Some responses of *Eryngium vesiculosum* Labill. to light intensity, daylength, and nitrogen.

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INTRODUCTION

Eryngium vesiculosum Labill. is a plant commonly found in sandy coastal flat in New Zealand. Some preliminary observations (pers comm. Veale) had suggested that flowering in this species might be controlled by the external environment especially with respect to daylength. Normally the mature plant has oblong and dissected leaves but in the shade the leaves tend to have less dissection and this change in leaf form serves as a useful feature for morphological investigations. Another interesting feature of the plant is that it produces runners like the strawberry plant. The plant also produces daughter plants on the runners and lateral branches. The production of these organs, which enables a large family of homogenous plants to be raised rapidly by vegetative propagation is another feature which would make this plant useful experimental material. The object of this study is to obtain some information on the effects of light intensity, daylength and nitrogen on flowering, leaf morphology and anatomy and plant growth.

Much work has been carried out on the effects of the three external factors used in this study on many plant species but none on E. vesiculosum. Because of the absence of such information on this plant, the review of literature includes other plant species thus making it more voluminous than otherwise would be. For obvious reasons the review has been limited to present only the salient responses of some plant species to light intensity, daylength and nitrogen.

CHAPTER I. REVIEW OF LITERATURE

1.1 PLANT RESPONSES TO LIGHT INTENSITY

Plants grown in different light intensities show characteristic and well known differences in growth and development. One of the first experiments on light intensity effects on plants was carried out by Lubimenko (1908) who found that the amount of dry matter production increased with increasing light intensity up to a certain maximum and then decreased. Other early investigators including Garner and Allard (1920), Popp (1920) and Shirley (1929) were in general agreement that under shading, plants were reduced in their growth.

A considerably detailed investigation of the effects of light intensity on the vegetative growth of 22 herbaceous species has been conducted by Blackman and his followers (Blackman and Rutter, 1948; Blackman and Wilson, 1951a, b; Blackman, Black and Kemp, 1955; Blackman and Black, 1959; Blackman, 1961). The growth affected by light intensity was evaluated in terms of relative growth rate and other attributes of classical growth analysis. They observed that changes in leaf area ratio and net assimilation rates over the range of 0.1 to 1.0 (full) sunlight were linearly related to the logarithm of the light intensity. The relative growth rate which is the product of leaf area ratio and net assimilation rate was curvilinearly related to logarithm of light intensity. This is in agreement with the recent finding of Wilson (1967). Using multiple regression analysis, he found that net assimilation rate increased linearly with radiation in rape (Brassica napus), sunflower (Helianthus annuus) and maize (Zeas mays). Further, he found the leaf area ratio to decrease and relative growth rate to increase with radiation.

In the discussion of the effect of external factors on growth attributes, the effect of plant age needs to be considered. Evidence on this effect is conflicting. Experiments have indicated that there were no significant trends in net assimilation rate with age during the vegetative phase (Heath, 1937; Blackman and Wilson, 1951a). Watson (1947) pointed out that where seasonal trends were observed these can be attributed to seasonal variations in climatic factors, rather than to plant age. The introduction of controlled environment opportunity which eliminates the effect of fluctuating external environmental factors enables investigators to examine more critically than before the effect of age on growth parameters. Throne (1960, 1961) found that relative growth rate, net assimilation rate and leaf area ratio of sugar beet, potato and barley to drift with time in growth rooms. Eagles (1969) working on two natural populations of Dactylis glomerata in controlled environments, found relative growth rate to decline with age. The net assimilation rate of wheat, on a leaf area basis was found to decrease with time under controlled conditions (Friend, 1969).

Light intensity can affect the morphology and anatomy of a leaf during its development. The generalisation has been made that high light intensity produces smaller leaves than those produced in the shade. This conclusion has been reached by many investigators, including Penfound (1931) for Polygonium and unwatered Helianthus, Blackman (1934) for tomato, Cormack and Gorham (1953, 1955) for Menziesia glabella, Lonicera glaucescens and Vicia americana and Newton (1963) for Cucumis sativus. In contrast Milthorpe (1943) observed that leaf area of flax declined with a decreasing light intensity. Similarly Milthorpe and Newton (1963) found that sun leaves of Cucumis sativus were larger in area than shade leaves.

The influence of light intensity on leaf shape has been studied by many investigators. Njoku (1956) found that Ipomoea caerulea plants already forming lobed leaves reverted to the production of entire leaves when transferred to deep shade. The length:breadth ratio is often used in the study of leaf shape. Bensink (1960) found that length:breadth ratio of lettuce decreased with an increase in light intensity. This finding agrees with the results of Abery (1943) on Lobelia dortmanna. Leaves formed on plants grown at low light intensity were longer than those grown at high light intensity for several species including Vicia americana Cormack (1955), wheat (Friend and Pomeroy, 1970) and Lolium (Wilson and Cooper, 1969).

Recent work on the effect of environmental conditions on leaf size had placed importance on cell number and cell size as two attributes in determining the ultimate size of the leaf. Humphries and Wheeler (1960) working with Phaseolus vulgaris leaf disks noticed that light increased cell size and cell division. They concluded that leaf expansion by illumination was caused partly by increased cell division and partly by increased cell size, but that increased cell size was more important than cell division. The conclusion was confirmed by Bulter (1963) on broad bean seedlings. In contrast Friend and Pomeroy (1970) found that high light intensity decreased epidermal cell length and number in the spring variety Marquis and winter variety Kharkov wheat. The effect of light intensity on mesophyll cells was similar to that on the epidermal cells. The upper and lower epidermal cell size of Menziesia glabella and Lonicera glaucescens are also reported to be increased with decreased light intensity (Cormack and Gorham, 1953).

Cormack and Gorham (1953) also studied the effect of light intensity on stomatal frequency. They found that stomata were more numerous per unit area in sun leaves than in shade leaves. This result is in agreement with the work of Cooper and Qualls (1967) on lucerne and birdsfoot trefoil, of Friend and Pomeroy (1970) on two varieties of wheat and of Bjorkman and Holmgren (1963) on Solidago virgaurea but is in contrast with the data of Penfound (1931) on Helianthus, of Cormack (1953) on Vicia americana and of Wilson and Cooper (1969) on Lolium.

Besides affecting stomatal frequency, shading also affects stomatal size. Leaves of Lolium grown in weaker light had much smaller stomata than those grown in stronger light (Wilson and Cooper, 1969). The work of Cameron (1969) on Eucalyptus fastigata showed that the abaxial surface of juvenile leaves of plants in 40% sunlight possessed smaller stomata than juvenile leaves from plants grown in full sunlight while intermediate leaves possessed largest stomata when shaded to 40% sunlight.

Literature on the effect of shading on chlorophyll content is limited and contradictory. Shade leaves of species E. fastigata (Cameron, 1969) lucerne and birdsfoot trefoil (Cooper and Qualls, 1967) contained significantly less chlorophyll per unit leaf area than sun leaves. On a unit leaf weight basis however, shade leaves had more chlorophyll (Cooper and Qualls, 1967). By contrast, Evans and Hughes (1961) found that the amount of chlorophyll per unit leaf area was roughly the same for all levels of shading, although the content on a dry weight or fresh weight basis showed a systematic increase with shading.

It is important to appreciate that light intensity may not be solely responsible for morphological and anatomical changes. Other factors are involved and are reviewed by Shields (1950). The effect of light intensity is often highly correlated with temperature. For example leaf size and shape are often affected by temperature. Friend et al. (1962) growing Marquis spring wheat in sand culture at different temperatures found that the greatest area of individual leaves was formed at 20°C. The effect of temperature on leaf shape have been observed by several investigators. Schwabe (1957) found that Chrysanthemum leaves were much more dissected at 17°C than at 27°C. The work of Fisher (1960) on Ranunculus hirtus showed that although light intensity had no effect on leaf shape, temperature had a pronounced effect. Leaves produced at 20°C were of the undivided form whereas at 10°C they were the deeply three-lobed form. Leaf shape in lettuce and Eucalyptus were reported to be regulated by temperature (Bensink, 1960; Scurfield, 1961).

1.2. PLANT RESPONSES TO PHOTOPERIOD

The great variety of plant processes influenced by photoperiod is enormous. Pigment synthesis, organic acid content of the leaves, accumulation of food reserves in leaf bases of bulbing plants or rhizomatous stems are influenced by photoperiod. Many morphological and anatomical phenomena are now known to be affected by daylength. Breaking of dormancy in seeds and buds, rooting of leafy cuttings, vegetative development, flowering and senescence are in many cases regulated by light duration.

Literature concerning the photoperiodic control of flowering has become voluminous, and excellent reviews are found in Hillman (1962), Salisbury (1963), Lang (1965), Searl (1965), Evans (1969) and others.

Flowering has been known to be regulated by developmental and environmental factors. That plants will not flower until they have passed a certain developmental stage have been recorded. Klebs (1913) established the term "ripeness to flower" to describe the apparent necessity for plants to produce a number of leaves before they are capable of flowering. Purvis (1934) introduced the concept of minimal leaf number as a measure of this develop mental phase. Minimal leaf numbers for several species have been determined by some investigators (Barber and Paton, 1952; Leopold and Guernsey, 1953; Holdsworth, 1956).

The control of flowering by daylength was discovered by Garner and Allard (1920). They were able actually to control flowering in some species and quantitatively to hasten or delay it in others. They grouped the photoperiodic responses of plants into three classes: short day plants (SDP), long day plants (LDP) and indeterminate plants. Among both short day plants and long day plants some species may be strictly dependent on daylength for flowering; while others may be only quantitatively hastened or delayed in flowering by the photoperiod.

The photoperiodic response of plants to flowering does not necessarily mean the influence is due to the specific length of the day. The influence may be due to the quantity of light being supplied. Influences of quantity of light on flowering is shown by Haupt (1958) on peas.

A large amount of work has been done to elucidate the mechanism of flower induction and the present understanding of this topic has been recently reviewed (Evans, 1969).

Plant vigour has been found to be influenced by photoperiod. Th.Fries (1918) comparing fresh and dry weight, leaf number etc. of several species grown in continuous daylight with those of 12 hour day under arctic conditions found in nearly all cases the largest plants were obtained in continuous daylight. Schwabe (1956) also found that reduction of the daily photoperiod had adverse effects on overall growth of Kalanchoe blossfeldiana, Xanthium pennsylvanicum, Hyoscyamus niger and Beta vulgaris. The effect of photoperiod on growth may be complicated by the fact that top and root growth may have different responses to daylength. There was no difference in the total growth of Bromus inermis in 13 and 18 hour daylengths, but top growth exceeded root growth in long days, while it was less than root growth in short days (Gall, 1947). In Festuca arundinacea photoperiod had no net effect on growth because greater tillering and better root growth under short days offset the better leaf growth under long days (Templeton, Mott and Bula, 1961).

The size and shape of the leaf may be influenced by daylength. Generally the largest leaf area was attained by a long day plant under short day conditions (Bunning, 1956). Conversely short day plants sometimes produced largest leaves under long days (Bunning, 1956; Schwabe, 1957). However, Borthwick, Parker and Scully (1943) working on the long day plant, Taraxacum kok-saghyz found that leaf area increased more in long than in short days. Banga (1952) also found that in long days the long day plant , red beet formed largest leaves. It appears there is no definite trend about photoperiod and leaf size.

Not all workers accept the view that light has an effect on leaf shape. Ashby (1948) proposed that some instances of hetero blastic development were due to a response of leaf shape to length of day. However Allsopp (1965) pointed out that although there were numerous instances of a marked effect of day length on leaf shape, yet the length of day can be of little fundamental significance in heteroblastic development, since in many plants the normal course of ontogeny was carried out even under constant lighting conditions. A few examples of daylength effect on leaf shape are cited here. Leaves of Sesamum orientale which normally were entire marginal and simple on 10 and 11 hour photoperiod, were compound on 15 and 16 hour photoperiod. (Sen Gupta and Payne, 1947). Binet (1958) found in Ulex europaeus that short days increased the formation of trifoliate leaves while long days favoured the production of simple leaves. Leaf shape in Chrysanthemum, Kalanchoe and Ipomoea were reported to be regulated by photoperiod (Ashby, 1950; Schwabe, 1956). The increased carbohydrate production under longer days might well be responsible for the changes in leaf shape rather than the length of day as such. The effect of increasing carbohydrates and daylength were not partitioned in these examples.

1.3. PLANT RESPONSES TO NITROGEN

The lack of nitrogen will seriously hamper the growth of plant organs, through a reduction of protein synthesis and the consequent restriction of meristematic activity is obvious. Conversely an ample supply of nitrogen would favour protein synthesis and thus increase meristematic activity, leading to luxuriant growth and generally increased vigour.

The effect of nitrogen on plant growth was studied by several investigators. Withrow (1945) demonstrated that in both long and short day plants, abundant nitrogen supply generally favoured heavier and taller plants. This observation was confirmed by Loustalot and Winters (1948) who found that a low nitrogen supply markedly depressed the growth of Cinchona ledgeriana seedlings. Ballard and Petrie (1936) found that increasing initial nitrogen supply caused at first a depression in dry weight. The depression passed off with time and provided the supply did not exceed some optimum value determined by the conditions and species, gave place to an increase. There is contradictory evidence on the response of net assimilation rate on nitrogen treatment. Gregory and Baptiste (1936) reported that up to the time of maximum leaf area, net assimilation rate was independent of nitrogen supply for barley. However when leaf area was decreasing from its maximum, the net assimilation rate of nitrogen deficient plants was less than that of plants supplied with complete nutrient (Mather, 1933). Gregory and Baptiste (1936) attributed this observation to the presence of a large number of senescent leaves in plants under nitrogen deficiency than in plants under complete nutrient. By contrast Ballard and Petrie (1936) observed that the Net assimilation rate can be increased by nitrogen treatment. William (1946) on Phalaris tuberosum found that net assimilation rate was not affected by nitrogen supply in the early stages of growth but increased significantly later even when leaf weight and area were still increasing. Watson (1947) found that nitrogen tr treatment increased the net assimilation rate of barley in the period before maximum leaf area and that of mangolds throughout the whole growth period. These few examples show that further analysis of this phenomenon is desirable.

There is general agreement among investigators on the effect of nitrogen on shoot:root ratios. One of the early experiments on nitrogen effect on shoot:root ratio was done by Turner (1922) who found that increasing nitrate concentration favoured a high shoot:root ratio for barley. This was confirmed by several investigators including Reid (1924, 1929 a,b), Weisman (1950) Curtis and Clarke (1950), and Siderie and Young (1950) who found that when nitrogen was low there was a decrease in shoot growth and an increase in root growth thus resulting in a low shoot:root ratio.

It is recognised that leaf form could be affected by the nitrogen supply. Arney (1952) found epidermal cells in Kale were about 10 percent larger in high nitrogen plants, but leaf size increased more than this. Morton and Watson (1948) confirmed this finding. Njoku (1957) studying the effect of mineral nutrition on leaf shape in Ipomoea caerulea found that nitrogen supply had the greatest effect, on a nodal basis, on leaf shape. The leaves of plants from a high nitrogen supply were less deeply lobed than those of the control plants.

The structural features of the leaf such as stomatal frequency, leaf thickness, cell number, cell size and chlorophyll content may be affected by nitrogen treatment. In potato leaves, nitrogen has no effect on cell size, but increased the cell number (Humphries and French, 1962). Nitrogen is essential in the structure of the protochlorophyll and chlorophyll molecules. It is well known that nitrogenous fertilisers produce remarkably green leaves, while a deficiency of nitrogen makes them yellow. Raper (1966) working with tobacco plants in the field found that plants grown under nitrogen stress not serious enough to cause acute deficiency were slightly yellow indicating a reduction in chlorophyll content. Greig, Motes and Al-Tikriti (1968) also reported that increased nitrogen supply enhanced the chlorophyll content in the spinach plant.

During the early 1900's there was much speculation about the importance of nutrition to flowering. Klebs (1903) and Kraus and Krybill (1918) considered that an excess of carbohydrates favoured flowering. In long photoperiod, it has been found that the macroscopic flower buds of long day plants often appear earlier in low nitrogen than in high nitrogen supply. This situation is examplified in long day plants, such as spinach (Knott, 1940) and mustard (El. Hinnawy, 1956). Conversely, certain short day plants such as Xanthium (Neidle, 1939) and soybean (Scully, Parker and Borthwick, 1945) in a short photoperiod favourable for flowering and deficient in nitrogen tend to develop macroscopic flower buds later than high nitrogen plants. In general an abundant supply of nitrogen appears to hasten flowering in short day plants and delay flowering in long day plants. There appears to be an important interaction of photoperiod and nitrogen on flowering, as shown by the work of Blake and Harris (1960). They reported that low nitrogen supply delayed flower initiation in carnation only in photoperiods unfavourable to flowering, namely short days. The effect of nitrogen on inflorescence initiation in grasses has also been investigated. Calder and Cooper (1961) found that high nitrogen supply promoted floral initiation in cocksfoot.

Though these reports indicated that nitrogen supply can affect time of flowering, they did not show whether the effect was through influences on flower development or flower initiation or both. Leopold (1951) stated that nitrogen had very little or no influence on floral initiation.

CHAPTER II THE PLANT

2.1 SOURCE

The plant Eryngium vesiculosum Labill. was grown from seeds harvested from clonal material maintained by Professor J.A. Veale, Department of Horticulture, Massey University, Palmerston North, New Zealand and originating from samples collected at Birdlings Flat, Canterbury.

2.2 THE PLANT

The plant has been described by a few investigators including Allen (1961). It is a small tufted perrenial herb belonging to the Umbelliferae. Diagram 2.1 shows the mature plant with its roots, leaves, runner, lateral branches and inflorescences.

2.2.1 The Root

The plant has a short rootstock and a number of long stout lateral roots (Plate Ia) Old roots are replaced by new ones as shown in Plate Ib. The roots are thickish (0.5cm.) end whitish in colour, turning to brown and black as they age.

2.2.2 The Leaf

The plant has numerous radical leaves arranged in a rosette on the crown and these are varied in size and shape. Varations occur between successive leaves within a single plant (Plate II). Variation of this kind, where the juvenile and adult forms of leaves are strikingly different, is referred to as heteroblastic development.

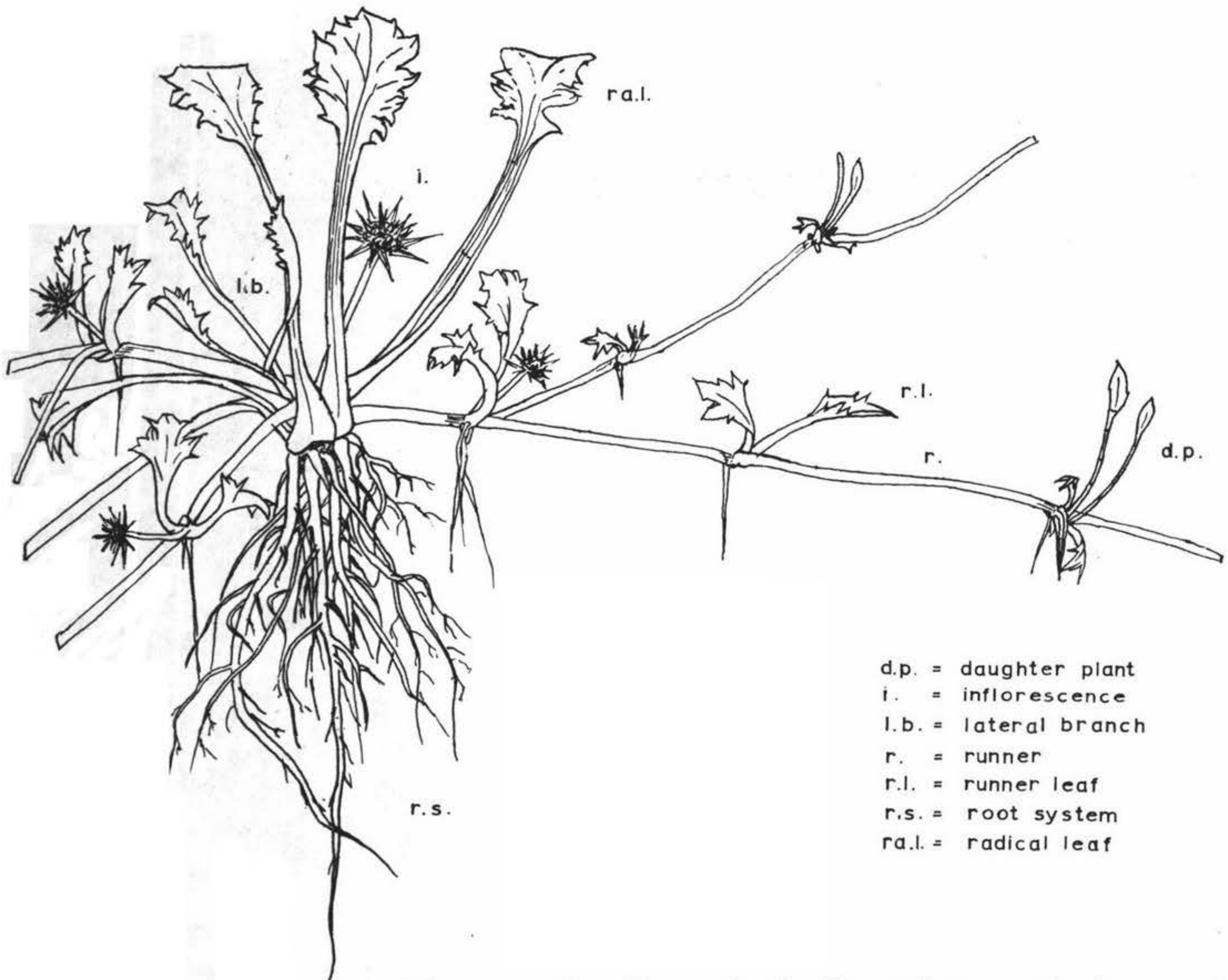


Diagram 2.1. The plant Eryngium vesiculosum Labill. X ½

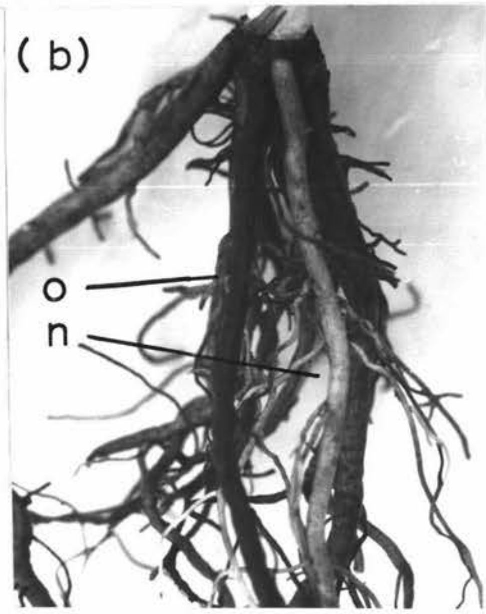


Plate Ia and b. (a) Root system showing the long stout lateral roots. X.75 (b) Old root (o) with new root (n) initiated beside it. X1.25

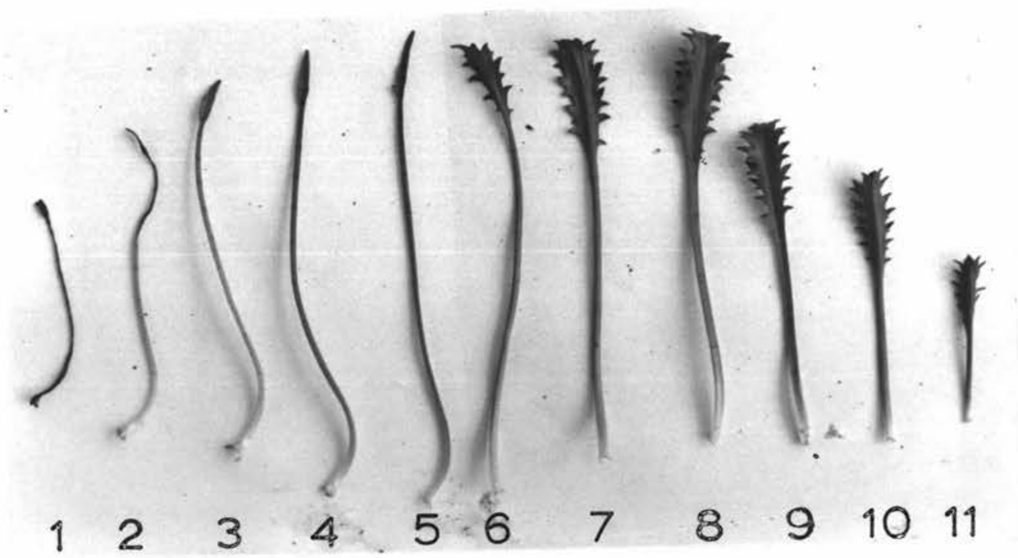


Plate II. Successive leaves within a single plant showing variation in leaf shape. X.50 [Highest number represents the latest leaf]

The juvenile leaves are narrow column almost filiform with their margins entire but these range through to the adult leaves which are somewhat rigid, lanceolate or oblanceolate with margins deeply toothed. The teeth are spinous and about 5mm. long. Plants growing in intense shade have the juvenile form of leaves (1-5, Plate II). The length of the leaves which narrows into a petiole extends from 4 - 15cm.. All have hollow midribs and are of a distinctive bluish green colour.

The pair of leaves at each node on the runner is smaller and has less teeth than the adult radical leaves (Plate III).

2.2.3 The Runner

As far as the author is aware the plant only produces runners after flower initiation, although some plants have been observed to produce runners without flow ring eventuating . (per comm. Veale.)

The runners 2 to 8 per plant are prostrate rooting at the nodes and reaching up to 60 cm. in length. A pair of leaves and a inflorescence are located at each node (Plate III) although sometimes the inflorescence at the node is abortive. Also each node may have a daughter plant (Plate IV).

2.2.4 The Inflorescence

The flowering axis is short and leafless arising from the central rosette and from the nodes of the runners. Each flowering axis terminates with a umbel (Plate V). The umbel is an indeterminate type of inflorescence whose flowers do not reach maturity simultaneously, the outer flowers opening before the inner. Each inflorescence, about 2.0 cm. in diameter, has

Plate III.

A pair of leaves at node of runner.

Note root initials at the node

Plate IV

A daughter plant with roots formed at the node of runner X1.0

Plate V

A hemispherical umbe on a short flowering axis. X1.25

20 to 40 flowers. No further leaves are produced on the central axis after the inflorescence is initiated and flowering is observed in natural habitat from November to February.

2.2.5 The Flower

Each flower is subtended by a lanceolate and spinous bract about 1.2 cm. long which is often longer than the flower and with hyaline wing forming a sheathing base (Plate VI).

The flower is actinomorphic with the calyx of five sepals adnate to the ovary. The sepals are erect and ovate with narrow hyaline winged margins while the corolla of five petals is apically inflexed. The petals which are white and about the length of the sepals have a keel projecting inwards and with margins protruding from between the sepals.

The five stamens which alternate with the petals arise from an epigynous disk which forms two lobed semicircular cushions surrounding the bases of the two filamentous styles. The anthers are two celled, dorifixed and hehiscing longitudinally while the stigmatic tip of each style is rounded (Plate VIb). The ovary is inferior and covered with overlapping vesicular scales as illustrated in Plate VI.

2.2.6 The Fruit

The fruit is a schizocarp compounded of two mericarps coherent and dehiscing by their commissure. Each fruit is oval with the persistant sepals, petals and styles at one end (Plate VIIa). The seed when mature has a dark brown testa

Plate VI (a) X90 (b) X90

(a) The flower subtended by a bract with hyaline sheathing base. Note the 5 stamens and 2 styles

(b) An older flower with its matured styles. Stigmatic tips are rounded and most of the stamens have dropped off. The Stamens mature earlier than the styles.

Plate VII (a) X90 (b) X90

(a) A schizocarp compound fruit with vesicular scales on it. The Calyx, corolla and styles are still at one end.

(b) Two mericarps dehise at their commissure.

covered with vesicular scales (Plate VIIb). Seeds did not show good germination just after collection but observed to do so after having been retained for a period of possible maturation.

2.3 VEGETATIVE PROPAGATION

The runner grows horizontally along the ground and forms a new plant at each node. The daughter plant at each node takes root but remains attached to the mother plant for some time. The connecting runner dies eventually and each daughter plant becomes an independent unit. The daughter plants which have rooted are dug and then transplanted.

Another method of vegetative propagation is be means of lateral branches. At the base of the main stem of the parent plant lateral branches or offsets may develop. These offsets are removed by cutting them close to the main stem. If the offset is rooted it can be potted as is done with any rooted cutting. If insufficient roots are present, the offset is placed in a suitable rooting medium and treated as a leaf stem cutting.

CHAPTER III CULTURAL ENVIRONMENT AND METHODS

3.1 CULTURAL ENVIRONMENT

3.1.1 The Glasshouse

All the experiments were conducted in a 12' x 18' Hartley glasshouse orientated in an east-west direction at the Plant Physiology Division, D.S.I.R., Palmerston North, New Zealand.

The glasshouse is equipped with thermostatically controlled vents, heaters and cooling system. This allows temperature to be controlled within about ± 3°C of the desired mean value.

3.1.2 The Temperature

In all the experiments the plants were maintained at an average day temperature of 25° ± 3°C and an average night temperature of 15° ± 3°C. Mean temperatures during the experimental periods are shown in Appendix I.

3.1.3 The Light Energy

The light energy data have been prepared from the records of the Plant Physiology Division Meteorological Station over the experimental period, the station being situated approximately 50 yards from the glasshouse. The amount of light energy falling on the plants inside the glasshouse was obtained directly from the measurement of total solar radiation outside multiplied by the appropriate transmission factor of the glass. Table of the solar energy for the various experimental periods is shown in Appendix I.

3.1.4 The Daylength

In all daylength experiments two daylengths of 16 hours (long) and 8 hours (short) were employed. The long daylength was achieved by extending the natural day with supplementary light of approximately 80 f.c. at plant level given from 4 a.m. to sunrise and sunset to 8 p.m. The short daylength was achieved by lowering the plants after 8 hours of daylight into a bay and covering it with a black light proof curtain. The difference in temperature in the long day and short day treatments did not vary more than 1°C (pers. comm. Hicks).

3.1.5 The Shadings

There are three levels of shading.

Shading I is the light intensity obtained in the glasshouse. Measurements (see Appendix IIa) indicated that the light in the glasshouse was about 77% of full daylight. For convenience in this investigation this level is referred to as 'full' daylight. The other two levels of shadings were obtained by means of two types of Sarlon material placed on light metal frames over the plants in the glasshouse.

The transmission levels of the two materials for Shadings 2 and 3 were measured by two methods. EEL Lightmaster with a self contained galvanometer calibrated in f.c. was used to provide a relationship with photometric units, while a Eppley pyrheliometer calibrated in cal/cm^2/day provided a relationship with energy units.

Using the EEL Lightmaster, measurements were made at different times during the day. A check reading was taken with the recording head exposed to "full" daylight and after pushing the recording head carefully under the material at the level of the plant leaves a second reading was taken. The readings are shown in Appendix IIb.

Readings recorded by the Eppley pyrheliometer at plant leaf level over one day is presented in Appendix IIb.

Comparison between readings of the EEL Lightmaster and the Eppley pyrheliometer showed that transmission in Shadings 2 and 3 determined by the two methods did not differ much. The transmission in the Shadings 2 and 3 based on the EEL Lightmaster was 52.7% and 42.8% respectively. Based on the Eppley pyrheliometer the corresponding figure was 51.0% and 42.1%.

The means of the readings from both methods were taken to be a good estimate of the transmission in Shadings 2 and 3, which were 51.9% and 42.4% respectively.

For use in this study, these figures were rounded off and referred to as 52% and 42% and the logarithm conversion were based on these latter figures.

Temperature variations in the shade were determined by measuring the soil temperature at different times of the day over a few days. At no time did the temperature vary more than 3°C in the different shadings (see Appendix III). This level of variation was not considered an important source of error in these investigations.

3.2 CULTURAL METHODS

3.2.1 Growing of the Plant

The seeds were sown in seed boxes in 3:1 peat and sand mixture respectively. After about 10 weeks when the seedlings attained 4-5 emerged leaves they were transplanted to 3-inch P.V.C. square pots containing well washed river sand. After about a fortnight in the propagating house the plants were assigned to treatments. They were matched into groups visually, the number depending on the experiment and each group was assigned to its treatment. Plants that were not used immediately were maintained in a vegetative state by keeping them in 8 hour daylength.

