INFLUENCE OF LIGHT ON INFLORESCENCE DEVELOPMENT AND SEED YIELD IN WHITE CLOVER (*TRIFOLIUM REPENS L.*)

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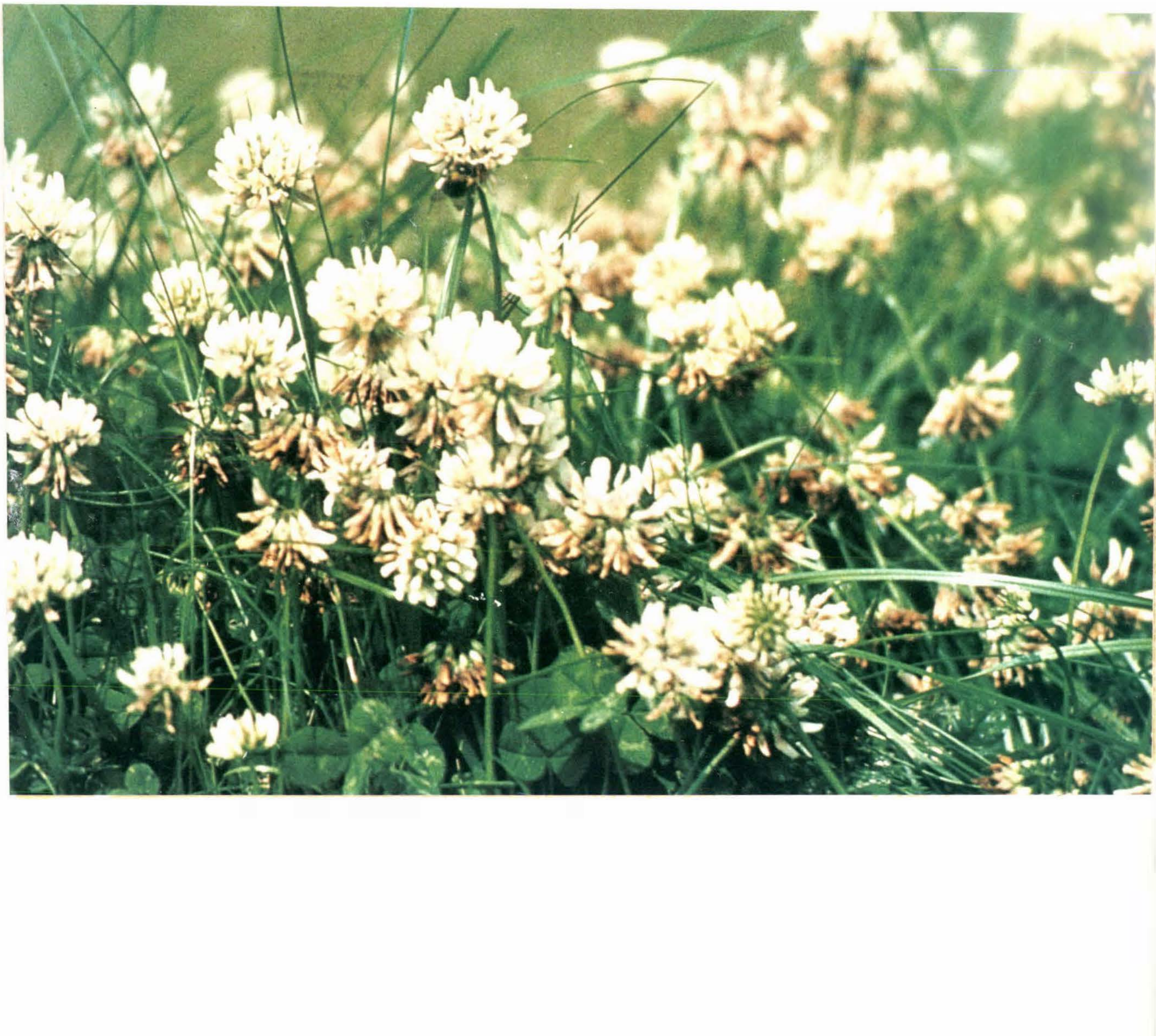
DEDICATION

To my parents, Sri Krishna Pasumarty and Seshi Rekha Pasumarty, without their encouragement and help this thesis could not have been written.



ABSTRACT

White clover (*Trifolium repens* L.) florets have the capacity to produce up to 6 seeds, yet normally the average number of seeds per floret is somewhere between 2 and 3.5. The cause of this low seed set is not known, but such an understanding is necessary as a basis for the development of improved management practices for seed production. Low light intensity has been implicated in the reduction of seed number per flower head and even in the abortion of developing flower heads. Therefore the present study examined the influence of light intensity on inflorescence development and seed yield of "Grasslands Huia" and "Grasslands Pitau" white clover (*Trifolium repens* L.). Investigations were also carried out to examine the effects of light intensity on sink strength of young flower heads with a view to understanding the mode of action of light.

Under controlled environmental conditions when plants were grown at a range of light intensities from 2000 to 10000 lux, the ovary length, number of florets per inflorescence, the size of the ovules within the carpel, percentage of fertile ovules and percentage of ovules setting seed in the plants grown at the lowest intensity were decreased by 18, 53, 13, 75% respectively compared with controls grown at the highest intensity. A stain-clearing technique was used to examine the cytoplasmic state of embryo sacs in intact, unfertilized, mature ovules. Ovules with fully formed embryo sacs containing a full complement of nuclei were classed as fertile ovule. Ovules with shrunken embryo sacs which lacked a full complement of nuclei were classed as sterile. Light intensity had no significant effect on ovule number. However, in the field, the young flower heads experience very low light levels due to shading by foliage canopy only when they are young. Within the canopy light intensities may be as low as 1% of full light even at midday when incoming radiation is most intense. To simulate field conditions in the glasshouse the inflorescences were shaded on otherwise fully lit plants by using either neutral shade or simulated shade light at wavelengths similar to those of light filtered through a leaf canopy. Low light had a slight effect on pollen fertility, the effect being significant only at some stages of inflorescence development. These stages of inflorescence development were synchronized with the development of pollen mother cells into pollen grain. Irrespective of the stage of inflorescence development, shading the inflorescence alone decreased the length of the ovary. The most striking observation was that even in good growing conditions only 70% of ovules formed in a flower head had fertile embryo sacs capable of setting seeds. The reduction in seed number per head was brought about by an increase in the number of florets aborting, and by a decrease in the percentage of ovules setting seeds. The close correlation between the percentage of apparently fertile ovules and the percentage of ovules setting seeds strongly suggests that this reduction was largely brought about by an increase in ovule sterility. The degree of ovule sterility was greatest when shade was applied to the inflorescence at the eighth node below the apex on a stolon. Shade treatments might have interfered with meiosis (formation of megaspores).

To examine the significance of these observations for seed production practices, field experiments were set up to determine to what extent and under what growing conditions flower head development and seed yield per head were influenced by canopy density and simulated overcast weather conditions in plants of "Grasslands Huia" and also "Grasslands Pitau". Field studies showed that flower heads developed in a dense canopy produced 37-39% fewer seeds per head than those formed in an open canopy. Some of this reduction was brought about by an increase in the number of florets aborting, but much of it was caused by a higher proportion of sterile ovules in dense canopies than in open canopies. Simulation of overcast weather by artificial shading also strongly affected the seed yield per flower head. When plants were shaded before pollination only, there was a 24-31% reduction in seed number per head; when shade was applied only after pollination there was a reduction of 25-28%. Therefore overcast weather conditions during early stages of inflorescence development or during the seed maturation period could lead to reduction in seed number per head. In the past, low seed number per flower head has been attributed to poor pollination. The results obtained in the present investigation showed that a high percentage of pollinated carpels contained sufficient pollen tubes for the fertilization of all ovules. The observation of a random seed set pattern and a positive correlation between the ovule fertility and the ovules setting seed also strongly suggest that pollination was probably not the limiting factor. Rather, the limiting factor appeared to be the degree of sterility of unfertilized ovules.

These results showed that there was a direct effect of light intensity on flower head development. Therefore investigations were carried out to study the influence of shade on the growth and sink activity of young flower heads and peduncles. Measurement of the peduncle elongation rate by using a linear voltage displacement transducer showed that when the inflorescence alone was shaded, peduncle elongation was higher than in the light. Translocation studies using a ^{11}C-labelling technique showed that shading the inflorescence alone had little effect on translocation of assimilates into the inflorescence, but induced a major change in partitioning of assimilates within it. Peduncle elongation induced by shade was accompanied by an increase in partitioning of photoassimilates to the peduncle, at the expense of the flower head.

