subtending raceme, together with the leaves and racemes above and below the fed leaf were removed, extracted and counted as previously described.

### Communication of assimilates between podded nodes

Plants of line 56/118/20 and Maris Bead were used.

The methods and materials used were the same as described for the previous experiment, except that a leaf subtending the fifth flowering node for line 56/118/20 and eleventh

flowering node Maris Bead, were allowed to assimilate 20 $^{14}CO_2$ per plant for three and a half hours. After the reaction had been quenched, the fed flowers and peduncle, all other flowers, peduncles and pods together with the apex and leaves which subtended racemes, and 1 cm portions of the stem above and below the fed leaf, were removed, extracted and counted as previously described.

Incorporation of $^{14}C$ into a plant of line 56/143/9 when in flower

The leaf on the 10th node of a plant of line 56/143/9 was allowed to assimilate 20 $\mu Ci$ $^{14}CO_2$ for three and a half hours as described previously. All racemes, leaves, 1 cm stem portions above and below the fed leaf and the apex were removed from the plant, extracted and counted as described previously.

Examination of the vascular system of stem and peduncle

Eosin was used to map the vascular traces of both stem and peduncles. The dye was either injected into one main vascular strand, with a fine hypodermic syringe or freshly cut stems were placed into a beaker of eosin solution and the dye was allowed to transpire up the stem. In addition the dye was applied to leaves and peduncles, from a parafilm (Gallenkamp, Ltd.) well constructed around them. Either the peduncle or the leaf was subsequently removed underneath the layer of eosin. A similar method was used with the dye fluorescein. To further examine phloem transport, however, fluorescein was applied directly to abrased leaves, within small plastic wells sealed into the leaf with paraffin wax. Sections were subsequently made from peduncles and stems and either examined under light (eosin) or fluorescent (fluorescein) microscopy. Alternatively eosin stained material was carefully

dissected under a stereoscopic dissecting microscope (Nikon SMZ 70).

### Clearing and staining techniques for examination of vascular strands

Vascular traces were examined by placing specimen peduncles in 50% lactic acid. This was gently heated for a few minutes until the tissue, except the vascular traces had become transparent. Samples were then mounted in dilute glycerol and examined under a stereoscopic dissecting microscope. Alternatively samples were stained with a 1:1 solution of concentrated sulphuric acid and phloroglucinol. Excess stain was removed with 95% ethanol, specimens were mounted in dilute glycerol and examined under the dissecting or light microscope.

## Environmental and Physiological experiments on inbred lines possessing the independent vascular supply characteristic

### The effect of various tripping treatments on flower abscission

Five replicates from lines 22 and 56/143/9 were subjected to the four treatments described in the previous tripping experiment. Each plant was subsequently scored for flower drop.

### The effect of application of 2,3,5-Triiodobenzoic acid (T.I.B.A.) on flower drop

In order to investigate the effect of TIBA on flower abscission ten plants each of Maris Bead and 56/118/20 were picked at random and sprayed at flowering with 100 mg/l TIBA in distilled water together with two-three drops of a wetting agent. The other ten plants of each plant type were sprayed with distilled water. All plants were subsequently scored for flower drop and pod set.

The effect of supplementary lighting and temperature on flower drop

Half of the seeds of Deiniol and of line 56/143/9 were grown in the cool glasshouse (maximum day temperature 15°C, minimum night temperature 10°C) the other half were grown in the warm glasshouse (maximum day temperature 25°C, minimum night temperature 15°C). Half the plants assigned to each greenhouse (seven plants of each variety), were also picked at random to receive supplementary lighting (Type SON/T). All plants were scored for flower drop and pod set as described previously.

# CHAPTER 3

## RESULTS OF VISUAL OBSERVATIONS AND ENVIRONMENTAL STRESS EXPERIMENTS USING COMMERCIAL CULTIVARS OF FABA BEANS

### Assessment of flower drop in commercial varieties

Most flower abscission occurred in all the cultivars tested when flowers had reached developmental stage 10. Flower drop within each raceme followed a similar pattern in all varieties examined, in that most pods set on proximal flower positions whereas at distal positions flowers usually abscind (Figures 3.1.1, 3.1.2, 3.2.1. 3.2.2, 3.3.1, 3.3.2). There was a progressive increase in flower drop on every raceme from the bottom of the plant to the top. The least amount of flower drop commonly occurred on inflorescences formed in the lower-middle flowering portion of plants (Figures 3.4.1, 3.4.2, 3.4.3, 3.5.1, 3.5.2, 3.5.3). In addition bud abortion frequently occurred on distal racemes. Overall flower abscission fluctuated considerably between different seasons (Table 3.1). Flower drop also varied between

Table 3.1 Average abscission of varieties in EEC trial

| Variety | Flower abscission (%) | | |
|---|---|---|---|
| | 1980 | 1981 | 1982 |
| Maris Bead | 48 | 52 | 62 |
| Kristall | 52 | 60 | 58 |
| Weirboon | 62 | 75 | 87 |
| Herz Freya | 37 | 55 | 66 |
| Montica | 57 | 72 | 87 |
| Deiniol | 36 | 52 | 61 |
| Strubes | 56 | 65 | 71 |
| Minica | 40 | 72 | 81 |

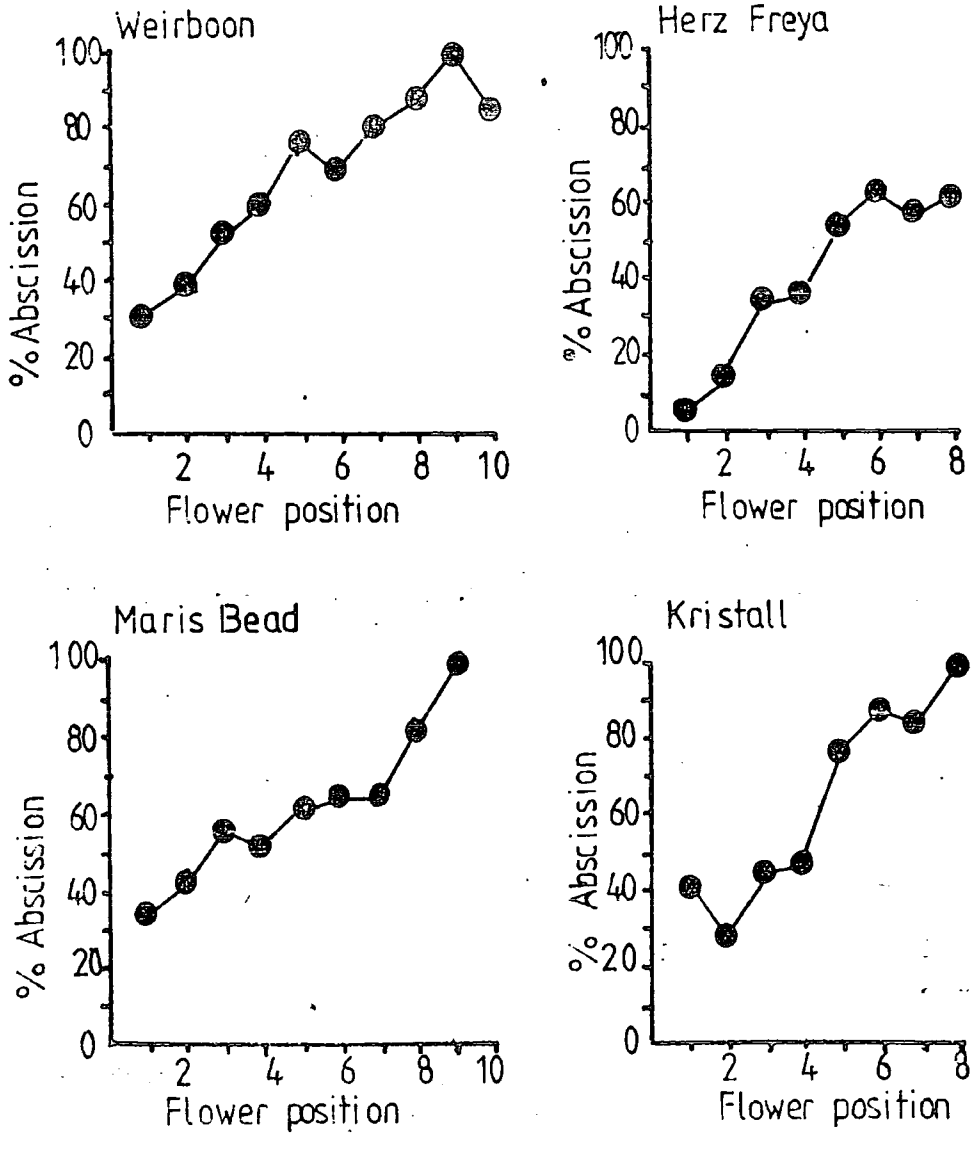



Figure 3.1.1: Flower abscission for varieties in the EEC trial 1980. Each value is an overall percentage.

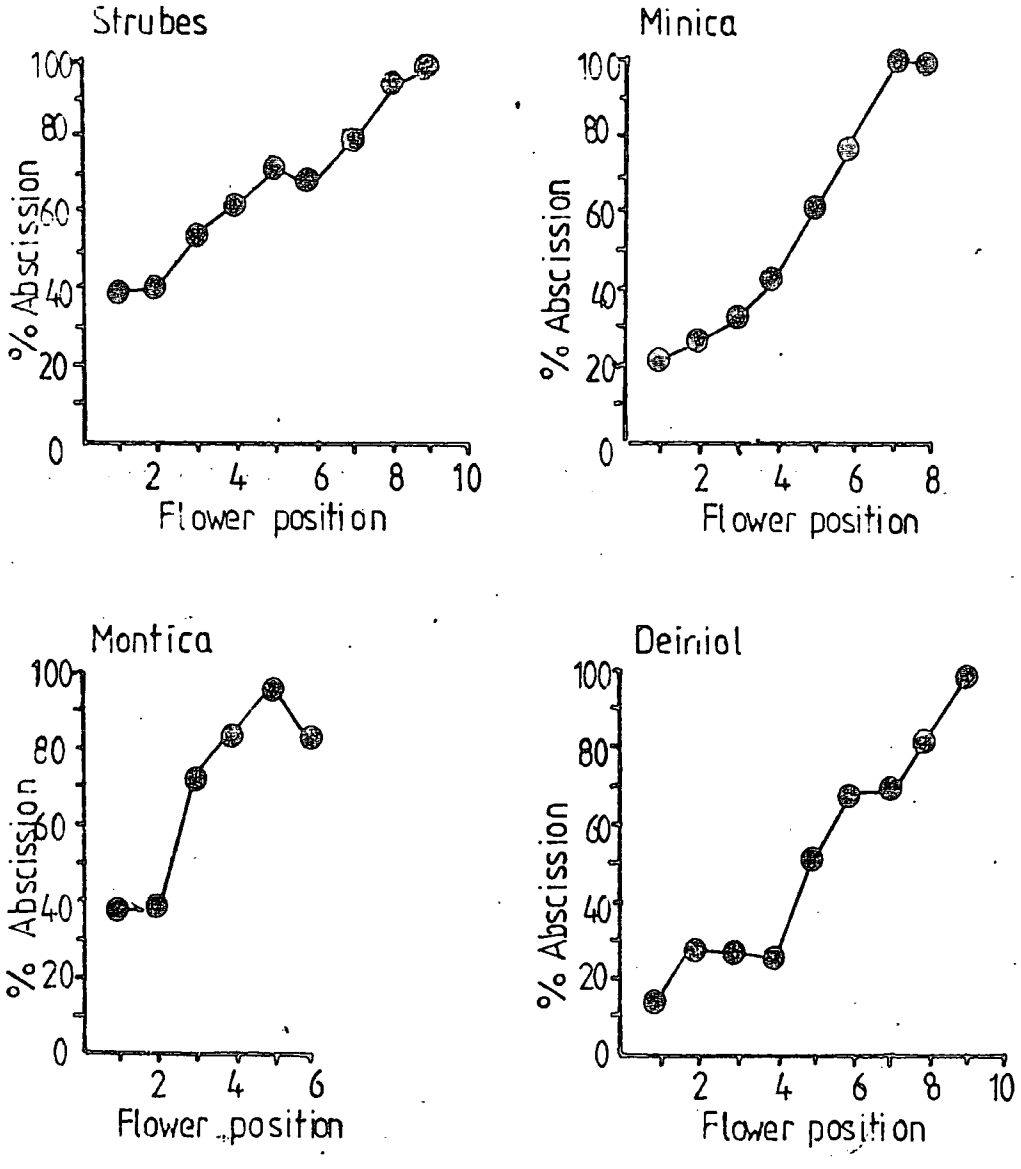



Figure 3.1.2: Flower abscission for varieties in EEC trial 1980. Each value is an overall percentage.

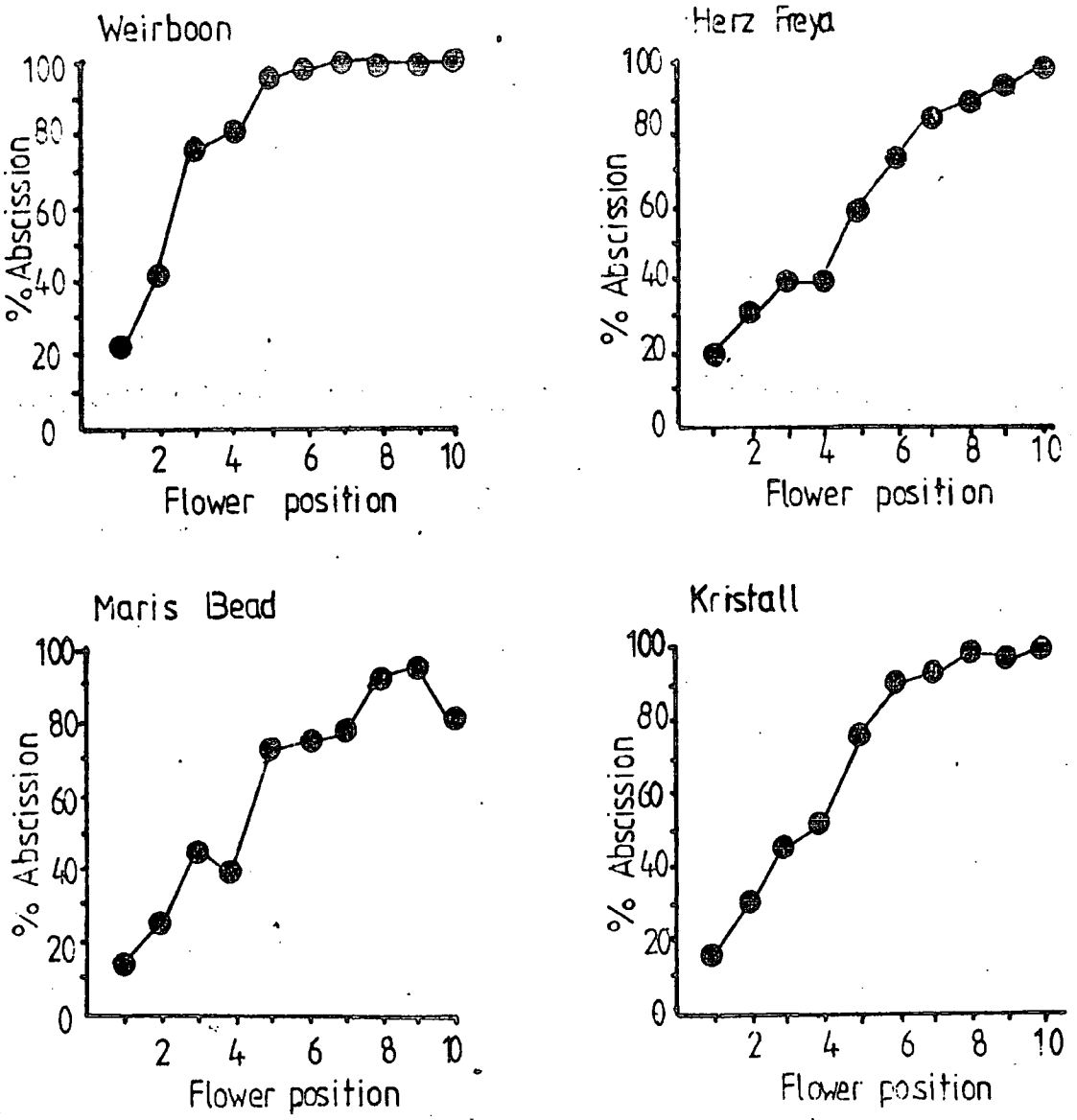



Figure 3.2.1: Flower abscission for varieties in EEC trial 1981. Each value is an overall percentage.

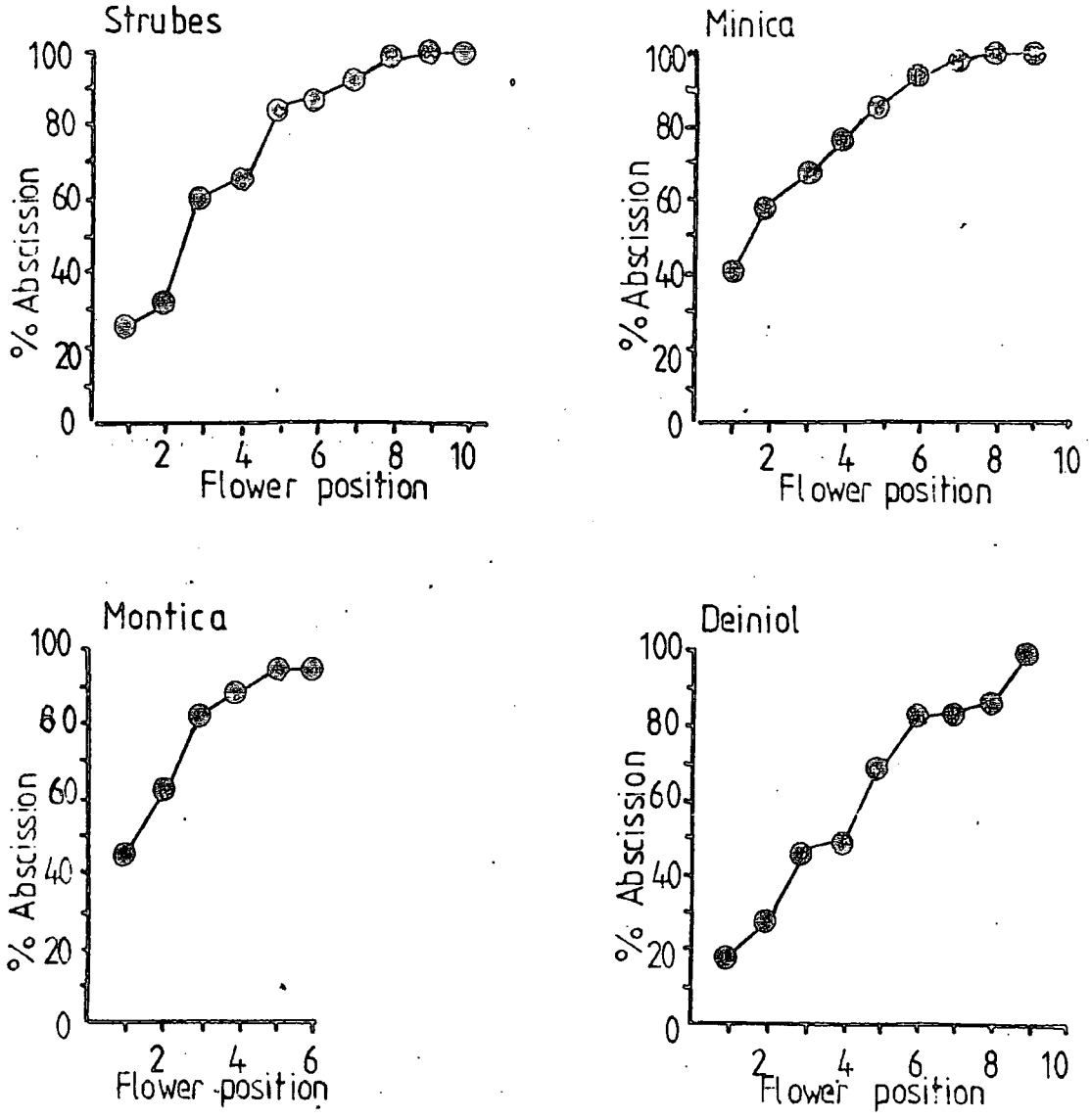



Figure 3.2.2: Flower abscission for varieties in EEC trial, 1981. Each value is an overall percentage.

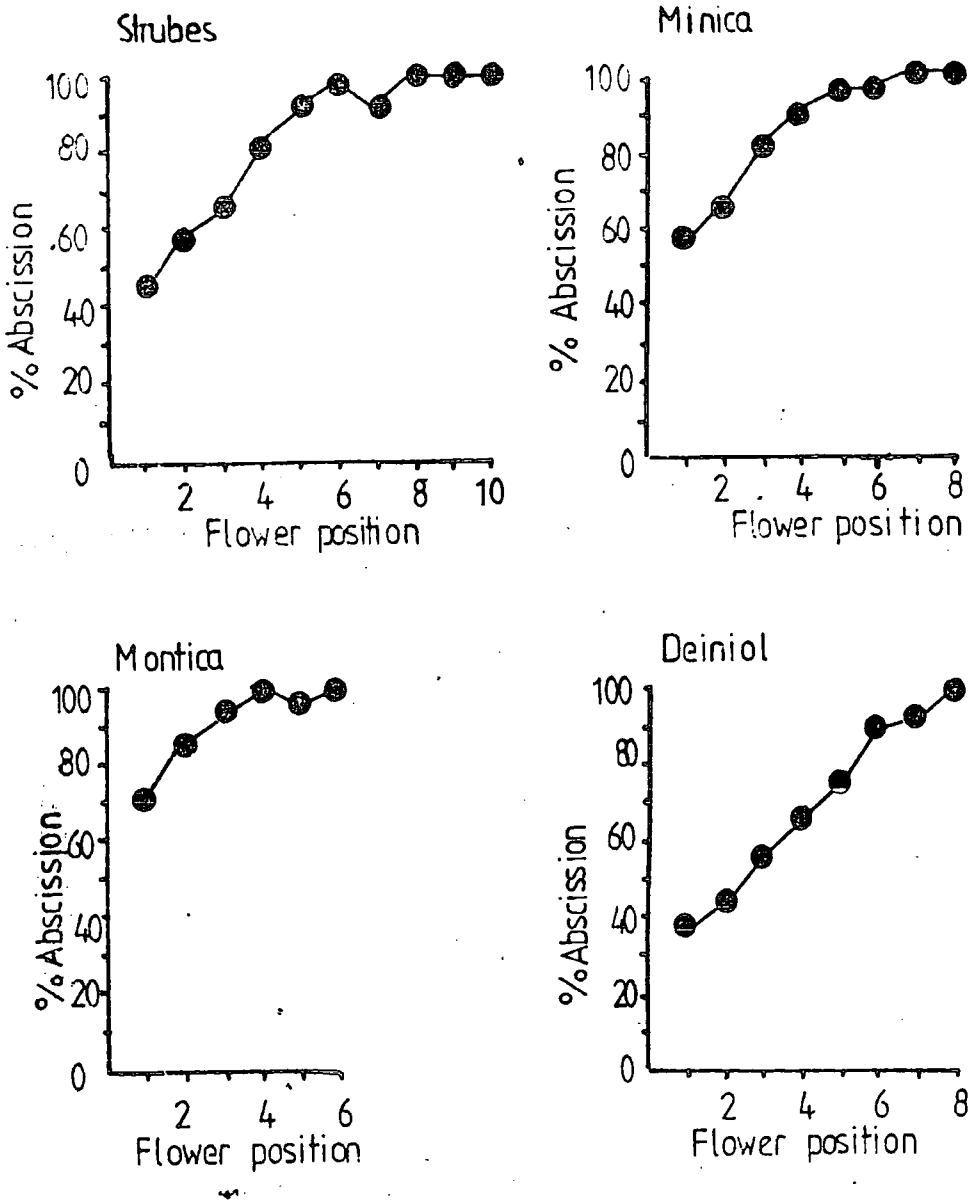



Figure 3.3.2: Flower abscission for varieties in the EEC trial 1982. Each value is an overall percentage.

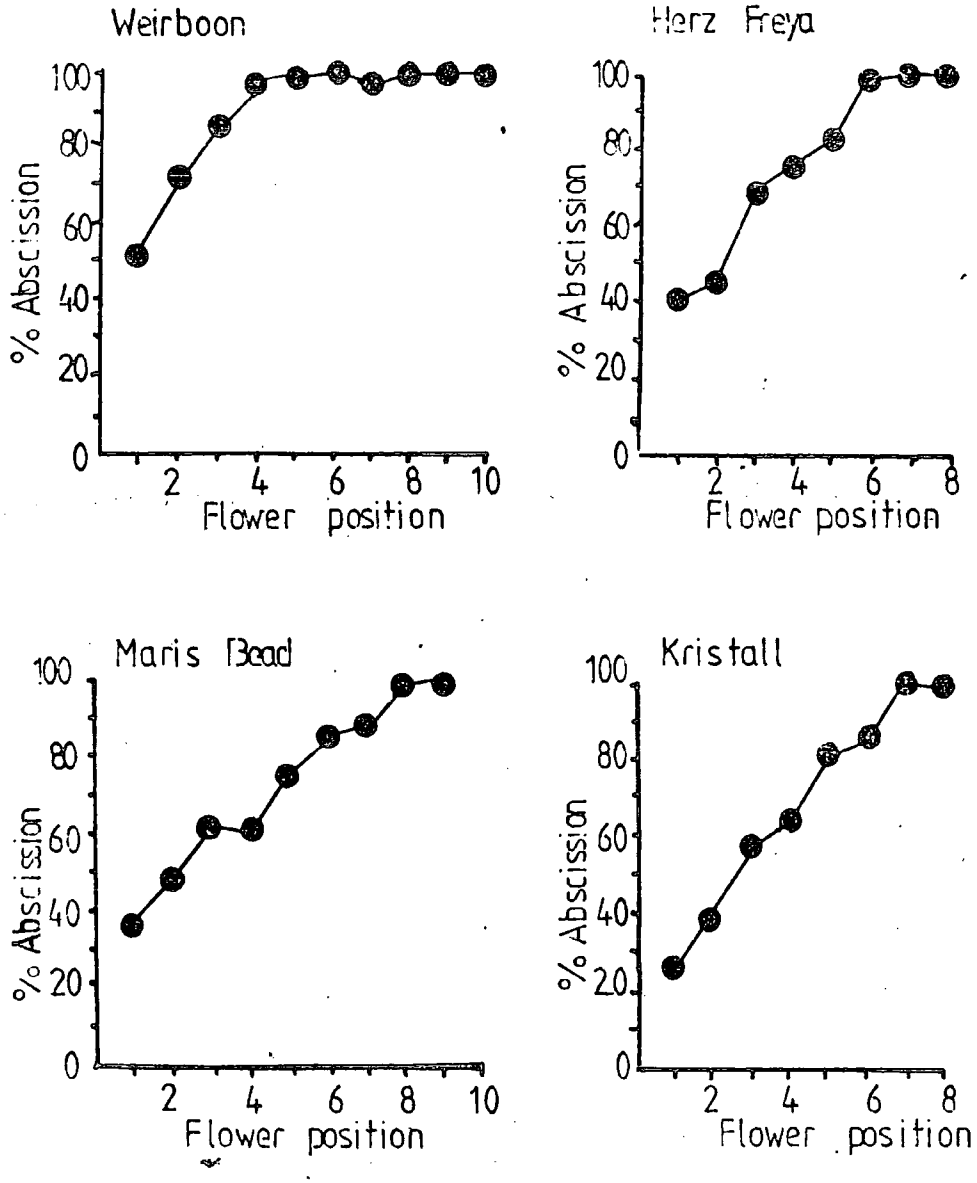



Figure 3.3.1: Flower abscission for varieties in EEC trial 1982. Each value is an overall percentage.

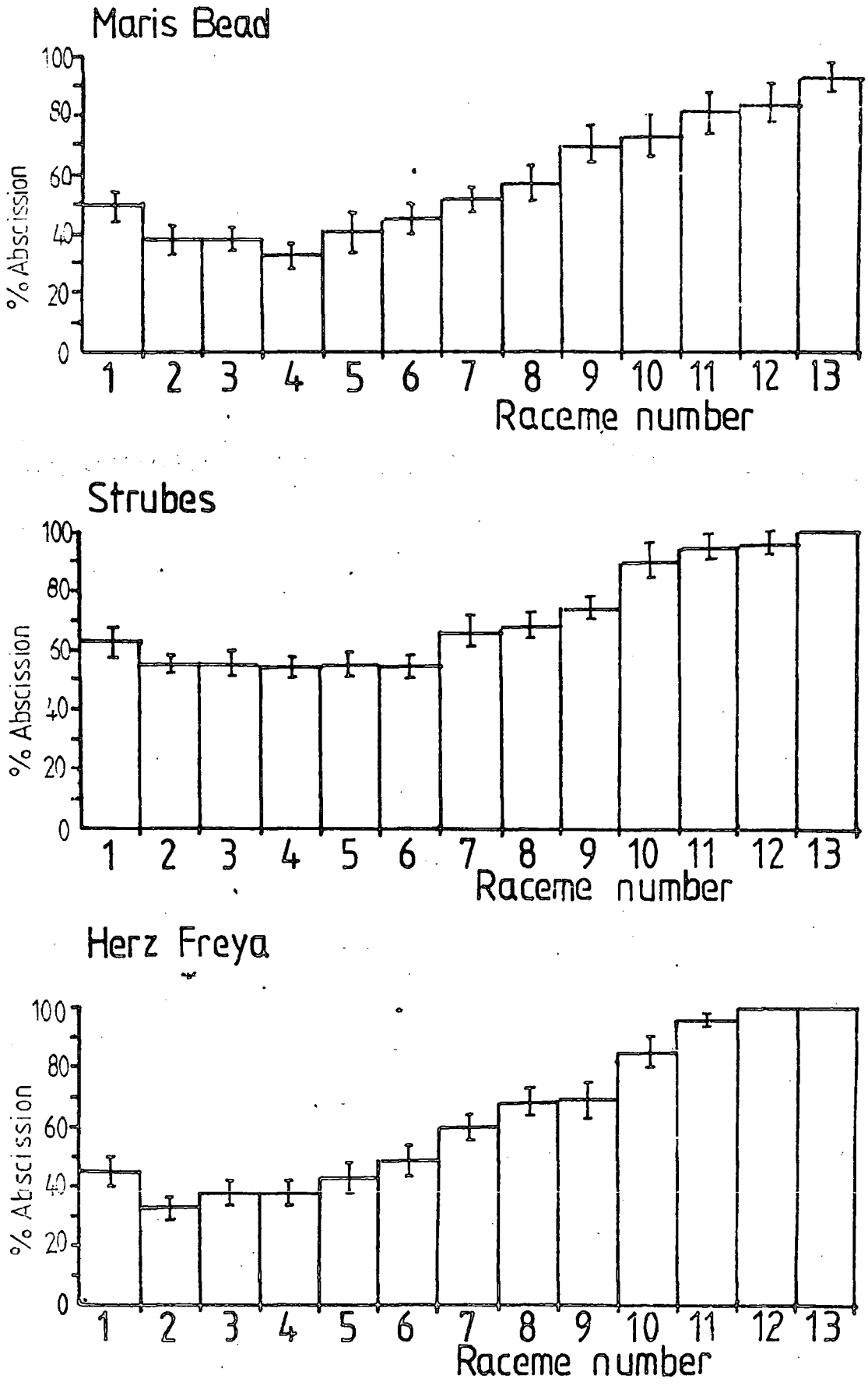



Figure 3.4.1: Flower abscission for varieties in the EEC trial 1981. Each value is an average percentage, standard errors are represented by a bar.

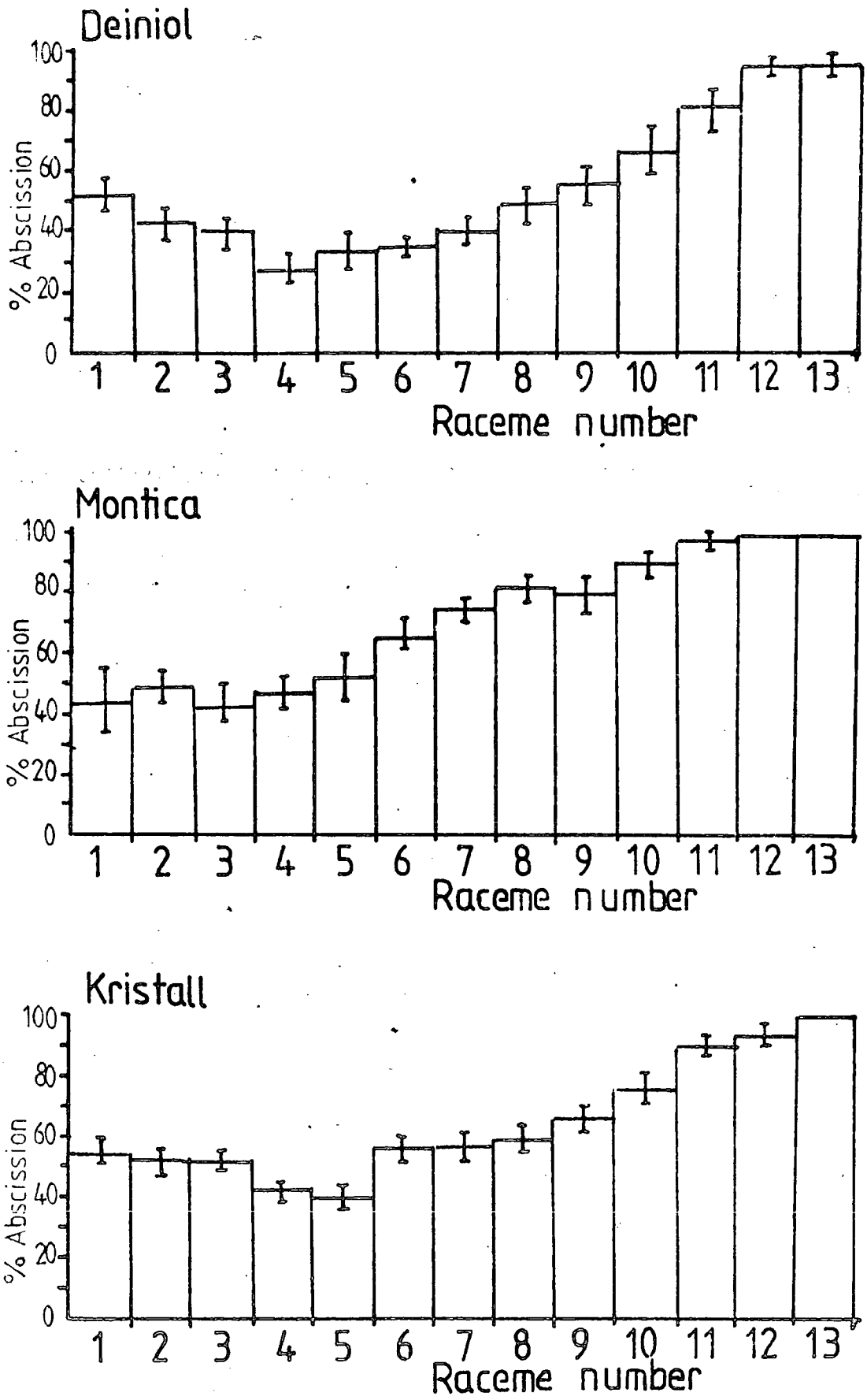



Figure 3.4.2: Flower abscission for varieties in the EEC trial, 1981. Each value is an average percentage, standard errors are represented by a bar.

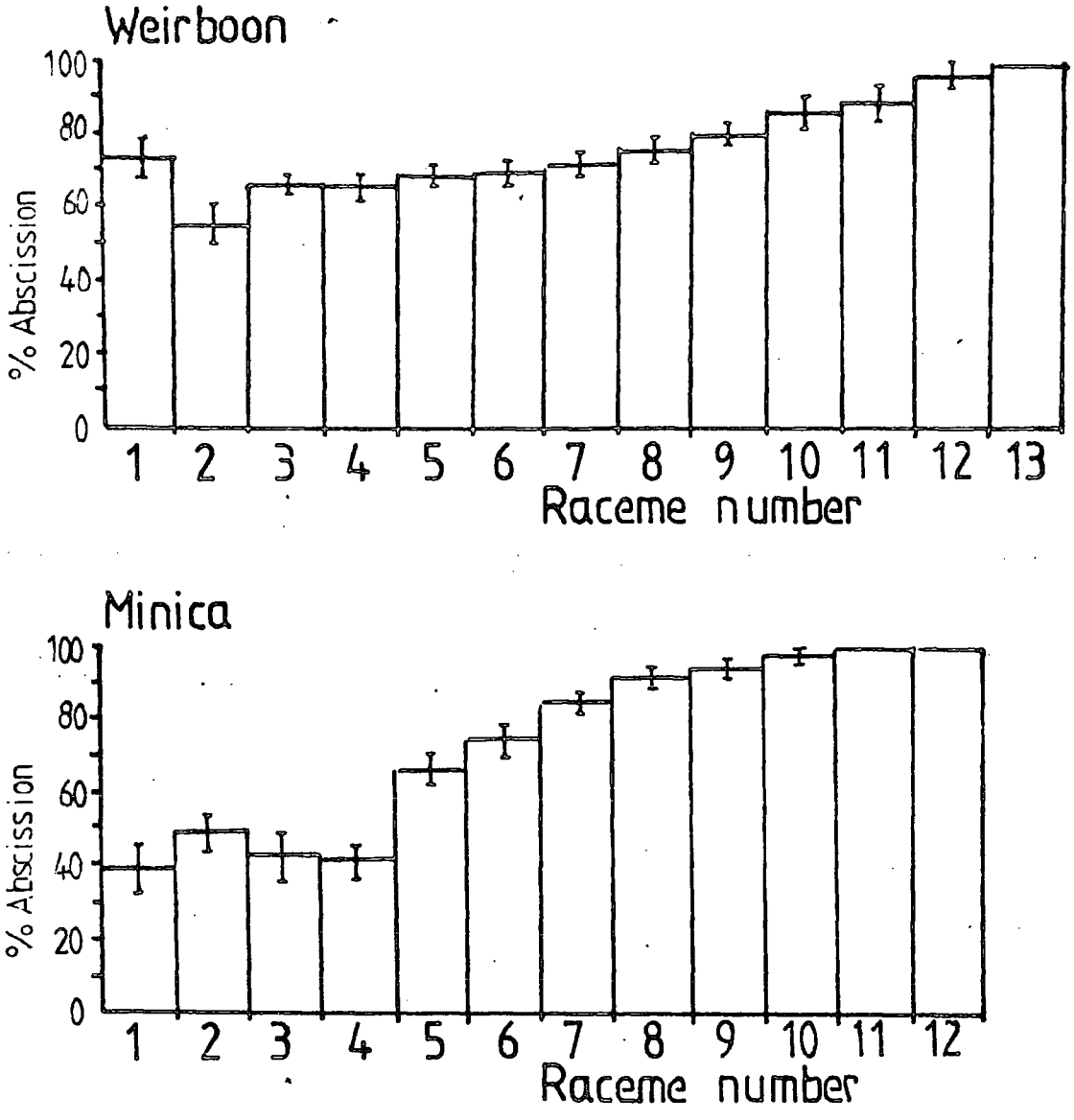



Figure 3.4.3: Flower abscission for varieties in the EEC trial, 1981. Each value is an average percentage, standard errors are represented by a bar.

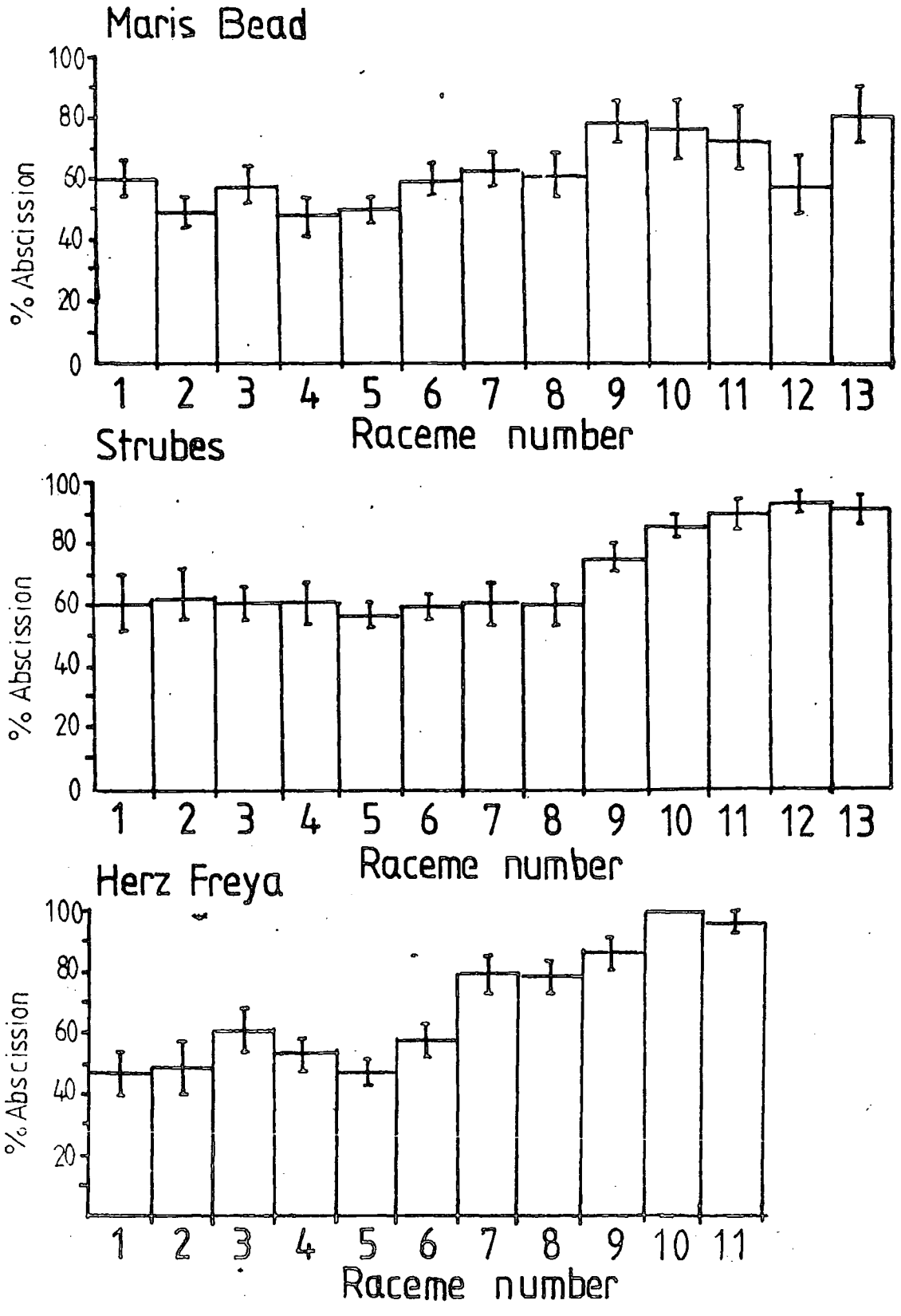



Figure 3.5.1: Flower abscission for varieties in the EEC trial, 1982. Each value is an average percentage, standard errors are represented by a bar.

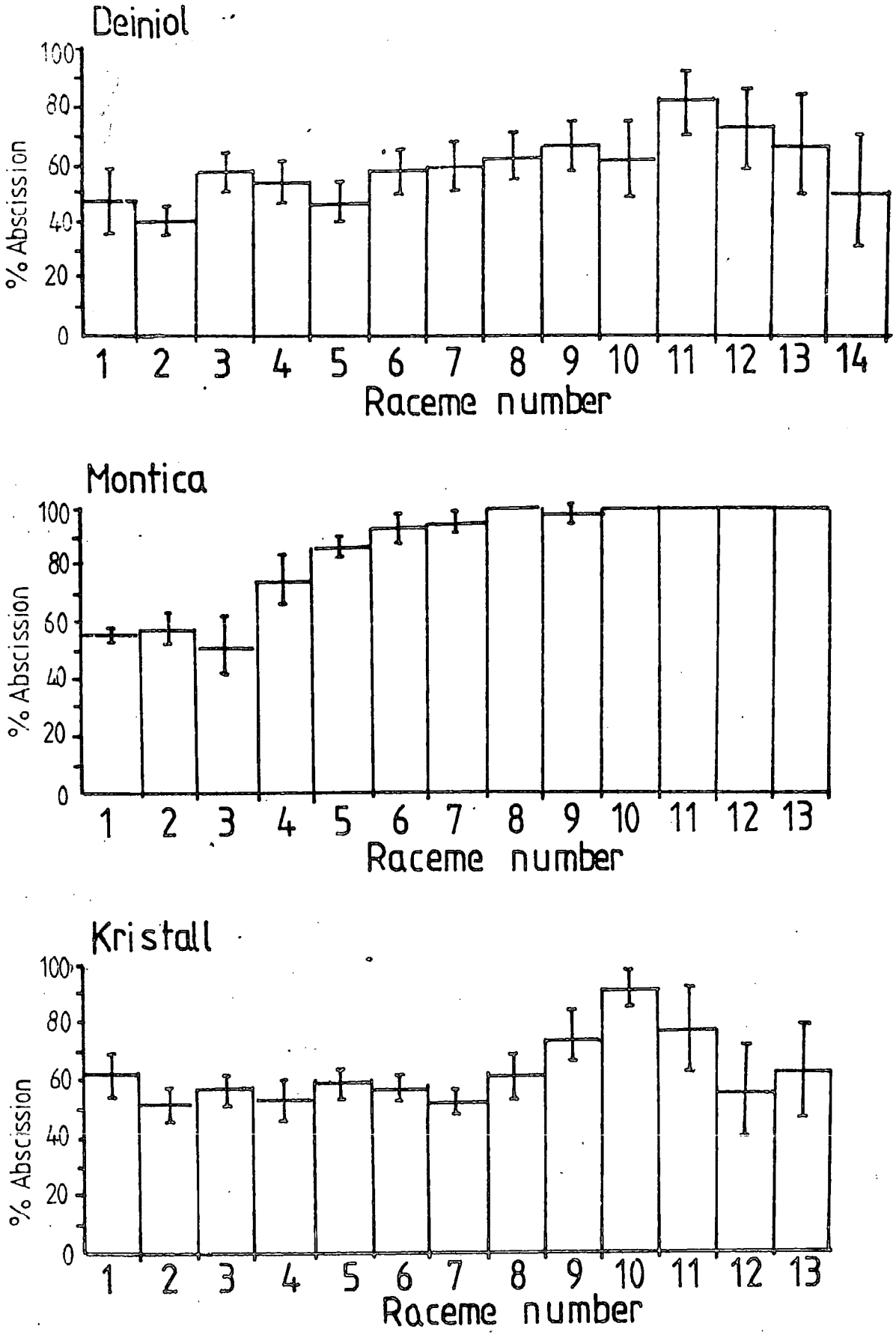



Figure 3.5.2: Flower abscission for varieties in the EEC trial, 1982. Each value is an average percentage, standard errors are represented by a bar.

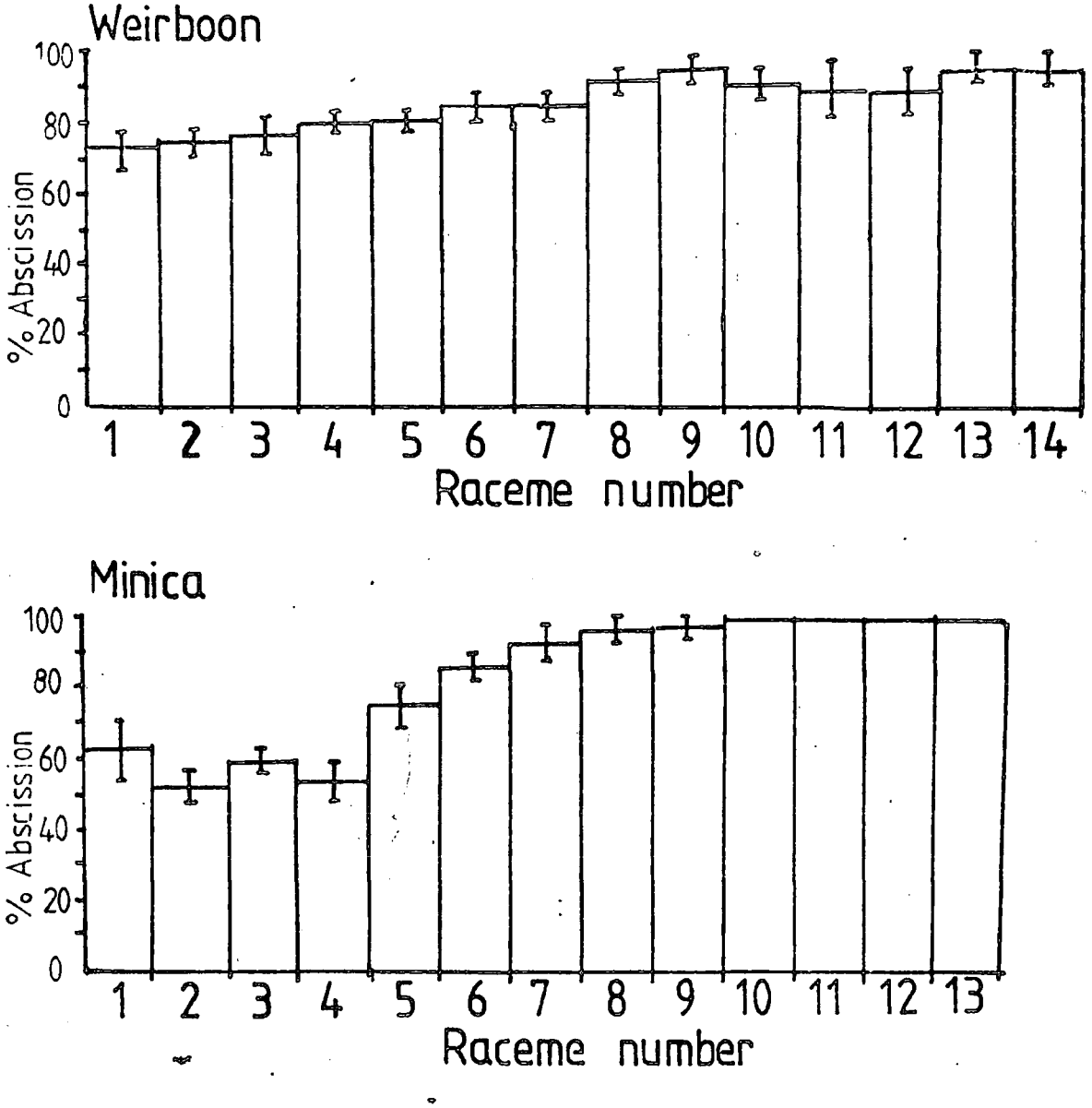



Figure 3.5.3: Flower abscission for varieties in the EEC trial, 1982. Each value is an average percentage, standard errors are represented by a bar.

cultivars and this may be due to genetic influence over flower abscission. Analysis of the yield components of these varieties (Table 3.2, 3.3) indicates that the number of pods reaching maturity is a function of seed weight. The varieties with the heaviest seed weight retain a lower number of mature pods in comparison to those with lower seed weights.

Environmental stress experiments

The influence of irrigation on flower drop

Irrigation had a most pronounced effect on growth of field beans. The indeterminate genotypes were on average 41 cm taller than equivalent non-irrigated plants; the determinate genotype, TI Col. was 23 cm taller (Table 3.5). Irrigated plants showed a greater amount of vegetative growth and their canopy cover was more dense so shading the lowest flowering nodes. They also continued flowering longer than the non-irrigated plants, in which flowering was confined to approximately 12 flowering nodes. Pod set took place approximately one week later than the non-irrigated plants, although flowering was not significantly delayed by irrigation (Figure 3.6).

Irrigated plants of all genotypes showed a significant increase in flower abscission, at each raceme (Figure 3.7.1, 3.7.2; Table 3.4). This followed a similar pattern to that observed previously in that the least abscission occurred on proximal flower positions and highest on distal positions. In comparison to non-irrigated plants much more flower drop occurred on lower and middle raceme positions. This might be expected, however, because abscission was greatest on distal positions of those plants not subjected to stress. Irrigation

Figure 3.6: Comparison of two plants of cv Maris Bead to show the effect of irrigation.

NI = Control, not irrigated;
I = Irrigated.


NI
I
Maris Bead

Table 3.2 Mean yield components of EEC joint field bean trial, PBI, Cambridge, 1980.

| Variety | Plant length (m) | Plant density/ $m^2$ | Pods/ plant | Seeds/ plant | Dry seed weight/ plant (g) | 1000 seed weight/g |
|---|---|---|---|---|---|---|
| Maris Bead | 1.118 | 20 | 15.4 | 50.5 | 18.2 | 405.4 |
| Deiniol | 1.116 | 20 | 13.1 | 47.6 | 17.2 | 406.4 |
| Kristall | 1.281 | 20 | 15.9 | 46.9 | 17.8 | 427.2 |
| Herz Freya | 1.136 | 20 | 18.3 | 39.7 | 14.5 | 408.2 |
| Strubes | 1.143 | 20 | 13.4 | 36.5 | 20.7 | 640.3 |
| Minica | 0.743 | 20 | 10.6 | 31.2 | 22.5 | 812.8 |
| Weirboon | 1.073 | 20 | 12.1 | 26.4 | 25.4 | 1083.9 |
| Montica | 0.578 | 20 | 8.6 | 22.0 | 15.1 | 779.8 |

Table 3.3 Mean yield components of EEC joint field bean trial, PBI, Cambridge, 1981

| Variety | Plant length (m) | Plant density/ $m^2$ | Pods/ plant | Seeds/ plant | Dry seed weight/ plant (g) | 1000 seed weight/g |
|---|---|---|---|---|---|---|
| Maris Bead | 1.174 | 20 | 21.9 | 62.3 | 19.4 | 311.4 |
| Deiniol | 1.102 | 20 | 19.0 | 55.4 | 19.6 | 353.8 |
| Kristall | 0.736 | 20 | 16.9 | 52.2 | 17.4 | 333.3 |
| Herz Freya | 1.188 | 20 | 19.7 | 59.0 | 17.8 | 301.7 |
| Strubes | 1.132 | 20 | 14.3 | 41.3 | 22.5 | 544.8 |
| Minica | 0.964 | 20 | 6.5 | 22.5 | 19.8 | 878.8 |
| Weirboon | 1.161 | 20 | 8.3 | 20.1 | 29.3 | 990.1 |
| Montica | 0.736 | 20 | 5.8 | 18.7 | 15.6 | 832.8 |

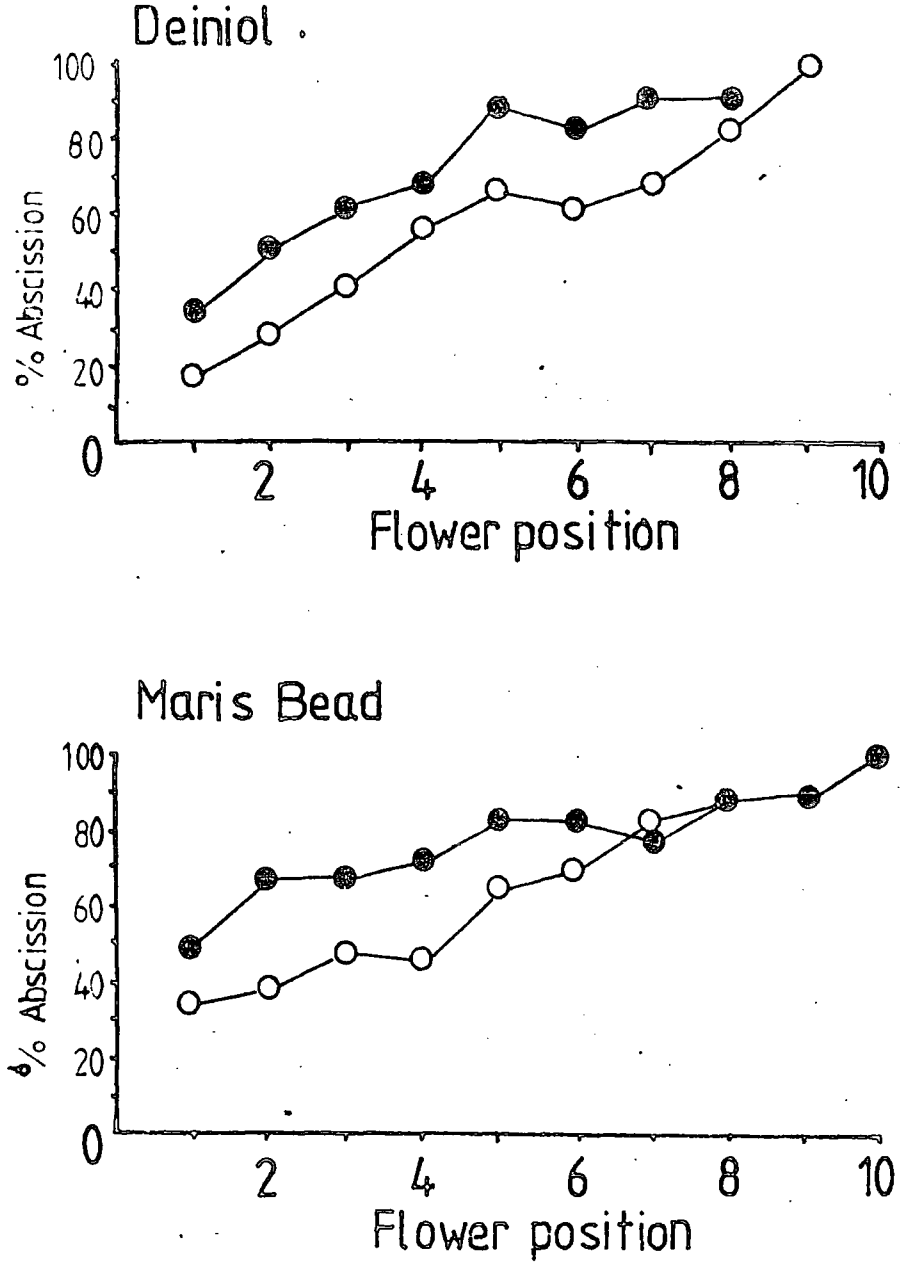



Figure 3.7.2: Influence of irrigation on flower abscission. Each value is an overall percentage,

o = unirrigated
o = irrigated plants.

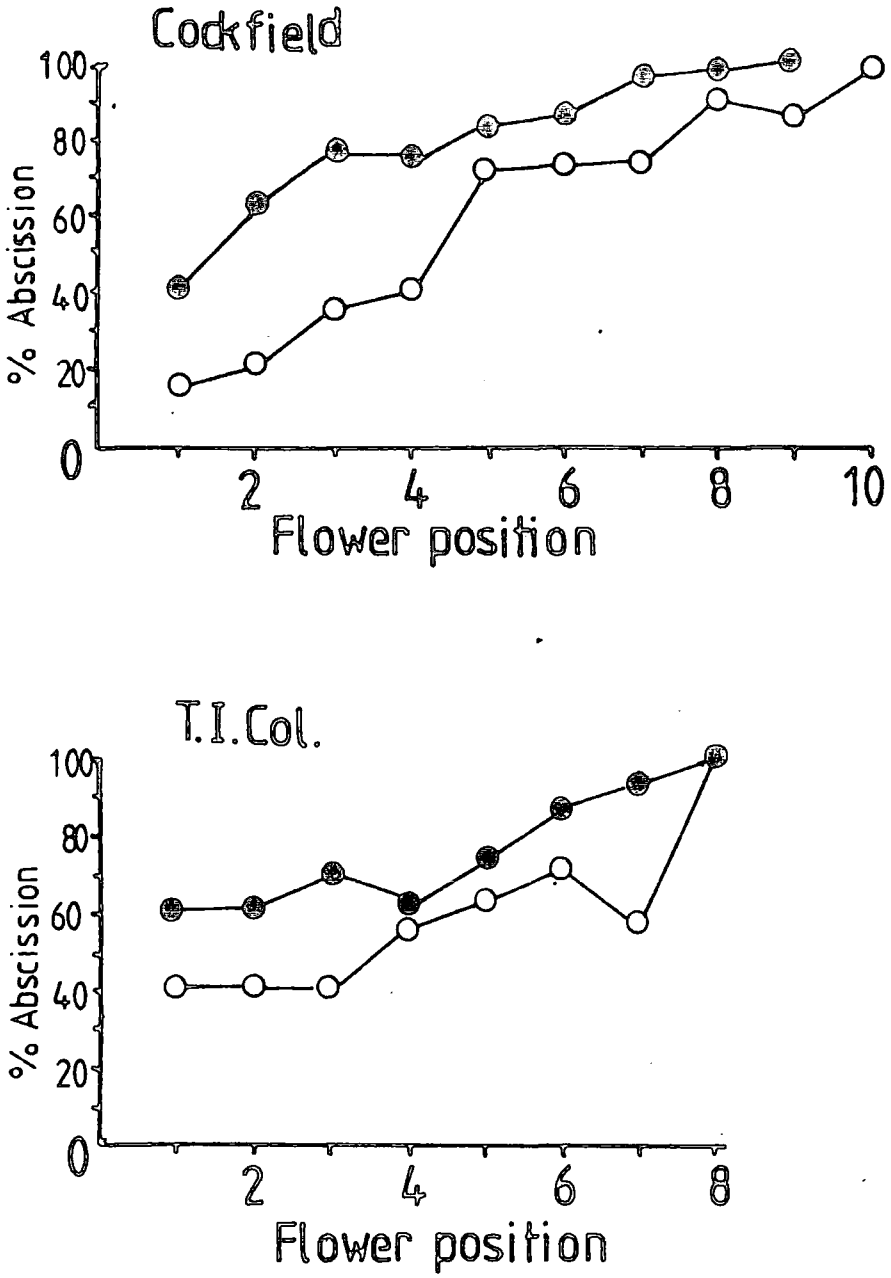



Figure 3.7.2: Influence of irrigation on flower abscission. Each value is an overall percentage,

o = unirrigated
o = irrigated plants.

Table 3.4 Comparison of percentage flower abscission of plants sampled from irrigated and non-irrigated plots

| Variety | Average Flower Abscission | | $\chi^2$, v = 1 |
|---|---|---|---|
| | Irrigated | Non-irrigated | |
| Maris Bead | 70.6 | 50.8 | 37.60 (P> 0.001) |
| Cockfield | 76.1 | 51.9 | 73.80 (P> 0.001) |
| Deiniol | 67.7 | 44.8 | 48.00 (P> 0.001) |
| TI Col. | 67.6 | 48.7 | 43.70 (P> 0.001) |

Table 3.5 Mean Height (cm) of plants in irrigation experiment

| Variety | Irrigated | Non-irrigated |
|---|---|---|
| Maris Bead | 170.8 | 114.1 |
| Cockfield | 157.5 | 117.5 |
| Deiniol | 143.3 | 115.8 |
| TI Col. | 86.6 | 63.3 |

Table 3.6 Total yield (in grams) of plants subjected to irrigation

| Variety | Irrigated | Non-irrigated |
|---|---|---|
| Maris Bead | 995 | 1865.0 |
| Cockfield | 1020 | 1750.0 |
| Deiniol | 435 | 945.0 |
| TI Col. | 916 | 1860.5 |

Each value is the mean of replicate plots.

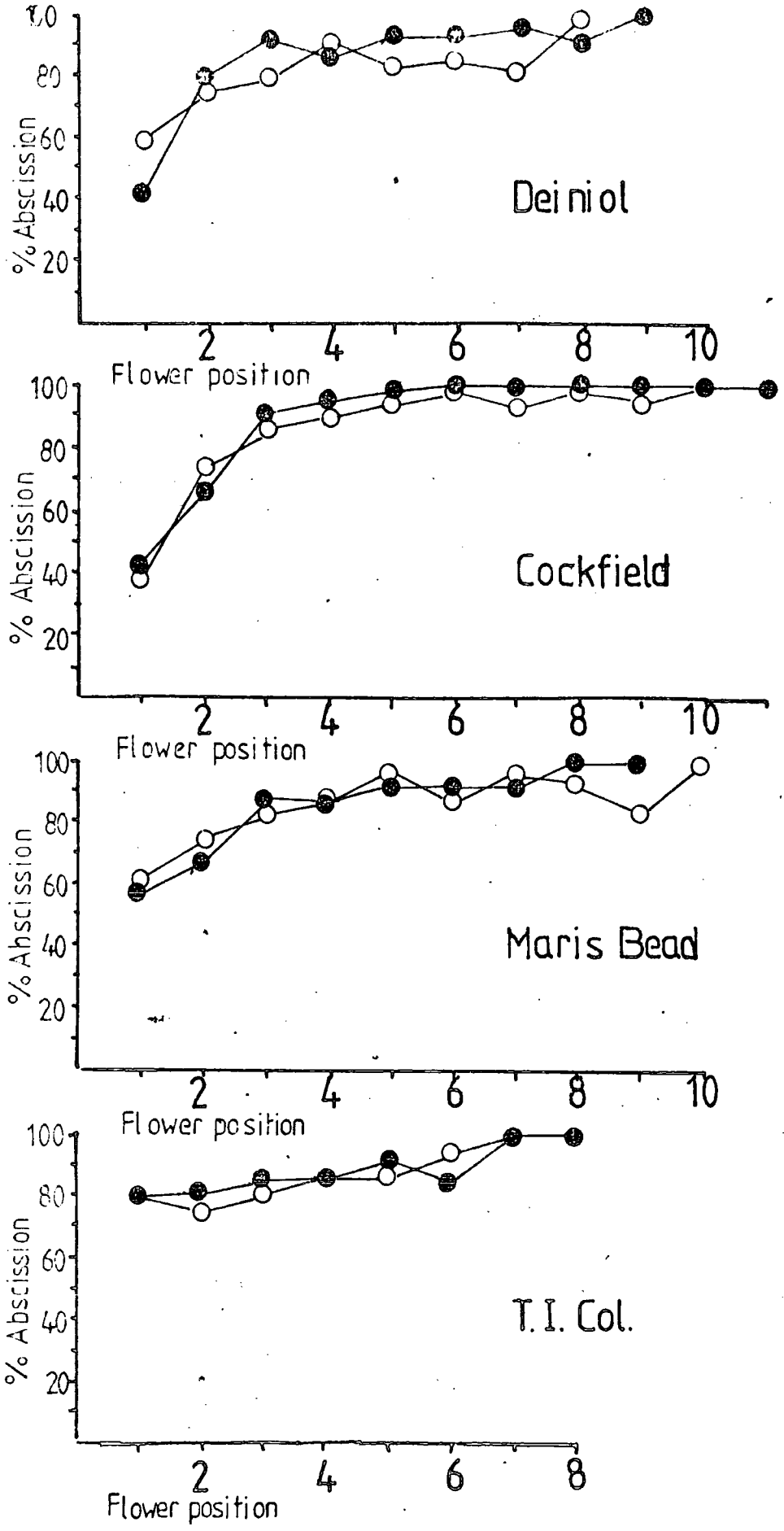



Figure 3.8: Influence of over-watering on flower abscission. Each value is an overall percentage.
● = treated plants; o = control plants.

depressed the final yield of all four cultivars to 52% of that of the unirrigated plants (Table 3.6).

The influence of overwatering on flower drop

Overwatering resulted in a slight increase in mean flower abscission within each raceme in all cultivars tested, that is except for Maris Bead plants (Figure 3.8). Only for Cockfield plants, however, was the increase significant (Table 3.7). This experiment illustrates the different response to waterlogging of pot bound plants to those grown in the field and subjected to irrigation. As the compost was waterlogged over the whole flowering period, it might be supposed that faba bean plants are quite tolerant to excess water when roots are so confined.

Water deficit and flower drop

In all cases the occurrence of water stress increased flower abscission within each raceme (Figure 3.9). This effect was more pronounced when applied to flowers at an earlier stage of flower development (Table 3.8). These results may indicate that plants are more sensitive to water deficit before they reach anthesis; at later developmental stages flowers appear able to cope better with the stress.

Influence of shading at different periods during flowering on flower abscission

Shading affected the growth of plants differently according to the stage of flowering at which the shading was applied. In all cases etiolation of the haulms was observed and resulted in very weak stems for treatments (a) and (b). The mean height of these plants, after the screens had been removed, did not vary significantly from that of control plants.