3.2.2 Nutrient

Nutrient used in all the experiments was the nitrate-type nutrient solution described by Hewitt (1966) with a few modifications. The high nitrogen nutrient solution containing 250 ppm N was obtained by the addition of NH_4NO_3 to Hewitt's nutrient solution. Calcium was supplied by $CaCl_2$ instead of $Ca(NO_3)_2$. The low nitrogen nutrient solution containing 57 ppm N was achieved by replacing $Ca(NO_3)_2$ with $CaCl_2$. In both solutions iron chelate was used instead of iron citrate. Because of the large volumes required, solutions were made up with tap water. The pH of the two solutions was 6.5. The composition of the two nutrient solutions is shown in Appendix IV.

3.2.3 Watering

The plants were watered on alternate days with their respective nutrient solutions. Enough solution was used to allow a small loss to drainage. Water alone was applied on other days when necessary to provide adequate moisture for the plants. Once a week water alone was used to leach accumulated salts.

CHAPTER IV. EFFECTS OF LIGHT INTENSITY AND NITROGEN ON E. VESICULOSUM.

4.1. ANATOMY AND MORPHOLOGY

4.1.1. Experimental Design

The experiment was designed to study the effects of different light intensity and nitrogen levels on leaf anatomy and morphology and involved eight blocks of a split-plot design. Each block was divided into three plots (main plots) of light intensity treatments (full, 52% and 42% daylight). The nitrogen treatments which were low (57 ppm N) and high (250 ppm N) were randomised within the main plots. Each subplot was a single pot-plant.

Four blocks were allocated for the determination of cell size, cell number, chlorophyll content and stomatal characteristics. The other four blocks were used for the determination of leaf shape.

4.1.2. Experimental Methods

4.1.2.1. Determination of Total Cell Number

The determination of cell numbers was by a maceration technique based on that developed by Brown & Rickless (1949). Samples for cell number determination consisted of two discs cut out of the fully expanded leaves with a known diameter cork borer. All samples were taken from leaves of corresponding age, i.e. leaf number 10. The discs were immersed in 4 ml. of 5% W/V solution of chromic acid and left overnight at room temperature. Next day they were broken up by shaking and finally completely macerated by drawing up and squirting back into the fluid approximately 150 times.with a pipette until the suspension was homogenous to the naked eye. In all cases this procedure was sufficient to ensure that most of the cells were separated and clumps did not contain more than half a dozen cells.

The number of cells in an aliquot of suspension was determined by using Fuchs Rosenthal haemocytometer with a griddled area of 9 $mm.^2$ and a depth of 0.2 mm., giving a volume of 1.8 $mm.^3$. The means of four aliquots of each suspension was used to calculate the total number of cells in the original solution. The suspension was shaken before each aliquot was taken by a pipette. The result was expressed as total number of cells per unit leaf area ($/mm.^2$). Total number of cells used in this text is defined as total number of epidermal and mesophyll cells.

4.1.2.2. <u>Preparation of Epidermal Strips</u>

The epidermal strips were prepared from fully expanded leaves of similar age. The leaves were blanched in boiling water for about 30 seconds and this treatment softened the tissues so that the epidermis could be peeled off with fine forceps. The adaxial epidermis was used because it was easier to peel than the abaxial.

The epidermal peels were washed in distilled water, stained in Harris haemotoxylin for 20 minutes, blued with tap water, washed with distilled water, dehydrated with 70%, 95%, 100% ethyl alcohol, cleared and mounted in Xam. The prepared slides were used for the determinations of cell size, stomatal size and frequency. For each plant one epidermal strip was prepared.

4.1.2.3. <u>Determination of Epidermal Cell Size</u>

Determination of cell size was made from measurements of epidermal cells using the prepared slides.

Three randomly sampled fields (avoiding the main vein) from each slide were photographed at X400. Photographs were used to estimate the cell area, Cell area was determined by measuring the area of groups of four cells by planimeter. The mean of three readings were taken in each case. Five groups of four cells were determined for each sample. Cell area was calculated knowing the magnification of the photographs. In all cases stomata were excluded in the measurements. The area was measured in square microns (μ^2).

4.1.2.4. Determination of Stomatal Frequency

Stomatal frequency was determined from the prepared slides of the epidermal strips.

Frequency counts were made with an X9 eyepiece and X45 objective giving a round field area of 0.038mm^2. Six randomly sampled fields per slide were counted. In all cases the main vein was avoided. Any incomplete stoma . in the field was counted as a whole stoma. The result was expressed in number of stomata per mm^2.

4.1.2.5. Determination of Stomatal Size

Stomatal length and breadth were determined from the prepared slides of the epidermal strips.

Ten stomata were measured at random using a microscope fitted with an ocular micrometer. Measurement was in microns (μ).

4.1.2.6. Determination of Chlorophyll Content

Since chlorophyll in detached leaves degrades quite rapidly when exposed to light at normal room temperature (Bray, 1960) plants in their pots were taken into the laboratory from the glass-house. The leaves were left intact on the plants until required for determination.

Samples of chlorophyll determination consisted of four discs cut with a known diameter cork borer of the fully expanded terminal leaves. The samples were always taken from the same region of each leaf i.e. at the centre of the leaf and on each side of the main vein. The weight of the discs was determined by weighing.

Chlorophyll from the samples was extracted by blending the discs in a MSE homogeniser for 8 minutes in a 4/1 (by volume) mixture of acetone and alcohol. The extract was transferred to a graduated centrifuge tube and its volume made up to 10ml.. The extract was centrifuged for about five minutes in a BTL Bench Centrifuge run at approximately 3000 r.p.m. In all cases 8 minutes was sufficient to blend the leaf discs and 5 minutes was adequate to clear the supernatant. A 10 mm. cell was filled with the supernatant and the optical density determined at wavelengths 645 mμ and 663 mμ in a Hitachi 101 spectrophotometer.

The amount of chlorophyll in the sample was calculated as:

$$\text{Chlorophyll a} = (12.7\ D\ 663 - 2.69\ D\ 645)\ \frac{V}{1000}\ \text{mg}$$

$$\text{Chlorophyll b} = (22.9\ D\ 645 - 4.68\ D\ 663)\ \frac{V}{1000}\ \text{mg}$$

$$\text{Total Chlorophyll} = (20.2\ D\ 645 + 8.02\ D\ 663)\ \frac{V}{1000}\ \text{mg}$$

where D is the optical density at the wavelength indicated and V is the volume of extract (Arnon, 1949). The result was expressed in chlorophyll content on unit leaf area basis ($mg./cm.^2$) and chlorophyll content on unit fresh leaf weight basis (mg./mg.).

The determination of chlorophyll a and b in a mixture by spectrophotometry assumes that neither pigment influences the specific absorption of the other. Van Norman (1957) found spectrophotometer method was less reliable when either chlorophyll a or b was about five times more concentrated than the other. In this investigation the ratio of chlorophyll a and b did not increase above 2 and this was not considered as an important source of error.

4.1.2.7. Determination of Leaf Size and Leaf Shape

Leaf size and leaf shape were taken on leaf number 8 to leaf number 12 inclusive. These leaves were detached from the plants and blue printed on Ammo-Positive Process Paper. The outlines of these leaves were used for the measurement of leaf length and breadth in cm.

The leaf length was measured from the tip of the lamina to the base of the petiole. The breadth was measured at the widest breadth of the lamina. In deeply toothed leaves, a line was drawn joining all the bases of the teeth before the widest breadth was measured, as illustrated in Diagram 4.1.

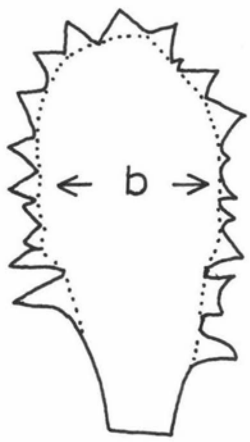


Diagram 4.1. Dotted line joins all the bases of the teeth before the widest breadth (b) was determined.

The length:breadth ratio was used to express the leaf shape. A set of photographs was also used to illustrate the leaf shape.

4.1.2.8. Analysis of Data

The experiment was a randomised split-plot design with three levels of light intensity, two levels of nitrogen and replicated four times. A standard form of analysis of variance was carried out for the split-plot design following Snedecor and Cochran (1968) To establish which mean differs significantly from other mean least significant difference test (LSD) was employed. Coefficient of variation was calculated for each analysis. An example of the method used is presented in detail in Appendix V.

4.1.3. Results

The means and standard errors of the raw data are in Appendix VII and the summaries of the analysis of variance are in Appendix VI. The light intensity x nitrogen interactions were not significant and will not be presented.

4.1.3.1. Total Cell Number and Epidermal Cell Size

The effects of nitrogen on total cell number and epidermal cell size were not statistically significant as shown in Table I.

a. Light intensity treatment

The effects of light intensity on total cell number and epidermal cell size are presented in Table I. The treatment had a statistically significant effect on total cell number ($P < 0.01$) and epidermal cell size ($P < 0.05$). It is evident from Table I that total cell number increased and epidermal cell size decreased. with increasing light intensity. Between the 52% and 42% daylight the difference was not statistically significant for total cell number and epidermal cell size. The effect of light intensity on epidermal cell size is illustrated in Plate VIII.

TABLE I

The main effects of light intensity and nitrogen on total cell number and epidermal cell size.

	Light intensity			Result	Nitrogen		Result
Character	Full	52%	42%	(5%LSD)	Low	High	
Total cell number (/mm^2)	6107	4954	4987	**+ (629)	5272	5428	n.s
Epidermal cell size (μ^2)	1085	1275	1302	* (174)	1228	1213	n.s

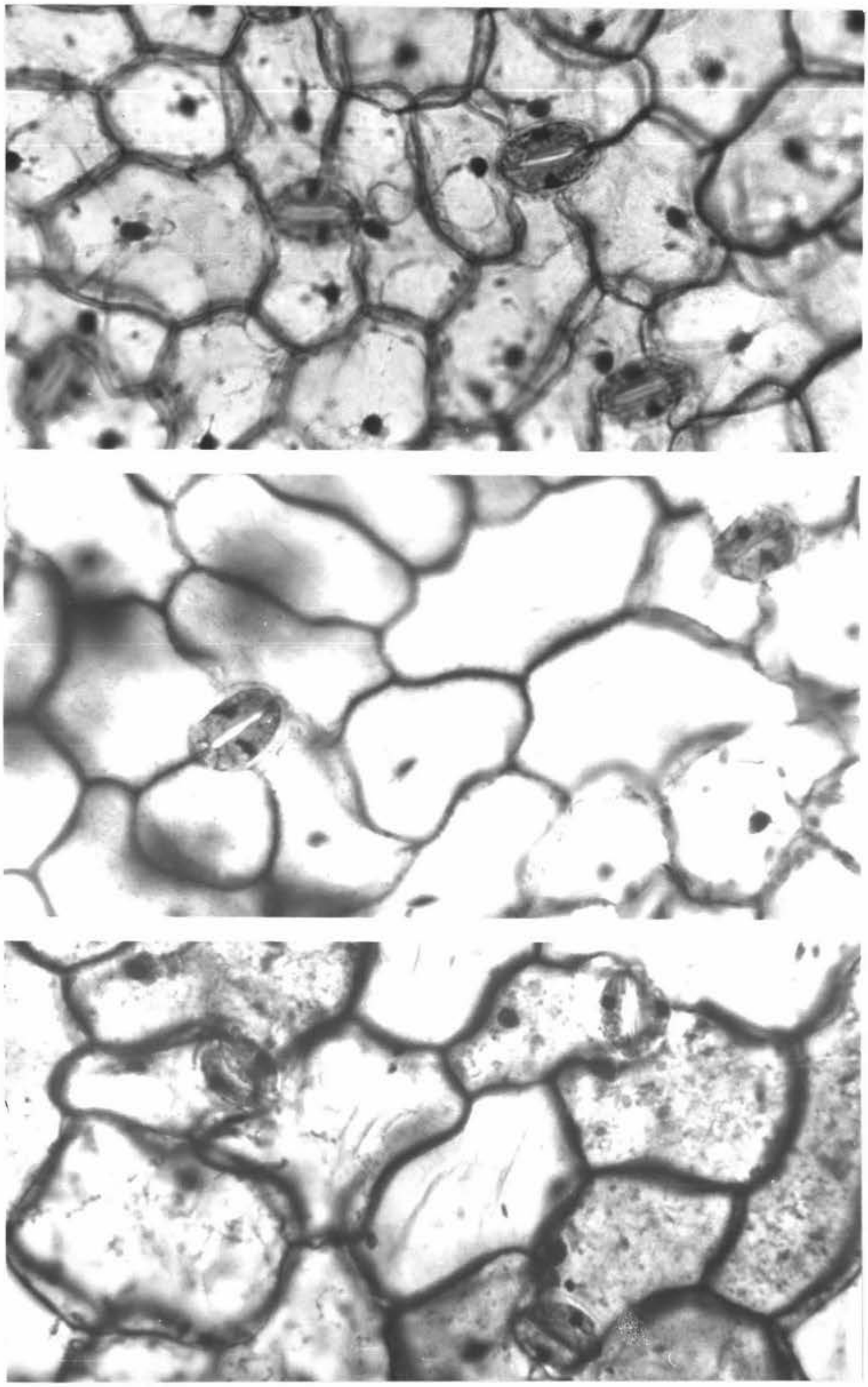
+ n.s P 0.05; * P 0.05; ** P 0.01. These notations will be used in the rest of the text.

4.1.3.2. Stomatal Characteristics

Increasing the nitrogen level from 57 p.p.m. to 250 p.p.m. had no significant effects on the stomatal length, the breadth and the frequency (Table II).

a. Light intensity treatment

Analysis of stomatal characteristics over different light intensities reveals a statistical difference in stomatal length ($P < 0.05$) and in stomatal frequency ($P < 0.05$) but not in stomatal breadth. Stomata from plants grown in full daylight were longer and more per unit area than those from plants grown in lower light intensities as shown in Table II. There was no statistically significant difference in stomatal length and frequency in 52% and 42% daylight.



(a)

(b)

(c)

Plate VIII. The effects of light intensity on upper epidermal cell size. (a) under full daylight, (b) under 52% daylight, (c) under 42% daylight (all X 570)

TABLE II

The main effects of light intensity and nitrogen on stomatal characterisitics.

	Light intensity			Result	Nitrogen		Result
Character	Full	52%	42%	(5%LSD)	Low	High	
Stomatal length (µ)	28.48	27.79	27.10	* (1.00)	27.63	27.96	n.s.
Stomatal breadth (µ)	18.92	17.48	17.48	n.s.	18.01	17.91	n.s.
Stomatal frequency ($/mm^2$)	378	299	320	* (44)	342	323	n.s.

4.1.3.3. Leaf Size and Leaf Shape

a. Light intensity treatment

Analysis of variance reveals that the treatment had statistically significant effects on leaf length ($P < 0.05$) and leaf length:leaf breadth ratio ($P < 0.01$) but had no significant effect on leaf breadth. Leaves from plants in full daylight were shorter and their length:breadth ratios were smaller than those from plants in lower light intensities (Table III). Between the lower light intensities the difference was statistically significant for the length:breadth ratio but not for the leaf length. The treatment effects are illustrated in Plate IX. It can be seen in Plate IX that low light intensity retarded the formation of dissected leaves. Leaf number 8 to leaf number 12 from plants grown under full daylight were more dissected than those from plants grown under 52% and 42% daylight.

TABLE III

The main effects of light intensity and nitrogen on leaf size and leaf shape.

Character	Light intensity Full	52%	42%	Result (5%LSD)	Nitrogen Low	High	Result
Leaf length (cm)	10.0	13.2	16.5	* (3.8)	13.0	13.6	n.s.
Leaf breadth (cm)	.60	.60	.65	n.s.	.60	.63	n.s.
Leaf length: breadth ratio	16.8	22.4	26.6	** (3.7)	21.8	22.8	n.s.

b. Nitrogen treatment

There was a trend that the high nitrogen level increased the leaf length, the breadth and the length:breadth ratio although the effect did not reach the 5% level of significance. The treatment appeared to have a slight influence on the degree of dissection of the leaves; low nitrogen tended to produce less dissected leaves (Plate IX).

4.1.3.4. Chlorophyll Contents.

a. Light intensity treatment

The treatment had no statistically significant effects on chlorophyll contents measured on a unit fresh leaf weight basis but had statistically significant effects on chlorophyll contents measured on a unit leaf area basis. It is evident from Table IVa that chlorophyll contents on a unit leaf area basis were higher

Plate IX (a) Full daylight

Plate IX a - c <u>The effects of light intensity and nitrogen levels on leaf size and leaf shape.</u>

(leaf number 8 to leaf number 12 from left to right)

LN = Low nitrogen HN = High nitrogen

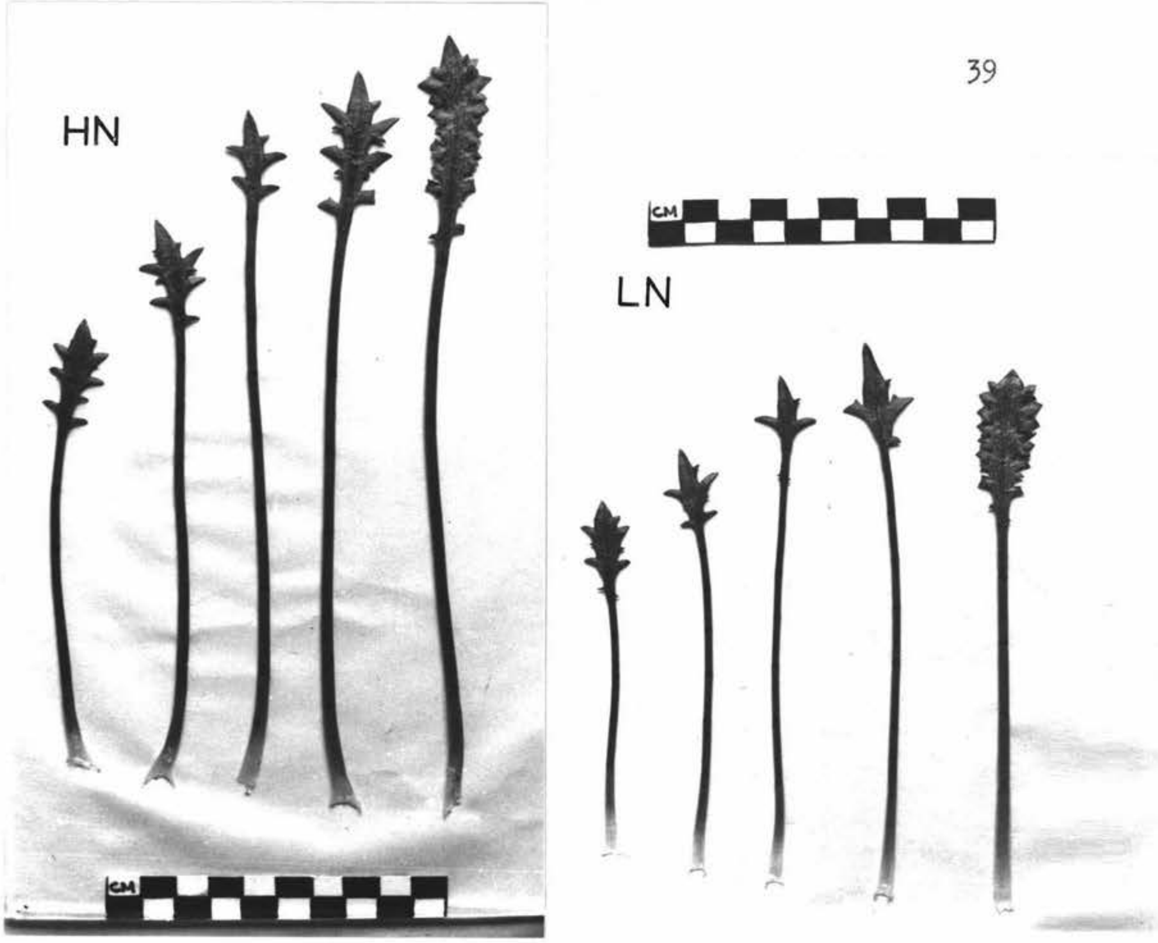


Plate IX (b). 52% daylight

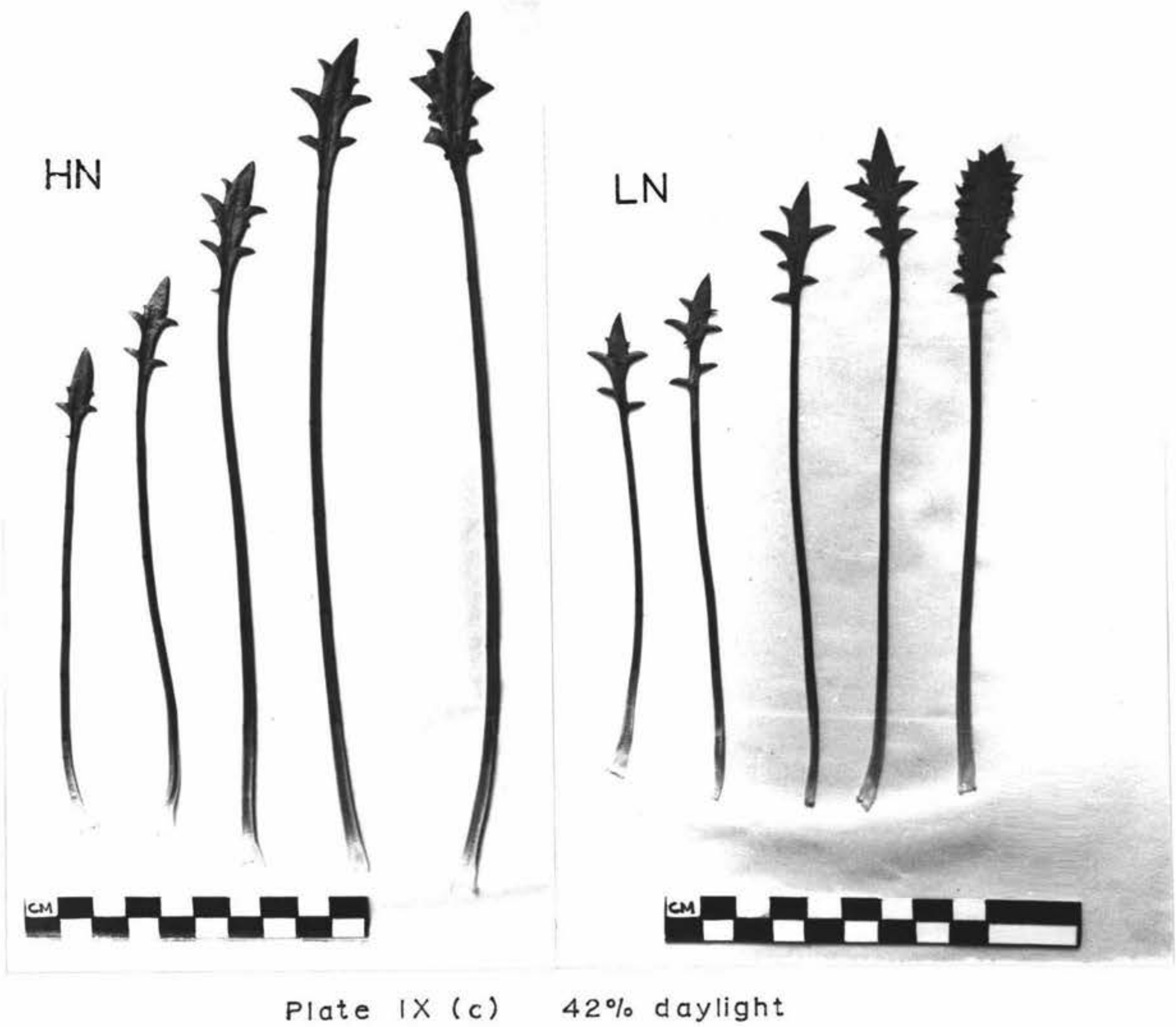


Plate IX (c) 42% daylight

from leaves in full daylight than from leaves in the lower light intensities. Only total chlorophyll and chlorophyll a were statistically significant ($P < 0.05$). Though the effects of light intensity on chlorophyll contents on a unit fresh leaf weight basis were not significant, a regular trend was observed in that the full daylight yielded a lower chlorophyll content than the lower light intensities. The treatment had no statistically significant effect on the chlorophyll a/b ratio.

TABLE IVa

The effects of light intensity on chlorophyll contents

	Light Intensity			Result
Character	Full	52%	42%	(5% LSD)
Chlorophyl a/b ratio	1.49	1.47	1.47	n.s.
Contents on unit leaf area basis (mg x 10^{-1}/cm^2)				
Total chlorophyll	.673	.619	.578	(.067)*
Chlorophyll a	.402	.368	.344	(.037)*
Chlorophyll b	.271	.251	.234	n.s.
Contents on unit fresh leaf weight basis (mg x 10^{-4}/mg)				
Total chlorophyll	13.07	13.60	13.41	n.s.
Chlorophyll a	7.79	8.09	8.14	n.s.
Chlorophyll b	5.28	5.51	5.27	n.s.

b. Nitrogen treatment

The treatment had statistically significant effects on chlorophyll contents on a unit leaf area basis ($P < 0.05$) and on the chlorophyll contents on a unit fresh leaf weight basis ($P < 0.01$). Leaves from plants of high nitrogen had more total chlorophyll, chlorophyll a and chlorophyll b on a unit fresh leaf basis and on a unit leaf area basis than those from plants of low nitrogen (Table IVb). Plants of high nitrogen had dark green leaves while those of low nitrogen had pale green leaves. The chlorophyll a/b ratio was not statistically significantly affected by increasing the nitrogen level from 57 ppm to 250 ppm.

TABLE IVb

The effects of nitrogen on chlorophyll contents

Character	Nitrogen Low	Nitrogen High	Result (5% LSD)
Chlorophyll a/b ratio	1.49	1.46	n.s.
Contents on unit leaf area basis ($mg \times 10^{-1}/cm^2$)			
Total chlorophyll	.585	.661	(.054)*
Chlorophyll a	.350	.392	(.032)*
Chlorophyll b	.235	.269	(.022)**
Contents on unit fresh leaf weight basis ($mg \times 10^{-4}/mg$)			
Total chlorophyll	11.82	14.90	(1.34)**
Chlorophyll a	7.06	8.96	(.83)**
Chlorophyll b	4.76	5.94	(.71)**

4.1.4. Discussion

The results of this study generally confirm the results and observations by several investigators (refer review of literature) on the effects of light intensity and nitrogen on leaf anatomy and morphology. Shading affects the epidermal cell size and total cell number (epidermal plus mesophyll cells) per unit leaf area in leaves of E. vesiculosum. The trend was for shading to increase epidermal cell area and to decrease total cell number per unit leaf area (Table I). Shading the plants in 42% daylight induced an increment in cell area of up to 20%. The result agrees with the data of Njoku (1956) and Forde (1966). Working on the adaxial epidermis of leaves of Ipomoea caerulea under five different light intensities Njoku found that cell area was largest at 74% daylight and that cell area at the other intensities (56%, 28% and 23% daylight) were intermediate between 75% and 100% daylight. Of closer agreement are the results of Forde who studied the effects of 100%, 70% and 20% of daylight on Lolium perenne and Dactylis glomerata. He reported that the abaxial epidermal cell area was increased by 95% for L. perenne and 54% for D. glomerata by shading to 20% daylight. His higher increase in cell area by shading compared to the result of this study could have been in part due to the different species of plants he had used and also partly due to his heavier shading.

Only the epidermal cell area was measured here. Considering the number of epidermal cells per unit leaf area can be estimated assuming that there is no great variation of cell size within the epidermis, it follows then that the effect of light intensity on cell number per unit leaf area would show an opposite trend to that observed for cell size, i.e. the shading decreases epidermal cell number per unit leaf area. This is in agreement with the findings of Njoku (1956) for I. caerulae.

In the study of the effects of cell size and cell number on leaf size several investigators (Ashby, 1948; Ashby & Wangermann, 1950; and Forde, 1966) measured the epidermal cells only. As the epidermal cells apparently ceased dividing before other cells of the leaf, observation of epidermal cells only suggest possible trends (Milthorpe & Newton, 1963) and thus counting all the cells in the leaf would present a more accurate method. For this reason the total cell number per unit leaf area was also determined in this study and was found to increase with increasing light intensity. This is in accord with the results of Humphries & Wheeler (1960) who studied the effects of light and darkness on cell division and cell expansion. Cell size was also determined in their experiment but not in this study. In their determination of cell size they assumed that the number of cells in the transverse direction remained constant. This assumption may not be true as several investigators (Hughes, 1959: Cormack & Gorham, 1953; Cameron, 1969) have observed from transverse leaf sections that sun leaves are thicker and have more palisade layers than shade leaves. It would be a fair assumption, from the deduction made from these anatomical differences, that total cell number per unit leaf area in sun leaves would be greater than that in shade leaves. Further, Wilson & Cooper (1969) found that shading decreased mesophyll cell number per unit leaf area.

E. vesiculosum had a greater number of stomata per unit leaf area when grown in the full daylight than in the 52% and 42% daylight (Table II). This result is in harmony with the findings of

Bjorkman & Holmgren (1963) on Solidago virgaurea, of Cooper & Qualls (1967) on Medicago sativa and Lotus corniculata, of Cormack & Gorham (1953) on Menziesia glabella and Lonicera glaucesens, of Friend & Pomeroy (1970) on Marquis and Kharkov wheat, but is in variance with the data of Penfound (1931) on Helianthus, of Wilson & Cooper (1969) on Lolium and of Cormack (1955) on Vicea americana. The stomatal length of E. vesiculosum was also affected by light intensity, being longer in sun leaves than in shade leaves, this was also reported by Wilson & Cooper (1969) on Lolium.

The formative effect of light intensity on leaf shape was similar to that reported by other investigators (refer review of literature). Plate IX shows the effect of light intensity through its effect on simplification of leaf shape. Leaves in full daylight were more dissected than those in lower light intensities. The leaf length:breadth ratio which is an expression of leaf shape was greater in shade leaves than in sun leaves; this was a result of a significant increase in leaf length but not in leaf breadth as a consequence of shading (Table III). Similar observations were reported by Talbert & Holch (1957), Njoku (1955), Bensink (1960) and Sanchez (1967) on different plants.

The view that under light a substance or substances responsible for the leaf shape is or are produced has been expressed by several investigators (e.g. Aberg, 1943). Aberg attributed the increase in the increase in the leaf length:breadth ratio with progressively lower light intensities in Lobelia dortmana to the carbohydrate balance which is affected by light intensity. Growth substances were also suggested to be responsible for the changes in leaf shape by several investigators (Njoku, 1958; Scurfield & Moore, 1958; Robbins, 1957, 1960). Njoku obtained a prolongation of the juvenile stage in which the leaves were entire to the adult stage in which the leaves were tri-lobed in I. caerulae with gibberellic acid and various other growth substances (e.g. Indole-3-acetic acid, α-naphthylacetic acid and 2.3,5 triiodobenzoic acid). Similar result was obtained by Robbins on Hedera but in Hedera it was the juvenile leaf that was lobed while the adult form was entire. Scurfield & Moore however found that gibberellic acid treated plants developed the adult type of foliage earlier than did the control plants. Allsopp (1965) in his review discussed the hormonal action on leaf shape and concluded that the observed changes in leaf form could be probably explained by the changes in carbohydrate level. In the gibberellic acid treatment the carbohydrate decreased because of the increased growth with consequent reduction in available sugar. His contention was supported by his experiments with aspetically cultured Marsilea drummondii in which the external supply of glucose has found to influence the leaf shape by increasing leaf segmentation. The carbohydrate balance could be a mechanism by which the leaf shape in E. vesiculosum is controlled. Shading reduced photosynthesis with consequent reduction in the amount of carbohydrate which in turn affected the leaf shape. Without further investigations the possibility of hormonal effects in leaf shape cannot be entirely neglected.

The study did not detect any significant effects of nitrogen level on the characters so far discussed. Arney (1952) working on marrow-stem kale also found that nitrogen level has practically no effect on epidermal cell size, thickness of palisade and mesophyll and stomatal frequency. In contrast the work of Morton and Watson (1948) showed that nitrogen increased cell number and also cell size of the palisade mesophyll. The leaf length, the breadth and the length:breadth ratio of leaves in high nitrogen were greater than those in low nitrogen although the statistical analysis revealed that these differences were not significant. Great variation in the above discussed characters may be possible from plant to plant and which may have a role in producing the insignificant difference. Another possibility may be that the two nitrogen levels used may not be adequately different for their effects to be detected statistically.