The results of this study suggest that one of the major advantages of the practice of defoliation at the time of closing the paddock for seed production is probably the enhancement of ovule fertility; and that decreased seed yield in duller, wetter summers is probably, at least in part, attributable to increased ovule sterility in the dense canopies formed under those conditions. Form this point of view, for best seed production an optimal management strategy would be to grow the crop as spaced plants with an open canopy rather than a denser sward with a closed canopy.

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CHAPTER.1

INTRODUCTION

White clover (***Trifolium repens*** **L.**) is known for its fluctuation in yield of seed. Seed yield is built up from several yield components which in turn are determined by a combination of plant and environmental factors. The seed production capacity of white clover represents the cumulative expression of four principal components: number of flower heads per unit area, number of florets per head, number of seeds per floret, and seed weight (Romero, 1985). These components all differ in their relative contribution to total seed yield and change with genetic variability within the species, as well as with the environmental conditions (Zaleski, 1961 and Thomas, 1981). Huxley et al. (1979) reported that the major components of yield in white clover were the number of inflorescences per unit area and seed weight which accounted for 40% and 59% of the diversity in seed yield respectively. According to these workers the number of seeds per floret (seed set) made a comparatively negligible contribution to seed yield and was independent of inflorescence number. Gaspar et al. (1981) and Haggar et al.(1963) determined correlation coefficients between seed yield and associated characters and found that seed yield was positively correlated with seed set, heads per branch and seed weight. Binek (1983) found that removal of up to 48% of inflorescences from the cultivar Podkowa did not reduce seed yield. Compensatory increase of 58-78% occurred in both number and weight of seed per head, although seed weight altered little (less than 8%). This evidence strongly suggests that seed set is also a major component of yield in white clover.

Under normal summer conditions only about 50% of ovules develop into seeds. Dessureaux (1951) analysed the seed setting abilities of Ladino clover clones by recording number of ovules per floret, seed set per floret and percentage of florets bearing seeds. He found that clones differ significantly in all three characters. Thomas (1981) reported that the mean number of ovules per floret in "Grasslands Huia" was 5.5. Clifford (1979) found the average number of seeds per floret to be only 2.2. A population of Ladino clover plants, in which the ovule number per ovary averaged 4.6, yielded an average of 2.4 seeds per floret under apparently optimal pollination conditions (Dessureaux, 1951). Romero (1985) reported that in "Grasslands Huia" the seed production of the plants was consistently poor compared with the potential yield which could be obtained with improved seed set. The level of set was only 30-40% of the potential seed set (2.0-2.3 out of a total of 6-7 ovules per ovary). In Ladino clover the percentage of ovules setting seed in different strains ranges from about 35 to 63% (Dessureaux 1951). Thus some plants have genetic potential for more than 50% seed set. Theoretically, by increasing seed set per ovary there is the potential for doubling seed yield under conditions of optimal head density. The reasons for poor seed set are not known despite such knowledge being highly desirable as a basis for the development of improved management practices for seed production. The present study is aimed at understanding the cause of this low seed set with a view to improving it by appropriate management techniques.

CHAPTER.2

LITERATURE REVIEW

Seed yield in white clover, or any other plant, depends on the total number of seeds per unit area. However, to some extent, yield is related to the number of inflorescences produced or inversely related to the percentage of total or partial abortion of inflorescences.

Inflorescences in white clover form at the stolon apices. In vegetative plants the youngest axillary buds appear in the axils of the third oldest leaf primordia at the apex of a stolon. With the transition to the reproductive state, buds form in the axils of the youngest leaf primordia (Thomas, 1980). These precocious axillary buds then grow into seed head primordia which usually emerge from their stipular sheaths a few days after the emergence of their subtending leaves. Flower initiation is controlled by temperature, photoperiod and genotype (Thomas, 1987).

The present review does not give any attention to the internal and environmental causes of the transition of plants from the vegetative to the reproductive phase (flower initiation) but deals with the factors influencing reproductive development once they have been initiated. Reproductive development will here be interpreted as encompassing flower development, fertilization, growth and ripening of the fruit including development of the seed structures from the fertilized ovules.

2.1 FLOWER DEVELOPMENT

In many plant species the rate of post-initiation flower development and the proportion of initiated flowers which reach anthesis depend on the environmental conditions before anthesis.

2.2 ENVIRONMENTAL FACTORS CONTROLLING FLOWER DEVELOPMENT

Environmental factors involved in flower development are many, but the major factors are:

2.2.1 Control by nutrition
2.2.2 Control by temperature
2.2.3 Control by water stress
2.2.4 Control by light.

2.2.1 Control by nutrition

Mineral nitrogen has a great influence on flower development. In ***Xanthium***, high N levels generally promote development of both male and female inflorescences (Neidle, 1939) and the number of days from the appearance of visible flower buds to anthesis is increased in N-deficient plants of ***Perilla*** and ***Kalanchoe*** (Kinet et al. 1985). In ***Triticum***, higher nitrogen levels increased spikelet number, proportion of fertile florets and then the degree of grain setting within spikelets (Langer and Liew, 1973). Inhibition of development by high N supply has been found in some species. A high concentration of soil nitrate decreased the yield and quality of flowers in ***Lilium*** (Lily) (Eastwood, 1952). In various monoecious species, and also in some dioecious and hermaphroditic species, femaleness is increased with N supply. Growing ***Cucumis sativus*** plants in a soil of high N content, increased the total number of flowers of both sexes, but the increase in number of female flowers exceeded that of male ones. Nitrogen nutrition is also found to influence sex expression in some dioecious species (Kinet et al. 1985). Tibeau (1936) grew hemp (***Cannabis sativa*** L.) plants in solution culture and found that with normal N levels there was a 65:35 female to male flower ratio. Plants supplied with high N (eight times normal) differentiated female flowers only and all those without N differentiated male flowers only. In the ***Lycopersicon***, N deficiency has little effect upon anther development but represses the female organ (Howlett, 1936). Bilderback (1972) observed that ***Aquilegia*** buds initiate carpels on high N media, but not on low N. Brevedan et al. (1978) found that applying N at the beginning of anthesis decreased soybean flower abscission from 55% to 45%, but N applied at the end of anthesis had no effect. This evidence clearly suggests that not only the quantity of N but also the time of application is important to get desirable effects.

Other elements such as K, P, Ca and B also influence flower development. In various species, male sterility is associated with copper and boron deficiency which would impair microsporogenesis at, or near, meiosis Kinet et al. (1985). Boron is one of the essential micro nutrients which is found to influence seed set. Most often it has been suggested that boron increases the pollen tube length, hastens pollen germination and also increases the pollen germination percentage. Johnson and Wear (1967) studied the effect of boron on white clover seed production. They observed that addition of boron increased the number of seeds per head while zinc, copper, manganese, and molybdenum showed no effect on seed yield.

2.2.2 Control by temperature

As with every other aspect of plant growth, temperature is important for flower and inflorescence development. Besides its action on the rate of development, temperature is critical in certain species, in determining normal development until anthesis or abortion of the buds.

2.2.2.1 Rate of development and abortion

Increase in temperature usually tends to speed development towards anthesis, the rate of morphological development of reproductive structures being more rapid at higher temperatures. This effect has been recorded in many species (Kinet et al. 1985). Higher temperatures reduce the time to anthesis in ***Tulipa*** (Dosser and Larson, 1981) and ***Iris*** (Fortanier and Zavenbergen, 1973). In ***Iris hollandica*** day and night temperatures affect time to anthesis indicating that the average daily temperature is the major factor determining the rate of flower development. The differential effect of air and soil temperatures has been investigated in few species. Generally, increase in both reduces the number of days to anthesis; in ***Iris*** (Fortanier and Zavenbergen, 1973) the effect of air temperature is, however, much greater. Harris and Scott (1969) varied independently the temperature of flower bud and other parts of the carnation plant and found that anthesis occurs four weeks earlier as a result of heating the bud, but is not significantly hastened by heating the lower part of the shoot. This result suggests that temperature-dependent processes take place in the flower bud rather than in the stem or leaves.