Table 3.7 Summary of results for overwatering experiment

| Variety and treatment | Percentage overall flower abscission | Percentage overall pods set | $\chi^2$, v = 1 |
|---|---|---|---|
| Deiniol | | | |
| Overwatered | 81.7 | 18.3 | 1.39 P< 0.1 |
| Control | 79.1 | 20.9 | |
| Cockfield | | | |
| Overwatered | 88.7 | 11.3 | 3.22 P> 0.1 |
| Control | 85.6 | 14.4 | |
| Maris Bead | | | |
| Overwatered | 83.1 | 16.9 | 0.17 P< 0.1 |
| Control | 84.2 | 15.8 | |
| TI Col. | | | |
| Overwatered | 83.9 | 16.1 | 0.56 P< 0.1 |
| Control | 82.1 | 17.9 | |

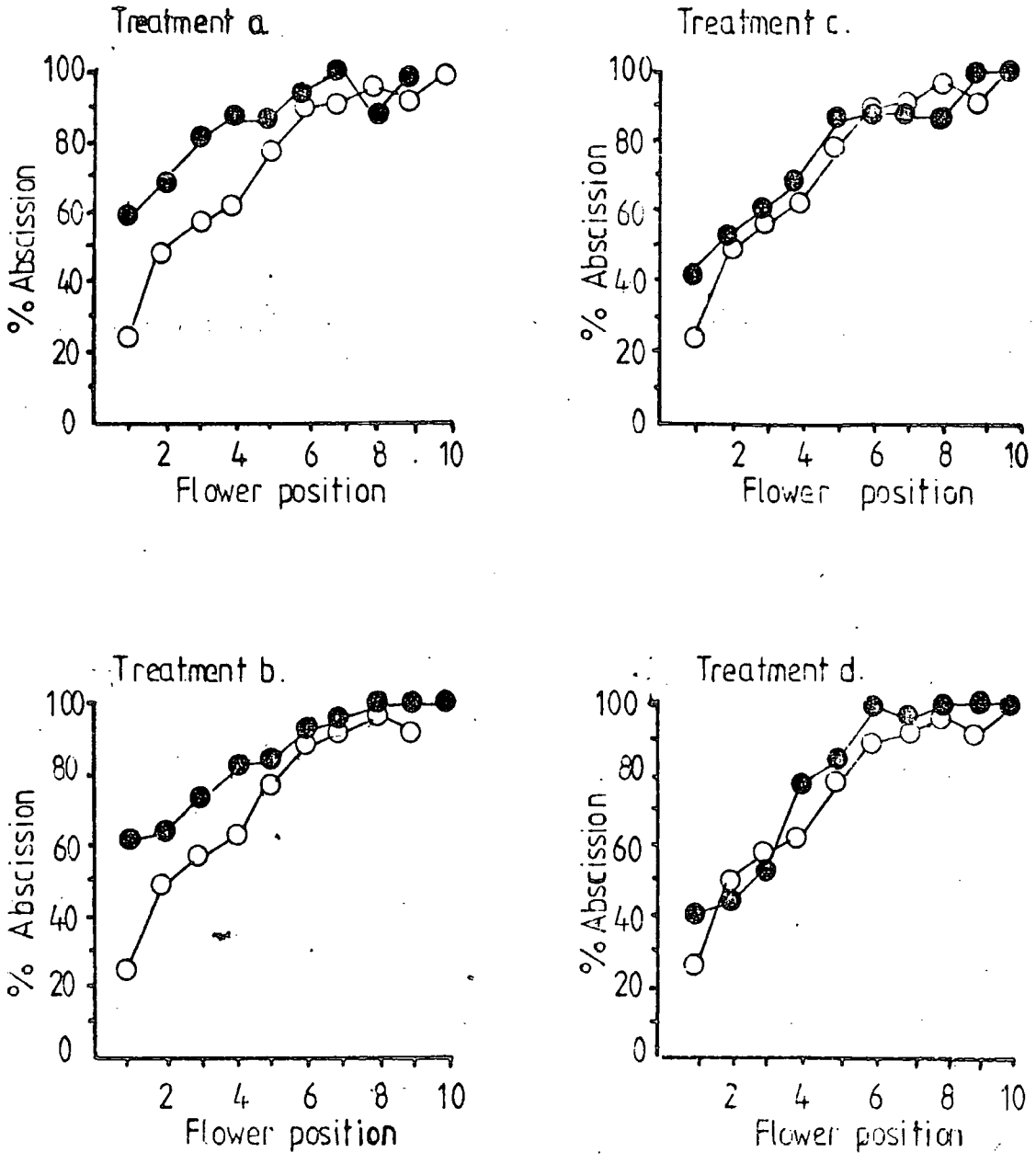



Figure 3.9: Influence of water stress on flower drop in variety Maris Bead. Each value is an overall percentage; ⊖ = treated plants; o = control plants.

Treatment (a) = first raceme, all flowers at stage 1

(b) = first raceme, all flowers at stage 5-6

(c) = first raceme, all flowers at stage 8-9

(d) = first two racemes, all flowers at stage 8-9.

Table 3.8 Summary of results for Maris Bead plants subjected to water deficit

| Treatment | Total % flowers dropped | Total % pods set | $\chi^2$, v = 1 |
|---|---|---|---|
| Control | 66.8 | 33.1 | |
| (a) | 81.2 | 18.8 | 14.28 (P > 0.001) |
| (b) | 79.2 | 20.8 | 13.10 (P > 0.001) |
| (c) | 72.2 | 27.8 | 7.73 (P > 0.01) |
| (d) | 72.4 | 27.6 | 1.56 (P < 0.1) |

Maris Bead and Cockfield subjected to treatment (c) were, on average, 22 cm and 26 cm taller than the control plants (Table 3.9). The determinate genotype, TI Col. did not vary significantly in height in control and other treatments.

Shading at treatment (a) slowed down the initiation of flowering racemes, but promoted bud abortion where initials had already formed. Once the screens were removed, flowering resumed and continued for approximately two weeks after plants in the control plots had set pods (Tables 3.10.1, 3.10.2, 3.11.1, 3.11.2). The indeterminate genotypes in treatment (b) had fewer flowering nodes than those of the control and other treatments (Table 3.12).

Treatments (b) and (c) applied to the indeterminate genotypes resulted in greater flower drop at all positions compared to control plants (Figures 3.10.1, 3.10.2). The determinate genotype (Figure 3.10.3) showed greater flower drop for all treatments. In all cases treatment (c) increased abscission to the greatest extent.

Shading resulted in higher flower drop on most inflorescences (Figures 3.11.1, 3.11.2, 3.11.3), for indeterminate varieties subjected to shading treatments (b) and (c). whereas the same varieties subjected to shading treatment (a) experienced a decrease in abscission at higher inflorescences, which was compensated for, however, by increased bud abortion (Table 3.11.2). For TI Col. abscission on every raceme was higher for treatments (a) and (c); treatment (b) had no significant effect of abscission.

Effect of cold shock on flower abscission

Cold shock differentially affected abscission according to the flowering stage and the cultivar tested (Table 3.14).

Table 3.9 Height of plants subjected to shading

| Variety and Treatment | Height (cm) |
|---|---|
| Maris Bead | |
| (a) | 101.2 (11.6) |
| (b) | 105.2 (14.1) |
| (c) | 134.2 (11.3) |
| Control | 112.5 (4.5) |
| Cockfield | |
| (a) | 110.5 (14.6) |
| (b) | 123.3 (7.9) |
| (c) | 148.3 (3.7) |
| Control | 121.8 (4.5) |
| TI Col. | |
| (a) | 93.7 (2.8) |
| (b) | 90.2 (7.7) |
| (c) | 97.3 (9.0) |
| Control | 85.3 (5.8) |

Each value is the average of three plants chosen at random from each subplot (nine plants in total). Standard deviation is in parenthesis.

Shading was applied to plants for two weeks from:

(a) bud initiation (b) first raceme flowers at stage 9

(c) first five inflorescences flowering, the first raceme possessed young pods.

Table 3.10.1 Fate of flowers and buds within racemes of Cockfield plants subjected to shading

| Flower Position | % flower drop | | % bud abortion | | % late flowering | | % pods set | |
|---|---|---|---|---|---|---|---|---|
| | (a) | Control | (a) | Control | (a) | Control | (a) | Control |
| 1 | 17.4 | 11.4 | 15.4 | 0.0 | 16.1 | 0.0 | 51.4 | 88.6 |
| 2 | 31.5 | 23.6 | 21.5 | 0.0 | 14.1 | 0.0 | 32.9 | 76.4 |
| 3 | 32.9 | 33.1 | 24.2 | 0.0 | 10.1 | 0.0 | 32.8 | 66.9 |
| 4 | 37.6 | 49.4 | 24.1 | 0.0 | 7.4 | 0.0 | 30.9 | 50.6 |
| 5 | 50.3 | 73.7 | 28.6 | 0.0 | 6.8 | 0.0 | 14.3 | 26.3 |
| 6 | 52.7 | 80.5 | 28.8 | 0.0 | 8.2 | 0.0 | 10.3 | 19.5 |
| 7 | 59.3 | 86.7 | 27.6 | 0.0 | 8.3 | 0.0 | 4.8 | 13.3 |
| 8 | 60.6 | 93.4 | 27.6 | 0.0 | 6.4 | 0.0 | 5.4 | 6.6 |
| 9 | 58.6 | 98.6 | 32.3 | 0.0 | 6.6 | 0.0 | 2.2 | 1.4 |
| 10 | 64.7 | 100.0 | 35.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Comparison of shading from bud initiation (a) to control plants, where no shading was given at all. All figures are average percentages.

Table 3.10.3 Fate of flowers and buds within racemes of Maris Bead plants subjected to shading

| Flower Position | % flower drop | | % bud abortion | | % late flowering | | % pods set | |
|---|---|---|---|---|---|---|---|---|
| | (a) | Control | (a) | Control | (a) | Control | (a) | Control |
| 1 | 16.4 | 19.0 | 9.6 | 0.0 | 20.6 | 0.0 | 53.4 | 81.0 |
| 2 | 29.4 | 29.1 | 14.4 | 0.0 | 22.0 | 0.0 | 34.2 | 70.9 |
| 3 | 34.2 | 39.3 | 17.1 | 0.0 | 17.1 | 0.0 | 31.6 | 60.7 |
| 4 | 38.3 | 45.5 | 16.4 | 0.0 | 15.8 | 0.0 | 29.5 | 54.5 |
| 5 | 52.9 | 70.4 | 16.6 | 0.0 | 13.8 | 0.0 | 16.7 | 29.5 |
| 6 | 62.9 | 73.7 | 18.5 | 0.0 | 9.7 | 0.0 | 8.9 | 26.3 |
| 7 | 71.0 | 80.0 | 15.8 | 0.0 | 7.9 | 0.0 | 5.3 | 20.0 |
| 8 | 65.2 | 85.7 | 13.0 | 0.0 | 17.4 | 0.0 | 4.4 | 14.3 |
| 9 | 40.0 | 100.0 | 0.0 | 0.0 | 40.0 | 0.0 | 20.0 | 0.0 |

Comparison of shading from bud initiation for two weeks (a) to control plants, where no shading was given at all, all figures are average percentages.

Table 3.11.1 Flowering nodes on which flowers were still in bloom after shading for two weeks from bud initiation (a) compared to control plants

| Node Number | Maris Bead | | Cockfield | | TI Col | |
|---|---|---|---|---|---|---|
| | (a) | Control | (a) | Control | (a) | Control |
| 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 4 | 6.6 (4.1) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5 | 6.0 (3.8) | 0.0 | 3.2 (0.0) | 0.0 | 0.0 | 0.0 |
| 6 | 13.0 (4.1) | 0.0 | 23.0 (6.1) | 0.0 | 0.0 | 0.0 |
| 7 | 11.1 (6.5) | 0.0 | 21.0 (5.8) | 0.0 | - | - |
| 8 | 29.0 (13.2) | 0.0 | 49.1 (7.5) | 0.0 | - | - |
| 9 | 12.4 (7.5) | 0.0 | 35.2 (5.9) | 0.0 | - | - |
| 10 | - | 0.0 | 16.0 (4.9) | 0.0 | - | - |
| 11 | - | 0.0 | 9.0 (6.5) | 0.0 | - | - |
| 12 | - | 0.0 | - | 0.0 | - | - |

Table 3.11.2 Flowering nodes on which flower abortion occurred after shading for two weeks from bud initiation (a), compared to control plants

| Node Number | Maris Bead | | Cockfield | | TI Col. | |
|---|---|---|---|---|---|---|
| | (a) | Control | (a) | Control | (a) | Control |
| 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | 2.4 (0.0) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 4 | 6.6 (4.5) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5 | 12.2 (6.1) | 0.0 | 0.0 | 0.0 | 8.3 (0.0) | 0.0 |
| 6 | 17.0 (7.1) | 0.0 | 0.0 | 0.0 | 30.0 (21.2) | 0.0 |
| 7 | 13.2 (8.2) | 0.0 | 2.2 (0.0) | 0.0 | - | - |
| 8 | 34.0 (7.1) | 0.0 | 12.3 (4.0) | 0.0 | - | - |
| 9 | 34.2 (6.3) | 0.0 | 26.1 (8.0) | 0.0 | - | - |
| 10 | 56.0 (12.0) | 0.0 | 34.1 (8.2) | 0.0 | - | - |
| 11 | - | 0.0 | 57.1 (9.5) | 0.0 | - | - |
| 12 | - | 0.0 | - | 0.0 | - | - |

Figures are average percentages. Standard errors in parentheses.

Table 3.12 Influence of shading on average number of racemes formed

| Variety | Treatment | | | |
|---|---|---|---|---|
| | (a) | (b) | (c) | (d) |
| Maris Bead | 9.7 (1.1) | 7.4 (1.3) | 10.2 (1.2) | 11.2 (1.1) |
| Cockfield | 10.3 (1.4) | 8.4 (1.2) | 10.5 (1.2) | 11.1 (1.1) |
| TI Col. | 5.1 (0.6) | 5.1 (0.7 | 5.0 (0.7 | 5.1 (0.6) |

Shading was applied for a two week period from bud initiation (a), flowers on first raceme at stage 9 (b), pod set on first raceme (c), and control no shading (d). Standard deviation is given in parenthesis.

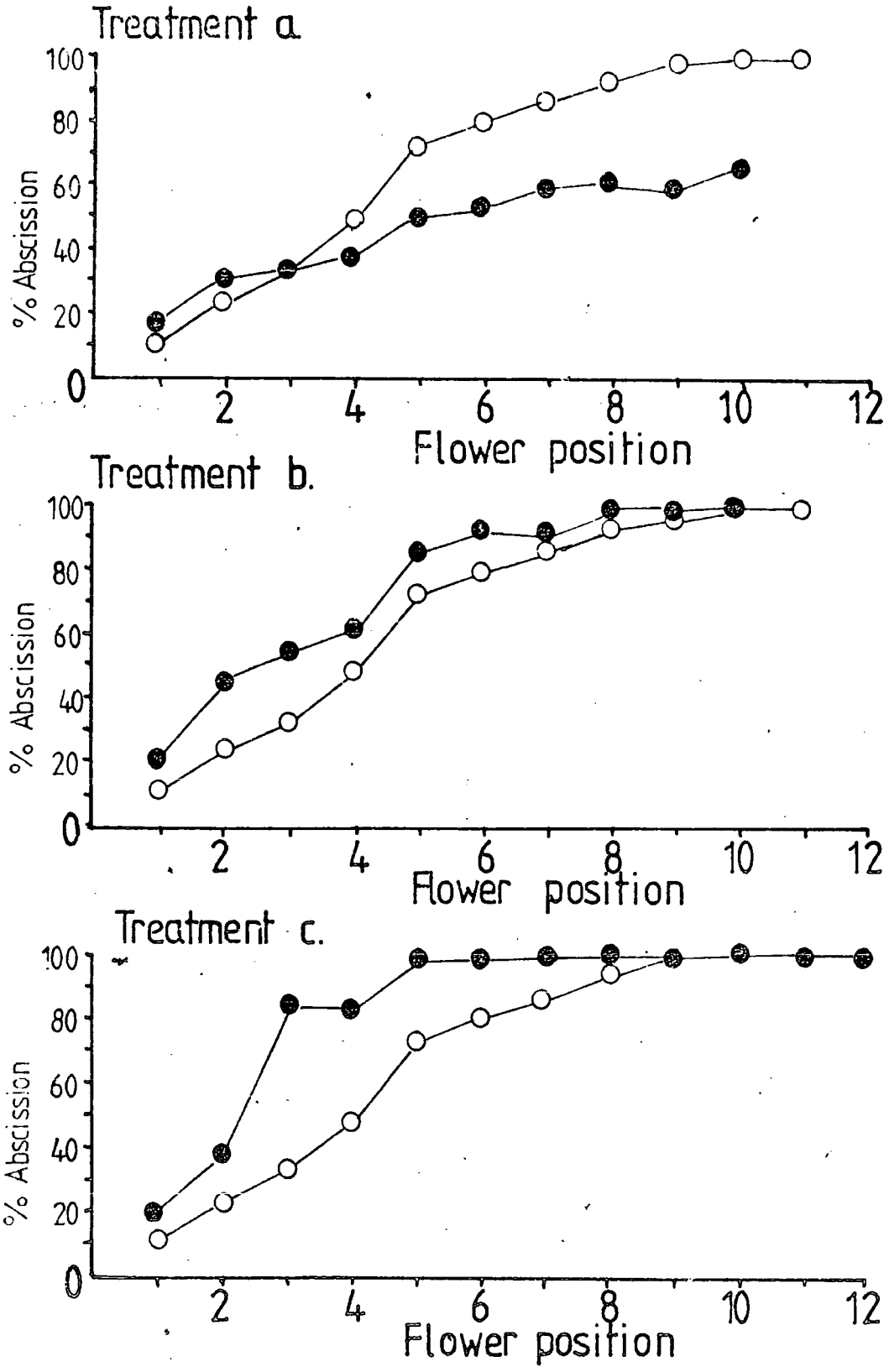



Figure 3.10.1: Effect of shading plants of variety Cockfield on flower abscission. Each value is an overall percentage; ● = treatment; o = control. Shading was applied from:
(a) bud initiation,
(b) first raceme flowers at stage 9,
(c) at least five inflorescences flowering, the first inflorescence possessed pods.

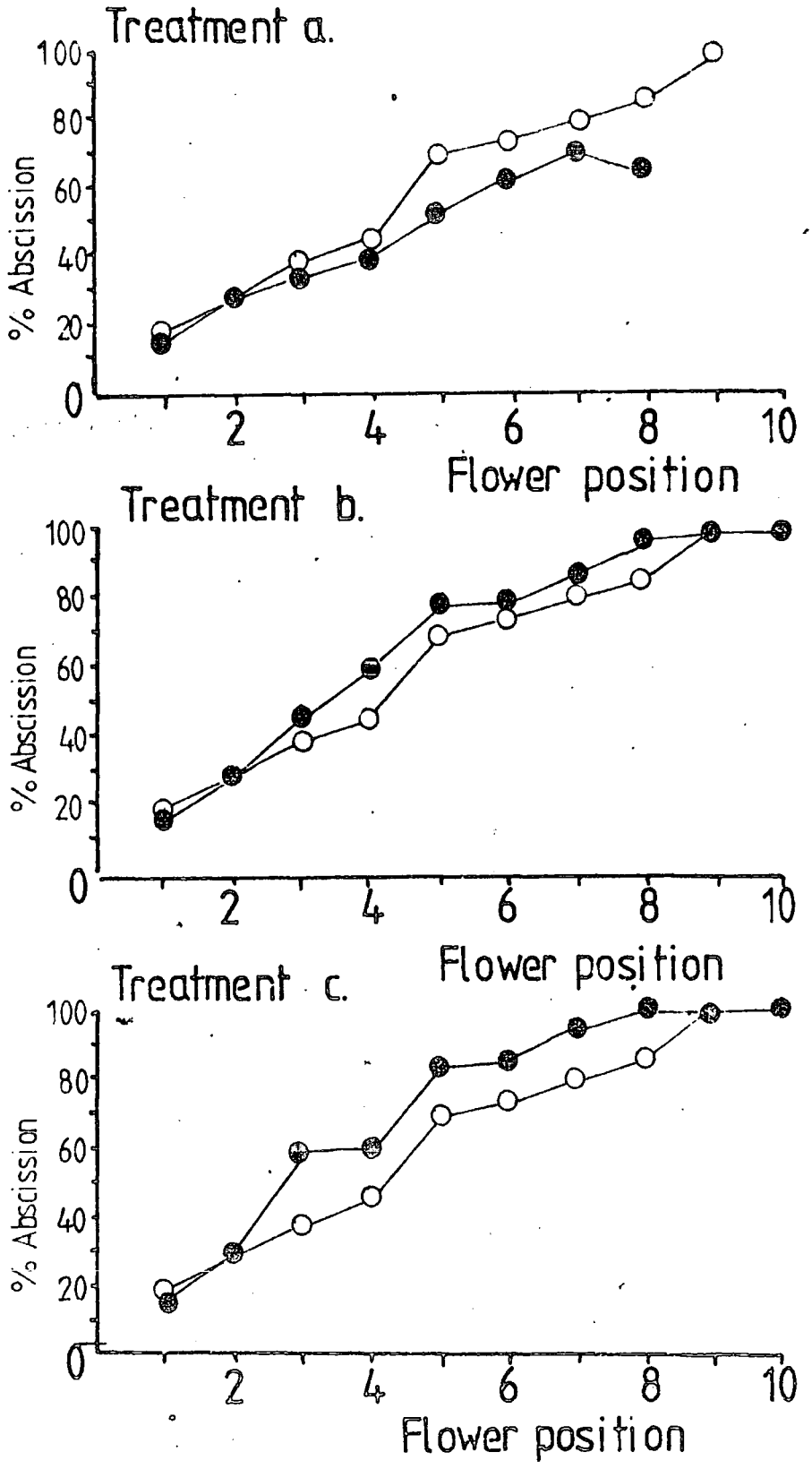



Figure 3.10.2: Effect of shading plants of variety Maris Bead on flower abscission. Each value is an overall percentage; ● = treatment; ○ = control. Shading was applied from:
(a) bud initiation,
(b) first raceme flowers at stage 9,
(c) at least five inflorescences flowering, the first inflorescence possessed pods.

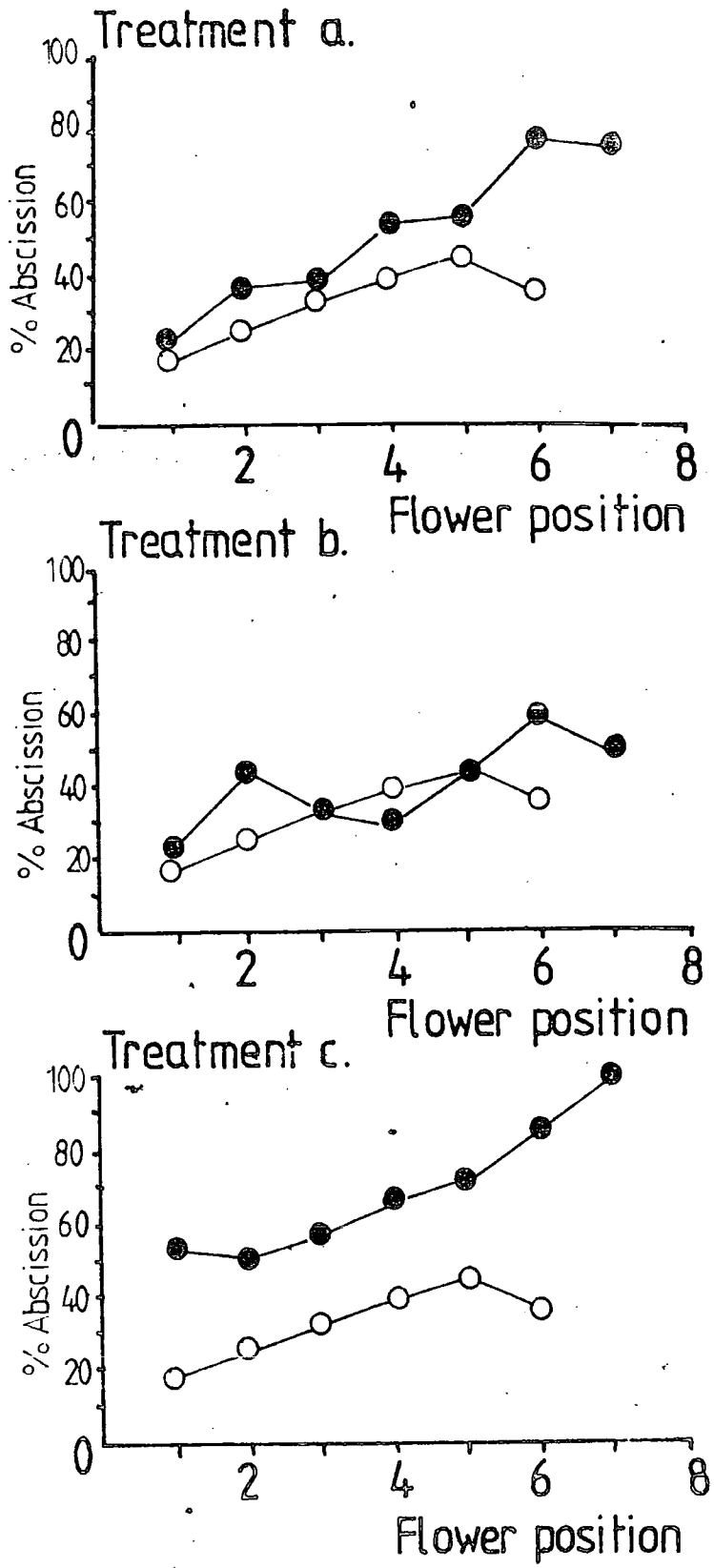



Figure 3.10.3: Effect of shading TI Col. plants on flower abscission. Each value is an overall percentage,
● = treatment
o = control

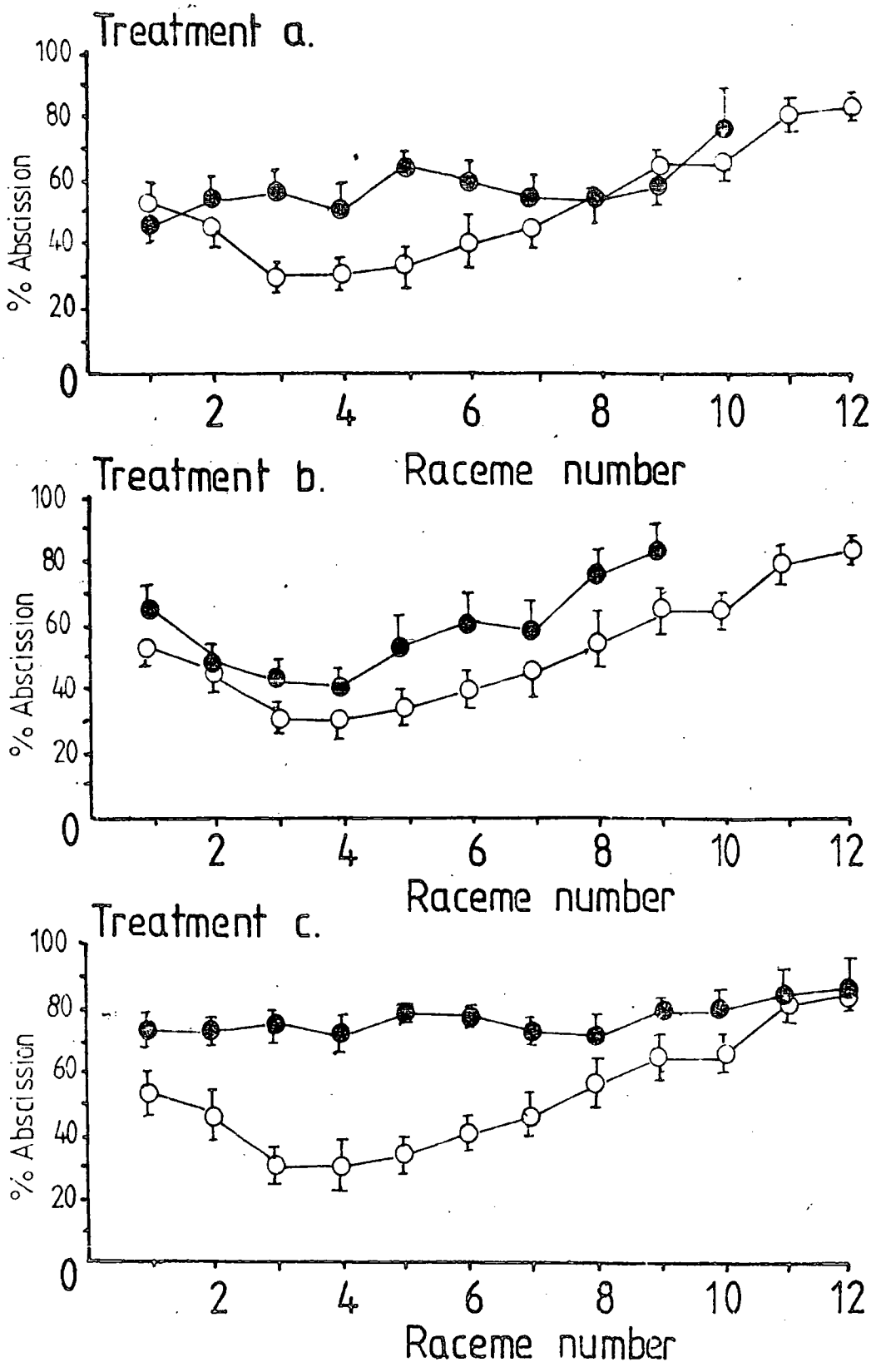



Figure 3.11.1: Influence of shading of Maris Bead plants on flower abscission. Each value is an average percentage,
● = treatment
o = control
standard errors are denoted by a bar.

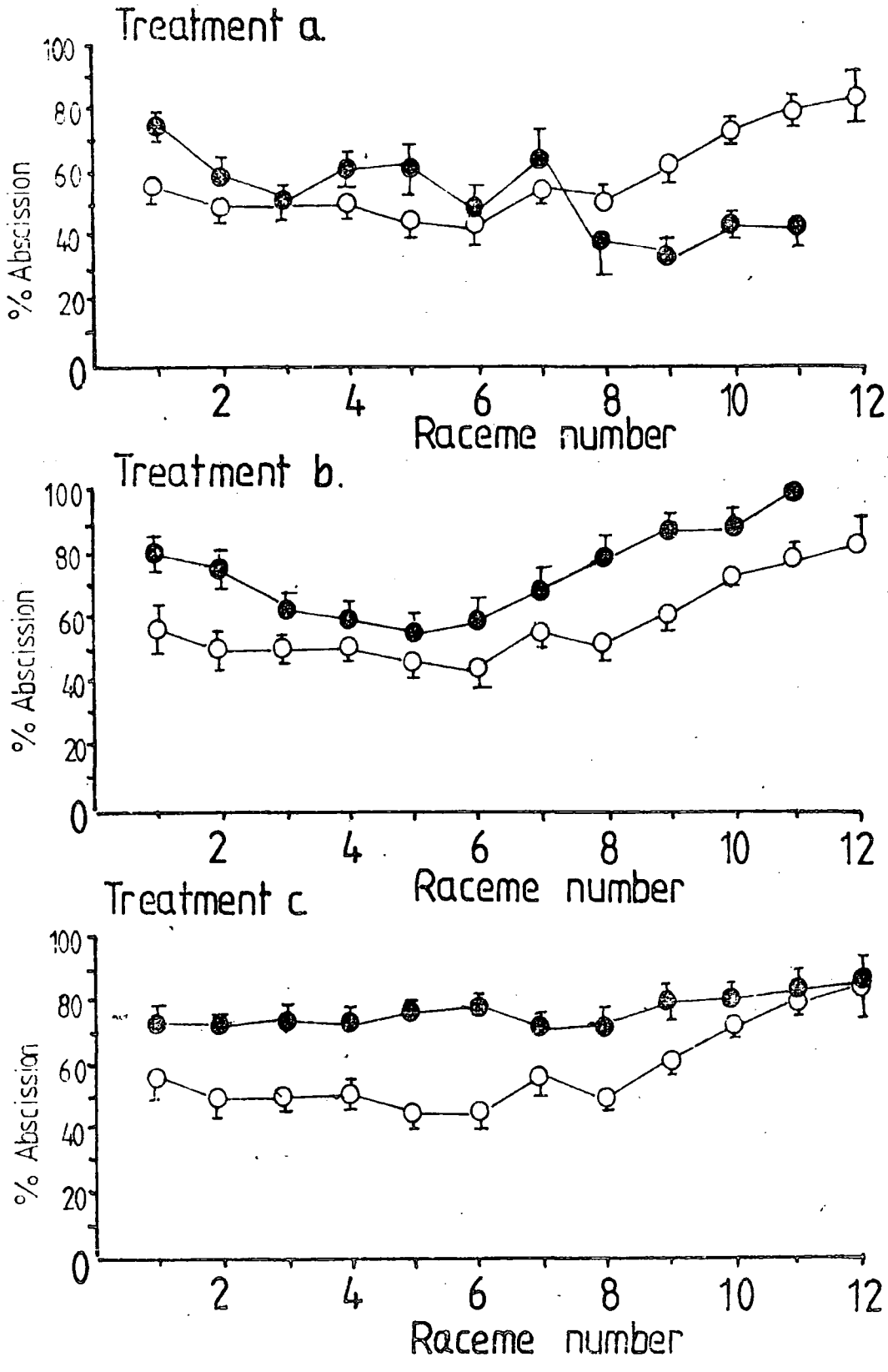



Figure 3.11.2: Influence of shading plants of variety Cockfield on flower abscission. Each value is an average percentage,
● = treatment
o = control
Standard errors are denoted by a bar.

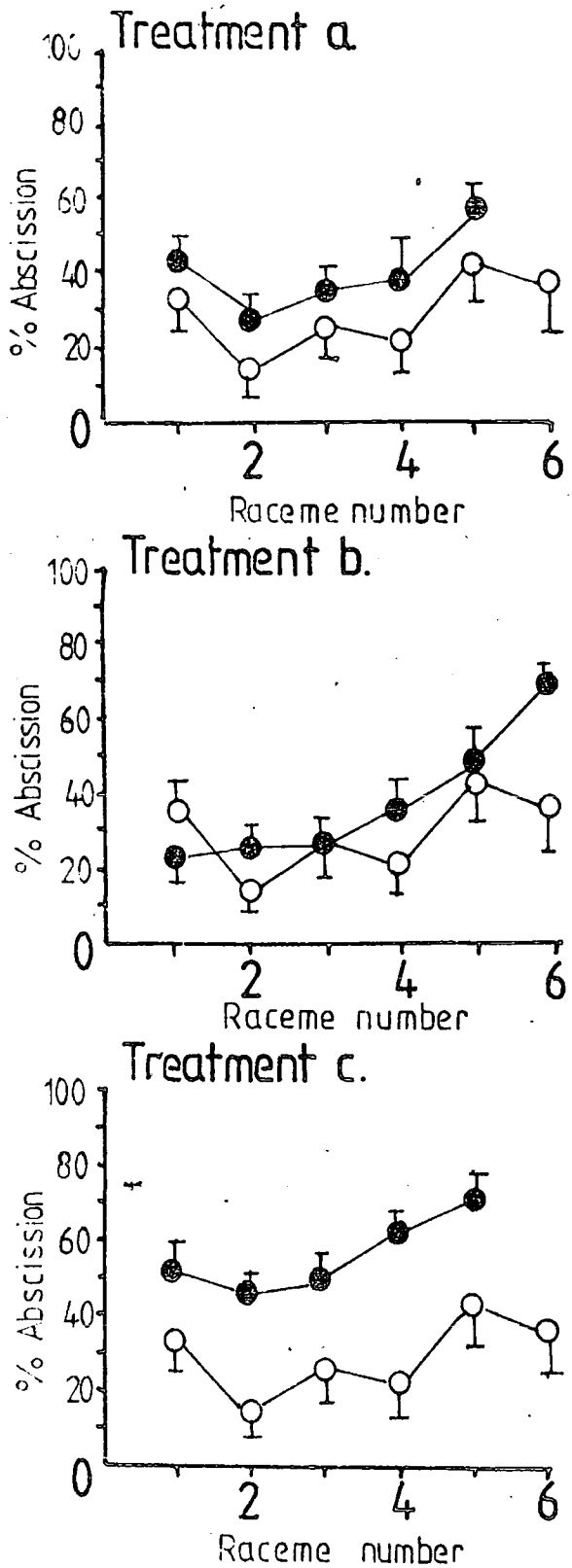



Figure 3.11.3: Influence of shading of plants of TI Col. on flower abscission. Each value is an average percentage,
● = treatment
o = control
Standard errors are denoted by a bar.

Table 3.13 Summary of results and Chi squared analysis for the shading experiment

| Variety and Treatment | Total flowers dropped (%) | Total pods set (%) | $\chi^2$, v = 1 |
|---|---|---|---|
| Maris Bead | | | |
| (a) | 395 (60.6) | 257 (39.4) | 16.48 (P > 0.001) |
| (b) | 415 (55.9) | 327 (44.1) | 5.02 (P = 0.025) |
| (c) | 623 (60.8) | 401 (39.2) | 22.67 (P > 0.001) |
| Control | 579 (50.6) | 564 (49.4) | |
| Cockfield | | | |
| (a) | 497 (65.5) | 262 (34.5 | 13.04 (P > 0.001) |
| (b) | 636 (68.5) | 292 (31.5) | 28.74 (P > 0.001) |
| (c) | 970 (77.3) | 284 (22.7) | 117.58 (P > 0.001) |
| Control | 807 (57.5) | 596 (42.5) | |
| TI Col. | | | |
| (a) | 130 (40.5) | 191 (59.5) | 7.41 (P > 0.01 < 0.05) |
| (b) | 103 (34.1) | 199 (65.9) | 1.06 (P < 0.1) |
| (c) | 201 (58.3) | 144 (41.7) | 53.20 (P > 0.001) |
| Control | 99 (30.3) | 228 (69.7) | |

Percentages in parentheses, all chi-squared values are derived from comparison of treatments and control plants.

Table 3.14 Summary of results for cold shock experiment

| Variety | Cold shock at stage 8-9 | Cold shock at stage 5-6 | Control | $\chi^2$ (v = 2) |
|---|---|---|---|---|
| Maris Bead | | | | |
| Flowers dropped | 83 (62.4) | 67 (57.7) | 63 (52.1) | 2.8 |
| Pods set | 50 (37.6) | 49 (42.3) | 58 (47.9) | (P< 0.1) |
| Cockfield | | | | |
| Flowers dropped | 86 (71.1) | 81 (60.0) | 104 (78.2) | 10.66 |
| Pods set | 35 (28.9 | 54 (40.0) | 29 (21.8) | (P > 0.05) |
| TI Col. | | | | |
| Flowers dropped | 4 (7.1) | 12 (17.4) | 40 (48.2) | 33.4 |
| Pods set | 52 (92.9) | 57 (82.6) | 43 (51.8) | (P > 0.001) |

Percentage figures are in parentheses.

Flower abscission within each raceme (Figure 3.12) was greater at distal flower positions of Maris Bead plants, subjected to cold shock. With Cockfield plants, cold shock reduced flower abscission at later flower stages more than at stage 5-6. With TI Col. plants, cold shock significantly reduced abscission for both treatments, however, when the shock was applied to plants with flowers at developmental stage 8-9, abscission at distal flower positions was similar to that of control plants. It appeared that flowers in this genotype are less sensitive to cold shock at earlier developmental stages, rather than those that have reached anthesis.

Frost damage and flower abscission

Frost during June 1980 damaged the apex of susceptible plants, rendering them topless. Three varieties were most affected; Weirboon, Strubes and Minica. A differential response occurred between cultivars. Minica and Weirboon affected by frost experienced increased flower abscission, especially on middle and upper inflorescence positions (Figure 3.13). Strubes, however, was not significantly affected by frost damage (Table 3.15).

The effect of plant density on flower abscission

Increasing plant density resulted in a highly significant increase in abscission (Table 3.16), especially on middle and distal raceme positions, proximal flower positions set most pods (Figure 3.14). Reducing density decreased flower abscission primarily on middle and distal flower positions. However, in all treatments flower abscission was always greatest at distal positions. Abscission (Figure 3.15) was generally lower on most racemes of plants spaced 45 cm apart as compared to those spaced 30 cm apart. Similarly flower

Table 3.15 Summary of data comparing normal (control) plants with those made topless by frost

| Variety | Plants made topless by frost | Control | $\chi^2$ (v = 1) |
|---|---|---|---|
| Minica | | | |
| Flowers dropped | 121 | 72 | 30.1 (P > 0.001) |
| Pods set | 60 | 116 | |
| Strubes | | | |
| Flowers dropped | 126 | 109 | 2.27 (P < 0.1) |
| Pods set | 111 | 71 | |
| Weirboon | | | |
| Flowers dropped | 185 | 168 | 15.00 (P > 0.001) |
| Pods set | 69 | 127 | |

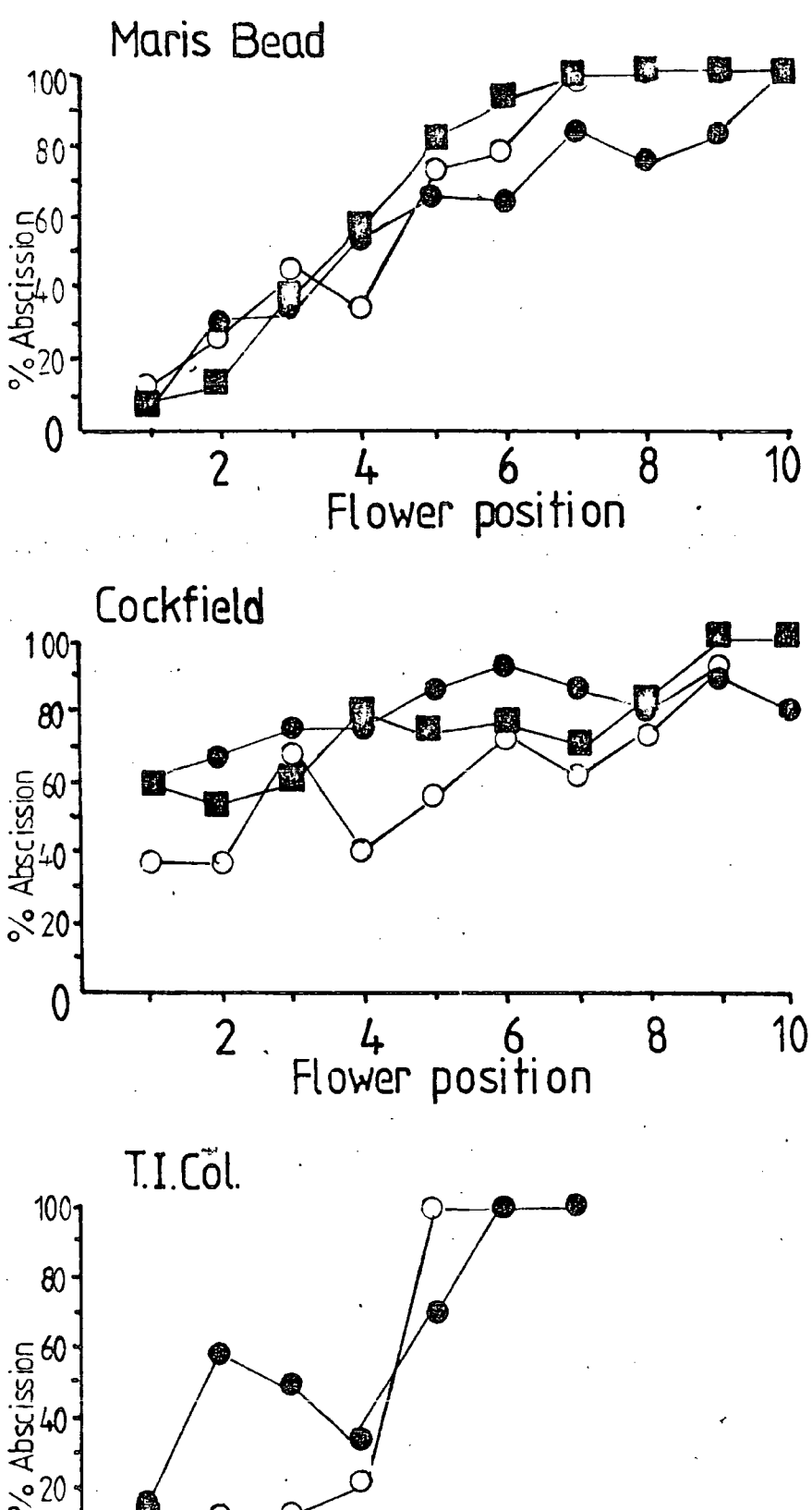



Figure 3.12: Influence of cold shock on flower abscission. The treatments were as follows: first flowering node had reached developmental stage 8-9 ( o ) and stage 5-6 ( ■ ) as compared to plants receiving no cold treatment (●). Each value is an overall percentage.

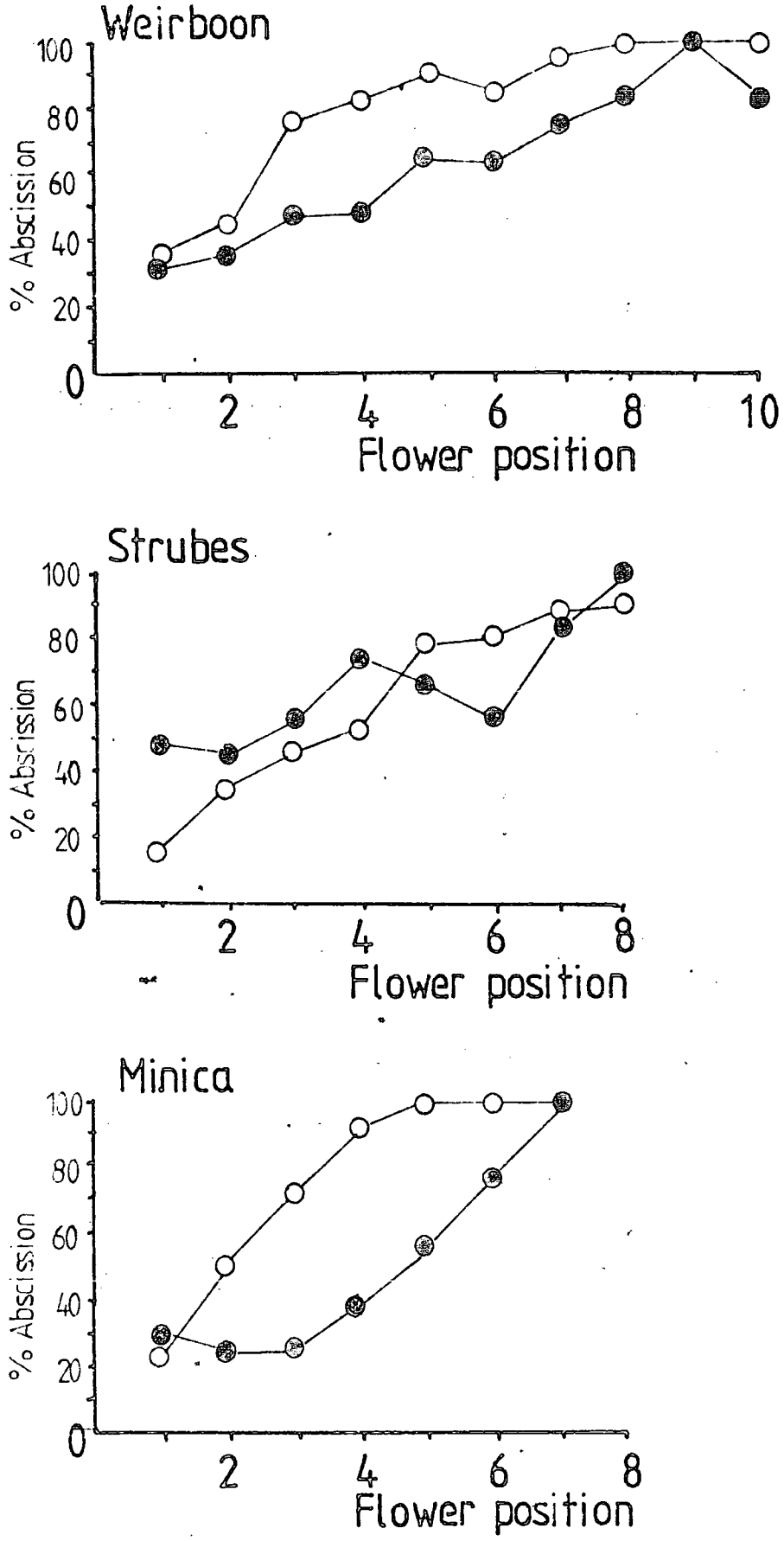



Figure 3.13: Flower drop response of three varieties of field beans made topless by frost damage. Each value is an overall percentage; o = control plants, o = frost damaged plants.

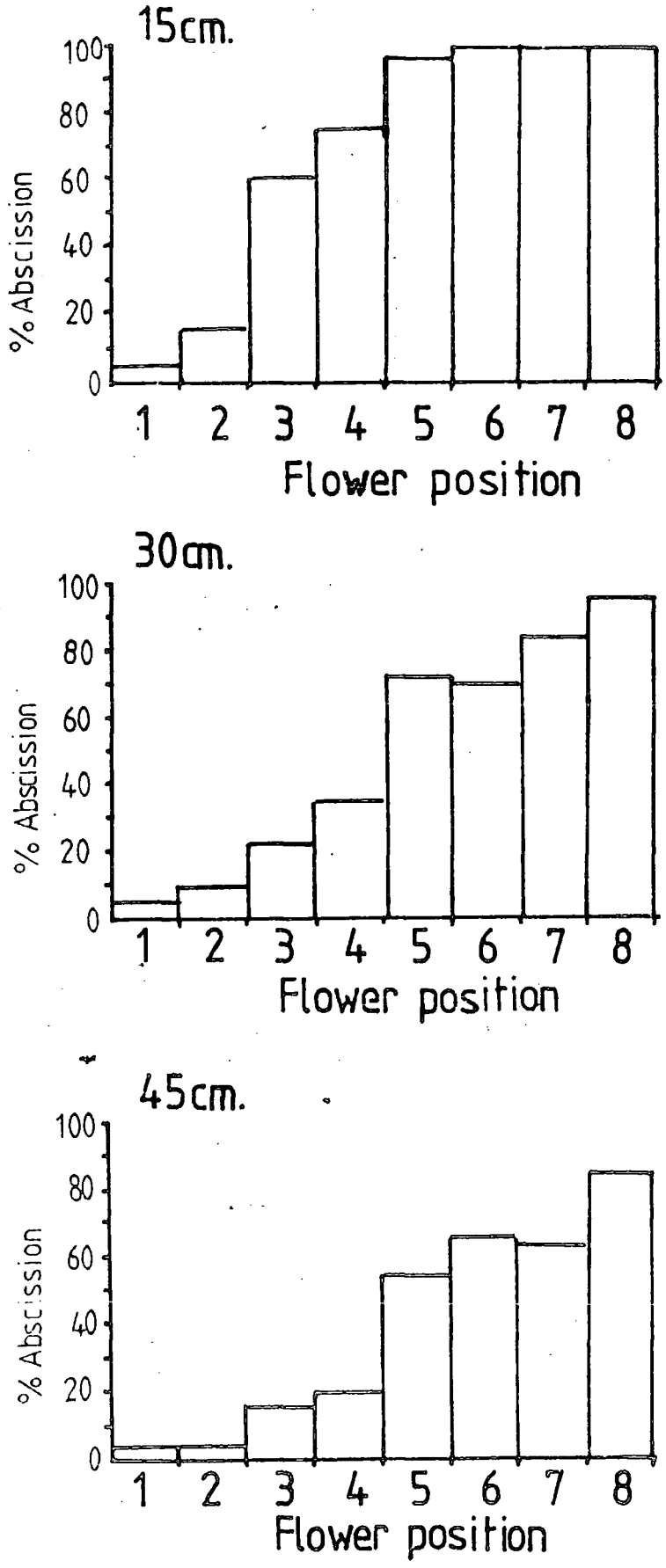



Figure 3.14: Average abscission of flowers as affected by density in variety Maris Bead. Each value is an overall percentage.

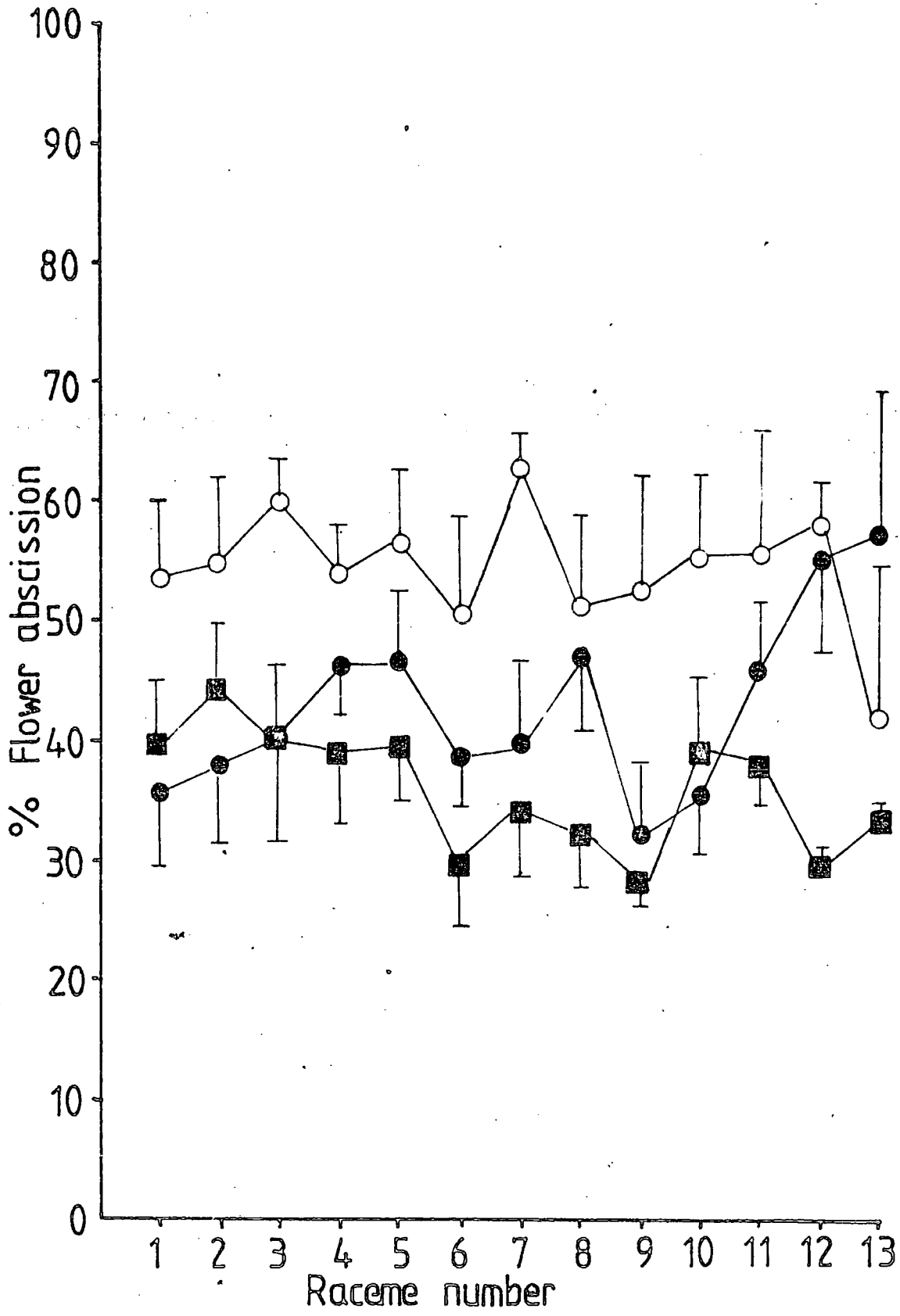



Figure 3.15: Influence of density on flower abscission for variety Maris Bead.
Soving distances, 15 cm (o), 30 cm (⊙) and 45 cm (■). Standard errors are represented by a bar.

Table 3.16 Summary of flowers dropped and pods set of Maris Bead plants grown at different densities

| | Seed spacing (cm) | | | $\chi^2$, v = 2 |
|---|---|---|---|---|
| | 15 | 30 | 45 | |
| Flowers dropped | 310 (57.1) | 260 (41.8) | 251 (53.6) | 58.9 (P> 0.001) |
| Pods set | 233 (42.9) | 362 (58.2) | 453 (64.4) | |

Percentage figures are in parentheses.

drop was higher over most racemes of plants spaced 15 cm apart compared to that of plants spaced 30 cm apart.

Analysis of the final yield components (Tables 3.17, 3.18) indicated that the number of mature pods per plant increased with decreasing density. However, the overall final yield decreases with decreasing density. This is an indication that seed yield in Maris Bead is a function of the number of pods per unit area, and not of the number of pods per plant.

Table 3.17 Total yield of Maris Bead from plots sown at different densities

| Seed spacing (cm) | Average total yield (g) of plots |
|---|---|
| 15 | 1414.4 |
| 30 | 730.2 |
| 45 | 426.5 |

Table 3.18 Yield components of Maris Bead grown at different densities

| Seed spacing (cm) | Average yield component | | | | |
|---|---|---|---|---|---|
| | Pod number | Seed number | Seed weight (g) | Number of seeds/pod | Weight of each seed (g) |
| 15 | 13.4 (1.1) | 40.1 (3.8) | 10.6 (0.9) | 3.0 | 0.264 (0.01) |
| 30 | 31.7 (2.9) | 82.2 (6.3) | 20.5 (1.9) | 2.6 | 0.249 (0.01) |
| 45 | 60.2 (10.4) | 126.0 (26.8) | 28.6 (5.9) | 2.1 | 0.227 (0.02) |

Standard error is given in parenteses.

# CHAPTER 4

## RESULTS OF OBSERVATIONS ON PHYSIOLOGICAL EXPERIMENTS PERFORMED ON COMMERCIAL VARIETIES AND INBRED LINES

### Introduction

Evidence presented in the previous chapter showed that for all the commercial varieties examined mean flower abscission within each raceme followed a similar pattern, in that least flower drop occurred on proximal raceme positions and most on distal receme positions. Application of various environmental stresses resulted in an increase in abscission, most of which occurred on middle and upper flower positions. The basic pattern of abscission, however, remained the same. In addition it was shown that plants are most sensitive to stress when flowers have reached anthesis. A notable exception to this was water deficit, where flowers appeared to be more susceptible at earlier developmental stages.

In this chapter the results of physiological experiments designed to elucidate possible reasons for the observed pattern of flower drop are described.

### The effect of tripping on flower drop

Tripping all flower positions on all axillary racemes significantly increased pod set in inbred lines T51 and T2 (Table 4.1). This increase was most pronounced in line T2. This might be expected as T51 displays a degree of auto-fertility and T2 autosterility. Maris Bead plants, conversely, exhibited no significant difference in flower abscission between those plants that had been tripped and those whose flowers had been left untripped.

Table 4.1 Summary of results for tripping experiment

| Variety and treatment | Flowers dropped (%) | Pods set (%) | $\chi^2$, v = 1 |
|---|---|---|---|
| T2 | | | |
| none tripped | 361 (82.6) | 76 (17.4) | 19.40 (P > 0.001) |
| all tripped | 342 (69.8) | 148 (30.2) | |
| proximal two flowers tripped | 388 (76.5) | 119 (23.5) | |
| distal two flowers tripped | 343 (75.9) | 109 (24.1) | |
| T51 | | | |
| none tripped | 219 (71.8) | 86 (28.2) | 3.01 (P > 0.1 < 0.05) |
| all flowers tripped | 155 (64.8) | 84 (35.2) | |
| Proximal two flowers tripped | 258 (70.7) | 107 (29.3) | |
| distal two flowers tripped | 190 (66.6) | 95 (33.4) | |
| Maris Bead | | | |
| none tripped | 421 (83.5) | 83 (16.5) | 2.20 (P < 0.1) |
| all flowers tripped | 387 (86.9) | 58 (13.1) | |
| proximal two flowers tripped | 322 (85.4) | 55 (14.6) | |
| distal two flowers tripped | 326 (82.7) | 68 (17.3) | |

Percentage figures are in parentheses, chi-squared analysis compares tripping with no tripping.

Tripping of proximal flower positions of plants of line T2 resulted in a similar degree of abscission within each raceme, compared to similar flower positions on control plants. An increase in flower drop was, however, experienced by those flowers situated on distal positions of treated plants (Figure 4.1.1). When the distal two flowers were tripped on each raceme less abscission occurred to those flowers, but greater abscission occurred to flowers situated on more proximal positions compared to equivalent positions on control plants (Figure 4.1.1). Similar, but less pronounced, effects on flower drop within each raceme, were observed on T51 and Maris Bead plants subjected to the same treatments (Figures 4.1.2, 4.1.3).

Flower abscission was greater on most racemes of line T2 when flowers were not tripped compared with flowers that had been tripped. Other treatments resulted in greater flower abscission at racemes situated on the upper reproductive portion of the plant (Figure 4.2.1).

Complete lack of tripping of plants of line T51 resulted in greater abscission on proximally situated racemes, but less abscission of flowers on inflorescences situated towards the top of these plants, relative to plants whose flowers had all been tripped (Figure 4.2.2). A similar, but less pronounced, effect was produced by plants where flowers situated distally on every raceme were tripped. Plants of line T51, where proximal flowers were tripped, experienced greater abscission at lower inflorescences, while there was little difference between abscission on inflorescences situated near to the top of plants compared with the control plants (with all flowers tripped).

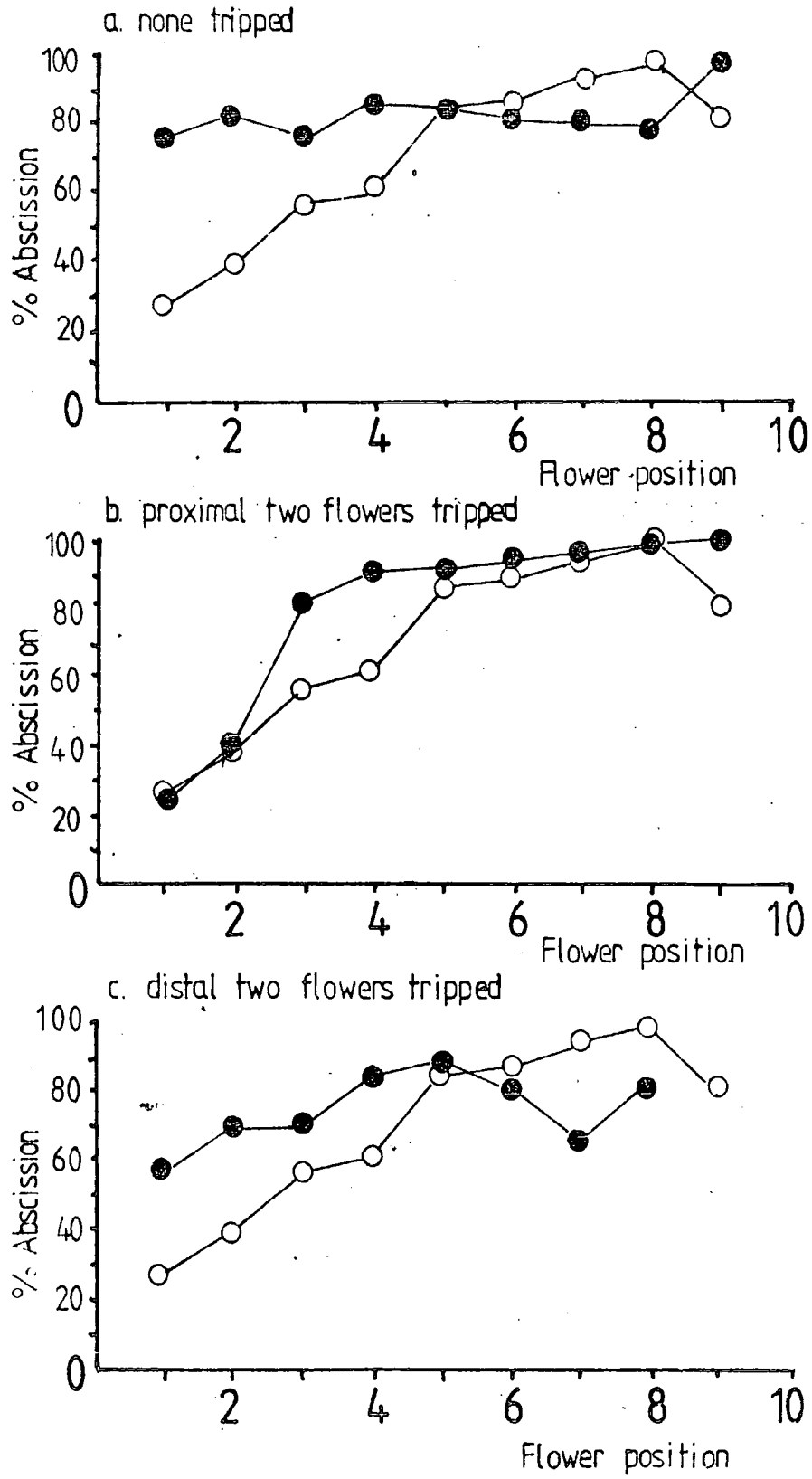



Figure 4.1.1: Effect of tripping on flower abscission, on inbred line T2.
Each value is an average percentage
o = control plants, all tripped
● = treatment plants

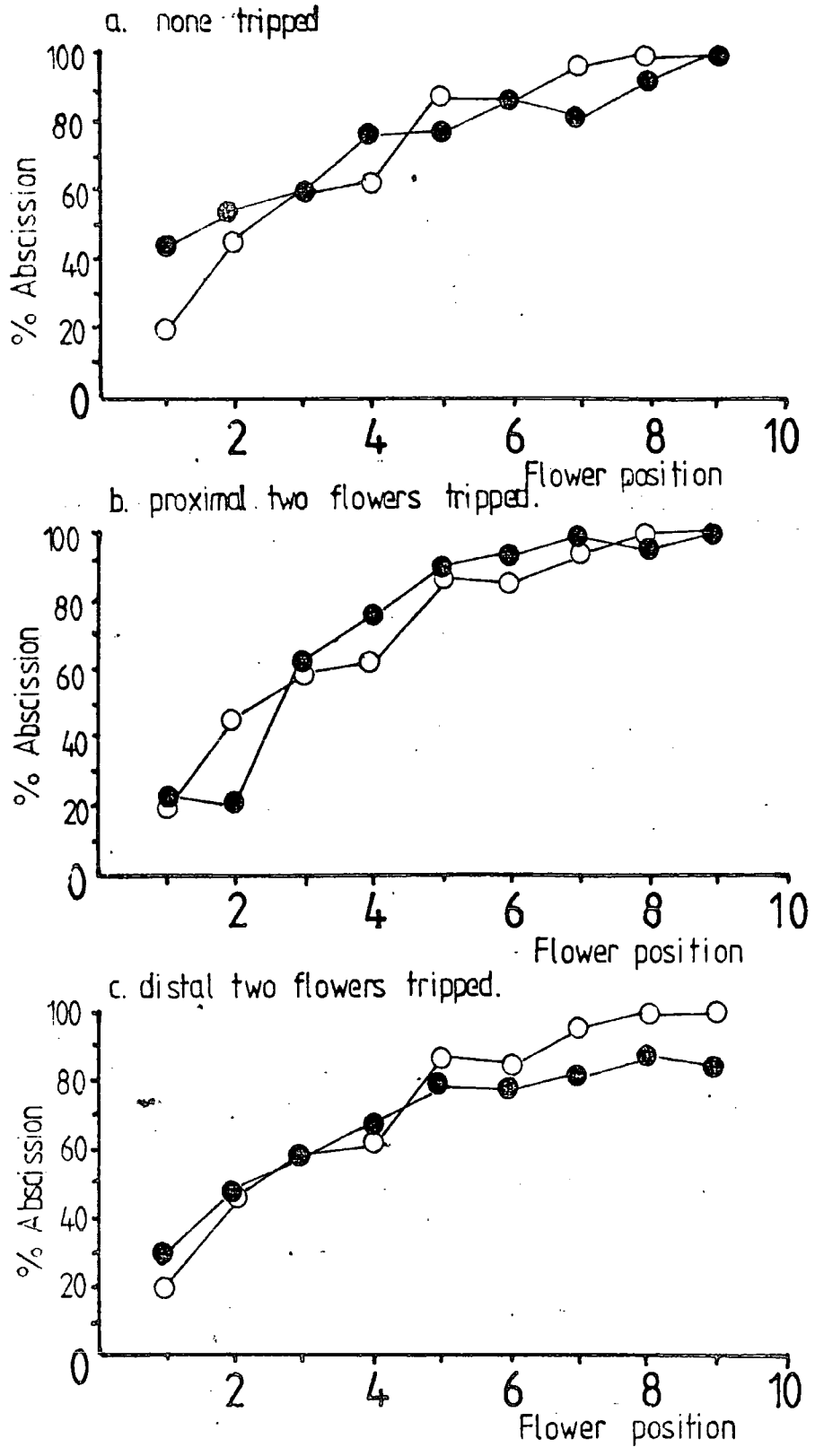



Figure 4.1.2: Effect of tripping on flower abscission on inbred line T51.
Each value is an average percentage.
o = control plants, all tripped
● = treatment plants.

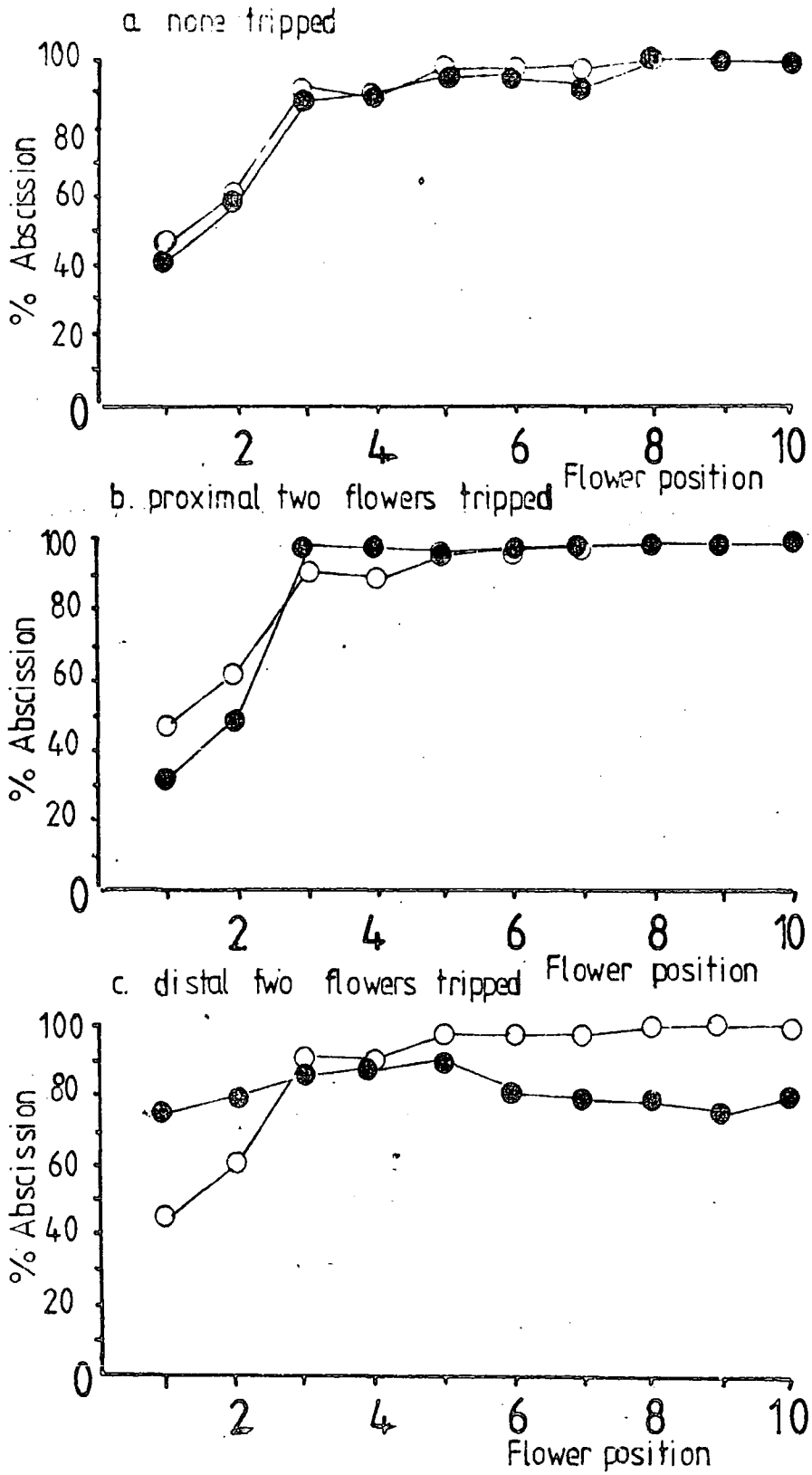



Figure 4.1.3: Effect of tripping on flower abscission of Maris Bead. Each value is an average percentage.
o = control plants, all tripped
⊖ = treated plants.