In the study of light intensity on chlorophyll contents the analysis of variance showed a statistically significant block effect. This has much to do with the procedure adopted for the determination of chlorophyll. Sampling was done throughout the day and error could have been introduced by this procedure as diurnal variations in chlorophyll content have been reported on some plants by Henrici (1926), by Bukatsch (1939) and by Bavrina (1959). This procedure may also introduce a further error. Different amounts of tissue are likely to be taken with the same cork-borer in comparing samples taken in the morning with those taken say at noon because of possible changes in water content. This error would bias the chlorophyll content result when expressed in unit fresh leaf weight basis.

Leaves of E. vesiculosum in full daylight had more chlorophyll per unit leaf area and less chlorophyll per unit fresh leaf weight than those in the lower intensities (Table IVa). The results of Cooper & Qualls (1967) on Medicago sativa and Lotus corniculatus and of Cameron (1970) on Eucalyptus fastigata confirm the results of this study. However the results should be taken with some reservation because it is difficult to decide whether the treatment was directly changing the chlorophyll content or merely appearing to change it as a result of a change in leaf anatimy. For example if the chlorophyll content is expressed on unit leaf area basis, the chlorophyll content may have been higher with sun leaves because the leaves are likely to increase in thickness with increasing irradiance, in contrast if the chlorophyll content is expressed on unit fresh leaf weight basis the chlorophyll content may be lower with sun leaves because the leaves are likely to increase in weight with increasing irradiance. Chlorophyll a/b ratio was not affected by light intensity because chlorophyll a and b changed proportionately with varying light intensity.

As expected nitrogen had a significant effect on chlorophyll content as it is an essential element in the structure of the protochlorophyll and chlorophyll molecules. Leaves from high nitrogen had more total chlorophyll, chlorophyll a and chlorophyll b expressed in unit leaf area and unit fresh leaf weight basis (Table IVb). The result is compatible with the general observation that plants grown in high nitrogen have greener leaves than those grown in low nitrogen (Raper, 1966). Further evidence of the positive effect of nitrogen on chlorophyll content is shown by the work of Greig, Motes & Al-Tikrite (1968) on spinach. The chlorophyll a/b ratio was not affected by the nitrogen treatment. Both chlorophyll a and b were affected by high nitrogen in the same direction and in proportionate amounts.

The effect of shading on leaf morphology and anatomy may not be solely due to the direct effect of light. Other factors, like water supply and temperature may be involved (Shields, 1950). In this study the temperature was almost the same in the three light intensities (see Appendix III) and plants were kept moist at all times. Hence all differences between leaves from full daylight and from 52% and 42% daylight would most likely be due to the light intensity effect. But there are other factors either uncontrollable in this study or unknown, that may play a part in producing an effect. The difference in the levels of shadings of 52% and 42% daylight was probably not adequately marked for their effects to be statistically different at the 5% level of significance in most cases.

4.2. PLANT GROWTH

4.2.1. Experimental Design

The objective of the experiment was to study the effects of three levels of light intensity and two levels of nitrogen and their interactions on plant growth with respect to dry weights and other attributes of classical growth analysis at six harvests.

The experiment was laid out on eight blocks of a split-plot design similar to the one described in section 4.1.1. In this experiment eight blocks were taken at each harvest. When harvest was taken as an additional factor the design was considered as a split-split-plot with harvest as a sub-sub plot. Each sub-sub plot was a single pot plant. The interval between two consecutive harvests was ten days. Harvest 1 was the base harvest.

4.2.2. Experimental Methods

4.2.2.1. Determination of Dry Weights, Leaf Area and Leaf Number

The plant was removed from the pot by washing out the sand with a slow jet of water, any sand still left was washed through a sieve which retained the roots. This procedure reduced the loss of roots. The plant was then divided into leaves and roots. The very small stem was included in the roots. A sub-sample (all leaves from plants of four blocks) was taken for the estimation of leaf area and the individual parts dried in an oven at 180°F for 24 hours and weighed in mg. Dry weight of whole plant (total dry weight), of all leaves (leaf dry weight) and of all roots (root dry weight) were determined. Before being weighed the material was cooled and stored in a dessicator.

Outlines of the sub-sample of fresh leaves were blueprinted on Ammo-Positive Process Paper and the area subsequently determined with a planimeter. The mean of three readings was taken in each case. From the area and dry weight of the sub-sample the ratio of leaf area to leaf weight also termed specific leaf area was derived and the total leaf area of whole plant (leaf area) was estimated in cm^2 using the ratio. The specific leaf area in cm^2/mg for four blocks was used for statistical analysis. Leaf number in the four blocks was also counted for statistical analysis.

4.2.2.2. Derivation of Variables

Since at each harvest the dry weight of whole plant, leaves, roots and the leaf area were obtained, several derived variables were calculated. The variables obtained were specific leaf area (SLA), root:top ratio, instantaneous leaf area ratio (LAR), leaf weight ratio (LWR), mean leaf area ratio ($\overline{LAR}$), net assimilation rate (NAR) and relative growth rate (RGR). SLA is the leaf area/the dry weight of the leaves, root:top ratio is the dry weight of the root/the dry weight of the leaves, LWR is the dry weight of the leaves/the total dry weight of the plant and LAR is the leaf area/the total dry weight of the plant. The other three variables $\overline{LAR}$, NAR and RGR are more difficult to derive and several formulae are available for their derivation. Radford (1967) had cited necessary conditions for the use of the various formulae.

The method of Fisher (1921) was employed to calculate the RGR of the whole plant. The formula is $\frac{\text{Log}_e W_2 - \log_e W_1}{t_2 - t_1}$ where W_2 and W_1 are the dry weights per plant at t_2 and t_1, the second and first harvest respectively. This formula assumes that dry weight varies without discontinuity throughout the interval t_1 to t_2. This condition is met in this study.

For the NAR the formula $\frac{(W_2-W_1)\ (\log_e L_2 - \log_e L_1)}{(L_2-L_1)\ (t_2-t_1)}$ was used where L_2 and L_1 are the leaf area per plant at t_2 and t_1 the second and first harvest respectively. The formula assumes that plant weight and leaf area are lineally related if not it will lead to an over estimation of NAR. However the error involved will be small if the leaf area at the second harvest is not more than 2 times that at the first (Coombe, 1960) as in this experiment.

For the $\overline{LAR}$ the following formula was used:

$$\overline{LAR} = \frac{(\log_e W_2 - \log_e W_1)}{(W_2 - W_1)} \frac{(L_2 - L_1)}{(\log_e L_2 - \text{Log}_e L_1)}$$

This formula assumes that both leaf area and total plant weight increased exponentially. (Radford, 1967). This occurs during short growth intervals as in this study.

4.2.2.3. Analysis of Data

Transformation of the raw data was considered before the analysis of variance was performed. The mean to range ratios of the raw data were no more constant than the ranges themselves. This was taken to suggest that transformation was not important and the data were analysed without transformation. The analysis of the raw data was performed at each harvest separately as a split plot design (see section 4.1.2.8.). In cases where harvest was considered as an additional factor its analysis of variance was performed as a split-split plot design following Federer (1955). Least significant difference test was employed to establish which mean differs significantly from other mean. Coefficient of variation was calculated for each analysis. The formula used is shown in Appendix V. All the data were analysed in terms of per unit plant.

4.2.3. Results

The results are expressed in the main body of the text either in graphic forms or in tables with their appropriate least significant difference. The summaries of the analysis of variance for all the data are in Appendix VIII. The means and standard error of the raw data are in Appendix IX.

4.2.3.1. Changes in Dry Weight

a. Light intensity treatment

The dry weight curves of the whole plant at each harvest graphed for the whole experimental period are presented in Figure 4.1 and those of the parts in Figure 4.2. The treatment had significant effects on the total dry weight at harvests 2,3,4 and 6 ($P < 0.05$) and at harvest 5 ($P < 0.01$) and root dry weight at harvest 2 to 6 ($P < 0.01$) but had no significant effect on leaf dry weight. From the graphs it is evident that total dry weight and root dry weight of plants from full daylight were heavier than of those from lower light intensities. The differences in total dry weight and root dry weight of plants from full and 42% daylight were significantly different ($P < 0.05$) at all harvests. Between full and 52% daylight the differences were significant for root dry weight at all harvests but were not significant in total dry weight at any harvest. Even between the 52% and 42% daylight there were significant differences in root dry weight at harvests 4,5 and 6 and in total dry weight at harvest 3. A regular trend that leaf dry weight of plants from 52% daylight was the heaviest among the three light levels was observed at harvest 3 to 6 although the differences were not statistically significant. Typical responses of plant to the three light intensities are shown in plate X. The graphs show that total, leaf and root dry weights increased with time.

b. Nitrogen treatment

Figures 4.3. and 4.4. show the effects of two nitrogen levels on dry weights of whole plant, of leaves and of roots.

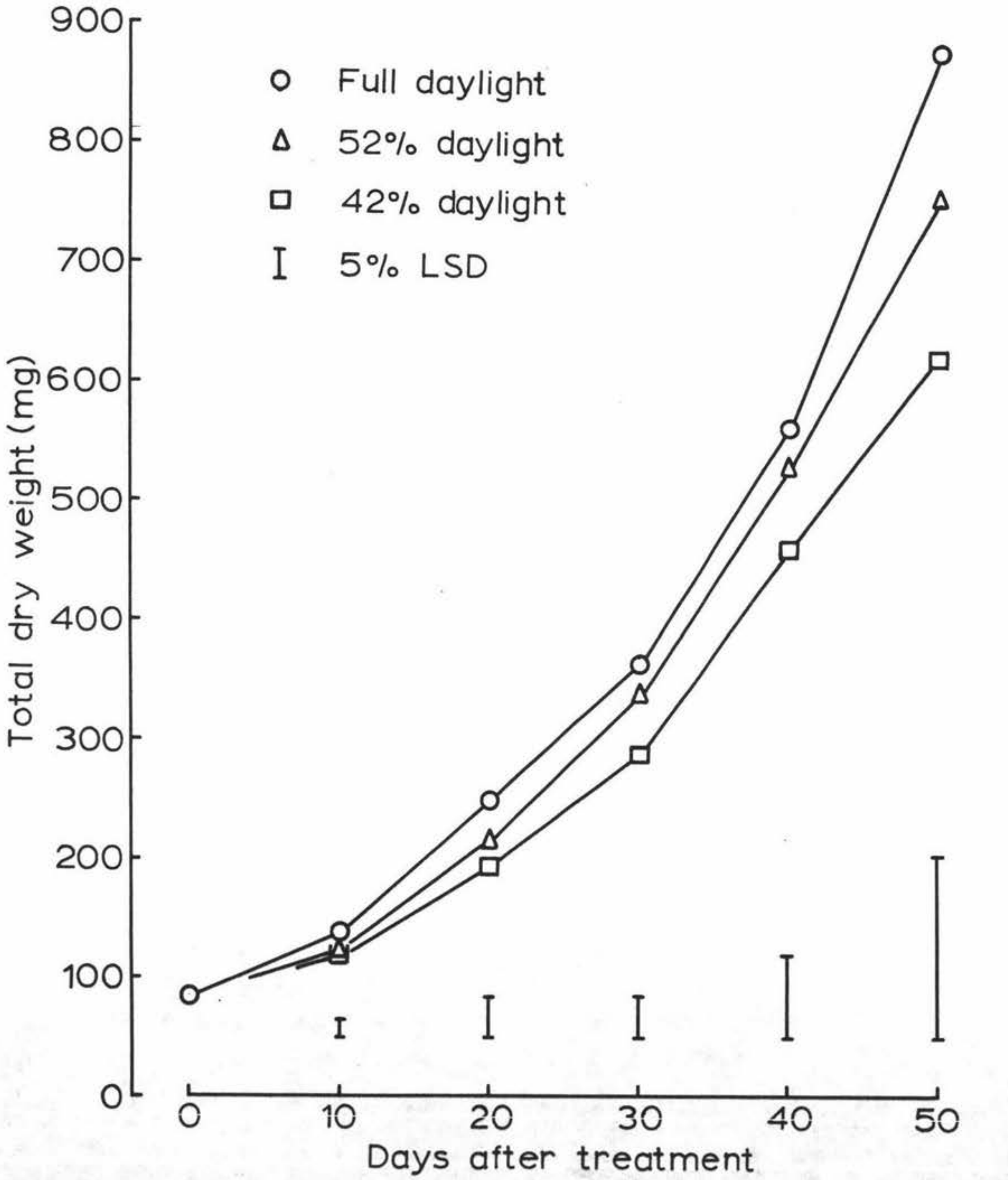


Fig.4.1. Effect of light intensity on the total dry weight.

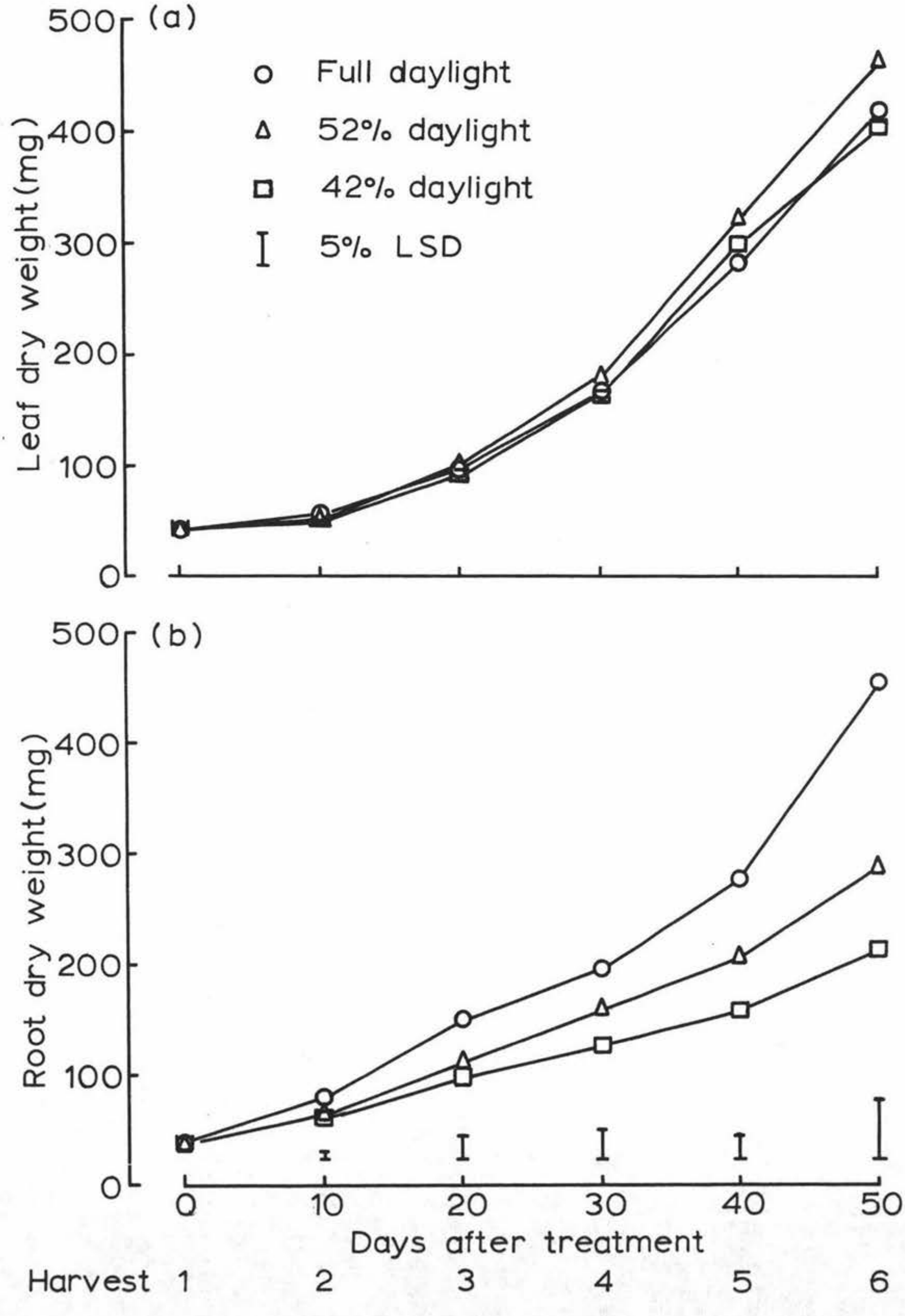
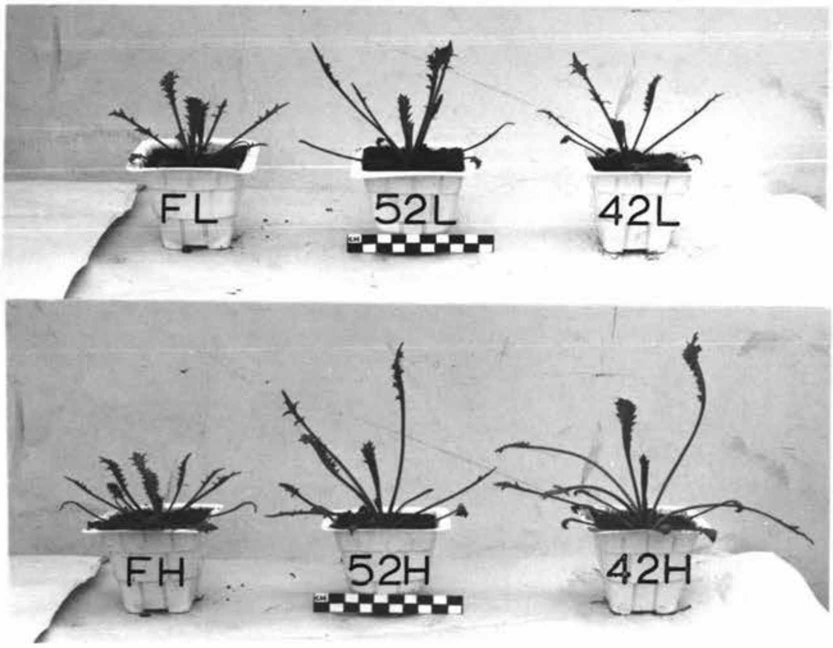
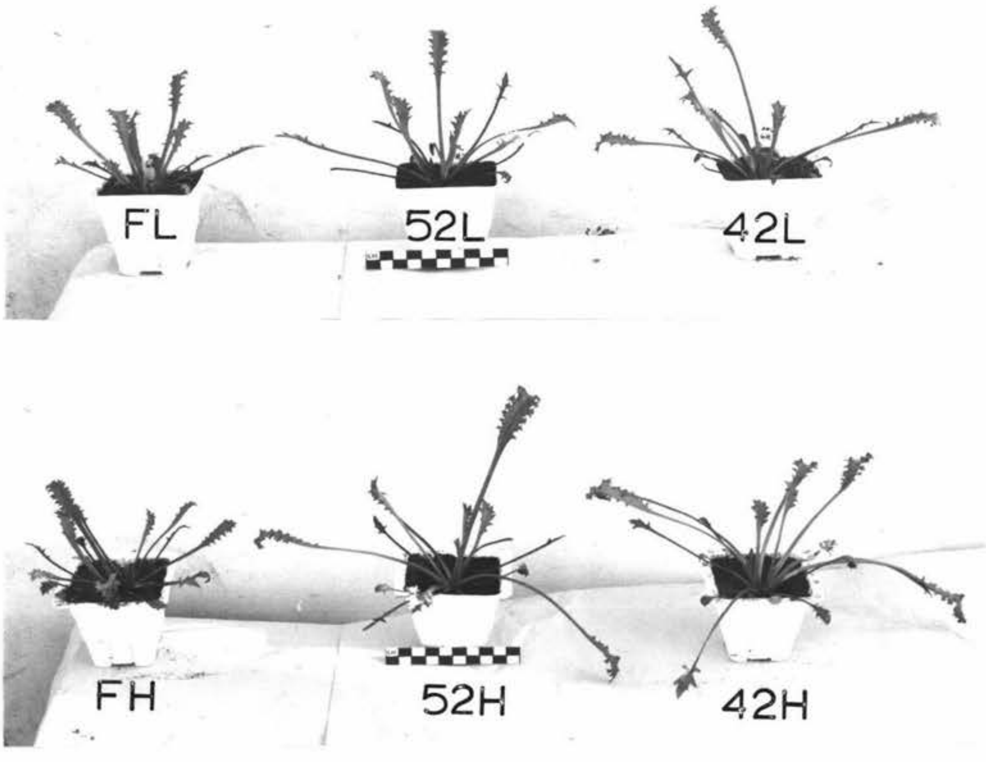


Fig.4.2. Effect of light intensity on (a) the leaf dry weight, and (b) the root dry weight.



(A)



(B)

Plate X. The effects of light intensity and nitrogen on plant growth at harvest 4 (A) and at harvest 6 (B).
F = Full daylight; 52 = 52% daylight; 42 = 42% daylight; L = Low N; H = High N.

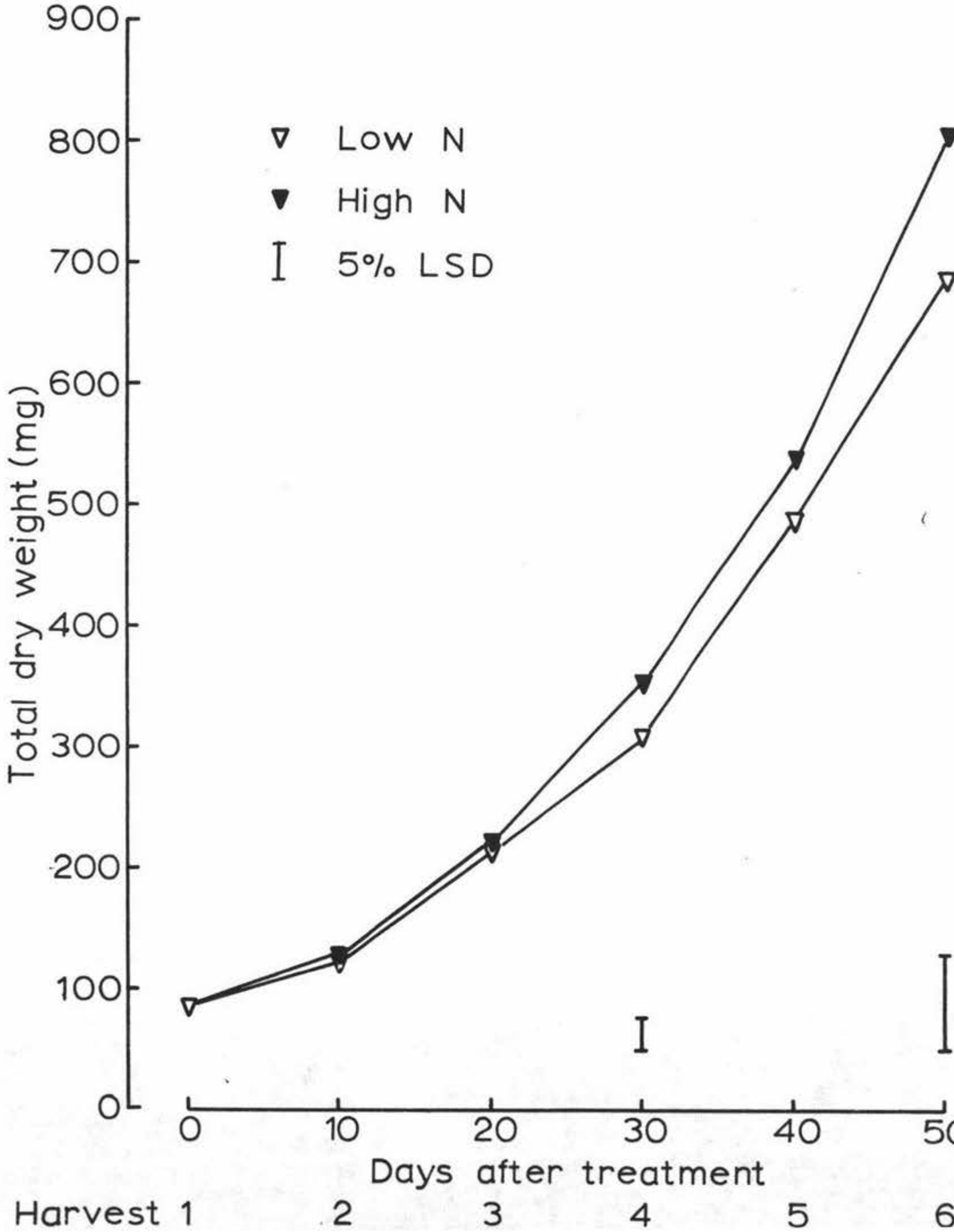


Fig.4.3. Effect of nitrogen on the total dry weight.

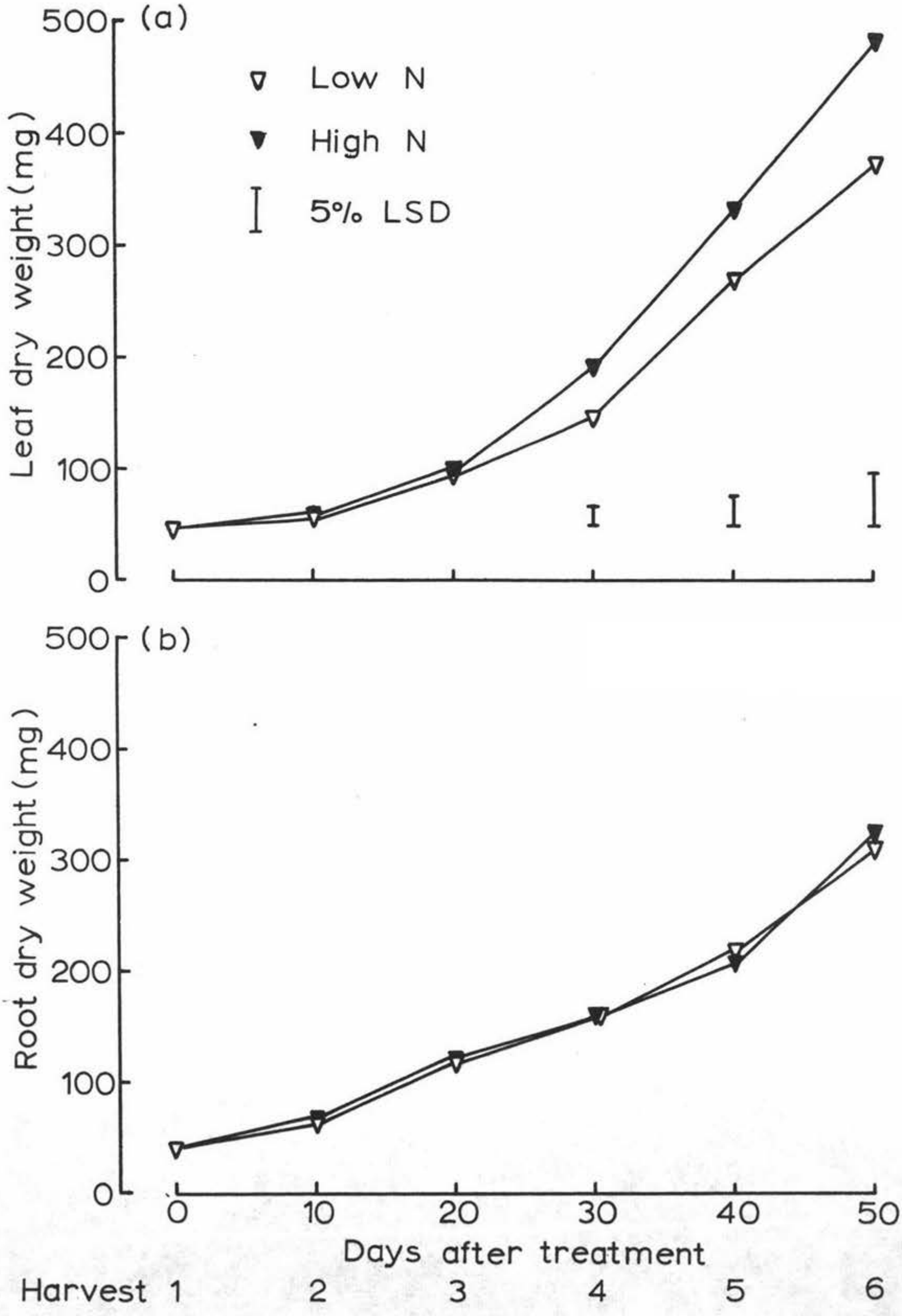


Fig.4.4. Effect of nitrogen on (a) the leaf dry weight, and (b) the root dry weight.

The treatment had significant effects on total dry weight at harvests 4 and 6 ($P < 0.01$) and on leaf dry weight at harvests 4, 5 and 6 ($P < 0.01$) but had no significant effect on root dry weight. Plate X shows the responses of the plant to nitrogen treatment. The graphs show that total dry weight and leaf dry weight were enhanced by high nitrogen. Although the result was not significant, heavier root dry weight associated with high nitrogen was observed.

c. Light intensity x nitrogen interaction

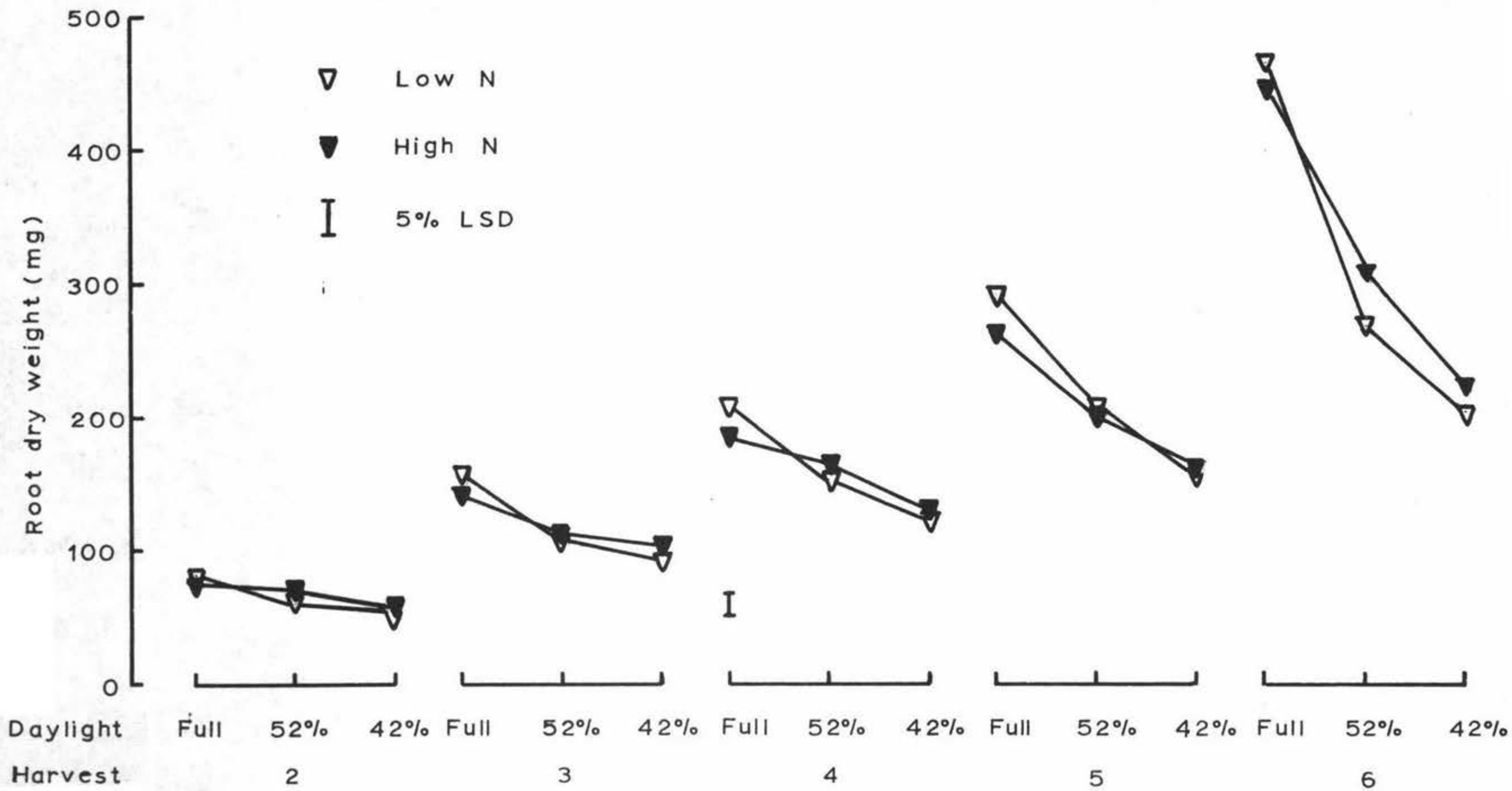
There was a significant interaction of light intensity and nitrogen on root dry weight at harvest 3. Under full daylight the root weight of plants grown in low nitrogen was significantly ($P < 0.05$) heavier than those grown in high nitrogen. Similar trends were observed at the other harvests (Figure 4.5). High and low nitrogen levels had little effect on the root dry weight of plants grown under low light intensities.