Thomas (1961b) reported that once an inflorescence was initiated in white clover, high temperatures and long days led to the most rapid growth and low temperatures and short days to the slowest. Thus the number of flower heads appearing in a given time was greatest in long days at high temperatures, conditions which give both most abundant initiation and most rapid head emergence.

High temperature prevents flowers from reaching anthesis in numerous species. Generally the higher the temperature, the higher the percent abortion. In ***Iris***, abortion of flowers is promoted by increasing both day and night temperatures but increase in day temperature is more detrimental than increased night temperature (Fortanier and Zavenbergen, 1973). The effect of air temperature is also greater than that of soil temperature in ***Iris*** (Fortanier and Zavenbergen, 1973). Low temperatures also induce abortion in some species: in ***Rosa***, for example, low air or soil temperature promotes "blind" shoot formation (Zieslin and Halevy, 1975).

Temperature was found to influence the growth and development of young ***Lycopersicon*** (tomato) plants. Calvert (1964a) found that temperature influenced growth rates of young tomato plants in poor light but its effect became less marked when light conditions improved. Later, Calvert (1969) showed that in winter-sown plants, high temperatures during later stages of flower development up to anthesis were associated with flower abortion. High temperatures during early growth, however, favoured later flower development. Calvert suggested that in summer flower buds readily develop over a wide range of temperatures, whereas in winter temperature becomes a critical factor, satisfactory flower development being largely dependent on the correct choice within a comparatively narrow range. In summary, it can be seen from this work that in general, abortion seems to be associated with low light levels and high temperatures during the critical stage from bud appearance to anthesis. In white clover, low light intensities and warm short photoperiods in particular seem to favour the abortion of inflorescences (Thomas, 1987).

2.2.2.2 Flower and inflorescence size

High temperatures which promote flower development frequently result in poor quality flowers. This is well known in bulbous plants (Rees, 1966). Detrimental effects of high temperature have been recorded in cereals, too. In ***Triticum***, Friend (1965) has found that the length of the developing inflorescence and of the ear and the number of spikelets are greatest at low temperature. Low temperature retards morphological development of the ear to a greater extent than that of the whole plant, prolonging the duration of ear development and spikelet initiation which result in more spikelets per ear at anthesis. Rawson and Bagga (1979) reported that low temperatures during pre-anthesis growth result in an increased number of grains set per ear suggesting that the proportion of fertile florets on each spikelet is increased. In general, the number of floral parts is markedly affected by temperature and low temperature during pre-anthesis growth resulting in an increased number of floral parts.

Environment has a strong effect on the size of the flower heads and their component parts in white clover. When plants on which inflorescences had already been initiated in cool natural winter days were transferred into controlled environment conditions (Thomas, 1961b), long days and low temperatures both independently increased the number of florets per flower head and the size of the florets as indicated by corolla length and ovule number per carpel. Thomas (1981) reported that the same genetic material growing in field plots in Palmerston North (N.Z) showed floret number per head to decrease from 61.9 in early November to 51.2 in January and corolla length to decrease from 10.8 to 9.35 mm over the same period. Number of florets per inflorescence was similarly found to decrease from spring to summer in field plots of "Grasslands Pitau" in New Zealand by Clifford (1979). Similar to other plants, low temperatures during pre-anthesis growth in white clover increased the number of florets per head.

2.2.2.3 Fertility

In various cereals, such as ***Sorghum bicolor*** and ***Oryza sativa***, low temperatures induce male sterility (Brooking, 1976). High temperatures, too, were found to affect male fertility adversely in various species, including maize, tomato, and wheat (Kinet et al. 1985). Pre-meiotic and/or meiotic stages are particularly sensitive. In ***Triticum***, the proportion of florets containing abnormal anthers is increased and pollen viability in normal anthers is reduced by exposing plants to 30^{o} C for 1 day only during the sensitive stage (Saini and Aspinall, 1982). Female fertility is also reduced by high temperature. In contrast, in white clover the percentage of aborted pollen grains was greatest at lower temperature (10^{o} C). No consistent difference was found between the "fertility" of pollen formed at 30^{o} and 20^{o} C, and in no case at these temperatures did pollen abortion exceed 26%. At 10^{o} C, however, three of the ten clones investigated produced more than 85% aborted pollen (Thomas, 1961a). It is general practice in commercial white clover seed production in New Zealand to" close" white clover paddocks in November or December (mid-summer). Flower heads providing the bulk of the seed crop are initiated just before or shortly after closure, viz. between late October and early November. These reach anthesis during December. Pollen fertility is high in heads emerging after paddock "closure", and it is unlikely to be a factor affecting seed yield per hectare.

Temperature strongly interacts with light conditions. In some photoperiodic plants a low temperature treatment can override the daylength requirement for flower development (Kinet et al. 1985). Low temperatures also counteract the detrimental effects of low light flux in various species suggesting that assimilate supply, redistribution, and utilization might be essential factors in mediating temperature effects (Kinet et al. 1985).

2.2.3 Control by water stress

Water status of the soil markedly influences flower development both quantitatively and qualitatively. In cereals, a period of water deficit generally affects both the production of new spikelets by the meristem and the differentiation of the spikelets already initiated (Kinet et al. 1985). In maize, water stress imposed during tassel initiation and early development reduces both the growth rate and the mature size of that structure (Damptey and Aspinall, 1976). It induces a marked degree of sterility in the spikelets on the lateral branches although spikelets on the main axis are generally fertile. Male gametogenesis in barley and wheat is also particularly sensitive to water status of the soil (Saini and Aspinall, 1981). Withholding water during the premeiotic and meiotic stages and immediately after meiosis induces pollen sterility in wheat, whereas female fertility is unaffected (Saini and Aspinall, 1981). The above evidence suggests that water stress reduces growth rate of florets and inflorescences and induces male sterility in cereals.

Korte et al. (1983) found that irrigation during flowering in soybean increased pod set, but they did not distinguish whether this was due to increase in flower number or percent seed set. Lord and Heslop-Harrison (1984) postulated that irrigation would enhance the expression of autofertility, and Stoddard (1986) found that irrigation of winter field beans was associated with an enhanced proportion of fertilized ovules within fertilized flowers. Water stress at anthesis can markedly reduce fertilization and grain set in most cereals (Slatyer, 1973). A brief period of wilting at anthesis in corn caused a 50% reduction in yield (Robins and Domingo, 1953). From the work of Robins and Domingo (1953) with corn, it appears that the germination of pollen or growth of pollen tubes from stigma to ovules might be impaired by a brief period of water stress at the time of anthesis.

Zaleski (1970) investigated the effect of irrigation on white clover seed production under U.K. conditions. In two out of three years, irrigation up to the beginning of flowering was beneficial for seed production. However, an excess of water, especially during the flowering period, increased the lush vegetative growth at the expense of inflorescence production and yield of seed. Under the drier conditions experienced in New Zealand, it is sometimes necessary to irrigate during flowering if the weather is such that the plants show signs of wilting (Jolly, 1958). Clifford (1980) reported that clover plants grown in moisture retentive soils or where irrigation was available gave highest yields.

2.2.4 Control by light

The importance of light for flower development has long been recognised. In early studies, where there is no clear distinction between the effect of daylength and light flux, interpretation of the data is difficult. Now, it is clearly established that both factors can independently influence flower development. In nature they are linked and both contribute to the daily light integral, an important parameter for various species (Kinet et al. 1985). For the present review, emphasis is given to irradiance and light integral (light flux).

Daylength is a major factor controlling flower development in many plants. It may act independently and also in conjunction with irradiance, since both contribute to the daily light integral. In photoperiodic species the optimum daylength for flower development is not necessarily the same as for initiation. Photoperiod not only affects the rate of development and the abortion of the reproductive structures, but it also has numerous morphogenetic effects. Daylength is a potent factor determining sex expression in various monoecious and dioecious species (Kinet et al. 1985). For instance, 50% of early planted male plants of hop have bisexual flowers at early stages of plant development whereas at later stages, at a time corresponding to the normal flowering season when days lengthen almost all flowers are male.