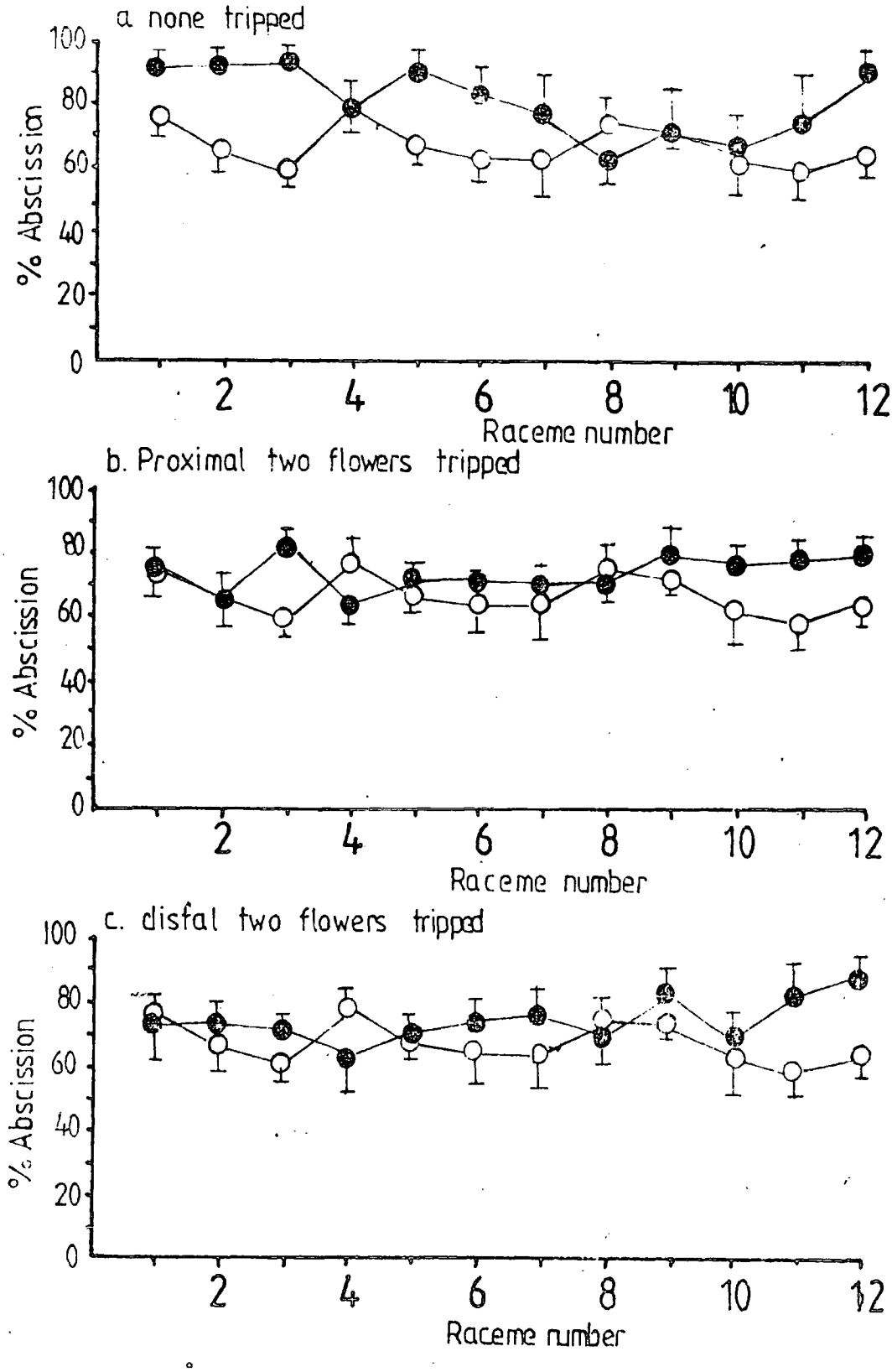



Figure 4.2.1: Effect of tripping on flower abscission of inbred line T2.
Each value is an average percentage.
Standard errors are represented by a bar.
o = control plants, all tripped
● = treatment plants.

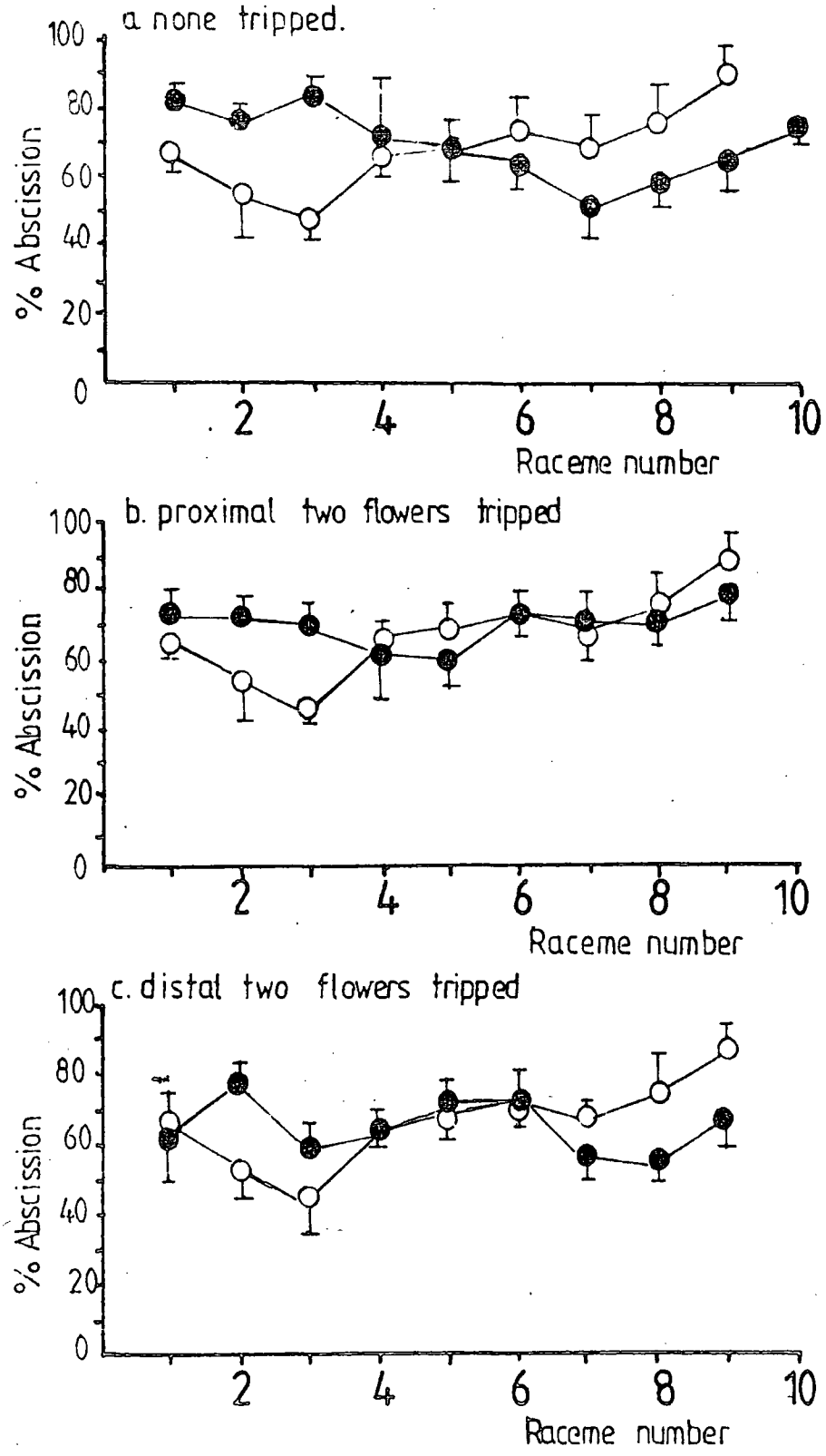



Figure 4.2.2: Effect of tripping on flower abscission of inbred line T51.
Each value is an average percentage.
Standard errors are represented as a bar.
o = control plants, all tripped
⊛ = treatment plants.

Flower abscission of Maris Bead plants subjected to all the treatments, was slightly greater over most racemes (Figure 4.2.3).

These results show that although tripping reduced abscission, especially for inbred line T2, it does not in itself alter the basic pattern of flower drop, which is still high on distal raceme positions, even when all flowers had been tripped. Tripping in itself is not necessary for pods to set, unless plants are completely autosterile.

Presence of pollen tubes in abscised flowers

Observations on tripped and untripped flowers (Table 4.2) showed that flowers which were tripped and possessed ovules with pollen tubes present, had still abscised. Flowers which had been left untripped, and since had dropped had no detectable pollen tubes present.

In addition it was subsequently observed (Table 4.3) that many more pollen tubes were detected near to ovules from untripped autofertile as opposed to autosterile lines.

These results show that, if pollen tubes are detected near to ovules, and if ovules are then regarded as fertilized, then lack of fertilization cannot be the sole reason for the failure of flowers to set pods.

Effect of growth regulators on abscission

Initial glasshouse experiments

Application of the ethylene inhibitor silver nitrate, to inbred lines 3, 4 and 5 resulted, on average, in a decrease in abscission within each raceme, especially at proximal flower positions (Figure 4.3). This decrease in abscission was significant in all lines examined (Table 4.4). Flower drop in treated plants, however, was still great, and followed

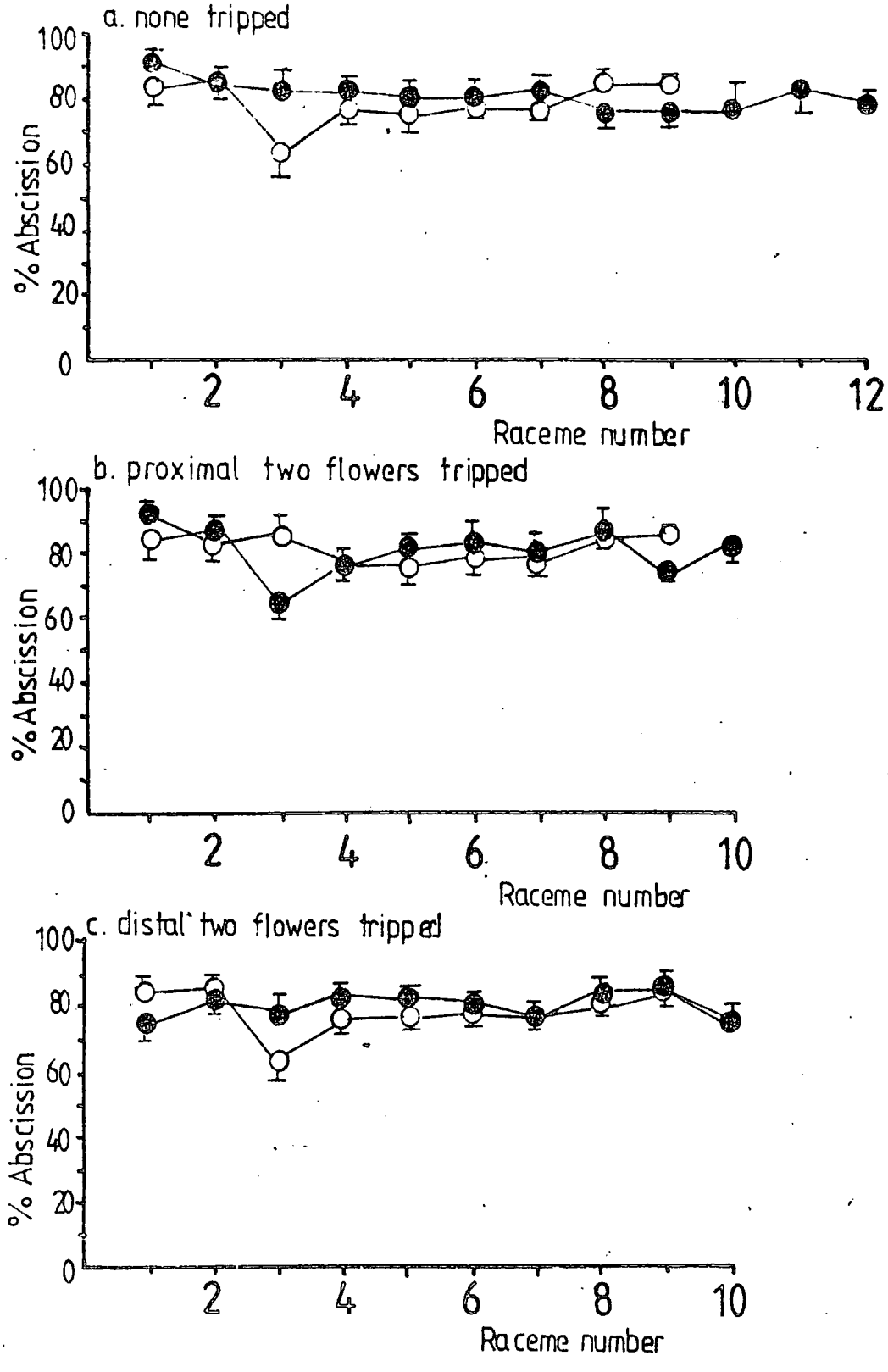



Figure 4.2.3: Effect of tripping on flower abscission of variety Maris Bead.
Each value is an average percentage.
Standard errors are represented as a bar.
o = control plants, all tripped
● = treatment plants.

Table 4.2 Percentage pollen tubes detected from abscised flowers of two inbred lines of field beans

| Inbred line | Pollen tubes detected | No pollen tubes detected |
|---|---|---|
| Line 5 | | |
| tripped | 50 | 50 |
| left untripped | 0 | 100 |
| Line 4 | | |
| tripped | 64.3 | 35.7 |
| left untripped | 0 | 100 |

Table 4.3 Percentage pollen tubes detected from abscised flowers of an autosterile and an autofertile inbred line of field beans

| Inbred line | Pollen tubes detected | No pollen tubes detected |
|---|---|---|
| Line 8 | 60.8 | 39.2 |
| Line T2 | 5.3 | 94.7 |

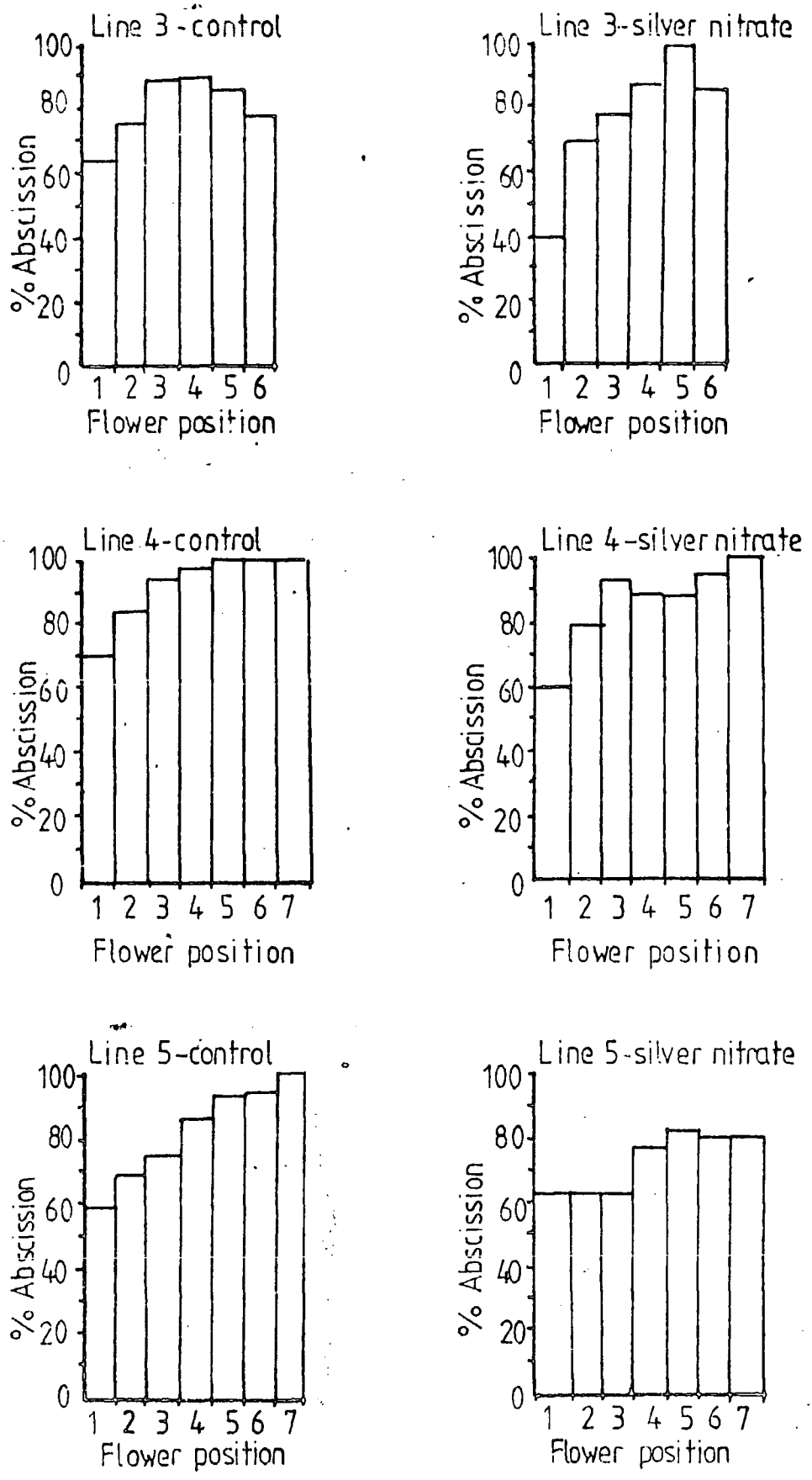



Figure 4.3: Flower abscission of inbred lines 3, 4 and 5 subjected to silver nitrate spraying as compared to control plants. Each value is an overall peecentage.

Table 4.4 Summary of results for silver nitrate experiment

| Variety and treatment | Control | Spraying with Silver Nitrate | $\chi^2$, v = 1 |
|---|---|---|---|
| Line 3 | | | |
| flowers dropped | 80.1 | 71.2 | 3.41 |
| pods set | | | (P > 0.1) |
| Line 4 | | | |
| flowers dropped | 91.0 | 84.2 | 4.28 |
| pods set | 9.0 | 19.1 | (P > 0.05) |
| Line 5 | | | |
| flowers dropped | 78.8 | 69.9 | 3.30 |
| pods set | 21.2 | 30.1 | (P > 0.1) |

Each value is an overall percentage.

the same pattern within each raceme, to that described previously. Flower abscission was reduced primarily on the middle racemes of treated plants (Figure 4.4).

Initial experiments with sodium benzoate, on Maris Bead plants, resulted in no significant change in flower abscission (Figure 4.5, Table 4.5).

Table 4.5 Summary of results for Sodium Benzoate experiment

| Treatment | Flowers dropped (%) | Pods set (%) | $\chi^2$, v = 1 |
|---|---|---|---|
| Control | 710 (83.2) | 143 (16.8) | 0.65 ($P < 0.1$) |
| Sodium benzoate treated | 895 (84.6) | 163 (15.4) | |

Percentage figures are in parentheses.

Field experiments

Deiniol, Minica and Montica plants, responded differently to the application of silver nitrate (Figures 4.6.1, 4.6.2, 4.6.3). Silver nitrate application significantly reduced flower drop in Deiniol and Montica plants, and slightly reduced flower drop in Minica plants, but not to a significant extent (Table 4.6).

Sodium benzoate application produced a slight reduction in abscission (Figures 4.6.1, 4.6.2, 4.6.3) although this was insignificant compared to the control plants (Table 4.6), this effect being most pronounced for Deiniol plants.

These results show that the smaller seeded variety, Deiniol, responded more readily to the application of silver

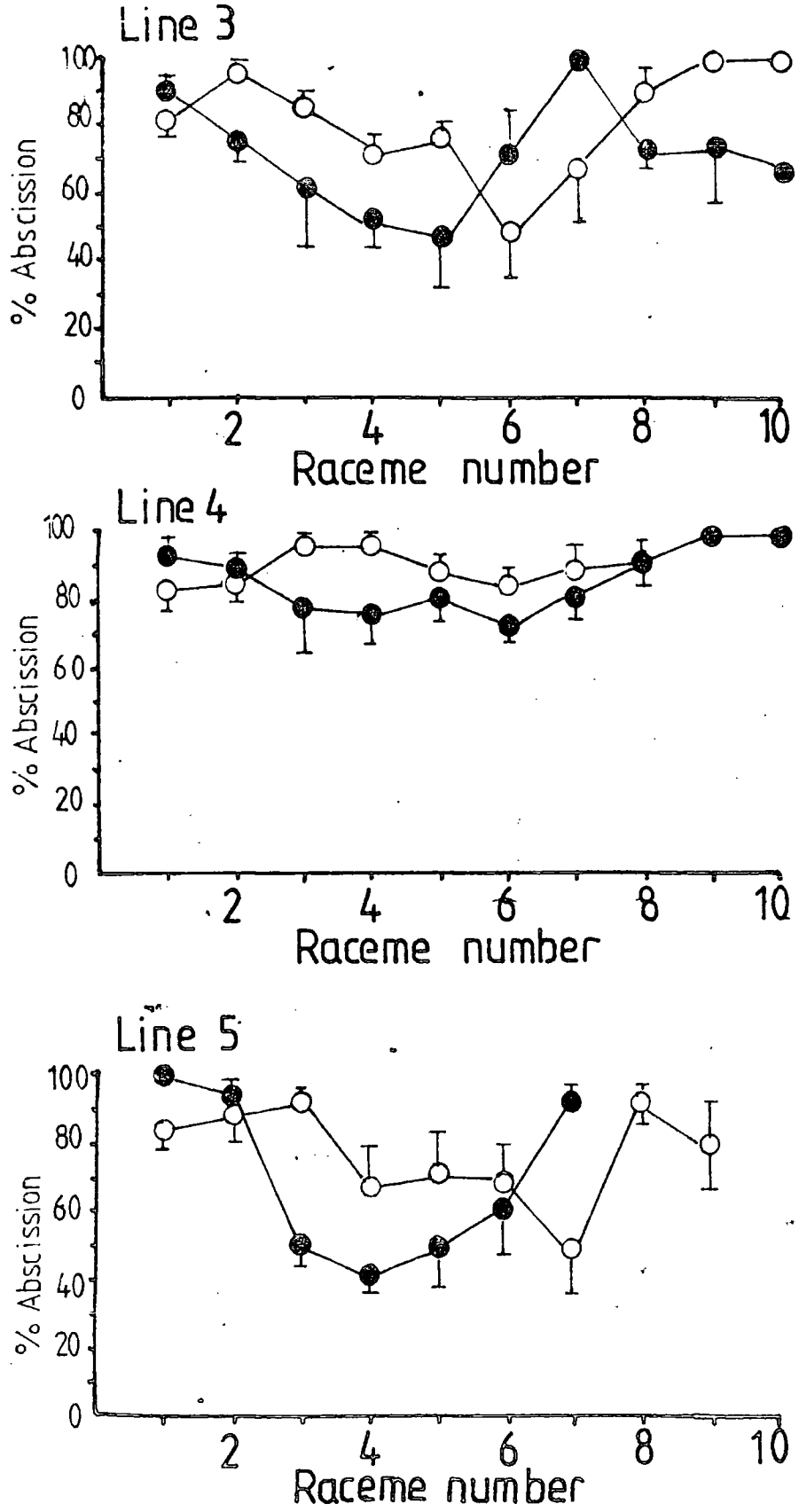



Figure 4.4: Flower abscission, for silver nitrate experiment. o = control plants; ● = silver nitrate sprayed plants Each value is an average percentage. Standard error is represented by a bar.

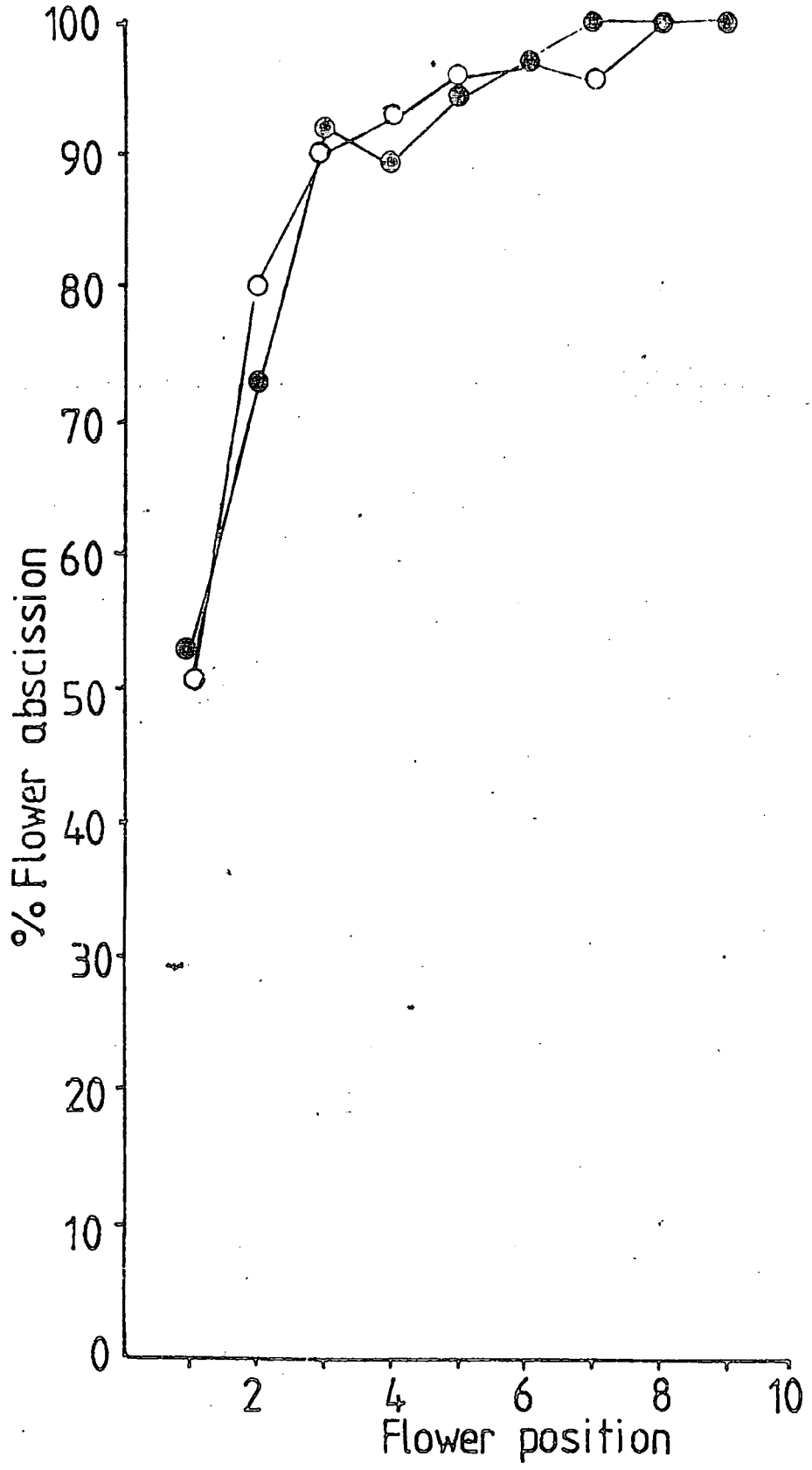



Figure 4.5: Flower abscission for Maris Bead plants sprayed with sodium benzoate.
o = control plants;
o = sodium benzoate sprayed plants
Each value is an overall percentage.

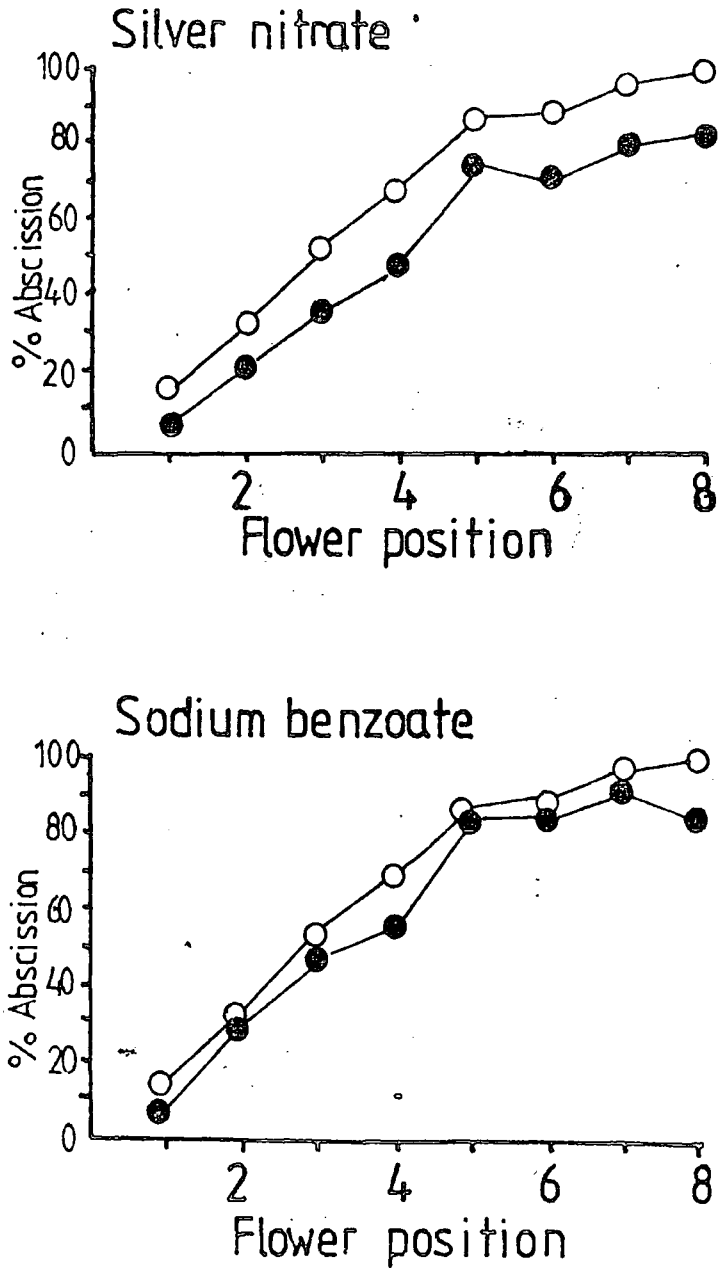



Figure 4.6.1: Influence of silver nitrate and sodium benzoate application on flower abscission for variety Deiniol. Each value is an overall percentage.
o = control plants
● = treatment plants.

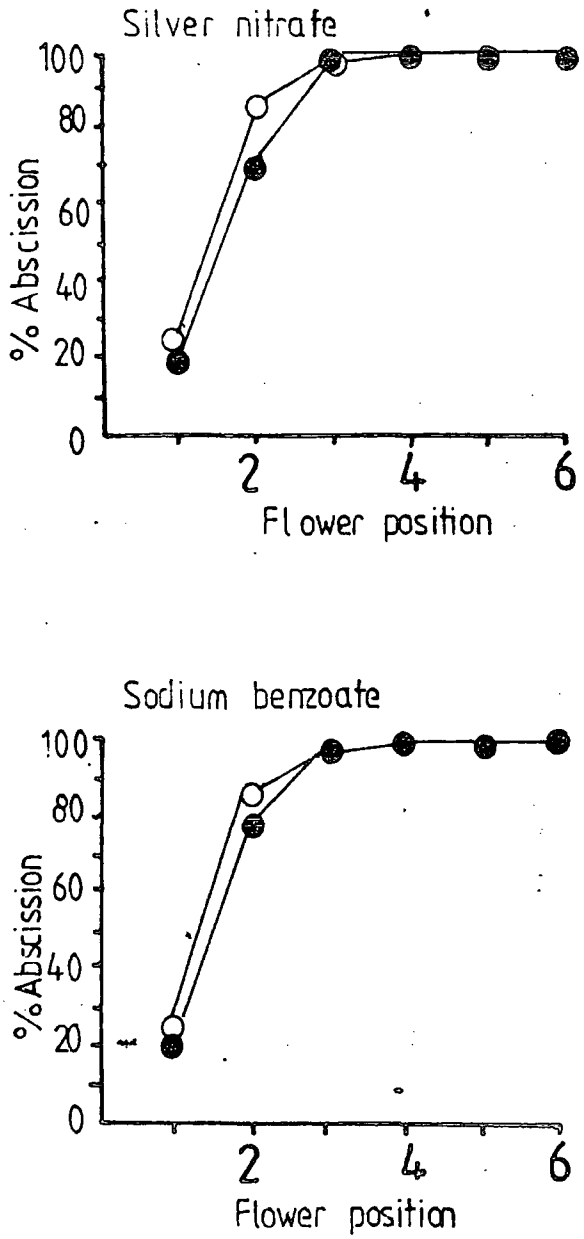



Figure 4.6.2: Influence of silver nitrate and sodium benzoate application on flower abscission for variety Montica. Each value is an overall percentage.
o = control
● = treatment.

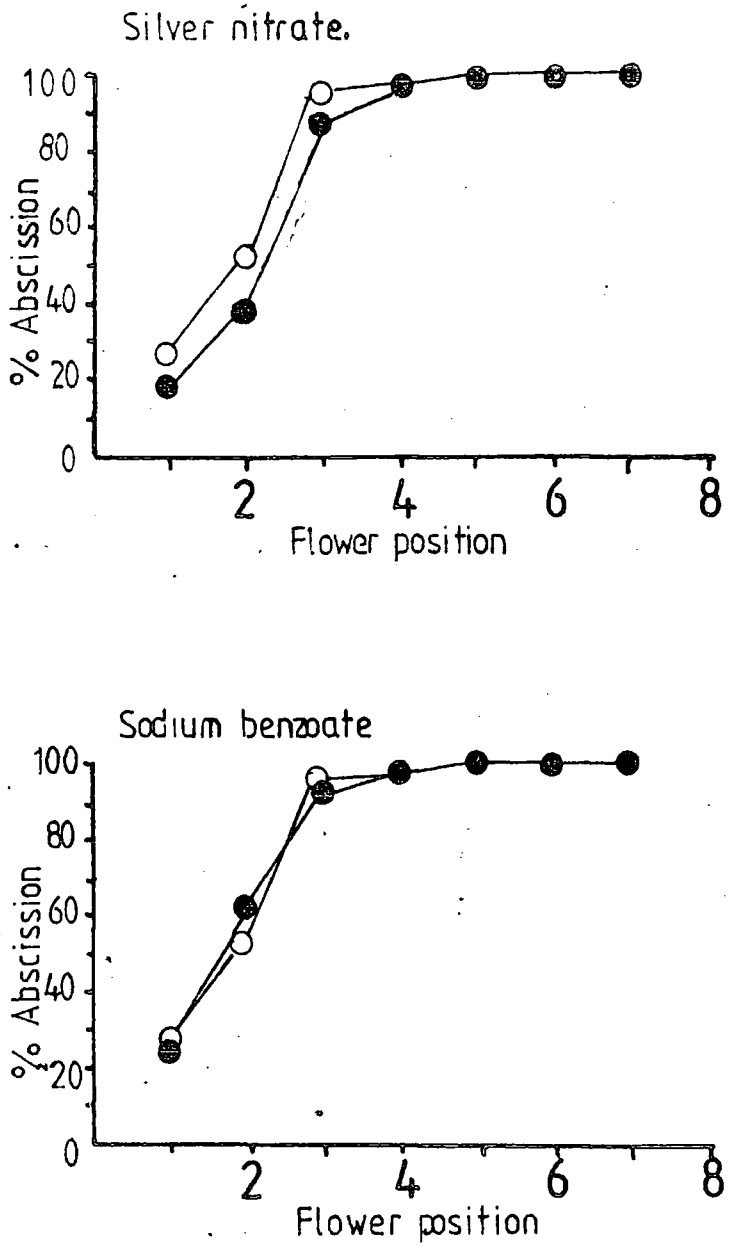



Figure 4.6.3: Influence of silver nitrate and sodium benzoate application on flower abscission for variety Minica. Each value is an overall percentage,
o = control,
● = treatment.

Table 4.6 Summary of results and chi-squared analysis for sodium benzoate and silver nitrate treated plants

| Variety and treatment | Total flowers dropped (%) | Total pods set (%) | $\chi^2$, v = 1 |
|---|---|---|---|
| Deiniol | | | |
| control | 848 (53.3) | 744 (46.7) | |
| silver nitrate treated | 395 (41.3) | 561 (58.7) | 34.12 ($P > 0.001$) |
| sodium benzoate treated | 513 (50.4) | 504 (49.6) | 1.98 ($P < 0.1$) |
| Montica | | | |
| control | 706 (77.4) | 206 (22.6) | |
| silver nitrate treated | 348 (72.0) | 135 (28.0) | 4.91 ($P > 0.05 < 0.025$) |
| sodium benzoate treated | 354 (73.9) | 125 (26.1) | 2.13 ($P < 0.1$) |
| Minica | | | |
| control | 420 (80.1) | 104 (19.9) | |
| silver nitrate treated | 190 (75.1) | 63 (24.9) | 2.58 ($P < 0.1$) |
| sodium benzoate treated | 184 (79.3) | 48 (20.7) | 0.07 ($P < 0.1$) |

Percentage values are in parentheses.
(controls involved twice as many plants).

nitrate. The larger seeded varieties responded to this chemical to a lesser extent, most of the observed reduction in flower abscission occurred mainly on the proximal flower positions.

Application of silver nitrate reduced flower drop to a similar extent over most axillary racemes (Figures 4.7.1, 4.7.2, 4.7.3). Application of sodium benzoate resulted in similar flower abscission to that experienced by control plants. The results with sodium benzoate, are not conclusive, but perhaps serve to indicate that silver nitrate produces a true effect on treated plants.

Analysis of yield components (Table 4.7) revealed that silver nitrate application increased the number of mature pods produced by plants of variety Deiniol. Little or no change in the number of mature pods was produced by plants of other varieties, subjected to this treatment. In all varieties examined, however, sodium benzoate treated plants had a greater average number of mature pods, seeds and the heaviest seed weight. This may indicate that sodium benzoate might have an effect on pod retention at later stages of pod development, rather than at the pod set stage.

It appears that silver nitrate by inhibiting the production of ethylene reduces flower abscission. This treatment, however, by itself does not alter the basic pattern of abscission, in that proximal flowers set most pods, and distal flowers drop.

Inflorescence removal in line T2 and its relationship to flower drop

Removal of the first axillary inflorescence had very little effect. Removal of the first two racemes, exceptionally,

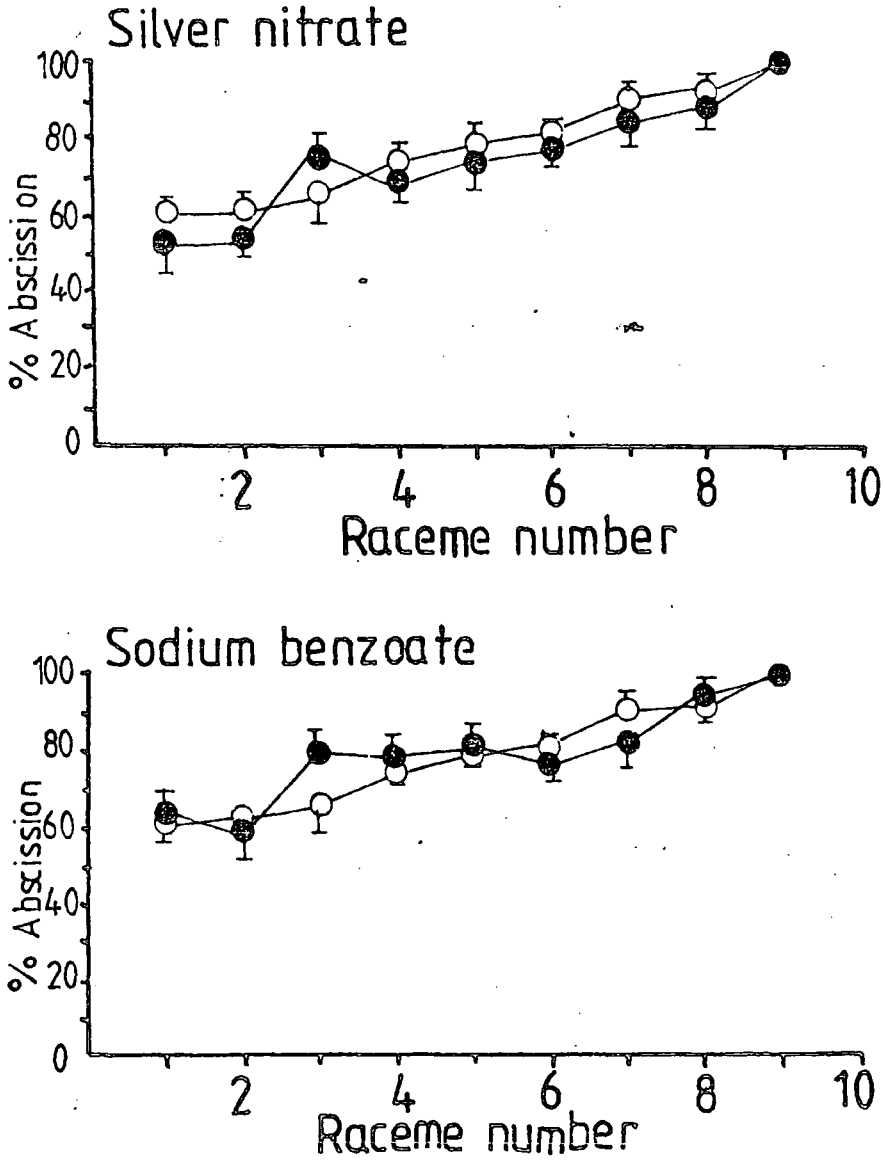



Figure 4.7.1: Influence of silver nitrate and sodium benzoate on flower abscission of Minica plants. Each value is an average percentage. Standard errors are represented by a bar.
● = treated plants,
o = control plants.

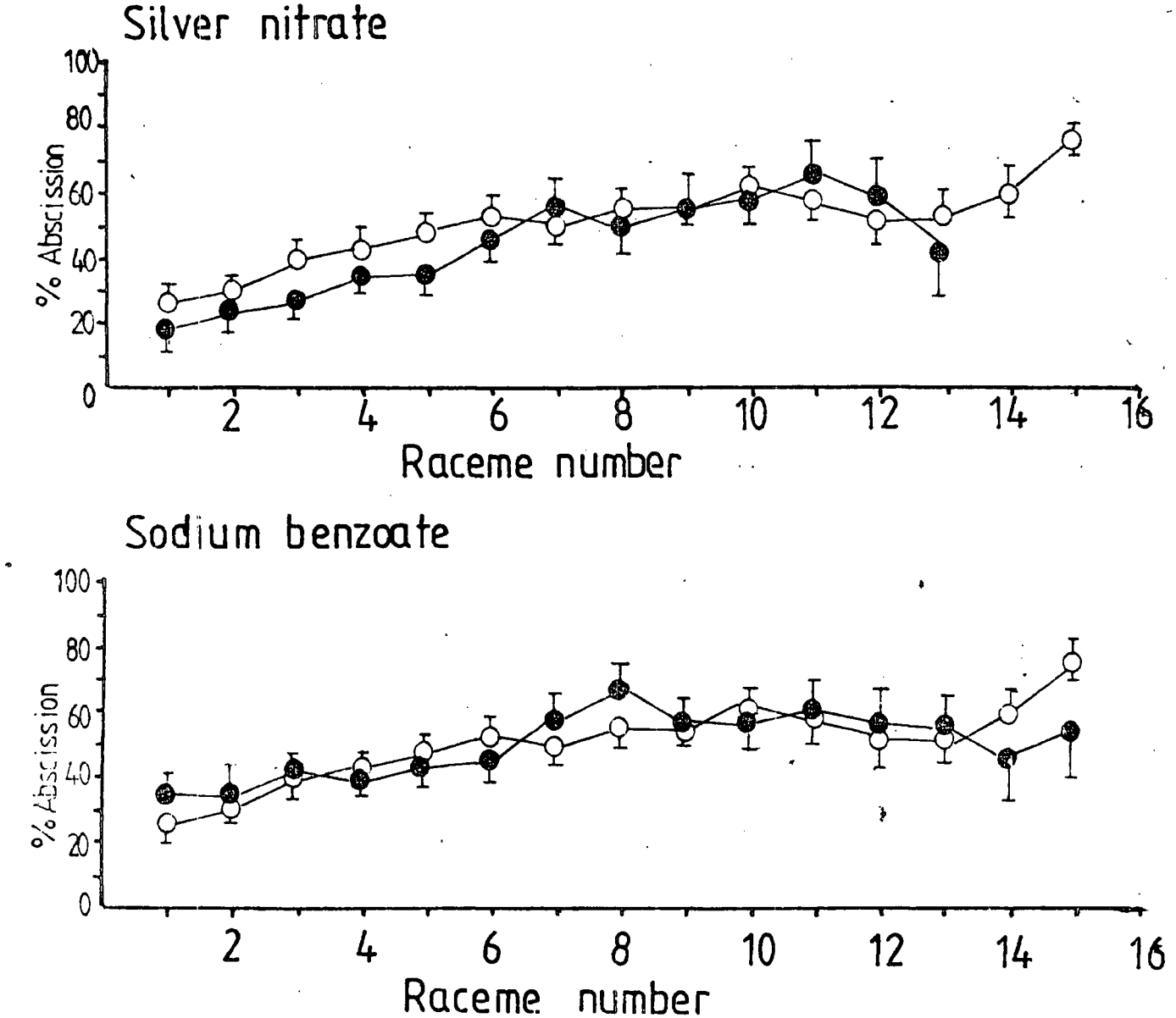



Figure 4.7.2: Influence of silver nitrate and sodium benzoate on flower abscission of Deiniol plants. Each value is an average percentage. Standard errors are represented by a bar.
(●) = treated plants; (o) = control plants.

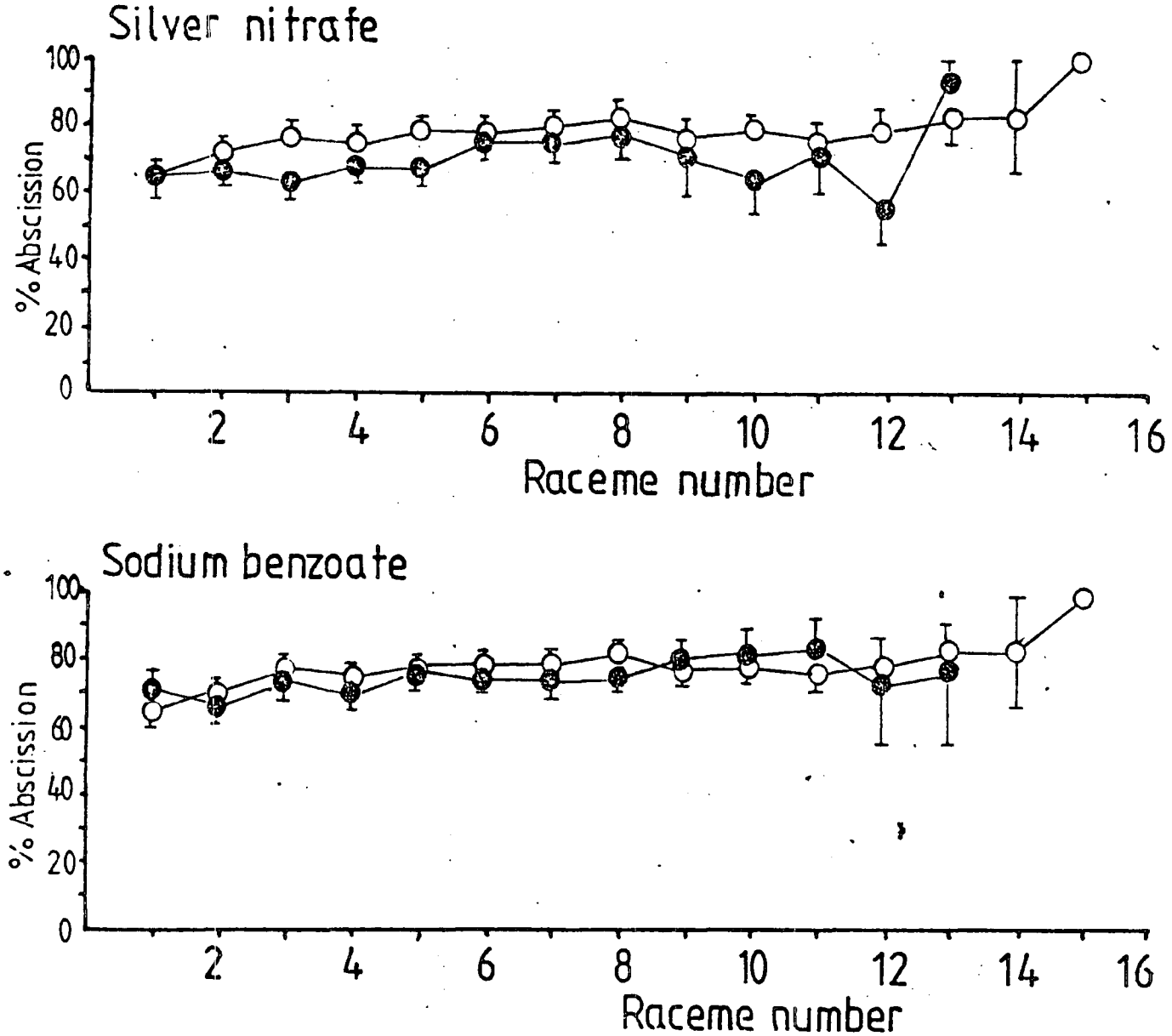



Figure 4.7.3: Influence of silver nitrate and sodium benzoate on flower abscission of Montica plants. Each value is an average percentage. Standard errors are represented by a bar.
(●) = treated plants; (o) = control plants.

Table 4.7 Average yield components of silver nitrate and sodium benzoate experiment

| Variety and treatment | Number of pods / plant | Number of seeds/plant | Total weight of seeds (g) | Weight of each seed (g) |
|---|---|---|---|---|
| Deiniol | | | | |
| control | 16.0 (1.39) | 42.7 (4.28) | 17.6 (1.82) | 0.40 (0.016) |
| silver nitrate treated | 18.0 (3.60) | 40.9 (8.61) | 14.3 (3.57) | 0.36 (0.023) |
| sodium benzoate treated | 18.8 (2.61) | 60.8 (8.29) | 24.6 (3.49) | 0.39 (0.019) |
| Minica | | | | |
| control | 4.8 (0.38) | 17.0 (1.56) | 17.3 (1.41) | 1.04 (0.051) |
| silver nitrate treated | 4.8 (0.33) | 18.0 (1.98) | 18.1 (1.83) | 1.02 (0.046) |
| sodium benzoate treated | 5.2 (0.77) | 22.8 (2.69) | 21.5 (1.82) | 0.96 (0.353) |
| Montica | | | | |
| control | 8.9 (1.04) | 26.7 (2.99) | 24.6 (3.36) | 0.93 (0.052) |
| silver nitrate treated | 7.6 (1.31) | 19.0 (3.83) | 20.7 (2.58) | 1.20 (0.122) |
| sodium benzoate treated | 11.2 (0.93) | 34.3 (3.95) | 29.0 (4.08) | 0.86 (0.067) |

Standard errors are in parentheses.

increased abscission within remaining racemes, especially at proximal flower positions. Removal of successively more racemes reduced abscission within the remaining inflorescences. On plants where six racemes had been removed, flower abscission within each raceme was reduced over most flower positions, except for those most distally situated. Even at these positions, however, flower drop was less compared to control plants. (Figure 4.8; Table 4.8).

All treatments (Figure 4.9) except where the first two inflorescences had been removed, caused a reduction in flower abscission on the majority of the remaining racemes.

Analysis of yield components (Table 4.9) revealed that compared to control plants, removal of the first and second racemes, resulted in a decrease in yield (i.e. number of mature pods produced). Plants which had the first three and four racemes removed, experienced an increase in yield. Subsequent removal of racemes above the fourth inflorescence, resulted in a progressive yield decrease.

These results indicate the strong effect that lower flowering nodes, which may possess half mature pods, exert on more distal inflorescences, which may have flowers at and still to reach anthesis. In addition, removal of the earliest formed inflorescences not only reduced flower abscission of subsequent racemes, but also enhanced the final yield of plants so treated.

Top removal (decapitation of the apex) and flower drop

Decapitation of the apex of $T_2$ plants, resulted in a significant reduction of abscission within remaining racemes (Figure 4.10, Table 4.10). When plants were decapitated after six flowering nodes had formed, the reduction of flower abscission at proximal raceme positions was accompanied by

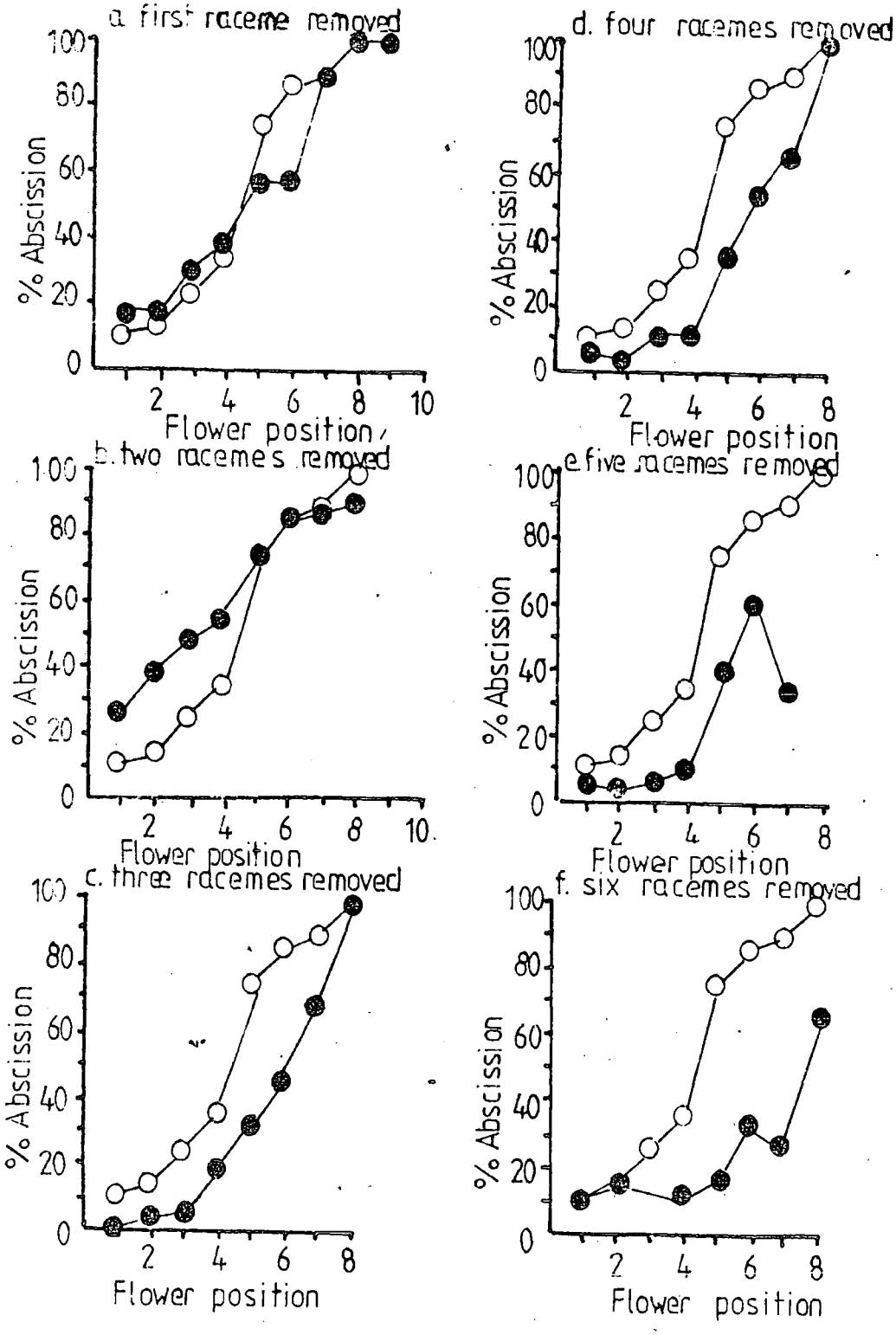



Figure 4.8: Influence of inflorescence removal on flower abscission. Each value is an overall percentage.
● = treatment plants
o = control plants..

Table 4.8 Summary of $T_2$ plants subjected to Inflorescence removal

| Treatment | Total % flowers dropped | Total % pods set | $\chi^2$ v = 1 |
|---|---|---|---|
| Control | 46.9 | 53.1 | |
| (a) first raceme | 43.8 | 56.2 | 0.89 ($P < 0.1$) |
| (b) two racemes | 58.4 | 41.6 | 10.34 ($P > 0.05$) |
| (c) three racemes | 23.0 | 77.0 | 40.97 ($P > 0.001$) |
| (d) four racemes | 26.0 | 74.0 | 31.69 ($P > 0.001$) |
| (e) five racemes | 18.6 | 81.4 | 44.10 ($P > 0.001$) |
| (f) six racemes | 18.5 | 81.5 | 34.09 ($P > 0.001$) |

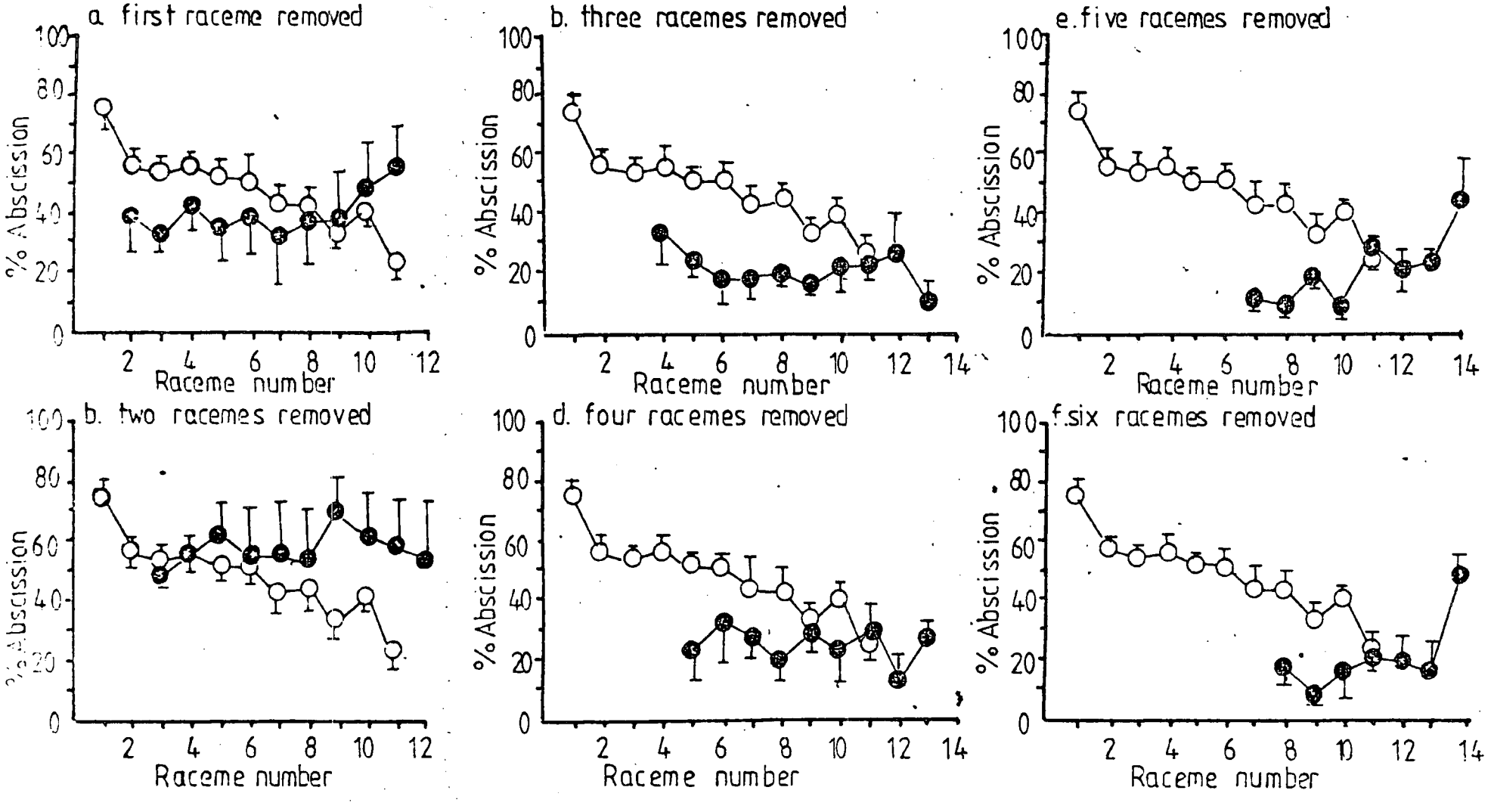



Figure 4.9: Influence of inflorescence removal on flower abscission.
Standard errors as bar. Each value is an average percentage.
● = treatment plants; o = control plants.

Table 4.9 Summary of average yield components of $T_2$ plants subjected to inflorescence removal

| Treatment | Number of pods | Number of seeds | Seed weight per plant (g) | Weight of each seed (g) |
|---|---|---|---|---|
| Controls | 13.4 (1.83) | 47.5 (6.47) | 9.1 (1.34) | 0.191 (0.011) |
| (a) first raceme removed | 12.2 (3.06) | 43.5 (11.11) | 9.7 (2.51) | 0.229 (0.019) |
| (b) two racemes removed | 9.3 (3.60) | 32.3 (13.34) | 5.4 (2.17) | 0.142 (0.028) |
| (c) three racemes removed | 13.5 (2.27) | 47.2 (7.45) | 8.5 (1.61) | 0.177 (0.007) |
| (d) four racemes removed | 22.6 (4.06) | 80.3 (14.31) | 15.5 (1.48) | 0.201 (0.016) |
| (e) five racemes removed | 12.0 (1.37) | 42.7 (0.04) | 5.1 (1.35) | 0.113 (0.017) |
| (f) six racemes removed | 11.0 (2.47) | 39.7 (8.32) | 6.6 (0.85) | 0.178 (0.022) |

Standard errors are in parentheses.

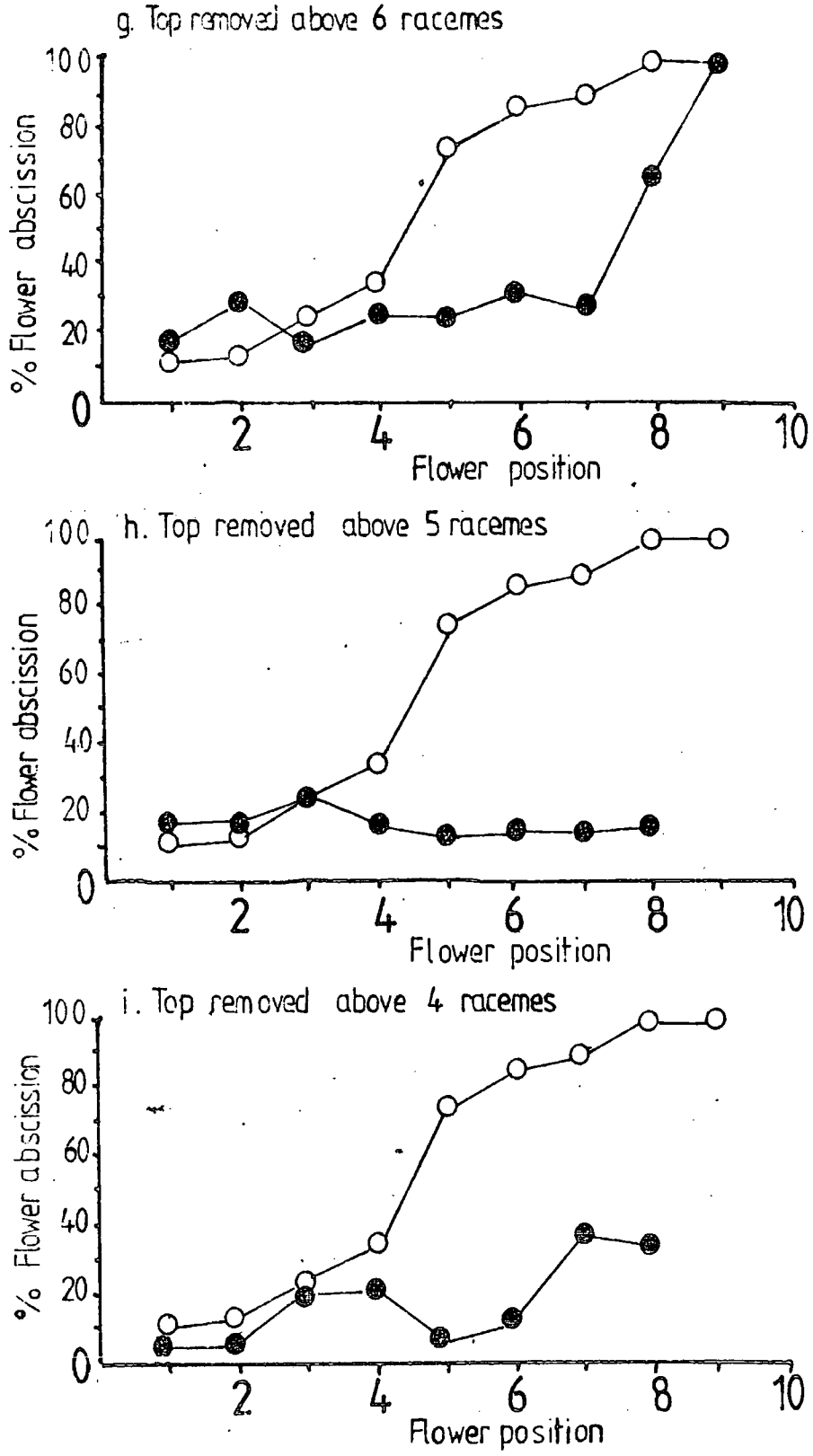



Figure 4.10: Influence of top removal on flower abscission for T2 plants. Each value is an overall percentage.
● = treatment plants,
o = control plants.

Table 4.10 Summary of results for $T_2$ plants subjected to top removal

| Treatment | Total % flowers dropped | Total % pods set | $\chi^2$, v = 1 |
|---|---|---|---|
| Control | 46.9 | 53.1 | |
| (g) Top removed after 6 flowering nodes | 25.9 | 74.1 | 22.63 (P> 0.001) |
| (h) Top removed after 5 flowering nodes | 16.1 | 83.9 | 41.86 (P> 0.001) |
| (i) Top removed after 4 flowering nodes | 14.8 | 85.2 | 41.15 (P> 0.001) |

a sharp increase in abscission of flowers on distal positions. A similar, although much less pronounced phenomenon was observed when tops were removed after four inflorescences had formed. When plants were decapitated after five flowering nodes, flowers on all positions experienced a similar low amount of abscission.

Analysis of yield components (Table 4.11) revealed, however, that the number of mature pods produced were reduced for all treatments. The number of mature pods was most drastically reduced, when plants whose tops had been removed after four inflorescences had formed.

These results indicate that decapitation of the apex can radically alter abscission within each remaining raceme. However the final yield figures indicate a detrimental effect of this type of treatment on the production of mature pods. This is perhaps due to the considerable reduction in leaf area resulting from the removal of the top of the plant.

The effect of surgical apex removal and decapitation on flower abscission

The apex of plants were removed above five raceme initials as distinct from decapitation of the apex after five axillary inflorescences had formed using plants of varieties Cockfield, Maris Bead and Deiniol. Decapitation of plants of these varieties was also performed as a comparison.

Both treatments resulted in a reduction of abscission within the racemes of all varieties tested. Removal of the apex had the most pronounced effect, which for the smaller seeded varieties Maris Bead and Deiniol, resulted in negligible abscission within remaining axillary racemes (Figures 4.11.1, 4.11.2). These results indicate a possible competitive effect

Table 4.11 Summary of average yield components of plants subjected to top removal and controls of inbred line $T_2$

| Treatment | Number of pods | Number of seeds | Seed weight per plant (g) | Weight of each seed (g) |
|---|---|---|---|---|
| (g) Top removed after six flowering nodes | 9.0 (1.27) | 31.0 (4.72) | 7.18 (1.48) | 0.227 (0.022) |
| (h) Top removed after five flowering nodes | 10.0 (2.42) | 34.5 (8.28) | 7.17 (0.62) | 0.234 (0.031) |
| (i) Top removed after four flowering nodes | 3.3 (0.72) | 10.0 (2.62) | 2.72 (0.78) | 0.274 (0.027) |
| Controls | 13.4 (1.83) | 47.5 (6.47) | 9.1 (1.34) | 0.191 (0.011) |

Standard errors are in parentheses.