4.2.3.2. Changes in Leaf Area

a. Light intensity treatment

Light intensity had significant effects on leaf area at harvests 2,3, and 6 ($P < 0.05$) and at harvests 4 and 5 ($P < 0.01$) and on SLA at all harvests ($P < 0.01$). The curves in Figure 4.6 show that plants grown under full daylight had significantly smaller leaf area and SLA than those grown under 52% and 42% daylight at all harvests. Between the 52% and 42% daylight the difference in leaf area was not significant and the difference in SLA was significant at harvest 4 ($P < 0.05$).

Fig.4.5. Light intensity X nitrogen interaction on root dry weight



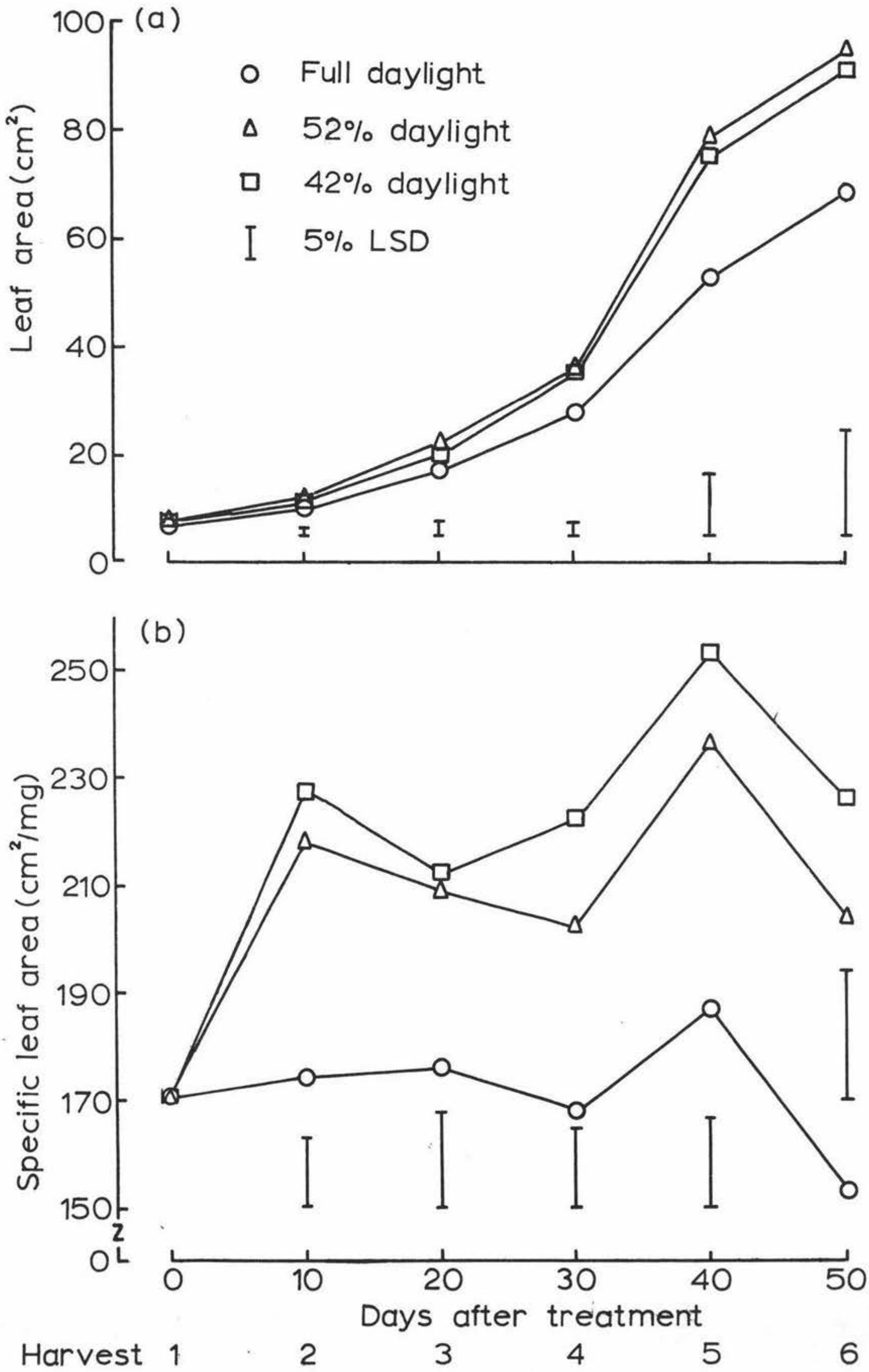


Fig.4.6. Effect of light intensity on (a) the leaf area and (b) the specific leaf area.

b. Nitrogen treatment

The treatment had no significant effect on SLA but had significant effect on leaf area at harvests 4, 5 and 6 ($P < 0.01$). Plants grown in high nitrogen had significantly larger leaf area than those grown in low nitrogen (Figure 4.7). Although at harvests 2 and 3 the treatment effect was not significant, high nitrogen associated with larger leaf area was observed.

c. Light intensity x nitrogen interaction

Apart from the result at the second harvest there was no significant interaction of light intensity and nitrogen on leaf area (Figure 4.8). At harvest 2 high nitrogen depressed the leaf area under full daylight but not under the lower intensities. At later harvests high nitrogen increased the leaf area under the three light levels. The interaction was also not significant on SLA. The results indicate that the influence of nitrogen levels on leaf area and SLA did not change with light intensities.

4.2.3.3. Changes in Dry Weight Proportions

a. Light intensity treatment

Light intensity had significant effects on root:top ratio ($P < 0.01$), LAR ($P < 0.01$) and LWR ($P < 0.01$) at all harvests as shown graphically in Figure 4.9. Plants from full daylight had higher root:top ratios than those from the lower light intensities. Between the 52% and 42% daylight the difference in the ratio was significant at harvests 5 and 6 but not at harvests 2, 3 and 4. The effects of the treatment on LAR and LWR were in contrast with that on root:top ratio. These two ratios decreased with increasing light intensity. The LARs of the plants grown in the three light

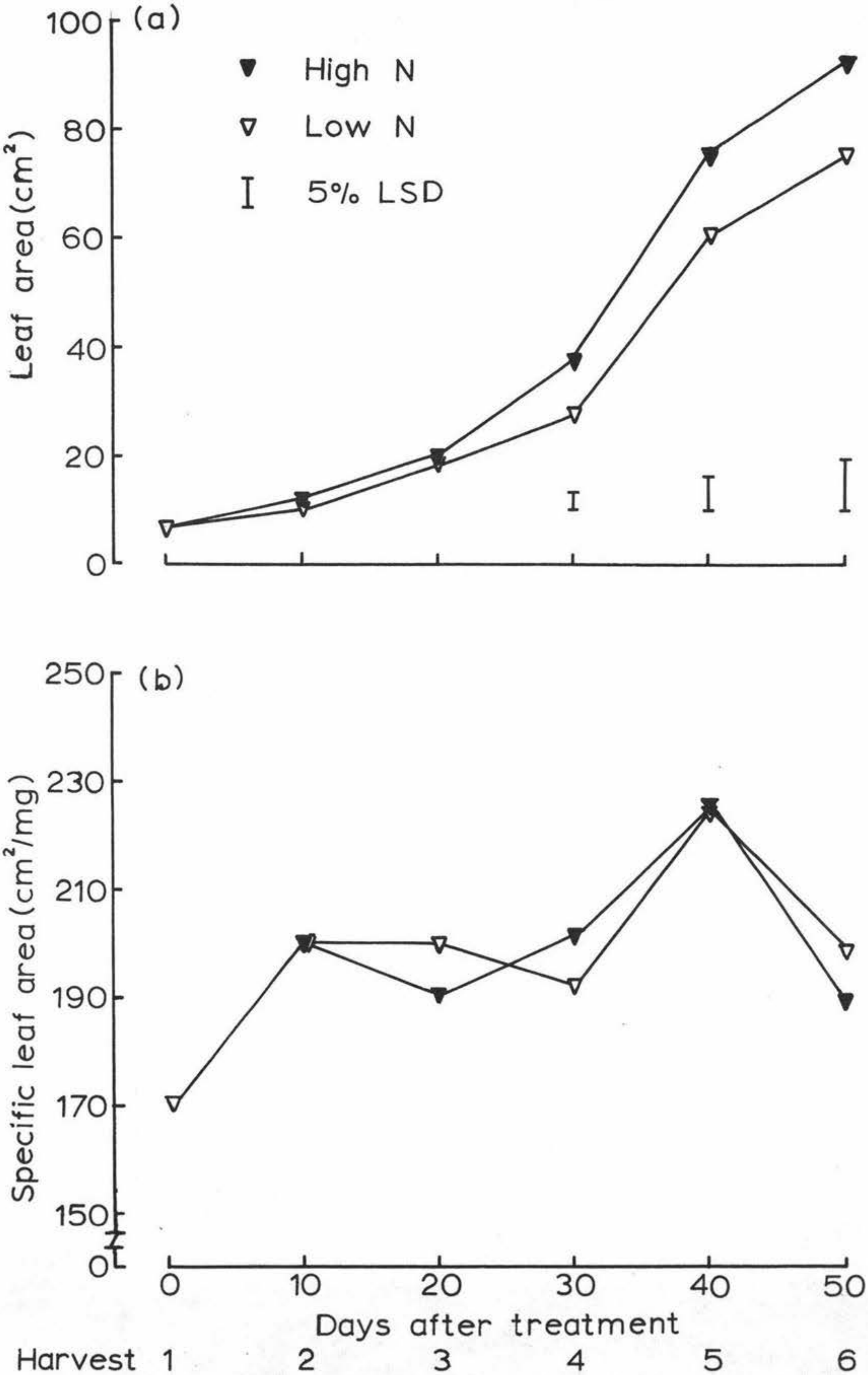
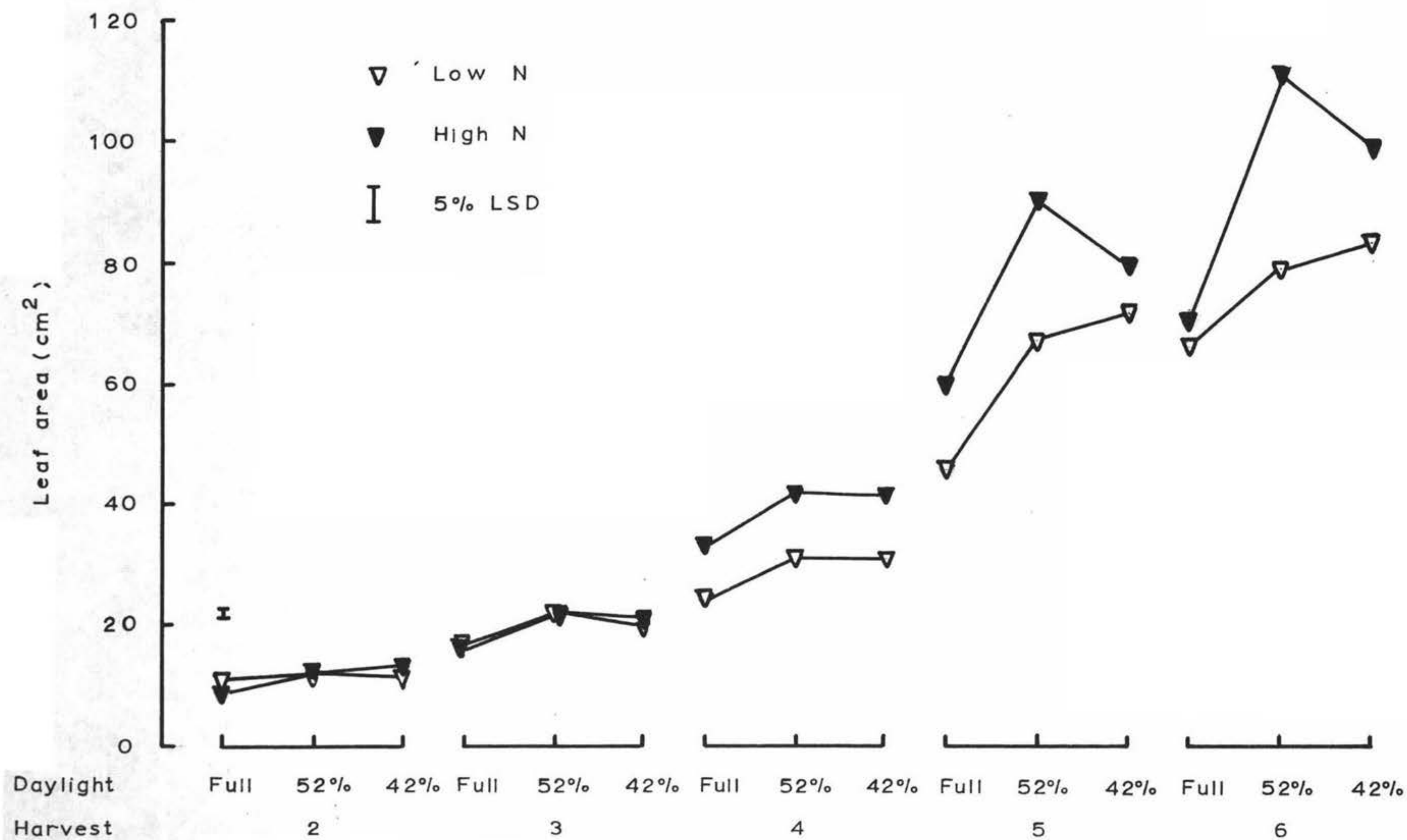


Fig.4.7. Effect of nitrogen on (a) the leaf area, and (b) the specific leaf area.

Fig.4.8. Light intensity X nitrogen interaction on leaf area



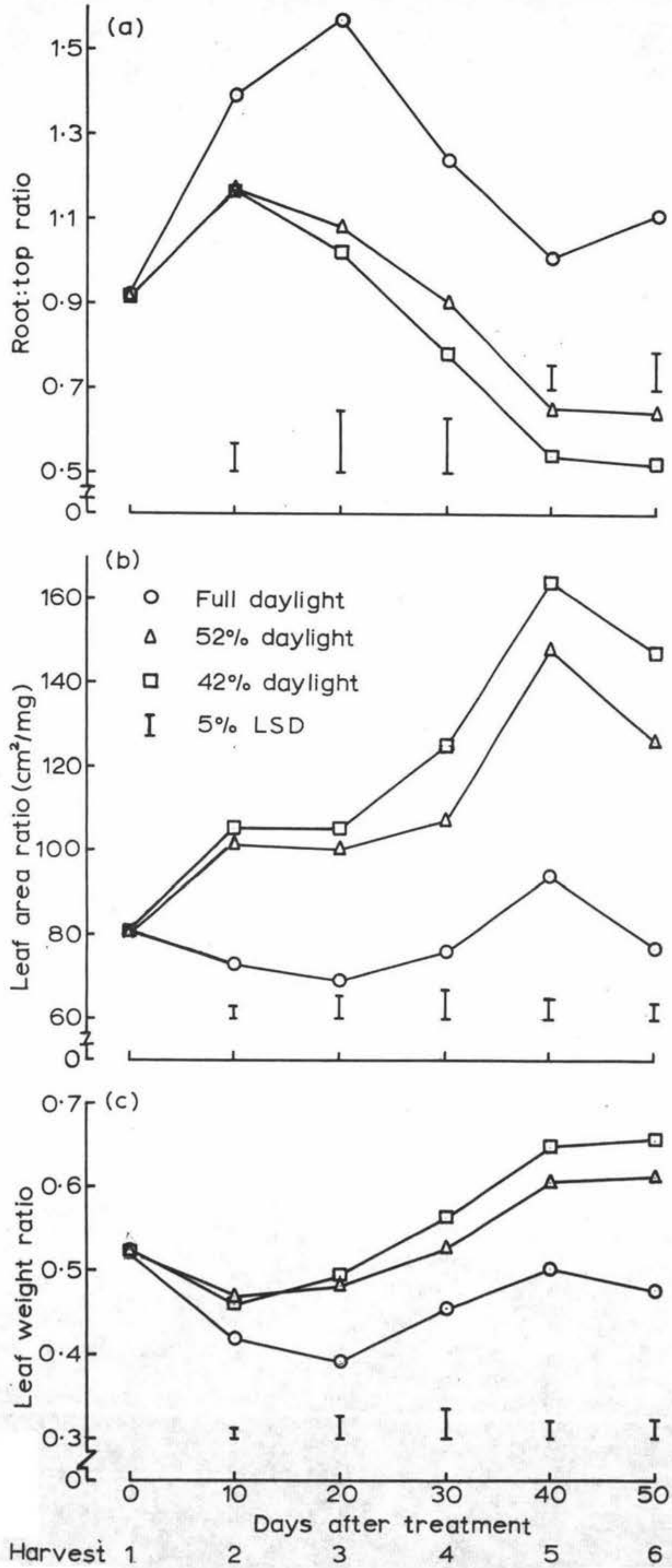


Fig.4.9. Effect of light intensity on (a) the root:top ratio, (b) the leaf area ratio, and (c) the leaf weight ratio.

levels were significantly different from one another at all harvests. The LWR from plants grown in full daylight was significantly lower than that from plants grown in lower light intensities. At harvests 4, 5 and 6 the LWRs from plants grown under 52% daylight were significantly lower than that from plants grown under 42% daylight.

b. Nitrogen treatment

Nitrogen had a negative effect on root:top ratio. From Figure 4.10 it is evident that high nitrogen significantly depressed the ratio at harvest 3 ($P < 0.05$) and at harvests 4, 5 and 6 ($P < 0.01$). In contrast the LAR and the LWR were significantly increased by high nitrogen level at harvests 4 and 5 ($P < 0.01$) for the former and at harvests 4, 5 and 6 ($P < 0.01$) for the latter. There was a negative effect of nitrogen on the LAR at harvest 3 ($P < 0.05$).

c. Light intensity x nitrogen interaction

The interaction effect of light intensity and nitrogen was significant on root:top ratio at harvests 3 and 5 ($P < 0.01$) and at harvest 4 ($P < 0.05$). As evident from Figure 4.11, the interaction indicates that the influences of the two nitrogen levels change with light intensities. High nitrogen depressed the ratio under the three light intensities but the depression was greatest under full daylight and least under 42% daylight. There were also important interaction effects of light intensity and nitrogen on the LAR and the LWR. The interaction effects were significant on the LAR at harvest 5 ($P < 0.01$) and on the LWR at harvests 3 and 5 ($P < 0.05$) as shown graphically in Figures 4.12 and 4.13 respectively. Under the three light intensities the ratios were increased by high

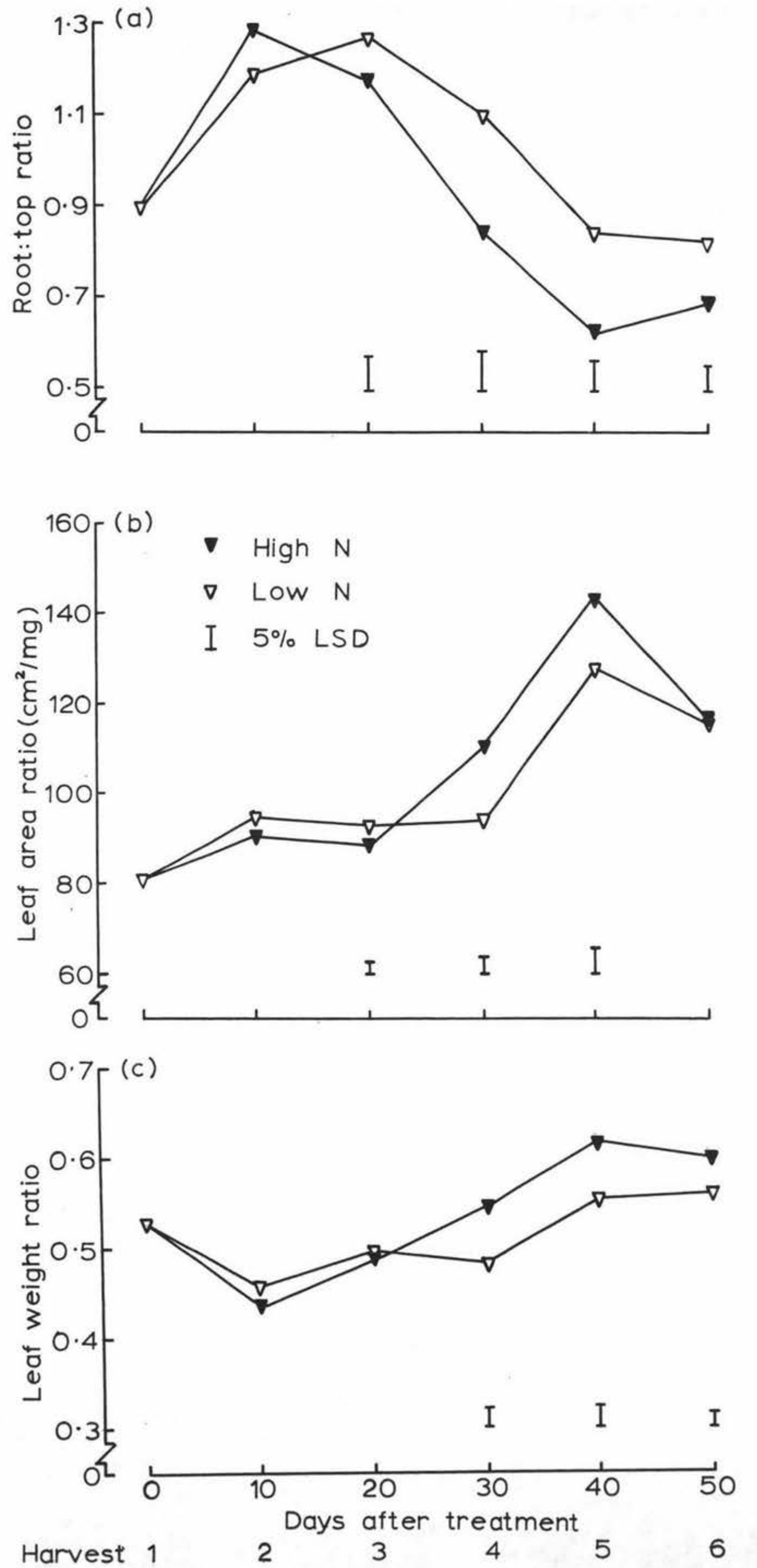


Fig.4.10. Effect of nitrogen on (a) the root: top ratio (b) the leaf area ratio, and (c) the leaf weight ratio.

Fig.4.11. Light intensity X nitrogen interaction on root:top ratio

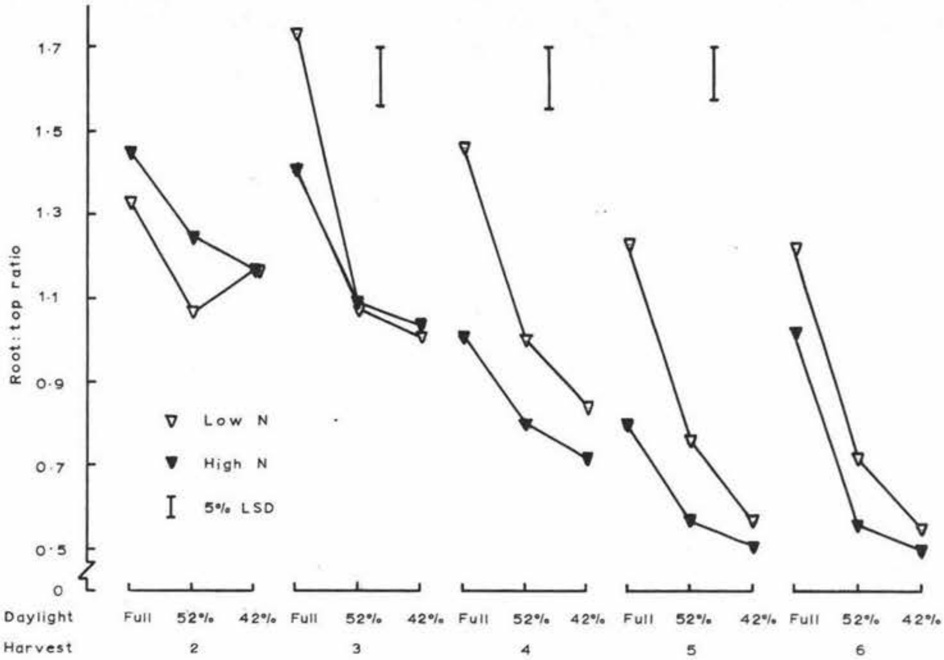


Fig. 4.12. Light intensity X nitrogen interaction on leaf area ratio

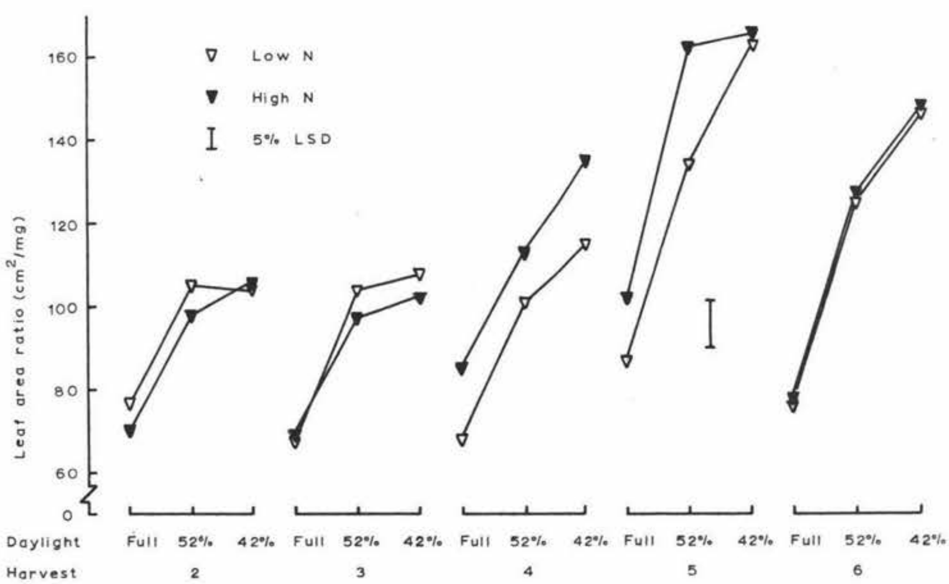
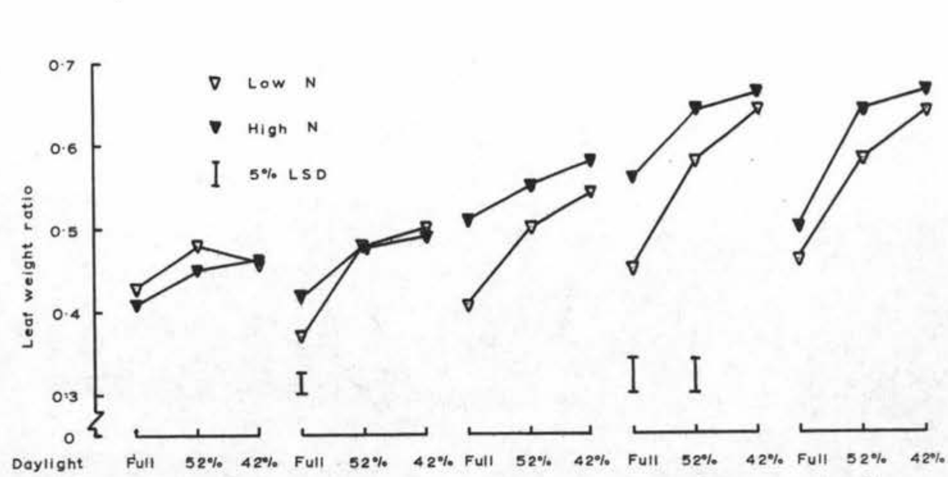


Fig.4.13. Light intensity X nitrogen interaction on leaf weight ratio



nitrogen (apart from harvests 2 and 3) but the increases were not the same at different light intensities.

4.2.3.4. Net Assimilation Rate, Mean Leaf Area Ratio and Relative Growth Rate

a. Light intensity treatment

The treatment effects on the three growth attributes are presented graphically in Figure 4.14. It is evident from the figure that light intensity had significant effects on the NAR at harvest intervals 1, 2 and 4 ($P < 0.05$) and at harvest interval 5 ($P < 0.01$) and on the $\overline{LAR}$ at all harvest intervals ($P < 0.01$) but had no significant effect on the RGR. The $\overline{LAR}$ of the plants grown in the three light intensities were significantly different from one another. In the case of the NAR plants grown in full daylight had significantly higher rates than those grown in lower light intensities. Between the 52% and 42% daylight the difference was not significant. Figure 4.14 also shows that the NAR reached the maximum at harvest interval 2 and then declined with time. A similar trend was observed for the R.G.R. The $\overline{LAR}$ behaved differently, it increased with time. A significant effect of light intensity on the RGR was detected when the mean values for the whole experimental period were compared as shown in Table V. The treatment depressed the RGR and the NAR ($P < 0.01$) but enhanced the $\overline{LAR}$ ($P < 0.01$).

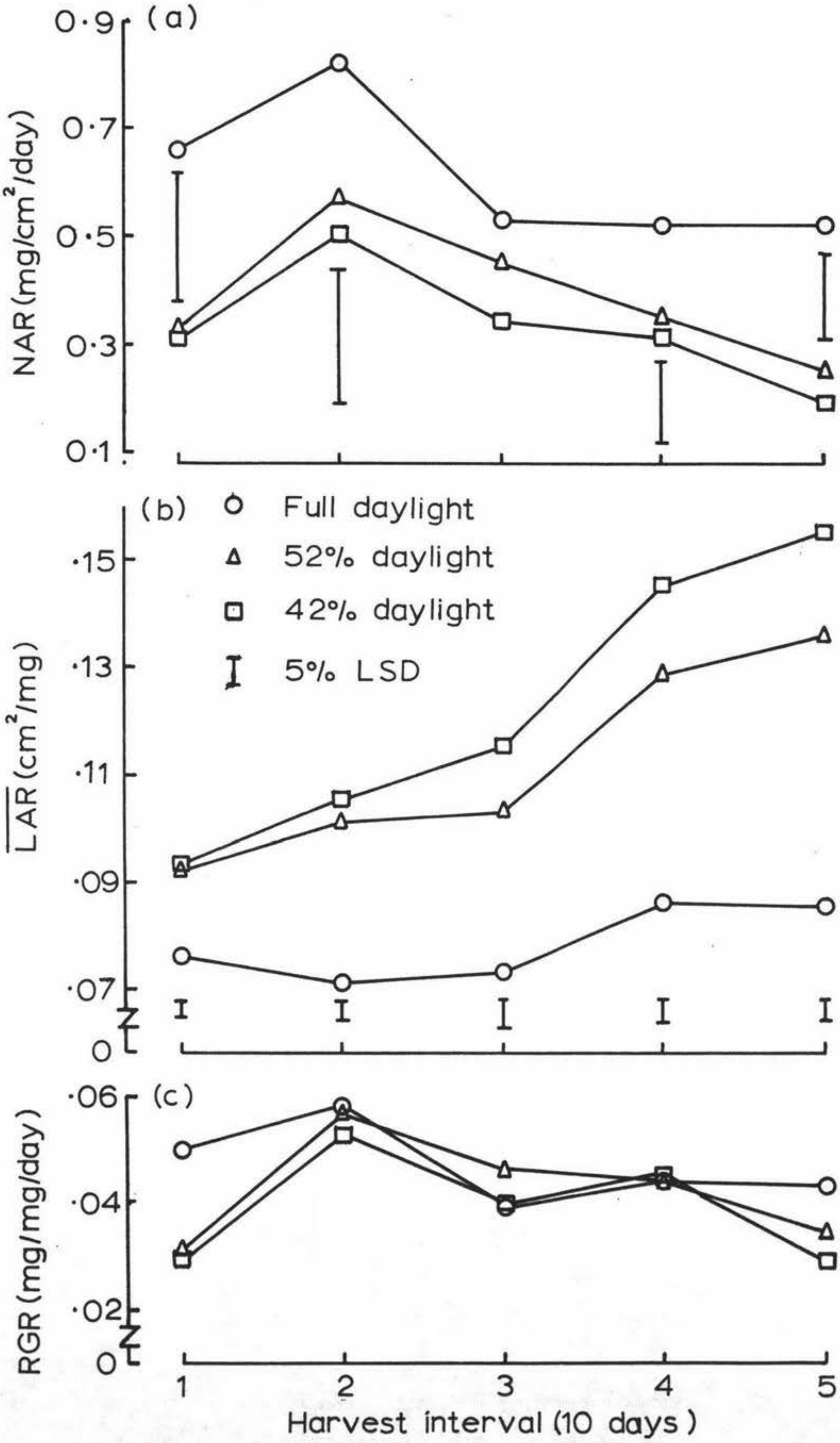


Fig.4.14. Effect of light intensity on (a) the net assimilation rate(NAR), (b) the mean leaf area ratio ($\overline{LAR}$), and (c) the relative growth rate (RGR).