2.2.4.1 Rate of development and abortion

Progress to anthesis is generally delayed by low light levels. Plants of ***Rosa*** cv. "Baccara" grown at 21^{o} C with continuous illumination at 12,000 lux flower about three weeks earlier than plants grown at 1,500 or 3,000 lux (Moe, 1972). The time interval from appearance of macroscopic tomato inflorescences to first anthesis is also shorter when the daily light supply is increased. This effect is independent of daylength (Kinet, 1977). Thorough investigation by Hughes and Cockshull (1971) of inflorescence initiation and early development as a function of light supply in ***Chrysanthemum*** cv "Bright Golden Anne" provides the basic data for a photosynthetically-based model of floral initiation and development.

Abortion of flowers or inflorescences seems to be one of the main developmental phases affected by light in several species including ***Rosa***, ***Lycopersicon***, ***Bougainvillea***, and some bulbous crops (Kinet et al.1985). In ***Rosa*** hybrids the annual light integral and flower production curves are nearly congruent (Post and Howland, 1946), the prime impacts of light being upon the number of flowers completing development to anthesis and the rate of development. ***Vitis*** "anlagen" require some minimum photon flux to develop into inflorescences rather than tendrils. In the cultivar "Sultana", shading the whole vine (73% reduction of sun light) for at least four weeks in the late spring (at which time inflorescence primordia of the next season are initiated) reduces the number of shoots with inflorescences (May, 1965). Clear recognition of the importance of photon flux for inflorescence development in ***Lycopersicon*** is shown by Calvert, 1964b and Cooper, 1964. This hypothesis has been tested by other workers. Verkerk (1964) demonstrated that additional lighting promoted development and fresh weight of the first inflorescence in November-sown plants. Rodriguez and Lamberth (1975) investigated the effects of additional lighting and plant density on flower development. They concluded that in winter grown tomato plants, a reduction in inter plant competition for light, reduced flower abortion, achieved either by increasing the space between plants or by additional lighting. Russell (1980) demonstrated a gradient in inflorescence development from 0 to 100% abortion in plants grown at 4.8 to 72 plants per m^2. Menzel and Simpson (1988) studied the effect of continuous shading on flowering in passionfruit. They found that heavy shading was associated with strong reduction in the number of floral buds. Furthermore, the number of open flowers declined with each reduction in irradiance. Not all floral buds developed fully. Shading reduced the chance of individual buds reaching anthesis, so that virtually no open flowers were observed at 2.1 MJ per m^2 per day. All studies reviewed here show that low irradiance or low light intensity reduces inflorescence development. There is evidence that in most species the inflorescence will be altered in form or abort prematurely at low light intensities.

Similarly, light intensity has a big influence on flower initiation and development in white clover. Low intensity (1,000-3,000 lux) may lead to a complete failure of floral initiation or to a complete abortion of buds and inflorescences, even under suitable temperatures and daylength (Zaleski, 1964). The same author found a highly significant positive correlation between light intensity and length of stolons and also number of inflorescences.

2.2.4.2 Time dependence of light intensity effects

A relatively narrow time dependence for low light-induced abortion has been found in several species by transferring plants to low light or darkness for a limited period at different stages of flower development. In ***Lycopersicon*** one critical stage in inflorescence development is after the appearance of macroscopic inflorescence buds (Calvert, 1969). At that time some reduction in the percent of inflorescences which develop to anthesis occurs with only two days exposure to low light flux indicating the extreme sensitivity of the inflorescences to reduced photosynthesis. A 26-day, 95% shade treatment inhibits inflorescence development in ***Gladiolus*** cv. "Sans Souci". Shillo and Halevy (1976) showed that plants are most sensitive to light conditions at the four to six leaf stage, at which time inflorescences are elongating, floret initiation is in full progress, and some florets have all floral organs formed; low irradiance during this period reduces both the percentage of flowering plants and floret number. Shading at a later stage causes only abortion of individual florets.

In all cases of abortion recorded in white clover, observations indicate that inflorescence development proceeds normally for approximately the first six plastochrons. Attempts to induce an arrest of development before this stage have failed. Thus the initial stimulus to inflorescence formation is apparently strong enough to carry development through six plastochrons. After this stage, however, environmental conditions do have an effect (Thomas, 1987). Later cessation of development results in the death of floret buds while they are still immature when the peduncle is about 2 cm long. In these cases there is little or no further development after the leaflets of the subtending leaf have unfolded. It is common for the upper florets of an inflorescence to abort while the lower ones develop fully (Thomas, 1987). Light intensity seems to be a major environmental factor associated with inflorescence abortion in white clover. Davies (1970) observed that for plants grown at low light levels (100-300 foot candles), flower induction failed or there was complete or partial abortion of buds and a reduced number of inflorescences. Growing the plants in a glasshouse with half the normal light also halved the number of inflorescences, but some clones suffered relatively more than others.

2.2.4.3 Sink vs source activation by high light intensities

In some plants at least a part of the light requirement cannot be explained as being a need for increased photosynthesis, although there is little question that higher photosynthetic activity in the source leaves is probably a major factor contributing to the promotion of flower development by high light intensities.

Heindl and Brun (1983) studied the effect of light and shade on abscission and ^{14}C-photoassimilate partitioning among reproductive structures in soybean and found that shading flowers and pods at the pulse node for any length of time before pulsing the subtending leaf tends to reduce sink strength of the flowers. Similarly Mor and Halevy (1980) reported a 50% reduction in the amount of ^{14}C-assimilates recovered in darkened young ***Rosa*** shoots compared to control shoots and they noted that shading caused a 13.5-fold decrease in relative specific activity (which was their measure of sink intensity). May (1965) observed in ***Vitis*** as in ***Rosa***, that high light intensities act at least in part, by enhancing sink activity of the developing inflorescence. For white clover, Pasumarty (1987) observed a 40% reduction in the amount of ^{14}C-assimilates recovered in shaded young flower heads compared to control flower heads.

In contrast, there is no evidence for irradiance-induced sink activation in ***Bougainvillea***, tomato, and ***Chrysanthemum*** (Kinet et al. 1985). Shading the inflorescence bud of tomato, from macroscopic appearance to anthesis does not prevent, nor even reduce the rate of development (Kinet et al 1985). When

Chrysanthemum shoot tips which contain developing inflorescences are shaded there is no reduction in development if the leaves are maintained in high light intensities (Kinet et al 1985).

Interactions between daylength and light intensity have been recorded in several photoperiodic species (Kinet et al. 1985). Sachs and Hackett (1969) demonstrated that high light not only promotes inflorescence development in ***Bougainvillea***, but may even override the photoperiodic signal so that this plant behaves then as a day neutral plant. White clover plants of S.184 produced more inflorescences under a high light intensity even under 15 hour daylength than the same plants under low light intensity and a long day (17 hour) (Zaleski, 1970). Experiments with light intensity suggest that when plants are grown in insufficient light there is a complete failure of floral induction or a complete or partial abortion of buds and inflorescences, even under suitable temperatures and daylength (Zaleski, 1970).

2.2.4.4 Fertility

In hermaphroditic species such as ***Lycopersicon***, ***Kalanchoe***, and ***Fragaria*** low light conditions induce male sterility (Howlett, 1936; Smeets, 1980). Howlett (1936) related this phenomenon in ***Lycopersicon*** to early degeneration of microspores with little pollen production as well as to poor germinability of pollen grains . In ***Fragaria*** cultivar "Sivetta", mature stamen number is reduced by 80% with three days of 75% shading. A similar shading treatment is without effect on stamen development in the cultivar "Karina" indicating that there is a strong cultivar dependence on light intensity for male sterility (Smeets, 1980). For white clover, Thomas (1961a) found that the pollen fertility was little affected by daylength and total light quantity.

There is no literature available on the effect of light, in particular, on the degree of sterility of unfertilized ovules in white clover.

2.3 FACTORS RESPONSIBLE FOR LOW SEED SET

The main factor determining seed production capacity in white clover, or any other plant, is the number of fertile ovules produced per plant. Provided that the ovules show a high level of fertility, the seed producing ability of plants will be strongly affected by the number of ovules formed. The number of ovules formed in a white clover plant is determined by the number of inflorescences, the number of florets per flower head and the number of ovules per floret. Given both high head density and large floret number per head, yield in white clover could be affected by the number of ovules setting seeds i.e seed number per floret. Under normal summer conditions only about 50% of ovules develop into seeds in white clover (Thomas, 1981). The reasons for poor seed set are not known. The factors which could theoretically be responsible for such a low percentage of ovules developing into seeds are:

1) Inadequate pollination
2) Pollen sterility
3) Pollen-stigma incompatibility
4) Ovule sterility
5) Fertilization failure
6) Post-fertilization abortion of developing seeds;
6a) Abortion of entire pod
6b) Abortion of developing seed in a pod

Most often low seed set has been attributed in the past either to poor pollination or to inadequate supply of nutrients for developing seed.