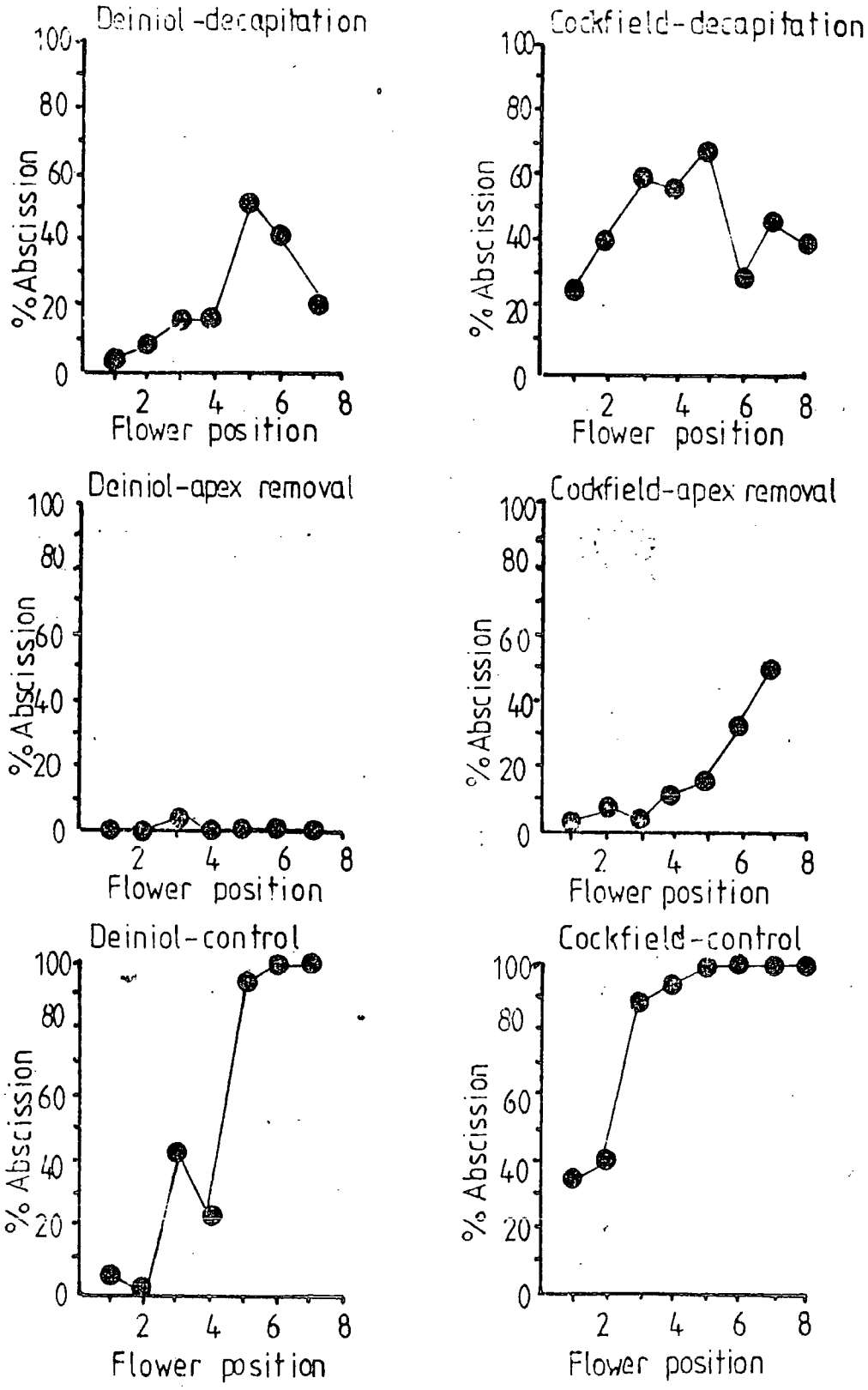



Figure 4.11.1: Effect of apex removal and decapitation on flower abscission. Each value is an overall percentage.

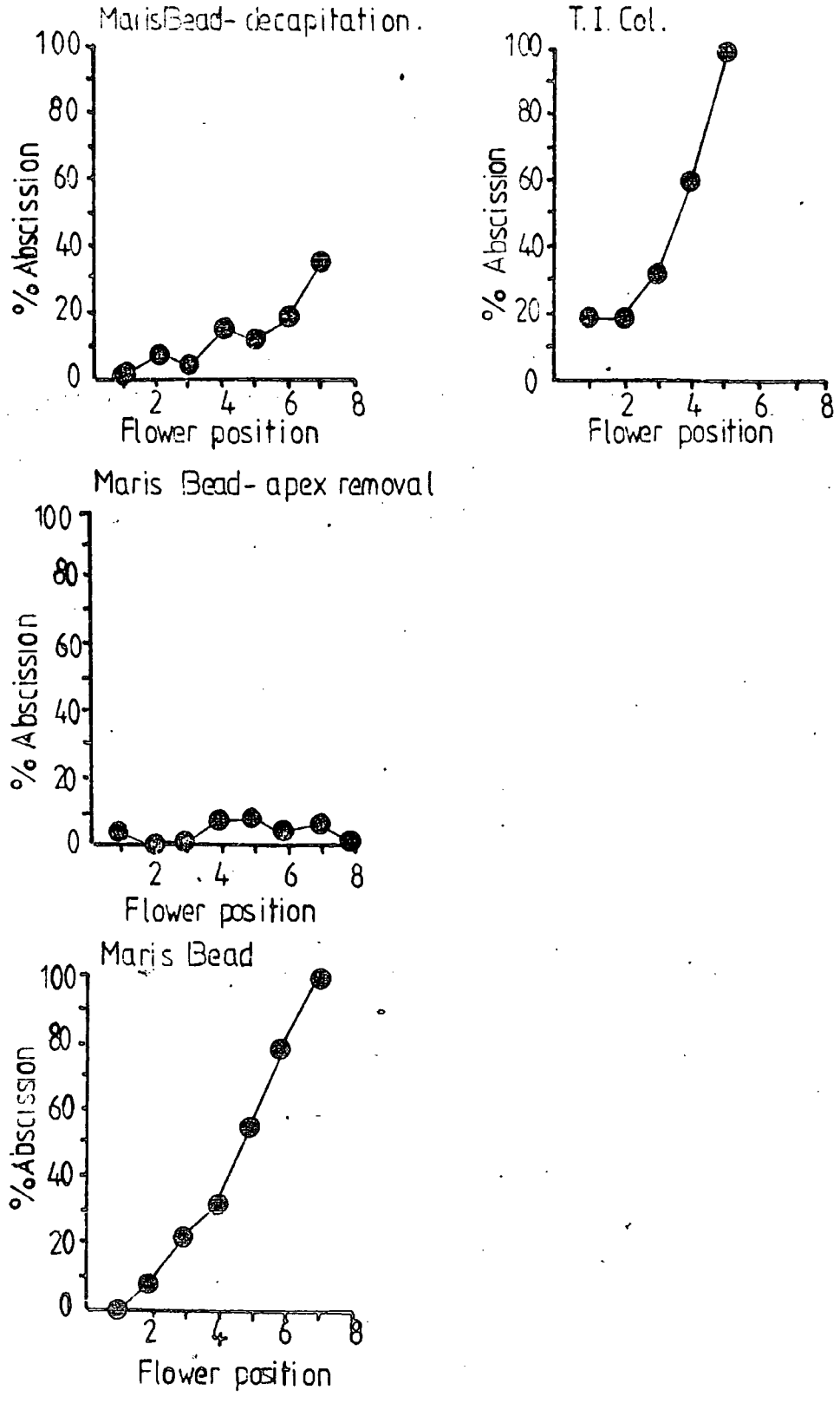



Figure 4.11.2: Effect of apex removal and decapitation on flower abscission. Each figure is an overall percentage.

between the apex and the reproductive parts of the plant. Apex removal could be regarded as mimicking the effect of the terminal inflorescence gene. However the reduction in abscission due to apex removal and decapitation was much more pronounced, than that experienced by TI Col., compared with control plants of the indeterminate genotypes. The genotype, TI Col, in fact, experienced a similar pattern of flower abscission to that of the control plants, in that distal flower positions had an abscission rate approaching 100%.

Abscission on remaining inflorescences (Figures 4.12.1, 4.12.2) was low, for all varieties tested, compared to the control plants. Subsequent pod drop was, however, very high compensating for the reduction in abscission (Table 4.12).

Control plants of Maris Bead and Deiniol yielded more mature pods over the same number of flowering nodes, compared to those plants subjected to apex removal and decapitation. Cockfield plants subjected to apex removal, produced slightly more mature pods and seeds, than control plants. Decapitated Cockfield plants produced slightly fewer pods, but slightly more seeds compared to control plants (Table 4.13).

The effect of leaf removal on flower abscission

All leaf removal treatments resulted in augmented flower abscission (Table 4.14). A greater increase was experienced by plants subjected to more severe leaf removal treatments.

When leaves on non-flowering nodes were removed (treatment (b) ), an increase in flower abscission within each raceme was experienced mainly on middle raceme positions compared to control plants. This pattern of abscission was emphasized by removing in addition alternate leaves on

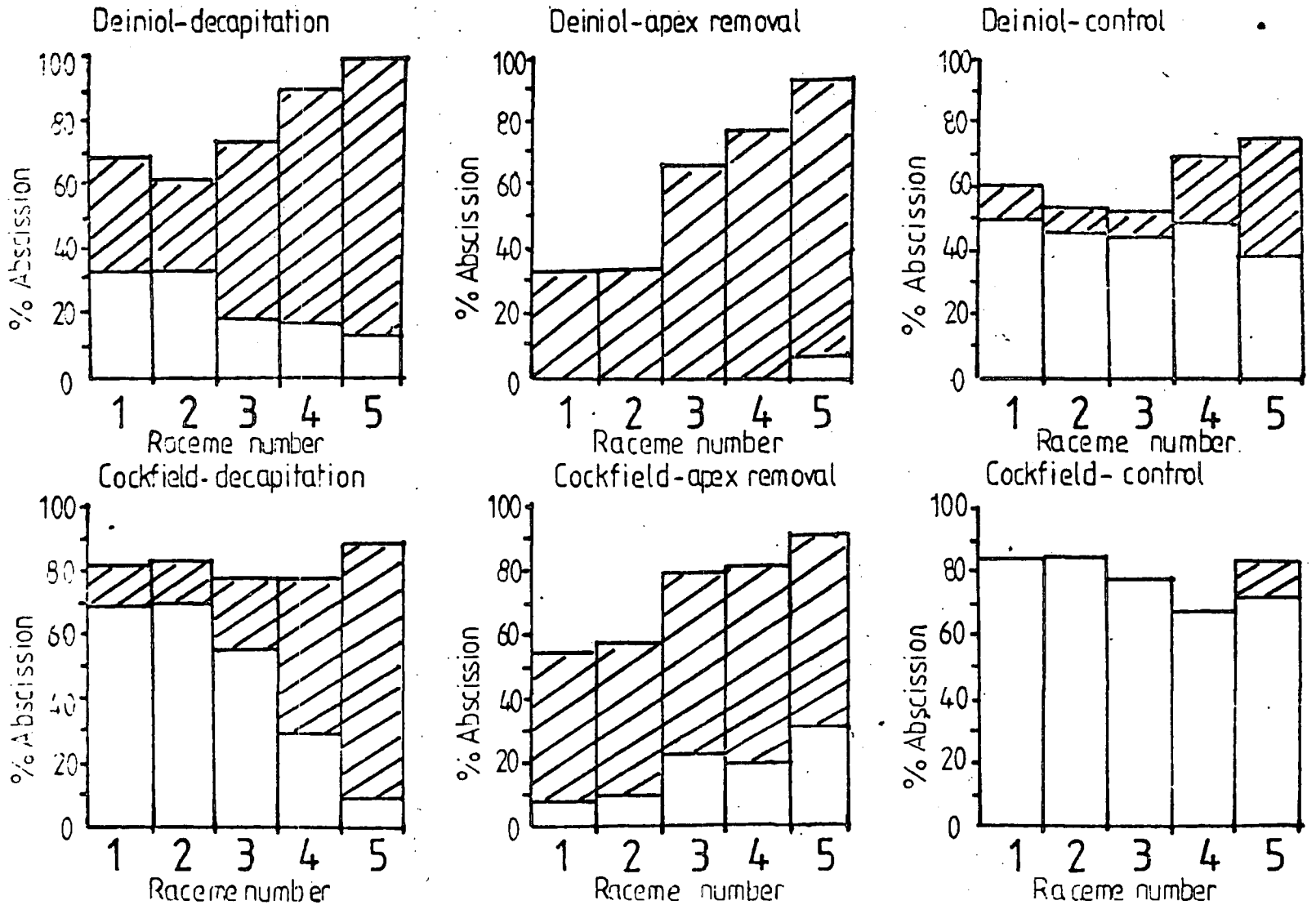



Figure 4.12.1: Effect of apex removal and decapitation on flower and pod abscission. Shaded area = young pod drop.

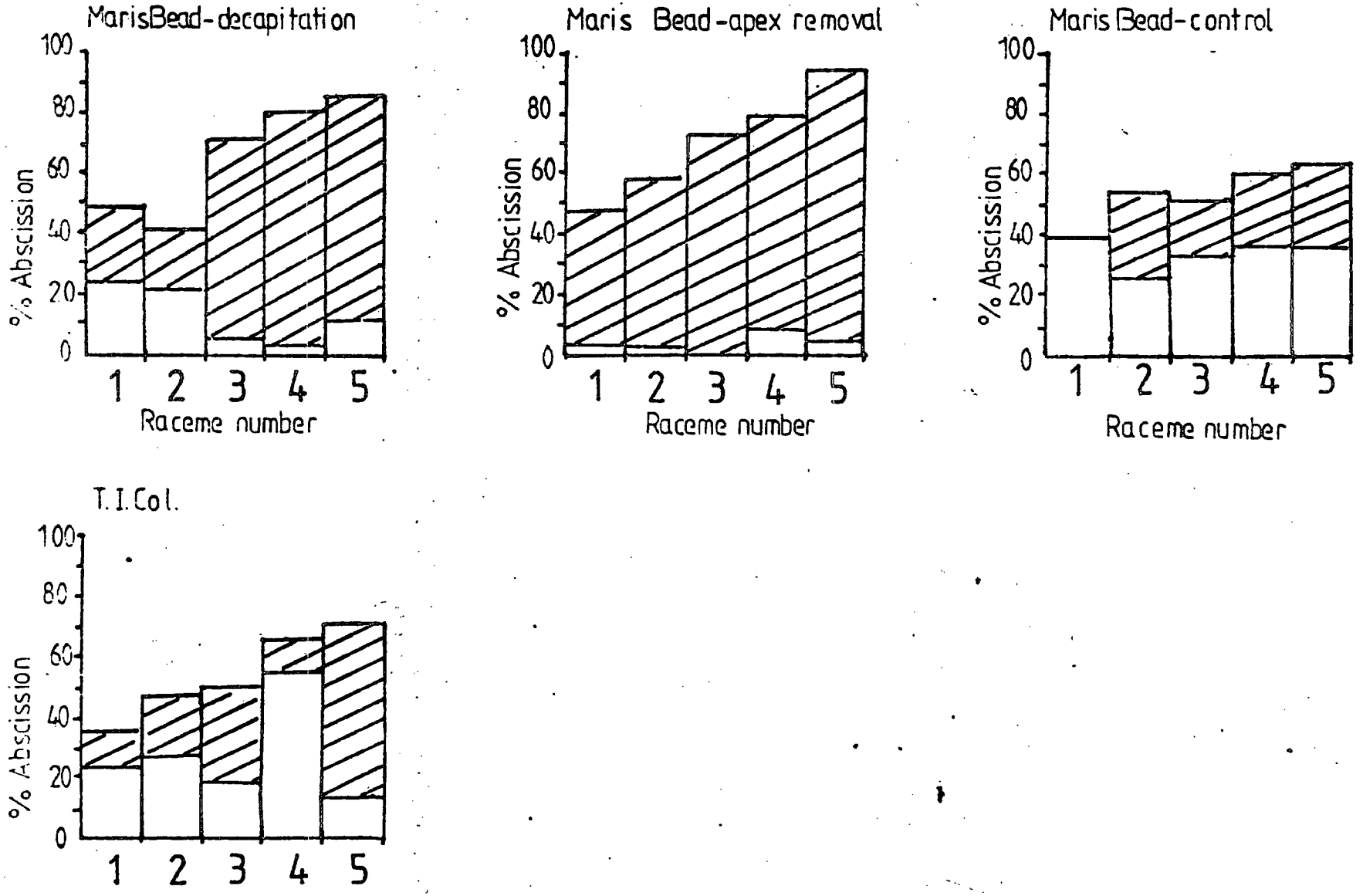



Figure 4.12.2: Effect of apex removal and decapitation on flower and pod abscission. Shaded area = young pod drop.

Table 4.12 Summary of results for apex removal and decapitation experiment

| Variety | Apex removal | Decapitation | Control | $\chi^2$ (v = 2) |
|---|---|---|---|---|
| Deiniol | | | | |
| Number of flowers dropped | 2 (1.40) | 33 (22.1) | 53 (44.9) | 73.1 (P> 0.001) |
| Number of pods set | 142 (98.6) | 116 (77.9) | 65 (55.1) | |
| Number of flowers and pods dropped | 118 (79.2) | 89 (61.8) | 73 (61.8) | 13.2 (P> 0.005) |
| Number of mature pods | 31 (20.8) | 55 (38.2) | 45 (38.2) | |
| Maris Bead | | | | |
| Number of flowers dropped | 7 (4.1) | 20 (12.3) | 48 (33.8) | 53.3 (P> 0.001) |
| Number of pods set | 162 (95.9) | 143 (87.7) | 94 (66.2) | |
| Number of flowers and pods dropped | 123 (72.8) | 109 (66.9) | 82 (57.7) | 7.8 (P > 0.025) |
| Number of mature pods | 46 (27.2) | 54 (33.1) | 60 (42.3) | |
| Cockfield | | | | |
| Number of flowers dropped | 29 (18.3) | 70 (43.5) | 98 (77.8) | 78.8 (P > 0.001) |
| Number of pods set | 129 (81.7) | 91 (56.5) | 28 (22.2) | |
| Number of flowers and pods dropped | 121 (76.6) | 131 (81.4) | 101 (80.1) | 6.1 (P > 0.05) |
| Number of mature pods | 37 (23.4) | 30 (18.6) | 25 (19.9) | |

Percentage values are in parentheses.
Each value is derived from 5 plants.

Table 4.13 Average yield components of apex removal and decapitation experiment

| Variety | Treatment | Mean pod numbers (S.E.) | Mean seed numbers (S.E.) | Mean seed weight (g) (S.E.) | Mean number of seeds/pod |
|---|---|---|---|---|---|
| Deiniol | Apex removal | 11.0 (1.0) | 31.6 (3.2) | 14.0 (1.5) | 2.9 |
| | Decapitation | 6.2 (0.9) | 16.2 (2.0) | 6.9 (1.0) | 2.6 |
| | Control | 11.2 (0.7) | 36.7 (2.2) | 19.4 (1.7) | 3.3 |
| Maris Bead | Apex removal | 9.2 (0.9) | 23.8 (2.9) | 9.9 (1.6) | 2.6 |
| | Decapitation | 10.8 (0.7) | 29.0 (2.4) | 13.0 (1.7) | 2.7 |
| | Control | 12.0 (0.6) | 39.0 (4.6) | 16.5 (1.7) | 3.2 |
| Cockfield | Apex removal | 7.2 (2.1) | 17.8 (5.2) | 12.6 (3.9) | 2.5 |
| | Decapitation | 6.0 (0.9) | 15.2 (2.5) | 8.8 (2.1) | 2.5 |
| | Control | 7.0 (0.7) | 15.0 (2.6) | 11.9 (2.1) | 2.1 |
| TI Col. | Control | 6.6 (1.2) | 18.6 (5.2) | 11.4 (2.6) | 2.8 |

Standard errors are in parentheses.

Table 4.14 Summary of results for leaf removal experiments on variety Cockfield

| Treatment | Total % flowers dropped | Total % pods set | $\chi^2$, v = 1 |
|---|---|---|---|
| (a) Control | 76.8 | 23.2 | |
| (b) | 81.0 | 19.0 | 3.62 ($P > 0.1 < 0.05$) |
| (c) | 82.3 | 17.7 | 6.67 ($P > 0.01 < 0.005$) |
| (d) | 85.5 | 14.5 | 18.35 ($P > 0.001$) |
| (e) | 86.3 | 13.7 | 16.31 ($P > 0.001$) |
| (f) | 97.7 | 2.3 | 98.04 ($P > 0.001$) |
| (g) | 82.3 | 17.7 | 6.29 ($P > 0.025 < 0.01$) |
| (h) | 86.4 | 13.6 | 18.24 ($P > 0.001$) |

(b) all leaves on vegetative nodes removed

(c) leaves on vegetative nodes not removed, but alternate leaves subtending racemes removed from first flowering node upwards

(d) as (c) but from second flowering node upwards

(e) as (c) but leaves from vegetative nodes also removed

(f) All leaves removed

(g) leaves only removed on vegetative nodes on the apical portion of the plant

(h) all leaves subtending inflorescences removed

flowering nodes (treatments (c), (d) and (e) ). Further, in these cases, more abscission occurred on proximal flower positions (Figure 4.13.1), although it is only at these proximal positions where an increase in flower abscission can take place, since flower abscission at distal raceme positions was close to 100%.

Removal of alternate leaves from the second flowering node upwards (treatment (d) ) produced a more pronounced effect on abscission within each raceme than removing alternate leaves from the first flowering node upwards (treatment (e) ). All other treatments, with the exception of treatment (f), where all leaves were removed produced similar increases in abscission within each raceme to that described above. Removal of all the leaves on plants, resulted in almost total abscission of all flowers at all raceme positions.

Abscission on distal inflorescences was less for control plants (Figures 4.14.1, 4.14.2) than for plants subjected to most of the leaf removal treatments.

There was no difference in total abscission between axillary racemes on the same plant subtending intact and removed leaves (treatments (c), (d) and (e) ) (Table 4.15). This is evidence that the recorded increase in flower abscission due to leaf removal is distributed over all inflorescences and not concentrated on racemes situated on the same nodes as the removed leaves.

Analysis of yield components (Table 4.16) revealed that for all treatments there was a reduction in the number of mature pods and seeds produced compared with control plants. The reduction was proportional to the severity of the treatment applied.

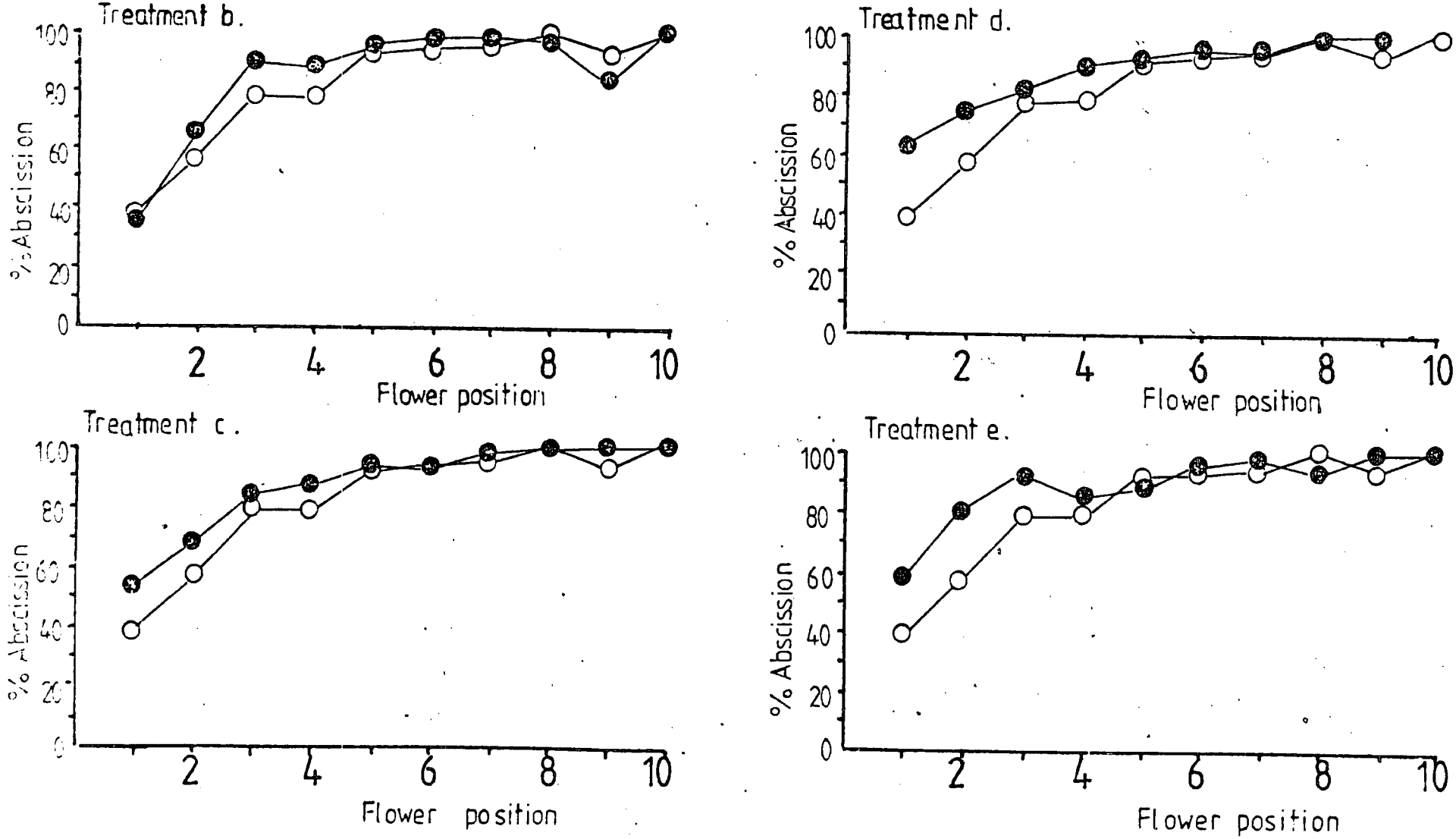



Figure 4.15.1: Influence of leaf removal on flower abscission of variety Cockfield. Each value is an overall percentage.
● = treatment plants, o = control plants.

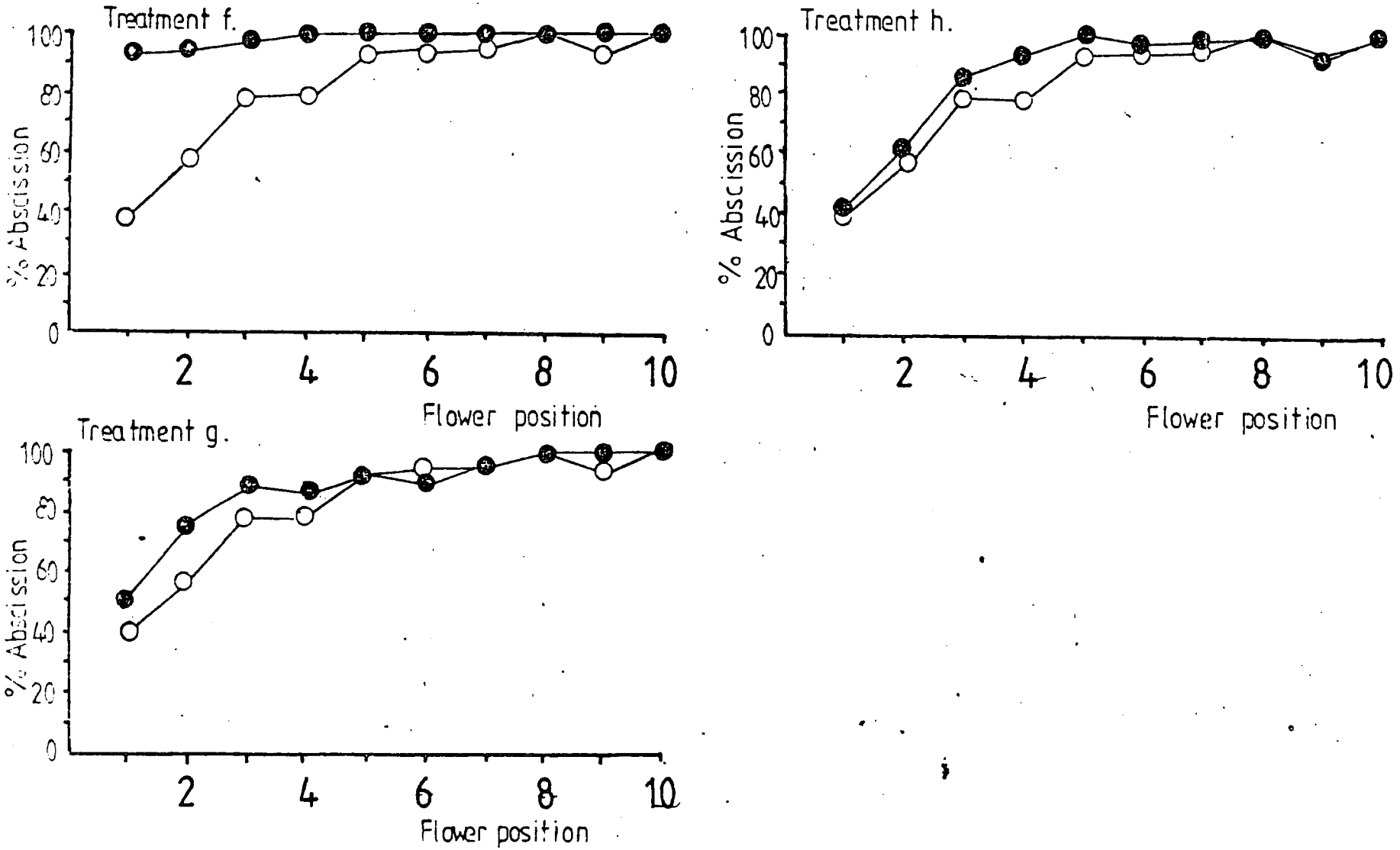



Figure 4.15.2: Influence of leaf removal on flower abscission for variety Cockfield. Each value is an overall percentage.
● = treatment plants; o = control plants.

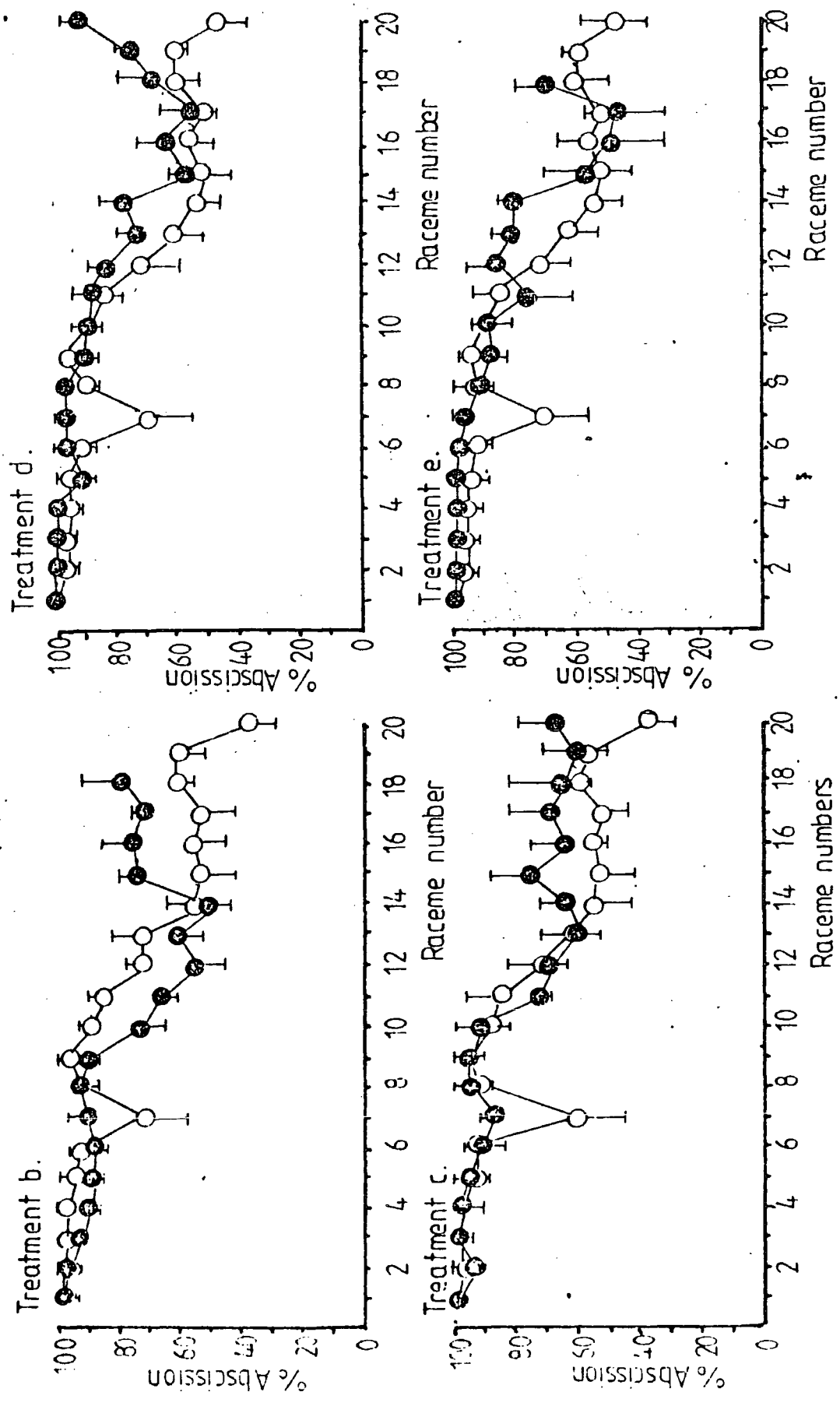



Figure 4.14.1: Influence of leaf removal on flower abscission for variety Cockfield. Each value is an average percentage. Standard errors are represented by a bar.
● = treatment plants; o = control plants.

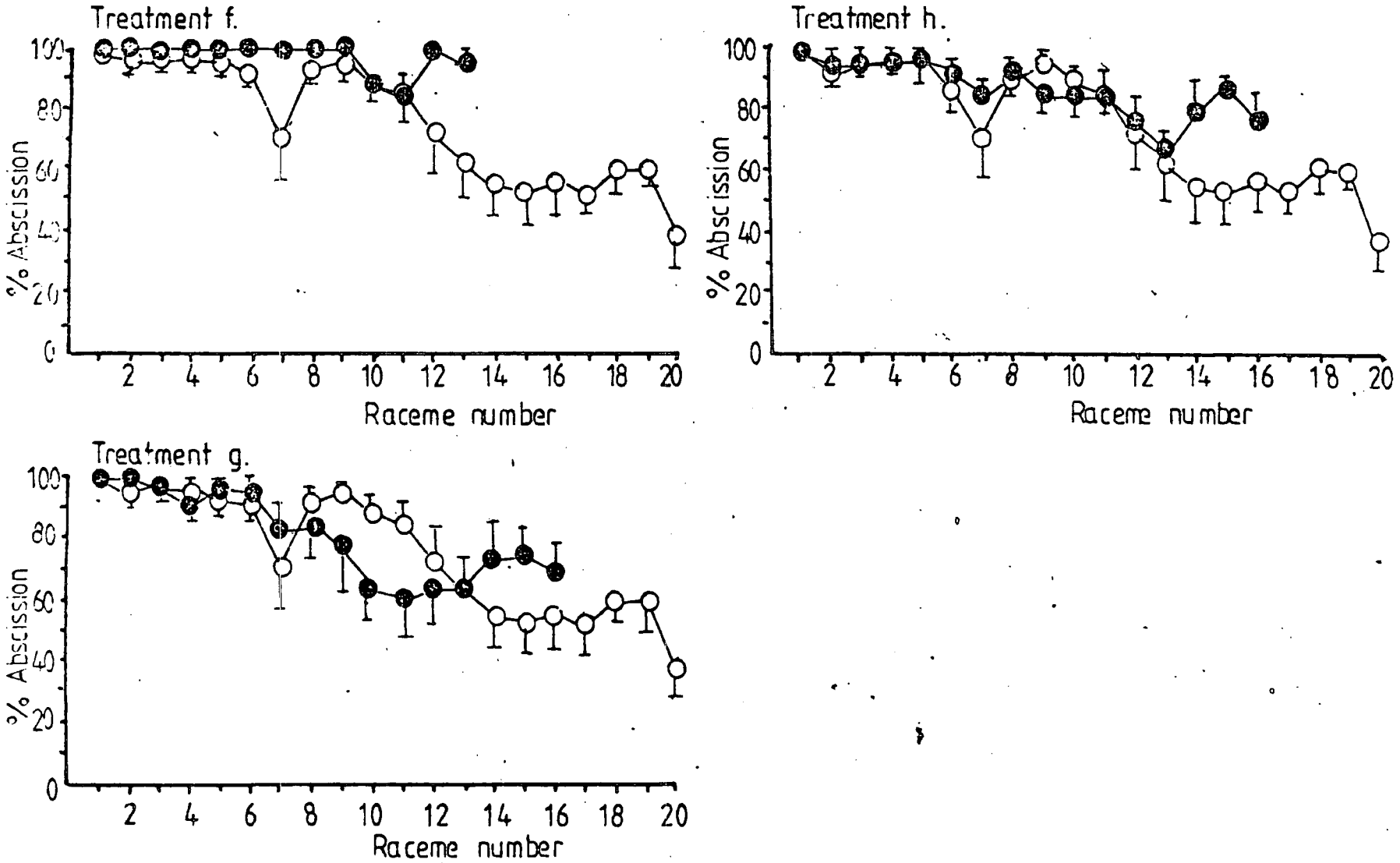



Figure 4.14.2: Influence of leaf removal on flower abscission for variety Cockfield. Each value is an average percentage. Standard errors are represented by a bar. ● = treatment plants; o = control plants.

Table 4.15 Comparison of overall flowers dropped and pods set between racemes subtending removed and intact leaves

| Treatment | | flowers dropped (%) | pods set (%) | $\chi^2$, v = 1 |
|---|---|---|---|---|
| (c) | intact | 279 (83.5) | 55 (16.5) | 0.58 |
| | removed | 269 (81.3 | 62 (18.7) | (P< 0.1) |
| (d) | intact | 306 (84.3) | 57 (15.7) | 1.96 |
| | removed | 306 (87.9) | 42 (12.1) | (P< 0.1) |
| (e) | intact | 190 (84.4) | 35 (15.6) | 1.29 |
| | removed | 207 (99.1) | 28 (11.9) | (P< 0.1) |

Percentage figures are in parentheses.

Table 4.16 Average yield components of Cockfield plants subjected to leaf removal

| Treatment | Pods/ plant | Seeds/ plant | Dry seed weight (g)/plant | Weight of each seed (g) |
|---|---|---|---|---|
| (a) Control | 8.0 | 23.4 | 13.1 | 0.59 |
| (b) | 7.0 | 20.8 | 12.4 | 0.61 |
| (c) | 5.0 | 16.8 | 10.0 | 0.55 |
| (d) | 4.9 | 14.6 | 8.4 | 0.60 |
| (e) | 3.2 | 8.0 | 4.9 | 0.77 |
| (f) | 0.0 | 0.0 | 0.0 | 0.00 |
| (g) | 3.4 | 12.0 | 6.7 | 0.67 |
| (h) | 2.5 | 7.4 | 5.4 | 0.88 |

For explanation of treatments see Table 4.14.

The effect of complete leaf shading on flower abscission

Shading leaves with silver foil instead of removing them resulted in their ultimate abscission.

All treatments caused, on average, an increase in flower abscission within each raceme and on every inflorescence, similar to that observed in the previous leaf removal experiment (Figures 4.15, 4.16, Table 4.17).

Comparison of overall flower abscission on racemes situated on nodes with shaded leaves to those on nodes with unshaded leaves (Table 4.18) indicated, as before, that the observed increase in abscission was distributed over all racemes. It was not confined to racemes situated on the same nodes as the shaded leaves.

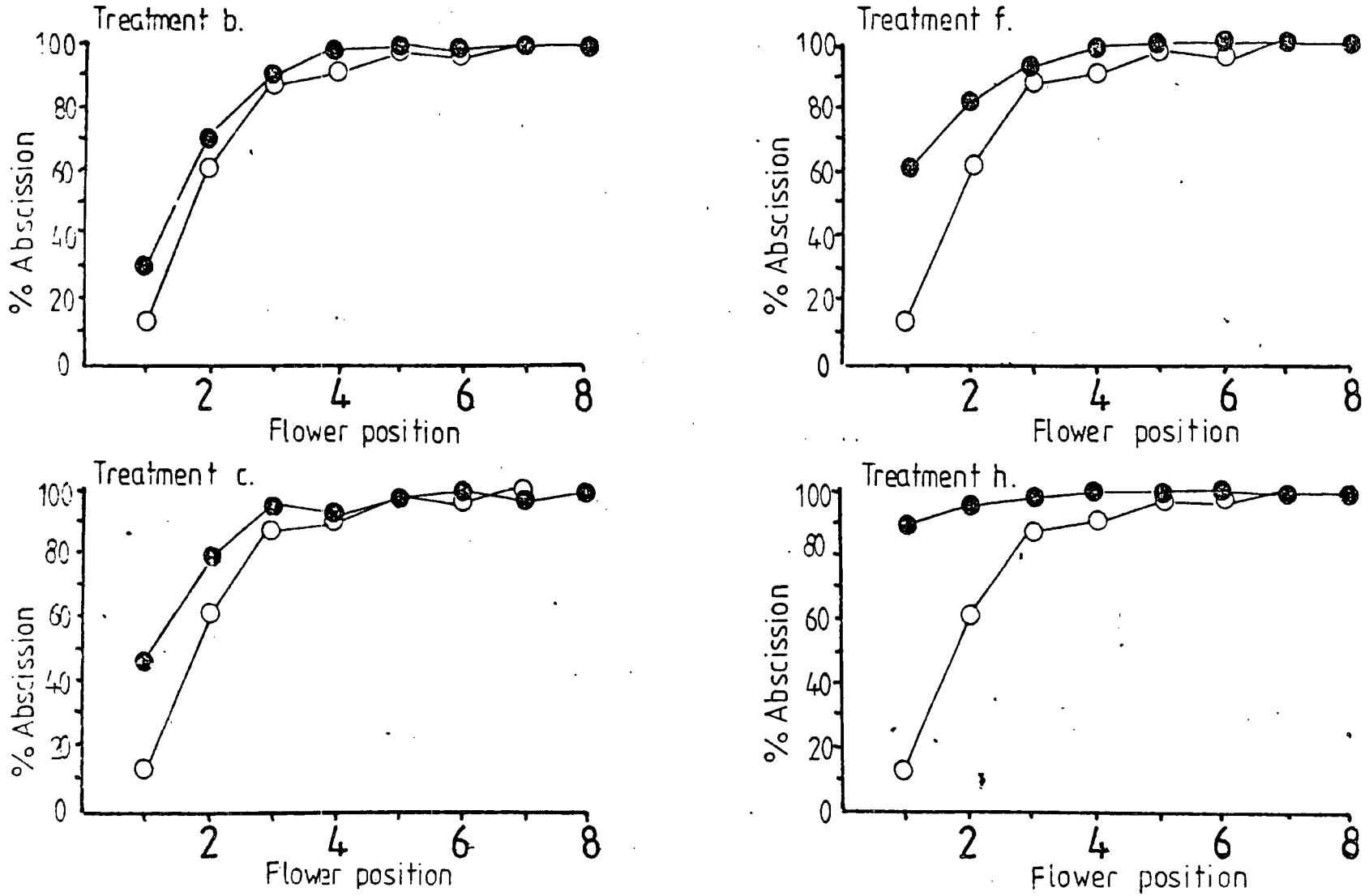



Figure 4.15: Influence of complete leaf shading on flower abscission of variety Deiniol. Each value is an average percentage. o = control; ● = treatment.

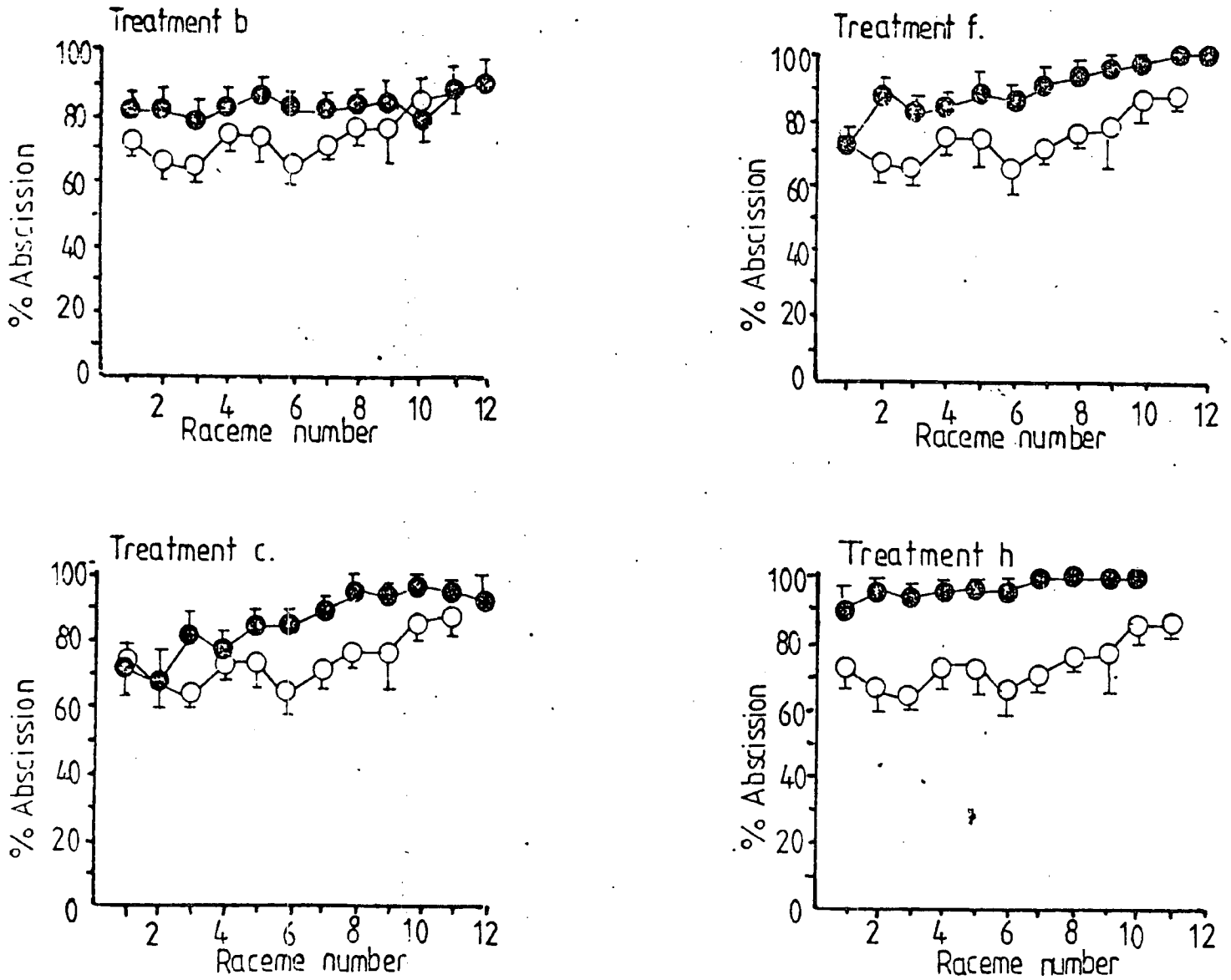



Figure 4.16: Influence of complete leaf shading on flower abscission of variety Deiniol. Each value is an average percentage. Standard errors are denoted by a bar. o = control values, ● = treatment values.

Table 4.17 Summary of results for complete leaf shading experiment

| Treatment | Total % flowers dropped | Total % pods set | $\chi^2$, v = 1 |
|---|---|---|---|
| Control | 74.6 | 25.4 | |
| treatment (b) | 84.1 | 15.9 | 10.64 (P > 0.005 < 0.001) |
| treatment (c) | 87.4 | 12.6 | 21.26 (P > 0.001) |
| treatment (h) | 90.9 | 9.1 | 36.48 (P > 0.001) |
| treatment (f) | 97.6 | 2.4 | 73.37 (P > 0.001) |

Table 4.18 Comparison of overall flowers dropped and pods set between racemes subtending shaded and unshaded leaves

| Treatment | Flowers dropped (%) | Pods set (%) | $\chi^2$, v = 1 |
|---|---|---|---|
| treatment (c) | | | |
| not shaded | 179 (86.5) | 28 (13.5 | 0.043 (P < 0.1) |
| shaded | 190 (87.1) | 28 (12.9) | |

Percentage figures are in parentheses.

# CHAPTER 5

# CELLULAR CHANGES IN THE FLORAL ABSCISSION ZONE OF FABA BEANS AND OTHER LEGUMES

## Introduction

Evidence given in Chapter 4 indicated that many fertilized flowers still abscise. Apex removal, decapitation and removal of proximally formed inflorescences all decreased flower drop. When apex removal and decapitation experiments were performed, however, high pod drop subsequently occurred, negating the observed reduction in flower abscission.

The mode and mechanism of flower shedding and pod set is, however, poorly understood in V. faba. In this chapter a description of the cellular changes occurring in the pedicel and peduncle associated with flower abscission and pod set is made.

## Morphology of the pedicel and peduncle at anthesis

At anthesis in line 22 the pedicel had a diameter of approximately 1 mm and was composed of five central vascular bundles (three large and two small) surrounded by six ranks of thin-walled cortical cells (Figure 5.1(a) ). The number and cross-sectional area of the vascular bundles varied between genotypes. At the pedicel/peduncle junction a collar of thick walled meristematic cells with dense cytoplasm surrounded the vascular bundles (Figure 5.1(b) ). Large reserves of starch were present in the sheath around the vascular bundles and in the parenchyma cells between them (Figure 5.1)c) ).

## Changes during early pod set

During pod set the pedicel underwent a massive increase in volume, with an approximately six-fold increase in diameter

Figure 5.1: Cellular changes of the pedicel/peduncle junction.

(a) Scanning-electron micrograph of the pedicel/peduncle junction in Vicia faba L. at anthesis, showing vascular arrangement in the pedicel X250.

(b) Tangential L.S. through the pedicel/peduncle junction X400.

(c) L.S. through pedicel/peduncle junction showing starch sheath around vascular tissue X500.

(d) Characteristically thick walls of parenchymatous cells within the pedicel vascular cylinder of a young pod X2300.

(e) Cell separation at the pedicel/peduncle junction immediately prior to flower shedding. Note that cells have separated along cell walls (darts) X2500.

(f) Development of the annular separation of cells at the pedicel/peduncle junction (darts) X400.

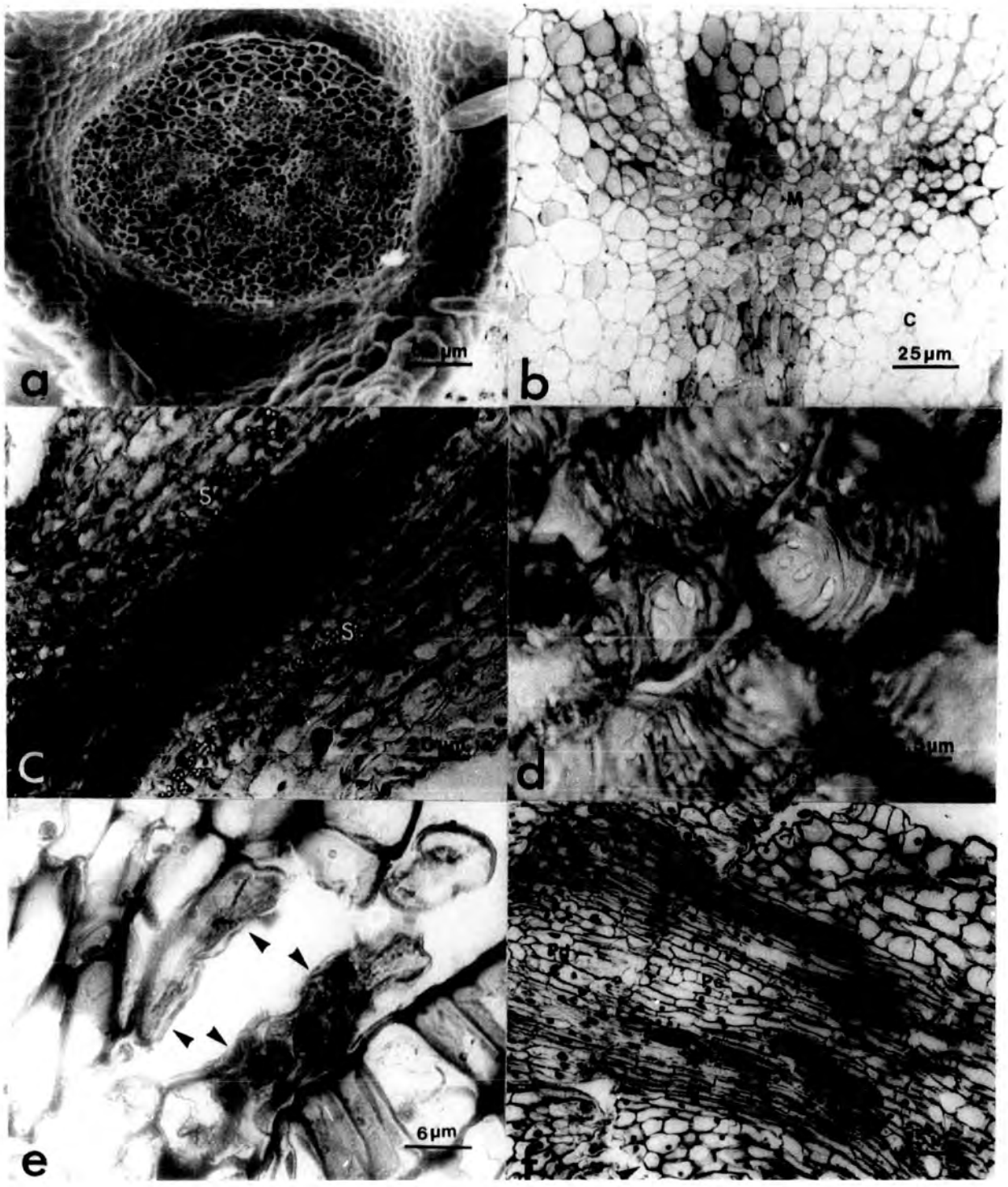

a
b
M
C
25µm
S
S
C
d
e
6µm
f

Plate abbreviations

Pc: Pedicel

Pd: Peduncle

Vb: Vascular bundle

M: Collar of thick walled meristematic cells

C: Cortical cells

S: Starch grains

Ep: Eipidermal cells

occurring between anthesis and the time of maximum pod fresh-weight. Cell expansion began immediately after fertilization and the cortical cells of the pedicel increased in diameter from 45 $\mu$m to 70 $\mu$m, in the first 72 h after pollination, concurrently with ovary swelling. After this phase of cell expansion stored starch could no longer be detected around the vascular bundles.

The above initial phase of cell expansion was followed by cell division and expansion in the collar of thick-walled meristematic cells at the pedicel/peduncle junction. The cross-sectional area of the vascular tissue increased approximately four-fold between anthesis and the development of a pod 2 cm long. A cylinder of vascular tissue was formed, penetrated by a narrow row of parenchyma cells. Parenchymatous cells within the vascular cylinder showed a characteristic wall thickening (Figure 5.1 (d) ).

Changes during flower shedding

The onset of flower abscission was initiated by the development of an annular split at the pedical/peduncle junction extending from the epidermis to the vascular bundles (Figure 5.1(f) ). This occurred by separation of cells rather than by cell fracture (Figures 5.1(e), 5.2(a) ). Ultrastructural examination of the peduncle surface after pedicel separation revealed that the separation of cells in the thickened meristematic collar had occurred by disruption of the middle lamella, leaving a primary wall enclosing the protoplasts of the exposed peduncle cells (Figure 5.2(b) ). Separation of the cellulose microfibrils was also apparent close to the middle lamella between cells of the surface layer (Figure 5.2(b), Star). The protoplasts of the surface layer

Figure 5.2: Light and transmission electron micrographs of peduncle immediately after abscission.

(a) Light micrograph of a toluidine blue stained 1 $\mu$m section of resin-embedded peduncle fixed immediately after flower abscission showing collar of meristematic cells and location of Fig. 2(b) X100.

(b) Electron micrograph of serial thin section to Fig. 2a showing detail of cells of exposed peduncle, separation of wall microfibrils near meddle lamella (star), numerous dictyosomes (circles) and fusion of vesicles with plasmalemma (arrows) X10,000.


(pedicel)
b
a
peduncle

cells contained a greater cytoplasm/vacuole ratio than adjacent cells within the peduncle. The cytoplasm contained numerous free and bound ribosomes, mitochondria with many conspicous cristae, plastids and many dictyosomes (circles Figure 5.2(b) ). The presence of numerous cytoplasmic vesicles and the appearance of the plasmalemma including fusion of vesicles (small arrows Figure 5.2(b) ) suggested that transport of vesicle-bound material across the membrane heterochromatin was conspicuously abundant in the nuclei of surface cells.

Flowers were shed shortly after the development of the annular split of cortical cells at the pedicel/peduncle junction. Scanning electron microscopy (SEM) of the peduncle abscission scar revealed that the ring of cortical cells had become enormously inflated (Figure 5.3(a) ). Light microscopy and SEM observations often revealed expanded coils of thickened xylem protruding from the abscission scar, where the inflation of the cortical cells had stretched the vascular connections until they had finally ruptured. Similar expanded cortical cells were observed on the abscised pedicel base (Figure 5.3(f) ).

Following pedicel separation the inflated cortical cells rapidly collapsed and a new epidermal layer eventually covered the scar left by the abscised flower (Figure 5.3(b) ).

The inflated cortical cells were also visible in free hand cut sections of peduncles and by manipulating the osmotic medium they could be made to expand or collapse.

Flower abscission in other legumes

The floral abscission scars on the peduncles of a range of legumes were examined and in every case expanded cortical

Figure 5.3: Scanning electron micrographs of the pedicel scar on the peduncle after flower shedding.

(a) Ring of cortical cells on the peduncle of V. faba L., immediately after separation of the pedicel. Note the fractured vascular bundle X230.

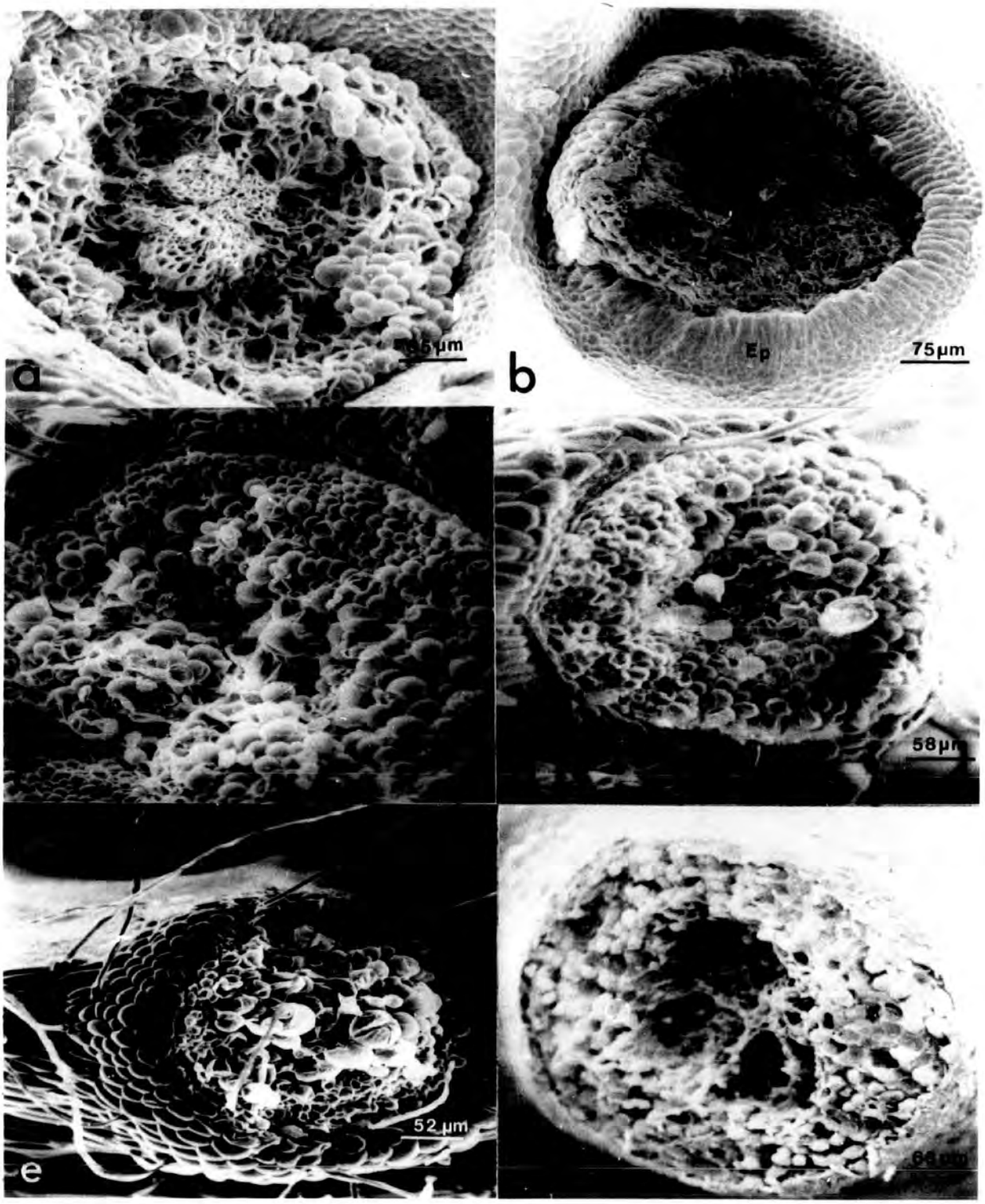
(b) Regrowth of epidermal cells over the pedicel scar of V. faba L., after collapse of inflated cortical cells X200.

(c) Pedicel scar on the peduncle of V. pannonica c.v. magledi, showing inflated cortical cells X200.

(d) Peduncle of Phaseolus vulgaris c.v. 519N, showing inflated cortical cells X260.

(e) Peduncle of Vicia villosa Roth. c.v. Kartali, showing inflated cortical cells X270.

(f) Cortical cells on the pedicel base of Vicia faba L. minor c.v. Maris Bead X250.


a
b
Ep
75µm
58µm
52 µm
e

cells, similar to those seen in V. faba were recorded (Figures 5.3(c), (d), (e) ).

Starch degradation and cell expansion during flower abscission and pod development in Vicia faba L.

Amylase was assayed by radial diffusion in an agarose gel (Figures 5.4, 5.5(a) ). Large reserves of starch were present in two rows of cells which form a sheath around the vascular bundles at anthesis (Figures 5.6(a), (b) ). Cell expansion occurred immediately after fertilization in both vascular and parenchyma tissues of the pedicel/peduncle region. After this phase of cell expansion starch could no longer be detected around the vascular tissue (Figure 5.6(c).

The disappearance of starch is concomitant with a dramatic rise in amylase concentration which occurred 12 h after pollination (Figure 5.5(b) ). This rise in amylase occurs at a critical point during flower development (anthesis) (Figure 5.7). After this increase has occurred the amylase concentration drops back to a lower level during further developmental stages.

The role of the enzymes pectin-methylesterase and pectinase in abscission

Pectin-methylesterase and pectinase were assayed by radial diffusion in an agarose gel (Figures 5.8, 5.9). Pectinase could be detected in pedicel/peduncle junctions at developmental stages 8 and 9 only (Figure 5.10). The greatest concentration of pectin-methylesterase in junctions was also detected at the same developmental stages (Figure 5.11). This enzyme, however, was also present at other developmental stages. In the case of pectinase, no detectable enzyme was present in abscised pedicel/peduncle junctions. In addition there was less pectin-methylesterase detectable in abscised

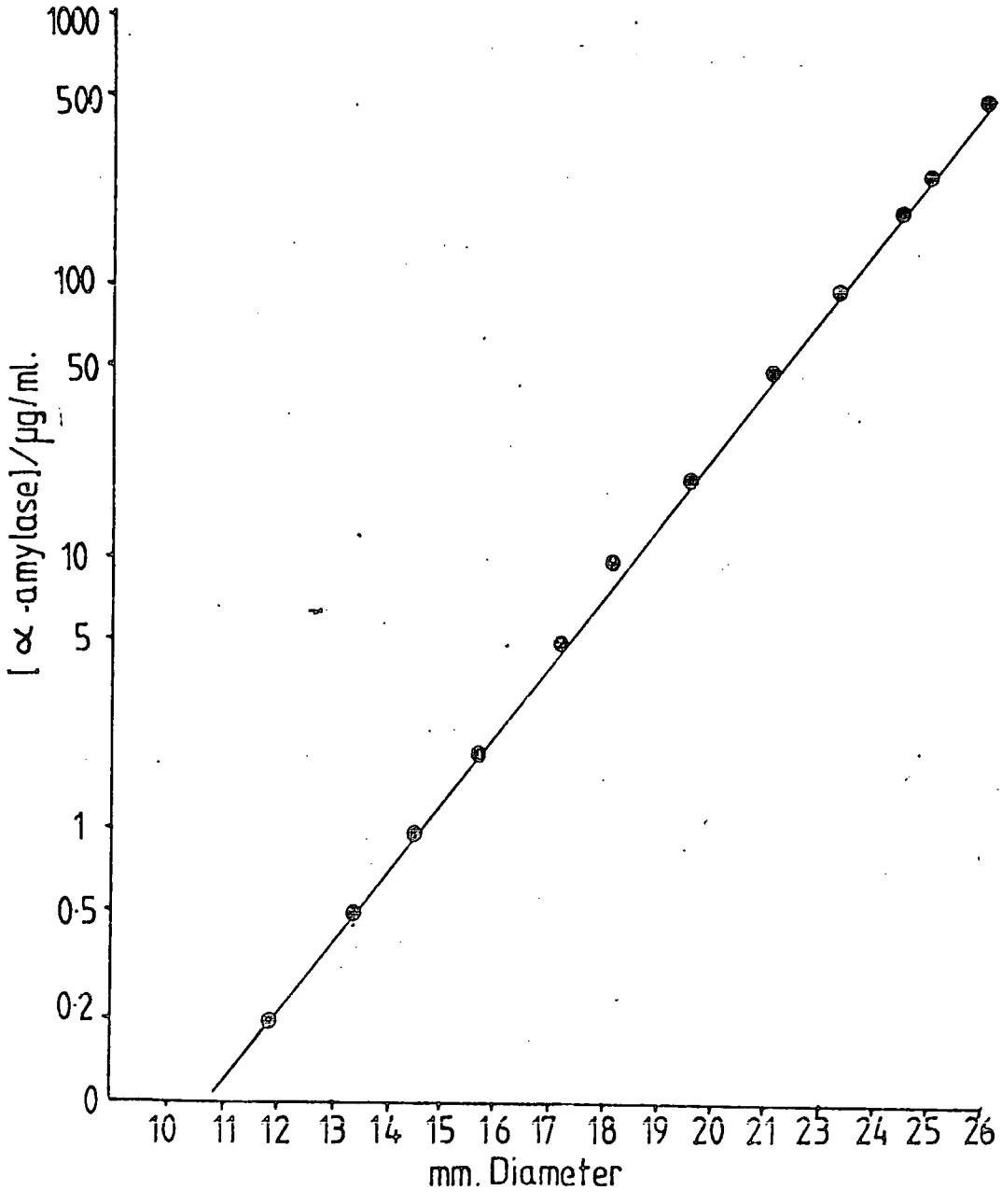



Figure 5.4: Standard curve for α-amylase assay.
Standard errors are denoted by a bar.

Figure 5.5: A = Standard curve for the $\alpha$-amylase assay.

B = Replicate samples of pedicel/peduncle junctions at different developmental stages.

h = flower left untripped at stage 8-9

b = stage 7

f = emasculated and pollinated at stage 5.