TABLE V

The main effects of light intensity on the NAR, the $\overline{LAR}$ and the RGR

Character	Daylight			Result
	Full	52%	42%	(5% LSD)
NAR ($mg/cm^2/day$)	.6088	.3887	.3291	** (.0382)
$\overline{LAR}$ (cm^2/mg)	.0780	.1117	.1224	** (.0021)
RGR (mg/mg/day)	.0467	.0423	.0388	** (.0035)

In Figure 4.15 the growth attributes are plotted against the logarithm of the light intensities for the five harvest intervals. The graph shows that the NAR increased with increasing light intensity. At all harvest intervals there was a close fit to a straight-line regression. Similarly the graph reveals that $\overline{LAR}$ was lineally related to the logarithm of the light intensities, but in contrast to the trend of NAR, the ratio increased with decreasing light intensity. The curves of RGR plotted against the logarithm of the light intensities show a good agreement between the observed values of the RGR and the values calculated from the quadratic equations obtained from the product of the appropriate regressions of NAR and $\overline{LAR}$. The regression equations are presented in Appendix X.

Figure 4.15 also shows that the regression lines are different for each harvest interval. The slopes of the regression lines of NAR and $\overline{LAR}$ changed with time. The shape of the RGR curves is different at each harvest interval. For the first

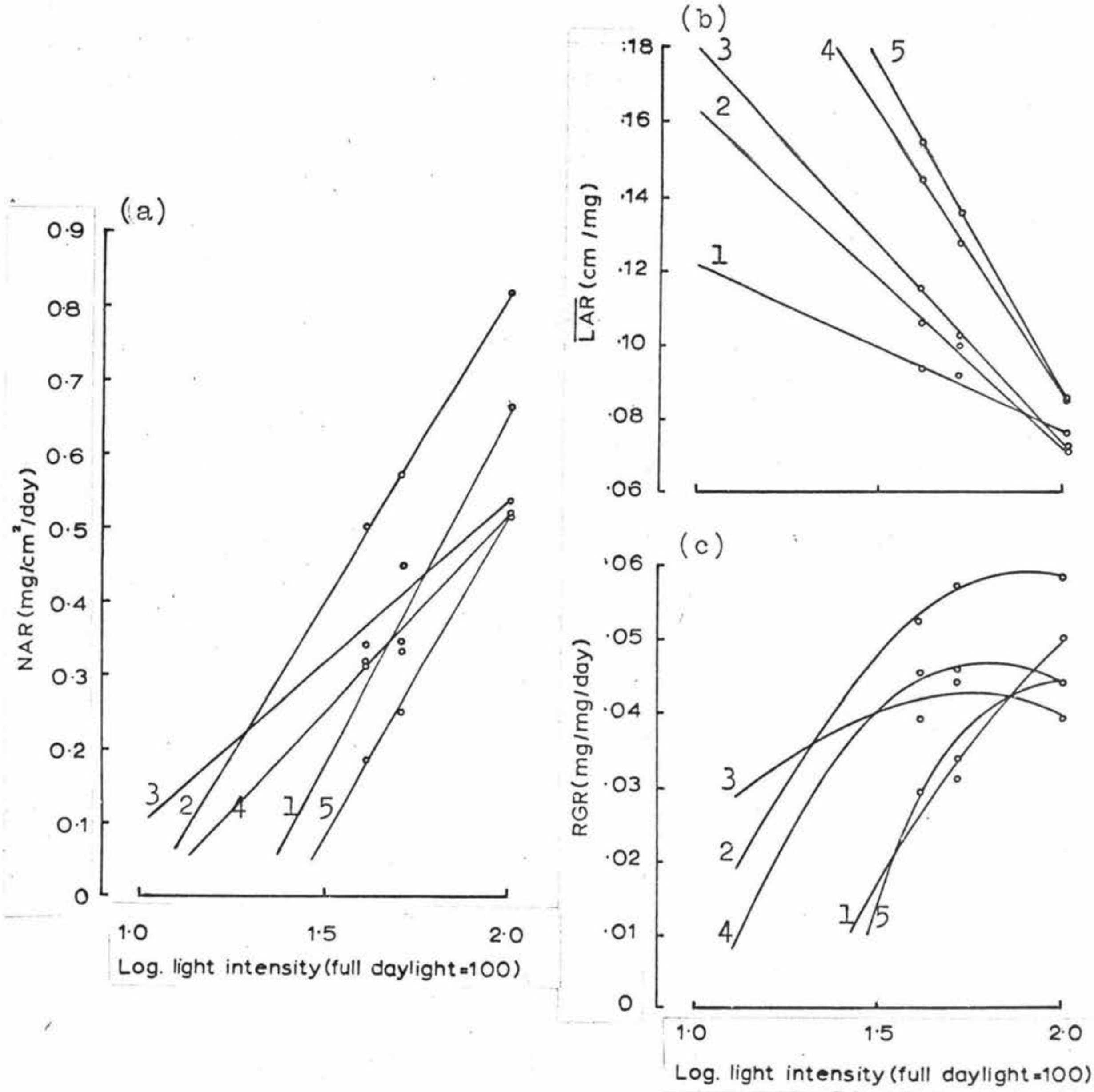


Fig.4.15. Effect of log. light intensity on (a) the net assimilation rate(NAR), (b) the mean leaf area ratio ($\overline{\text{LAR}}$), and (c) the relative growth rate(RGR). 1,2,3,4,and 5 refers to harvest intervals 1(23/4-2/4), 2(2/4-12/4),3(12/4-22/4),4(22/4-2/5) and 5(2/5-12/5) respectively.

interval the curve indicates that the maximum RGR would be obtained at full daylight while the rest of the curves suggest that the maximum is achieved at intensities below full daylight.

b. Nitrogen treatment

Apart from harvest intervals 1 and 2 high nitrogen significantly increased the $\overline{LAR}$ ($P < 0.01$). At harvest interval 1 the treatment was not significantand at harvest interval 2 the treatment decreased the ratio (Figure 4.16). The reason for the decrease is not clear, but perhaps it may be a consequence of the ammonium toxic effect discussed in section 4.2.4. Nitrogen had no significant effects on the NAR and the RGR at every harvest interval although the rates were observed to increase with increased nitrogen (Figure 4.16). However when the mean values for the whole experimental period were compared high nitrogen significantly enhanced the RGR ($P < 0.01$) but still not the NAR (Table VI).

TABLE VI

The main effects of nitrogen level on the NAR, the $\overline{LAR}$ and the RGR

Character	Low nitrogen	High nitrogen	Results (5%LSD)
NAR ($mg/cm^2/day$)	.4346	.4499	n.s
$\overline{LAR}$ (cm^2/mg)	.1016	.1065	** (.0015)
RGR (mg/mg/day)	.0410	.0442	** (.0015)

c. Light intensity x nitrogen interaction

The interaction of light intensity x nitrogen had no significant effects on the NAR and the RGR but had significant effect on the $\overline{LAR}$ at harvest interval 5 ($P < 0.01$) as shown graphically in Figure 4.17. Under any one light intensity, high nitrogen increased the $\overline{LAR}$ but the increase was greatest under 52% daylight. At the other harvest intervals a similar trend was observed although it was not statistically significant at the 5% level.

4.2.4. Discussion

Of the effects of light intensity on changes in dry weight, only that of leaf dry weight was not significantly affected by varying light intensity. Plants in full daylight had heavier total dry weight than those in lower light intensities (Figure 4.1.) because of their heavier root weight (Figure 4.2.). The reduction of root dry weight by shading is probably related to reduced carbohydrate production by the top as a result of lowering the light intensity. Since root depended on the leaves for sugar it is not unexpected that root dry weight decreased with the reduction in light intensity. Further the number of sieve tubes and their capacity for sugar transport may be limiting in plants grown under shade. This could also account for the reduction in root dry weight observed with decreasing light intensity. Gist and Mott (1958) also found that root growth was more affected by decreasing light intensity than was top growth. This is in accord with the studies of Blackman and Rutter (1947, 1948) which showed that shading had no effect on leaf weight during the plants active growth phase.

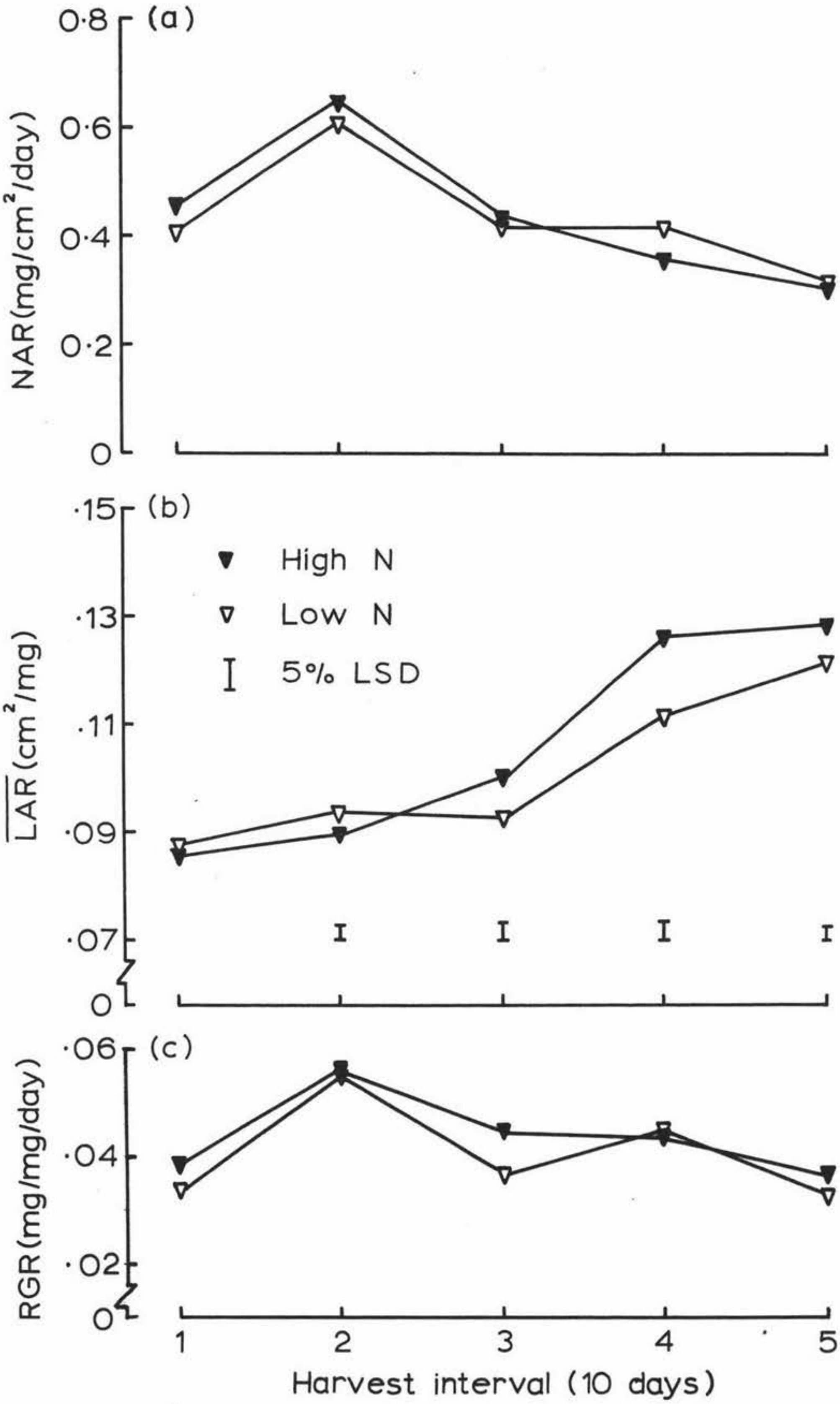
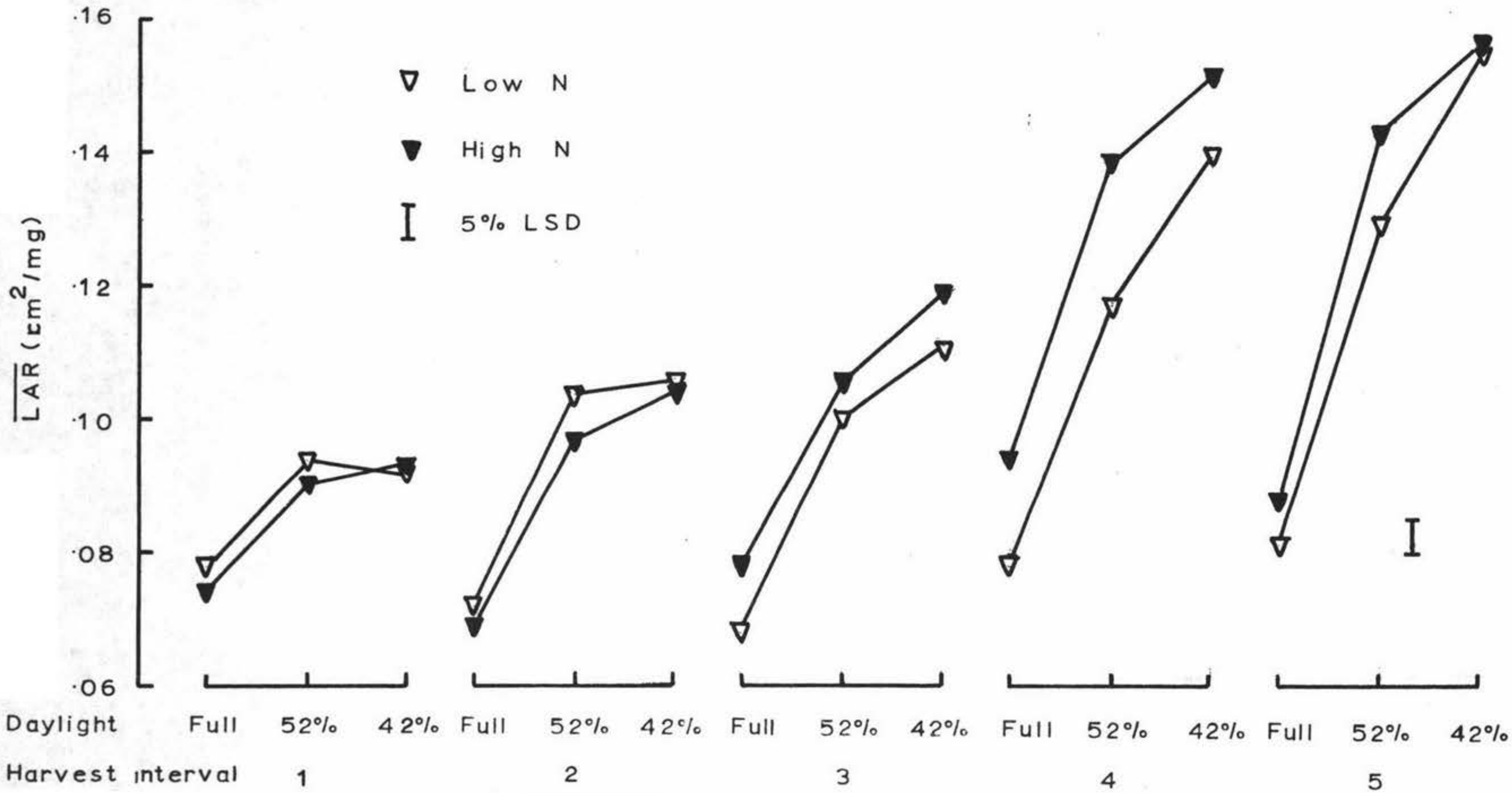


Fig. 4.16. Effect of nitrogen on (a) the net assimilation rate(NAR), (b) the mean leaf area ratio ($\overline{LAR}$), and (c) the relative growth rate(RGR).

Fig. 4.17 Light intensity X nitrogen interaction on mean leaf area ratio ($\overline{LAR}$)



In spite of the independence of light intensity and leaf dry weight there was a significant increase in leaf area by shading (Figure 4.6a). As the leaf weight of the three light levels was not significantly different from one to another, the significant difference in leaf area could be explained by the leaf thickness as measured by the ratio of leaf area to leaf weight, i.e. the SLA. Figure 4.6b shows that the SLA increased with decreasing light intensity. This response is confirmed by the results of Mitchell (1953) and of Bean (1964). Difference in SLA represents real difference in leaf thickness. Friend et al. (1962) obtained a close correlation of SLA with the actual leaf thickness as measured by a micrometer. Leaf number was not an important contributing factor to the increase in leaf area by shading as shown in Table VII. In fact the table shows that the leaf number was slightly lower

TABLE VII

The main effect of light intensity on leaf number

Harvest	2	3	4	5	6
Full daylight	9.1	12.3	14.1	18.0	17.4
52% daylight	9.0	12.0	14.6	18.0	17.9
42% daylight	9.1	12.0	13.9	16.8	16.6
Results	n.s.	n.s.	n.s.	n.s.	n.s.

under 42% daylight than under the two higher light intensities. The lower leaf number under 42% daylight could be an explanation for the smaller leaf area in 42% daylight than in 52% daylight (Figure 4.6a).

The amount of photosynthetic tissue present per unit of plant weight as measured by the LAR was observed to decreace with increasing light intensity. The LAR is dependent on changes in leaf thickness expressed in SLA and changes in leafness of the plant expressed in LWR. A change in LAR could be attributed to either SLA or LWR or both. In this study SLA and LWR were found to decrease with increasing light intensity as shown in Figures 4. 4.6(b) and 4.9(c). A low SLA indicates a smaller leaf area formed from the same amount of dry matter and a low LWR indicates a decrease in amount of leaf dry matter in relation to the total dry matter. The decrease in the LAR under increasing light intensity found in this study was attributed to both SLA and LWR with SLA being the dominant component. The present finding is in variance with the data of Blackman (1961) and Evans and Hughes (1961) which showed that LWR played only a negligible part in decreasing the LAR, but agrees with that of Friend et al (1965) and Cline (1966) which showed that the LAR was dependent on both the SLA and the LWR.

Figure 4.7(a) shows that the distribution of dry weight between root and top is strongly influenced by varying light intensity. The higher root:top ratio in full daylight was entirely due to heavier root weight as top weight was not affected by light intensity (Figure 4.2(a)). The result can be explained by the fact that under low light intensity there would be a reduction of available carbohydrate. The leaves being nearest to the source of carbohydrate will be able to benefit most and root growth will be relatively retarded.

The effects of light intensity on the NAR and the $\overline{\text{LAR}}$ are compensatory in that high intensities increased the NAR but decreased the $\overline{\text{LAR}}$ in consequence the RGR was more or less constant (Figure 4.14). However when the mean values for the whole experimental period were compared high light intensity depressed the $\overline{\text{LAR}}$ and increased the NAR and the RGR (Table V). Similar results were obtained by Wilson (1967) on different plant species. The work of Thorne and Evans (1964) on sugar beet and spinach beet could offer an explanation to the decrease in the NAR under low light intensities. By some grafting experiments they found that the greater NAR in sugar-beet was associated with a greater root system than spinach beet. The sugar beet root probably increased the NAR by providing a better sink for assimilates than spinach beet root. That more sinks are conducive to increased photosynthesis has also been reported by Humphries and Thorne (1964). In this study, plants in low light intensities had less roots than those in the full daylight and hence could be associated with decreased photosynthesis. Components of the leaves such as stomatal frequency and chlorophyll content are found in section 4.1. to be influenced by light intensity. These changes are likely to affect photosynthesis (El-sharkawy and Hesketh, 1964; Sestak and Bartos, 1962).

The result of this study conforms to the reports of Blackman and his followers that the NAR and the $\overline{\text{LAR}}$ are lineally related to the logarithm of the light intensity (Figure 4.15). In this experiment prior to the treatments the plants received full daylight and in consequence modification for the plants takes some time before full adaptation to the changing light intensity is completed. The slopes of the $\overline{LAR}$ regression line in Figure 4.15(b) measures the adaptation of the plant. At the start of the experiment the slope of the regression line for the three light intensities is the same and parallel to the light axis. At any harvest taken before the plants are fully adapted to each light intensity the slope of the regression line will be intermediate between zero and the value when the full potential for adaptation is completed. From Figure 4.15(b) it is evident that the slope of the regression line of each harvest interval is greater than that of the preceding one. Adaptation has almost completed for E. vesiculosum at harvest interval 4 as the slopes of the regression lines at harvest intervals 4 and 5 did not differ much. The changing shape of the RGR curves in Figure 4.15(c) is another indication of the adaptation of the plant to changing light intensity. In the first harvest interval the curve indicates that the maximum growth rate would be achieved at light intensity at full daylight or higher, while for the rest of the harvest intervals the curves show that the maximum has shifted to an intensity below full daylight.

There has been a controversy among investigators whether or not NAR drifts with time. In recent years several critical studies on this issue have been undertaken. The work of Thorne (1960, 1961) indicated that NAR declined with time independent of environment. Investigations of Evans and Hughes (1961) carried out in the glasshouse on I. parviflora showed that NAR decreased to only a small extent before self shading occurred. The data of this study indicated a significant decrease of NAR with time as shown in Table VIII.

TABLE VIII

The effects of harvest interval on the NAR, the $\overline{LAR}$ and the RGR

Harvest interval (a)	1	2	3	4	5	Result (5% LSD)
NAR ($mg/cm^2/day$)	.4340	.6282	.4402	.3923	.3163	(**.0764)
$\overline{LAR}$ (cm^2/mg)	.0870	.0919	.0968	.1194	.1251	(**.0012)
RGR (mg/mg/day)	.0365	.0557	.0412	.0443	.0353	(**.0071)

(a) Harvest interval was considered an additional factor and analysis of variance was performed on a split-split plot design (Appendix X).

The table also shows that the RGR declines with time and the $\overline{LAR}$ increases with time. The RGR result is in accord with the data of Thorne (1960) but the $\overline{LAR}$ result is in contrast with the data of Thorne (1960).

The decline of NAR with time could be attributable to a decrease in the LWR and or a decrease in the $\overline{LAR}$. In this study these two attributes increased with time and could not be the contributing factors. Hence the fall in the NAR could be probably caused by self shading and or some internal factors. As this study was not performed in controlled environment the change in growth attributes with time could be influenced by varying temperature and solar radiation. The variations of these two invironmental factors with time were not great (Appendix I) and would not be inportant factors in this study.

The effect of varying the nitrogen supply on plants has been studied by many investigators. In E. vesiculosum increasing the level of nitrogen from 57 ppm to 250 ppm significantly increased the total dry weight and leaf dry weight but not the root dry weight. The failure of the high nitrogen to promote root growth could be explained by the light intensity x nitrogen interaction. From Figure 4.5 it is shown that high nitrogen depressed root growth for the five harvests in full daylight but not in the 52% and 42% daylight. The negative effect of high nitrogen in full daylight could nullify the positive effect of high nitrogen in the lower intensities thus giving a mean value of the high nitrogen effect little different from the value of the low nitrogen effect (Figure 4.4). The lack of significant difference between low and high nitrogen on total dry weight at harvest 5 is attributable to the depressing effect of high nitrogen in full daylight being greater than the promotive effect of high nitrogen in the lower intensites. On looking through the raw data (the means given in Appendix IX) it was found that high nitrogen also depressed leaf dry weight at harvest 2 and total dry weight at harvests 2,3,4 and 5 in full daylight but not in 52% and 42% daylight. Ballard and Petrie (1936) and other investigators (cited by Ballard and Petrie, 1936) also obtained depression of plant growth by high nitrogen.

The depressing effect of high nitrogen could be an ammonium toxicity effect. In this study the source for low nitrogen was from nitrate and the source for high nitrogen was from nitrate and ammonium. It is known that plants under prolonged application of ammonium as a source of nitrogen leads to severe physiological and morphological disorders resulting in retarded growth and in some cases death. Behnett et al (1964) comparing the effect of nitrate and ammonium on growth in sand culture found that the effect of ammonium on growth was striking. Root and top dry weights of corn was greater when nitrate supplied the nitrogen than when nitrogen was supplied by ammonium. Roots of plants grown in ammonium form were darker and appeared poorer than roots grown on the nitrate form. The decreased growth by ammonium source of nitrogen could be explained by its disruptive effects on various apects of plant metabolism (Krogman et al., 1959; Avron, 1960; Trebot et al., 1960; and Puritch and Barker, 1967).

With reference to the result of this study the depressing effect of high nitrogen could be explained by ammonium toxicity. The absence of the toxic effect under low light intensity could be due to the fact that ammonium could not reach the toxic level because its uptake was limited by low light energy. Tokimasa and Suetomi (1958) noted that the NH_4-N absorption by wheat was retarded by shading. Ballard and Petries (1936) explained their depression as a result of a toxic effect. It is likely that ammonium toxicity can be rul d out as their source of nitrogen was from nitrate. However the results of some of the investigators cited by Ballard and Petrie could be associated with ammonium toxicity as their sources of nitrogen were from ammonium.

The increase in leaf weight by high nitrogen which agrees with data of other investigators (e.g. Arney, 1952) could be the result of two factors. High nitrogen could induce more leaf number or the individual leaves were greater in size or both. The second factor was mainly responsible in this study as nitrogen had little effect on leaf production except at harvest 4 as shown in the Table IX. The result agrees with the work of Langer (1959) on timothy. The increase in leaf area by increased nitrogen

TABLE IX

The main effect of nitrogen level on leaf number

Harvest	2	3	4	5	6
Low N	9.1	11.9	13.3	17.4	17.3
High N	9.1	12.3	15.1	17.8	17.3
Result (5% LSD)	n.s.	n.s.	*(1.3)	n.s.	n.s.

level was due to increased leaf weight and not to leaf thickness as there was no treatment effect on SLA (Figure 4.7).

Since nitrogen has no significant effect on SLA, it follows that the variation in the LAR will be proportional to changes in the LWR. Both the LAR and the LWR were significantly enhanced by increasing the nitrogen level from 57 ppm to 250 ppm. Hence the increase in the LAR was due to more material going to the formation of leaves than to other organs of the plant.

E. vesiculosum plants grown in 57 ppm nitrogen had a higher root:top ratio than those grown in 250 ppm nitrogen. This observation is in accord with the works of other investigators (e.g. Sidens and Young, 1950). The result could be explained by the reduced top growth in low nitrogen. The top growth utilises a relatively small proportion of the carbohydrate synthesised during photosynthesis and the excess is translocated to the root system. Greater root growth becomes possible through the use of the translocated carbohydrate. If high nitrogen is available the top growth increases and less amount of carbohydrate is available for root growth and consequently a reduction in the root:top ratio. Other interpretations such as hormone balance may be involved (Bosemark, 1954).

Increasing the nitrogen level from 57 ppm to 250 ppm did not significantly affect the NAR. This observation agrees with the result of Gregory and Baptiste (1936) on barley, of Ballard and Petrie (1936) on Sudan grass and of Crowther (1934) on cotton but is in contrast with the data of Ballard and Petrie (1936) on wheat, of Watson (1947) on barley and mangolds and of Langer (1959) on timothy. As an explanation for his results Crowther (1934) suggested that the internal level of nitrogen was not influenced very much by the high or low nitrogen treatment because the internal nitrogen level was in a sense self-regulated.

Langer (1959) obtained significant nitrogen effect on the NAR in his 1957 experiment when the mean values of the NAR for intervals of 4 weeks were compared but not in his 1956 experiment. He explained the inconsistent results to the different levels of nitrogen used in both experiments. In the 1956 experiment 30 ppm and 150 ppm of nitrogen were used and the lowest level was not adequately low to obtain any significant effect. The lowest level of nitrogen in the 1957 experiment was 6 ppm, while the others were at 30 ppm and 150 ppm. The lowest nitrogen level used in this study was 57 ppm which may not be low enough to influence the NAR to the 5% significant level.

In the 1956 experiment of Langer nitrogen treatment of RGR was not significant but in the 1957 experiment low RGR was consistently associated with low nitrogen (6 ppm) but this was only during the period between weeks 8 to 12 when nitrogen effect could be significantly detected. The $\overline{\text{LAR}}$ was also significantly increased by high nitrogen between weeks 8 and 10.

There were indications in this study that the RGR was significantly influenced by nitrogen, but because of high variability no significant difference was observed. However a significant difference ($P < 0.01$) appeared when the mean values for the whole experimental period were compared (Table VI). The NAR was still not significantly influenced by nitrogen when mean values for the whole experimental period were compared although high nitrogen had higher value. The $\overline{\text{LAR}}$ was more sensitive to changing nitrogen levels. It was significantly increased by high nitrogen at harvest intervals 3, 4 and 5. The increase in the RGR with high nitrogen was due mainly to the increase in the $\overline{\text{LAR}}$.

Most of the interactions observed in this study may be due to the toxic effect of high nitrogen on growth in full daylight. In Figure 4.8 the significant interaction of light intensity and nitrogen on leaf area at harvest 2 was due to the initial depressing effect of high nitrogen on leaf area under full daylight. Similarly the significant interaction effects root:top ratio and LWR could be due to the same cause. Taking the root:top ratio first, the general effect of increasing nitrogen level was an increase in root weight but the top weight increased more in proportion hence there was a lower root:top ratio as observed in the three light intensities in Figure 4.11. However in the full daylight the high nitrogen depressed root weight instead of increasing it thus resulting in a greater reduction in the root:top ratio as shown in the graph at harvests 3, 4 and 5. At harvest 6 there was no significant interaction effect most probably because the toxic effect had worn off with time. It may be too early at harvest 2 for the nitrogen effect to be evident as suggested by the lack of significant interaction at this harvest. The interaction effect on the LWR in Figure 4.13 may be accounted for by similar explanation.

The interaction of light intensity and nitrogen have significant effects on the LAR and the $\overline{\text{LAR}}$ at harvest 5 and at harvest interval 5 respectively. The interactions cannot be explained by the toxic effect. The combination of high nitrogen and 52% daylight at that particular time had far greater promotive effect on the ratios than at any other time, this could be a possible explanation.

The significant block effect which occurred in some cases was due to the varying sizes of the plants used at the start of the experiment and with time, when the plants matured and were more uniform the effect disappeared.

Chapter V EFFECTS OF DAYLENGTH AND NITROGEN ON E. VESICULOSUM

5.1. MORPHOLOGY AND PLANT GROWTH

5.1.1 Experiment design

There were two experiments involved in the study of morphology and growth in response to daylength and nitrogen. In both experiments the plants were of about the same physiological and morphological age (4 leaves per plant). Experiment 5.1 (I) was laid out on a factorial combination of two daylength treatments, "short" (8 hours) and "long" (16 hours) and two nitrogen levels, "low" (57ppm N) and "high" (250ppm N). One bay was used for the short day and one for the long day treatment. Within daylength bays there were seven blocks of each nitrogen treatment.

At the beginning of the experiment, representative plants were dissected to ensure strictly vegetative growth before plants were assigned to the treatments. During the experiment, a record was kept of the percentage of plants with macroscopic inflorescence buds on three arbitary dates (32, 52 and 77 days after treatment) and of the number of emerged leaves per plant at five arbitary dates (0,32,52,60 and 77 days after treatment). At the harvest 77 days after treatment each treatment was represented by seven blocks of single pot plant. The number of flowers per inflorescence, the number of teeth per leaf, the number of runners per plant and the length of the leaves were determined. The dry weights of the plants of four blocks were determined and the plants of the other blocks were preserved for future anatomical investigation.

Experiment 5.1 (II) was laid out on a factorial design similar to Experiment 5.1 (I) except more blocks were used to allow for two harvests. For the first harvest 28 days after treatment four blocks were taken for each treatment. The growing points of all the plants were examined under a dissecting microscope. The dry weights of leaves and roots and the leaf area were determined. The final harvest was taken at 91 days after treatment. The dry weights of radical leaves, runner leaves, runner stem, lateral branches, inflorescences and roots, the leaf area (radical and runner separately), the number of runners per plant and the number of flowers per inflorescence were determined. The flowering time (definition in section 5.2.2.1) was also recorded. For each treatment seven blocks of single pot plant were taken.

5.1.2. Experimental Methods

5.1.2.1 Determination of Dry Weights and Leaf Area

The method used was as described in Section 4.2.2.1

5.1.2.2 Determination of Leaf Length, Leaf Dissection and Number of Emerged Leaves per Plant

The length of leaf number 4 to number 11 inclusive in the plant was measured at the harvest. Each leaf was detached from the plant and its length measured from tip of lamina to the base of the petiole. Earlier leaves were not used because when the plants were assigned to their treatments these leaves were fairly long and would most probably response less to the treatment. To assure that the leaves had attained their maximum growth when measured leaves later than leaf number 11 were not taken.