2.3.1 Pre-fertilization events

Pollination is an important determinant for any seed crop, since it must take place, whether automatically within the flower or assisted by an insect, before fertilization can occur and seed can form. Potentially, therefore, pollination is a factor which can influence seed yield.

Many factors are involved in the complex processes which lead to fruit setting, though basically there are only two reasons why a flower may fail to set fruit.
(1) Lack of pollination: This may be due to an absence of pollen (eg; as caused by male sterility) or to some breakdown in the mechanism by which pollen is transferred to the stigma (eg; lack of pollinating insects).

(2) Failure of pollen to effect fertilization: This may be due to sterility of pollen grains and/or egg cells or the pollen tubes may be incapable of growing down the style (incompatibility).

2.3.1.1 Compatibility

The majority of flowering plants, being hermaphrodite, produce fertile male spores (pollen) and fertile female gametes(eggs) but many species are unable to reproduce sexually by self-pollination; they are self-incompatible. White clover has a well developed genetic gametophytic self-incompatibility mechanism (Atwood, 1941) with only a small proportion of plants in a population being quite strongly self-compatible. Cross-pollination is thus essential for significant seed set within a population and the most important natural pollinating agents responsible for this are bees (Erith, 1924).

Although the anthers of a floret dehisce before its petals have fully elongated, autogamous self-pollination is infrequent (Thomas, 1987). Seed set without the aid of an external agent does occur regularly in some genotypes (Atwood, 1941). Within a population of plants in which the transfer of pollen from anthers to stigmas by an external agent was prevented, the average number of seeds set per flower head was found to be only 0.5 by Atwood (1941) and 2.7 by Scullen (1952). When florets were artificially self-pollinated, either by hand pollination or by gently rolling the heads between thumb and fingers (rubbing), the amount of seed set was increased (Thomas, 1987). Atwood (1941) obtained an average of 5.7 seeds per head by hand self-pollinating or by rubbing. Harberd (1963) found 3% of selfed carpels set seed. Other workers have had less success however, Ware (1925) obtained virtually no seed by selfing and Williams (1931) found seed set in only 25% of selfed plants. The differences reported probably result largely from differences in temperature and genotype.

The degree of self-incompatibility shown by white clover plants, although determined primarily by the genetic make-up, may be modified by environmental factors. One factor influencing the degree of self-incompatibility of white clover is temperature. Temperature has a strong influence on pollen germination and pollen tube growth. Comparing plants at constant temperatures of 15, 25 and 35^{o} C, Chen and Gibson (1973) found that germination of pollen grains on compatible and incompatible stigmas was faster at high temperatures, but detected no differences between germination rates in compatible and incompatible crosses at any temperature. Rates of pollen tube growth were also strongly influenced by temperature. From the time of compatible cross-pollination, pollen took 20 h to grow down the style to reach the ovary at 15^{o} C compared with only about 2 h at 35^{o} C. At all three temperatures the pollen tube growth following incompatible self pollination was slower than after cross pollination. Pollen tubes reached the ovules at each temperature in compatible crosses, but only did so at 35^{o} C in incompatible self pollination. No fertilization occurred after incompatible self pollination at 15 and 25^{o} C, but seed was set at 35^{o} C. These responses to temperature suggest possible causes of the discrepancies between the results obtained by different workers, and between results obtained by the same workers in different years.

The interaction between the stigma and pollen germination in white clover has yet to be elucidated. According to Atwood (1943) there are two "interference zones" active in preventing the growth of the incompatible pollen, one on the stigma and the other about three quarters of the distance down the style. Pollen on the stigma does not germinate until the cuticle covering the stigma papillae is ruptured and releases the mucilaginous secretion beneath. Presumably pollen germination in "rubbed" flower heads results from the combined release of mucilaginous matter by mechanical damage and the transfer of pollen grains from dehisced anthers to stigma surfaces. Visits by bees would have a similar effect to manual "rubbing" (Thomas, 1987). The report by Rinderer et al. (1981) that the intensity of honey bee movement during visits to flower heads was positively correlated with seed set also suggests the importance of mechanical damage in stimulating pollen germination.

2.3.1.2 Lack of pollinating insects

As white clover is self-incompatible, cross-pollination is essential for significant seed set. Pollination is more straightforward in white clover than for red clover and lucerne, as the tripping mechanism is simpler to operate and the corolla tube is short so that florets can be visited easily by a wide range of insects (Davies, 1970). The flowers are frequently visited by honey bees and bumble bees, and pollination is generally successful. After a visit, the parts of the floret return to their original position (Thomas, 1987). New Zealand results suggest that one bee-hive per 3.2 hectares is ample (Palmer-Jones et al. 1962), although overseas results indicate that a higher concentrations of one hive per 1.2-1.6 hectare of crop are desirable (Haggar and Holmes, 1963). It may be that the cooler climate necessitates an increased number of bees per hectare. It is generally observed that even under optimum conditions for pollination the number of seeds set per floret is often rather low. Romero (1985) found that differences in climate were reflected in the number of seeds formed per floret (seed set). Following the generally less favourable climate during pollination at Palmerston North in 1982/83 seed set was low i.e approximately one seed per floret. In the next year 1983/84 more suitable conditions during pollination were reflected in a seed set of approximately two seeds per floret (and up to 3.5). The second year results compare favourably with values recorded by Clifford (1979). Nevertheless, even the highest values obtained are a poor result considering that generally six ovules are present per floret (Thomas, 1981). This suggests that even under apparently good climatic conditions for pollination and seed set, considerable wastage occurs between potential and final seed number per floret. This also indicates that factors apart from bee activity might affect seed set in white clover.

2.3.1.3 Sterility

In the event of optimum conditions for pollination, low seed set may be due to sterility. Self-incompatibility differs from self-sterility in that viable ovules and pollen grains are produced but, owing to genetic factors, pollen tubes are incapable of growing down the style of the genotype that produces them. The same pollen, however, is capable of growing down the style of certain other varieties and effecting fertilization. The term "sterility" refers to the failure to produce viable pollen grains or egg cells. Sterility is sometimes due to the failure of either the male or female organs of the flower to develop fully, giving rise to plants showing either male or female sterility.

Heslop-Harrison (1971) divided male sterility into 3 broad groups, based upon the developmental stage at which an abnormality first occurs: 1) abnormality prior to meiosis; 2) aberrant sporogenesis or gametogenesis; and 3) aberrant anthesis or anther dehiscence. Abnormalities prior to meiosis include the stamens being absent, aborted or not differentiated so that no microspores or gametes are produced. Aberrant sporogenesis involves abnormalities in the developing pollen grains and also in the tapetum. In aberrant anthesis or anther dehiscence, normal spores and gametes are produced but some abnormality inhibits their dispersal.

Partial male sterility has been reported in ***Petunia***, ***Brassica***, ***Lycopersicon***, ***Gossypium***, and ***Glycine max*** (Carlson and Williams, 1985). In most of these species, high day temperatures result in the expression of male sterility. Stelly and Palmer (1980) found that male sterility was decreased and fertility enhanced by high temperatures in homozygous partial male sterile soybean plants. Control of the expression of partial sterility appears to be unique to the species in question. Sterility in ***Lycopersicon*** (Rick and Boynton, 1967) and ***Petunia*** (Izhar, 1975), can be induced by short exposure to high temperatures prior to meiosis. In tomato, the effect could be restricted to a single branch by treatment with the critical temperature (Rick and Boynton, 1967). Hashimoto and Yamamoto (1976) reported that a certain degree of sterility would result from low temperature (15^{o} C) treatment of fertile ***Glycine max*** 7 to 11 days before flowering. Graybosch and Palmer (1984) have indicated that sterility in partially male sterile soybean plants occurs as a result of aberrations at specific stages of microgametogenesis. In some species, there appears to be an optimum temperature regime for the expression of male sterility (Carlson and Williams, 1985).