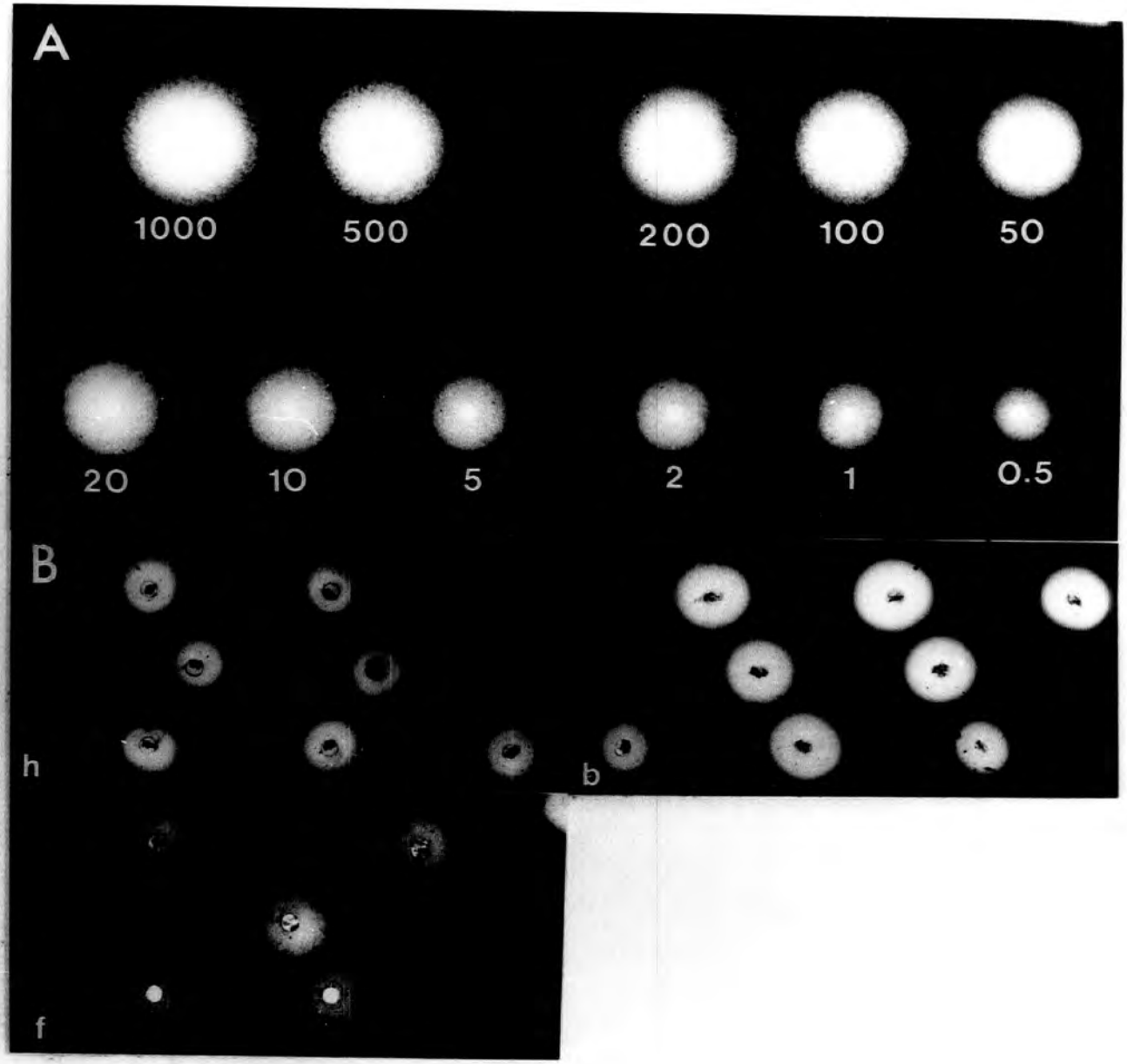
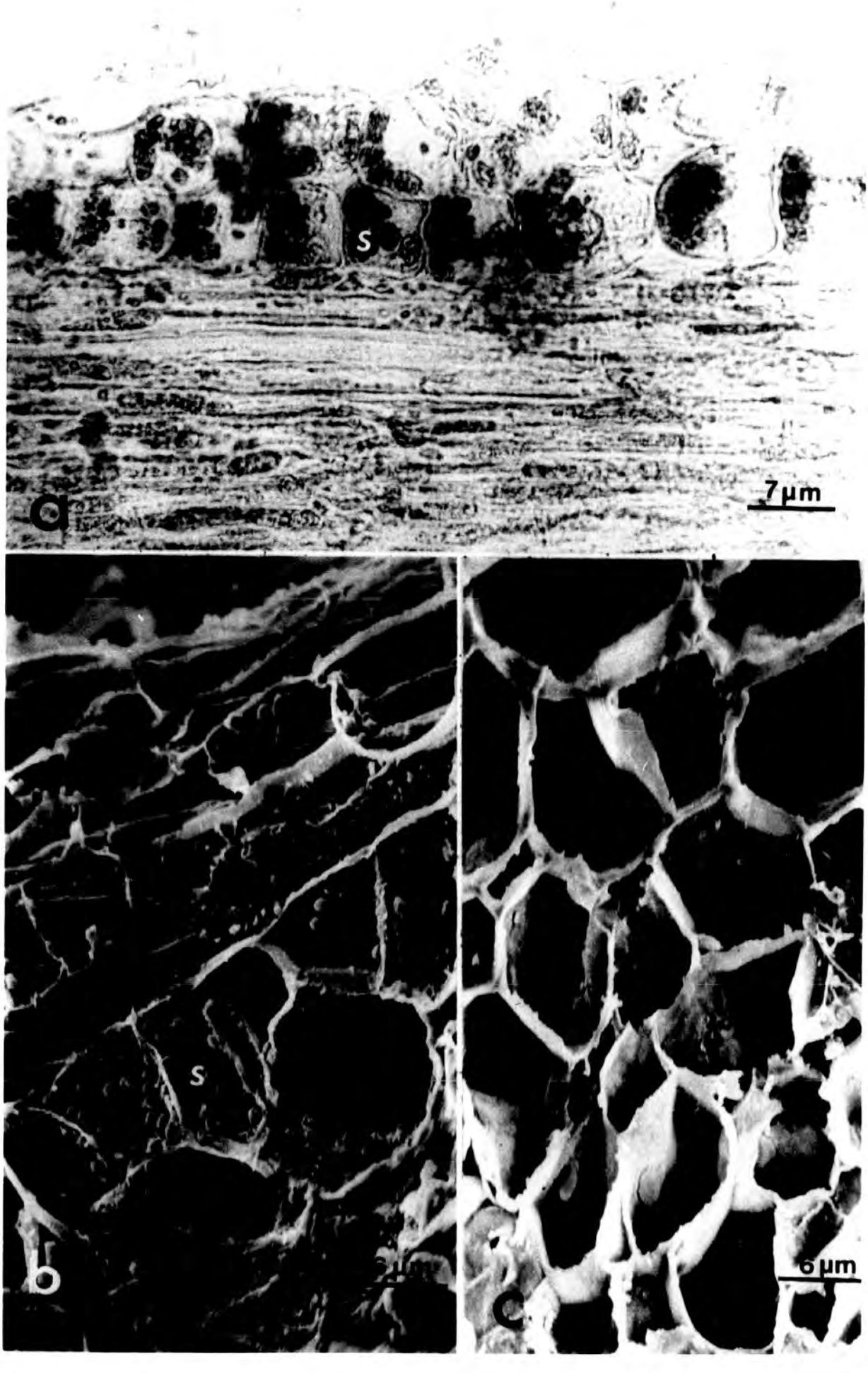

A
1000
500
200
100
50
20
10
5
2
1
0.5
B
h
b
f

Figure 5.6: (a) Double layer of starch containing cells around a vascular bundle of a pedicel/ peduncle junction at anthesis X2000.

(b) Scanning electron micrograph of (a), X2100.

(c) Scanning electron micrograph of a pedicel/ peduncle junction at early pod set, note lack of starch grains X2100.

All specimens are of Vicia faba L. minor inbred line 22.


S
7 µm
a
S
b
6 µm
c

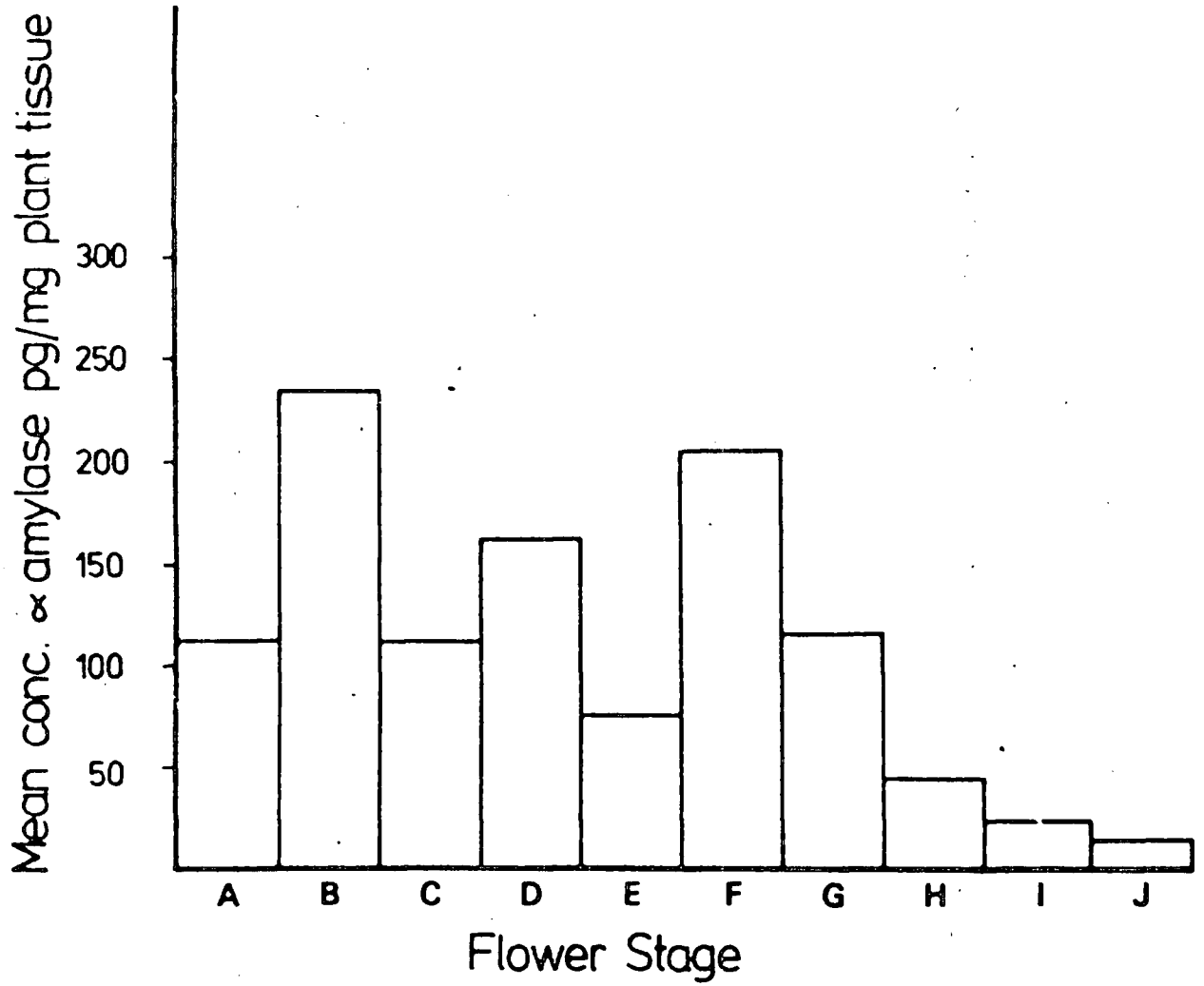



Figure 5.7: Change in ∝-amylase concentration in the pedicel/peduncle junctions of flowers at different developmental stages.

A = stage 6; B = stage 7; C = stage 8; D = stage 8/9; E = stage 9; F = emasculated and pollinated at stage 5; G = emasculated only at stage 5; H = flower left untripped at stage 8/9; I = abscised flower; J = early pod set.

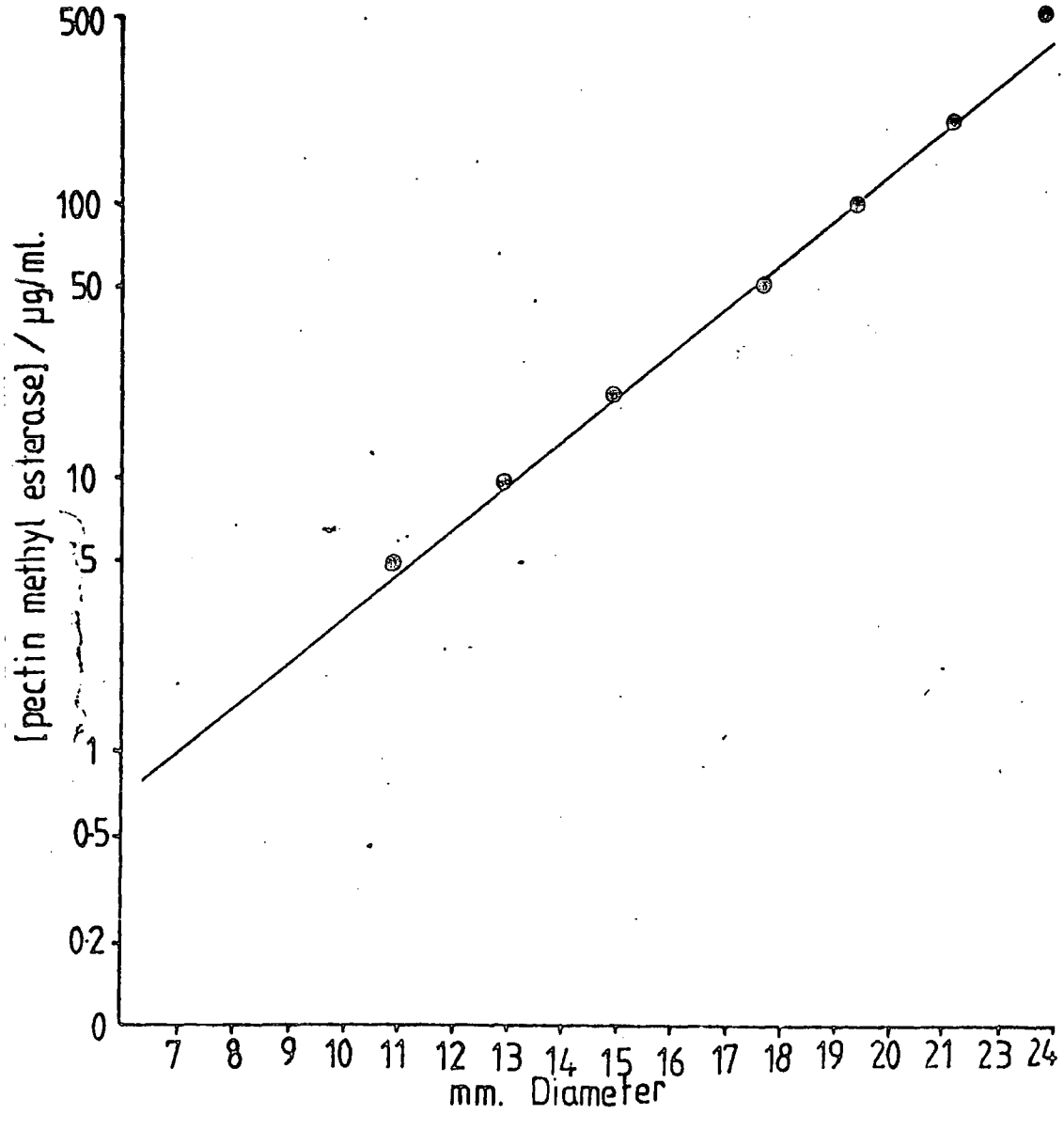



Figure 5.8: Standard curve for pectin methyl esterase. Standard errors are denoted by a bar.

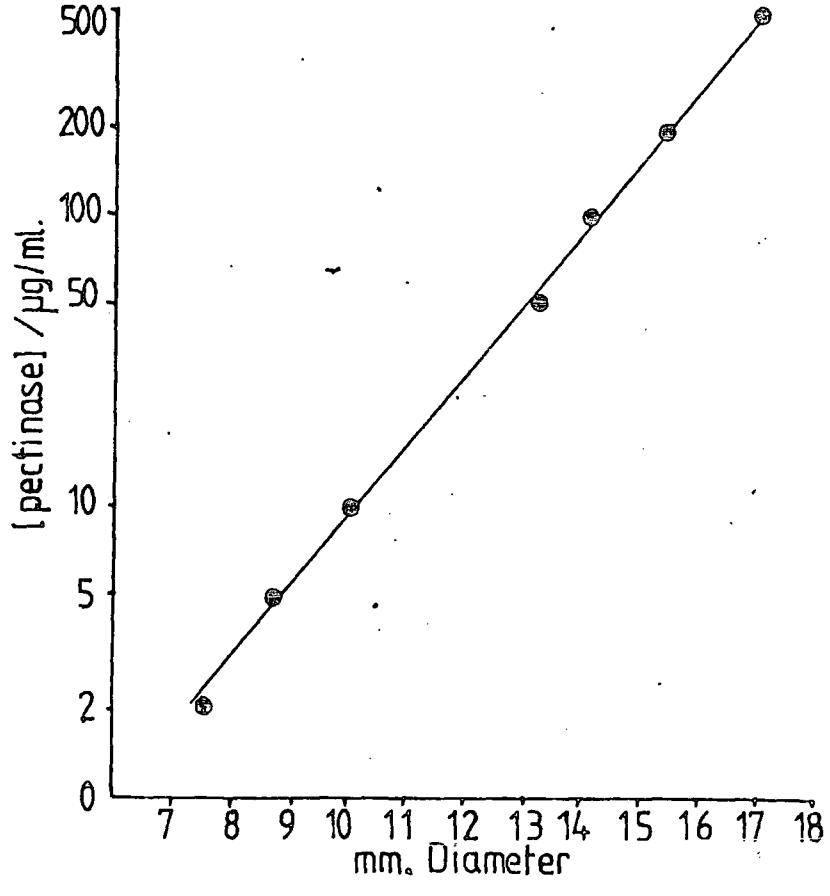



Figure 5.9: Standard curve for pectinase assay. Standard errors are denoted by a bar.

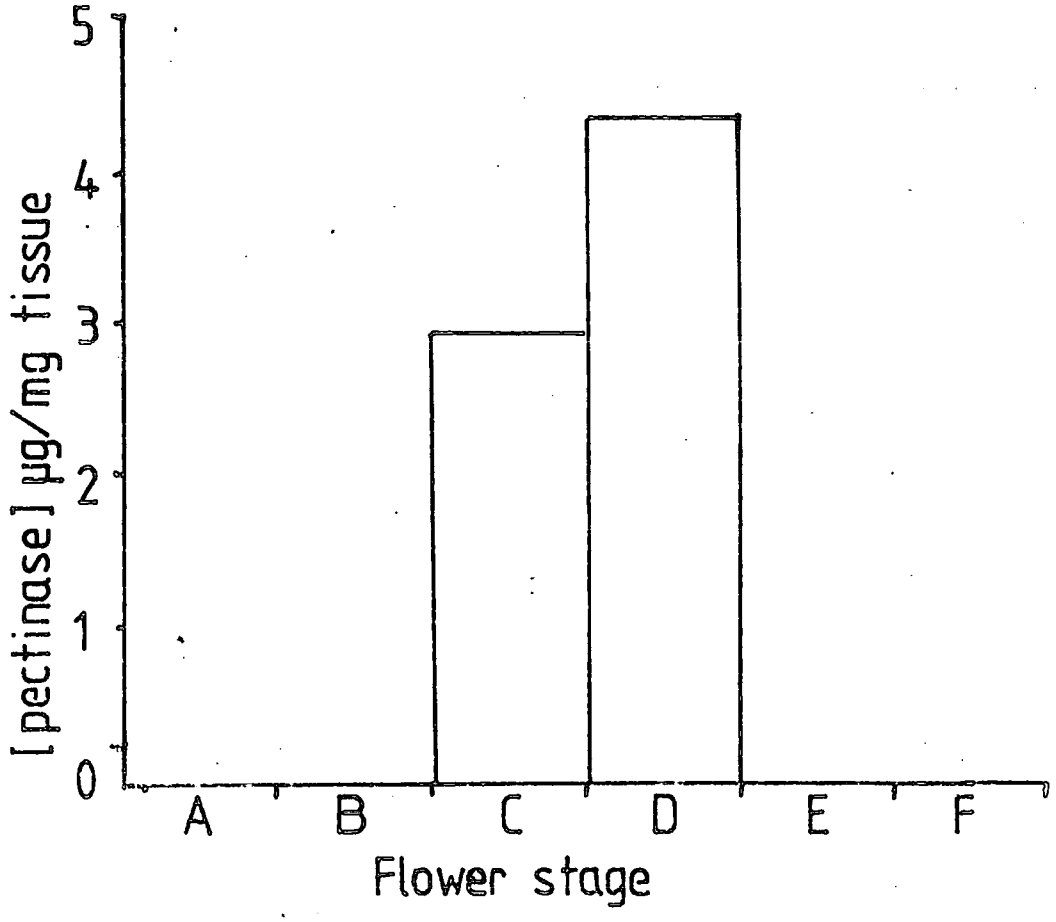



Figure 5.10: Concentration of Pectinase, within pedicel/peduncle junction of cultivar STW at different developmental stages. Each concentration is an average of 10 replicates.

A = stage 6, B = stage 7, C = stage 8, D = stage 9; E = stage 20, abscised; F = early pod set.

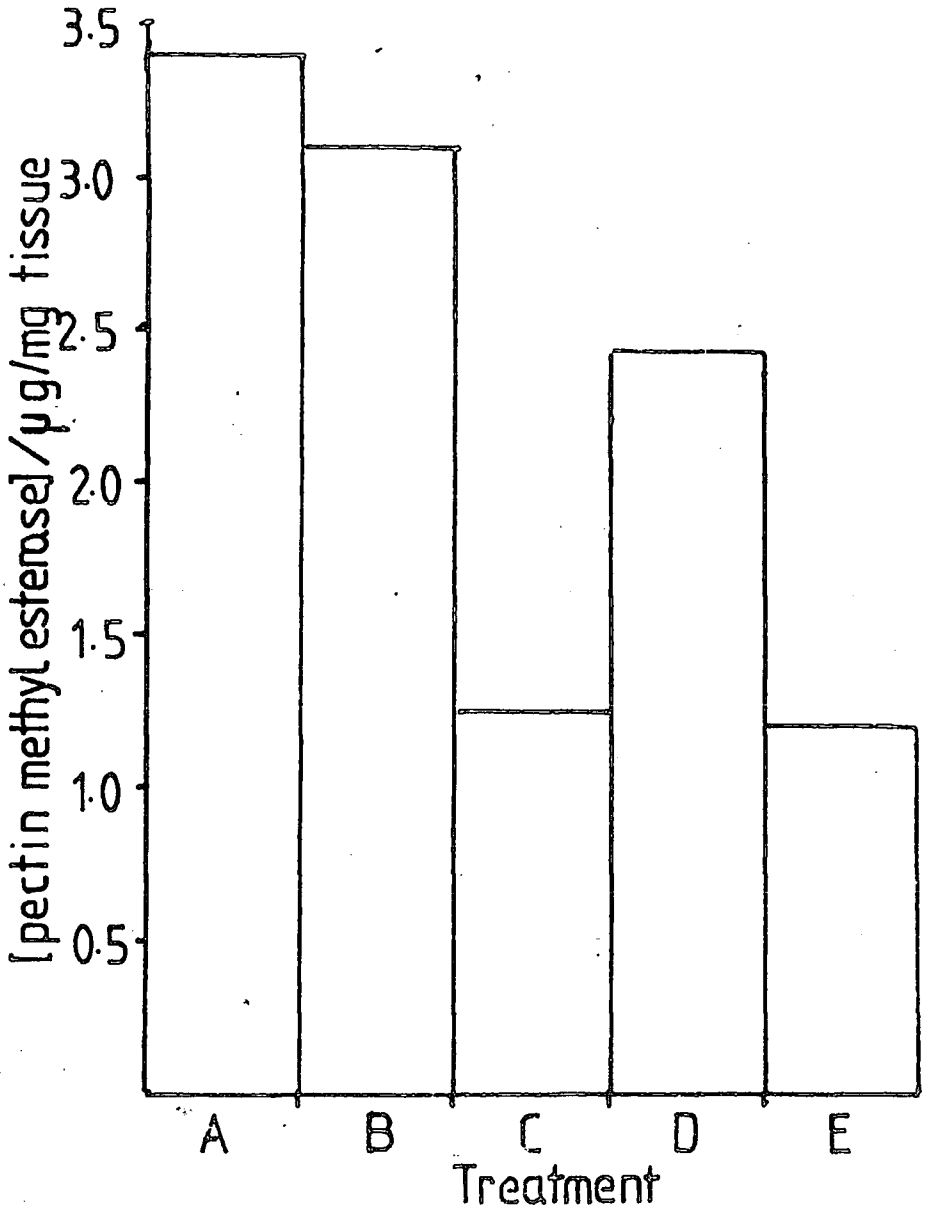



Figure 5.11: Concentration of Pectin methyl esterase, within pedicel/Peduncle junctions of variety STW at different developmental stages. Each concentration is an average of 10 replicates.

A = stage 8; B = stage 9; C = stage 10; D = early pod set; E = stage 10, abscised.

flowers than at developmental stages 8 and 9; although at pod set a greater concentration of this enzyme was detected than at abscission.

# CHAPTER 6

# FLOWER REMOVAL EXPERIMENTS : RESULTS

## Introduction

As described in Chapter 4 the removal of lowermost inflorescences effects reduced abscission within each raceme and at every raceme, on subsequently formed axillary inflorescences. As observed in Chapter 5, once pollination has taken place, many enzymic and cellular changes occur in the pedicel/peduncle junction, which lead either to successful pod set or abscission. In this chapter the effect of removing proximally situated flowers on the subsequent development of pods, which are more distally situated within the same raceme, is described.

## The effect of removal of the proximal flower within each raceme on flower drop

Removal of the proximal flower on each raceme on plants of Cockfield and Maris Bead resulted in reduced flower abscission on the three positions distal to the removed flower. Abscission within each raceme of plants of genotype TI Col. was, on average, not reduced by this treatment (Figure 6.1).

Plants of Cockfield and Maris Bead displayed a reduction in flower drop at every single raceme, under this treatment. Plants of TI Col. on the other hand, experienced greater flower abscission than the control plants (Figure 6.2).

Overall abscission for entire plants of variety Cockfield subjected to the above treatment was significantly less than that for the control plants. Whereas for plants of Maris Bead this treatment resulted only in a slight reduction in flower abscission. In contrast TI Col. plants under this

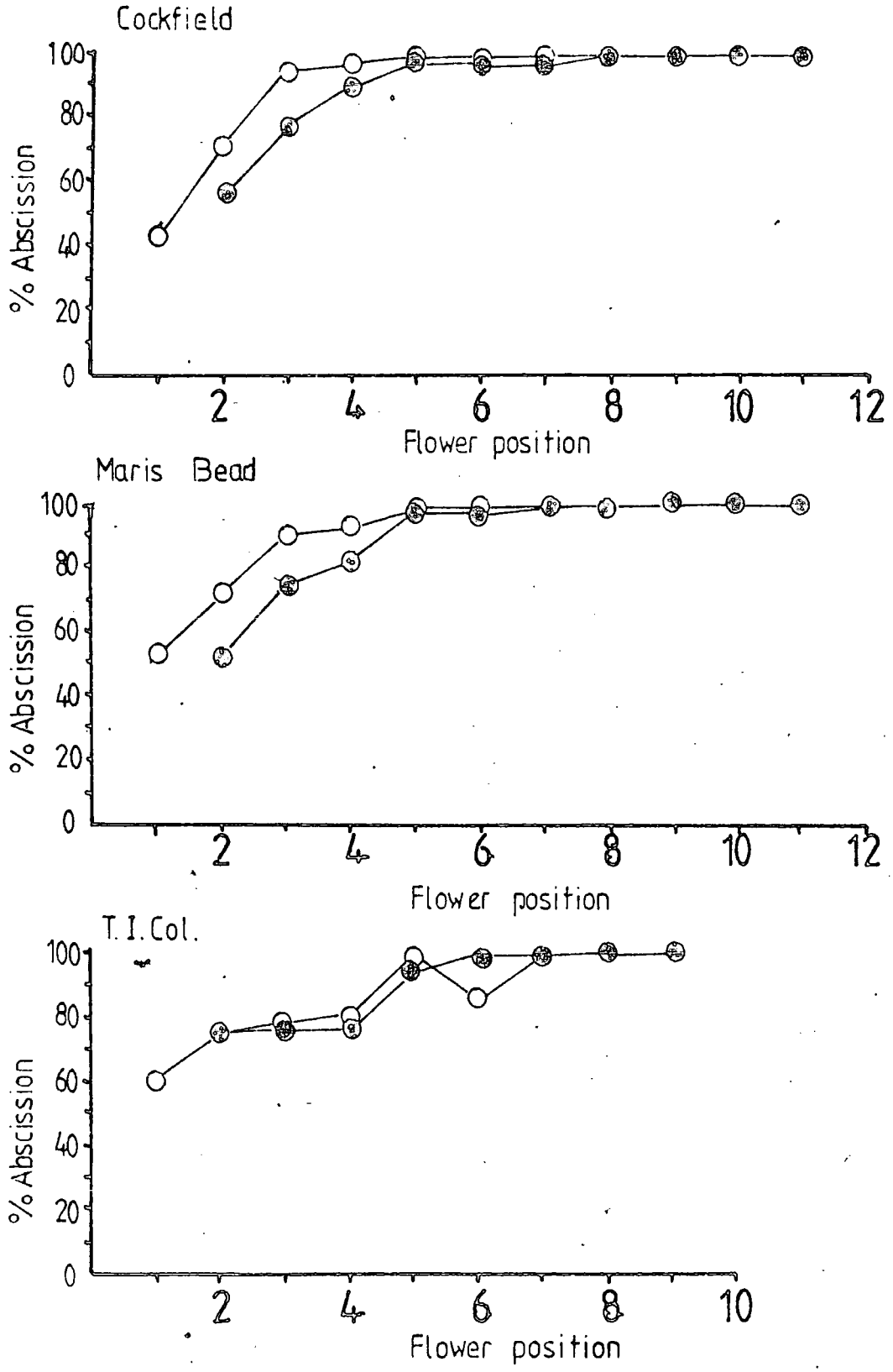



Figure 6.1: Influence of proximal flower removal on flower abscission. Each value is an overall percentage. o = proximal flower removed; o = control plants.

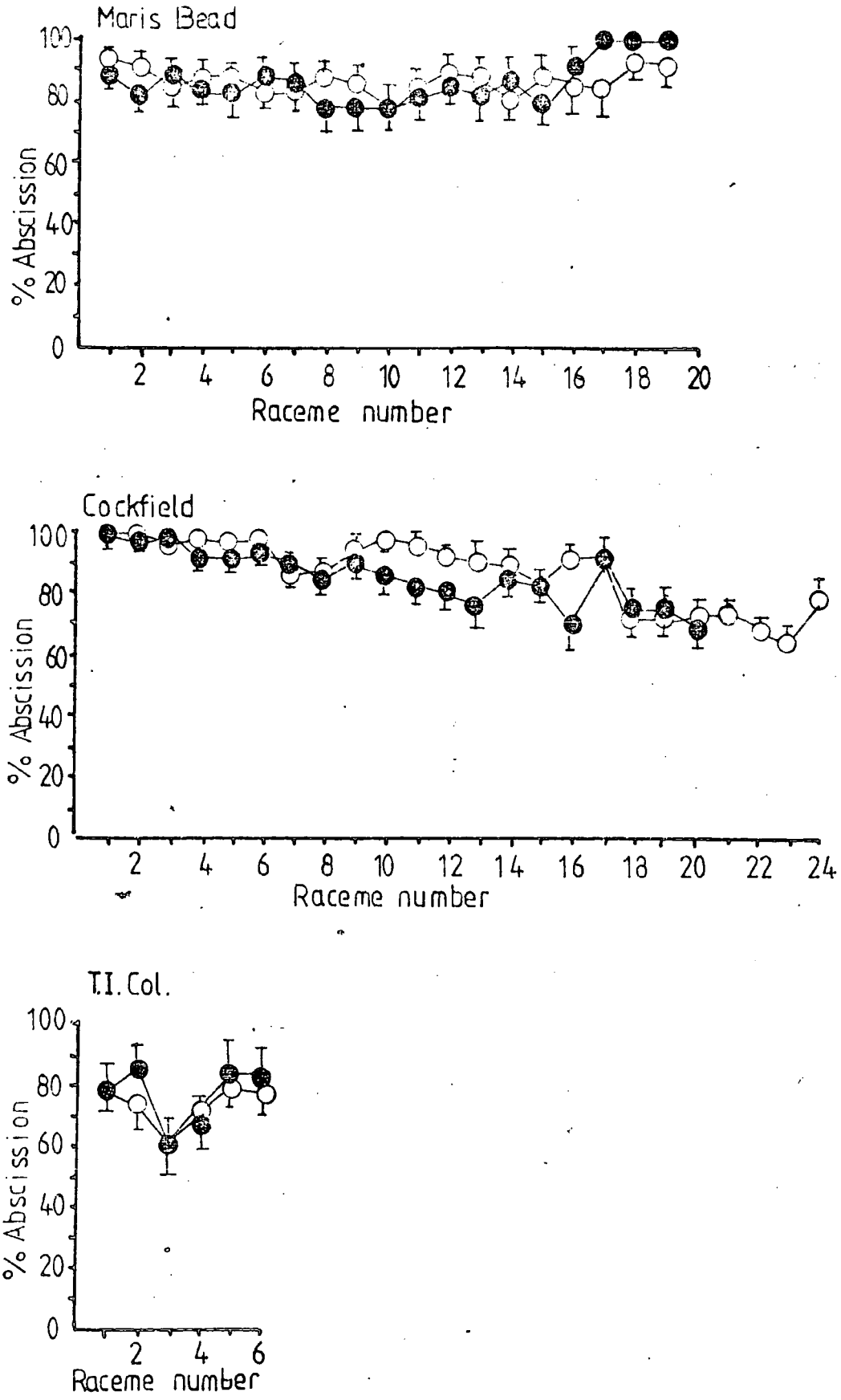



Figure 6.2: Influence of proximal flower removal on flower abscission. Each value is an average percentage. Standard errors are shown as a bar. ● = proximal flower removed; o = control plants

treatment experienced a slight increase in overall abscission In contrast, TI Col. plants under this treatment experienced a slight increase in overall abscission (Table 6.1).

Removal of flowers on flower drop of Deiniol plants

Removal of the proximal two, three or four flowers within each raceme resulted in a substantial reduction in abscission of remaining flowers (Figure 6.3). Of the remaining flowers, those more proximally situated experienced less abscission compared to those situated at more distal positions.

For all flower removal treatments (Figure 6.4) a reduction in abscission, at all but the most distally situated and most proximally situated racemes, occurred. In all cases a highly significant reduction in overall flower drop was experienced for all flower removal treatments (Table 6.2).

All the above flower removal treatments resulted in an equal development of young pods on raceme positions distal to the removed flowers. In addition these experiments show that flowers on all receme positions are capable of setting a pod (Figure 6.5).

The effect of removal of proximal flowers on flower drop using variety Cockfield

When proximal flowers were removed (Figure 6.6) abscission of the remaining flowers within each raceme was reduced compared to control plants. Using this variety, however, the effect was most pronounced when the first two flowers were removed. This reduction was concentrated on the remaining flowers which were most proximally situated (Table 6.3).

Abscission was reduced over most inflorescences when

Table 6.1 Summary of results for varieties subjected to proximal flower removal

| Variety and treatment | Flowers dropped (%) | Pods set (%) | $\chi^2$, v = 1 |
|---|---|---|---|
| Cockfield | | | |
| Control | 1016 (88.7) | 129 (11.3) | 2.95 (P> 0.1) |
| Proximal flower removed | 1141 (86.4) | 179 (13.6) | |
| Maris Bead | | | |
| Control | 1134 (88.1) | 152 (11.9) | 1.59 (P< 0.1) |
| Proximal flower removed | 945 (86.5) | 148 (13.5) | |
| TI Col. | | | |
| Control | 171 (76.7) | 52 (23.3) | 0.61 (P< 0.1) |
| Proximal flower removed | 132 (80.0) | 33 (20.0) | |

Percentage values are in parentheses.

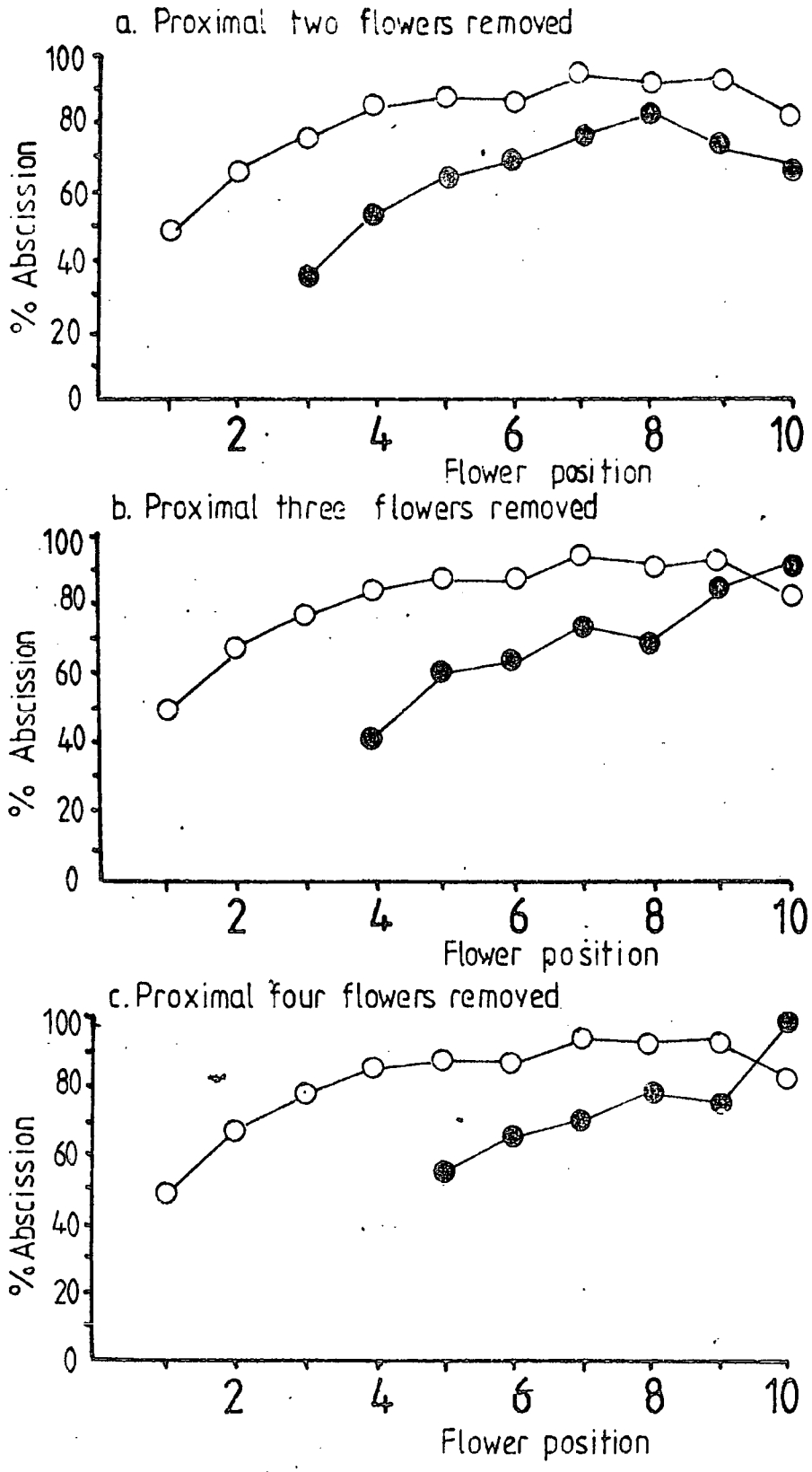



Figure 6.3: Effect of proximal flower removal on abscission for variety Deiniol. Each value is an overall percentage.
⊙ = treatment plants; o = control plants.

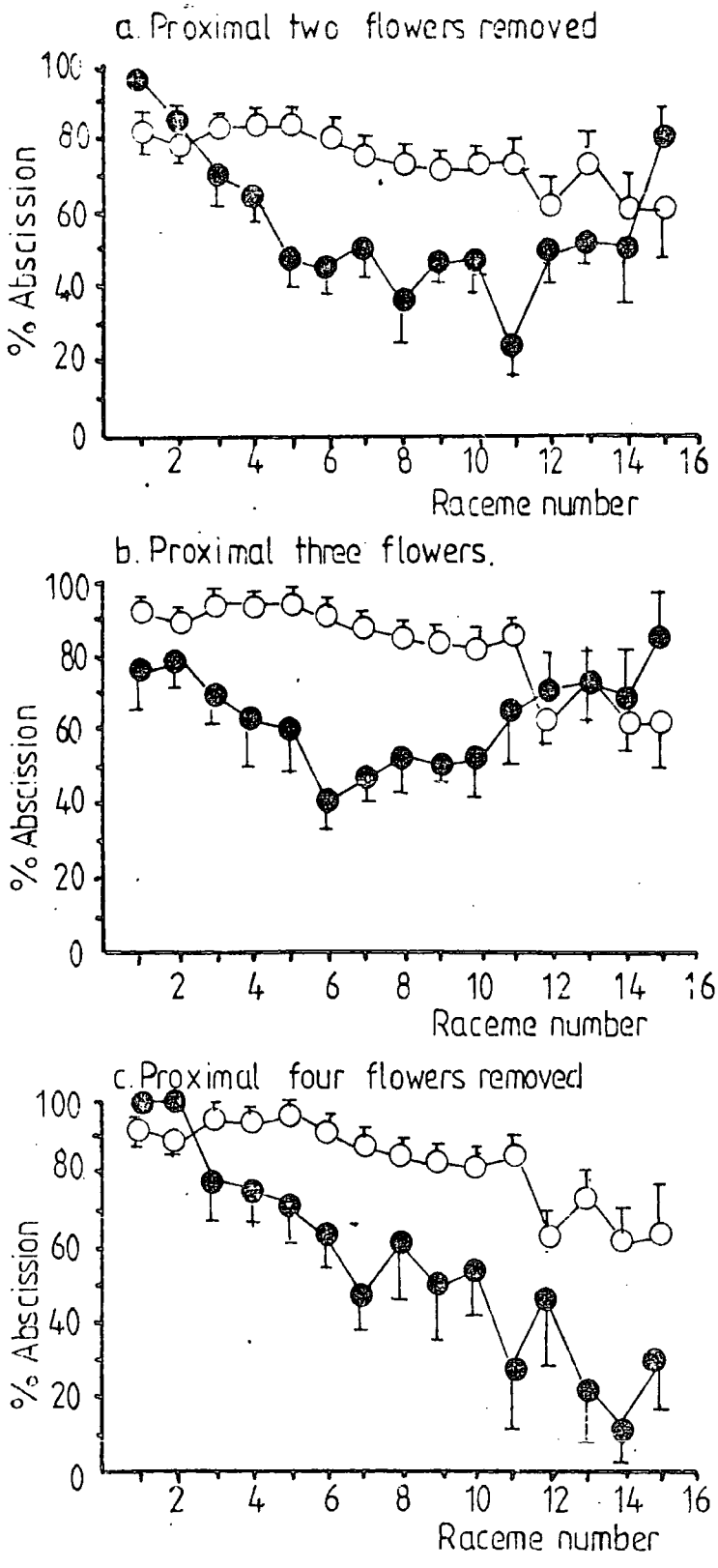



Figure 6.4: Influence of proximal flower removal on flower abscission for variety Deiniol. East value is an overall percentage. ⊛ = treatment plants; o = control plants.

Table 6.2 Summary of flowers dropped and pods set for variety Deiniol, subjected to proximal flower removal

| Flower positions where flowers removed | Flowers dropped (%) | Pods set (%) | $\chi^2$ (v = 1) |
|---|---|---|---|
| 1,2 | 377 (63.4) | 218 (36.6) | 55.47 (P> 0.001) |
| 1,2,3 | 331 (63.4) | 191 (36.6) | 50.94 (P> 0.001) |
| 1,2,3,4 | 278 (65.6) | 146 (34.4) | 33.91 (P> 0.001) |
| Control | 1016 (79.5) | 262 (20.5) | |

Figure 6.5: Effect of flower removal on pod set in Vicia faba L.
a = control, no flower removal;
b = proximal two flowers removed;
c = proximal three flowers removed;
d = proximal four flowers removed.

a
b
c
d

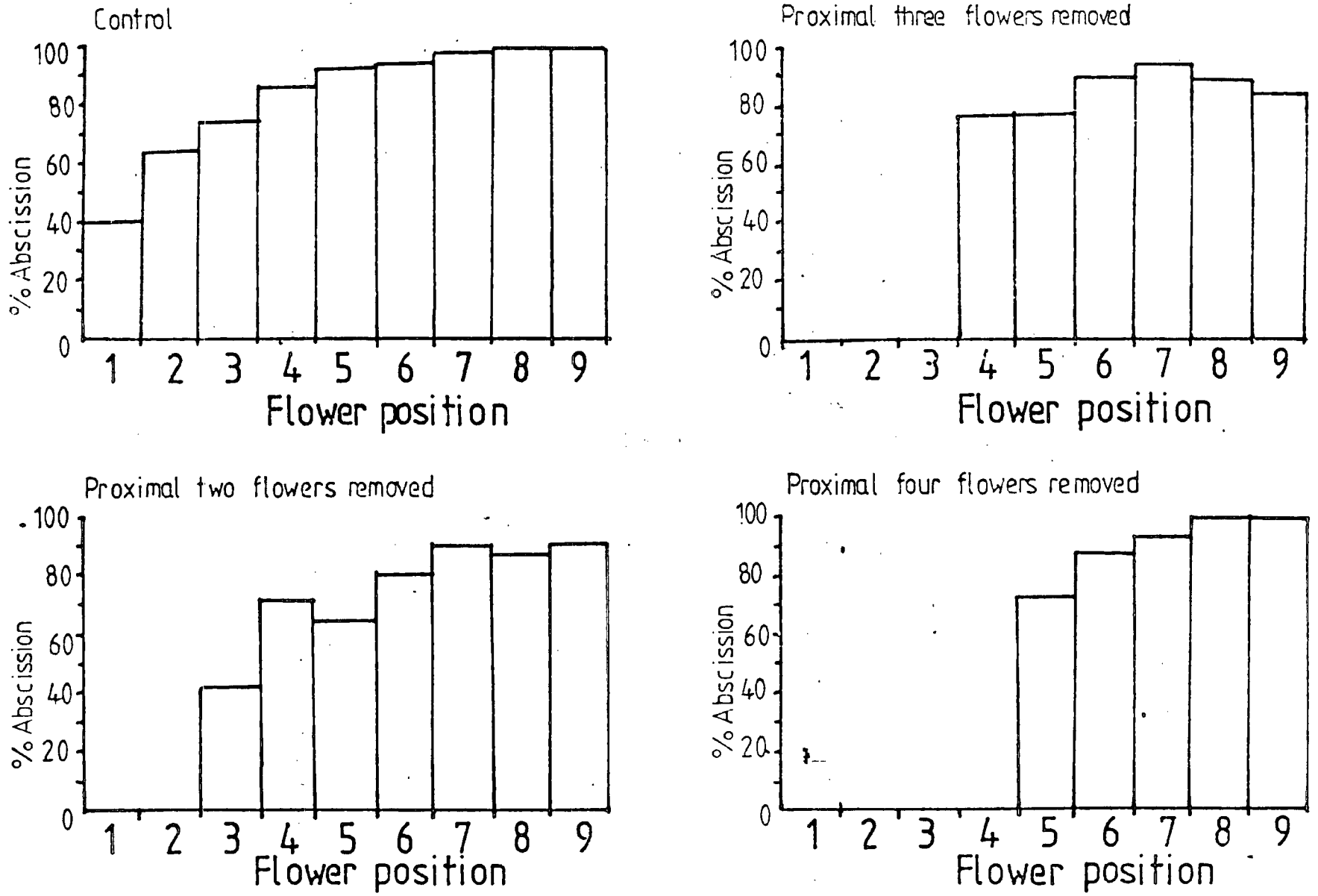



Figure 6.6: Influence of proximal flower removal on flower abscission in variety Cockfield. Each bar is an overall percentage.

Table 6.3 Influence of removing proximal flowers on abscission within racemes for variety Cockfield

| | Percentage reduction in flower drop compared with control plants | | | | | | |
|---|---|---|---|---|---|---|---|
| Positions of flowers removed | Floral position 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 1,2 | 32.2 | 15.8 | 28.0 | 13.4 | 8.2 | 12.5 | 9.5 |
| 1,2,3 | - | 9.0 | 15.7 | +3.7 | 4.0 | +11.4 | +15.4 |
| 1,2,3,4 | - | - | 20.1 | 6.0 | 5.4 | 0.0 | 0.0 |

the first two flowers on each raceme were removed. Reduction in abscission was, however, concentrated on the more proximally situated inflorescences when the first three and four flowers on each raceme were removed (Figure 6.7).

A reduction in abscission over the entire plant only occurred when the first two flowers were removed on each raceme. Other treatments resulted in an overall increase in flower abscission (Table 6.4).

Effect of removal of proximal flowers on flower drop of plants grown in the field

Plants of variety Cockfield subjected to removal of one, two or three flowers on racemes, all showed reduced abscission of remaining flowers on each raceme compared to control plants (Figure 6.8). Maris Bead plants subjected to flower removal treatments all experienced reduced flower abscission within each raceme. This effect was least pronounced when the proximal flower was removed, and most when the proximal three flowers were removed (Figure 6.9). TI Col. plants which were subject to the above flower removal treatments, resulted in reduced flower abscission within each raceme on the flower situated immediately above the removed flower(s). However on more distal positions, a rapid increase in abscission was experienced (Figure 6.10).

Flower abscission at every raceme of plants of Cockfield, subjected to removal of the proximal flower and the proximal two flowers of each raceme, was reduced over the inflorescences situated on the lower flowering portion of the plants. Where three flowers had been removed on each raceme, however, higher flower abscission occurred on racemes on the lower flowering portion of plants, and approximately the same

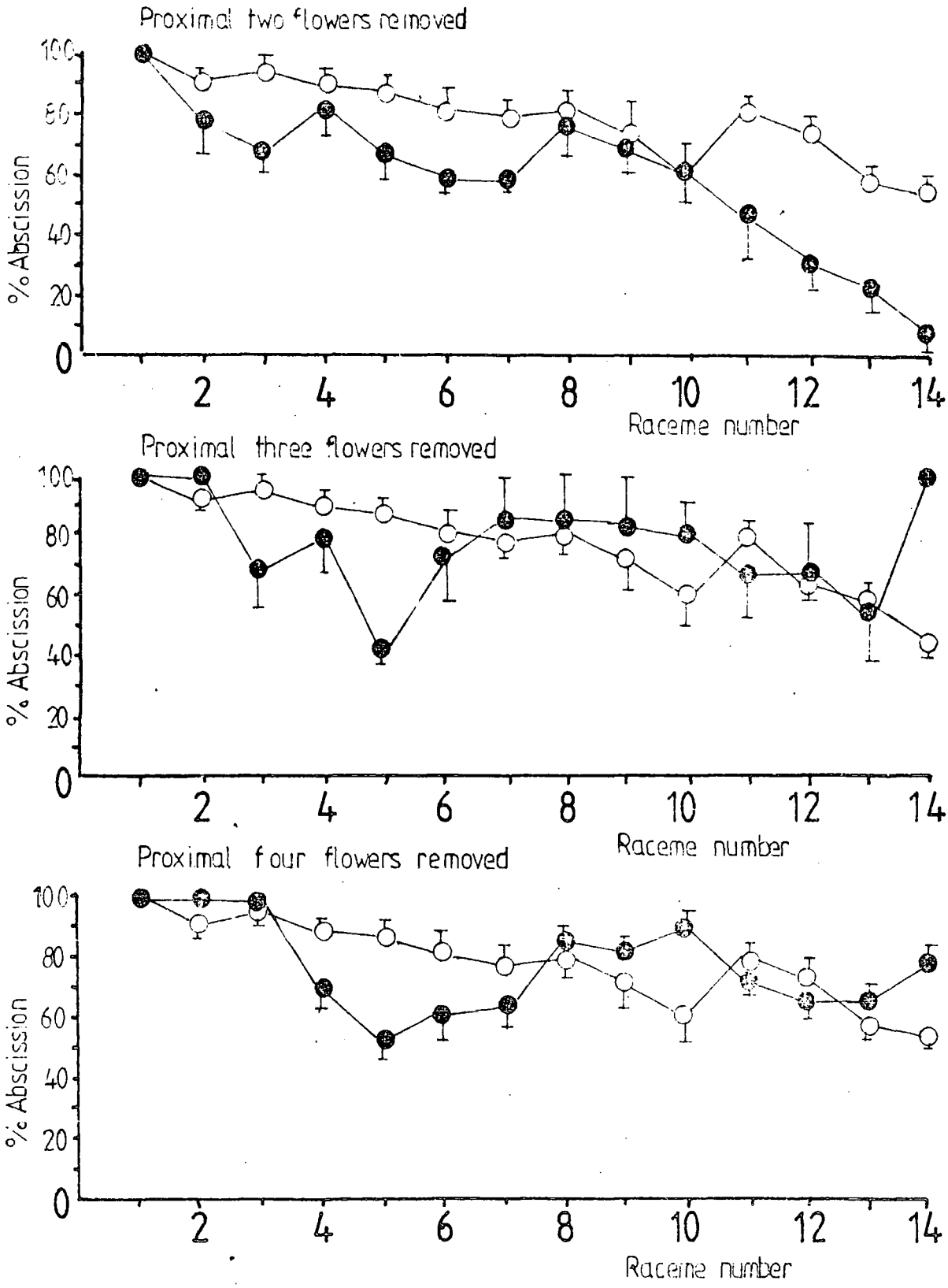



Figure 6.7: Influence of proximal flower removal on flower abscission for variety Cockfield. ● = treatment plants; o = control plants. Each value is an average percentage. Standard errors are represented by a bar.

Table 6.4 Summary of flowers dropped and pods set for variety Cockfield, subjected to proximal flower removal

| Flower positions where flowers removed | Flowers Dropped (%) | Pods set (%) | $\chi^2$ (v = 1) |
|---|---|---|---|
| 1,2 | 300 (72.3) | 115 (27.7) | 8.77 (P > 0.005 < 0.001) |
| 1,2,3 | 253 (84.9) | 45 (15.1) | 2.72 (P < 0.1) |
| 1,2,3,4 | 225 (86.5) | 35 (13.5) | 4.67 (P > 0.05 < 0.025) |
| Control | 454 (80.3) | 111 (19.7) | |

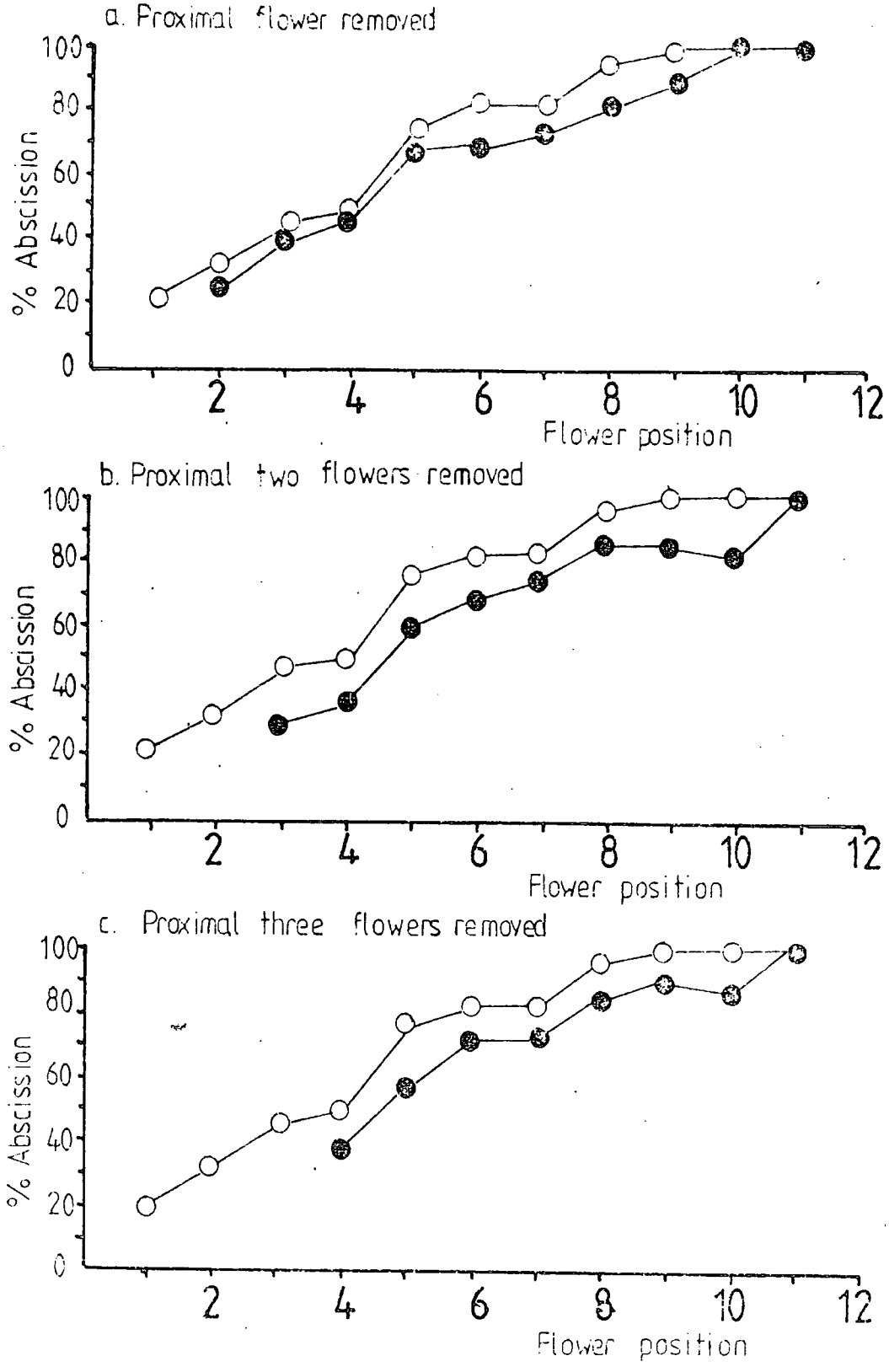



Figure 6.8: Effect of proximal flower removal on flower abscission for variety Cockfield. East value is an overall percentage. ⊙ = treatment plants; o = control plants.

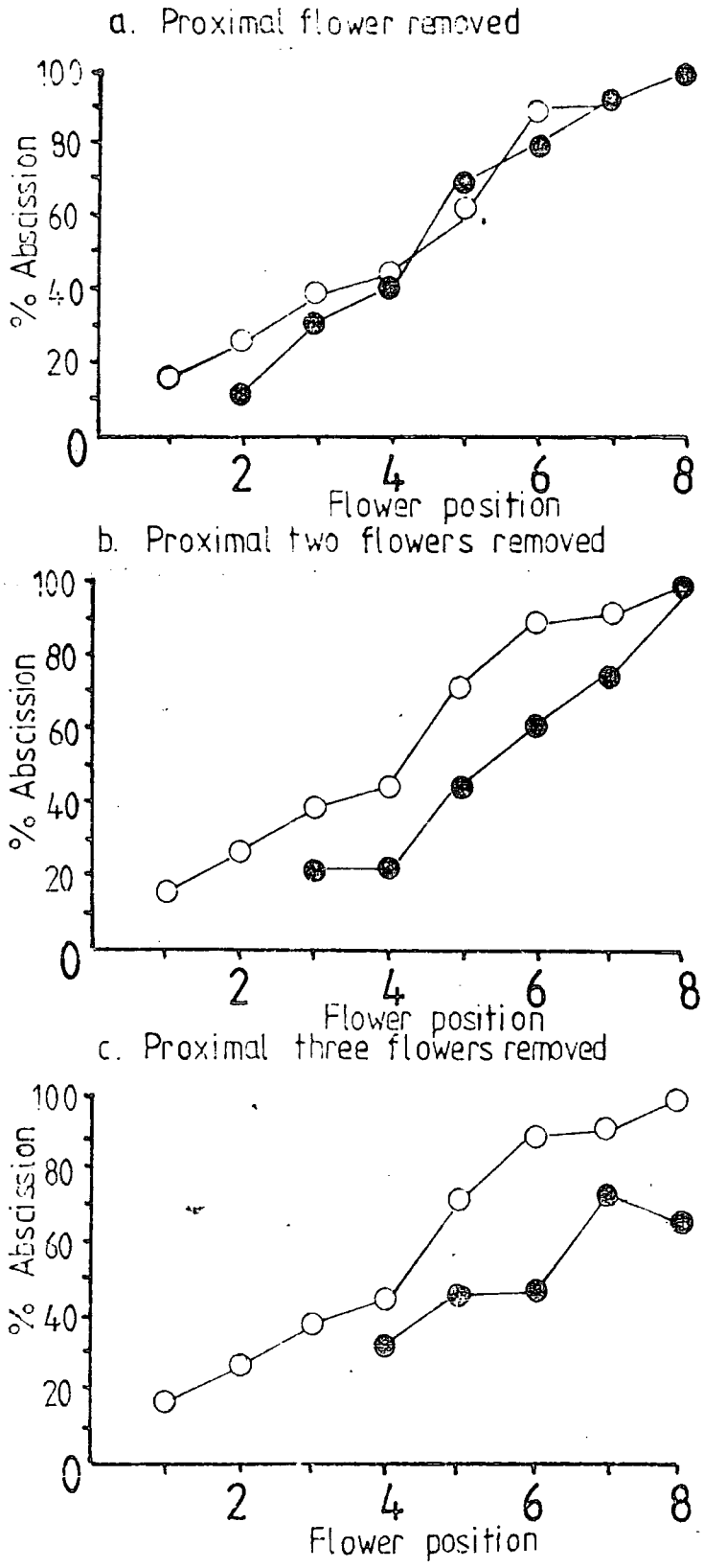



Figure 6.9: Effect of proximal flower removal on flower abscission for Maris Bead. Each value is an overall percentage. ⊛ = treatment plants; o = control plants.

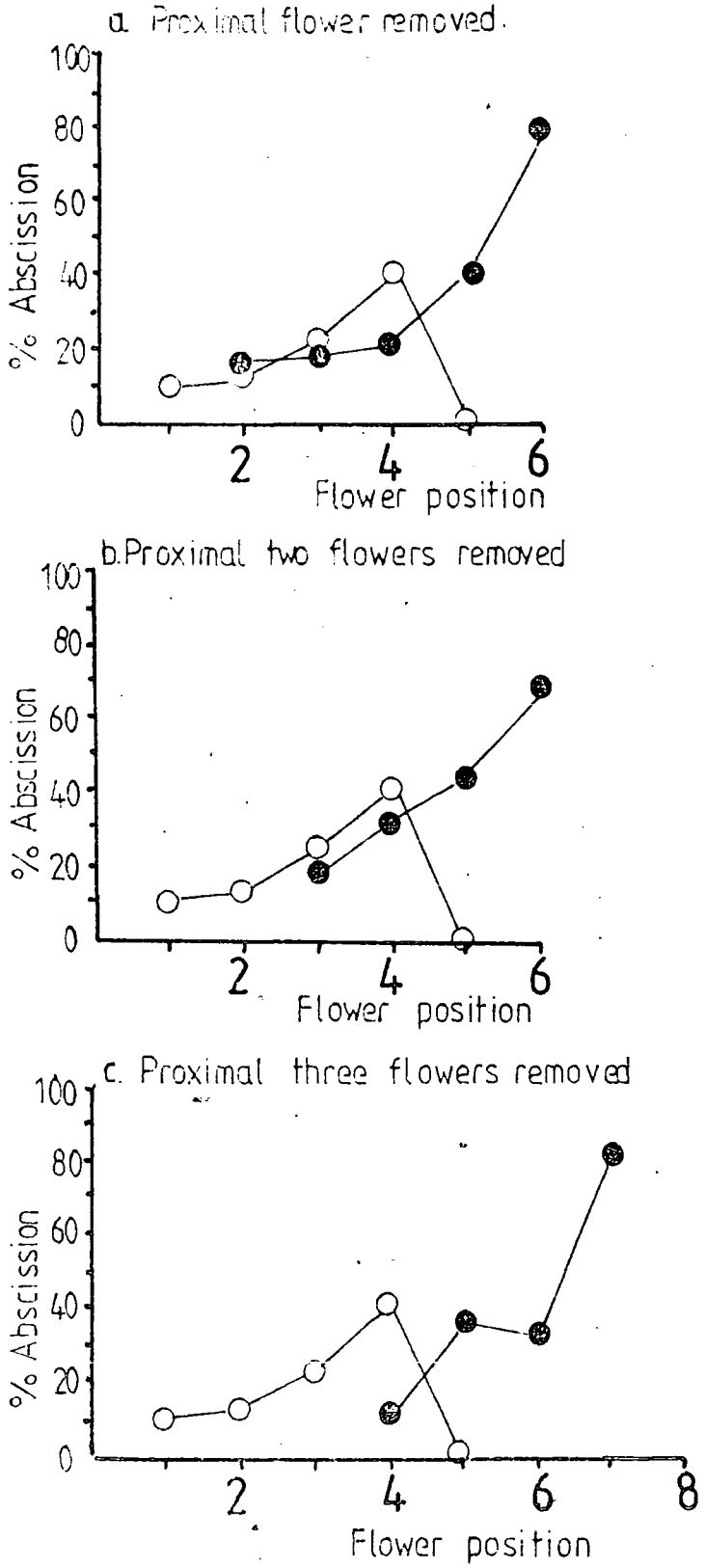



Figure 6.10: Effect of proximal flower removal on flower abscission for variety TI Col. Each value is an overall percentage. ● = treatment plants; o = control plants.

abscission of flowers occurred on the upper flowering portion as the control plants (Figure 6.11).

Abscission on each raceme of Maris Bead plants, subject to proximal flower removal, was greater on the first formed inflorescences, but slightly less on the subsequent racemes compared with control plants. This pattern of flower abscission was maintained when plants were subjected to removal of the first two flowers on each raceme, however, the reduction in abscission was much greater on racemes situated on the upper flowering portion of plants. A similar, but less pronounced pattern of abscission was displayed by plants subjected to removal of the first three flowers on each raceme (Figure 6.12). Flower abscission on inflorescences of TI Col. plants was generally higher on the first formed raceme, lower on middle racemes and higher, again, on distal racemes and the terminal inflorescence, for all treatments, compared to control plants (Figure 6.13).

Flower abscission, overall (Table 6.5), was significantly reduced for Maris Bead plants subject to removal of the proximal two flowers on each raceme. Abscission was significantly reduced compared to control Cockfield plants when subject to proximal and first two flower removal for each raceme. TI Col. plants, subjected to flower removal treatments all experienced an overall increase in abscission, which increased with increasing flower removal, compared to the control plants.

An increase in yield of Maris Bead plants was effected by the removal of the proximal two flowers on each raceme. This was also true for Cockfield plants subject to removal of the first three flowers on each raceme (Table 6.6).

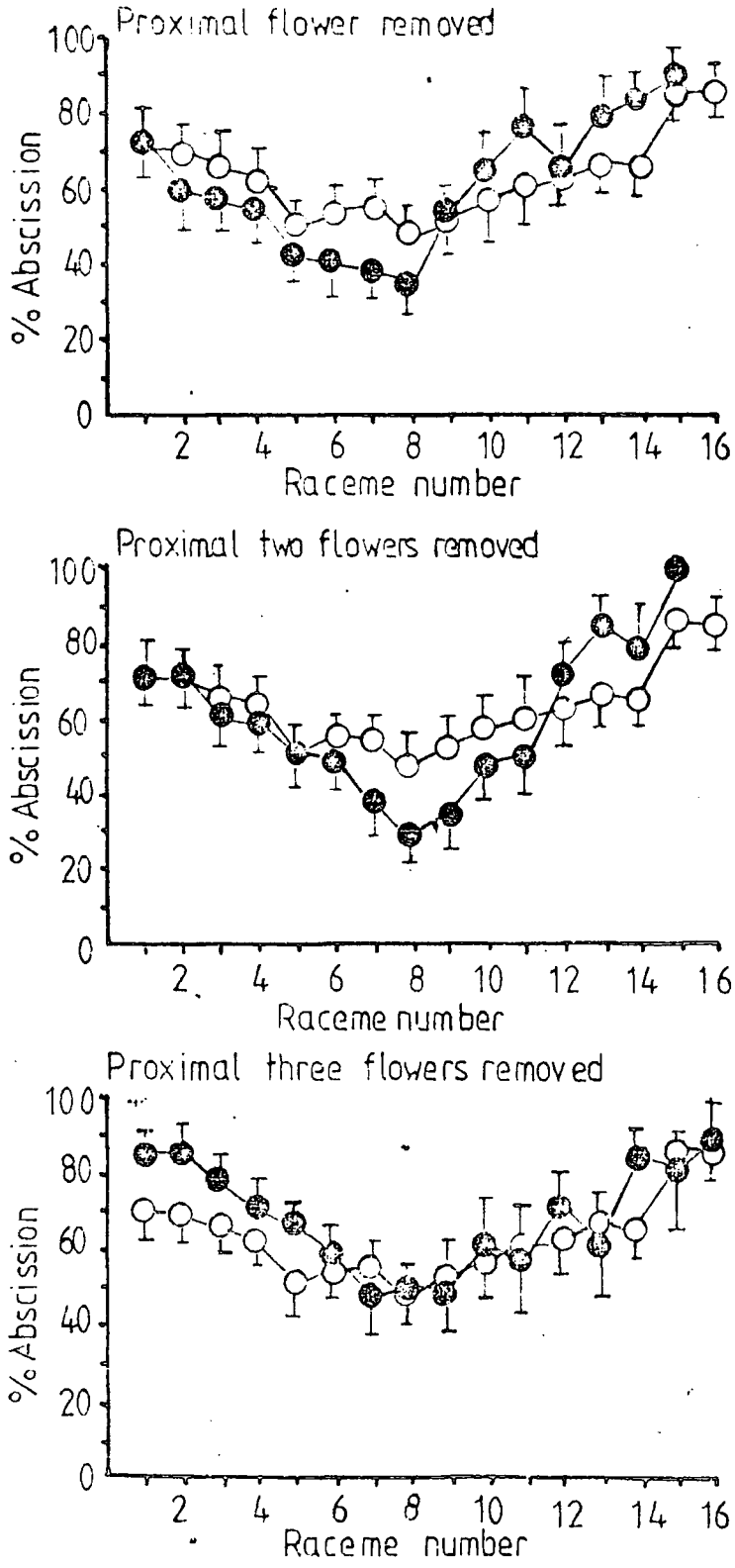



Figure 6.11: Influence of flower removal on flower abscission of variety Cockfield.
Each value is an average percentage.
o = control; ⊙ = treatment.
Standard errors are represented by a bar.