The leaf dissection is expressed as the number of teeth per leaf. The more teeth per leaf the more dissected was the leaf. Leaf dissection was determined on leaf number 6 to number 13 inclusive per plant. These leaves were chosen because the earlier leaves were all entire and in order to have uniform entries per treatment leaves later than leaf number13 were not taken as in some blocks only thirteen leaves were in a plant while in others more leaves were available.

Number of leaves emerging from the central axis was counted on each plant. An emerged leaf is defined in this study as one that can be observed with the naked eye.

5.1.2.3 Analysis of Data

The data were analysed as a 2 x 2 factorial design. Least significant difference test and co-efficient of variation were calculated for each analysis. An example of the method used is in Appendix XI. All analyses were performed on the original scale in terms of per unit plant.

5.1.3 Results

The results are expressed in the main body of the text either in tables or graphs with their appropriate least significant difference. Analysis of variance is tabulated with mean squares and significance levels in Appendix XII.

Plate XI(a) shows the plants at 45 days after treatment in Expt. 5.1 (I). There were no infloresences or runners visible at this stage. Plants under long days were bigger than those under short days. In Experiment 5.1 (II) the plants at the first harvest (28 days after treatment) had just initiated inflorescence primordia under long days and were vegetative under short days. At the final harvest at 91 days after treatment the long day treated plants were flowering and producing runners and the short days were still vegetative as illustrated in Plate XI (b)

A. MORPHOLOGY

5.1.3.1 Leaf Length and Leaf Dissection

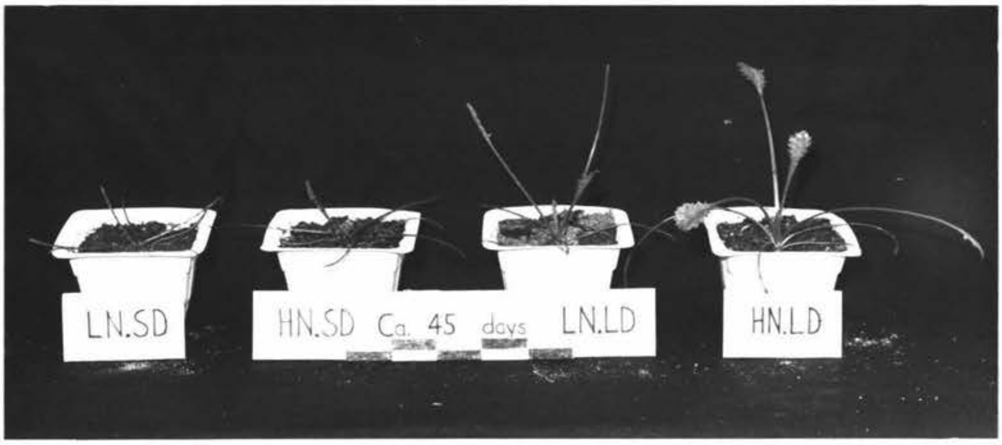
(a) Daylength treatment

Daylength had statistically significant effects on leaf length ($P<0.01$) and leaf dissection ($P<0.01$). The length of the leaf and the number of teeth per leaf were increased by increasing the daylength from eight hours to sixteen hours as shown in Table X.

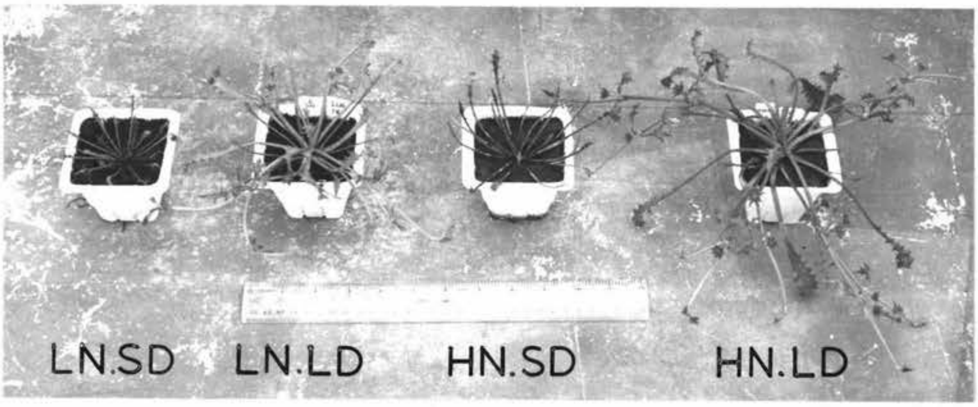
TABLE X

The main effects of daylength and nitrogen on leaf length and leaf dissection

Treatment		Leaf Length (cm.)	No. teeth/leaf
Daylength:	short	7.6	4.1
	long	13.5	23.8
Result (5% LSD)		** (.4)	** (1.5)
Nitrogen:	Low	10.0	10.6
	High	11.1	17.3
Result (5% LSD)		** (.4)	** (1.5)



(a)



(b)

PLATE XI Effects of two nitrogen levels (57ppm N = LN , 250ppm N = HN on E. *vesiculosum* grown in 8·hour daylength (SD) and 16 hour daylength (LD) at (a) 45 days and (b) 91 days after start of the experiment.

(b) Nitrogen treatment

Increasing the nitrogen level from 57ppm to 250ppm had statistically significant effects on leaf length ($P<0.01$) and leaf dissection ($P<0.01$). The higher nitrogen level was associated with longer and more dissected leaves (Table X).

(c) Leaf Position

Leaf length and leaf dissection were statistically influenced ($P<0.01$) by leaf position. Leaf length was longer as the leaf was inserted further from the base and this reached its maximum value at leaf number 7 and then slowly declined (Table XI). The degree of dissection of the leaf increased as the leaf was at a further position from the base and it attained its maximum dissection at leaf number 10 and then decreased as shown in Table XI.

TABLE XI

The main effects of leaf position on leaf length and leaf dissection

Leaf position	Leaf length (cm.)	No. teeth/leaf
4	8.9	-
5	10.1	-
6	11.1	1.4
7	12.0	4.1
8	11.4	14.5
9	10.9	19.6
10	10.4	21.0
11	9.8	20.9
12	-	16.5
13	-	13.4
Result (5% LSD)	** (.8)	** (3.5)

(c) Interactions

The interaction of daylength and leaf position had statistictically significant effects on leaf length ($P<0.01$) and leaf disection ($P<0.01$). At all the leaf positions the length of the leaf was greater in long days but the increase in length was not equal for all the positions. The increase was progressively greater until leaf number 7 and then slowly declined as graphically illustrated in Figure 5.1 (a). In short days the leaf length was relatively constant from leaf number 4 to 11. The curves of the interaction effect on leaf dissection show that in short days the degree of dissection did not change much after leaf number 9 whereas in long days it increased rapidly from 2.3 teeth in leaf number 6 to 35.2 teeth in leaf number 9. It reached the plateau at leaf number 10 (36.7 teeth) and then rapidly decreased to 20.9 teeth in leaf number 13 as shown in Fugure 5.1 (b).

There was also a significant interaction effect of nitrogen and leaf position on leaf length ($P<0.01$) but not on leaf dissection. At the lower leaf positions the effect of increasing the nitrogen level from 57ppm to 250ppm was negligible but became significant at higher positions because of a more rapid decline in the leaf length in the lower nitrogen level (Figure 5.1 (a)). The high and low nitrogen level affected the leaf dissection in a similar trend (Figure 5.1 (b)). Both increased the number of teeth per leaf with increasing leaf position and after reaching the plateau at the same leaf number, began to decline.

The effect of daylength and nitrogen interaction on leaf length and leaf dissection was significant at the 1% level. Table XII shows that with both nitrogen levels long day increased the leaf length and the number of teeth per leaf but the increase was higher

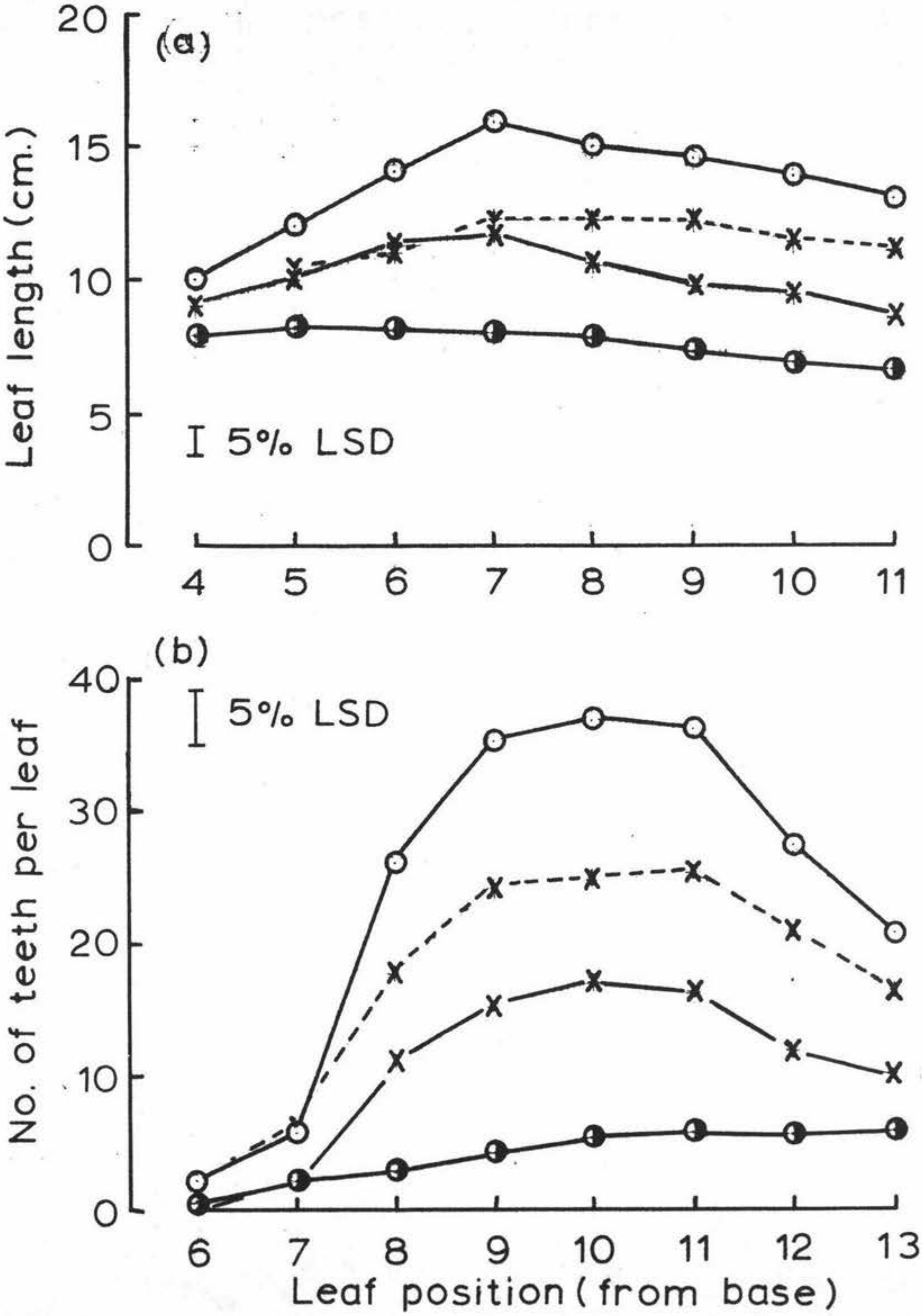


Fig.5.1. Effects of leaf position, daylength, and nitrogen on (a) the leaf length, and (b) the number of teeth per leaf.
o—o (long day), ◐—◐ (short day)
x---x (high N), x—x (low N)

with high nitrogen (6.9cm, 24.5 teeth) than with low nitrogen (5.1 cm and 15.0 teeth).

TABLE XII

The interaction effects of daylength and nitrogen on leaf length and leaf dissection

Daylength	Leaf length (cm.) Low N	Leaf length (cm.) High N	No. teeth/leaf Low N	No. teeth/leaf High N
Long	12.5	14.6	18.1	29.6
Short	7.4	7.7	3.1	5.1
Result (5% LSD)	** (.6)		** (2.2)	

5.1.3.2 Number of Emerged Leaves per Plant

(a) Daylength treatment

Data illustrated in Figure 5.2. (a) shows that the number of emerged leaves per plant tended to be slightly higher under long days although the difference did not reach the 5% level of significance. However a significant difference ($P<0.01$) was detected at 77 days after treatment; leaf number in short day treated plant was higher than that of long day treated ones. The observation was not unexpected as under long days the plant by then were flowering and consequently had stopped initiating leaves whereas under short days the plants were vegetative and continued to initiate leaves.

(b) Nitrogen treatment

Increasing the nitrogen level from 57 ppm to 250 ppm significantly increased the leaf number per plant at 52 ($P<0.01$), 66 and 77 days ($P<0.05$) after treatment (Figure 5.2 (b)). At 32 days

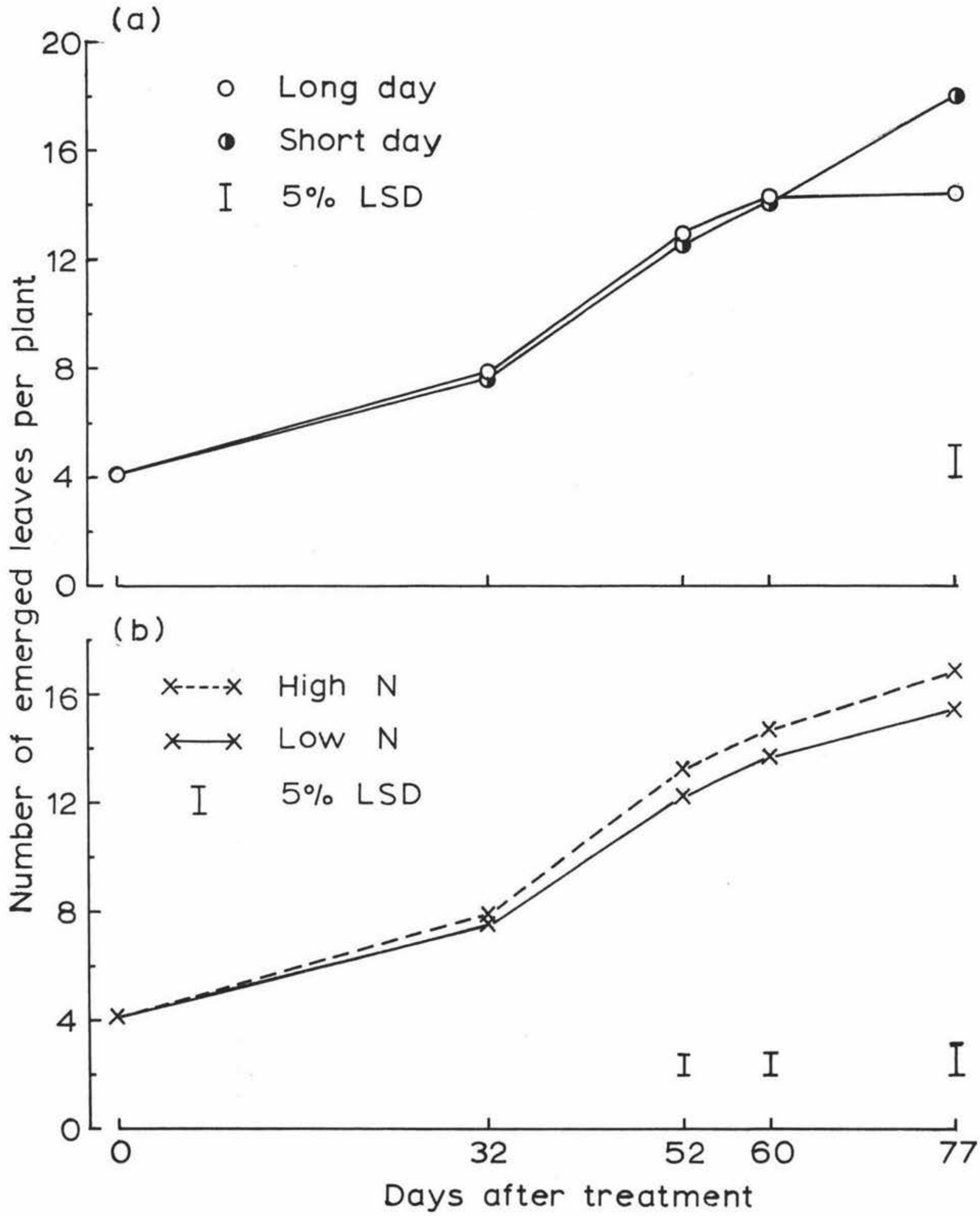


Fig.5.2. Effect of (a) daylength and (b) nitrogen on number of emerged leaves per plant.

after treatment, the effect of high nitrogen was not yet promotive to cause the difference in leaf number to be statistically detected.

(c) Daylength X nitrogen interaction

There was no significant daylength X nitrogen interaction effect on leaf number per plant except at 77 days after treatment. High nitrogen increased leaf number under both daylength treatments but the increase was much greater under short days (2.58 leaves) than under long days (.28 leaves). This is because the long day treated plants which had flowered did not produce further leaves on the control axis where short day treated plants which were still vegetative, continued their leaf production on the central axis.

B. GROWTH

5.1.3.3. Changes in Dry Weight

(a) Daylength treatment

When subjected to analysis of variance the differences between total dry weights and leaf dry weights within the two daylength treatments were found to be significant above the 5% level. The treatment effect on root dry weight was not statistically significant. Total dry weight was significantly increased by increasing the daylength from 8 hours to 16 hours when harvested at 28 ($P<0.05$) 77 and 91 ($P<0.01$) days after treatment (Table XIII). The great difference in total dry weight of plants grown in long days and of those grown in short days at 77 and 91 days after treatment was attributable to the extra weight contributed by the runners, lateral branches and inflorescences in the long-day treated plants. Plants grown in short days were vegetative and did not produce runners and lateral branches. Table XIII shows that when total dry weight was estimated only from roots and radical leaves the treatment effect was still significant ($P<0.05$) at 91 days after treatment. At 77 days after treatment top dry weight of plants was not separated into runner weight, lateral branch weight, inflorescence weight and radical leaf weight.

TABLE XIII

The maim effects of daylength on changes in dry weight (mg)

Character	Daylength Long	Short	Result (5% LSD)
28 days after treatment			
Total dry weight	293.00	221.20	* (70.53)
Leaf dry weight	121.57	78.60	** (28.09)
Root dry weight	171.43	142.60	n.s.
77 days after treatment			
Total dry weight	794.26	382.86	** (120.18)
Leaf dry weight	545.45	139.84	** (75.73)
Root dry weight	248.80	243.02	n.s.
91 days after treatment			
Total dry weight	2915.72	835.38	** (196.59)
(1)	946.23	835.38	* (104.73)
Leaf dry weight	763.35	254.86	** (101.29)
(2)	326.14	254.86	* (61.29)
Root dry weight	620.08	580.52	n.s.

(1) excluding weights from runners, lateral branches and inflorescences

(2) excluding weights from leaves of runners and lateral branches

Long day treated plants had heavier leaf dry weight than short day treated plants. At 77 and 91 days after treatment the heavier leaf weights were mostly attributed to leaves from runners and lateral branches although leaf weight estimated on radical leaves alone was still significantly heavier in long day treated plants than short day treated plants.

(b) Nitrogen treatment

The treatment effect was not evident 28 days after treatment. On later harvests high nitrogen yielded significantly heavier total dry weight ($P<0.01$) and leaf dry weight ($P<0.01$) at 77 days after treatment and significantly heavier total dryweight ($P<0.01$), leaf dry weight ($P<0.05$) and root dry weight ($P<0.01$) 91 days after treatment (Table XIV). The treatment effects were still significant when the total dry weight was estimated only from roots and radical leaves and when the leaf dry weight was estimated from the radical leaves alone (Table XV). High nitrogen had a depressing effect on root weight at 28 days after treatment although the effect was not statistically significant. The observation could be attributable to an experimental error or to an ammonium toxicity effect (see 4.2.4. Discussion) as the source of high nitrogen was from nitrate and ammonium whereas the source of low nitrogen was from only nitrate. The possibility that the toxic effect diminished with time was suggested by the positive effect of high nitrogen at 77 and 91 days after treatment.

TABLE XIV

The main effects of nitrogen on changes in dry weight (mg.)

Character	Nitrogen Low	Nitrogen High	Result (5% LSD)
28 days after treatment			
Total dry weight	256.43	257.76	n.s.
Leaf dry weight	95.74	104.43	n.s.
Root dry weight	160.69	153.34	n.s.
77 days after treatment			
Total dry weight	481.52	695.59	** (120.18)
Leaf dry weight	247.73	437.57	** (75.73)
Root dry weight	233.79	258.03	n.s.
91 days after treatment			
Total dry weight	1531.45	2219.64	** (196.59)
(1)	773.61	1008.00	** (104.73)
Leaf dry weight	407.20	611.01	** (101.29)
(2)	244.73	336.27	* (61.29)
Root dry weight	528.88	671.72	** (79.06)

For (1) and (2) refer Table XIII footnote

(c) Daylength X nitrogen interaction

The interaction effects of daylength and nitrogen were significant on leaf dry weights at 28 ($P<0.05$), 77 ($P<0.01$) and 91 ($P<0.05$) days after treatment and on total dry weight at 77 days and 91 days ($P<0.01$) after treatment. There was no significant interaction effect on root dry weights (Table XV). When the total dry weight and leaf dry weight at 91 days after treatment were estimated from root and radical leaves and from radical leaves respectively the interaction effects were no longer significant as shown in Table XV. Thus the significant interaction effects observed at 77 and 91 days after treatment could be an artifact produced by the presence of runners, lateral branches and inflorescences in long day treated plants. The interaction observed

TABLE XV

The interaction effects of daylength and nitrogen on changes in dry weight (mg)

Character	Daylength	Nitrogen Low	Nitrogen High	Result (5% lsd)
28 days after treatment				
Leaf dry weight	Long	101.86	136.28	* (39.72)
	Short	89.63	67.57	
77 days after treatment				
Total dry weight	Long	584.59	1003.92	** (169.97)
	Short	378.45	387.27	
Leaf dry weight	Long	355.32	735.59	** (107.11)
	Short	140.14	139.55	
91 days after treatment				
Total dry weight	Long	2363.08	3468.36	** (278.00)
	Long (1)	847.41	1045.05	n.s.
	Short	699.81	970.75	
Leaf dry weight	Long	603.69	923.01	* (143.25)
	Long (2)	278.75	358.21	n.s.
	Short	210.72	299.01	

For (1) and (2) refer Table XIII footnote

on leaf dry weight at 28 days after treatment could be caused by the ammonium toxicity effect of high nitrogen; the toxic effect being greater under short days than under long days.

5.1.3.4 Root:Top Ratio

(a) Daylength treatment

The treatment had statistically significant effect on root: top ratio at 28, 77 and 91 days after treatment ($P<0.01$). Plants grown in long days had lower ratios than those grown in short days (Table XVI).

TABLE XVI

The main effects of daylength and nitrogen on root: top ratio

Treatment			Days after treatment	
		28	77	91
Daylength:	Short	1.836	1.836	2.325
	Long	1.441	.508	.281
Result (5% LSD)		** (.167)	** (.287)	** (.257)
Nitrogen:	Low	1.696	1.214	1.344
	High	1.581	1.130	1.262
Result (5% LSD)		n.s.	n.s.	n.s.

The low ratios in long days observed 77 and 91 days after treatment indicated that the top growth exceeded root growth. This was expected as by this time the long day treated plants were flowering and producing runners and lateral branches. This would explain the interesting trend that in long days the ratio decreased with time whereas in short days the ratio was more or less constant with time (Table XVI).

(b) Nitrogen treatment

Nitrogen had no statistically significant effect on the ratio. However a regular trend was observed that the ratio decreased when nitrogen level was increased as evident from Table XVI.

(c) Daylength X Nitrogen interaction

There was a significant interaction on the ratio at 28 days after treatment ($P<0.01$). High nitrogen had a negative effect on the ratio in long days and a positive effect on the ratio in short day (Table XVII). A similar trend was observed on the ratio at 77 days after treatment although the

TABLE XVII

The interaction effect of daylength and nitrogen on root: top ratio

Days after treatment	Daylength	Low	High	Result (5% LSD)
28	Long	1.615	1.268	** (.235)
	Short	1.777	1.895	
77	Long	0.648	0.369	n.s.
	Short	1.781	1.891	
91	Long	.319	.243	n.s.
	Short	2.369	2.281	

interaction was not significant. At 91 days after treatment the interaction effect on the ration was also not significant and high nitrogen had depressing effect on the ratios under both daylengths. The significant interaction observed at 28 days after treatment could have resulted from the initial ammonuim toxic effect of high nitrogen.

5.1.3.5. Leaf Area

(a) Daylength treatment

An analysis of variance of the leaf area showed no statistically significant difference betweem the leaf area for the two daylength treatments at 28, and 91 days after treatment when the area was estimated from radical leaves alone. However when leaf area from the runners and lateral branches was included, increasing the daylength from 8 hours to 16 hours significantly increased the area ($P<0.01$) as shown in Table XVIII. This would be expected as runners and lateral branches which were only produced by long day treated plants as observed in this study contributed about 60% of the 1632.78cm^2 of leaf area.

TABLE XVIII

The main effects of daylength and nitrogen on leaf area (cm2)

Treatment		Days after treatment 28	91	91 (3)
Daylength:	Short	14.56	635.41	635.41
	Long	19.37	1632.78	696.62
Result (5% LSD)		n.s.	** (15.99)	n.s.
Nitrogen:	Low	17.84	61.96	37.85
	High	16.09	100.06	57.30
Result (5% LSD)		n.s.	** (15.99)	**(10.02)

(3) figures in this column exclude the leaf area from leaves of runners and lateral branches.

(b) Nitrogen treatment

The treatment had a statistically significant effect on the area at 91 days after treatment ($P<0.01$) but not at 28 days after treatment. From Table XVIII it is evident that plants grown in high level of nitrogen had larger leaf areas irrespective of whether the area was estimated from radical leaves alone or from radical leaves plus runner leaves and leaves from lateral branches than those grown in low nitrogen at 91 days after treatment.

5.2 FLOWERING, NUMBER OF FLOWERS PER INFLORESCENCE AND NUMBER OF RUNNERS PER PLANT

5.2.1 Experimental Design

The experiment was performed to investigate the effects of two nitrogen levels on the formation of flower primordia, number of flowers per inforescence and number of runners per plant. It was laid on a completely randomised block design. Representative plants were dissected and no floral primordia were found in any of the plants before allocating them to their treatments. Eighty single pot plants were assigned to eight blocks under long days. Half the plants in each block were fed with high nitrogen and the other half with low nitrogen. All the plants were of similar physiological age although the leaf number ranged from 23 - 48 with a mean of 32.8 $\pm$ .5.

One plant per block was examined under a dissecting microscope on the 25th, 26th, 27th and 29th day after treatment. The inflorescence primordium was scored according to the various stages defined in section 5.2.2.1. The remaining plants were continued with their treatments to determine the flowering time (definition see section 5.2.2.1). The number of flowers per inflorescence and runner number per plant were also counted and recorded.

5.2.2 Experimental Methods

5.2.2.1 Criteria of Flowering

Flowering may be measured by several methods based either on observation of macroscopic buds or flowers or microscopic floral primordia. Some methods used by various workers is reviewed by Lang (1965). The following methods were used in this study.

(a) Plants were examined at some arbitary time and a record was made as whether or not they have flowered. The results are expressed as a percentage of flowering plants compared to the total replication of a treatment.

(b) The flowering time was determined. This is measured as the number of days from the commencement of the treatment to the day of opening of the first flower in the inflorescence. The flower is said to be opened when the stamens can be seen with the naked eye.

(c) At some arbitary time plants were micro dissected and arbitary stages of floral development were assigned to these plants. The rate of floral development recorded by a stage score system is illustrated in Plate XIII and defined below.

Stage 0: Relatively small vegetative growing point

Stage 1: Growing point hemispherical in shape and clearly visible

Stage 2: Inflorescence primordium recognizible with its base constricted

Stage 3: Flower primordia visible on the inflorescence primordium, covering not more than a quarter of the inflorescence primordium

Stage 4: Flower primordia covering three quarters of the inflorescence primordium

Stage 5: Flower primordia covering the whole inflorescence primordium except the tip.

Stage 6: Flower primordia covering the whole inflorescence primordium.

Stage 7: Inflorescence primordium surrounded with bracts.

5.2.2.2. Determination of Number of Flowers per Inflorescence

Inflorescences were detached from the flowering axes and taken to the laboratory. Flowers in each inflorescence were removed from the inflorescence with a sharp scalpel and counted.

5.2.2.3. Analysis of data

The data were analysised as a completely randomised block design following Snedecor and Cochran (1968). Co efficient of variation was calculated for each analysis and least significant difference test was used to establish difference between means. Data from Experiment 5.1 (I) and from Experiment 5.1 (II) in the previous section 5.1 were analysed in conjunction with the current Experiment 5.2. The original scale was used in all the analyses.

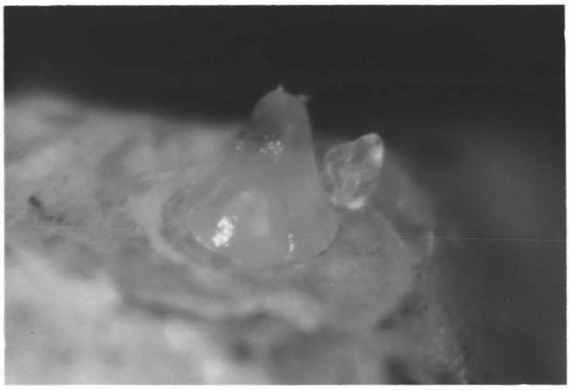
5.2.3. Results

The means and standard errors of the raw data are in Appendix XIV and summaries of analysis of variance in Appendix XV.

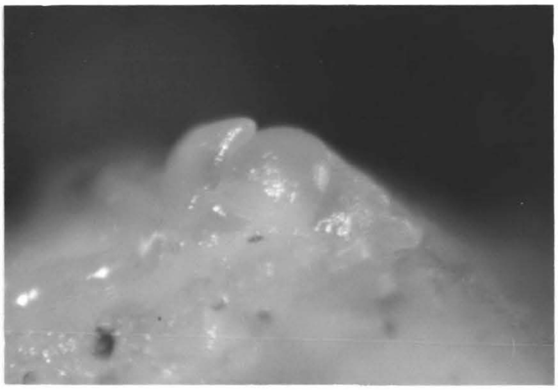
5.2.3.1. Flowering

Appearance of macroscopic inflorescence bud

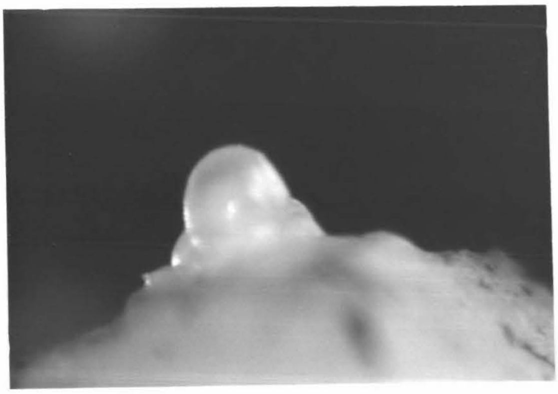
Flowering as determined by the appearance of macroscopic inflorescence bud was observed at 52, 60 and 77 days after treatment.