Thomas (1961a) studied the effect of environment on the average percentage of aborted pollen in the anthers of the florets sampled from ten clones of "Grasslands Huia" white clover. He found that the degree of pollen abortion was greatest at the lowest temperature (10^{o} C), but there was no clear influence of daylength. He also found that there was no consistent difference between the "fertility" of pollen formed at 30^{o} C or 20^{o} C. This effect of temperature was reflected in a parallel study of pollen abortion in a population of wild white clover growing outside in Palmerston North. In these plants pollen sterility decreased from 41% in August (Southern winter) to 7% in February (Southern summer) (Thomas, 1981). These results suggest that pollen fertility is an unlikely cause for low seed set in white clover.

Johns and Palmer (1982) studied the flower development in mutant soybean plants (T-269) with low seed set. Observations of the gynoecium of mature flowers revealed that megasporogenesis and megagametogenesis were normal but other features of ovule ontogeny were not. They concluded that low seed set on T269 plants was due both to a lack of self pollination, brought about by the abnormal petal development preventing staminal tube elongation, and to partial female sterility. The degree of aberration varied even within a carpel, but they estimated that at least 75% of the ovules were too aberrant to be functional. Sedgley (1989) observed the ovule and seed development in ***Eucalyptus woodwardii*** from anthesis to capsule maturity and found that of a mean of 280 ovular structures per ovary, 79 were sterile, 160 were apparently fertile, and 41 were abnormal. The sterile ovules consisted of outer and inner integument only. The normal ovules had an embryo sac with two synergid cells, an egg cell, and a central cell with one or two nuclei. The abnormal ovules showed reduced, multiple, or bisected embryo sacs. These results show that about 43% of ovules formed in an ovary were sterile and were not capable of forming seed. Carapetian and Rupert (1989) compared the development of safflower ovules and female gametophytes in fertile and genetically sterile genotypes. Fertile plants formed normal anatropous ovules with eight-nucleate embryo sacs. Sterile plants also formed normal ovules, but apparently with a delayed initiation of meiosis which was subsequently arrested at metaphase 1. Embryo sacs did not form in sterile florets except for the rare occurrence of uninucleate embryo sacs which began to degenerate before anthesis. The above results strongly suggest that timely initiation of meiosis is important for formation of functional megaspores and any factor which interferes with meiosis might cause ovule sterility.

Though megaspore development is an important factor controlling seed set in many species, no information exists with regard to ovule sterility and to the influence of environment on ovule sterility in white clover. Povilaitis and Boyes (1956) have made the interesting observation that, in red clover, ovule and pollen fertility appear to go hand in hand, and a similar situation appears to exist in ***Matthiola*** (Semeniuk, 1958). This suggests that environment might influence the ovule fertility as environmental conditions seem to control the expression of male sterility in many species (Carlson and Williams, 1985). If this is the case in white clover, with optimal conditions for pollen fertility also being optimal for ovule fertility, then low temperatures would lead to ovule sterility and temperatures within the range 20^{o} C to 30^{o} C would result in optimal fertility (Thomas, 1961a).

2.3.2 Fertilization failure

Pechan (1988) reported that failure of fertilization was the major factor limiting the number of seeds per pod in oil seed rape (***Brassica napus***). Sufficient pollen germination on a stigma did not guarantee full seed set and even when pollen tubes were present at the micropylar region, ovules were not penetrated. He suggested that there may therefore be a "barrier" between the pollen tube and the ovule which could prevent fertilization. It is possible that a chemical is produced by the ovule and emitted through the micropyle to facilitate pollen tube entry into the ovule. The precise origin of such a chemical is uncertain but it has been suggested by Rosen (1975) that it might originate in the synergids. Wilms (1981) has shown that in ***Spinacia*** immature ovules cannot be penetrated by pollen tubes but in mature ovules, subsequent to pollination, synergids release substances which dissolve the middle lamellae of the nucellus in the micropyle region thus allowing pollen tubes to penetrate the ovule. Pechan's (1988) observation that in the normal process of ovule fertilization in ***B.napus***, one of the two synergids degenerates but in unfertilized ovules both remain intact, strengthens the theory that synergids may be important in the fertilization process.

2.3.3 Post fertilization events

2.3.3.1 Pod abortion

In legumes, in particular ***Vicia faba***, carpels of half the fertilized flowers retained on the plant fail to develop into mature pods. Jacquiery and Keller (1980) have investigated this phenomenon in detail using ^{14}C labelling techniques and have identified a critical phase in early pod development during which pods are unable to compete successfully with other assimilate sinks. Gates et al. (1981) reported that failure of pod development in ***Vicia faba*** is associated with failure of vascular development in the pedicel and peduncle. But to date there are no reports indicating that pod abortion or floret abortion after fertilization is a problem in white clover.

2.3.3.2 Zygote and seed abortion

In ***Vicia faba*** there are commonly four ovules per ovary, and those ovules at the basal end of the carpel abort most frequently (Kambal,1969 and Champan et al. 1979). It is likely that this is either a result of failure of pollen tubes to traverse the whole length of the ovary and fertilize ovules nearest the pedicel, or the result of inter- ovule competition resulting from fertilized ovules nearest the stigma having a temporal developmental advantage. Differences in time of fertilization as little as one hour have been shown to induce inter-ovule competition for assimilates and lead to abortion of ovules fertilized later in ***Phaseolus vulgaris*** (Weinstein, 1926). In white clover, Atwood (1943) suggested that abortion of some developing fertilized ovules might arise as a result of competition for nutrients within the inflorescence. Unpublished X-ray photographs obtained by Romero (1985) showing the number of ovules forming seeds in individual florets in white clover, clearly demonstrate that abortion of ovules is not a result of failure of pollen tubes to traverse the whole length of the ovary, as ovule abortion is not confined to the distal portion of the carpel. The conclusion from these studies is that the time of fertilization or lack of nutrients might induce inter-ovule competition and lead to abortion of developing fertilized ovules in white clover.

2.4 SEED DEVELOPMENT

Development of an ovule into a seed proceeds as a response to fertilization. Environmental conditions before anthesis influence both the number and size of flowers and inflorescences in a crop, and thus determine the potential number of seeds. Environmental conditions at anthesis and during the next few days then determine how many seeds are set. The factors involved in seed development are many, but the major factors are as follows:

2.4.1 Control by temperature
2.4.2 Control by water stress
2.4.3 Control by nutrition
2.4.4 Control by light

2.4.1 Control by temperature

Sofield et al. (1977) observed that the duration and the rate of grain growth in wheat can vary substantially depending on cultivar and environmental conditions. Temperature exerts the greatest effect on duration, whereas both temperature and illuminance influence the rate of grain growth. They found that a rise in temperature of 15° C, from 15/10° C to 30/25° C (day/night), reduced the duration of grain growth by about two-thirds. The above results suggest that high temperatures in the field after anthesis would impose a major limitation on wheat yield through reduction of the duration of grain growth. They also found that environmental conditions (light and temperature) after anthesis influenced grain set or retention in wheat. High temperature reduced it slightly, and low illumination to a greater degree. The effect of high temperature and low light, both of which reduced grain size, was dependent on the floret position in the spikelet. Under low irradiance the grains in the upper florets were affected more than those in basal positions, a response that might be expected where there is competition for assimilates. High temperatures, on the other hand, reduced final grain size in all florets, which suggests that they directly affected the synthetic processes in the grain rather than the availability of assimilates.

The reproductive period (flower initiation to seed maturation) of cowpea is composed of overlapping periods of development of individual fruits, each lasting about 19 days. The longer the reproductive period, the greater the number of fruits that mature and the larger the yield (Wien and Summerfield, 1984). Summerfield et al. (1978) studied the responses to increased day temperature on duration of reproductive period and found that by increasing day temperature from 27° to 33° C, all but one of 22 cultivars experienced a 20-60 per cent reduction in reproductive period, culminating in reduction in yield. Cool temperatures, especially at night, during the boll development period in cotton, delay the development and maturation of the young fruit in the "top" crop (Delouche, 1980).