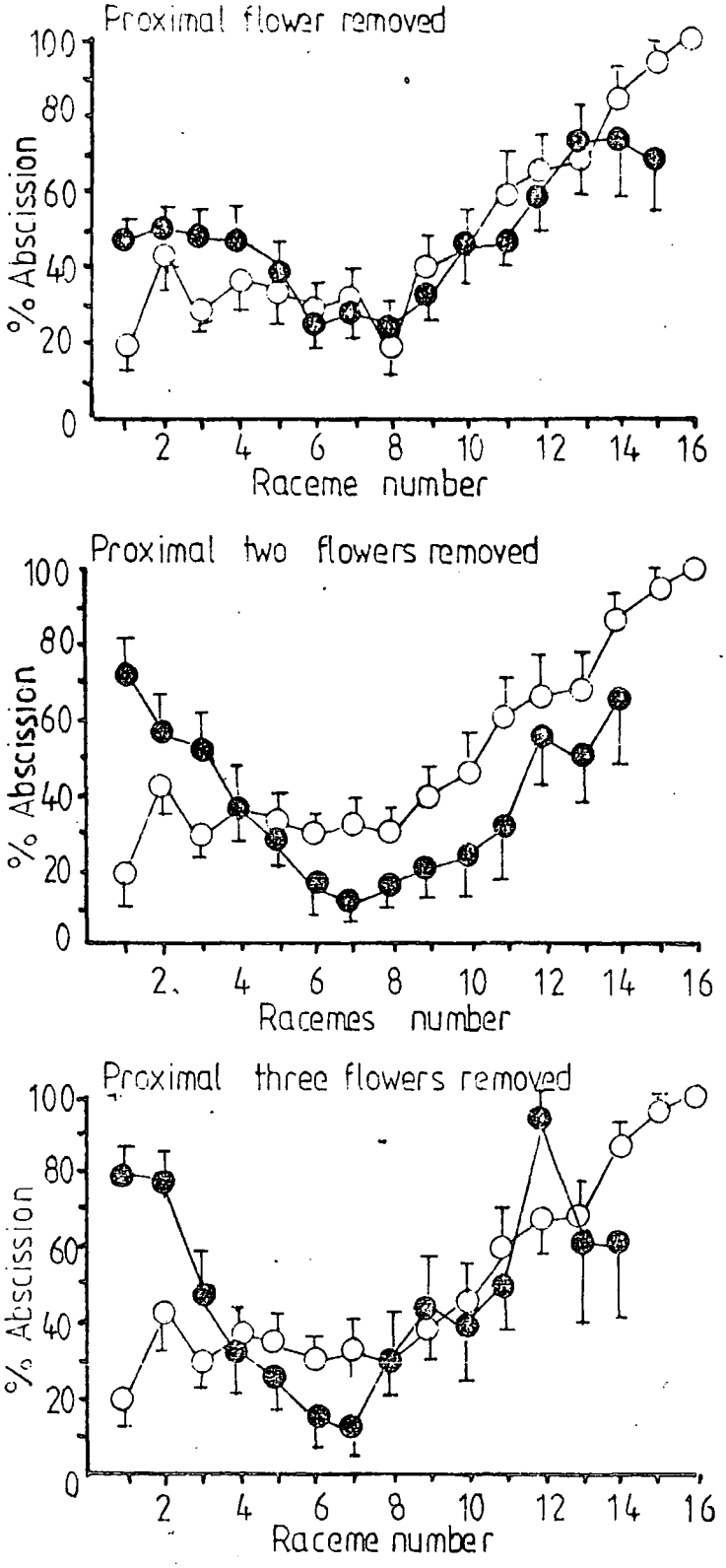



Figure 6.12: Influence of flower removal on flower abscission of variety Maris Bead. Each value is an average percentage. o = control; ● = treatment; Standard errors are represented by a bar.

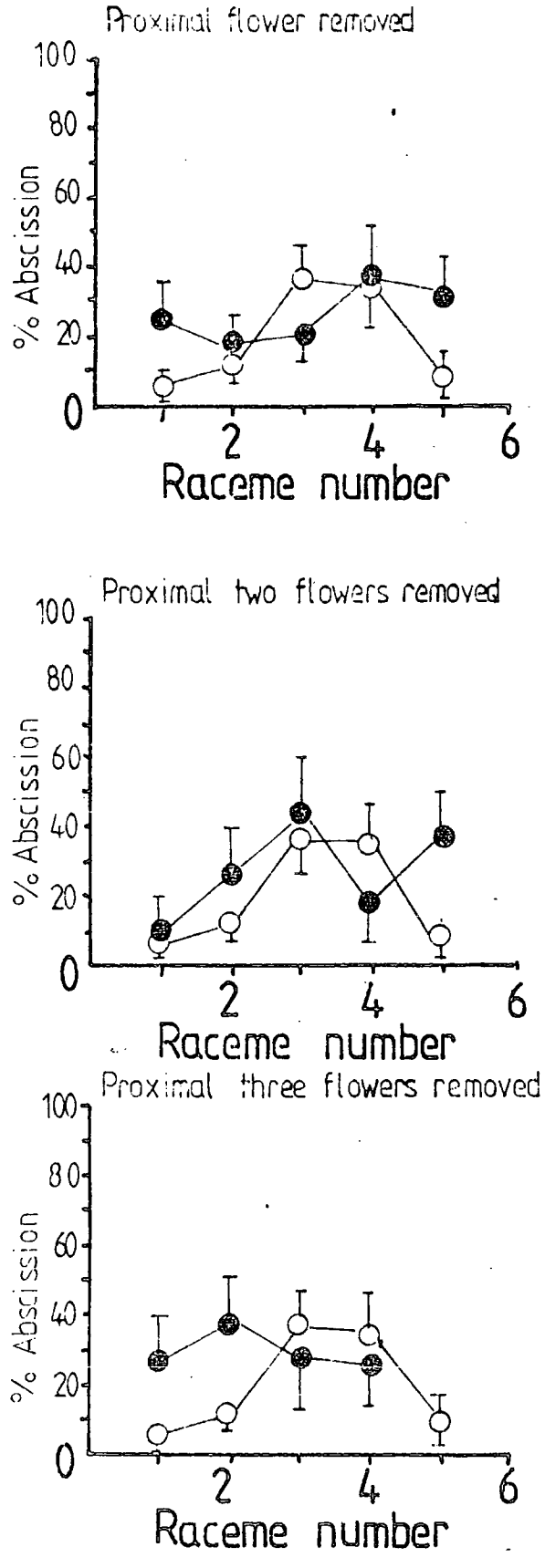



Figure 6.13: Influence of flower removal on flower abscission of variety TI Col. Each value is an average percentage.
o = control, ● = treatment;
Standard errors are represented by a bar.

Table 6.5 Summary of results for proximal removal experiments

| Variety and treatment | % flowers dropped | % Pods set | $\chi^2$, v = 1 |
|---|---|---|---|
| Maris Bead | | | |
| Control | 44.1 | 55.9 | |
| a) Proximal flower removed | 47.1 | 52.9 | 1.25 ($P < 0.1$) |
| b) Proximal two flowers removed | 27.3 | 72.7 | 4.99 ($P > 0.05 < 0.025$) |
| c) Proximal three flowers removed | 45.3 | 54.7 | 0.14 ($P < 0.1$) |
| Cockfield | | | |
| Control | 62.6 | 37.4 | |
| a) Proximal flower removed | 55.7 | 44.3 | 9.10 ($P > 0.005 < 0.001$) |
| b) Proximal two flowers removed | 57.2 | 52.8 | 5.49 ($P > 0.025 < 0.01$) |
| c) Proximal three flowers removed | 66.1 | 33.9 | 1.94 ($P < 0.1$) |
| TI Col. | | | |
| Control | 19.6 | 80.4 | |
| a) Proximal flower removed | 27.4 | 72.6 | 2.65 ($P < 0.1$) |
| b) Proximal two flowers removed | 29.7 | 70.3 | 2.92 ($P > 0.1 < 0.05$) |
| c) Proximal three flowers removed | 32.0 | 68.0 | 4.32 ($P > 0.05 < 0.025$) |

Table 6.6 Average yield components of flower removal experiment

| Variety and treatment | Number of pods | Number of seeds | Total weight of seeds (g) | Weight of each seed (g) |
|---|---|---|---|---|
| Maris Bead | | | | |
| Control | 26.6 (4.14) | 75.4 (14.86) | 31.9 (7.02) | 0.40 (0.031) |
| Proximal flower removed | 27.4 (1.93) | 81.4 (7.47) | 29.9 (2.13) | 0.38 (0.024) |
| Proximal two flowers removed | 22.5 (2.67) | 76.0 (8.03) | 30.4 (3.40) | 0.40 (0.016) |
| Proximal three flowers removed | 16.7 (3.10) | 51.0 (9.70) | 23.4 (7.39) | 0.47 (0.046) |
| Cockfield | | | | |
| Control | 24.4 (1.34) | 76.6 (3.35) | 42.0 (2.82) | 0.55 (0.019) |
| Proximal flower removed | 21.5 (2.51) | 61.8 (9.31) | 30.1 (19.34) | 0.45 (0.058) |
| Proximal two flowers removed | 32.5 (2.27) | 98.6 (11.00) | 59.5 (5.34) | 0.61 (0.014) |
| Proximal three flowers removed | 15.4 (2.61) | 41.6 (8.67) | 23.2 (5.48) | 0.54 (0.033) |

Standard errors are in parentheses.

Effect of distal flower removal within racemes on flower abscission

Cockfield plants subject to removal of the distal two or three flowers on each raceme, resulted in no change in flower drop within each axillary raceme (Figure 6.14, Table 6.7).

Table 6.7 Summary of results for distal flower removal with variety Cockfield

| Treatment | Flowers dropped (%) | Pods set (%) | $\chi^2$ ($v = 1$) |
|---|---|---|---|
| Control | 322 (88.7) | 41 (11.3) | |
| Distal two flowers removed | 329 (87.9) | 45 (12.1) | 0.09 ($P < 0.1$) |
| Distal three flowers removed | 312 (88.6) | 40 (11.4) | 0.0008 ($P < 0.1$) |

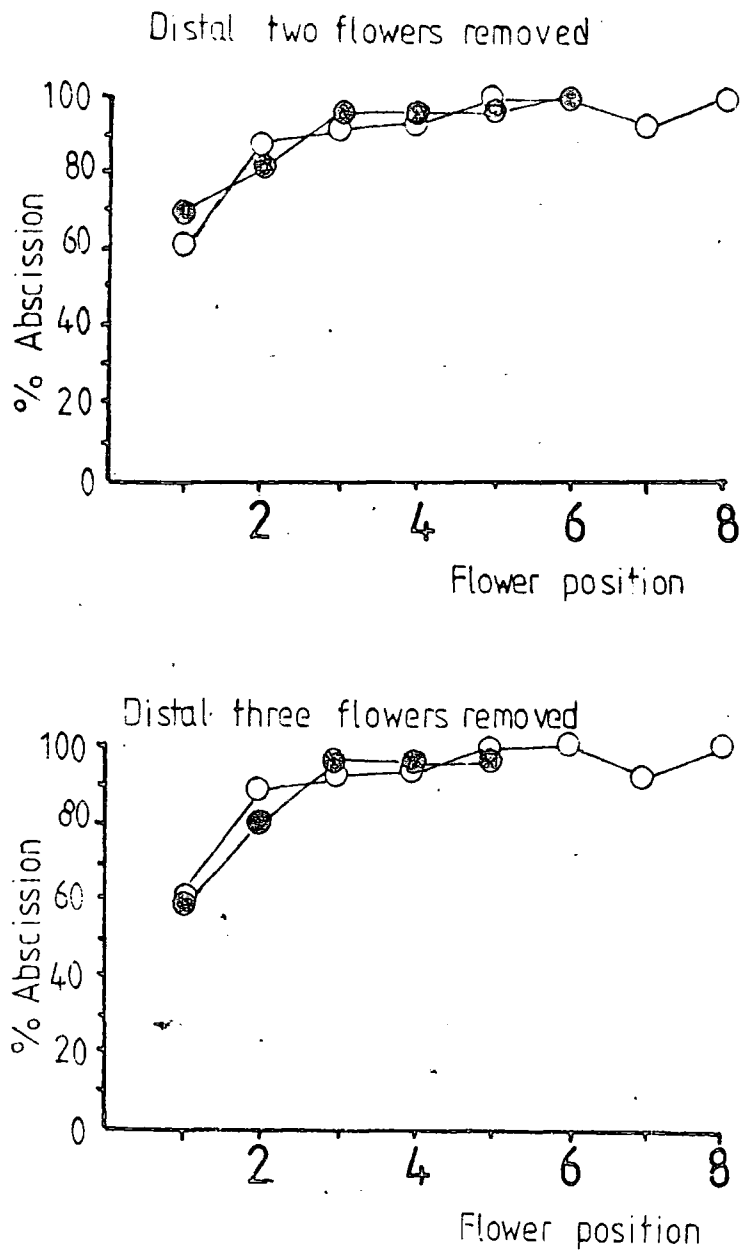



Figure 6.14: Influence of distal flower removal on flower abscission of variety Cockfield. o = treatment plants; o = control plants. Each value is an overall percentage.

# CHAPTER 7

## VISUAL OBSERVATIONS ON PLANTS WITH DIFFERING FLORAL AND PLANT ARCHITECTURES. RESULTS OF EXPERIMENTS TO ELUCIDATE DIFFERENCES IN VASCULAR ANATOMY BETWEEN PLANTS EXHIBITING LOW FLOWER ABSCISSION AND THOSE OF COMMERCIAL VARIETIES

### Observations of flower drop on plants grown in the glasshouse exhibiting different floral and plant architectures

Flower abscission was less in all (inbred) lines derived from the cross 22 x 21 compared to that experienced by commercial varieties. In most cases, however, fewer flowers were produced on each raceme in the former case.

Of those lines possessing a terminal inflorescence, some exhibited quite a high flower drop, while others did not. Plant lines exhibiting high flower drop, i.e. 56/134/7, 56/143/18, 56/109/7, 56/143/7, 56/109/15, 56/98/10, also had a pattern of abscission similar to that observed previously: proximal flowers abscised the least, distal flowers the most. Other lines, notably 56/134/13, 56/117/1 and 56/107/1 exhibited low abscission, and the pattern of flower drop within each raceme, was on average, altered. Most flower abscission occurred at middle raceme positions, while less drop was observed at positions proximal and distal to these. Plants of lines 56/107/4, 56/143/13, 56/134/14, 56/143/7 exhibited approximately similar flower drop at all positions, except the most distal one, at which the flower invariably abscised. This pattern of flower drop is exemplified by plants of line 56/143/13, where approximately 25% of the flowers dropped on the first four flower positions, but every flower dropped on the fifth position (Figures 7.1.1, 7.1.2, 7.1.3).

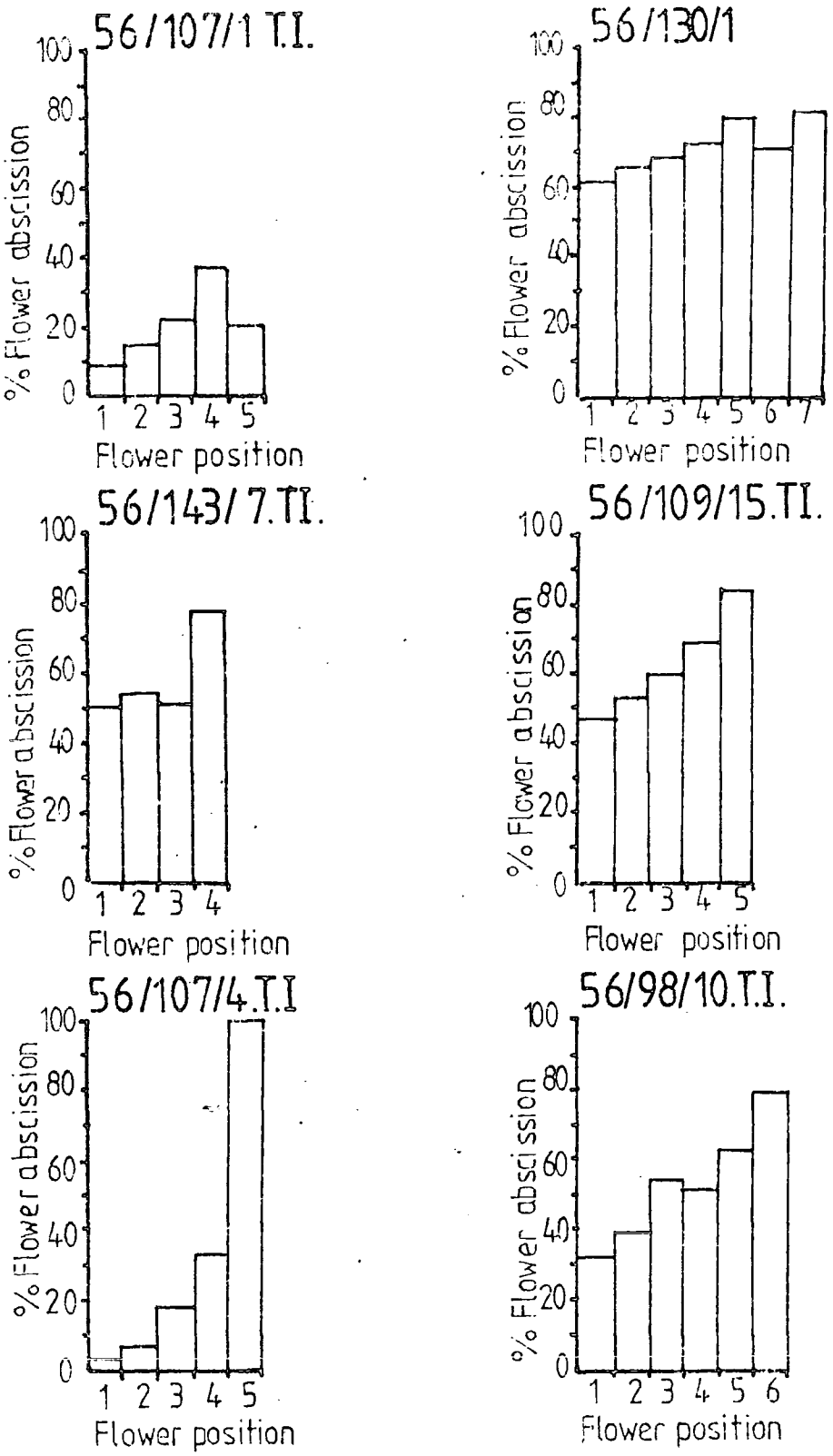



Figure 7.1.1: Flower abscission of inbred lines with different floral and plant architectures. All plants were obtained from the crossing programme. Each value is an overall percentage.

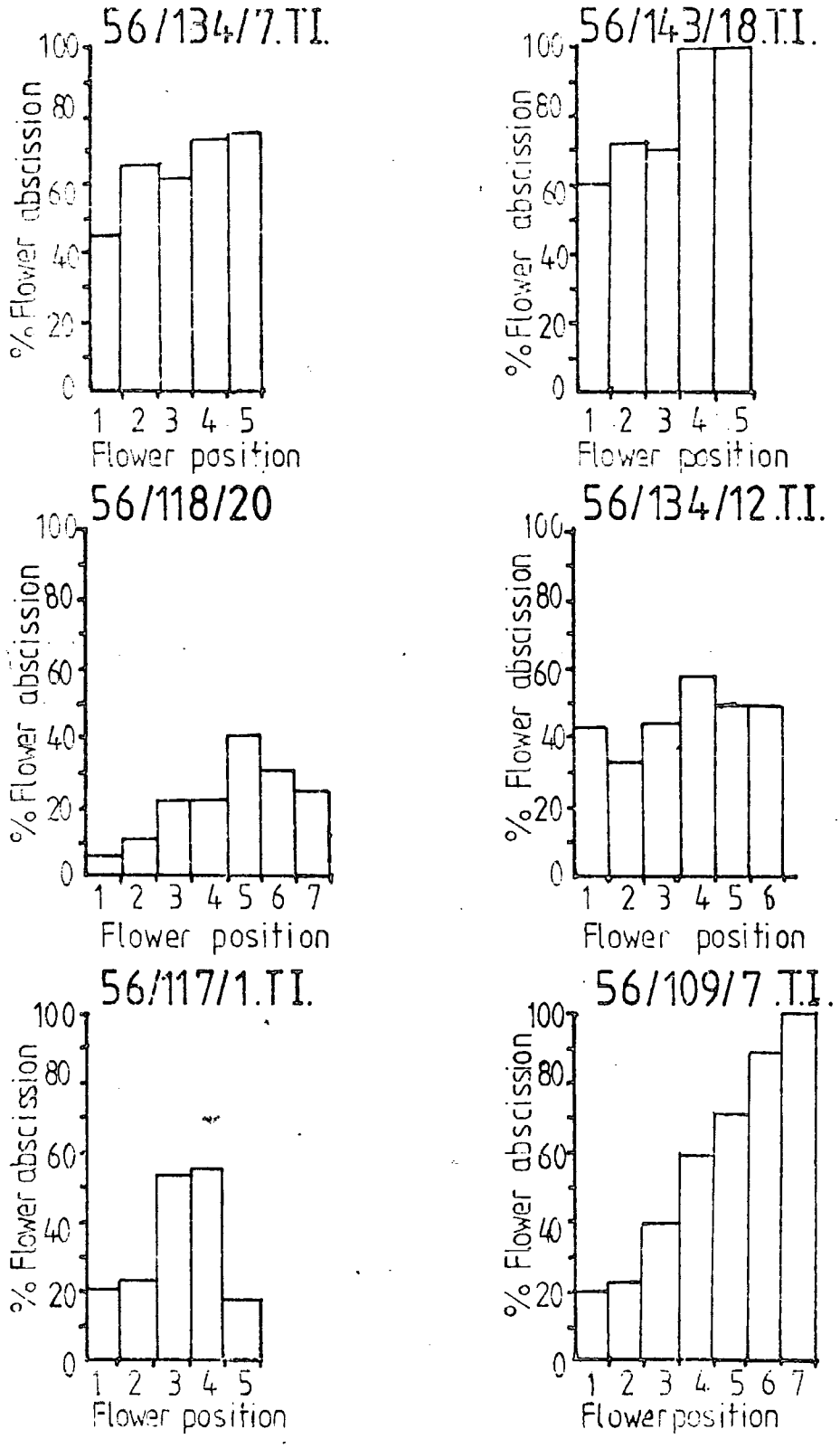



Figure 7.1.2: Flower abscission of inbred lines with different floral and plant architectures. All plants were obtained from the crossing programme. Each value is an overall percentage.

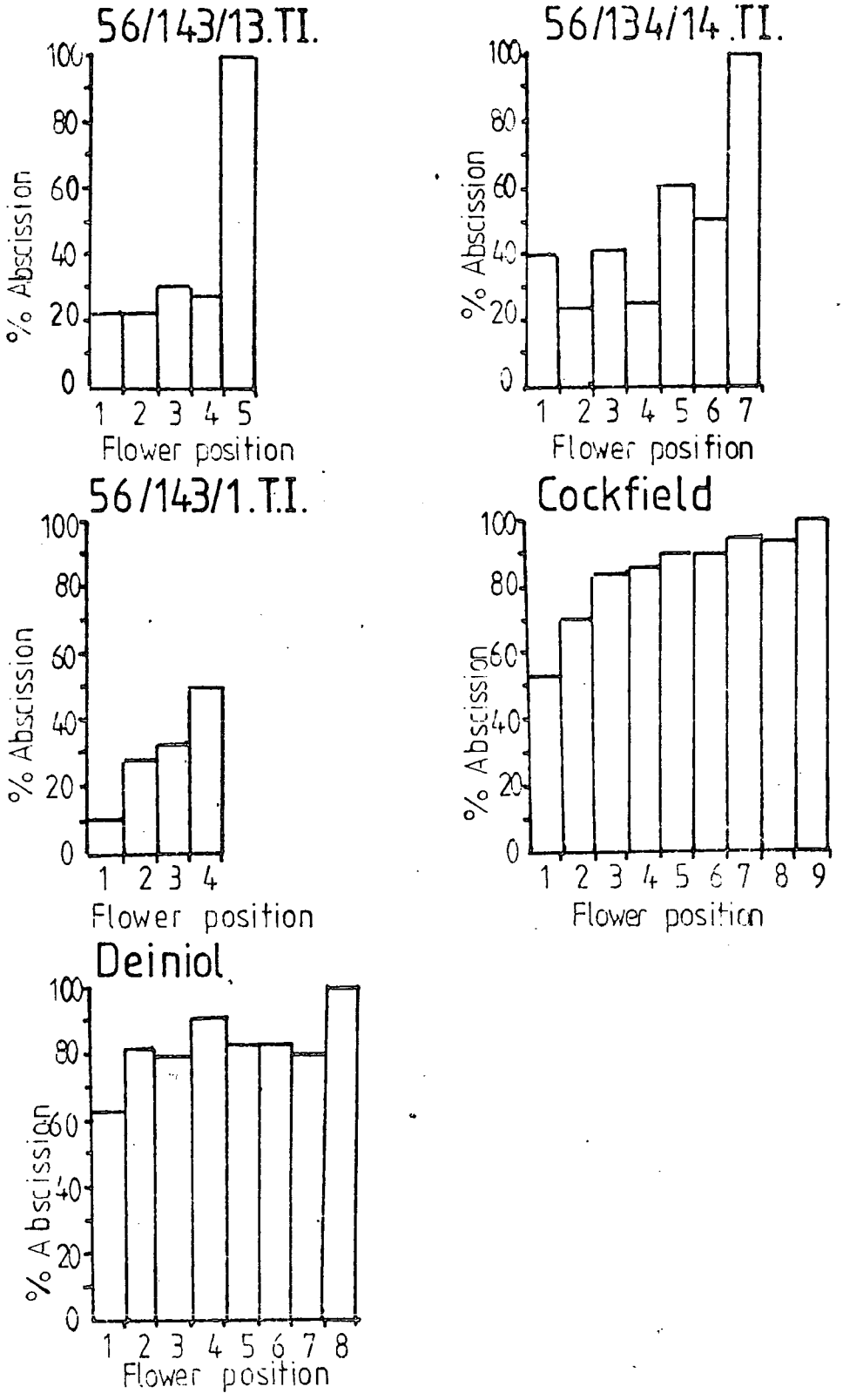



Figure 7.1.3: Flower abscission of inbred lines with different floral and plant architectures, and those of commercial varieties. All plants, except for commercial cultivars, were obtained from the crossing programme. Each value is an overall percentage.

Flower abscission for most determinate lines gradually increased from the proximal axillary raceme to the terminal raceme. One line of plants, 56/117/1, however, exhibited greatest flower abscission, on the proximal axillary inflorescences, and least on the terminal inflorescence. Two lines of plants, 56/107/1 and 56/107/4, exhibited low flower abscission on all racemes (Figures 7.2.1, 7.2.2, 7.2.3, 7.2.4).

Assessment of flower drop for plants of the indeterminate lines 56/130/1 and 56/118/20 (Figures 7.1.1, 7.1.2) revealed a considerable difference in abscission within each raceme, although both lines have a similar plant architecture. Both lines of plants possessed, on average, more flowers on each raceme, compared to the terminal - inflorescence lines. These plants could be termed semi-determinate, in that flowering abruptly stops after 10 - 14 racemes have been produced, but a further few vegetative nodes are produced before growth ceases. Flowers within a particular raceme attained anthesis almost synchronously.

Flower abscission on plants of line 56/130/1 was similar on most raceme positions, varying from 62% of the flowers situated on the proximal raceme position to 82% of flowers situated on the distal raceme position. Flower abscission on plants of line 56/118/20 was low at most flower positions. This varied from 6% of flowers at the proximal raceme position to a peak of 41% at position 5 going down to 25% at position 7.

Flower abscission considerably fluctuated over racemes on plants of line 56/130/1. High flower abscission occurred on proximal racemes, this declined on racemes situated in the middle flowering portion of the plant but increased on sub-

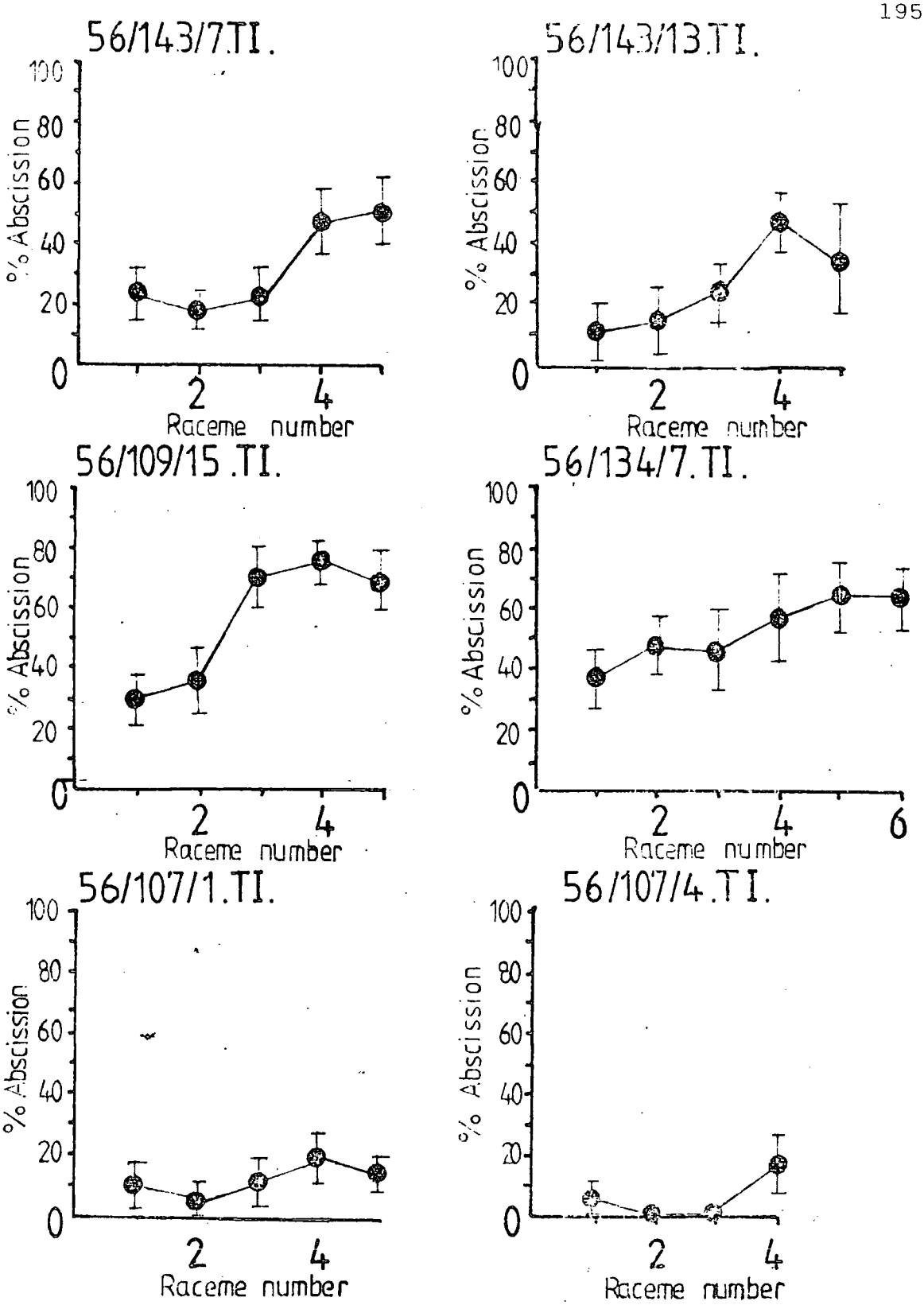



Figure 7.2.1: Flower abscission of inbred lines with different floral and plant architectures. Each value is an average percentage. Standard errors are represented by a bar.

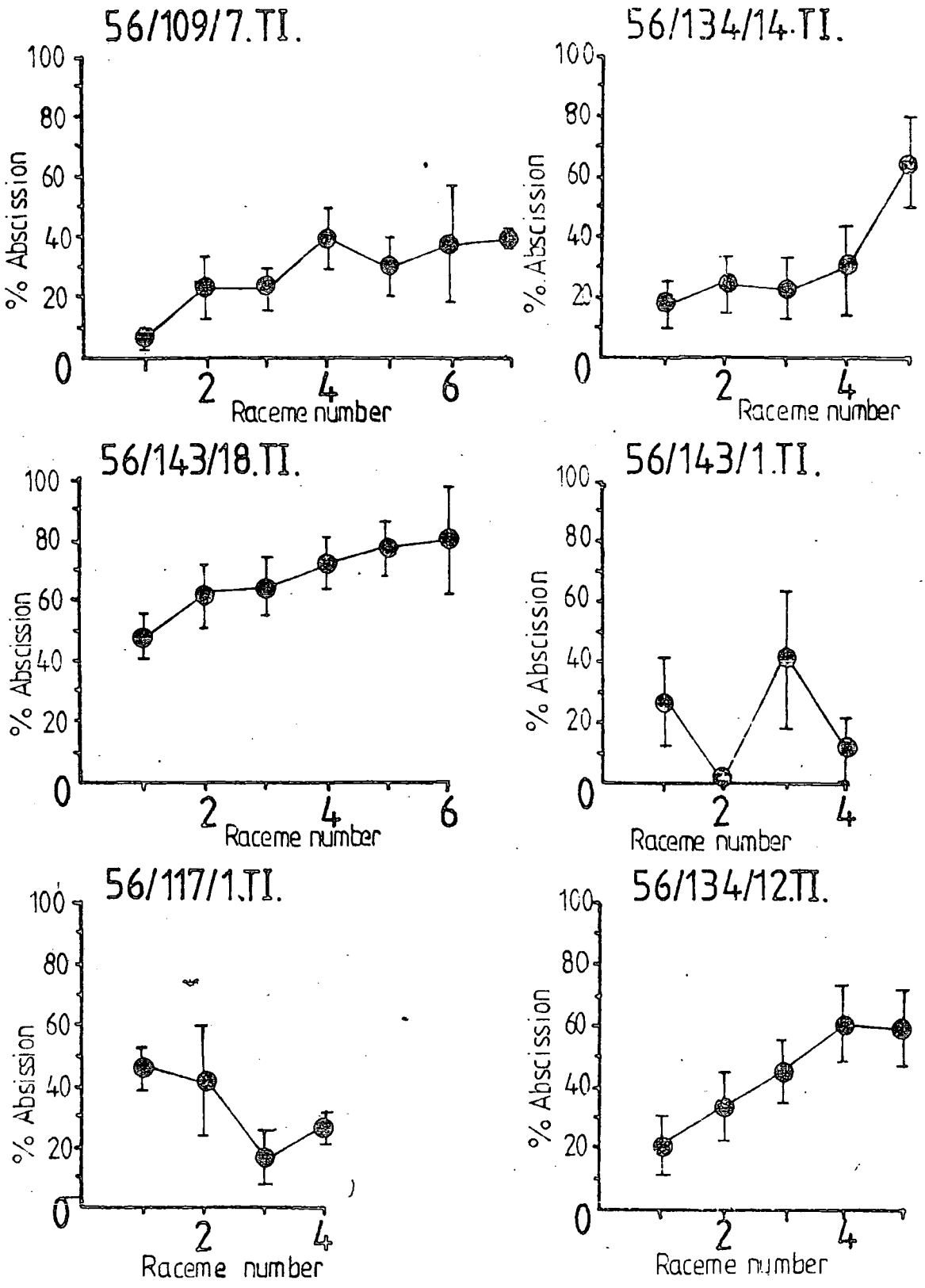



Figure 7.2.2: Flower abscission of inbred lines with different floral and plant architectures. Each value is an average percentage. Standard errors are represented by a bar.

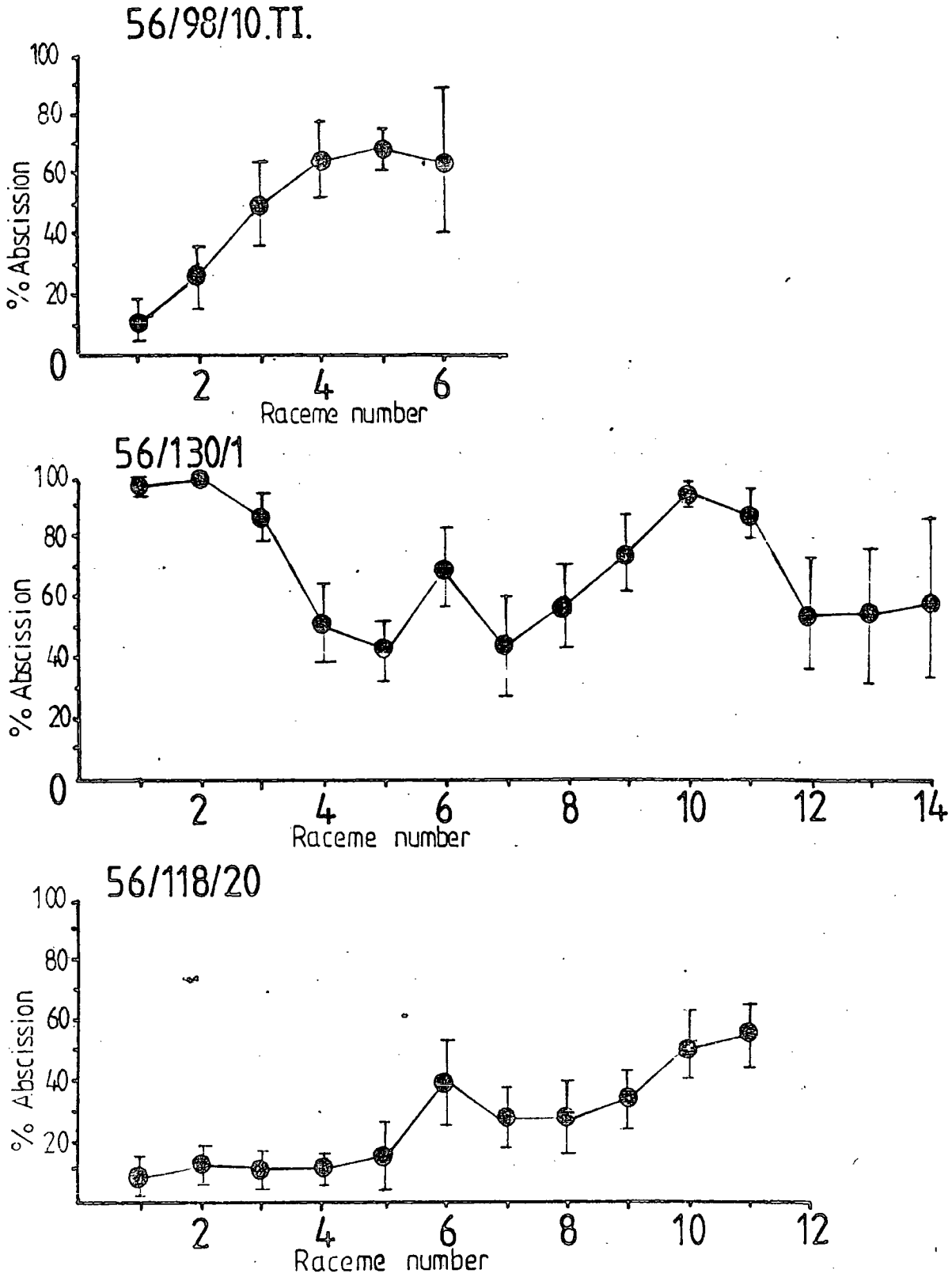



Figure 7.2.3: Flower abscission of inbred lines with different floral and plant architectures. Each value is an average percentage. Standard errors are represented by a bar.

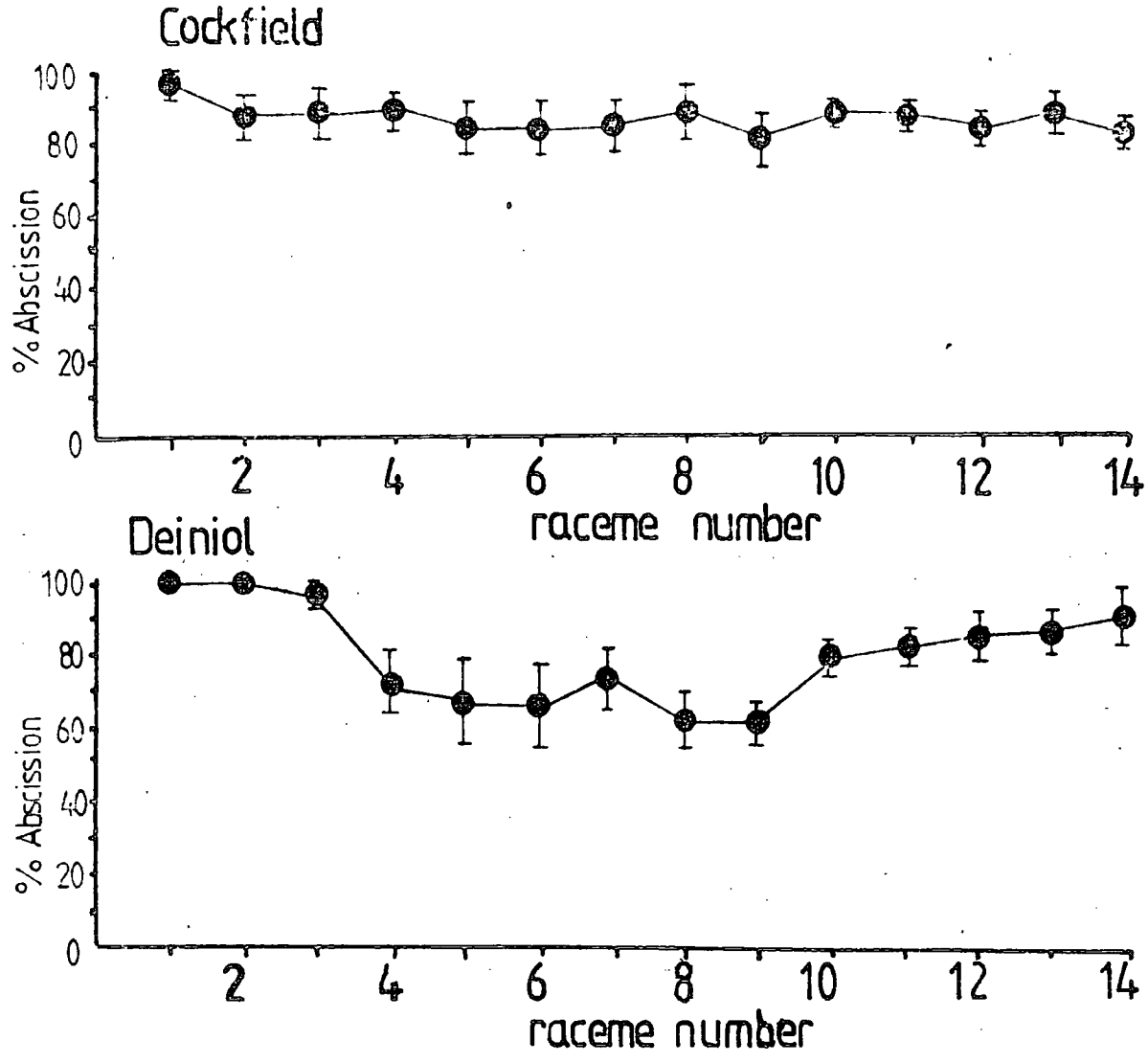



Figure 7.2.4: Flower abscission of commercial varieties Cockfield and Deiniol.
Each value is an average percentage.
Standard errors are represented by a bar.

sequent racemes, and declined again on the most distal inflorescences. Plants of line 56/118/20 exhibited, on average, similar flower abscission on each of the first five racemes, above these inflorescences a gradual increase in flower abscission was observed (Figure 7.2.3).

Overall flower abscission on plants of line 56/118/20 was 23%; whereas on line 56/130/1 it was 71%. The terminal inflorescence lines 56/107/1 and 56/107/4 exhibited less overall flower abscission than plants of line 56/118/20 (Table 7.1). On the former lines, however, less flowers are produced on each raceme and flowering is confined to an average of five inflorescences.

Visual observations of field grown plants with different floral and plant architectures

Plants of inbred line 56/62/F segregated for flower colour between those possessing wing petals with black spots and those with completely white flowers. Both types exhibited a pattern of flower abscission within each raceme, similar to that described previously, that is proximal flowers experienced less abscission than the distal ones. Less flower abscission was observed on plants possessing white flowers. Plants of line 56/14/F also exhibited a similar pattern of flower abscission within each raceme. Plants of line 56/107/1-4 segregated for flower colour and for the terminal inflorescence character. The indeterminate plants, with different flower colours, exhibited greater flower abscission, especially at distal raceme positions, than plants of the same line possessing a terminal inflorescence. Plants possessing flowers with yellow spotted wing petals, exhibited less abscission over all raceme positions, than

Table 7.1 Summary of average percentage abscission and pods set of plants with differing floral and plant architectures and commercial varieties

| Inbred line or variety | Flower abscission | Pods set |
|---|---|---|
| 56/143/13 | 74.3 | 25.7 |
| 56/134/14 | 64.4 | 35.6 |
| 56/143/1 | 74.6 | 25.4 |
| 56/134/7 | 59.3 | 40.7 |
| 56/118/20 | 23.0 | 77.0 |
| 56/117/1 | 32.2 | 67.7 |
| 56/143/18 | 68.4 | 31.6 |
| 56/134/12 | 42.3 | 57.7 |
| 56/109/7 | 38.2 | 61.8 |
| 56/107/1 | 16.2 | 83.8 |
| 56/130/1 | 71.0 | 29.0 |
| 56/143/7 | 53.6 | 46.4 |
| 56/143/13 | 33.1 | 66.9 |
| 56/109/15 | 59.2 | 40.8 |
| 56/107/4 | 13.1 | 86.9 |
| 56/98/10 | 47.4 | 52.6 |
| Cockfield | 82.0 | 18.0 |
| Deiniol | 80.3 | 19.7 |

plants possessing other flower colours. In contrast, for the terminal inflorescence plants of line 56/107/1-4 flower drop was similar at most raceme positions. This was especially so for plants with black spotted wing petals.

Plants of line 56/134/12 & 14, which all possessed a terminal inflorescence experienced low flower abscission at proximal raceme positions, and greater abscission at distal positions.

The terminal inflorescence line 56/143/13-18, exhibited similar flower drop at most inflorescence positions except for the most distally situated flower on each raceme, which exhibited greater drop.

The two lines 56/118/20 and 56/143/9 were semi-determinate in growth habit and had almost synchronous flowers within a particular raceme. Both lines of plants exhibited low flower drop within each raceme. The abscission experienced by flowers of plants of line 56/118/20 varied, on average, from 5% on the proximal flower position to 44% on the most distal position. The proximal flower on racemes of plants of line 56/143/9 had an abscission rate of 12%, in contrast to 38% for flowers situated on the distal raceme position (Figures 7.3.1, 7.3.2, 7.3.3).

Plants of line 56/118/20 exhibited, on average, a similar, low level of flower abscission over most racemes. Plants of line 56/143/9, on average, exhibited low flower abscission on most inflorescences, except those situated on the upper flowering position of the plants (Figures 7.4.1, 7.4.2, 7.4.3).

Overall flower abscission was lowest in both semi-determinate lines. Plants of line 56/62/F with white flowers

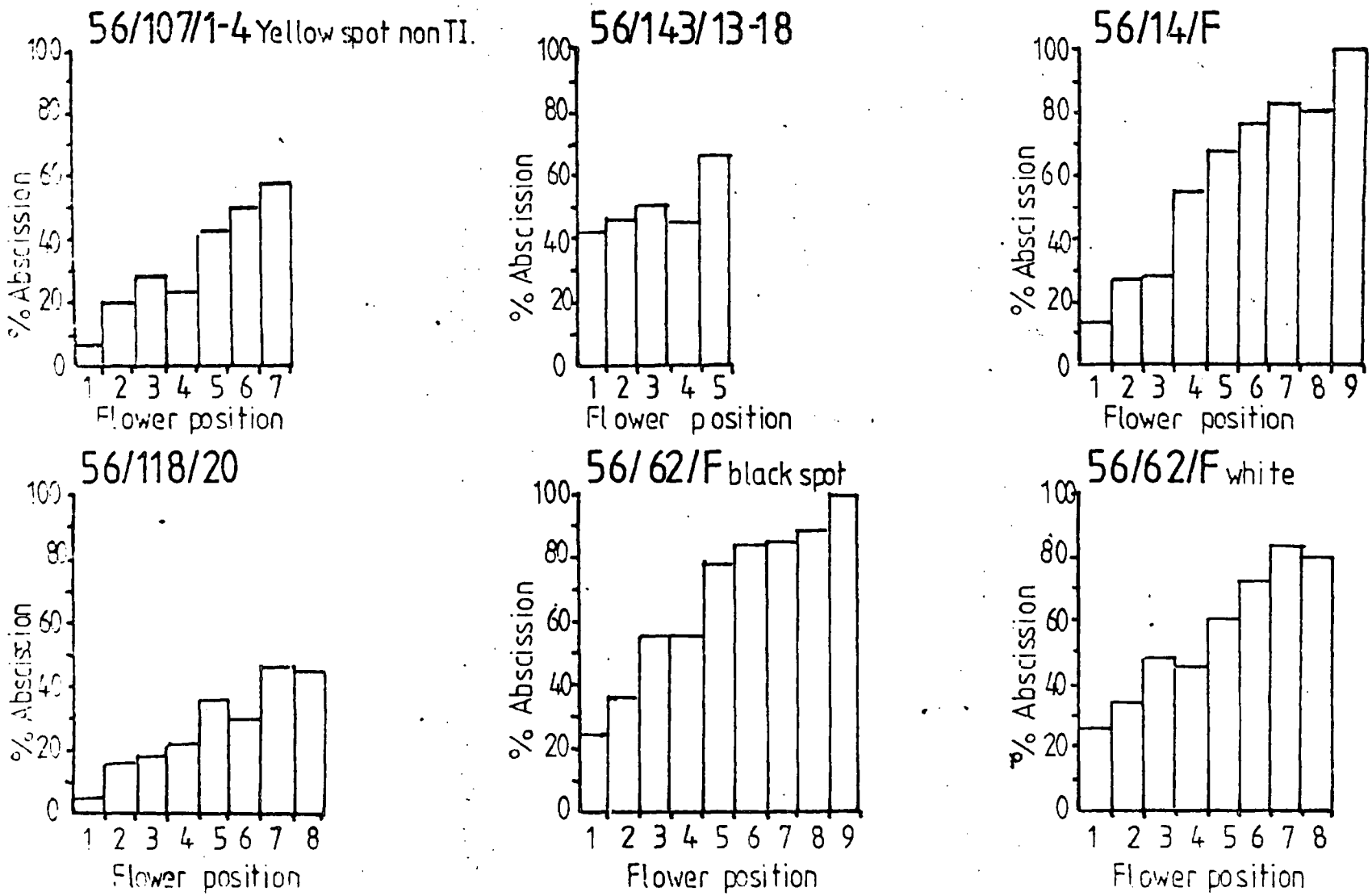



Figure 7.3.1: Flower abscission of F5 inbred lines.
All plants were grown at the PBI during 1981.
Each value is an overall percentage.

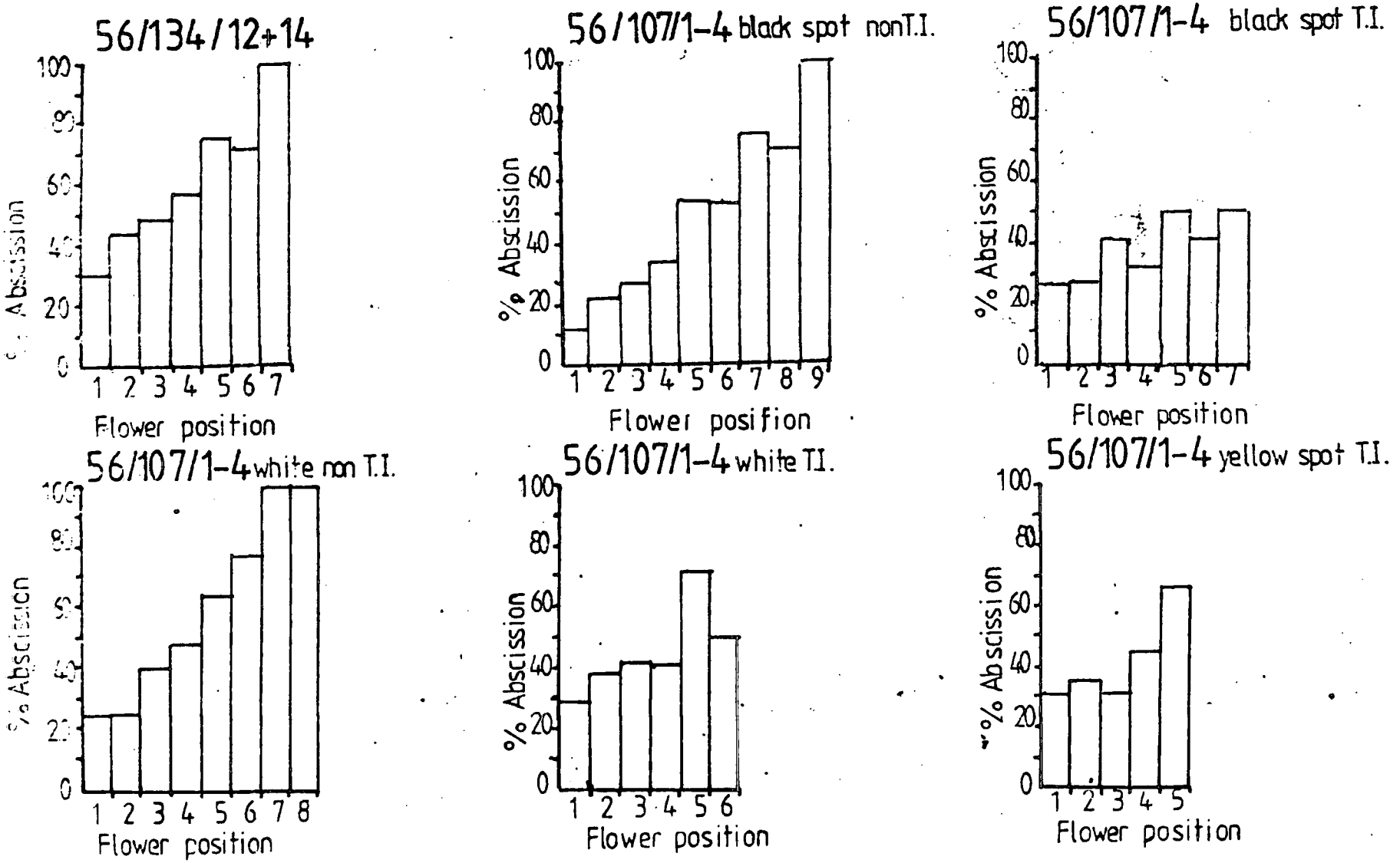



Figure 7.3.2: Flower abscission of F5 inbred lines.
All plants were grown at the PBI during 1981.
Each value is an overall percentage.

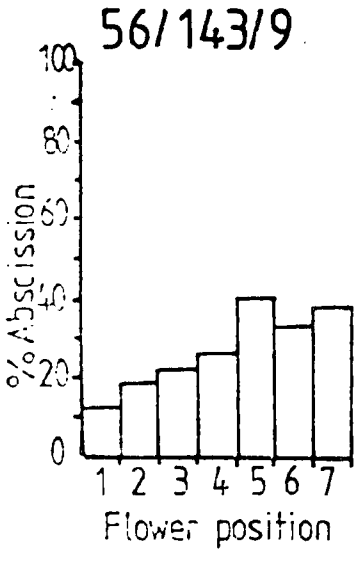



Figure 7.3.3: Flower abscission of F5 inbred lines.
All plants were grown at the PBI during 1981.
Each value is an overall percentage.

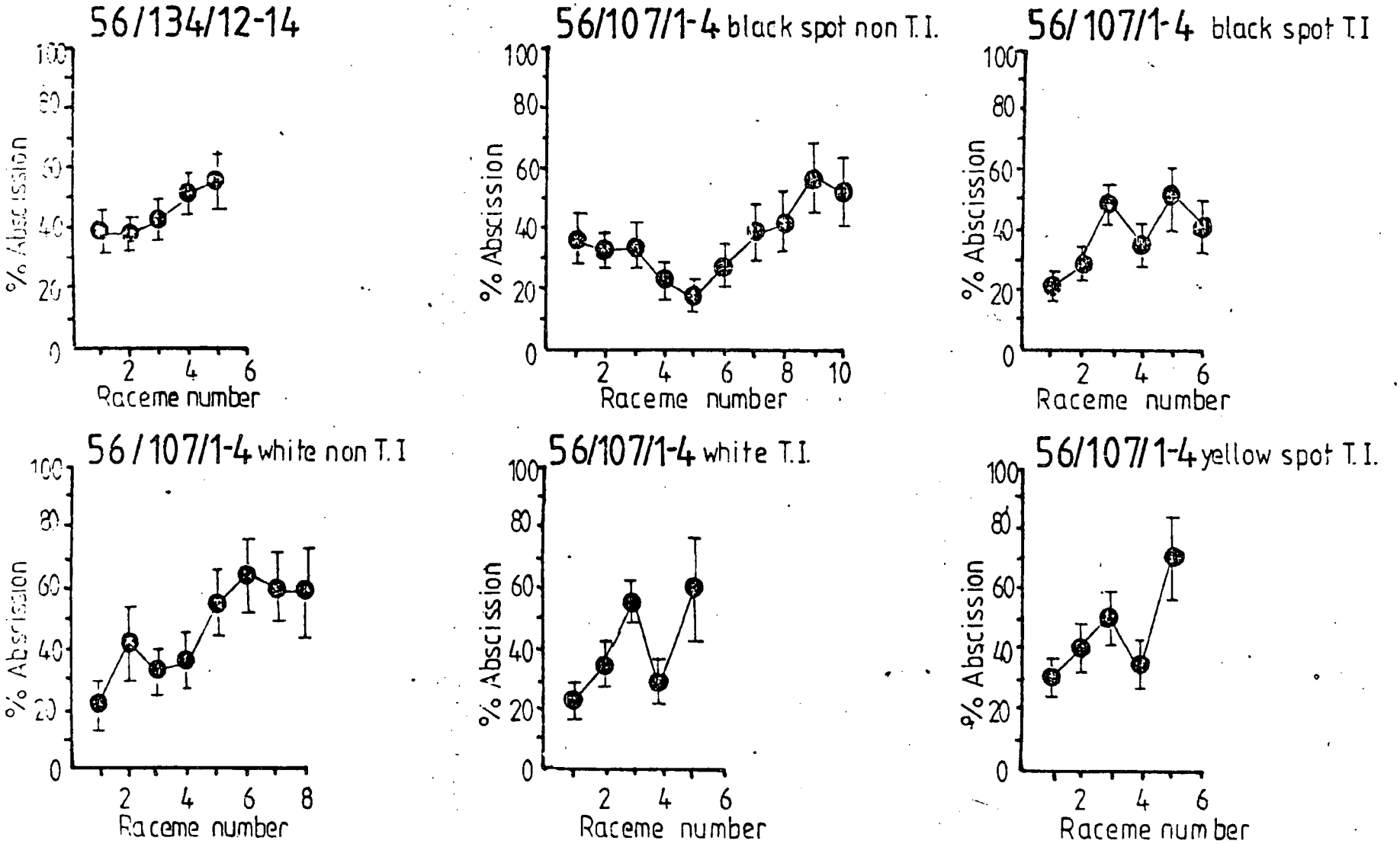



Figure 7.4.1: Flower abscission of F5 inbred lines.
Material was derived from the crossing programme and grown at the PBI during 1981.
Values are average percentages and standard errors are represented by a bar.

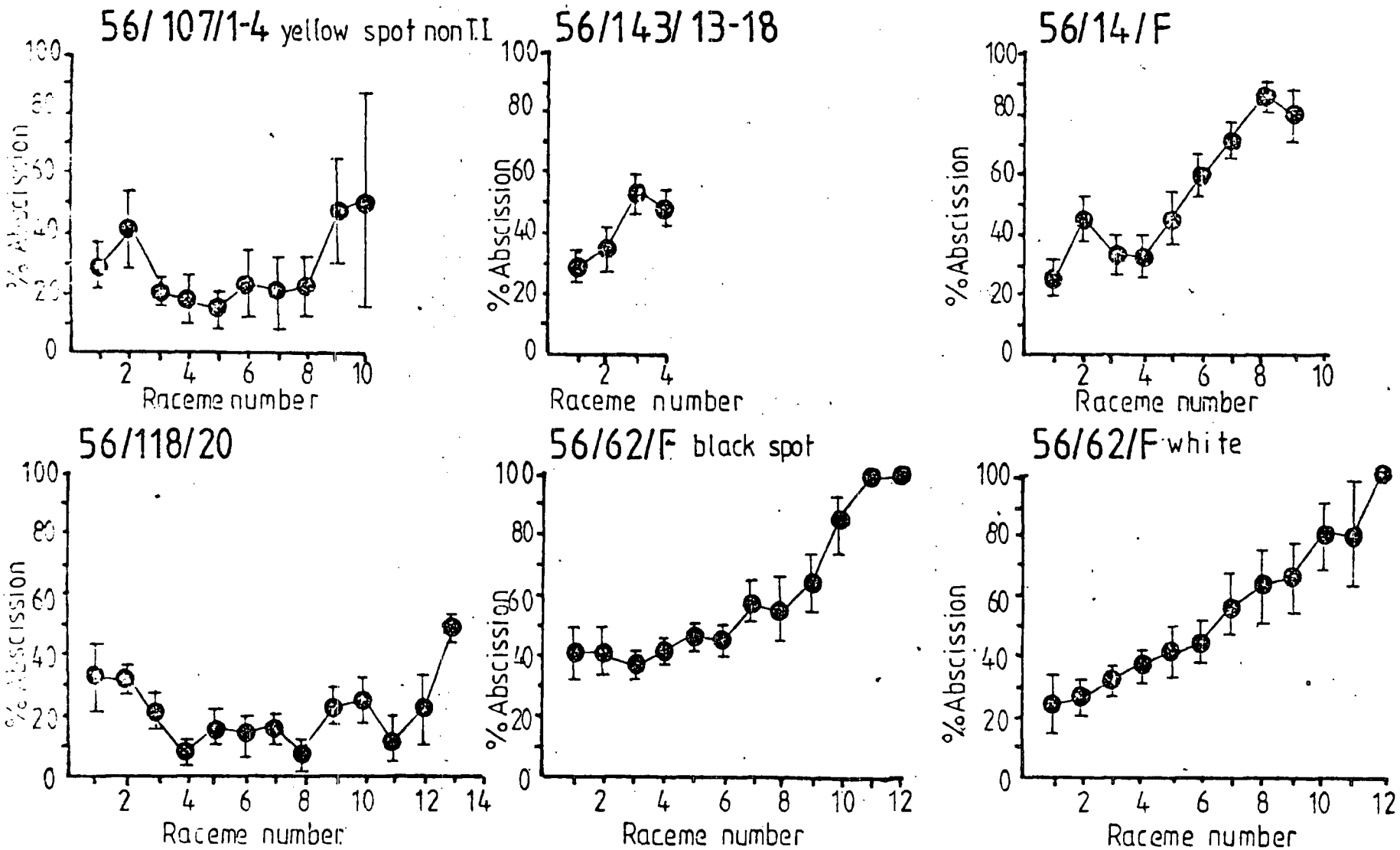



Figure 7.4.2: Flower abscission of F5 inbred lines.
Material was derived from the crossing programme and grown at the PBI during 1981.
Values are average percentages, and standard errors are represented by a bar.

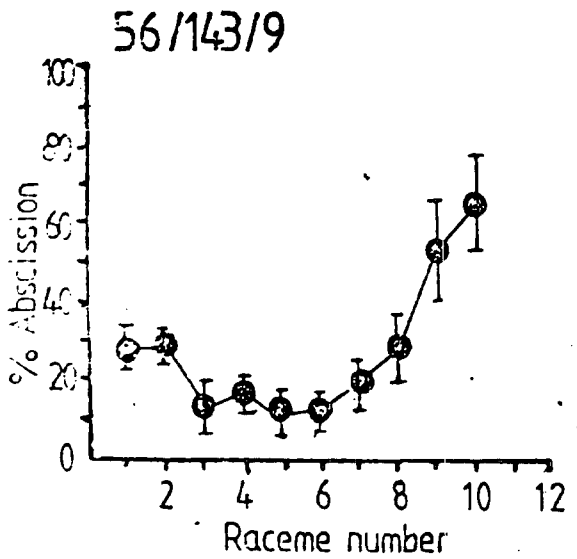



Figure 7.4.3: Flower abscission of F5 inbred lines.
Material was derived from the crossing programme and grown at the PBI during 1981.
Values are average percentages and standard errors are represented by a bar.

exhibited a significant reduction in flower drop, compared to plants of the same line possessing flowers with black spotted wing petals. Comparison between plants of line 56/107/1-4 displaying indeterminate growth, but with differing flower colour and those possessing flowers with black spotted wing petals, showed that overall flower abscission was higher in the plants with white flowers. Plants possessing flowers with yellow spotted wing petals exhibited significantly less overall flower abscission than plants possessing flowers with black spotted wing petals. No difference in overall flower abscission was observed between flowers of different colours, possessing the terminal inflorescence character in plants of line 56/107/1-4 (Table 7.2).

Analysis of yield components revealed that plants of line 56/118/20 produced the highest number of mature pods, but the seeds were smaller (and lighter) than those of other lines. The highest yield, in terms of the mean weight of seed produced per plant, was exhibited by plants of line 56/107/1-4 with an indeterminate growth habit possessing wing petals with a black spot. Plants of the semideterminate line 56/143/9, produced quite a high number of mature pods, but this was exceeded by indeterminate plants of line 56/107/1-4 possessing flowers with black or yellow spotted wing petals (Table 7.3).

Results of observations comparing the transport of the dye eosin within the vascular tissue of plant lines exhibiting low flower drop to commercial varieties

The proximal flower on racemes of Deiniol plants was removed at the pedicel/peduncle junction and eosin was fed into the vascular tissue via the cut surface. It was observed that eosin rapidly appeared in flowers distally situated on

Table 7.2 Summary of results obtained from $F_5$ inbred lines

| line | flowers dropped | pods set | $\chi^2$, v = 1 (where appropriate) |
|---|---|---|---|
| 56/134/12 + 14 | 250 (36.1) | 443 (63.9) | |
| 56/143/13-18 | 182 (40.3) | 269 (59.7) | |
| 56/14/F | 306 (47.7) | 336 (52.3) | |
| 56/118/20 | 116 (19.9) | 468 (80.1) | |
| 56/143/9 | 147 (25.9) | 419 (74.1) | |
| 56/62/F | | | |
| (a) black spot | 38 (51.4) | 359 (48.6) | |
| (b) white | 287 (44.2) | 362 (55.8) | 7.71 ($P > 0.005 < 0.001$) |
| 56/107/1-4 | | | |
| (a) black spot non TI | 229 (34.2) | 440 (65.8) | |
| (b) white non TI | 157 (44.8) | 193 (55.2) | 11.03 ($P > 0.001$)* |
| (c) black spot TI | 160 (33.0) | 325 (67.0) | |
| (d) white TI | 170 (37.1) | 288 (62.9) | 1.76 ($P < 0.1$) |
| (e) yellow spot non TI | 86 (26.5) | 238 (73.5) | 5.95 ($P > 0.025 < 0.005$)* |
| (f) yellow spot TI | 97 (33.7) | 191 (66.3) | 0.039 ($P < 0.1$) |

* as compared to 56/107/1-4, black spot non TI.

Overall percentages are given in parentheses.

Table 7.3 Yield components of $F_5$ inbred lines

| Line | Average yield component per plant | | | |
|---|---|---|---|---|
| | Pod Number | Seed Number | Seed weight (g) | Weight of each seed |
| 56/134/12 + 14 | 5.8 (0.89) | 11.7 (2.07) | 3.6 (0.69) | 0.30 (0.022) |
| 56/143/13-18 | 6.2 (1.04) | 9.4 (2.61) | 3.4 (1.09) | 0.31 (0.036) |
| 56/14/F | 9.7 (0.76) | 25.5 (1.58) | 14.1 (1.72) | 0.56 (0.066) |
| 56/118/20 | 25.6 (3.25) | 48.1 (9.13) | 10.9 (2.29) | 0.22 (0.012) |
| 56/143/9 | 16.7 (1.39) | 39.1 (3.07) | 10.0 (0.85) | 0.25 (0.0006) |
| 56/62/F | | | | |
| (a) black spot | 12.1 (1.53) | 32.4 (3.70) | 17.5 (2.27) | 0.54 (0.043) |
| (b) white | 10.1 (1.74) | 22.7 (3.75) | 9.4 (1.65) | 0.36 (0.034) |
| 56/107/1-4 | | | | |
| (a) black spot non TI | 19.1 (2.37) | 48.3 (6.91) | 23.6 (3.86) | 0.48 (0.029) |
| (b) white non TI | 13.0 (2.45) | 30.5 (6.74) | 16.1 (3.83) | 0.51 (0.058) |
| (c) black spot TI | 11.1 (1.20) | 20.2 (2.27) | 8.8 (1.33) | 0.42 (0.036) |
| (d) white TI | 9.7 (0.92) | 14.6 (2.56) | 3.95 (0.59) | 0.29 (0.016) |
| (e) yellow spot non TI | 23.1 (6.06) | 44.7 (13.34) | 15.6 (4.30) | 0.37 (0.018) |
| (f) yellow spot TI | 5.2 (0.83) | 9.80 (1.81) | 3.59 (0.79) | 0.33 (0.024) |

Standard errors are in parentheses.

the racemes. However, in some cases, no eosin was detected in flowers at positions 2 and 3, or 3 and 4 within a raceme.

When the same treatment was applied to the cut tissue at the second flower position within a raceme, in no case was eosin detected in the flower situated at the first raceme position. In most cases, the dye rapidly appeared in flowers situated on more distal raceme positions. In some cases, however, there was no transport of dye to flowers situated on the third position. In one case, no dye was observed in the fifth position within the raceme (Table 7.4.1).

When this experiment was repeated with plants of line 56/143/9 irrespective of which flower position was fed with eosin and with few exceptions no dye appeared at all in any of the other flowers on the raceme (Table 7.4.2).

Transport of $^{14}CO_2$ within racemes of plants of line 56/143/9, compared to that in racemes of inbred line T51

The proximal flower on a raceme of inbred line T51 and line 56/143/9 was fed with $^{14}CO_2$, and flowers situated on other raceme positions were subsequently assayed for the presence of incorporated $^{14}C$. This revealed that most $^{14}C$ was incorporated in the fed flower of line T51, but substantial $^{14}C$ was also detected in the second and third flowers on the raceme. In addition some radioactivity was detected in flowers situated at positions 5, 6 and 7 (Figure 7.5.1). The fed flower of line 56/143/9 contained most of the incorporated $^{14}C$. Very little radioactivity appeared in flowers situated at other positions on the raceme (Figure 7.5.2).