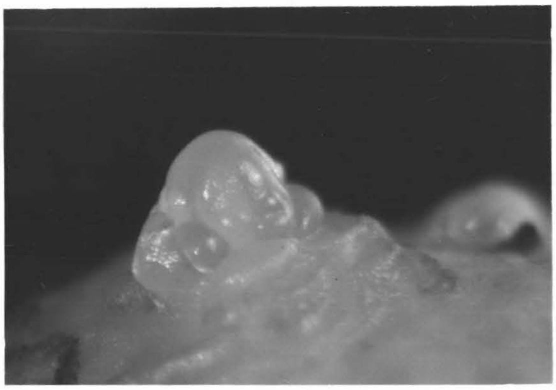
Stage 0



Stage 1

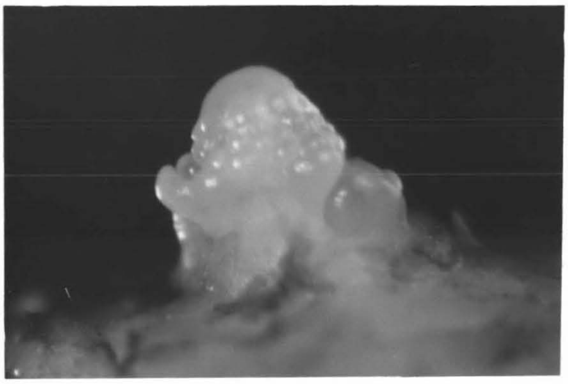


Stage 2

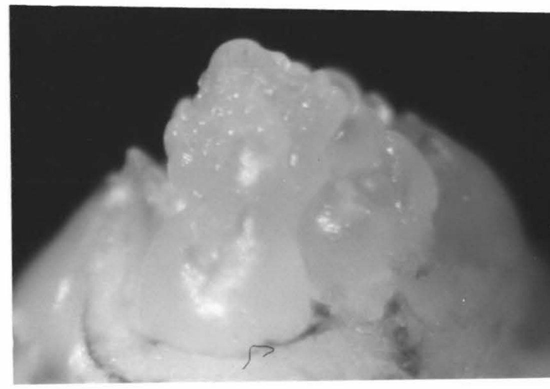


Stage 3

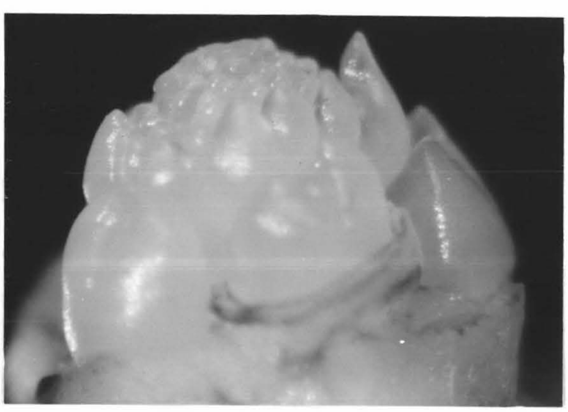
PLATE XIII Developmental stages of the inflorescence primordium X 400. For defination see text.



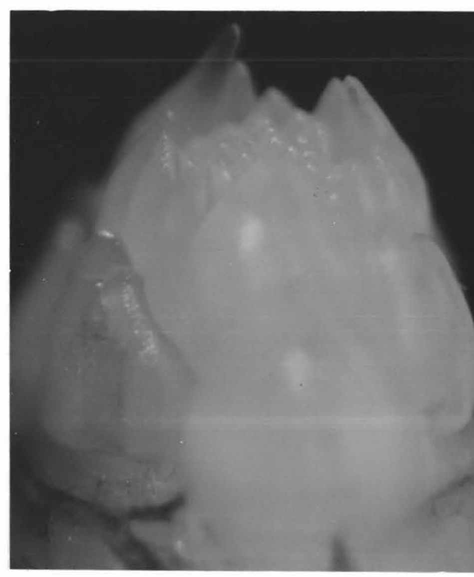
Stage 4



Stage 5



Stage 6



Stage 7

PLATE XIII Continued

The results are presented in Table XIX. The results indicated that plants flower d under long day and remained vegetative under short day. Further under long day plants fed with high nitrogen appeared to have earlier appearance of inflorescence buds than those fed with low nitrogen.

TABLE XIX

The effect of daylength and nitrogen on flowering

Daylength	Nitrogen	Percentage of plants with macroscopic inflorescence bud at days after treatment		
		52	60	77
Long	Low	28.6	71.4	100
	High	71.4	85.7	100
Short	Low	0	0	0
	High	0	0	0

(ii) Flowering time

Flower time was not statistically significantly affected by increasing the nitrogen level from 57ppm to 250 ppm in experiment II of section 5.1. However the present experiment showed that by increasing the nitrogen level the flowering time was significantly delayed ($P<0.01$). The average time of flowering time under low nitrogen was 90.13 days as compared to 95.38 days under high nitrogen (Appendix XIV).

(iii) Inflorescence development

An analysis of variance of the inflorescence development showed no significant differences between developmental stages for the two nitrogen levels when determined at 25, 26, 27 and 29 days after treatment.

5.2.3.2 Number of Flowers per Inflorescence

The data for the number of flowers per inflorescence were obtained from Experiment I and II in section 5.1 and the present experiment. When subjected to analysis of variance the difference between the number of flower per inflorescence in the high and low nitrogen treatments were found to be not significant at the 5% level for all the three experiments. (Appendix XIV.)

5.2.3.3 Number of Runners per Plant

Increasing the nitrogen level from 57 ppm to 250 ppm had no significant effect on runner number per plant in Experiment I and II in Section 5.1 and the present experiment. Comparing the results between experiments an interesting point can be noticed. Plants in the present experiment which were physiologically and morphologically older than those of the other two experiments had more runners per plant than those of the other experiments.

5.1.2.4 Discussion

It has been demonstrated by several investigators that leaf shape was affected by daylength. Ashby (1950) observed that in Ipomoea caerulea lobing of leaves in long days were more pronounced than those in short days. Similar findings were noted by Sen Gupta and Payne (1947) and Sanchez (1967) on different plant species. The results of this study showed that leaf length and the degree of leaf dissection were also increased by long day treatment (Table X). Independently of the effect of daylength , leaf position also influenced leaf shape. There was a progressive increase in leaf length and degree of leaf dissection from leaf position to leaf position up the main axis followed by a decline after reaching the maximum values (Table XI). This phenomenon has been observed by some investigators (eg. Ashby, 1950, Borrill, 1959, Aspinal and Paleg, 1964). According to Krenke's hypothesis (Ashby, 1948) the increasing amount of dissection or lobing of the leaves was associated with physiological age. The result of this study appeared to support this hypothesis as short day treatment delayed maturity through the process of inhibiting flowering while long day treatment hastened the process of maturity by promoting flowering. The findings of Ashby (1950) on I. caerulea in which both leaf shape and flowering were controlled by daylength did not support the Krenke's hypothesis. Borrill (1959) and Thomas (1961) noted that the heteroblastic changes were linked with floral development. These observations would be compatible with Krenke's hypothesis. The significant effect of leaf position on leaf shape in this study was probably an artifact as a result of long day treatment. The variation of leaf shape from leaf position to position was not significant under short days but was significant under long days.

There was a general tendency for number of emerged leaves per plant to increase with increasing daylength. The data of Friend et al (1962) of different plant species also showed that the rate of leaf production was increased by increasing the daylength. The increased number of emerged leaves per plant under long days could be caused by floral initiation under inductive long days. This would confirm the findings of Schwabe (1959), and of Langer and Bussell (1964) which showed that the increased rate of leaf production was associated with floral initiation.

The result of this study showed that both growth and flowering of E. vesiculosum were markedly influenced by daylength. Plants grown in long-days were significantly heavier than those grown in short-days (Table XIII). The heavier weight was due to the increase in top growth while the root growth was not significantly affected by daylength treatments. In the vegetative stage the top weight comprised of the radical leaves alone and in the reproductive stage the top included runners, lateral branches and inflorescences besides the radical leaves. Thus it was not unexpected that top weight of long-day treated plants in their reproductive stage was very much heavier than that of short-day treated plants which remained vegetative. The contribution made by the various organs towards the total plant weight of the long-day treated plants is shown in Table XX. However even when the weights estimated from radical leaves and roots for total dry weight and radical leaves alone for leaf dry weight were considered the long-day treated plants were still significantly heavier than those short-day treated plants though at a lower level of significance. The works of Friend et al (1967) on wheat and of Hughes and Evans (1963) on Impatiens parviflora also showed that increasing the daylength increased dry matter accumulation. Under long days the top growth exceeded root growth in E. vesiculosum resulting in a decline in root: top ratio (Table XVI). The result is similar to the findings of Gall (1947) on *Bromus inermis*.

TABLE XX

The percentage distribution of dry plant weights of long day treated plant at 91 days after treatment

Organs	Low nitrogen	High nitrogen
Radical leaves	12.0	10.7
Runner leaves	6.6	8.3
Runner stems	36.6	42.0
Lateral branches	7.0	7.5
Inflorescences	11.8	12.0
Roots	24.0	19.5

Leaf area was also found to be significantly affected by daylength treatment. Increasing the daylength from 8 hours to 16 hours resulted in an increased leaf area irrespective whether the area was estimated from radical leaves together with leaves from runners and lateral branches or from radical leaves alone. At 28 days after treatment the difference did not reach the 5% significance level possibly because the interval from start of the treatment to harvest was not adequately long enough for the daylength effect to become evident statistically. Further there was a initial depression caused by the high nitrogen treatment. The result contradicts the report of Sch wabe (1957) on *Xan.thium pennsylvanium*, *Beta vulgaris*, *Hyoscyamus niger* and tomato but is in agreement with the data of Arney (1956) and of

Gosselink and Smith (1967) on strawberry and of Friend et al (1967) on wheat.

Daylength had a very marked effect on flowering of E. vesiculosun. Plants grown in short days did not flower while those grown in long days were reproductive. In the photoperiodic control of flowering the length of the uninterrupted dark period is one of the important factors. It is generally accepted that the phytochrome for red (P 730) is promotive to flowering in long day plants and inhibitory to flowering in short day plants. The effect of short day is assumed to mediate through the P730 amd P660 (phytochrome red) balance. At long dark periods a level of P730 is established by thermal reversion of P730 → P660 which no longer inhibits flowering in short day plants and is too low to promote flowering of long day plants. Further the presence of high P660 checks reactions leading to hormone production (possibly flowering hormone or hormones) or may led to its inactivation or destruction.

Most of the growth response to increase in daylength in this study is similar to those reported for light intensity in the previous chapter. The effects of daylength may mediate mainly through changes in total radiant energy rather than the photoperiodic effect as such. The term daylength in this text is used as a general description without any suggestion as to which of the factors ie. light duration, light intensity or light quantity involved in it are caus al.

The significance of the nitrogen treatment on plant growth has already been discussed in the previous chapter (4.2.4 Discussion) and that of the daylength and nitrogen interaction had been dealt in the results. It is only necessary to add a few comments. Increasing the nitrogen level from 57ppm to 250ppm generally increased the dry weights and the leaf area but decreased the root: top ratio as found in the previous chapter. A initial depression of growth by high level of nitrogen was also observed which disappeared with time. High nitrogen enhanced root growth at 91 days after treatment; this positive effect of nitrogen was not observed in the previous chapter probably because of the shorter period of growth. The leaf shape expressed as leaf length or the number of teeth per leaf was significantly influenced by nitrogen levels which confirmed the findings of the previous chapter although the leaf shape was described in terms of length: breadth ratio. Increasing the nitrogen level from 57ppm to 250ppm increased the number of emerged leaves per plant. This confirms the general trend observed in the pervious chapter that high nitrogen increased leaf number (Table IX).

The influence of nitrogen levels in flowering was determined by a few criteria. Scoring the inflorescence primordium after a period of time was used and was found to be not significantly affected by nitrogen levels used in this study. However the score indicated that plants in low nitrogen appeared to initiate inflorescence primordia earlier than in high nitrogen. This would lead to the expectation that the low nitrogen treated plants would have shorter flowering time than the high nitrogen treated ones. The use of flowering time as an index of flowering is subject to error. The development of primordia to flowers is obviously influenced by environmental factors thus a longer flowering time does not necessarily indicate later floral initiation but may indicate slower development after initiation. The stage score system on microscopic inflorescence primordium is more satisfactory but may still be influenced by conditions during the development of the inflorescence primordium rather than conditions causing induction. However the error involved may be considered unimportant as the time period involved for the factors to act is short.

The results of Experiment 5.1 (II) showed that the high nitrogen treated plants had shorter flowering time than the low nitrogen treated whereas in Experiment 5.2 the reverse was observed. The conflicting results of Experiment 5.1. (II) amd of Experiment 5.2. may be caused by the different physiological and morphological age of the plants used in the two experiments. It is generally known that at flowering, a general internal redistribution of nutrients is initiated. In Experiment 5.1 (II) where young plants were used the nutrient available from redistribution from the small number of leaves and the small root system may be limiting the rate of development of primordia to flowers. Thus an external source of high nitrogen would be benefical to a faster rate of development from inflorescence primordia to flowers. In Experiment 5.2. where much older and bigger plants were used, the nutrient available from redistribution from the larger number of leaves and the bigger root system is not limiting the rate of flower development. Thus plants grown in a high level of nitrogen had no advantage over those grown in low nitrogen level. But since low nitrogen treated plants tended to have earlier inflorescence primordium initiation it is a reasonable assumption that their flowering time would be shorter than that of high nitrogen treated plants. Further investigations are necessary to confirm or refute this explanation.

The effects of nitrogen level on the number of flowers per inflorescence and the number of runners per plant were found to be not significant. Way and White (1968) working on strawberry plants found that the number of flowers per inflorescence was not influenced by nitrogen treatment in his 1963 experiment but that in the 1964 experiment nitrogen had a tendency to decrease the flower number per inflorescence. An interesting feature arose from these observations. The older plants used in Experiment 5.2. had higher values for number of flowers per inflorescence and for number of runners per plant than the younger plants in Experiment 5.1 (I) and Experiment 5.1.(II). If the promotive effect of using older plants on these two attributes persists on further investigations this feature may have practical implications in relation to other plant species where higher number of flowers per inflorescence and higher numbers of runners per plant would be an advantage. A case in mind would be the strawberry plant.

CHAPTER VI CONCLUSION AND SUMMARY

6.1 CONCLUSION

In the light of the results obtained from this study, the following conclusions can be drawn.

(a) Features of the plant apparently unaffected by the three external factors under study.

(i) The total cell number, epidermal cell size and stomatal characteristics were unaffected by nitrogen. Leaf morphology is measured in leaf length, leaf breadth and leaf length:breadth ratio was also independent of nitrogen.

(ii) The dry weight of roots was unaffected by daylength and it is little affected by nitrogen.

(iii) The dry weight of leaves was unaffected by light intensity.

(iv) The number of flowers for inflorescence and the number of runners per plant were unaffected by nitrogen.

(b) Features of the plant apparently affected by the three external factors under study.

(i) The total cell number, epidermal cell size, stomatal characteristics and chlorophyll content were effected by light intensity. Nitrogen also influenced the chlorophyll content. Leaf length, leaf dissection and number of leaves per plant were effected by daylength and nitrogen while the leaf length and leaf length:breadth ratio were affected by light intensity.

(ii) The total dry weight and leaf dry weight were dependent on daylength and nitrogen whereas total dry weight and root dry weight were affected by light intensity.

(iii) The root:top ratio was affected by day length, light intensity and nitrogen.

(iv) The leaf area was affected by nitrogen and light intensity; it was slightly affected by daylength.

(v) Flowering was dependent on daylength whereas the effect of nitrogen on flowering time was inconclusive.

(vi) The NAR, RGR AND $\overline{LAR}$ were affected by light intensity; nitrogen effected the $\overline{LAR}$ and the RGR but not the NAR.

6.2 SUMMARY

Some morphological aspects of E. vesiculosum are presented together with an account of the method of vegetative propagation of the plant. Determination procedures and methods are also described.

The influence of light intensity (Full 52% and 42% daylight), daylength (8 hours and 16 hours) and nitrogen

(57 ppm N and 250 ppm N) on the leaf anatomy and morphology and the plant growth and flowering was investigated in a number of glasshouse experiments.

A decrease in light intensity reduced the total cell number, the stomatal frequency and the stomatal length but increased the epidermal cell size. Leaf length and leaf length:breadth ratio were depressed by high light intensity. The result of this study showed in general that, in the whole plant and its parts, increasing the light intensity resulted in an increase in dry weight. The NAR was greater the higher the light intensity; the RGR followed a similar trend but the $\overline{LAR}$ was less the higher the light intensity. The plant showed a marked adaptation to growth in shade by the compensating increase of leaf area with decrease in light intensity.

Leaf anatomy was little affected by nitrogen treatment. An increase in chlorophyll occurred with high light intensity and high nitrogen. High nitrogen also increased the leaf length and leaf dissection. The dry weight of whole plant, the leaf dry weight and the leaf area were in general increased by increases in nitrogen level. The $\overline{LAR}$ and the RGR were found to be higher with high nitrogen level while no treatment effect was detected for the NAR.

Increasing the daylength from 8 hours to 16 hours induced flowering. Under short days flowering was inhibited. Plants grown under long days were heavier as a whole than those grown under short days. Long days increased the leaf length, leaf dissection and root:top ratio.

Other aspects of changes in dry weight proportions in response to light intensity and nitrogen were also presented. Mechanisms by which light intensity, nitrogen and daylength might regulate growth were discussed.

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APPENDIX I

Mean Temperature and Mean Light Energy During the Experimental Period

Experiment	Date	Temperature °C			Light Energy
		Min.	Max.	Average	cal./cm^2/day.
Effects of light intensity and nitrogen on:					
1. Anatomy and Morphology	27/3-22/5	14.2	24.2	19.2	174
2. Growth	23/3-2/4	16.0	26.7	21.5	204
	2/4 -12/4	14.7	25.6	20.2	193
	12/4-22/4	14.5	24.5	19.5	168
	22/4-2/5	13.9	24.8	19.4	172
	2/5 -12/5	13.3	21.9	17.6	146
Effects of daylength and nitrogen on:					
1. Growth					
Expt.5.1(I)	23/3-8/6	14.3	24.3	19.3	149
Expt.5.1(II)	8/7 -13/10	12.0	23.4	17.7	169
2. Flowering					
Expt. 5.2	26/3-25/6	14.2	24.2	19.2	164

APPENDIX II

Light Transmission of Shadings 1, 2 and 3

(a) Light transmission of Shading 1 (i.e. glasshouse) measured by Eppley pyrheliometer. (Values expressed as a percentage of the unshaded figures).

	Unshaded (outside glasshouse)	Shading 1 (inside glasshouse)
	100	75.6
	100	74.2
	100	79.5
	100	82.8
	100	80.3
	100	68.5
Mean	100	76.8 ± 2.1

(b) Light transmission of Shadings 2 and 3. (Values expressed as a percentage of Shading 1).

Instrument	Shading 1 ('full' daylight)	Shading 2	Shading 3
EEL Lightmaster	100	54.1	44.8
	100	54.3	43.5
	100	51.2	41.3
	100	51.7	42.7
	100	52.2	42.2
Mean	100	52.7 ± .6	42.9 ± .6
Eppley pyrheliometer	100	51.0	42.1

APPENDIX III

Soil Temperature under the Shadings 1, 2 and 3

Day	Time	Shading 1 °C	Shading 2 °C	Shading 3 °C
1	8.30 a.m.	14.0	14.0	14.5
	1.00 p.m.	23.0	21.0	21.0
	3.00 p.m.	23.5	22.0	22.0
2	8.45 a.m.	14.0	14.0	14.0
	Noon	25.0	23.0	23.0
	4.00 p.m.	28.0	25.5	25.0
	5.30 p.m.	27.0	24.5	25.0
	8.00 p.m.	18.0	18.0	18.0
3	1.30 p.m.	24.0	23.0	22.0
4	9.00 a.m.	16.0	15.0	15.0
	Noon	21.0	20.0	20.0

APPENDIX IV

Composition of Nutrient Solutions

HIGH NITROGEN NUTRIENT SOLUTION (250 p.p.m. N)

Salts	Weight in grams/100 litres	p.p.m.
Major nutrients:		
KNO_3	40.4	N57 K156
$CaCl_2$	44.4	Ca160 Cl284
NH_4NO_3	55.1	N193
$MgSO_4 7H_2O$	36.8	Mg36 S48
$NaH_2PO_4 2H_2O$	20.8	Na31 P41
Minor nutrients:		
Iron chelate	1.84	Fe2.8
$MnSO_4 4H_2O$	0.223	Mn0.55
$CuSO_4 5H_2O$	0.025	Cu0.064
$ZnSO_4 7H_2O$	0.029	Zn0.065
H_3BO_3	0.186	B0.33
$(NH_4)_6Mo_7O_{24}4H_2O$	0.0088	Mo0.048

LOW NITROGEN NUTRIENT SOLUTION (57 p.p.m.N)

Salts	Weight in grams/100 litres	p.p.m.	
Major nutrients:			
KNO_3	40.4	N57	K156
$CaCl_2$	44.4	Ca160	Cl284
$MgSO_4 7H_2O$	36.8	Mg36	S48
$NaH_2PO_4 2H_2O$	20.8	Na31	P41
Minor nutrients:			
Iron chelate	1.84	Fe2.8	
$MnSO_4 4H_2O$	0.223	Mn0.55	
$CuSO_4 5H_2O$	0.025	Cu0.064	
$ZnSO_4 7H_2O$	0.029	Zn0.065	
H_3BO_3	0.186	B0.33	
$(NH_4)_6Mo_7O_{24} 4H_2O$	0.0088	Mo0.048	

APPENDIX V

A method used in the analysis of variance of split plot design of total cell number

1. Analysis Table

Source of variation	df	ss	ms	F	Result
Main plots					
Light	2	6887068	3443534	12.99	* *
Blocks	3	3939321	1313107	4.95	*
Main plot error	6	1590250	265042		
Sub plots					
Nitrogen	1	146797	146797	.23	NS
Nitrogen x Light	2	372658	186329	.30	NS
Sub plot error	9	5682755	631417		
Total	23	18618849			

11 Table of all items: Total Cell number (/mm^2)

		BLOCKS			
Light	Nitrogen	1	. 2	3	4
Full	Low	5883	6657	5529	6369
	High	6192	6856	5794	5573
		12075	13513	11323	11942
52%	Low	4689	5264	4069	4777
	High	6237	5352	4821	4423
		10926	10616	8890	9200
42%	Low	5640	4180	3782	6414
	High	5109	6170	4202	4401
		10749	10350	7984	10815
TOTAL		33750	34479	28197	31957

Cont'd . . . .

III Table of Light x Nitrogen: Total Cell Number ($/mm^2$)

LIGHT	NITROGEN LOW	NITROGEN HIGH	TOTAL
Full	24438	24415	48853
52%	18799	20833	39632
42%	20016	19882	39898
TOTAL	63252	65130	128383

1. Correction: $C = (128383)^2/24 = 686758112$
2. Total: $(5883)^2 + \ldots + (4401)^2 - C = 18618849$
3. Main plots: $(12075)^2 + \ldots + (10815)^2/2 - C = 12416639$
4. Light: $(48853)^2 + \ldots + (39898)^2/8 - C = 6887068$
5. Blocks: $(33750)^2 + \ldots + (31957)^2/6 - C = 3939321$
6. Main plot error: 12416639 - (6887068 + 3939321) = 1590250
7. Sub-classes in Light x Nitrogen tabe: $(24438)^2 + \ldots + (19882)^2/4 - C$ = 7406523
8. Nitrogen: $(63252)^2 + (65130)^2/12 - C = 146797$
9. Light x Nitrogen: 7406523 - (6887068 + 146797) = 372658
10. Sub-plot error: 18618849)- (12416639 + 146797 + 372658) = 5682755

IV Least significant differences (LSD)

LSDs are calculated for main plot treatments, sub-plot treatments and for interaction effects.

The symbols in the following formulas are:

Ea = MS for main plot error = 265042

Eb = MS for sub-plot error = 631417

r = number of blocks = 4

a = number of main plot treatments = 3

b = number of sub-plot treatments = 2

ta = t valve for d.f. for main plot error

tb = t valve for d.f. for sub-plot error.

The subscripts .05 and .01 designate the 5% and 1% levels of significance.

LSD for differences between light means.

$ta_{.05}(6\ d.f.) = 2.447$

$ta_{.01}(6\ d.f.) = 3.707$

$$LSD = ta\sqrt{\frac{2\ Ea}{r\,b}} = ta\sqrt{\frac{(2)(265042)}{(4)(2)}} = ta\ (257)$$

$LSD_{.05} = 2.447\ (257) = 629$ cells per mm^2

$LSD_{.01} = 3.707\ (257) = 953$ cells per mm^2

Cont'd . . . .

LSD for differences between nitrogen means.

$tb_{.05}(9 \text{ d.f.}) = 2.262$

$tb_{.01}(9 \text{ d.f.}) = 3.250$

$$LSD = tb\sqrt{\frac{2\ Eb}{ra}} = tb\sqrt{\frac{(2)(631417)}{(4)(3)}} = tb(324)$$

$LSD_{.05} = 2.262\ (324) = 733$ cells per mm^2

$LSD_{.01} = 3.250\ (324) = 1053$ cells per mm^2

LSD for differences between nitrogen means at the same light level.

$$LSD = tb\sqrt{\frac{2\ Eb}{r}} = tb\sqrt{\frac{(2)(631417)}{4}} = tb(562)$$

$LSD_{.05} = 2.262\ (562) = 1271$ cells per mm^2

$LSD_{.01} = 3.250\ (562) = 1827$ cells per mm^2

v Coefficient of variation (C.V.)

Coefficient of variation was calculated using the following formula.

$$C.V. = \frac{\sqrt{Eb}}{\overline{X}} \times 100\%$$

where Eb = M.S. for sub-plot error

and $\overline{X}$ = means of the sum of all the values in the analysis.

APPENDIX VI

Analysis of variance

Character		Total cell number (/mm^2)		Epidermal cell size (/µ^2)		Stomatal frequency (/mm^2)		Stomatal length (/µ)		Stomatal breadth (/µ)	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Main plots											
Light	2	34435	**	11178	*	13441	*	3.8226	*	5.477	ns
Blocks	3	13131	*	7326	ns	4805	ns	.7426	ns	3.657	ns
Main plot error	6	2650		2043		1428		.6647		1.086	
Sub plots											
Nitrogen	1	1467	ns	136	ns	2147	ns	.6402	ns	.060	ns
Nitrogen x Light	2	1863	ns	304	ns	1951	ns	.7558	ns	.538	ns
Sub plot error	9	6314		1332		729		.2855		1.253	
C.V.		14.9		9.5		8.1		1.9		6.2	

APPENDIX VI (contd)

Analysis of variance

Character		Leaf Length (cm)		Leaf Breadth (cm)		Leaf Length : Breadth	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F
Main plots							
Light	2	84.18	*	.0062	ns	192.09	**
Blocks	3	3.23	ns	.0167	ns	29.98	ns
Main plot error	6	9.45		.0201		9.30	
Sub plots							
Nitrogen	1	2.04	ns	.0073	ns	.32	ns
NitrogenxLight	2	1.11	ns	.0223	ns	16.92	ns
Sub plot error	9	4.96		.0196		23.12	
C.V.		18.8		22.6		21.9	

APPENDIX VI (contd)

Analysis of variance

Character		Chloro. content on unit area (mg x 10^{-1}/cm^2)						Chloro. content on unit fresh weight - (mgx.10/mg)							
		Total chloro.		Chloro. a		Chloro. b		total chloro.		Chloro. a		Chloro. b		Chloro. a/b	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Main plots															
Light	2	.01803	*	.00667	*	.00274	ns	.584	ns	.2787	ns	.1518	ns	.0007	ns
Blocks	3	.02491	*	.00816	*	.00458	*	10.157	**	2.8068	**	2.3742	*	.0034	ns
Main plot error	6	.00303		.00091		.00074									
Sub plots															
Nitrogen	1	.03480	*	.01092	*	.00673	**	56.730	**	21.6467	**	18.2908	**	.0035	ns
Nitrogen x light	2	.00522	ns	.00200	ns	.00077	ns	.116	ns	.0311	ns	.3207	ns	.0007	ns
Sub plot error	9	.00340		.00124		.00056		2.093		.6970		.4973		.0022	
C.V.		9.4		9.5		9.4		10.8		10.4		13.2		3.2	

APPENDIX VII

Each mean with standard error is from measurement of 4 blocks

Character	Full daylight		52% daylight		42% daylight	
	Low nitrogen	High nitrogen	Low nitrogen	High nitrogen	Low nitrogen	High nitrogen
Total cell number (/mm^2)	6110 ± 251	6104 ± 282	4700 ± 246	5208 ± 392	5004 ± 618	4971 ± 445
Epidermal cell area (/μ^2)	1104 ± 36	1066 ± 63	1294 ± 77	1256 ± 43	1287 ± 145	1317 ± 71
Stomatal frequency (/mm^2)	371 ± 12	386 ± 18	322 ± 23	276 ± 17	333 ± 27	307 ± 23
Stomatal length (/μ)	28.27 ± .42	28.70 ± .42	27.35 ± .26	28.23 ± .50	27.27 ± .21	26.93 ± .15
Stomatal breadth (/μ)	19.27 ± .36	18.57 ± .79	17.40 ± .65	17.57 ± .73	17.37 ± .61	17.60 ± .59
Leaf length (cm)	9.8 ± 1.6	10.3 ± 1.3	12.6 ± .6	13.9 ± 1.0	16.6 ± 1.9	16.5 ± .7
Leaf breadth (cm)	.65 ± .07	.56 ± .07	.54 ± .04	.66 ± .08	.61 ± .04	.69 ± .11
Leaf length:leaf breadth	15.0 ± .7	18.6 ± 1.4	23.3 ± .9	21.6 ± .9	27.0 ± 2.2	26.0 ± 4.6
Total chlorophyll(mgx10^{-1}/cm^2)	.634 ±.005	.711 ±.063	.555 ±.057	.682 ±.043	.565 ±.017	.590 ±.031
Chloro. a (mgx10^{-1}/cm^2)	.381 ±.005	.422 ±.035	.330 ±.034	.406 ±.024	.338 ±.012	.350 ±.018
Chloro. b (mgx10^{-1}/cm^2)	.253 ±.005	.289 ±.028	.225 ±.024	.277 ±.019	.228 ±.005	.241 ±.013
Total chloro (mgx10^{-4}/mg freshwt)	11.52 ± .40	14.62 ± 1.33	11.95 ± .63	15.26 ± .43	12.00 ± .27	14.82 ± 1.35
Chloro a (mgx10^{-4}/mg freshwt)	6.92 ± .19	8.67 ± .76	7.11 ± .38	9.07 ± .24	7.15 ± .20	9.13 ± .69
Chloro (")	4.61 ± .22	5.95 ± .58	4.84 ± .28	6.19 ± .21	4.85 ± .10	5.69 ± .78
Chlorophyll a : b	1.51 ± .05	1.47 ± .03	1.47 ± .05	1.47 ± .03	1.48 ± .03	1.46 ± .02

APPENDIX VIII

Analysis of variance of total dry weight (mg)

Harvest		2		3		4		5		6	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	7	1496.0	*	4076	ns	12955	**	19254	ns	7201	ns
Light (L)	2	2174.6	*	11206	*	23073	**	45682	*	26532	*
Error (a)	14	449.4		2115		2074		8647		4138	
Nitrogen(N)	1	187.8	ns	790	ns	21903	**	33227	ns	18103	**
LXN	2	721.2	ns	626	ns	1330	ns	691	ns	3888	ns
Error (b)	21	349.3		913		2095		38839		1782	
C.V.		14.9%		13.8%		13.9%		38.3%		17.8%	

Analysis of variance of leaf dry weight (mg)

Harvest		2		3		4		5		6	
Source	D.F.	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	7	289.47	*	1163.0	*	5468	**	8578	ns	1509	ns
Light (L)	2	79.17	ns	329.4	ns	1174	ns	5873	ns	2494	ns
Error (a)	14	88.01		402.9		350		4494		1909	
Nitrogen(N)	1	.01	ns	735.0	ns	23366	**	48669	**	14179	**
LXN	2	211.52	ns	47.6	ns	486	ns	3359	ns	2127	ns
Error (b)	21	71.81		279.4		1002		2333		670	
C.V.		15.2%		16.9%		18.6%		16.0%		19.0%	

Analysis of variance of root dry weight (mg)

Harvest		2		3		4		5		6	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	7	526.7	*	1037	ns	1982	ns	2453	ns	1299	ns
Light (L)	2	1468.2	**	11525	**	19295	**	56483	**	24729	**
Error (a)	14	156.8		844		1160		952		530	
Nitrogen(N)	1	188.9	ns	1	ns	24	ns	1469	ns	239	ns
LXN	2	179.4	ns	818	ns	1333	*	1137	ns	333	ns
Error (b)	21	152.1		238		369		1609		414	
C.V.		17.8%		12.9%		12.0%		18.8%		20.2%	

Analysis of variance of leaf area (cm^2)

Harvest		2		3		4		5		6	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	7	130.86	*	456.43	*	21.46	**	49.42	ns	104.88	ns
Light (L)	2	235.44	*	932.13	*	35.02	**	304.26	**	3389.1	*
Error (a)	14	39.61		160.67		13.3		25.53		70.49	
Nitrogen (N)	1	.112	ns	.083	ns	121.0	**	257.06	**	3460.3	**
LXN	2	126.37	*	18.94	ns	5.1	ns	247.1	ns	747.3	ns
Error (b)	21	28.59		10.60		33.7		12.55		269.8	
C.V.		14.7%		16.6%		17.3%		16.2%		19.4%	