2.4.2 Control by water stress

Slatyer (1973) reviewed the effect of water stress on grain yield in cereals. The grain yield or weight per grain is influenced by both pre-flowering and post flowering conditions. In almost all cases, however, the post flowering stage is important (Slatyer, 1973). Yield development requires the accumulation of photosynthates in the grain. The two sources of these assimilates are photosynthesis in the ear itself and translocation from elsewhere in the plant. Although photosynthates accumulated prior to anthesis contribute to grain filling, by far the greatest contribution is usually from photosynthesis after anthesis by the ear, leaves and stem (Allison and Watson, 1966). Asana (1966) demonstrated, in wheat, that virtually all the increase in dry weight after anthesis is associated with grain filling. Clearly, water stress by reducing photosynthesis at grain filling or seed development can lead to large reduction in yield. Wardlaw (1969) has shown, in ***Lolium***, that there is little effect of water stress on translocation of assimilates in the conducting tissue itself, but he has pointed out that translocation out of the leaves is slowed and prolonged by water stress. This phenomenon, combined with evidence that water stress hastens rather than slows maturation in wheat (Fischer, 1973), and with evidence that there is a direct effect of stress on photosynthesis in the ear as well as in the leaves, contributes to lower grain weight in water stressed plants (Slatyer, 1973). Because grain filling is a relatively rapid process and because most of the increase in plant weight after anthesis involves grain development, it follows that reduced photosynthesis at any point of the post-anthesis stage may have an effect on grain weight in wheat which may not be compensated by activity at other stages of grain filling (Fischer, 1973).

Laohasiriwong (1982) observed in soybeans that water stress from the start of anthesis through to the beginning of seed maturity severely reduced yield by decreasing the number of pods per plant and slightly decreasing seed weight. Momen et al. (1979) found that the greatest seed yield reduction in soybean due to moisture stress occurred during pod filling stage. Yield losses of 50% have been reported in field-grown soybean at 10% available soil water (Doss et al. 1974) and about 40% in growth-chamber-grown soybean when leaf water potential was down to -2 to 3 Mpa (Sionit and Kramer, 1977). Sionit and Kramer indicated that stress at this stage produced the smallest seeds and shortened the length of the maturation period.

Zaleski (1970) reported that, in white clover, irrigation was beneficial for seed production when applied at the beginning of flowering. He also observed that an excess of water either from natural precipitation or irrigation during the flowering period increased the vegetative growth at the expense of head production and yield of seed. Under the drier conditions experienced in New Zealand it is sometimes necessary to irrigate during flowering if the weather is such that the plants show signs of wilting (Jolly, 1958). Clifford (1986) studied the effect of irrigation on seed yield and some yield components of "Grasslands Kopu" white clover and found that irrigation increased seed yield by 53%. The observed increase, in part, was attributable to a 4% increase in seed weight and 27% reduction in ovule abortion. He suggested that timing of water application to white clover seed crops should ensure (a) that only sufficient moisture is maintained to enable continuing growth, and thereby flowering (initiation), and (b) that the permanent wilting point is never reached, thus avoiding increased ovule abortion caused by poor plant nutrition.

2.4.3 Control by nutrition

Brevedan et al. (1978) illustrated the importance of the nitrogen nutrition of the soybean plant during the period from anthesis to pod set. An increase in N supply to the soybean plant during this flowering period increased the seed yield in the green house by 40% and in field studies by 22% to 32%. The N supply to the plant seemed to be of much less importance during the pod filling period than during the flowering period. The maximum demand for nitrogen in ***Vicia*** bean is associated with pod and seed development (Cooper et al. 1976). McEwen (1970) found that the greatest increase in yield in response to a heavy dressing of nitrogen was obtained by applying it at or after anthesis. Johnson and Wear (1967) reported that the addition of boron at the rate of 561 g/ha significantly increased white clover seed production. This response was due entirely to an increase in the number of seeds per seed head. As with most legumes, white clover requires adequate levels of lime, phosphate, sulphur, and molybdenum (Scott, 1977).

2.4.4 Control by light

The effects of light on yield can be separated into those during early vegetative growth and differentiation of the reproductive organs on the one hand, and those which follow and determine the extent to which they are filled on the other. Photosynthesis is the dominant process in the later stage. Assimilates stored earlier in the life of the plant usually contribute little to growth of seeds, which depends mainly on concurrent photosynthesis (Evans, 1973). When supplied with abundant water and nutrients, a high yielding cultivar of ***Oryza*** in the Philippines increased its yield with increasing radiation during grain filling, the correlation co-efficient between them being 0.713 (de Datta and Zarate, 1969). It has long been known that mutual shading of leaves and competition for light is especially evident in soybean. Under field conditions, the middle and bottom soybean leaves do not reach their photosynthetic potential, because of both shading and an overall degeneration of metabolic activity with age. Apparent photosynthesis can be increased by artificially lighting the bottom of the soybean canopy. Johnston et al. (1969) revealed that the apparent photosynthetic rate of bottom leaves increased 73% and of middle leaves increased 41% when plants were illuminated by fluorescent lamps at three canopy levels 23,46, and 69 cm above the soil surface. The treated plants had more seeds, nodes, pods, branches, pods per node, and seeds per pod than untreated plants. Light enrichment increased seed yield of the bottom, middle, and top canopy position by 30, 20, and 2% respectively, compared to controls. Schou et al. (1978) also found a 40 percent yield increase, mostly from lower nodes, in light enriched plants. They indicated that light enrichment increased the yield by increasing the number of pods which were formed and retained. Research on light reduction or shading in soybean provides complementary results to light enrichment studies. Seed yield from shaded plants was observed to be lower than from normal plants (Schou et al. 1978), especially when shading occurred during the early seed filling period (Baharsjah et al. 1980). All findings from light enrichment and shading studies strongly suggest the need for more photoassimilates to enhance pod retention and seed development. In other words, yield increase in soybean is limited by the availability of photoassimilates during seed development. Romero (1985) observed that the average number of seeds per floret in white clover was 0.1-1 in the 1982/83 growing season and suggested that the low seed number per pod was probably a reflection of the strong winds and excessive rainfall during the flowering period. In the following season (1983/84) the level of seed set (2.0-2.3) was higher. These differences were thought to reflect the effect of climatic conditions on pollinator activity and subsequent seed set. Possibly, in the writer's opinion, a difference in light intensity caused by the weather could also cause variation in seed number per floret through seed abortion. Atwood (1943) suggested that abortion of some developing fertilized ovules might arise as a result of competition for nutrients within the inflorescence.

Observations that poor weather leads to low seed yields per flower head are frequently held, with the suggestion that this is a result of lower activity of bees as pollinators (van Bogaert, 1977). However it is equally likely that bad weather might affect any or most of the factors listed above. Various other observations have suggested that the number of seeds set might be limited by the availability of nutrients for seed development. Under "low nutrient conditions" it is suggested that competition between florets in a flower head or ovules within a floret might lead to the abortion of developing seeds. The assumption has been made regarding the nutrient competition hypothesis that nutrient shortage results in post-fertilization abortion of developing seeds. It is important to realize, however, that all the observations on which this hypothesis is based could be explained equally well by proposing that fertility of ovules is adversely affected by poor nutrient conditions. Data available to date do not allow us to distinguish between these alternatives. The present investigation looks at the possibility that the low seed set per floret might be the result of ovule sterility.

Roberts (1979) showed a 50% increase in seed yield of white clover at 60 cm spacing compared to a broadcast sowing. This was attributed to increased inflorescence production and a higher number of seeds per inflorescence. Mohamed (1981) compared the seed yield components between plants established as single individuals with 90 cm spacing and in swards. In swards, floret number per flower head was reduced by 17% compared with similar plants established at 90 cm spacing. Similarly he noted that the seed set per floret was reduced by 13% under sward conditions. He suggested that decrease in seed yield per inflorescence in swards may be a consequence of increased inter and/or inter-plant competition. In the present writer's opinion, such decreased yield may be due to intra-plant competition for light resulting from the low light intensities at stolon levels in the sward compared with similar plants established at 90 cm spacing.

Because it is known that low light intensity can lead to total abortion of developing white clover flower heads (Zaleski, 1964) and that the number of seeds per floret in a "good" (warm, dry, sunny) summer is often up to 50% higher than in a "bad" (cool, rainy, dull) summer (Romero, 1985), the present study was undertaken to determine the influence of light intensity on flower head development and seed yield components in this species.