Table 7.4.1 Presence or absence of transported Eosin into flowers following proximal flower removal for cultivar Deiniol

| Treatment & flower position | Raceme replicate | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| (a) | | | | | |
| 1 | Removed | Removed | Removed | Removed | Removed |
| 2 | X | / | X | X | / |
| 3 | / | / | X | X | X |
| 4 | / | X | / | / | X |
| 5 | / | / | / | / | / |
| 6 | - | / | / | / | / |
| (b) | | | | | |
| 1 | X | X | X | X | X |
| 2 | Removed | Removed | Removed | Removed | Removed |
| 3 | / | / | X | / | X |
| 4 | / | / | / | / | / |
| 5 | / | X | / | / | / |
| 6 | - | / | - | - | / |

(a) first flower or (b) second flower on a raceme was removed

/ = presence of transported Eosin

X = absence of transported Eosin

Table 7.4.2 Presence or absence of transported Eosin into flowers following proximal flower removal for line 56/143/9

| Treatment and flower position | Raceme replicate | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| (a) | | | | | |
| 1 | Removed | Removed | Removed | Removed | Removed |
| 2 | X | X | / | X | X |
| 3 | X | X | X | X | X |
| 4 | X | X | X | X | X |
| 5 | X | X | X | X | X |
| 6 | X | - | - | X | / |
| (b) | | | | | |
| 1 | X | X | X | X | X |
| 2 | Removed | Removed | Removed | Removed | Removed |
| 3 | X | X | X | X | X |
| 4 | X | X | X | / | X |
| 5 | X | X | X | X | X |
| 6 | X | / | X | X | X |

(a) first flower or (b) second flower on a raceme was removed

/ = presence of Eosin X = absence of transported Eosin

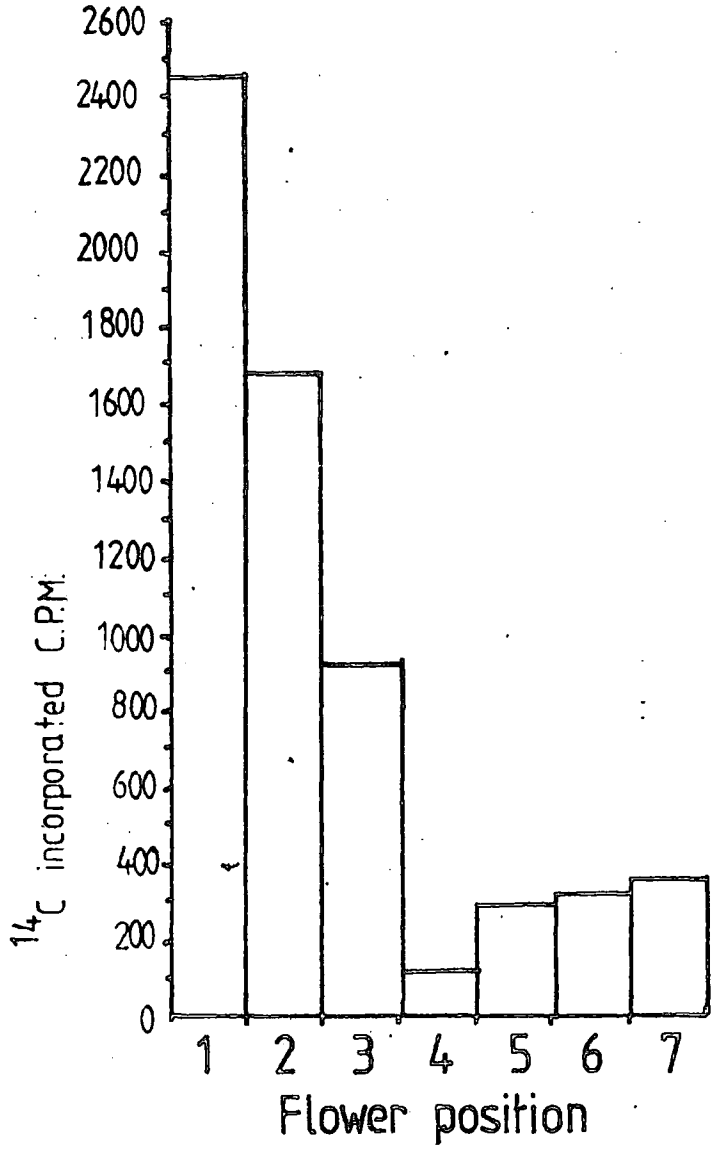



Figure 7.5.1: °Distribution of $^{14}C$ after feeding the proximal flower of a raceme of inbred line T51.

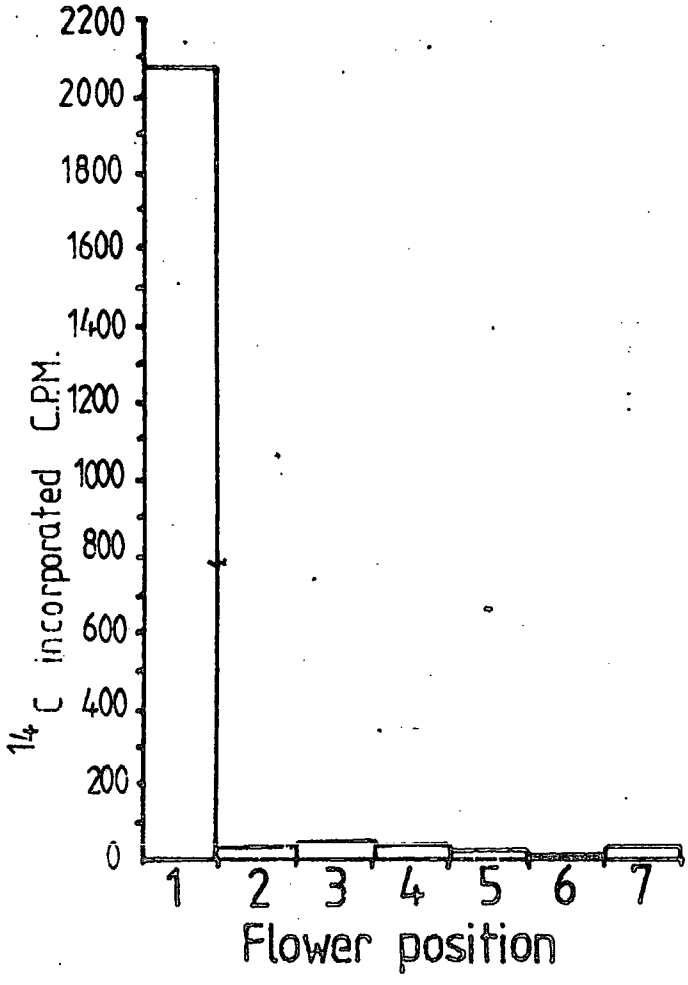



Figure 7.5.2: Distribution of $^{14}C$ after feeding the proximal flower of a raceme of line 56/143/9.

Results of experiments to compare the development of the pedicel/peduncle junctions of variety Maris Bead and line 56/118/20

The differentiation of vascular tissue within the pedicel/peduncle junction of flowers situated on different positions within a raceme was measured, by assaying the concentration of peroxidase within these junctions, using a gel diffusion enzyme assay (Figure 7.6).

Proximal Maris Bead junctions, of flowers at developmental stage 10 had greater peroxidase concentrations than junctions of flowers, at the same developmental stage, situated at distal raceme positions. Junctions of flowers, of plants of line 56/118/20, at developmental stage 10 at all raceme positions all exhibited a similar, low concentration of peroxidase.

Comparison of Deiniol pedicel/peduncle junctions at all raceme positions showed that it was the proximal two junctions that had a higher peroxidase concentration, which increased from developmental stage 10 to pod set. No such increase in peroxidase concentration was observed in pedicel/peduncle junctions of flowers at more distal raceme positions. Indeed many flowers at the more distal raceme positions were beginning to abscise. Comparison of the above concentrations with those found at junctions of line 56/143/9 showed that there was less peroxidase at the proximal flower positions of line 56/143/9, but the enzyme concentration increased at all raceme positions between developmental stage 10 to pod set (Figure 7.7).

These results show that pedicel/peduncle junctions of proximally situated flowers of varieties Maris Bead and Deiniol have a developmental advantage over flowers, at the

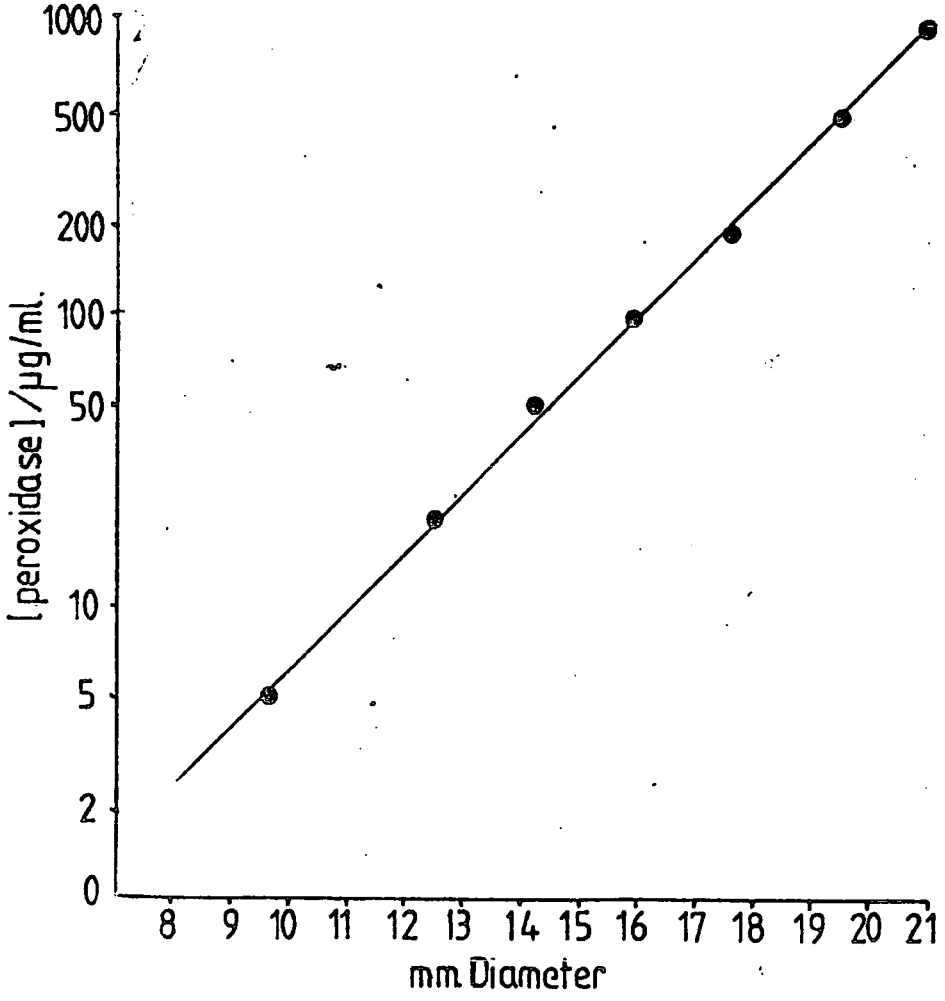



Figure 7.6: Standard curve for peroxidase assay. Standard errors denoted as a bar.

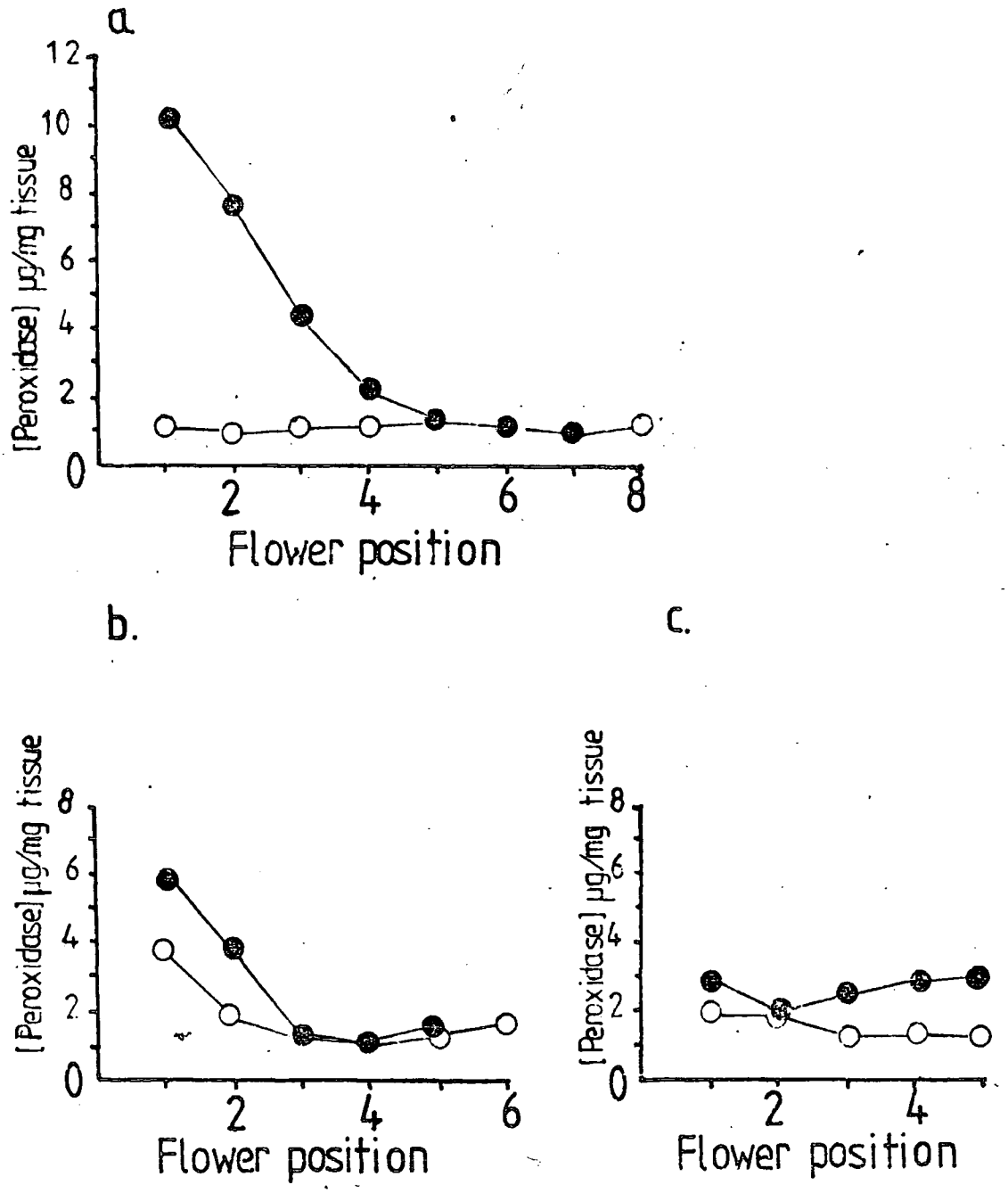



Figure 7.7: Estimation of peroxidase concentration in pedicel peduncle junctions at each flower position and at different developmental stages. Each value is an average of 10 replicates.

(a) Comparison of Maris Bead (●) of junctions from flowers at stage 10 at the proximal position and at stage 9 at the distal position and 56/118/20 all flowers at stage 10.

(b) Comparison of Deiniol junctions at Stage 10 (o) and at pod set (●).

(c) Comparison of 56/143/9 junctions at stage 10 (o) and at pod set (●).

same developmental stage, situated at more distal raceme positions. However, pedicel/peduncle junctions of flowers of lines 56/118/20 and 56/143/9 differentiate evenly, but more slowly, over all raceme positions.

Examination of the vascular anatomy within racemes of commercial varieties and those lines exhibiting low flower abscission

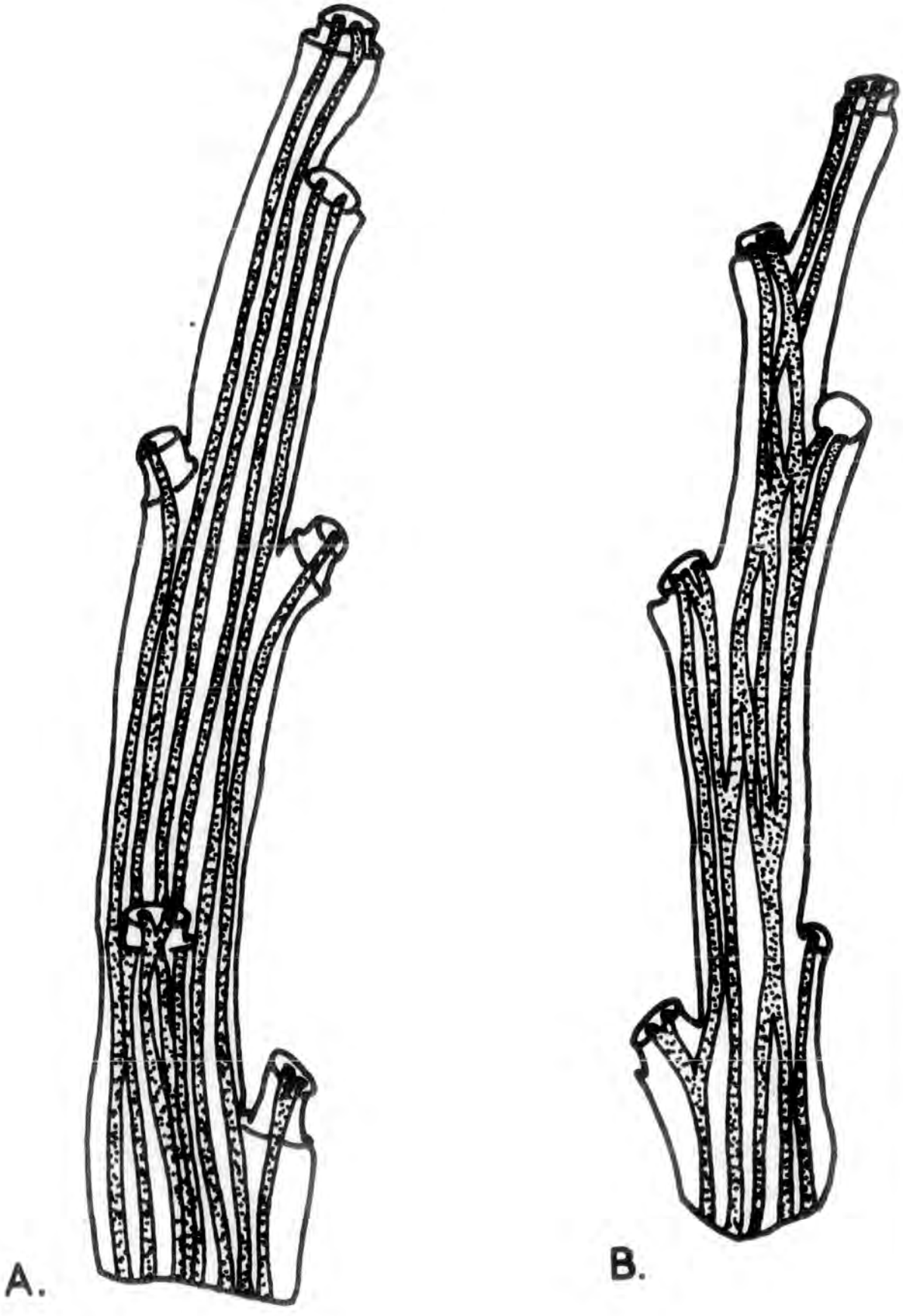
Racemes obtained from plants of Maris Bead, Deiniol, Cockfield and the terminal genotype TI Col. and inbred lines 56/118/20 and 56/143/9 were sectioned and examined under fluorescent and light microscopy. In addition squashes of whole racemes from these lines were also examined. In all commercial varieties and the determinate genotype TI Col, in most cases, it was observed that the vascular trace of the proximal flower position was independent of the other flowers. In some cases the vascular trace supplying the second flower was also independent of the other flowers. In many cases the vascular strands leading to the second and third flower positions were connected to those traces supplying the flowers situated on more distal positions. It was also clear that much variation of this general vascular architecture occurred in commercial varieties.

Examination of the vasculature of racemes from plants of lines 56/143/9 and 56/118/20, revealed that the vascular tissue supplying proximal flowers was, in the majority of cases, independent of that supplying flowers situated at other raceme positions. Again, however, some variation from this vascular architecture was also observed (Figure 7.8).

The above evidence suggests that the observed reduction in flower drop in lines 56/143/9 and 56/118/20, is due to the

Figure 7.8: A. Diagram of peduncle, showing independent vascular supply to each flower.

B. Typical branched pattern of vascular supply.


A.
B.

particular vascular architecture within the raceme of these plants (see Figure 7.9).

The incorporation of $^{14}CO_2$ into racemes from fed leaves

Leaves of Maris Bead and line 22 plants were fed with $^{14}CO_2$ (Figures 7.10.1, 7.10.2). The results show that most $^{14}C$ was incorporated into the raceme on the same node as the fed leaf. Very little $^{14}C$ was incorporated in leaves and racemes elsewhere.

Distribution of $^{14}C$ in pods and leaves of line 56/143/9 and commercial variety Deiniol

In a plant of variety Deiniol much $^{14}C$ was incorporated into the raceme on the same node as the fed leaf. $^{14}C$ was, however, also incorporated in pods developing on the lower portion of the plant. This incorporation was selective, in that most of the $^{14}C$ only appeared in the pods and peduncles situated 2, 4, 6 and 8 nodes below the fed leaf. In addition, $^{14}C$ only appeared in the pods and peduncles, not in the leaves. Little or no $^{14}C$ was incorporated into pods and peduncles 1, 3, and 7 nodes below the fed leaf. (No raceme was formed at the 5th node below the fed leaf in the specimen used). (Table 7.5; Figure 7.11).

A similar pattern of $^{14}C$ incorporation was observed in plants of line 56/143/9 (Table 7.6, Figure 7.12). In both cases there was very little incorporation of $^{14}C$ in organs above the fed leaf. However, a small amount of $^{14}C$ was incorporated into the apex of both plants. This incorporation was greater in Deiniol plants.

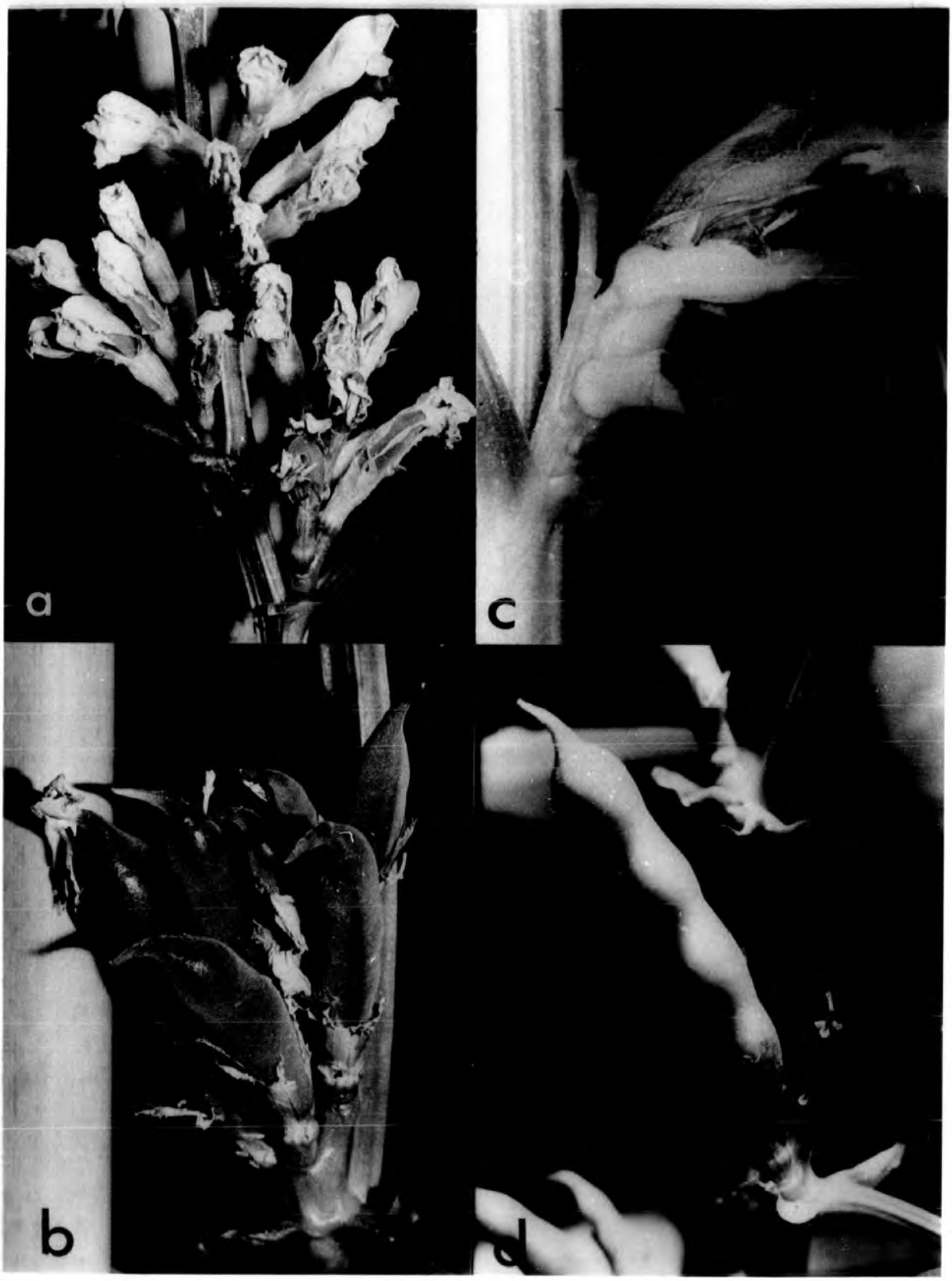
Feeding a leaf of a plant of line 56/143/9 when in flower, but with no pods set on the plant with $^{14}CO_2$, resulted in $^{14}C$ being incorporated into alternate and flowers above the fed leaf. Much more radioactivity appeared in the apex of the plant (Fig. 7.13).

Figure 7.9: (a) Early pod set in independent vascular supply line 56/143/9.

(b) Pods maturing on a raceme of inbred line 56/143/9.

(c) Early pod set on a raceme of commercial variety Cockfield.

(d) Maturing pod on a raceme of commercial variety Cockfield.


a
c
b
d

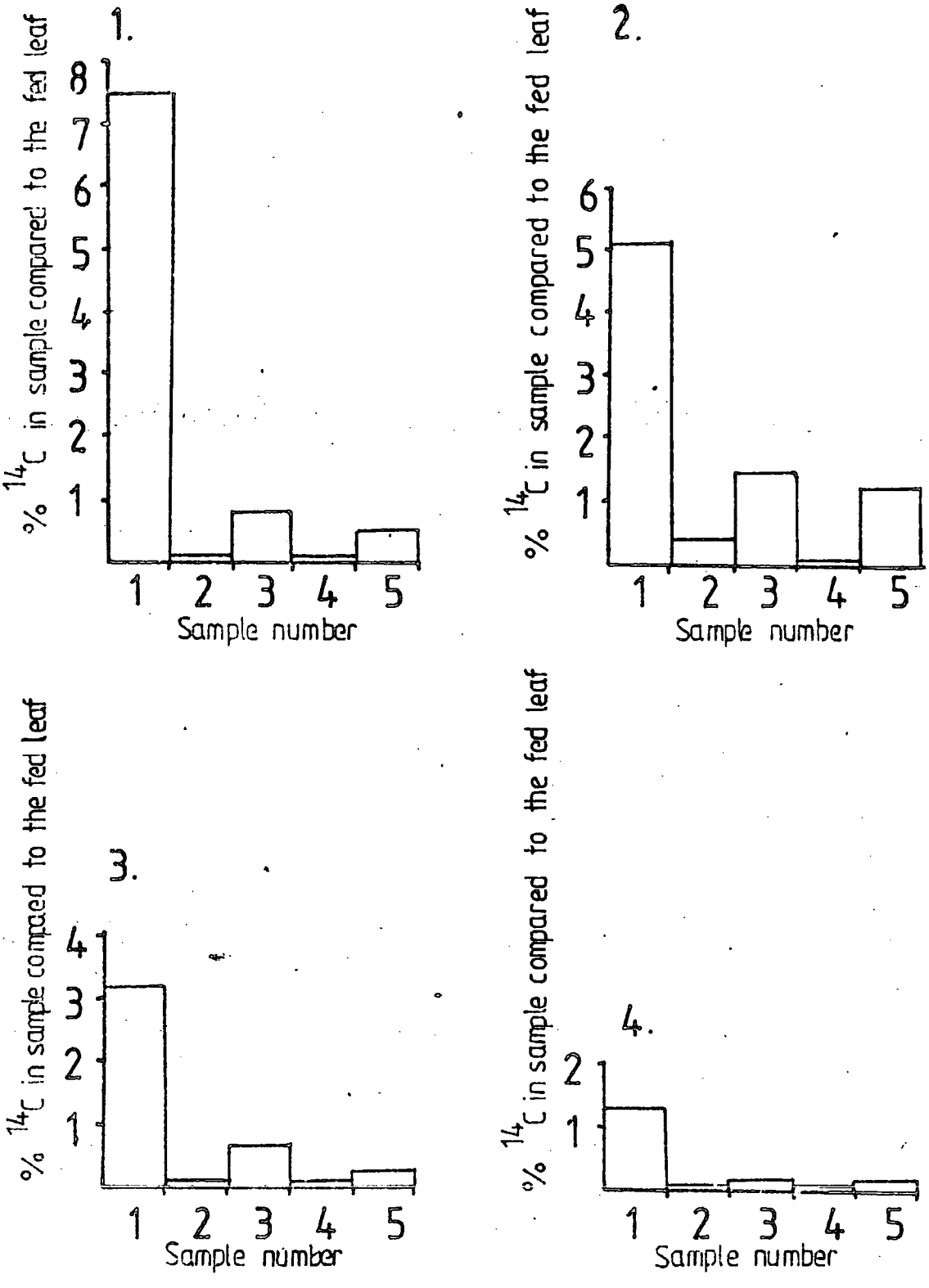



Figure 7.10.1: Incorporation of $^{14}C$ into racemes and leaves of cultivar Maris Bead.

1 = fed raceme; 2 = leaf above fed leaf; 3 = raceme about fed leaf; 4 = leaf below fed leaf; 5 = raceme below fed leaf.

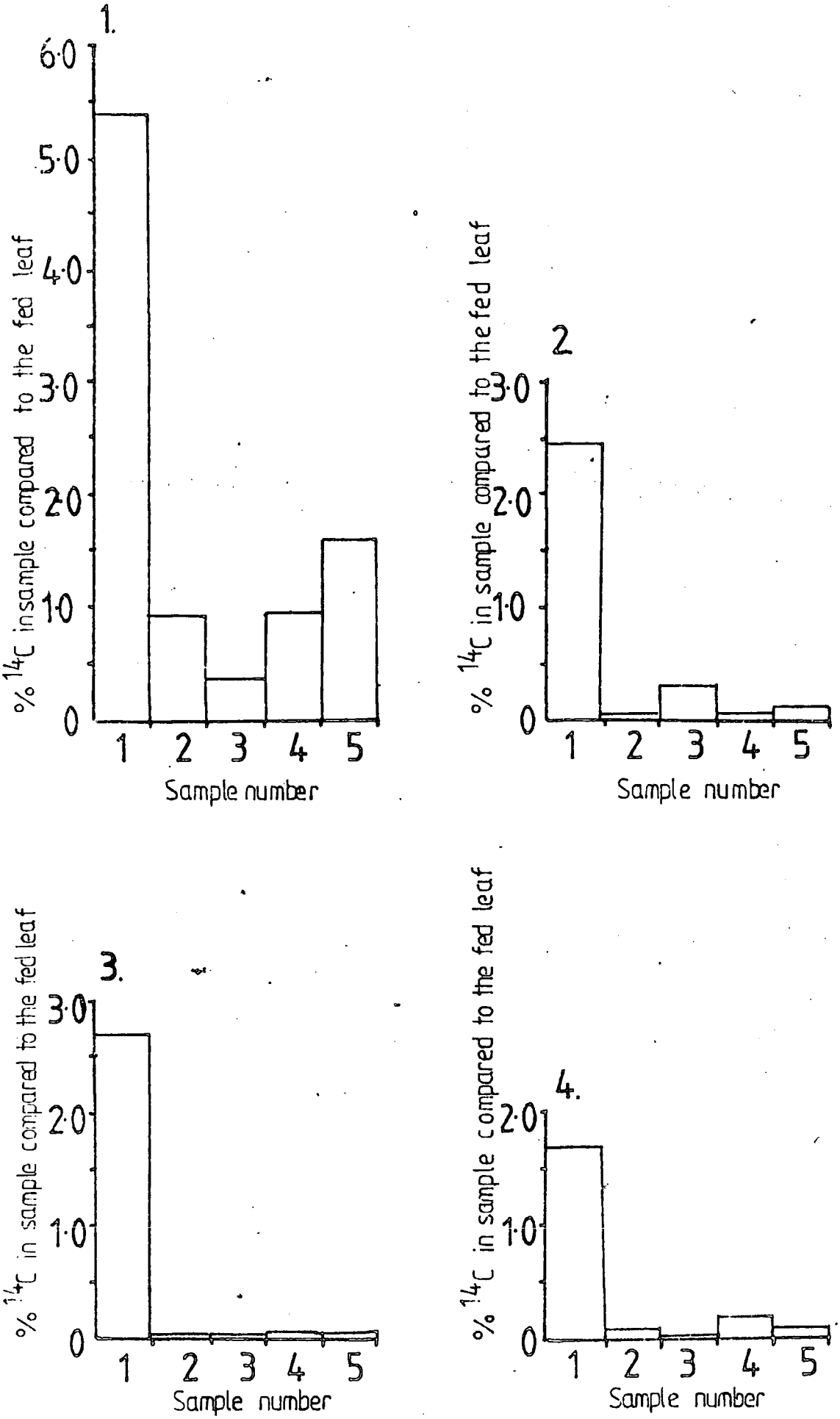



Figure 7.10.2: Incorporation of $^{14}C$ into racemes and leaves of line 22.
1 = fed raceme; 2 = leaf above fed leaf;
3 = raceme above fed leaf; 4 = leaf below fed leaf;
5 = raceme below fed leaf.

Table 7.5 Explanation of samples for $^{14}C$ feeding experiment with Deiniol (see Figure 7.11)

| Sample number | Explanation |
|---|---|
| 1 | 1 cm section of stem above fed leaf. |
| 2 | 1 cm section of stem below fed leaf. |
| 3 | Pods, flowers and raceme subtending fed leaf. |
| 4 | Leaf below fed leaf. |
| 5 | Flowers, pod and node (Pod 2 cm) |
| 6 | 2nd lead below fed leaf. |
| 7 | Pods and raceme (Pod 3 cm). |
| 8 | 3rd leaf below fed leaf. |
| 9 | Pod and raceme (Pod 3.5 cm). |
| 10 | 4th leaf below fed leaf. |
| 11 | Pod and raceme (Pod 5 cm). |
| 12 | 5th leaf below - no raceme. |
| 13 | 6th leaf below fed leaf. |
| 14 | Pod and raceme (Pod 5.5 cm) |
| 15 | 7th leaf below fed leaf. |
| 16 | Pod and raceme (Pod 6 cm) |
| 17 | 8th leaf below fed leaf. |
| 18 | Pod and raceme (Pod 6 cm) |
| 19 | Total % radioactivity of leaves, flowers and racemes and apex above fed leaf. |

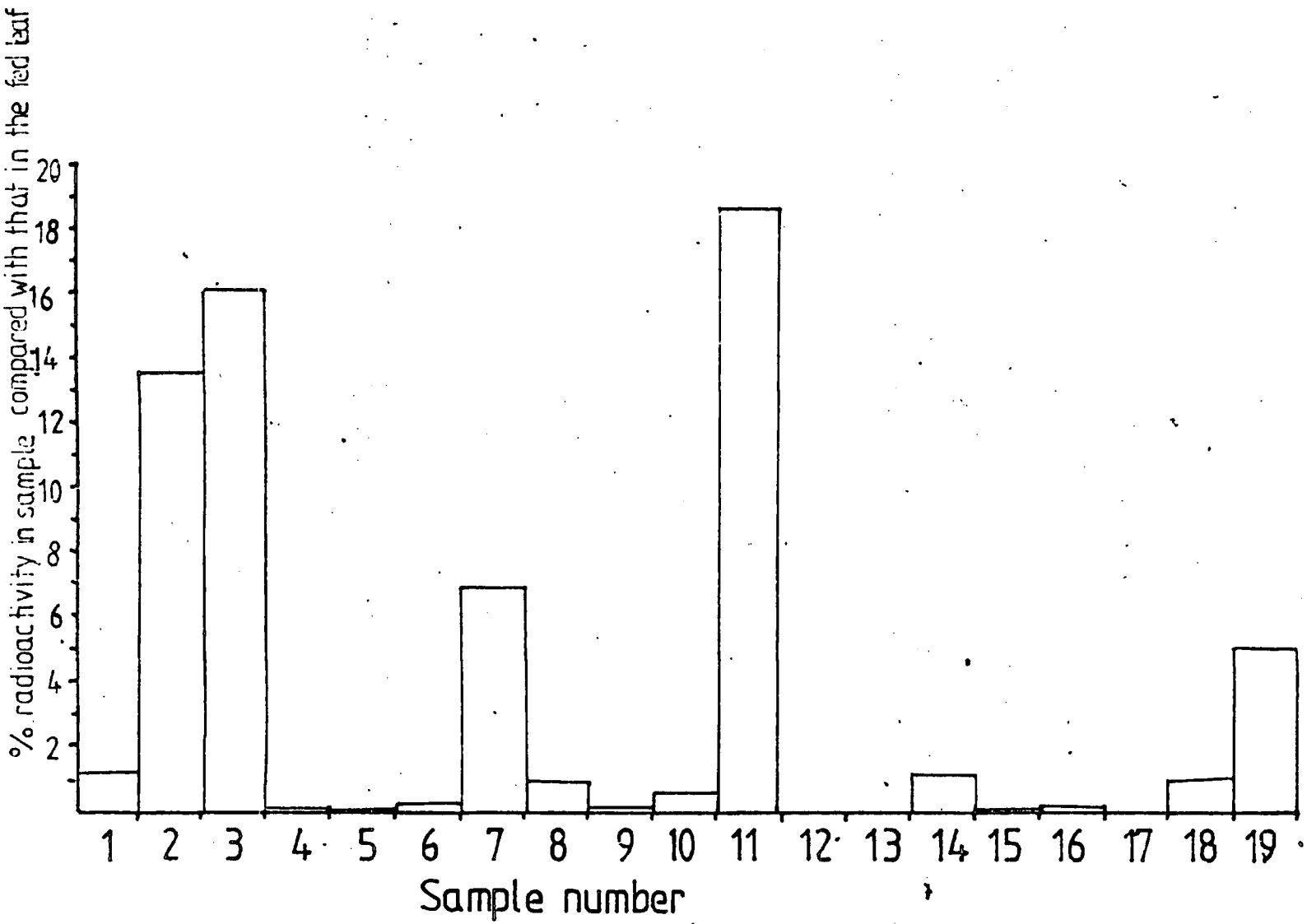



Figure 7.11: Percentage radioactivity incorporated into pods and leaves of cultival Deiniol. For explanation of samples see accompanying table.

Table 7.6 Explanation of samples for $^{14}C$ feeding experiment with 56/143/9 (see Figure 7.12)

| Sample number | Explanation |
|---|---|
| 1 | 1 cm section of stem above fed leaf. |
| 2 | 1 cm section of stem below fed leaf. |
| 3 | Pods, flowers and raceme subtending fed leaf. |
| 4 | Leaf below fed leaf. |
| 5 | Flowers, pods and raceme (Pods 1 cm) |
| 6 | 2nd leaf below fed leaf. |
| 7 | Pods and raceme (Pods: 2 x 3 cm,<br>1 x 2 cm below) |
| 8 | 3rd leaf below fed leaf. |
| 9 | Pods and raceme (Pods: 1 x 3 cm<br>1 x 1 cm) |
| 10 | 4th leaf below fed leaf. |
| 11 | Pods and raceme (Pods: 2 x 4 cm<br>1 x 2 cm<br>1 x 3 cm<br>1 x 1 cm) |
| 12 | 5th leaf below fed leaf. |
| 13 | Pods and raceme (Pods 1 x 5 cm,<br>1 x 2 cm<br>2 x 1 cm) |
| 14 | Total % radioactivity of leaves, flowers, racemes and apex above fed leaf. |

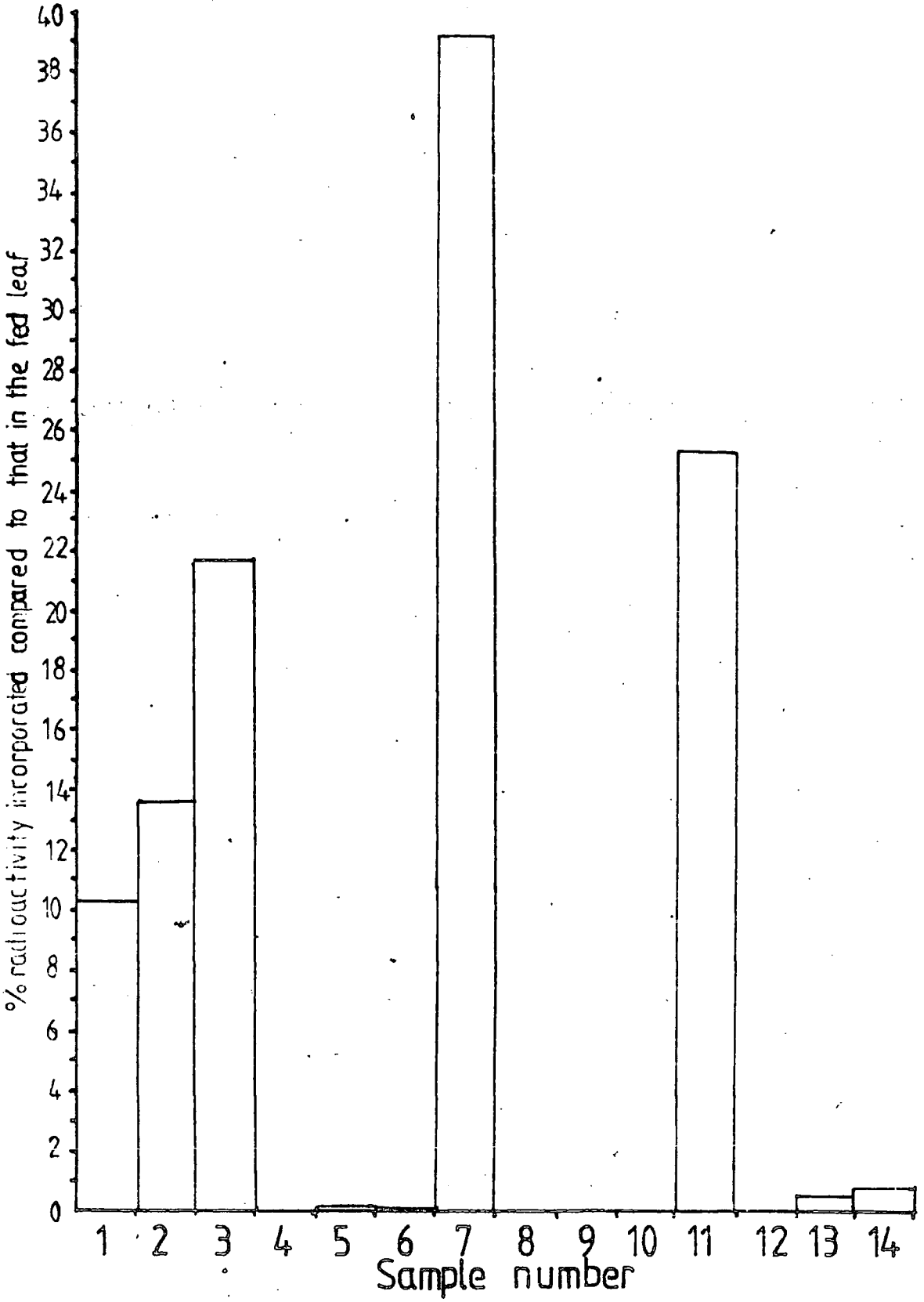



Figure 7.12: Percentage radioactivity ($^{14}C$) incorporated into pods and leaves of line 56/143/9. For explanation of samples see accompanying table.

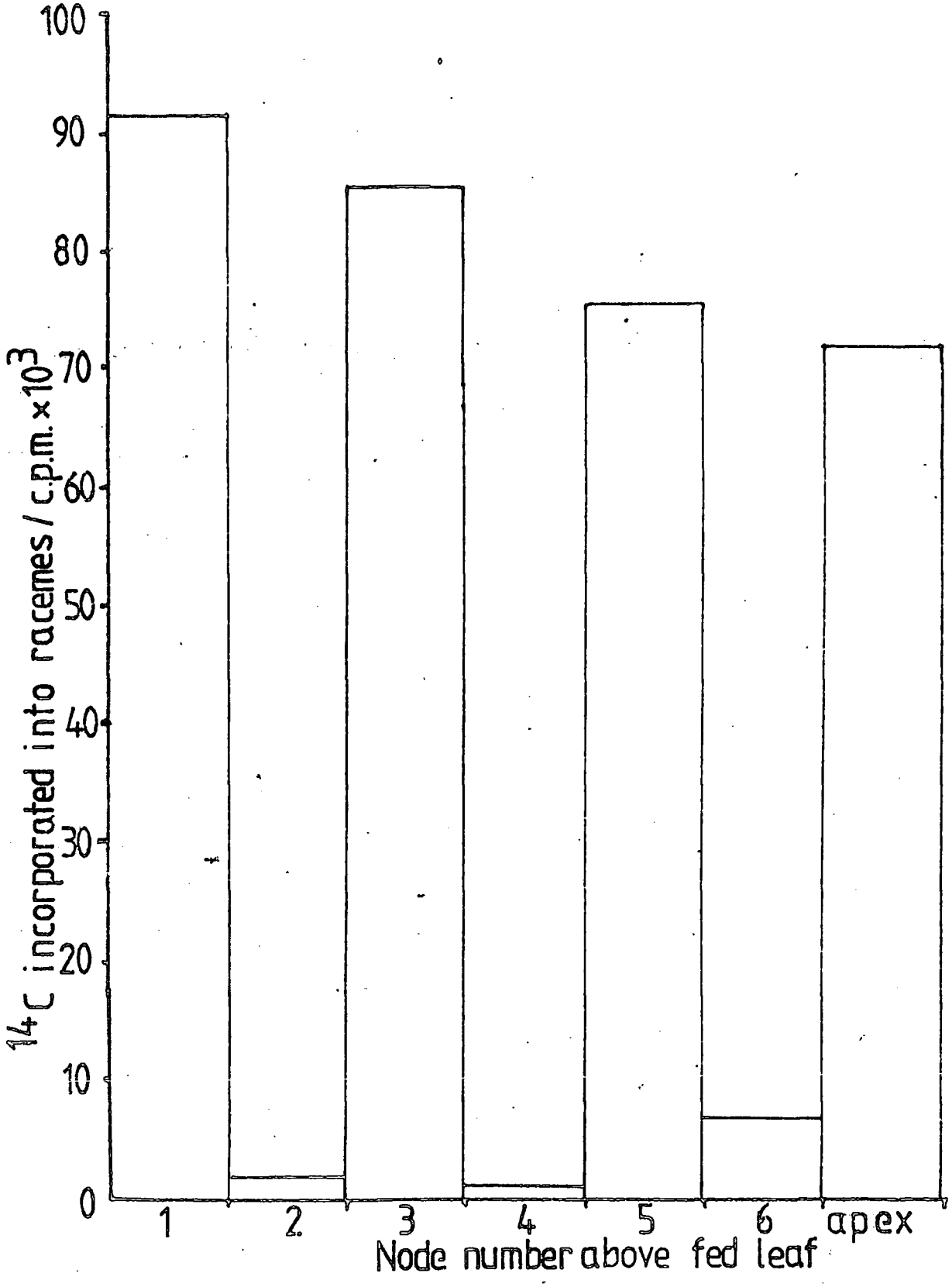



Figure 7.13: Incorporation of $^{14}C$ into flowers and racemes of line 56/143/9 when flowering, with no pods set on plant.

Vascular supply of the stem feeding leaves and racemes of Vicia faba

Ecsin and fluorescein were allowed to transport from the cut tissue resulting from removal of a raceme, on plants of Maris Bead, Deiniol, Cockfield, 56/143/9, and 56/118/20. The dyes appeared in racemes, not the leaves, situated on alternate nodes, above that of the fed raceme. There was no transport of dye into peduncles situated below the fed tissue.

Feeding the cut tissue of excised leaves with eosin, or the abraded surface of leaves with fluorescein, resulted in the appearance of either dye in alternate leaves and petioles above and below the fed leaf. No dye, however, appeared in any of the axillary racemes of fed plants.

Serial sections of the stem of Vicia faba plants

Serial sections of the varieties and inbred lines listed above were taken and examined after feeding with eosin and fluorescein.

The leaves of Vicia faba are arranged in two orthostiches; leaf 3 positioned over leaf 1. Each leaf is supplied by only three vascular bundles, 2 lateral and 1 median bundle. Leaf 1 is supplied by the median bundle, this branches at the node, and this branch traverses two internodes to feed into leaf 3. There is no connection between this bundle and the vascular traces supplying leaf 2. The course of the two lateral bundles supplying a leaf is essentially similar to that of the median trace, except that the first two internodes are traversed in isolation, inside the wings of the stem.

More importantly, it appears that the vascular strands supplying leaves are separate to those supplying the raceme. Two lateral and two median strands supply a peduncle. Just

before entering the peduncle, at a node, these vascular bundles branch extensively. Branches from these strands also supply alternate racemes, situated further up a plant. Vascular strands on the opposite orthostiche, supply the other racemes (Figures 7.13 7.14, 7.15).

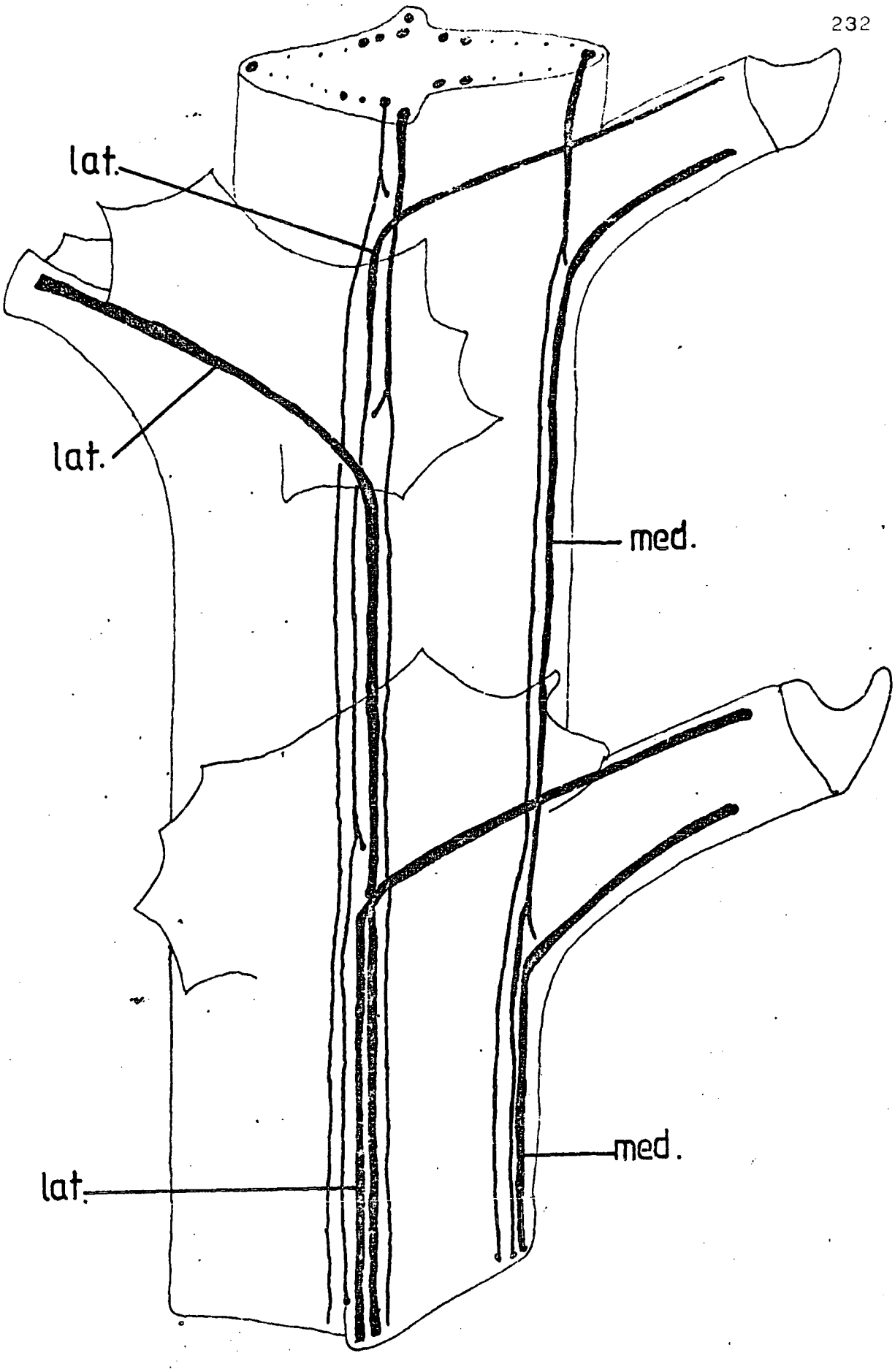



Figure 7.13: Outline of major leaf traces in Vicia faba: lat = lateral traces
med = median traces.

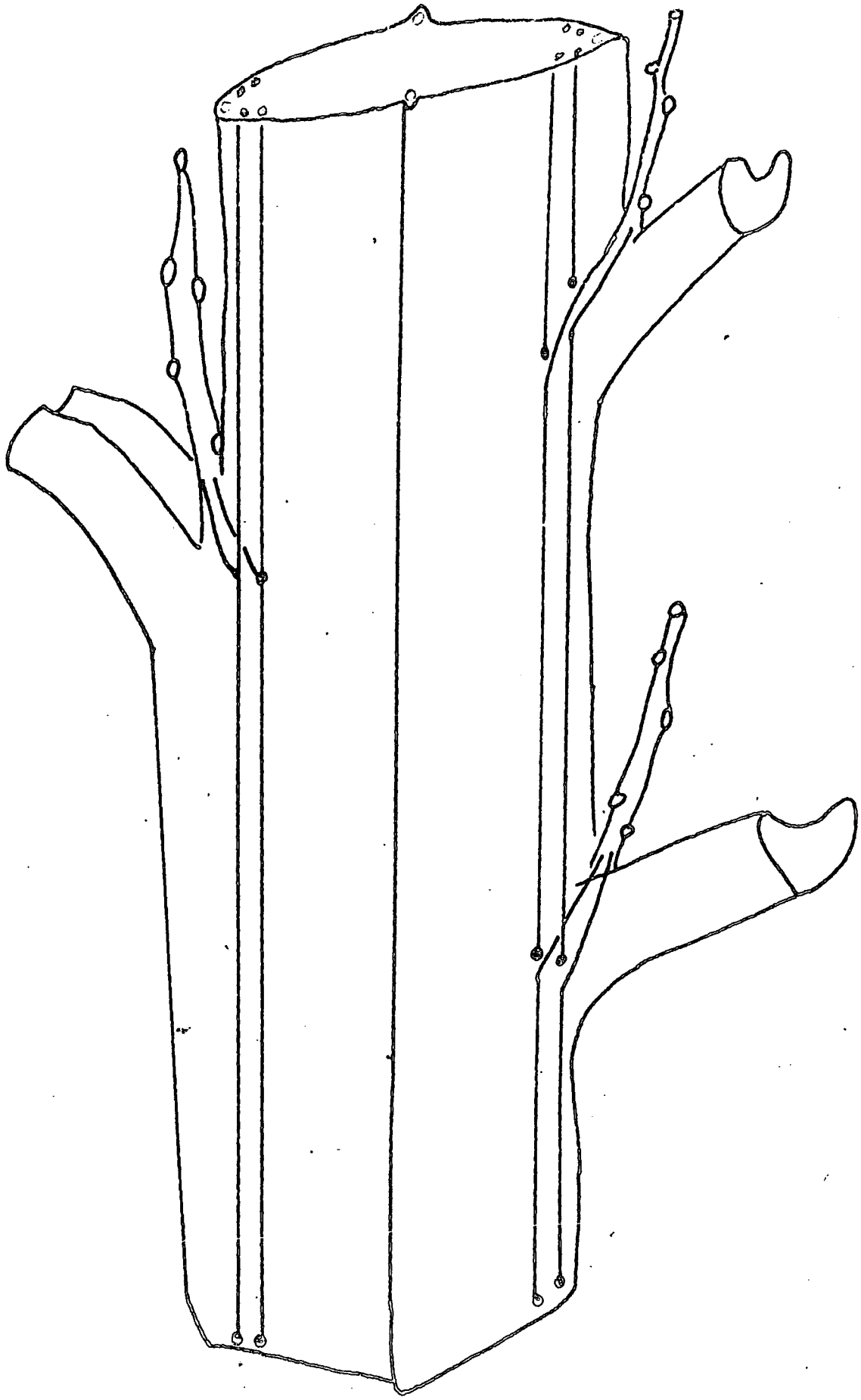


Figure 7.14: Outline of major peduncle traces in Vicia faba L.

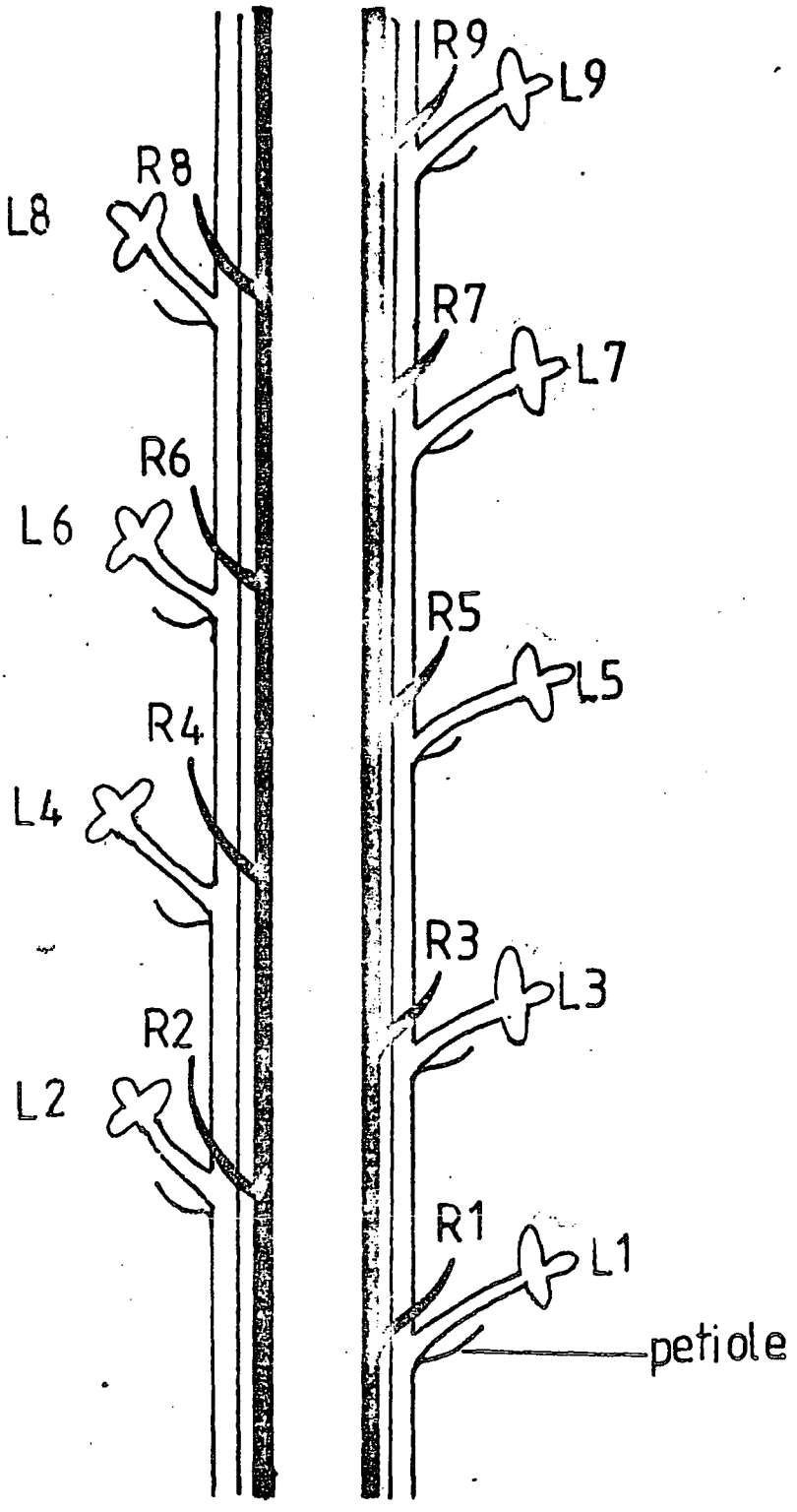



Figure 7.15: Diagram of the vascular supply of leaves and racemes of a Vicia faba plant. L = leaf; R = raceme.

# CHAPTER 8

## RESULTS OF INITIAL EXPERIMENTS TO ASSESS THE EFFECTS OF ENVIRONMENTAL AND PHYSIOLOGICAL STRESSES AND THE APPLICATION OF A GROWTH REGULATOR TO PLANTS EXHIBITING THE INDEPENDENT VASCULAR SUPPLY CHARACTERISTIC

### Effect of supplementary lighting and of temperature on flower drop

Plants were either grown in a warm or cool glasshouse, with or without supplementary lighting.

Flower abscission at most raceme positions of Deiniol plants was greater when they were grown in warm conditions, than when they were grown in a cool glasshouse (Figure 8.1). Flower drop in both cases was greater on plants grown with supplementary lighting than those grown without additional lighting. In all cases flowers situated at more proximal raceme positions set most pods, while those situated more distally frequently abscised (Figure 8.1).

Plants of (inbred) line 56/143/9 experienced low flower abscission at all raceme positions, when they were grown in the cool glasshouse, with or without any supplementary lighting (Figure 8.2). Higher flower abscission occurred on plants grown under warm conditions although this was much lower than that experienced by plants of variety Deiniol. When 56/143/9 plants were subjected to both warmth and additional lighting they experienced a similar flower drop to Deiniol plants; but less in percentage terms (Figure 8.2).

Flower abscission was increased at most inflorescences of Deiniol plants when subjected to additional lighting regardless of the ambient growth temperature (Figure 8.3). Here, flower abscission at all racemes was higher in plants grown in warm conditions than in cool conditions.

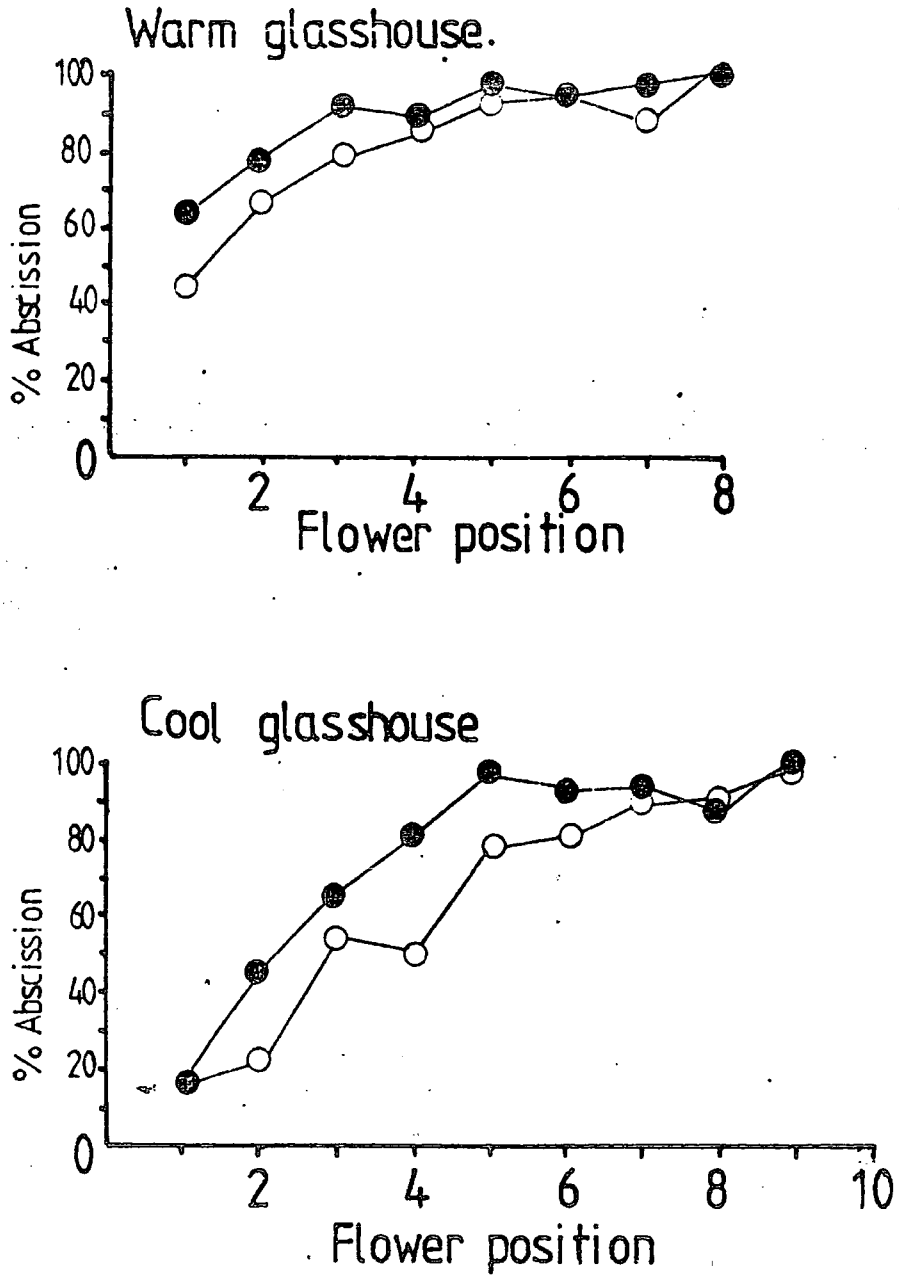



Figure 8.1: Influence of supplementary lighting and temperature on flower abscission for cultivar Deiniol. Each figure is an average percentage;
o = no supplementary lighting,
● = supplementary lighting.

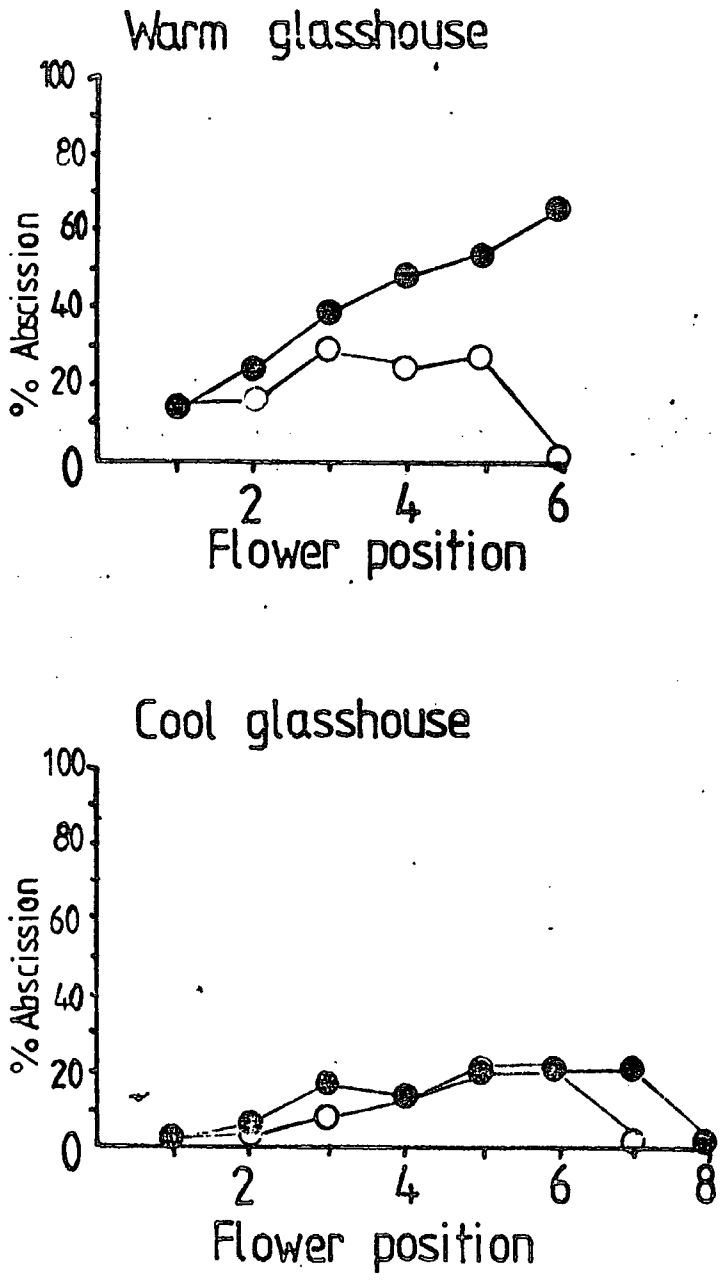



Figure 8.2: Influence of supplementary lighting and temperature on flower abscission for line 56/143/9. Each figure is an average percentage.
o = no supplementary lighting,
● = supplementary lighting.