Analysis of variance of specific leaf area (cm^2/g)

Harvest		2		3		4		5		6	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	3	85.6	ns	528.3	ns.	220.8	ns	283.5	ns	521	ns
Light (L)	2	6401.5	**	3221.9	**	5896.9	**	9435.9	**	11082	**
Error (a)	6	106.6		226.5		151.0		202.5		388	
Nitrogen (N)	1	.8	ns	1217.0	ns	533.0	ns	.1	ns	500	ns
LXN	2	92.8	ns	84.5	ns	292.0	ns	511.9	ns	343	ns
Error (b)	9	234.7		281.8		188.5		336.0		302	
C.V.		7.4%		8.4%		7.0%		8.1%		9.0%	

Analysis of variance of root : top ratio

Harvest		2		3		4		5		6	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	7	.03332	*	.0459	ns	.04635	ns	.00732	ns	.0122	ns
Light (L)	2	.26373	**	1.4584	**	.88610	**	.97606	**	1.5167	**
Error (a)	14	.00947		.0372		.02887		.00812		.0130	
Nitrogen (N)	1	.11900	ns	.1082	*	.78515	**	.57729	**	.2049	**
LXN	2	.03595	ns	.1470	**	.11677	*	.15478	**	.0216	ns
Error (b)	21	.03723		.0189		.02146		.01481		.0083	
C.V.		15.5%		11.2%		15.0%		16.4%		12.0%	

Analysis of variance of leaf area ratio (cm^2/g)

Harvest		2		3		4		5		6	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	7	54.5	*	62.6	ns	95.5	ns	44	ns	26	ns
Light (L)	2	4782.3	**	6203.5	**	9585.1	**	21289	**	20461	**
Error (a)	14	15.2		56.9		84.6		42		31	
Nitrogen (N)	1	200.5	ns	188.5	*	3406.2	**	2680	**	21	ns
LXN	2	122.4	ns	78.3	ns	150.9	ns	663	**	1	ns
Error (b)	21	64.9		25.7		43.9		96		33	
C.V.		8.7%		5.6%		6.5%		7.3%		4.9%	

Analysis of variance of leaf weight ratio (mg/mg)

Harvest		2		3		4		5		6	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	7	.00132	*	.00169	ns	.00270	ns	.00083	ns	.00081	ns
Light (L)	2	.00987	**	.04993	**	.04733	**	.09038	**	.13736	**
Error (a)	14	.00034		.00144		.00219		.00085		.00098	
Nitrogen (N)	1	.00456	ns	.00258	ns	.04705	**	.04909	**	.01942	**
LXN	2	.00151	ns	.00394	*	.00411	ns	.00800	*	.00165	ns
Error (b)	21	.00148		.00069		.00613		.00174		.00081	
C.V.		8.6%		5.8%		15.2%		7.1%		4.9%	

Analysis of variance of leaf number

Harvest		2		3		4		5		6	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	3	.72	ns	3.83	ns	3.82	ns	4.94	ns	3.60	ns
Light (L)	2	.04	ns	.17	ns	1.17	ns	4.17	ns	3.17	ns
Error (a)	6	.43		1.47		.94		2.11		.89	
Nitrogen (N)	1	0	ns	.66	ns	18.38	*	.66	ns	.04	ns
LXN	2	.38	ns	.67	ns	2.00	ns	.17	ns	.17	ns
Error (b)	9	.25		1.00		2.01		1.22		1.79	
C.V.		5.5%		8.3%		11.6%		6.3%		7.7%	

Analysis of variance of net assimilation rate (mg/cm^2/day)

Harvest interval		1		2		3		4		5	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	7	.18838	ns	.01257	ns	.01781	ns	.03686	ns	.08947	ns
Light (L)	2	.61582	*	.44885	*	.15305	ns	.18515	*	.50149	**
Error (a)	14	.10795		.11278		.08680		.03701		.04324	
Nitrogen (N)	1	.03218	ns	.02128	ns	.02816	ns	.04114	ns	.00064	ns
LXN	2	.05648	ns	.20065	ns	.013616	ns	.00567	ns	.06611	ns
Error (b)	21	.03861		.07293		.02456		.05668		.05437	
C.V.		45.3%		43.0%		35.6%		60.7%		73.7%	

Analysis of variance of mean leaf area ratio (cm^2/g)

Harvest interval		1		2		3		4		5	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	7	.00003	ns	.00003	ns	.00004	ns	.00004	ns	.00002	ns
Light (L)	2	.00137	**	.00553	**	.00758	**	.01478	**	.02094	**
Error (a)	14	.00002		.00003		.00005		.00004		.00003	
Nitrogen (N)	1	.00005	ns	.00018	*	.00068	**	.00301	**	.00065	**
LXN	2	.00003	ns	.00003	ns	.00002	ns	.00006	ns	.00013	**
Error (b)	21	.00002		.00003		.00003		.00004		.00002	
C.V.		4.7%		5.7%		6.0%		5.5%		3.8%	

Analysis of variance of relative growth rate (mg/mg/day)

Harvest interval		1		2		3		4		5	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	7	.00137	ns	.00016	ns	.00016	ns	.00034	ns	.00090	ns
Light (L)	2	.00216	ns	.00017	ns	.00024	ns	.00001	ns	.00088	ns
Error (a)	14	.00096		.00102		.00073		.00042		.00038	
Nitrogen (N)	1	.00021	ns	.00000	ns	.00082	ns	.00001	ns	.00020	ns
LXN	2	.00054	ns	.00017	ns	.00017	ns	.00013	ns	.00156	ns
Error (b)	21	.00025		.00060		.00025		.00047		.00052	
C.V.		43.3%		43.9%		38.3%		48.8%		64.6%	

APPENDIX VIII

Analysis of variance of NAR, $\overline{LAR}$, and RGR (Harvest interval taken as a factor)

Character		NAR ($mg/cm^2/day$)		$\overline{LAR}$ (cm^2/mg)		RGR (mg/mg/day)	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F
Blocks	7	.0422	ns	.0001	ns	.0003	ns
Light (L)	2	1.7366	**	.0428	**	.0012	*
Error	14	.0253		.0000		.0002	
Nitrogen (N)	1	.0140	ns	.0014	**	.0006	**
LXN	2	.0192	*	.0000	ns	.0001	ns
Error	21	.0050		.0000		.0000	
Harvest (H)	4	.6362	**	.0140	**	.0032	**
HXL	8	.0419	ns	.0018	**	.0005	ns
HXN	4	.0273	ns	.0007	**	.0001	ns
HXNXL	8	.0356	ns	.0000	**	.0003	ns
Error	168	.0730		.0000		.0006	
C.V.		61.10%		4.06%		57.51%	

APPENDIX IX

Means and standard errors of raw data

Each mean with standard error is from measurement of 8 blocks

Character	Harvest	Treatment					
		Full daylight		52% daylight		42% daylight	
		Low nitrogen	High nitrogen	Low nitrogen	High nitrogen	Low nitrogen	High nitrogen
Total dry weight (mg)	1	base harvest :	86.27 ± 4.56				
	2	143.88 ± 3.02	132.49 ± 3.61	115.90 ± 3.22	126.72 ± 2.42	109.78 ± 2.81	122.34 ± 2.73
	3	249.22 ± 6.06	244.41 ± 5.94	210.95 ± 6.14	219.93 ± 5.81	184.12 ± 4.31	204.29 ± 3.27
	4	350.14 ± 9.08	378.15 ± 8.90	306.66 ± 5.64	386.09 ± 7.42	270.84 ± 6.18	307.88 ± 8.88
	5	530.30 ± 13.14	592.94 ± 8.27	498.13 ± 14.21	555.61 ± 9.16	437.86 ± 14.55	475.60 ± 9.53
	6	854.19 ± 21.64	896.19 ± 27.49	635.43 ± 16.64	868.10 ± .03	570.82 ± 17.88	664.63 ± 25.92
Leaf dry weight(mg)	1	base harvest :	45.32 ± 1.55				
	2	61.77 ± 1.17	53.92 ± 1.40	55.41 ± 1.26	56.89 ± 1.52	50.20 ± 1.11	56.67 ± 1.49
	3	91.42 ± 2.38	102.35 ± 3.16	102.12 ± 3.34	106.24 ± 3.23	91.64 ± 2.19	100.08 ± 1.36
	4	143.28 ± 4.37	193.41 ± 7.22	154.30 ± 3.85	205.14 ± 4.47	147.24 ± 3.37	178.65 ± 5.10
	5	238.46 ± 6.54	330.63 ± 5.11	289.91 ± 10.54	354.56 ± 7.29	280.32 ± 10.08	314.56 ± 7.02
	6	390.44 ± 11.02	449.23 ± 16.32	367.96 ± 8.58	560.35 ± 19.64	367.72 ± 11.28	442.64 ± 18.06
Root dry weight(mg)	1	base harvest :	40.95 ± 1.64				
	2	82.11 ± 2.13	78.57 ± 2.28	60.48 ± 1.99	69.83 ± 1.28	59.58 ± 1.74	65.67 ± 1.39
	3	157.80 ± 3.83	142.06 ± 3.48	108.83 ± 3.05	113.69 ± 2.90	92.48 ± 2.25	104.22 ± 2.05

Means and standard errors of raw data

Root dry weight(mg)	4	206.87 ± 4.88	184.74 ± 3.95	152.35 ± 2.44	164.63 ± 3.29	123.60 ± 3.47	129.23 ± 4.03
	5	260.71 ± 6.75	262.32 ± 5.24	208.01 ± 4.80	201.05 ± 3.73	157.54 ± 5.17	161.04 ± 2.61
	6	463.76 ± 11.92	446.96 ± 12.70	267.47 ± 8.41	307.75 ± 8.50	203.10 ± 7.07	221.99 ± 8.14
Total leaf area(cm²)	1	base harvest :	7.00 ± .02				
	2	11.00 ± .21	9.18 ± .24	12.02 ± .27	12.43 ± .33	11.30 ± .25	13.00 ± .34
	3	16.97 ± .44	16.97 ± .52	21.95 ± .72	21.39 ± .65	19.88 ± .47	20.69 ± .28
	4	23.34 ± .73	32.61 ± 1.22	30.99 ± .77	41.79 ± .91	30.95 ± .71	41.59 ± 1.19
	5	46.21 ± 1.27	60.24 ± .93	67.35 ± 2.45	90.13 ± 1.85	71.51 ± 2.57	78.61 ± 1.75
	6	65.79 ± 1.86	70.13 ± 2.55	79.19 ± 1.85	110.67 ± 3.88	83.36 ± 2.56	98.49 ± 4.02
Specific leaf area (cm²/gm)	1	base harvest :	169.3 ± 7.7				
	2	178.0 ± 2.9	170.0 ± 1.4	217.2 ± 5.9	218.9 ± 4.0	224.2 ± 10.7	229.3 ± 8.6
(mean is from measurement of 4 blocks)	3	186.3 ± 3.9	165.2 ± 6.6	215.2 ± 7.1	201.8 ± 7.9	216.3 ± 2.0	208.2 ± 16.8
	4	166.9 ± 8.1	169.4 ± 5.6	201.3 ± 8.2	203.6 ± 2.5	210.0 ± 4.3	233.4 ± 9.1
	5	190.2 ± 3.9	183.3 ± 5.2	227.1 ± 12.8	245.6 ± 9.6	256.0 ± 9.3	247.1 ± 6.3
	6	154.6 ± 15.1	151.7 ± 10.2	215.8 ± 7.6	191.6 ± 7.6	225.9 ± 4.8	225.6 ± 9.0
Root: top ratio	1	base harvest :	.908 ± .027				
	2	1.334 ± .079	1.454 ± .016	1.076 ± .016	1.258 ± .028	1.177 ± .013	1.174 ± .018
	3	1.735 ± .058	1.419 ± .032	1.085 ± .021	1.091 ± .024	1.014 ± .012	1.039 ± .012
	4	1.464 ± .018	1.016 ± .037	1.005 ± .019	.881 ± .010	.846 ± .018	.724 ± .012
	5	1.124 ± .011	.799 ± .016	.743 ± .017	.578 ± .015	.571 ± .012	.516 ± .004
	6	1.201 ± .021	1.018 ± .017	.723 ± .010	.561 ± .008	.553 ± .008	.506 ± .006

APPENDIX IX (contd)

Means and standard errors of raw data

Net assimilation rate ($mg/cm^2/day$)	1	.7029 ± .0343	.6175 ± .0335	.2713 ± .0401	.3939 ± .0317	.2504 ± .0394	.3685 ± .0408
	2	.7559 ± .0435	.8783 ± .0360	.5757 ± .0428	.5643 ± .0338	.4900 ± .0203	.5054 ± .0238
	3	.5056 ± .0294	.5627 ± .0363	.3965 ± .0268	.4985 ± .0230	.3458 ± .0174	.3321 ± .0203
	4	.5335 ± .0525	.4965 ± .0238	.3987 ± .0220	.2967 ± .0130	.3325 ± .0147	.2960 ± .0141
	5	.5911 ± .0504	.4444 ± .0390	.1929 ± .0168	.2994 ± .0200	.1762 ± .0183	.1943 ± .0147
Mean leaf area ratio (cm^2/mg)	1	.0783 ± .0006	.0744 ± .0005	.0940 ± .0005	.0905 ± .0008	.0919 ± .0005	.0933 ± .0005
	2	.0719 ± .0005	.0694 ± .0006	.1039 ± .0006	.0971 ± .0009	.1059 ± .0005	.1036 ± .0006
	3	.0679 ± .0004	.0776 ± .0012	.0999 ± .0008	.1053 ± .0007	.1114 ± .0007	.1188 ± .0007
	4	.0777 ± .0004	.0938 ± .0008	.1179 ± .0010	.1375 ± .0009	.1390 ± .0008	.1507 ± .0006
	5	.0814 ± .0005	.0884 ± .0006	.1292 ± .0005	.1424 ± .0008	.1540 ± .0008	.1558 ± .0004
Relative growth rate (mg/mg/day)	1	.0545 ± .0025	.0453 ± .0024	.0256 ± .0038	.0360 ± .0032	.0232 ± .0035	.0346 ± .0038
	2	.0545 ± .0033	.0616 ± .0031	.0601 ± .0045	.0543 ± .0034	.0521 ± .0022	.0520 ± .0023
	3	.0340 ± .0020	.0440 ± .0032	.0388 ± .0026	.0527 ± .0027	.0386 ± .0019	.0396 ± .0024
	4	.0413 ± .0040	.0459 ± .0021	.0471 ± .0027	.0411 ± .0019	.0462 ± .0022	.0446 ± .0022
	5	.0476 ± .0040	.0393 ± .0035	.0248 ± .0021	.0424 ± .0028	.0274 ± .0029	.0304 ± .0023
Leaf weight ratio	1	base harvest :	.5264 ± .1105				
	2	.4318 ± .0141	.4084 ± .0075	.4834 ± .0112	.4467 ± .0158	.4601 ± .0082	.4617 ± .0106
	3	.3666 ± .0075	.4173 ± .0157	.4821 ± .0132	.4816 ± .0147	.4975 ± .0084	.4913 ± .0083
	4	.4070 ± .0086	.5055 ± .0261	.5011 ± .0134	.5539 ± .0087	.5448 ± .0160	.5814 ± .0109
	5	.4477 ± .0065	.5584 ± .0143	.5769 ± .0165	.6366 ± .0155	.6384 ± .0135	.6599 ± .0051

APPENDIX IX (contd)

Means and standard errors of raw data

Leaf weight ratio	6	.4565 ± .0120	.4976 ± .0134	.5816 ± .0095	.6417 ± .0094	.6449 ± .0099	.6644 ± .0070
Leaf number	1	base harvest	: 6.4 ± .2				
(mean is from measurement of 4 blocks)	2	9.3 ± .5	9.0 ± .0	8.8 ± .5	9.3 ± .3	9.3 ± .3	9.0 ± .0
	3	11.8 ± .3	12.8 ± .9	12.0 ± .4	12.0 ± .6	12.0 ± .4	12.0 ± .6
	4	12.8 ± .3	15.5 ± 1.3	14.3 ± .5	15.0 ± .7	13.0 ± .6	14.8 ± .5
	5	18.0 ± .8	18.0 ± .0	17.8 ± .9	18.3 ± .9	16.5 ± .5	17.0 ± .9
	6	17.3 ± .8	17.5 ± .9	18.0 ± .4	17.8 ± .8	16.5 ± .7	16.8 ± .5
Instantenous leaf area ratio (cm^2/g)	1	base harvest	: 81.27 ± 1.11				
	2	76.87 ±.88	69.54 ± .45	104.85 ± .86	97.62 ± 1.22	103.62 ± .65	105.90 ± .86
	3	68.06 ± .49	96.21 ± .83	103.63 ± 1.00	96.97 ± 1.05	107.93 ± .64	101.56 ± .61
	4	67.75 ± .51	85.24 ± 1.56	100.63 ± .95	108.80 ± .62	114.53 ± 1.19	135.36 ± .90
	5	92.12 ± .44	101.75 ± .92	134.03 ± 1.35	161.84 ± 1.40	162.87 ± 1.22	164.93 ± .45
	6	75.51 ± .72	77.69 ± .74	125.17 ± .72	126.74 ± .65	146.22 ± .79	147.85 ± .55

APPENDIX X

Equations for the regression of NAR, $\overline{LAR}$ and RGR on logarithm of the light intensity.

Harvest interval		Regression equation	Coef. of correlation
NAR	1	Y= .9567x - 1.2626	.9842
	2	= .8297x - .8432	.9996
	3	= .4550x - .3667	.9389
	4	= .5313x - .5500	.9967
	5	= .8753x - 1.2361	.9979
$\overline{LAR}$	1	Y= .1668 - .0450x	.9765
	2	= .2540 - .9014x	.9926
	3	= .2876 - .1076x	.9986
	4	= .3862 - .1504x	.9989
	5	= .4424 - .1789x	.9996
R6R	1	$Y= - .2106 + .2164x - .0431x^2$	
	2	$= - .2142 + .2878x - .0758x^2$	
	3	$= - .1055 + .1704x - .0490x^2$	
	4	$= - .2124 + .2879x - .0799x^2$	
	5	$= - .5469 + .6083x - .1566x^2$	

APPENDIX XI

A method used in the analysis of variance of factorial design with two factors. e.g. Total dry weight (mg) Expt 5.1 (I).

I. Analysis of variance table

Source of variation	d.f.	ss	ms	F	Result
Blocks	3	40419	13473	1.19	n.s
Treatments	3	1028818	342939	30.37	**
Nitrogen (N)	1	183310	183310	16.23	**
Daylength (D)	1	676988	676988	59.95	**
Interaction (N) x (D)	1	168520	168520	14.92	**
Error	9	101626	11292		

II Table of all items

		Blocks			
Daylength	Nitrogen	1	2	3	4
Long	Low	665.03	574.28	618.11	481.05
	High	944.98	960.69	1054.98	1055.53
Short	Low	363.11	572.17	375.08	203.44
	High	341.26	561.40	278.91	367.50
Total		2314.38	2668.04	2326.97	2107.52

III Table of nitrogen x daylength

	Nitrogen		
Daylength	Low	High	Total
Long	2338.36	4015.68	6354.04
Short	1513.80	1549.07	3062.87
Total	3852.16	5564.75	9416.91

1. Correction: $C = (9416.91)^2/16 = 5542387$
2. Total $(665.03)^2 + \ldots + (367.50)^2 - C = 1170863$
3. Treatments: $(2338.36)^2 + \ldots + (1549.07)^2/4 - C = 1028818$
4. Blocks $(2314.38)^2 + \ldots + (2107.52)^2/4 - C = 40419$
5. Nitrogen: $(3852.16)^2 + (5564.75)^2/8 - C = 183310$
6. Daylength $(6354.04)^2 + (3062.87)^2/8 - C = 6769.88$
7. Interaction: 1028818 - (183310 + 676988) = 168520
8. Error: 1170863 - (1028818 + 40419) = 101626

IV **Least significant difference (LSD)**

Between nitrogen means.

$$LSD = t\sqrt{2(Ea)/rq}$$

where t = t value for d.f. for error

Ea = mean sum error

r = no. of blocks

q = no. of N. levels

$$LSD = t\sqrt{2(11292)/8}$$

$$LSD_{.05} = 2.262 \times 53.13 = 120.18$$

Between daylength means.

$$LSD = t\sqrt{2(Ea)/rp}$$ where p = no. of daylength leve[ls]

$$LSD = t\sqrt{2(11292)/8}$$

$$LSD_{.05} = 2.262 \times 53.13 = 120.18$$

Interaction effects (N x D)

$$LSD = t\sqrt{2(Ea)/r}$$

$$= t\sqrt{2(11292)/4}$$

$$LSD_{.05} = 2.262 \times 75.14 = 169.97$$

V **Coefficient of variation (C.V.)**

Formula used is $\dfrac{\sqrt{Ea}}{\overline{X}} \times 100\%$

where $\overline{X}$ is the mean of all the values in the analysis of variance.

APPENDIX XII

Analysis of variance of leaf length and number of teeth per plant

Character		Leaf length (cm)		No. teeth/leaf	
Source	d.f.	m.s.	F	m.s.	F
Blocks	6	28.34	**	102	**
Leaf Position (P)	7	26.35	**	1567	**
Daylength (D)	1	1987.31	**	21903	**
Nitrogen (N)	1	76.62	**	2572	**
Interaction (P x D)	7	26.51	**	1001	**
Interaction (P x N)	7	8.99	**	39	ns
Interaction (D x N)	1	43.92	**	1277	**
Interaction (P x D X N)	7	2.72	ns	45	ns
Error	186	2.47		34	
C.V.		14.9		41.8	

Analysis of variance of number of emerged leaves per plant

Days after treat.		32		52		60		77	
Source	d.f.	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	6	1.119	ns	3.453	ns	1.833	ns	3.203	ns
Nitrogen (N)	1	.571	ns	7.001	*	6.036	*	14.286	*
Daylength (D)	1	.143	ns	1.286	ns	.036	ns	89.287	**
N x D	1	.571	ns	.142	ns	2.892	ns	9.142	*
Error	18	.262		.976		1.071		2.266	
C.V.		6.5		7.7		7.2		9.2	

APPENDIX XII (contd)

Analysis of variance of dry weights, root: top ratio and leaf area (28 days after treatment)

Character		Total dry wt.		Leaf dry wt.		Root dry wt.		Root: top ratio		Leaf area	
Source	df	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	3	1283	ns	256	ns	456	ns	.0165	ns	7.25	ns
Nitrogen (N)	1	7	ns	302	ns	216	ns	.0529	ns	12.16	ns
Daylength (D)	1	20622	*	7386	**	3325	ns	.6225	**	92.30	ns
N x D	1	11143	ns	3779	*	1943	ns	.2158	**	51.37	ns
Error	9	3889		617		1490		.0216		19.50	
C.V.		24.3		24.8		24.6		9.0		26.0	

Analysis of variance of dry weights and root: top ratio (77 days after treatment)

Character		Total dry wt.		Leaf dry wt.		Root dr. wt.		Root: top ratio	
Source	df	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	3	1347	ns	193	ns	5779	ns	.0344	ns
Nitrogen (N)	1	18331	**	14416	**	2349	ns	.0287	ns
Daylength (D)	1	67699	**	65807	**	134	ns	7.0490	**
N x D	1	16853	**	14505	**	879	ns	.1509	ns
Error	9	1129		448		2392		.0648	
C.V.		18.1		19.5		19.9		21.8	

Analysis of variance of dry weights, root: top ratio and leaf area (91 days after the treatment)

Character		Total dry wt.		Leaf dry. wt.		Root dry wt.		Root: top ratio		Leaf area	
Source	df	m.s.	F	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	6	172	ns	173	ns	3122	ns	.069	ns	416	ns
(a)		6167	ns	6613	ns					169	ns
Nitrogen (N)	1	3315	**	2908	**	14284	**	.047	ns	10161	**
(a)		38455	**	58655	*					2648	**
Daylength ( )	1	30295	**	18099	**	1096	ns	29.256	**	35527	**
(a)		8601	*	35566	*					134	ns
N x D	1	1218	**	934	*	1120	ns	.000	ns	1788	ns
(a)		945	ns	74	ns					50	ns
Error	18	61		163		992		.105		406	
(a)		1739		5957						159	
C.V.		13.2		25.1		16.6		24.9		24.9	
(a)		14.8		26.6						26.5	

(a): figures in (a) rows are obtained from analysis of variance of values derived from radical leaves and roots only.

APPENDIX XIII

Means and standard errors of raw data

Each mean with standard error is from measurement of 7 blocks

Character	Treatment			
	Long day		Short day	
	Low nitrogen	High nitrogen	Low nitrogen	High nitrogen
Leaf Length(cm)				
Leaf position 4	9.9 ± .6	10.2 ± .8	8.0 ± .5	7.7 ± .3
5	11.7 ± .6	12.4 ± .7	8.3 ± .5	8.0 ± .2
6	14.1 ± .5	14.0 ± 1.0	8.2 ± .8	8.1 ± .4
7	15.4 ± 1.1	16.5 ± .8	7.9 ± .8	8.0 ± .4
8	13.4 ± 1.3	16.4 ± .6	7.6 ± .8	7.9 ± .6
9	12.6 ± .8	16.4 ± 1.7	6.8 ± .6	7.8 ± .5
10	11.9 ± .9	15.6 ± 1.0	6.7 ± .6	7.2 ± .4
11	10.9 ± .6	15.0 ± .5	6.0 ± .4	7.2 ± .3
No. of teeth/leaf				
Leaf position 6	.1 ± .2	4.4 ± 4.3	0 ± 0	1.1 ± .6
7	2.4 ± .6	9.4 ± 6.5	1.4 ± .5	3.1 ± 1.2
8	21.1 ± 1.4	31.0 ± 4.6	1.4 ± .3	4.4 ± 1.0
9	27.0 ± 2.2	43.2 ± 4.5	3.3 ± .8	5.0 ± .7
10	30.0 ± 1.4	43.4 ± 2.5	4.3 ± .4	6.4 ± 1.0
11	28.0 ± 1.5	44.4 ± 3.0	4.9 ± .8	6.4 ± .8
12	19.4 ± 1.5	35.3 ± 3.8	4.6 ± .7	6.6 ± 1.0
13	16.2 ± 1.5	25.6 ± 2.8	4.6 ± .7	7.3 ± 1.0
No. emerged leaves per plant				
Days after treatment				
0	base harvest	: 4.18 ± .14		
32	7.57 ± .37	8.14 ± .26	7.71 ± .18	7.71 ± .18
52	12.43 ± .43	13.57 ± .37	12.14 ± .59	13.00 ± .49
60	14.14 ± .26	14.43 ± .30	13.43 ± .69	15.00 ± .31
77	14.29 ± .18	14.57 ± .30	16.71 ± .75	19.29 ± .87

APPENDIX XIII (contd)

Means and standard errors of raw data

Character	Long day: Low nitrogen	Long day: High nitrogen	Short day: Low nitrogen	Short day: High nitrogen
28 days after treatment (Mean of 4 blocks)				
Total dry wt(mg)	265.94 ± 36.16	320.05 ± 25.21	267.25 ± 33.35	195.47 ± 84.55
Leaf dry wt. (mg)	101.86 ± 13.45	136.28 ± 11.68	89.63 ± 14.14	67.57 ± 30.54
Root dry wt. (mg)	164.08 ± 23.36	178.77 ± 14.41	157.29 ± 21.07	127.90 ± 5.84
Root: top ratio	1.615 ± .085	1.268 ± .054	1.777 ± .085	1.895 ± .054
Leaf area (cm^2)	18.45 ± 2.44	20.29 ± 1.68	17.23 ± 2.72	11.90 ± .54
71 days after treatment (Mean of 4 blocks)				
Total dry wt. (mg)	584.59 ± 39.17	1003.92 ± 29.80	378.45 ± 75.50	387.27 ± 21.84
Leaf dry wt. (mg)	355.32 ± 25.55	735.59 ± 34.74	140.14 ± 32.27	139.55 ± 30.80
Root dry wt (mg)	229.27 ± 18.23	268.33 ± 10.12	238.31 ± 43.37	247.72 ± 30.39
Root: top ratio	.648 ± .044	.369 ± .030	1.781 ± .133	1.891 ± .192
91 days after treatment (Mean of 7 blocks)				
Total dry wt (mg)	2363.08 ± 115.74	3468.36 ± 166.04	699.81 ± 64.91	970.95 ± 75.67
(1)	847.41 ± 40.14	1045.05 ± 68.67	699.81 ± 64.91	970.95 ± 75.67
Leaf dry wt (mg)	603.69 ± 44.46	923.01 ± 79.53	210.72 ± 21.36	299.01 ± 26.17
(2)	278.75 ± 21.16	358.21 ± 46.22	210.72 ± 21.32	299.01 ± 26.17
Root dry wt (mg)	568.66 ± 46.70	671.51 ± 33.61	489.09 ± 47.96	671.94 ± 55.66
Root: top ratio	.319 ± .032	.242 ± .013	2.369 ± .182	2.281 ± .145
Leaf area (mg)	89.59 ± 6.60	143.67 ± 12.38	34.33 ± 3.48	56.45 ± 4.94
(2)	41.37 ± 3.14	58.15 ± 6.80	34.33 ± 3.48	56.45 ± 4.94

1) excluding weights from runners, lateral branches and inflorescence

2) excluding weights or area from leaves of runners and lateral branches

APPENDIX XIV

Mean values with standard errors, number of plants (between brackets)of the flowering time (f.t.), the developmental stage of the inflorescence primordurim (i.p.) the number of flowers per inflorescence (f.i.) and the number of runners per plant (r.p.)

Experiment No.	Character	Low nitrogen	High nitrogen
5.2	f.t.	91.2 ± 1.0 (10)	95.4 ± .7 (8)
5.1 (II)	f.t.	83.0 ± 1.5 (7)	80.7 ± 1.5 (7)
5.2	i.p. (25 days)	2.4 ± .5 (8)	1.6 ± .3 (8)
	(26 days)	3.4 ± .3 (8)	3.2 ± .4 (8)
	(27 days)	3.4 ± .3 (8)	3.5 ± .3 (8)
	(29 days)	4.9 ± .3 (8)	4.9 ± .3 (8)
5.1 (I)	f.i.	22.1 ± 1.4 (7)	23.6 ± .8 (7)
5.2	f.i.	41.6 ± 1.5 (10)	41.0 ± 1.1 (8)
5.1 (II)	f.i.	29.7 ± .9 (7)	29.1 ± 1.7 (7)
5.1 (I)	r.p.	4 ± 0 (7)	3.9 ± .7 (7)
5.2	r.p.	5.5 ± .4 (10)	5.8 ± .6 (8)
5.1 (II)	r.p.	3.7 ± .3 (7)	4.3 ± .2 (7)
		19.9 ± .6 (27)	21.1 ± .6 (27)

APPENDIX XV

Analysis of variance of flowering time, number of flowers per florescence and number of runners per plant in (a) Expt. 5.1 (II). Expt.5.2 (b) Expt. 5.1. (I) and (c) Expt. 5.2. and inflorescence development in (d).

(a)

		Flowering time		No. flowers/inf.		No.runners/pl[t]	
Source	df	m.s.	F	m.s.	F	m.s.	F
Blocks	6	11.619	ns	11.571	ns	.333	ns
Treatment	1	18.286	ns	1.143	ns	1.143	ns
Error	6	18.952		13.476		.476	
C.V.		5.3		12.5		17.3	

(b)

		No. flowers/inf.		No. runners/pt	
Source	df	m.s.	F	m.s.	F
Blocks	6	7.119	ns	.238	ns
Treatment	1	7.143	ns	.077	ns
Error	6	9.643		.238	
C.V.		11.6		12.4	

(c)

		Flowering time		No.flowers/inf.		No.runners/pt	
Source	df	m.s.	F	m.s.	F	m.s.	F
Blocks	7	11.14	**	16.54	ns	.960	ns
Treatment	1	110.25	**	12.25	ns	.250	ns
Error	7	.68		9.39		2.960	
C.V.		8.9		7.3		30.6	

(d)

Days		25		26		27		29	
Source	df	m.s.	F	m.s.	F	m.s.	F	m.s.	F
Blocks	7	1.14	ns	1.29	ns	1.28	ns	.54	ns
Treatment	1	2.25	ns	.25	ns	.07	ns	0	ns
Error	7	1.11		0.82		.42		.86	
C.V.		52.7		27.9		49.8		50.2	