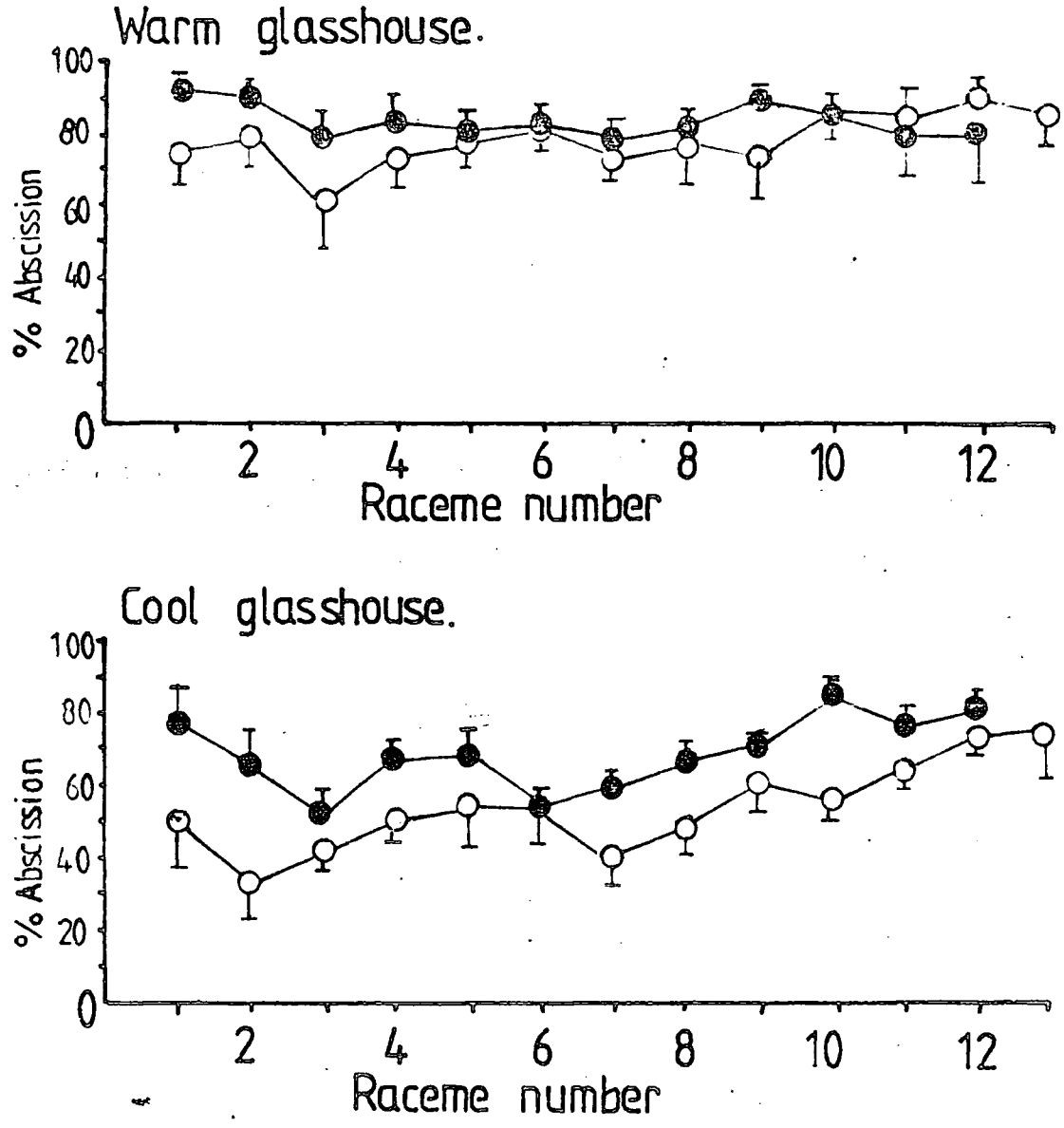



Figure 8.3: Influence of supplementary lighting and temperature on flower abscission for variety Deiniol. Each figure is an average percentage.
o = no supplementary lighting,
o = supplementary lighting.
Standard errors are represented by a bar.

Flower abscission on most racemes of 56/143/9 plants grown in warm conditions was greater than those grown in cool conditions (Figure 8.4). Growing these plants in warm conditions, with supplementary lighting, resulted in a further increase in abscission on racemes situated above the fourth flowering node, Supplementary lighting had very little effect on abscission of flowers of 56/143/9 plants grown under cool conditions. In all conditions the flower abscission experienced on all racemes of plants of line 56/143/9 was less than that experienced by Deiniol plants.

Overall flower abscission, increased when both plant types were grown under supplementary lighting, whether in warm or cool conditions. Overall abscission of flowers also increased for both types of plants, when grown in warm rather than cool conditions. The highest flower abscission, however, experienced by plants of line 56/143/9 grown in warm conditions and with supplementary lighting, i.e. most stress, was 20% less than that observed in Deiniol plants grown under the least stress, i.e. cool conditions and no supplementary lighting (Table 8.1).

The effect of tripping on flower drop

All tripping treatments had relatively little effect on flower abscission at most flower positions of plants of line 22 (Figure 8.5). The pattern of flower abscission differed from that observed in commercial varieties. Flowers abscised most frequently at raceme positions 3 and 4 with little or no flower abscission occurring at other raceme positions (Figure 8.5). Flowering was confined to an average of six nodes and flower abscission appeared to be independent of the position of the tripped flowers (Figure 8.6). Overall

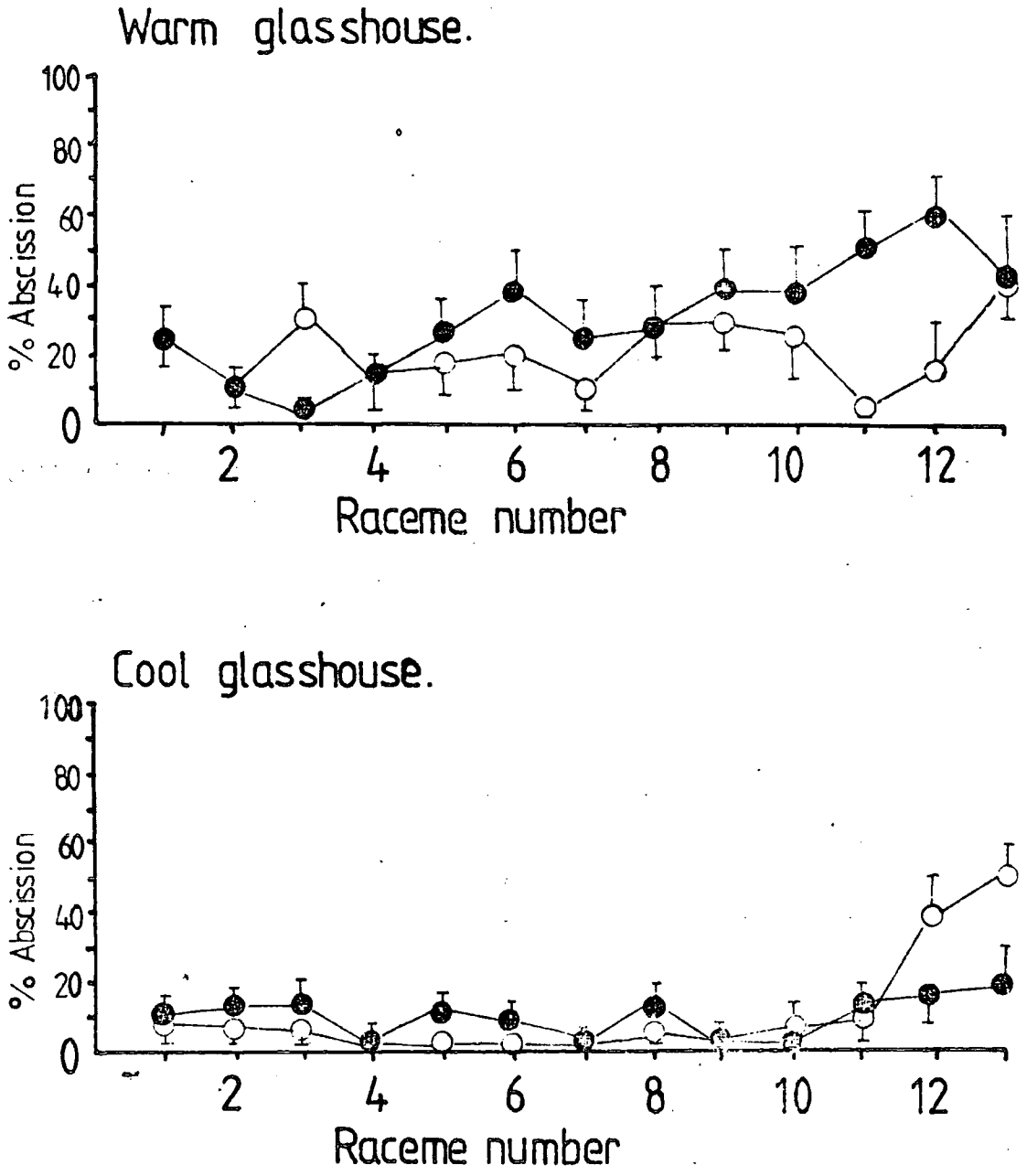



Figure 8.4: Influence of supplementary lighting and temperature on flower abscission for line 56/143/9. Each figure is an average percentage.
o = no supplementary lighting,
⊙ = supplementary lighting.
Standard errors are represented by a bar.

Table 8.1 Summary of results for temperature and lighting experiment

| Cultivar and treatment | Flowers dropped (%) | Pods set (%) |
|---|---|---|
| Deiniol | | |
| Warm glasshouse | | |
| supplementary lighting | 551 (86.1) | 89 (13.9) |
| no supplementary lighting | 390 (75.3) | 128 (24.7) |
| Cool glasshouse | | |
| Supplementary lighting | 436 (68.3) | 202 (31.7) |
| No supplementary lighting | 412 (56.5) | 317 (43.5) |
| 56/143/7 | | |
| Warm glasshouse | | |
| Supplementary lighting | 165 (36.8) | 283 (63.2) |
| No supplementary lighting | 88 (20.8) | 335 (79.2) |
| Cool glasshouse | | |
| Supplementary lighting | 77 (13.1) | 510 (86.9) |
| No supplementary lighting | 55 (9.4) | 527 (90.6) |

Percentage figures are in parentheses.

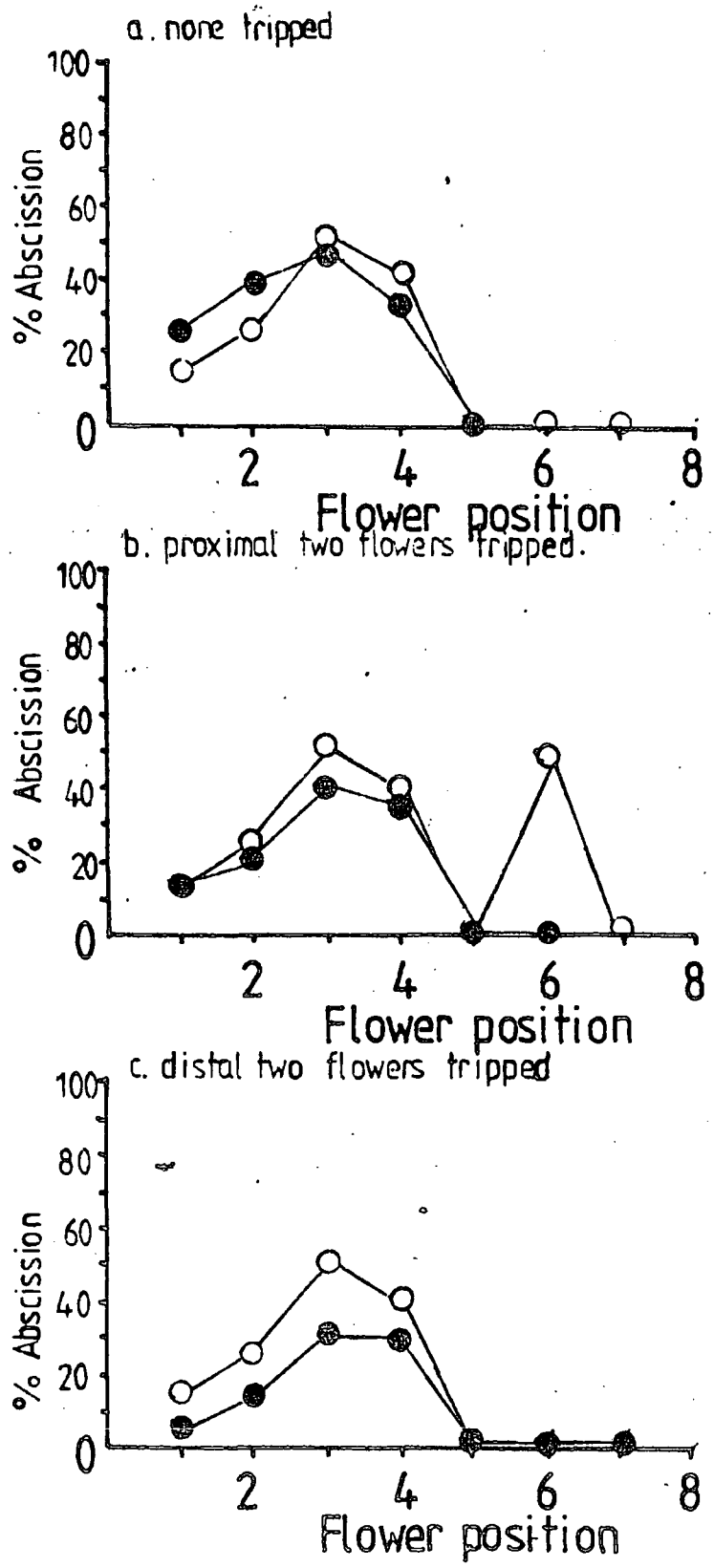



Figure 8.5: Effect of tripping on flower abscission on line 22 (STW). Each value is an overall percentage. o = control plants, all tripped, ● = treated plants.

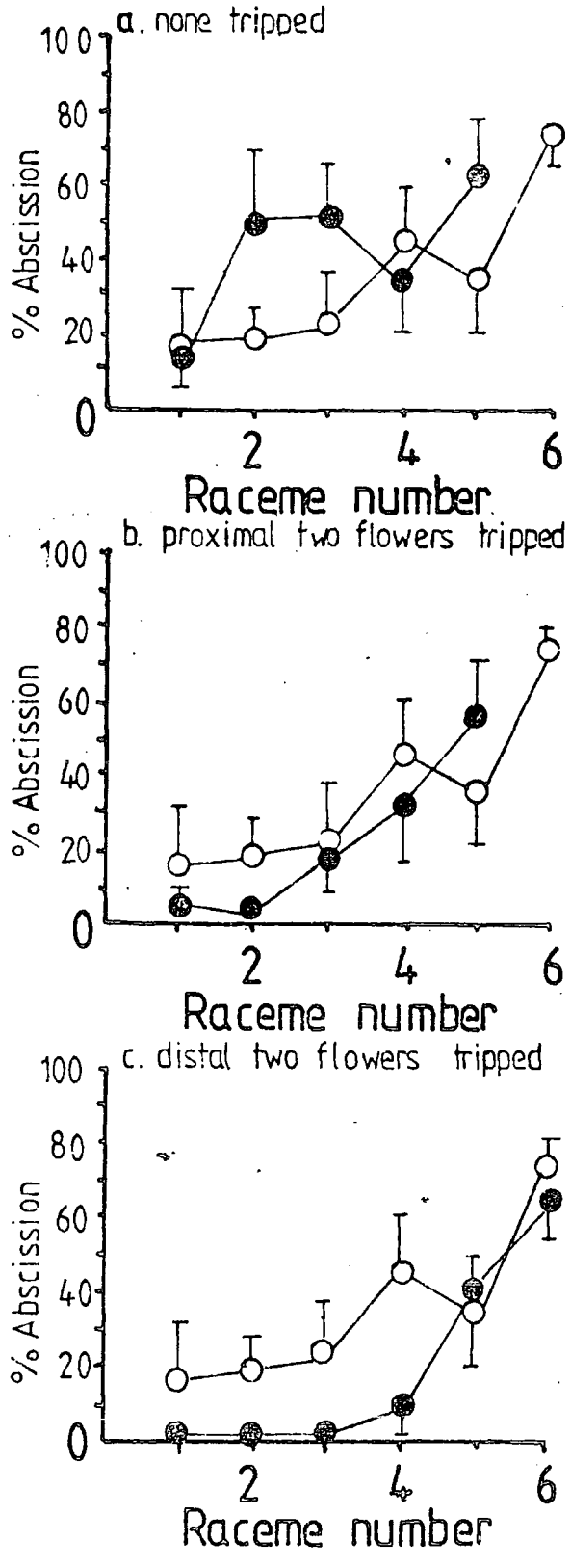



Figure 8.6: Effect of tripping on flower abscission on line 22. Each value is an average percentage. Standard errors are represented as a bar.
o = control plants, all tripped,
⊙ = treatment plants.

flower drop was greatest for plants whose flowers were not tripped. However, the difference between the overall flower drop in tripped and untripped plants was insignificant (Table 8.2).

In plants of line 56/143/9 (Figure 8.7), there was no discernible difference in flower abscission at all raceme positions, between plants, all of whose flowers had been tripped (Controls) and those with no flowers tripped. Similarly, tripping just the two most distally situated flowers on each raceme made no difference to the flower drop pattern, relative to the controls (Figure 8.7). Greater abscission at most raceme positions occurred when the proximal two flowers on each raceme were tripped, than in the control plants (Figure 8.7).

Tripping made no difference to flower abscission over all racemes for plants of line 56/149/9, except that greater abscission at every raceme, when the proximal two flowers on each raceme were tripped (Figure 8.8).

Overall flower abscission was greater when flowers were not tripped, although this increase was insignificant (Table 8.2).

Application of the anti-auxin 2,3,5 Triiodobenzoic acid (TIBA) to plants of Maris Bead and the inbred line 56/118/20

Application of 2,3,5 triiodobenzioc acid (TIBA) to plants that were in flower, resulted in a marked twisting of stems, petioles and young leaves situated near to the apex of the plants. These symptoms were more evident in Maris Bead plants. In addition, elongation of the peduncle and pedicel/peduncle junction was observed in both plant types.

Table 8.2 Summary of results for tripping experiment

| Variety and treatment | Flowers dropped (%) | Pods set (%) | $\chi^2$, v = 1 |
|---|---|---|---|
| Line 22 | | | |
| none tripped | 36 (36.4) | 63 (63.6) | 1.37 (P< 0.1) |
| all flowers tripped | 43 (29.2) | 104 (70.8) | |
| proximal two flowers tripped | 34 (25.9) | 97 (74.1) | |
| distal two flowers tripped | 24 (17.6) | 112 (82.4) | |
| Line 56/143/7 | | | |
| none tripped | 154 (38.0) | 251 (62.0) | 0.48 (P< 0.1) |
| all flowers tripped | 122 (35.6) | 221 (64.4) | |
| proximal two flowers tripped | 184 (48.5) | 195 (51.5) | |
| distal two flowers tripped | 132 (37.5) | 220 (62.5) | |

Percentage figures are in parentheses. Chi-squared analysis compares tripping with no tripping.

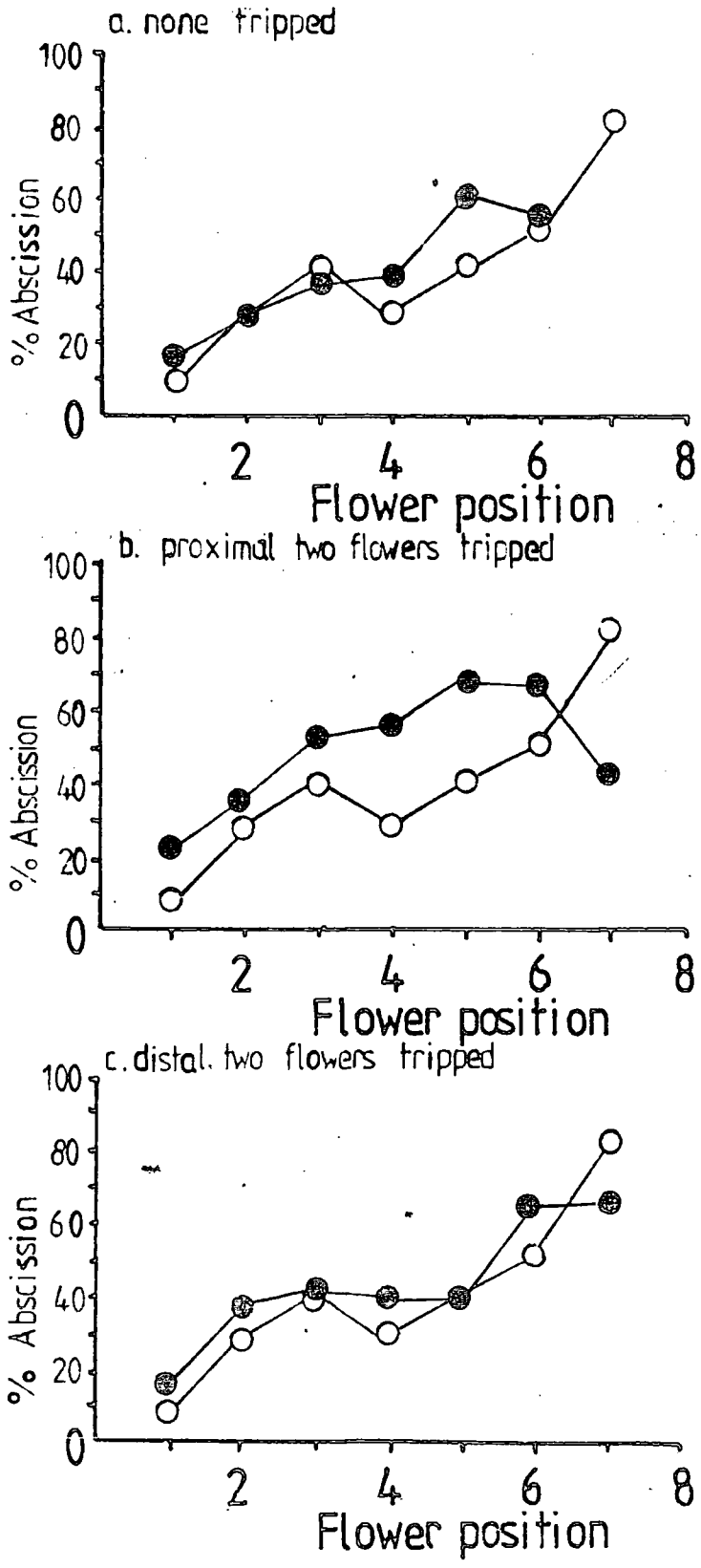



Figure 8.7: Effect of tripping on flower abscission of line 56/143/9.
Each value is an overall percentage.
o = control plants, all flowers tripped,
o = treatment plants.

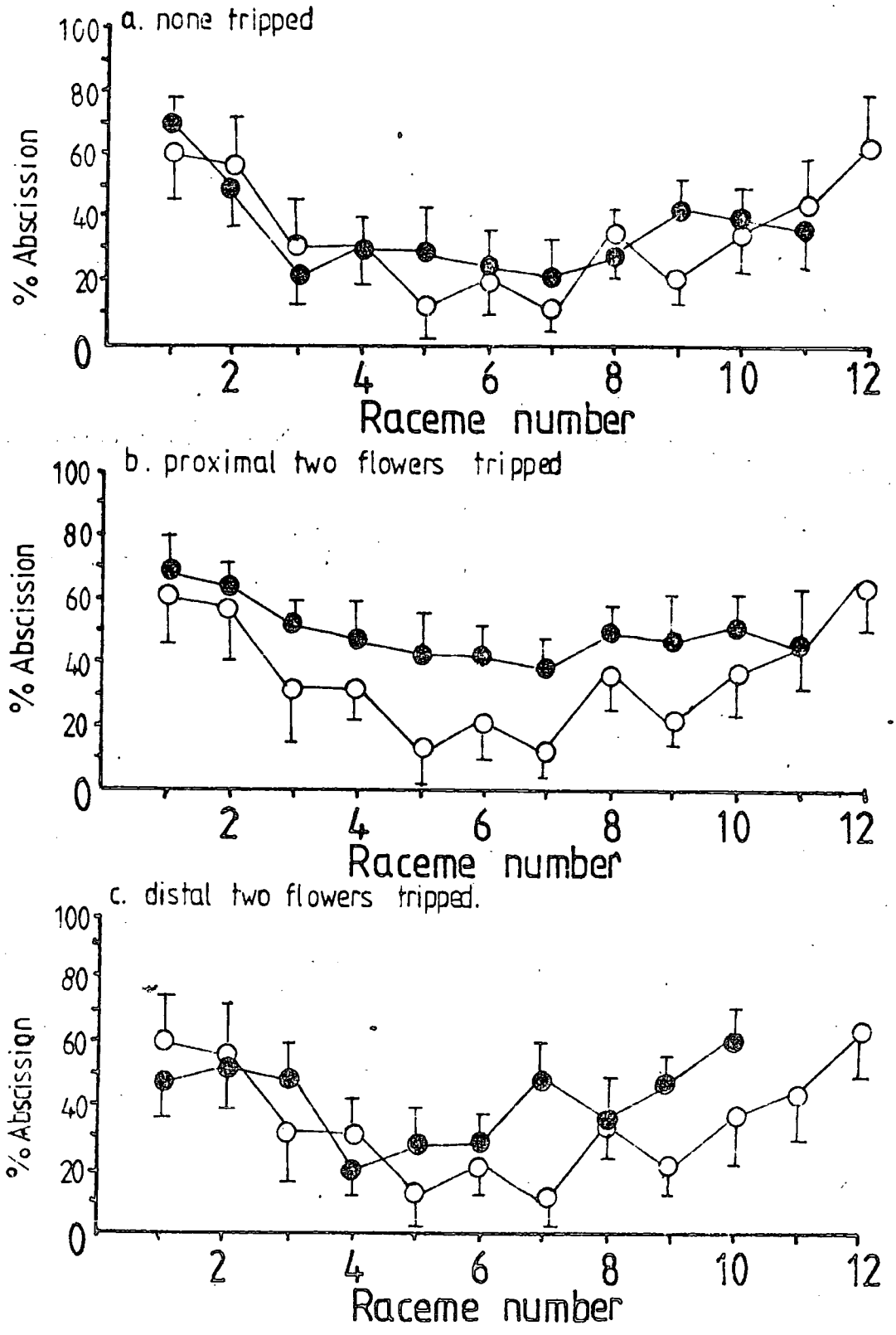



Figure 8.8: Effect of tripping on flower abscission on line 56/143/7. Each value is an average percentage.
o = control plants, all tripped,
o = treatment plants.

There was a marked reduction in apical dominance observed in Maris Bead, although this was not apparent in plants of line 56/118/20.

When TIBA was applied to Maris Bead plants, fewer flowers dropped, on average, at all raceme positions above the first two (Figure 8.9). In contrast TIBA had no effect on the observed flower drop at all raceme positions in plants of line 56/118/20 (Figure 8.9).

Flower abscission at all racemes experienced by TIBA sprayed Maris Bead plants was generally less than in control plants. There was little difference to flower abscission at all racemes between plants of line 56/118/20 sprayed with TIBA and those which were not (Figure 8.10).

Overall flower abscission in line 56/118/20 was not significantly affected by application of TIBA. In contrast there was a significant reduction in overall abscission in Maris Bead plants when sprayed with TIBA (Table 8.3).

Here again, plants of line 56/118/20 (sprayed or unsprayed with TIBA) experienced much less flower abscission than plants of variety Maris Bead.

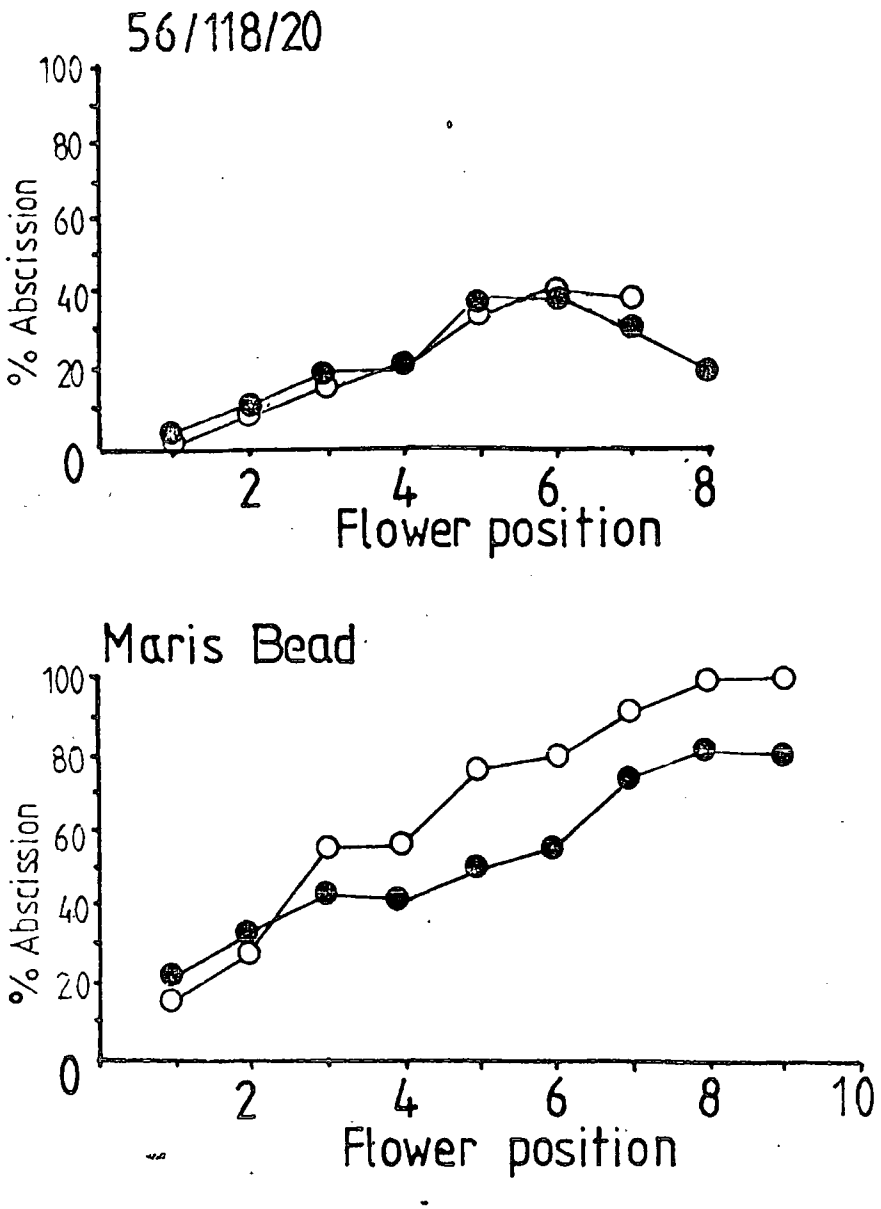



Figure 8.9: Effect of T.I.B.A. application on flower abscission.
o = control plants,
⊙ = T.I.B.A. treated plants.
Each figure is an overall percentage.

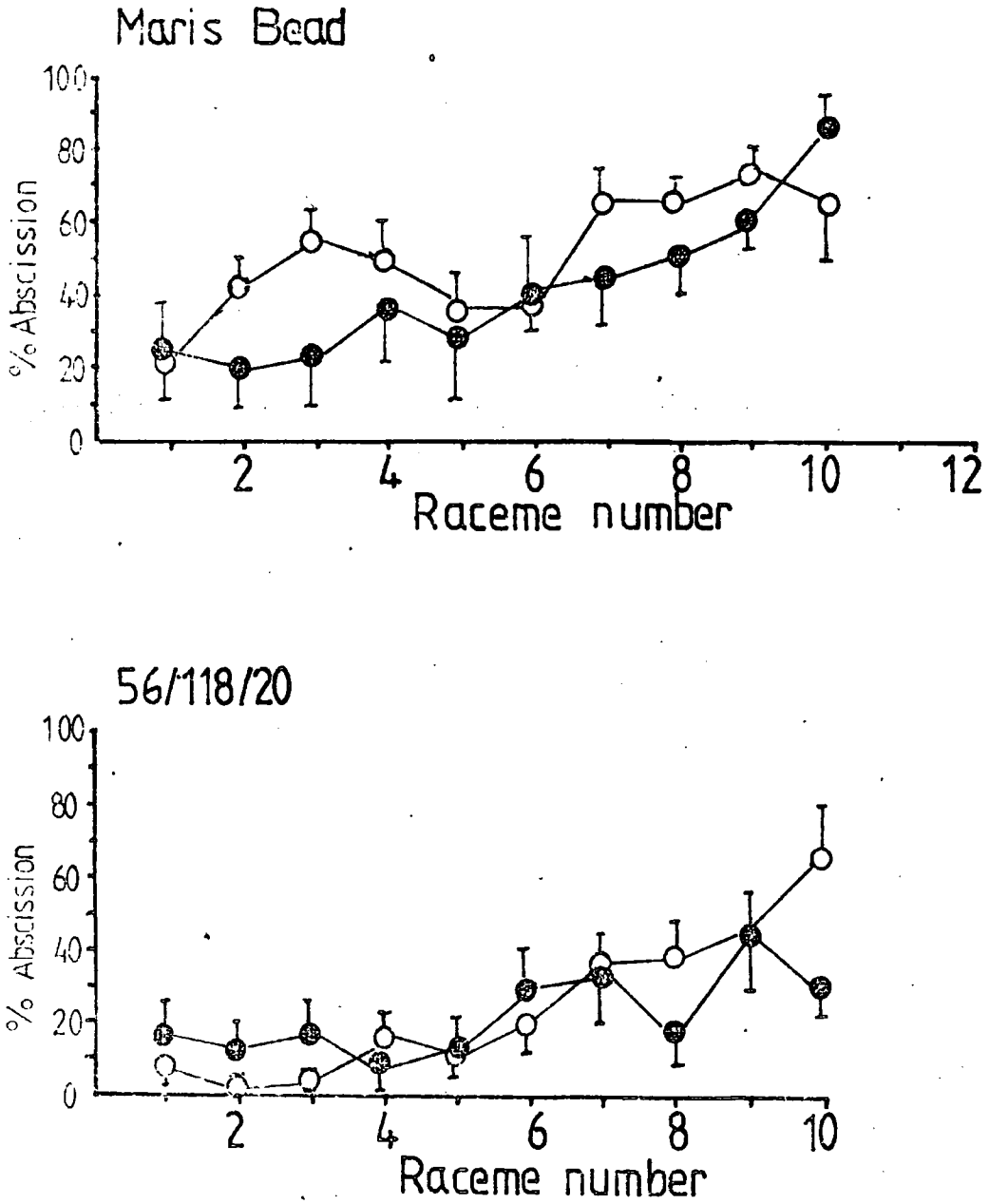



Figure 8.10: Effect of T.I.B.A. application on flower abscission.
o = control plants,
● = T.I.B.A. treated plants.
Each figure is an average percentage.
Standard errors represented by a bar.

Table 8.3 Summary of results for application of TIBA to plants

| Variety and Treatment | Total flower drop (%) | Total pods set (%) | $\chi^2$, v = 1 |
|---|---|---|---|
| 56/118/20 | | | |
| Control | 86 (21.3) | 318 (78.7) | 0.07 ($P < 0.1$) |
| TIBA treated | 91 (22.1) | 321 (77.9) | |
| Maris Bead | | | |
| Control | 212 (55.6) | 169 (44.4) | 7.07 ($P > 0.01 < 0.005$) |
| TIBA treated | 217 (46.5) | 250 (53.5) | |

# CHAPTER 9

# DISCUSSION

Flowers that least frequently abscind on a faba bean axillary inflorescence are those at proximal positions and the degree of shedding increases regularly at each successive distal position. A similar situation has been shown to exist in yellow lupin, Lupinus luteus L. (Van Steveninick, 1957, 1958, 1959), cowpea, Vigna unguiculata L. walp. (Ojehomon, 1972), pigeon peas, Cajanus cajan (Sheldrake, Narayanam and Venkataratnam, 1979) and soybean, Glycine max L. (Huff and Dybing, 1980).

Most pods set on nodes on the lower-middle flowering position of a branch, and the majority of flowers produced on the more distal racemes abscind. This is similar to the situation reported for pigeon peas, Cajanus cajan (Sheldrake Narayanam and Venkataratnam, 1979).

Cultivar differences for flower abscission have also been shown to exist between spring faba bean varieties (Table 3.1). Similar differences between inbred lines of faba beans have been reported by Sekurali, Frauen and Paul (1978), who proposed that this was due in part to differences in autofertility and also to differences in the hormonal status between lines, as it had previously been found that internal ethylene levels differed between the autosterile line (CH4) and the autofertile line(CH182) used in the above study (El-Beltagy and Hall, 1975). Cultivar differences in flower abscission bave also been shown to exist between varieties of Soybean (Van Shaik and Probst, 1958a and b; Weibold, Ashley and Boerma, 1981).

Considerable variation in flower abscission also occurs over different seasons between the same cultivars of faba beans, giving an indication of the influence of the environment on flower drop, and the contribution this phenomenon plays in the overall yield instability of the crop.

The application of a variety of environmental stresses, in general, increased flower abscission on treated plants. Irrigation increased flower abscission and depressed the final yield of plants. Such an effect on flower shedding has also been reported by El-Rahman, et al (1980). It is important here to recognize that it is the rate and timing of irrigation that affects the response of the plant and it is a high irrigation frequency that results in increased flower abscission. Irrigation in measured quantities, when necessary, has a beneficial effect on yield (Stolp, 1955; McEwen et al, 1981). The purpose of this experiment was, however, to demonstrate the adverse effect of wet conditions on flower drop. In all genotypes subjected to this stress, including a determinate genotype, TI Col., more flower abscission occurred by most flowers, situated on all flower positions of a raceme. Most pods set, however, on proximal positions within an inflorescence. It was also evident that irrigation favoured vegetative growth at the expense of reproductive growth.

A possible explanation for increased flower abscission due to irrigation has been proposed by Rowland, Bond and Parker (1982), in that such treatment decreases the proportion of flowers fertilized, even though both irrigated and unirrigated plots had equal access to bee visitation, because

most water was applied during the evening. The proposed inhibition of fertilization acted either through an inhibition of pollen germination or by a chemical signal acting either through the stigmatic exudate found by Paul, et al (1978) or by chemical inhibitors in the style or micropyle.

An alternative explanation is offered here, however, in that irrigation alters the competitive balance between apex, vegetative parts and the reproductive parts of the plant so limiting the amount of assimilates reaching fertilized flowers, thus allowing abscission promoting substances to cause changes in the pedicel/peduncle junctions of flowers leading ultimately to their abscission. The assertion that assimilates are limiting to reproductive structures when subject to irrigation is supported by the observation that pod development was delayed compared to those on non-irrigated plants. In addition, as the proximal positioned flowers are those that are most likely to set pods, such assimilate that is available must be channelled preferentially to these flowers, enabling a pod to be set. Hormones may also influence increased abscission and this is supported by El-Beltagy and Hall (1975), who showed that either a lack or excess of water caused a substantial increase in internal ethylene concentrations which correlated well with the abscission of flowers or pods. Chapman, Fagg and Peat (1979) also concluded that any treatment that increased within plant competition in V. faba predisposed that plant to flower drop and premature pod drop, whereas decreased competition had the opposite effect.

Overwatering of pot bound plants had little effect on flower abscission, although this was considerable in both treatment and control plants. This experiment, however,

illustrates the difference in response to additional water of pot bound compared with field grown plants. Jones (1963), concluded that V. faba plants were quite tolerant to periods of waterlogging. However, Taha and Drennan (1979) have reported that periods of severe waterlogging of plants reduced leaf expansion, promoted the early senescence of older leaves, suppressed root growth and increased pod drop. All these effects it was concluded were entirely due to oxygen deficiency in the soil induced by waterlogging.

Imposition of water deficit at different times during flowering increased flower shedding, especially when applied at the earlier stages of flower development (Figure 3.8). It appeared that field beans were more tolerant to a drought stress applied during full flowering, rather than at the initiation of flowering. These results complement those of El-Nadi (1969, 1970) who reported that although water stress at flowering increased flower drop, the effect on overall yield was most severe if water was withheld at the time of early pod set. In addition those results agree with El-Beltagy and Hall (1974, 1975) and Farah (1981) who showed that high moisture stress at early phases of reproductive development severely reduced yields. While recognizing that the duration of water stress affects subsequent flower abscission and that it is essential that adequate water be available for the growth and development of young pods, a tolerance to a drought period during full flowering, could confer advantages in the field. One advantage is that such a drought period would also coincide with the appearance of abundant insect pollinators. In addition a period free from rain, but not so severe as to cause undue stress on the plants during flowering and early

pod set, would have the effect of inducing a more determinate growth habit, so avoiding the detrimental effects experienced by plants subject to irrigation. With water stressed plants, in all cases it was again the flowers situated on proximal flower positions that set most pods, distal flowers on control and stressed plants' racemes frequently all abscind. The above suggested environmental conditions during the flowering period might ameliorate flower abscission, but such factors are not, however, the root cause of flower shedding.

The application of a short low temperate shock (Figure 3.11) in general had little or indeed a beneficial effect on flower drop. The determinate genotype TI Col., with flowers at early developmental stages, experiences dramatically reduced flower drop in response to a cold shock. Gehriger (1980) showed that flowers subject to cold stress stayed on longer but fell in a pattern similar to the control plants. This appeared to be true for the indeterminate varieties examined, but for the determinate genotype, more pods set. A short cold shock may have the effect of slowing down the metabolic processes leading to flower drop. As indicated in Chapter 1, unfavourable competition occurs between the apex, vegetative parts and reproductive parts of the plant. Cold shock might have the effect of decreasing this competition, and once the indeterminate plants were removed to normal temperature conditions, the competition between apex and racemes resumed. Such competition in determinate plants can only occur between leaves, roots and the racemes, so reducing competition further by a cold shock may allow for increased pod set on racemes of treated plants.

Frost damage rendered plants topless, because the sensitive apex died back. Flower drop increased in two of the

varieties so affected (Figure 3.12). One variety (Strubes), however, experienced reduced flower drop. This could indicate that there may be some genotypic variation for cold tolerance in spring faba beans. Flower abscission, however, in such plants was still high, especially by flowers situated at distal raceme positions. The work of Herzog (1978a, b; 1979) has given some insight into the differences in cold hardiness of spring and winter type faba beans and also into the mechanism of cold hardiness. Cold hardiness is apparently not a permanent character of a genotype and its appearance depends upon the photoperiodic and temperature conditions in which the plant is made to grow. Genotypes, however, do differ in their ability to acquire winter hardiness. It appears that frost susceptibility and induction of frost tolerance are relative to the rate of growth and metabolic activity and state of tissue differentiation. Spring types seem to have a faster rate of growth and assimilation than winter types and thus they show more frost suceptibility. These studies indicate that frost tolerance is related to dry matter content of the tissue and the rate of ontogenic differentiation. The possibility exists for using these parameters in selecting genotypes for increased potential for frost tolerance (Saxena, 1981). In the present study, however, frost damage seems to effect increased flower abscission, but again it appears that the cause of flower drop is not entirely due to such a factor.

Growing plants of V. faba at increased density also increased flower abscission, especially on middle and distal flower positions, on each and every raceme. Consequently less mature pods were produced on each plant grown at high density.

Seed yield, however, increased with increasing density. These results agree with those of Hodgeson and Blackman (1956), who showed that as planting density increased, branching, number of nodes per stem, number of flower bearing nodes and the number of pods reaching maturity all decreased. Ishag (1973) found that there were 40% more pods per plant and seed yield per plant increased by 50% when plants were grown at low density as opposed to those grown at high density. However, the dense population had one third higher yield. Hodgeson and Blackman (1956) found that yield increased with density over a range 11-67 plants/$m^2$. Similar results have also been reported by Seitzer and Evans (1973), Soper (1952), Sprent, Bradford and Norton (1977), Barry and Storey (1979) and Keller and Burkhard (1981). The general view is that with current commercial varieties, seed yield is a direct function of the number of mature pods per unit area, and not the number of mature pods per plant.

The results presented in Chapter 3 show that the proximal flowers of racemes of plants grown at high density are most likely to set a pod, whereas if plants are grown at lower density more pods will set at higher raceme positions. However flowers at the most distal positions still frequently abscind. This experiment is further evidence that by increasing within and between plant competition, will effect an increase in flower shedding. One of the consequences of increased density, is increased mutual shading of leaves, especially on the lower portion of plants. This may contribute to the limitation of carbon substrates and hence lead to increased abscission. Hodgeson and Blackman (1957), however, showed that shading basal nodes led to more pods at the top of the stem, while

defoliation of the lower half of the plant depressed pod development in the upper part. They concluded that the effects due to plant density were more likely to be caused by an altered balance of hormones and assimilates within plants, rather than by mutual shading between plants. This proposition has since been supported by Sprent, Bradford and Norton (1977) and El-Zahab, Al-Barbawy and Nidawy (1981).

Flower abscission, due to shading of plants at an early stage in flowering, was reduced in racemes positioned higher up the plant. This was compensated for, however, by increased bud abortion on higher racemes of indeterminate varieties. The abortion of buds before anthesis is a commonly observed phenomenon in the crop, but is generally of less importance than flower shedding. It occurs as evidenced by this experiment, when photosynthetic assimilates are limiting. In plants grown under normal field conditions, it occurs predominantly at lower flowering nodes, when assimilate supply is constrained by insufficient photosynthetic leaf area early in the life of the crop and at apical nodes when competition with pods for assimilates occurs in the latter stages of crop growth. Shading the determinate genotype, TI Col., from early flowering, resulted in increased flower shedding, but little bud abortion, because of the limited number of inflorescences formed in this genotype. Shading of plants during mid-flowering resulted in increased flower abscission, and also in the restriction of the number of flowering nodes produced (Table 3.12). There was therefore no compensation for the observed increase in flower drop, confirming results obtained by Hodgeson and Blackman (1957). Shading plants from full flowering resulted in greatly increased

flower abscission. Plants so shaded were not spindly, but grew taller, with increased amounts of vegetative growth relative to control plants. Plants appeared to compensate for the decreased availability of light by increasing the amount of leaf area available for photosynthesis. It therefore appears that shading at this stage, alters the competitive balance within plants, favouring vegetative growth, which was similar to that observed to occur by plants subject to irrigation. Again, if assimilates are limited to reproductive structures, this will have a detrimental effect on flower retention, by enabling, especially on middle and distal racemes, the processes leading to flower drop to occur. Shading at earlier periods during flowering also has an adverse effect on pod set, and it is concluded that if carbon substrates are limited, flower abscission will increase. Flower abscission, however, by control plants, not subject to undue stress, is still quite acute. Therefore other factors than carbon substrates alone, must be responsible for the observed abscission of flowers.

One of these factors especially for plants grown at high density or in large fields is that not all flowers are tripped by pollinating insects. Tripping of all flowers of an autofertile and an autosterile line together with a commercial variety, Maris Bead (Figures 4.1.1, 4.1.2, 4.1.3) did indeed improve pod set, especially in the autosterile line. These results agree with those of Free and Williams, 1976. Much flower abscission still occurred on plants whose flowers had been tripped. It therefore appears that flower shedding is not prevented by ensuring that all flowers are tripped. similar concludions were made by Kambal (1969) and Kambal,

Bond and Toynbee-Clarke (1976). A further factor that could affect flower drop, is that not all tripped flowers are fertilized. A recent study (Rowland, Bond and Parker, 1982), indicated that only 48% of flowers sampled had at least one fertilized ovule. A further study still in progress at the PBI, however, has cast doubt on this result and initial findings have indicated that between 80-90% of faba bean flowers are in fact fertilized (Stoddard, Lockwood and Bond, pers. comm.). Failure of fertilization has been eliminated as a major contributory factor leading to high levels of flower shedding in Glycine max (Abernathy et al, 1977). The results of Kambal (1969), Chapman, Fagg and Peat (1979). Gates et al (1981) as well as those presented in Table 4.2 have demonstrated that many abscised V. faba flowers are fertilized. This is evidence to support the proposition that fertilization failure is not the sole cause of flower shedding in V. faba.

Silver nitrate is reported to be an ethylene inhibitor (Veen, 1969; Beyer, 1979) and its application did reduce flower abscission in treated plants, although flower drop in these plants was still quite acute. In addition the anti-auxin TIBA is reported to enhance the competitive ability of the inflorescences, diminish that of the apex and also lower endogenous ethylene concentration, which is believed to be responsible for the initiation of abscission layers (Newaz and Lawes, 1980; Chapman and Sadjadi, 1981). This compound significantly decreased flower abscission in Maris Bead plants (Figure 8.9). These results may indicate that there is some form of hormonal control over flower abscission, although when such chemicals are applied exogenously, a total elimination of flower shedding does not occur.

The acropetal development of successive racemes appears to be an important factor influencing flower abscission. Removal of the first formed axillary racemes resulted in a decrease in flower drop within racemes situated higher up the stem (Figure 4.8). Removing such racemes, thus removed a potential source of maturing pods, which could monopolise nutrients and may, in addition, transport abscission accelerating substances to more distally situated racemes, with flowers that are at, or have still to reach anthesis. The removal of proximal racemes, also allows more leaf area to become available for remaining racemes, which may be an additional factor that ensures that more pods set.

The apex of indeterminate plants is also important in promoting competition between it and the developing fruits. If the apex or the top of the plant is removed then many more pods will set on remaining racemes (Figures 4.10, 4.11.1, 4.11.2). This effect has been shown to be most effective when five raceme initials or inflorescences are left to develop (Chapman, Guest and Peat, 1978). Although more pods set, these subsequently drop in a pattern similar to that of flower drop in control plants (Figures 4.12.1, 4.12.2). Such a treatment might be envisaged as mimicking the effect of the ti gene. TI Col. plants, however, experienced flower drop in a similar pattern and react to environmental stress in a similar way to that of indeterminate varieties. Therefore an alteration of the plant to a determinate growth habit will not in itself ensure that flower abscission is eliminated.

Decreasing within plant competition by decapitation, apex removal and removal of proximal racemes, predisposes the plant to decreased flower drop and increased pod set. Similar

conclusions have been reached by Crompton, Lloyd-Jones and Hill-Cottingham (1981), who showed that as V. faba is an indeterminate species, the first set pods will compete for assimilates with growing roots and the stem apex, as well as with developing pods further up the stem. It has been shown that the apex imports assimilates from the leaves until the end of the flowering period and it appears that competition between apex and fruits is responsible for premature pod drop (Jacquiery and Keller, 1978a, b, 1980; Gehriger and Keller, 1980). However, a distinction must be made here between pod drop and flower drop. Vicia faba pods do not become active sinks until they reach the length of about 4 cm (Jacquiery and Keller, 1980 and references therein). Most flower abscission has already occurred on a raceme, before pods have reached this length. Although small, developing pods will be present together with flowers on acropetally developing racemes. It therefore appears that much flower abscission is initiated by factors other than competition for assimilates. However, the unfavourable competition for assimilates between the apex and fertilized flowers and in addition, the proposed preferential transport of such assimilates to proximally situated flowers augments the underlying processes leading to abscission.

Further, in investigating the role of carbon assimilates in flower abscission, removing or completely shading leaves from the vegetative portion of the plant resulted in increased flower abscission (Figures 4.13.1, 4.13.2, 4.14.1, 4.14.2, 4.15, 4.16) indicating the contribution of these leaves to the development of pods on the plant, similar results were obtained by Hodgeson and Blackman (1957). Alternate leaf

removal from the reproductive area of the plant also increased flower drop, but this effect was distributed over all racemes of a plant, and not concentrated on racemes subtending the removed leaves. A similar conclusion was drawn by Crompton, Lloyd-Jones and Hill-Cottingham (1981) who showed that there was no exclusive relationship between a leaf and supply of assimilates of beans developing on the same node. McEwen (1972) showed that defoliation of all leaves fron nodes at which pods were developing reduced yield by only 20%. It was subsequently shown that the amount of $^{14}C$ fixed by partially defoliated plants was similar to that of intact plants, although the amounts of fixed carbon exported from source leaves to other plant parts decreased in partially defoliated plants. This shows that leaves can compensate to a certain extent for reduced leaf area, although not by increasing the percentage of fixed carbon to other plant parts (Ismail and Sagar, 1981b). Carbon assimilates were exported from the fed leaf primarily to the subtending inflorescence (Figures 7.10, 7.11), however, beans developing on this inflorescence did not have exclusive access to these assimilates, they are also exported to alternate peduncles, with developing pods below the fed leaf. There is, however, no direct connection between the vascular traces supplying racemes and leaves (Figure 7.10). The results of shading and leaf removal experiments, however, indicate that increased flower abscission due to leaf removal was spread evenly over all axillary racemes of the plant. It could be envisaged that interaction between racemes act on two levels: in the short term there is only communication of assimilates between alternate racemes on plants, but in addition there is a slower export of carbon

from the leaves into the stem and then to all racemes of a plant. It has been shown that labelled sugars were probably stored elsewhere other than the fed leaf and were remobilized during the night to supply the fruit (Kipps and Boulter, 1973). This view is upheld by subsequent investigation, which showed that after 24 h less than 40% of the $^{14}C$ initially fixed had been exported from the leaves. It was the stem which was the primary sink for the exported carbon (Ismail and Sager, 1980) The presence of pods reduced the retention of the exported $^{14}C$ by the stem. It therefore appears that the stem acts as a temporary sink for assimilates especially when there is no demand for them elsewhere (Ismail and Sager, 1981b). The strong competition between young grains or pods and vegetative parts, which is evident during the entire flowering period may be explained by this temporary storage of assimilates in the stem and leaves, which is only relieved at the end of flowering when developing grains receive more than 10% of the $^{14}C$ fixed by the stem (Jacquiery and Keller, 1980). The changing patterns of stored carbon in V. faba has been studied by Bertholdsson (1980), who showed that during the vegetative growth most $^{14}C$ was found in the expanding leaves of the preproductive area and the stem within the first five to six nodes. As the plant grew older there was less fixed carbon in leaves, although there was no change of that in the stem. During early pod set most $^{14}C$ was in the stem and apex, the developing pods also becoming sinks. When seed development commenced there was retranslocation of $^{14}C$ to the seeds, leaves, stem and pods. During late pod development the lower stem, the temporary sink, was used to fill the seeds. The arrangement of the vascular strands,

however, needs also to be taken into account when assessing pod set on subsequent racemes.

From the results so far discussed there is a standard pattern of flower drop within a raceme of many present day varieties of faba beans, in that proximal flowers set pods and distal flowers invariably abscind. Flower abscission is affected by various environmental stresses, which primarily results in increased shedding, especially of the more distally situated flowers. Flower drop is decreased by application of silver nitrate, TIBA and to a limited extent, by ensuring that all flowers are tripped on each successive raceme, particularly if the line displays autosterility. However, in all cases, much flower drop still occurs. In addition many abscised flowers have pollen tubes present, so fertilization failure is not the sole reason for flower drop. Apex removal and decapitation dramatically reduced flower drop, on remaining racemes indicating the unfavourable competition between the apex and the reproductive organs. However, small pods subsequently drop on plants subjected to these treatments in a pattern similar to that of flower drop. Inflorescence removal of proximally situated axillary racemes also reduced flower abscission indicating the competition between these racemes and those situated more distally on the stem. Removal or completely shading leaves, also accentuated flower abscission over all racemes of the plant. Results of radiocarbon feeding experiments, showed that developing pods became strong sinks for assimilates. Communication of such assimilates between podded racemes only occurred, however, between alternate racemes on a plant, at least in the short term. So, it is apparent that any alteration of assimilate balance within a

plant will predispose that plant to increased flower drop. However, as small pods are not strong sinks and most assimilates at this stage are stored in a temporary sink, the stem, then it is apparent that other factors, rather than just carbon assimilates are responsible for much flower abscission. For this reason, in order to better understand the cellular and enzymatic changes leading to abscission, an anatomical study was undertaken.

Results of anatomical studies (Chapter 5) showed that the earliest cellular change detected in the pedicel tissues after fertilization was an enlargement of cortical cells, which coincided with the disappearance of starch stored in these cells at the time of anthesis. The detected rise in $\alpha$ - amylase activity in pedicels immediately after pollination may mobilize the conversion of starch to soluble sugars. This can be used by the cells of the pedicel/peduncle cortex for one of two functions: if a pod is set the starch reserves are used for differentiation of the vascular tissue at this junction, if pod set is unsuccessful, however, then cellular events leading to abscission proceed, mobilized starch would increase the osmotic potential of the sap in cells surrounding the abscission zone. This would cause a concentration gradient to be set up, leading to an influx of fluid from cells adjacent to the cortical cells of the abscission zone. In addition the detection of increased activity of pectinase and pectin methyl esterase in the pedicel/peduncle cortex at flower stages 9 and 10, would also perhaps lead to a weakening and ultimate dissolution of the middle lamella. This, combined with cell expansion, strands ultimately push the flower off rupturing the vascular

Sacher (1957) observed that during abscission in Coleus leaves movement of cellular fluids into the walls of the separation layer caused the intercellular spaces to fill with liquid. Sexton (1976) measured the osmotic potential of abscission cells of Impatiens sultani which indicated that the concentration of the sap had increased. Moreover, Sexton, Jamieson and Allan (1977) observed that cellular collapse sometimes occurred in explants if they are induced to abscise in contact with water. Inflated cortical cells have been reported in leaf abscission zones of Impatiens sultani (Sexton, 1976), Malus sp. (MacDaniels, 1937), Citrus fruits (Scott, Shroeder and Turrell, 1948), and dupelets of Rubus idaeas (Mackenzie, 1979). Such cells are extremely fragile, and it was found to be important that an isotonic fixative should be employed during specimen preparation. Hypotonic or hypertonic solutions caused the cortical cells to burst or shrink respectively, in both cases giving the appearance of cell fracture under SEM examination, although floral abscission of V. faba and other legumes is clearly the result of cell separation along cortical cell middle lamellae. Sexton and Redshaw (1981) concluded that this mechanism of abscission also operates in many other species.

As mentioned previously, Jacquiery and Keller (1980 and references therein) have shown that V. faba pods do not become active sinks and compete successfully for assimilates with the growing stem apex, until they reach the length of 4 cm. This lag may be dependent on the differentiation of vascular tissue before developing pods can become functional sinks.

Pod development in V. faba is not dependent on fertilization (Chapman, Fagg and Peat, 1979) and the developmental changes in the pedicel associated with pod set can be induced merely by growth of pollen tubes in the style (Gates, unpublished results). Stead and Moore (1979), demonstrated that corolla abscission in Digitalis purpurea was stimulated by pollen tube growth in the style, also Deurenburg (1976) and Gillissen (1976) have indicated that there is communication of a hormonal stimulus from the style to other flower organs. It seems likely that changes in the walls of the pedicel cortex may be initiated via a hormonal stimulus from pollen tube growth in the style.

The results of this ultrastructural study suggest that flowers are actively shed after a series of clearly defined cellular and enzymatic changes at the junction of the pedicel and peduncle.

Removal of flowers situated on proximal positions (Chapter 6) resulted in the development of pods situated at more distal positions. This situation was most apparent when the proximal two or three flowers had been removed. These results show that distal flowers are capable of setting pods, and that they are not destined to abscise, as long as the first formed flowers are removed. It is apparent that the first formed pods communicate a chemical signal to flowers on the same raceme, which promotes these flowers to abscind. A similar situation has been reported to occur in Lupinus luteus (Van Steveninick, 1957, 1958, 1959), Lupinus angustifolium (Farrington and Pate, 1981), Vigna unguiculata (Ojehomon, 1972), Cajanus cajan (Sheldrake, Narayanan and Venkataratnam, 1977), Glycine max (Tayo, 1977),

Phaseolus vulgaris (Tamas et al, 1979) and Brassica napus L. (Tayo and Morgan, 1979).

The nature of this chemical signal is unclear. Van Steveninick (1957, 1958, 1959) proposed that it was some form of inhibitor, Huff and Dybing (1980) extracted substances from immature soybean pods that replaced the pods in promoting shedding at more distal floral positions. One possible contender for this chemical signal is believed to be abscisic acid (ABA), (Porter and Van Steveninick, 1966, Cornforth, et al, 1966). However, ABA did not significantly promote shedding of soybean flowers when applied in lanolin in place of the lowermost flowers on a raceme. Endogenous ABA were at peak levels just preceding abscission in yellow lupin, Lupinus luteus (Porter, 1977) but it was found that the concentration of ABA was not related to the degree of shedding, and application of ABA by injecting it into racemes in situ failed to increase shedding. Huff and Dybing (1980) showed that indole acetic acid (IAA) was the only plant hormone that promoted shedding, but side effects of bending of racemes, suppression of petal opening and hastening of abscission cast doubt on a role of endogenous IAA in initiating shedding. El-Beltagy and Hall (1975) found that high endogenous levels of ethylene correlated well with abscission of flowers or pods in V. faba but they could find no clear connection between any particular developmental process and endogenous levels of auxin or inhibitors. Spraying plants with silver nitrate or TIBA, however, reduced flower shedding, implying that both auxin and ethylene do have a role to play in the promotion of abscission, but the exact nature of the signal that initiates abscission remains unresolved.

Observations on determinate inbred lines from the crossing programme (Chapter 7), indicated that some of these lines displayed decreased flower drop, others did not. This is in line with observations already made of the genotype TI Col. under various environmental stresses, which resulted in a pattern of flower drop similar to that already described and that the determinate growth habit, in itself, will not reduce flower drop.

Two lines from observations made in the glasshouse (56/130/1 and 56/118/20) displayed a semi-determinate growth habit. One of these lines (56/130/1) displayed a high level of flower drop, while the other line (56/118/20) exhibited little flower drop on all racemes. It is apparent from these observations that because both lines displayed similar plant architecture, a possible reason for the difference in flower drop behaviour of the two lines is that an alteration in vascular architecture either within each raceme or between nodes had occurred.

A further line displaying reduced flower drop (56/143/9) was subsequently found and this was grown in field trials, together with 56/118/20 and plant lines displaying different plant architectures. These two lines displayed minimal flower drop when grown in the field.

Examination of the vascular architecture of the two lines that exhibited low flower drop with those of commercial varieties, indicated that the lines with low flower drop had a modified inflorescence vascular architecture compared to that of commercial varieties. Results of $^{14}C$ feeding studies, comparing the communication between racemes showed that a similar pattern of communication between alternate peduncles

occurred in lines with lower flower drop and commercial varieties (Figure 7.10, 7.11). It could be concluded, that as there has been no change in the gross vasculature of the stem, then interaction between alternate racemes has a small effect on flower drop, compared to the interactions within individual racemes. However, this might not be the case if a consideration of the factors influencing pod drop were to be made.

The vasculature of lines exhibiting low flower drop, was such that the vascular strands supplying each flower were independent of each other flower on a raceme. In contrast, the vascular supply to flowers within a raceme of commercial varieties examined was interconnected. In the majority of cases the first formed flower was independent of other flowers, while the second and third flowers were connected to other flowers via the vascular strands.

The independent vascular supply characteristic prevented any communication between basal flowers and apical flowers, as evidenced by eosin and radiocarbon feeding studies. There was, on the other hand, ready communication between flowers on the same raceme of commercial varieties not possessing this characteristic.

The elucidation of the arrangement of the vascular supply in racemes of commercial varieties correlates well with the observed pattern of flower drop in such genotypes. The proximal flower of such racemes, is the one that most often possesses an independent vascular supply, and it is this flower that most often sets a pod, even under environmental stress. In addition removal of the proximal flower on each raceme of commercial cultivars resulted in only a

slight or no reduction in abscission (Table 6.1). When the proximal two or three flowers were removed from racemes then a highly significant reduction in abscission occurred. It is apparent that it is the second and third formed pods, in many cases that communicates a chemical signal to more distally situated flowers, which initiates the events leading to flower abscission. It must be emphasised that much variation in vascular architecture occurred in commercial varieties and in some cases the proximal flower was also connected to other flowers on a raceme. The vascular arrangement of commercial varieties is also compatible with the assertion that limiting carbon assimilates accentuates flower abscission. Such an arrangement of the vascular strands leads to an uneven distribution of assimilates, as flowers on more distal positions would have to share assimilates with more proximally situated flowers. Whereas the proximal flower will receive proportionally more assimilate than other flowers. An adequate supply of assimilates is required for the differentiation of vascular tissue at the pedicel/peduncle cortex, which is an essential prerequisite for successful pod set. If distal pollinated flowers receive a smaller proportion of assimilate than more proximally situated flowers, then vascular differentiation of the pedicel/peduncle cortex would be slowed down, and this combined with the communication of an abscission promoting signal from small proximally set pods would lead to the cellular and enzymatic changes leading ultimately to flower drop. The circumvention of the above physiological interactions, by independent vascular supply not only prevents the communication of an abscission promoting signal to distal flowers, but should

also lead to an increased lag phase between fertilization and expansion of the ovary, while the vascular tissue of the pedicel/peduncle junctions of all flowers are differentiating. This would be expected because assimilates are now being shared equally between 6 or 7 flowers rather than just 1 or 2 flowers, which has hitherto been the case. Evidence for this comes from investigations into peroxidase levels in pedicel/peduncle junctions (Figure 7.7). Peroxidase was chosen as an enzymatic marker for flower development because the enzyme has been associated with growth and differentiation (Galston and Davies, 1969; Van Fleet, 1959; Huff and Dybing, 1980). These experiments indicated that for commercial varieties the activity of peroxidase was greater in junctions derived from flowers at the proximal raceme positions, and increased at these positions as development continued. No such increase in peroxidase activity occurred in junctions obtained from flowers situated at distal raceme positions. This is evidence of the developmental advantage that the first set pods have over flowers on more distal positions. Similar observations have been made between soybean ovaries, most likely to set pods compared to those most likely to abscind (Huff and Dybing, 1980). In lines possessing the independent vascular supply characteristic, peroxidase activity is lower at the same developmental stage in junctions from flowers at all positions, compared to that observed in junctions derived from commercial varieties. Peroxidase activity, however, increases in junctions derived from all flower positions from stage 10 to pod set. This could be taken as evidence that the pedicel/peduncle junctions are differentiating more slowly, but evenly, at all flower

positions in these lines. One flower does not have a developmental advantage over others on the same raceme.

In addition to the circumvention of the physiological interaction between young pods and flowers within the same raceme by independent vascular supply, such inbred lines also possess a "semi-determinate" character. This results in flowering on 10-14 racemes, which then abruptly ceases with the production of a few vegetative nodes. This is in contrast to commercial varieties which, especially when subject to warm and moist conditions, can flower almost continuously up until harvest. The maintenance of a strongly competitive apex has a detrimental effect on flower drop, as previously discussed. The semi-determinate character would help to reduce this interaction without resort to the more extreme terminal inflorescence mutants.

Initial stress experiments comparing lines possessing the independent vascular supply characteristic with those of commercial varieties (Chapter 8) indicate that many pods will set in independent vascular supply lines even under a high level of stress. Flower abscission, however, did increase in line 56/143/9, particularly when subject to warm conditions and supplementary lighting. It has been found in tomato flowers (Levy, Rabinovitch and Kedar, 1978) that high temperatures induce the drop of buds and flowers, and this is the result of a lack of fertilization. This itself was affected by a number of factors. Under high temperature, gametogenesis was disturbed and gamete viability reduced, so less pollen was produced in the flower. High temperature also affected the germination and elongation of the pollen tube into the style and this also inhibits

fertilization. As tomato flowers are self-pollinated anything reducing or preventing self-pollination will result in lower fruit set. As all flowers were tripped in V. faba in this experiment (as in every other experiment), it is possible that a similar inhibitory response to fertilization occurred in faba beans. If fertilization is not successful in V. faba then flower drop will proceed, irrespective of the vascular architecture of the plant.

The effect of different tripping treatments (Figures 8.6, 8.7) on inbred line 22 and 56/143/9 had very little effect on pod set, such differences that were apparent seemed to be due to differences in abscission behaviour between plants, possibly due to variations in raceme vascular architecture, which as already stated can occur between individual plants. The lack of response to tripping could be an indication of autofertility in these lines. Inbred line 22 also displays autofertility, although it was affected to some extent by the tripping treatments (Figure 8.6).

Spraying plants with the antiauxin TIBA had no effect on plants possessing the independent vascular supply character, although it significantly decreased abscission in commercial variety Maris Bead. This is a strong indication of the influence of a hormonal signal in promoting flower shedding in varieties not possessing independent vascular supply. The lack of communication of such a signal between flowers on plants with an independent vascular supply, enables a high degree of pod set.

In conclusion, the current varieties of V. faba may be regarded as only semi-domesticated (Hawtin , 1981) and

have many of the reproductive characteristics of successful weeds. Indeterminate growth, coupled with acropetal anthesis within racemes and the shedding of flowers induced by pod set are common growth characteristics of wild Vicia species and many other taxonomically and ecologically diverse species (Stephenson, 1981 and references therein). Such a strategy leads to a continuous supply of flowers and limited pod set. This might be expected to maximise the possibility of production of recombinants in allogamous species, especially in environments where resources are limiting.

In contrast, the agricultural requirement for high levels of pod setting and uniform pod maturation induces whole plant "self-destructive" senescence according to the concept of Sinclair and De Wit (1975). Ideotypes with high pod set achieved by the selection of independent vascular supply to flowers and synchronous pod development induce a more determinate growth habit, without resort to the more extreme terminal inflorescence mutants. They will result in crop yield being limited by the ability to supply assimilate to the developing sinks, rather than by sink capacity as has hitherto been the case (McEwen, 1972). Unlike determinate mutants the ideotype described above will retain some capacity to differentiate leaves in response to sink demand or to replace photosynthetic capacity lost due to leaf damage.

The narrow genetic base and limited genetic variability in the faba bean crop have been cited as a limiting factor in the improvement of the crop (Bond, 1976). While this is true of current selection criteria, there is a considerable untapped reservoir of anatomical and physiological variation

within the reproductive tissues of V. faba, as evidenced by results presented in Chapter 7. Such variation is now beginning to be recognised (Hobbs and Burnett, 1982). Different breeding techniques can be used to exploit different components of genetic variability, so that differences in genetic architecture between populations of faba beans could be taken into account when planning plant breeding programmes. Advantage could then be taken of interactions between breeding methods and populations that are phenotypically but not genetically similar.

It is possible to rapidly screen for vascular anatomy and tissue distribution by utilizing epi-illumination fluorescence microscopy (Gates and Oparka, 1982) and light microscopy, allowing the selection of vascular characteristics for the improvement of reproductive performance in the crop.

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