White clover has horizontally placed leaf blades borne at the top of thin erect petioles. When matured, adjacent leaves are sufficiently close to one another to form a distinct canopy. Light intensities beneath the foliage canopy of a white clover seed crop are often as low as 1% even at midday when incoming radiation is most intense. Flower heads of white clover emerge from the stolon apices in the axils of the youngest leaves. At this stage all their florets have been initiated and they are borne on very short peduncles (Thomas, 1987). Over the next few days the flower heads continue to grow and are gradually raised above the stolon by elongation of their peduncles. In a dense canopy this post-emergence growth takes place for several days in heavy shade before the flower heads are raised above the foliage. In other species (e.g soybean), however, there is evidence that shading the organs reduces their ability to draw assimilates from the source leaves as described in section 2.2.4.3. It is thus possible that shading may also affect the sink activity of white clover inflorescences. The present study was undertaken to determine the extent to which the growth and sink activity of young flower heads and peduncles is influenced by the shaded conditions that exist within dense white clover canopies.

SECTION A

Influence of low light on ovule fertility and seed set.

Growth room Experiments.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Plant material

Two genotypes of "Grasslands Huia" white clover (***Trifolium repens*** L.) were used in experiments: clones A and C. Both clones were selected by Thomas (1962, 1979). They differ in several respects, clone C having larger leaves and shorter stolon internodes than clone A. Clone A flowers more profusely than Clone C. In the present investigation all plants were uninoculated.

3.1.2 Propagation and Plant Maintenance

The departmental glasshouse was used for propagating plants for all experiments. As temperature is one of the major parameters which affects the growth of the plant, the maximum and minimum temperatures were measured. In summer, the maximum daytime temperature was generally in the range 28-33^{o}C and minimum night temperatures about 15-17^{o}C. The maximum daytime temperatures during autumn were generally in the range 25-30^{o}C and minimum night temperatures were around 15^{o}C.

Light intensity within the glasshouse was measured on one occasion on a sunny day at 10.30 a.m. in mid-January, to compare the intensity inside the glasshouse with outside. Light intensities were measured with a LI-COR LI-188B meter using a quantum probe: serial number Q 316-7309. This measured quantum flux (μE m^{-2} sec^{-1}) at photosynthetically active wavelengths (380-700nm). Measurements were taken at the same height as leaf laminae. The average light intensity inside the glasshouse was 1459 μE m^{-2} sec^{-1}, whereas the intensity outside the glasshouse in full sunlight was 1767 μE m^{-2} sec^{-1}. The plants in the glasshouse received about 85% of the daylight.

Plants were multiplied by taking tip cuttings from the stock plants. The best stolons to use as cuttings were found to be young and elongating stolons with at least two visible nodes, those of the youngest and next to youngest unfolded leaves. The plants were covered with a plastic sheet in the glasshouse to avoid direct sunlight and to provide a humid environment for two weeks to prevent them from wilting. They were then transferred to high light intensities in the glasshouse where they were watered frequently.

The potting mixture consisted of one part of peat and one part of sand. For every 1000 cm^3 of potting mixture, 170 cm^3 of lime, 35 cm^3 of slow release fertilizer (Osmocote), and 25 cm^3 superphosphate were added.

Two systems of watering were used. In one the plants were sub-irrigated by standing pots in a tray of water. This system of watering was used in summer. One problem with this system was that the level of water had to be closely watched to make sure that plants were not standing permanently in a pool of water. The distribution of water in the tray also had to be watched to make sure that all plants received adequate water. The other system was daily or twice daily application of water to the plant by pouring water into the pot and allowing it to soak down. This system of watering was used in autumn.

Aphids, especially pea and blue green lucerne aphids, and red spider mites are sometimes a problem. To cope with these pests, pesticide sprays were used. Aphids were usually controlled with Maldison 50 (50% Maldison; Yates); and a synthetic Permethrin (48% pirimiphos-methyl and 2.5% Permethrin ;1 ppm;Ambush, ICI Tasman). Mites were effectively controlled by Neoron 50 (50% Bromopropylate; 2 ppm; Ciba-Geigy). The pesticides were applied, either by watering or spraying.

3.2 METHODS:

3.2.1 Cytological technique to observe ovule fertility

The florets which reached anthesis were fixed in formalin/acetic/alcohol (FAA; 95% ethanol:water:39-40% formaldehyde:glacial acetic acid,10:7:2:1 by volume) until required for examination. Florets were stored in FAA for three months. Plant material can be effectively stored in this solution for up to four months (Young et al. 1979). Pistils were dissected from the flowers and transferred through the following schedule for specimen preparation.

3.2.1.1 Hydration

Before staining, the FAA fixed pistils were hydrated by transferring the material through a series of hydration steps as follows: 50% ethanol; 25% ethanol; distilled H_2O (two changes). The pistils were placed in a watch glass and each step was conducted using 5 ml liquid for at least 15 minutes. Solutions were changed with a Pasteur pipette. Finally the pistils were left in water for 24 hours.

3.2.1.2 Staining and destaining

The hydrated pistils were stained in Mayer's haemalum for 24 hours. Mayer's haemalum stains both cytoplasmic contents and the nucleus of a cell. To remove the stain from the cytoplasm while staining the nucleus the stained pistils were destained with 1.25% acetic acid. To achieve proper contrast the pistils were destained for 32 hours as described in Appendix 1. Destained pistils were rinsed in tap water for 24 hours to remove any remaining stain and destain materials on the pistils.

3.2.1.3 Dehydration

In the present study methyl salicylate was used as a clearing agent. Methyl salicylate is water insoluble so that destained pistils had to be dehydrated before clearing. The pistils were dehydrated by transferring them through a series of dehydration steps as follows: 25% ethanol; 50% ethanol; 70% ethanol; 95% ethanol; 100% ethanol (two changes). The pistils were placed in a watch glass and each step was conducted using 5 ml liquid for at least 15 minutes. Finally dehydrated pistils were left in 100% ethanol for 8 hours to ensure that they were properly dehydrated.

3.2.1.4 Clearing

Dehydrated pistils were optically cleared with methyl salicylate by transferring them through a series of clearing steps as follows: ethanol:methyl salicylate 2:1; ethanol:methyl salicylate 1:2; 100% methyl salicylate (three changes). The pistils were placed in a watch glass, and each step was conducted using 5 ml liquid for at least 15 minutes. Stained and cleared pistils were stored in vials containing methyl salicylate at room temperature.

To prepare slides, ovules were dissected out of ovaries and mounted in methyl salicylate. The ovules were covered with 22x50 mm wide coverslips and examined using Nomarski interference optics. A light green filter was used for viewing.

3.2.2 Pollen fertility

Pollen fertility was assessed by examination of a sample of microspores from each floret mounted on a glass slide and stained with Snow's alcoholic carmine (Snow, 1963). The stain was prepared as follows: to 15 ml of distilled water in a small beaker, 4g of certified carmine powder and 1 ml of concentrated HCl were added. The contents were mixed well and boiled gently for about 10 minutes while stirring frequently. After the contents had cooled down, 95 ml of 85% ethanol was added and then filtered. Snow's alcoholic carmine stains the cytoplasmic contents of the pollen grain. Pollen grains devoid of contents were classed as sterile and those containing cytoplasm as fertile.

Growth room Experiments.

CHAPTER 4

EFFECT OF LIGHT INTENSITY ON INFLORESCENCE DEVELOPMENT AND SEED YIELD

4.1 INTRODUCTION

The potential seed yield of white clover is determined firstly, by the number of flower heads initiated and secondly, by the efficiency of the pollinating agents. Whether pollination leads to fertilization depends largely upon the condition of the flower heads before anthesis. The primary developmental factors influencing the potential seed yield are those controlling the initiation and growth of reproductive organs (Thomas, 1961a). In conditions suitable for initiation, the seed production capacity is determined by the number of flower heads initiated, the number of florets per flower head which develop to anthesis, the number of ovules in each floret and the fertility of pollen and embryo sacs.

Light intensity has been shown to be an important factor affecting the amount of flowering of white clover. Zaleski (1970), working with "S-100" white clover under controlled conditions, has indicated that one of the most important factors determining the initiation of floral primordia and inflorescence development is the amount of light available. Thomas (1961a, 1987) found that low light intensities and short warm photoperiods in particular seem to favour the abortion of inflorescences. Zaleski (1964) found that when plants are grown in low light intensities, many of their flower heads aborted either completely or partially, thereby reducing the number of functional florets per head.

The observations of Zaleski (1964) and Thomas (1961a, 1987) on flower head abortion in low light intensities/ warm short days have led to this investigation. The present investigation was undertaken to determine the effects of light intensity on inflorescence development, the number and size of ovules in carpels, the fertility of embryo sacs and the average number of seeds per floret.

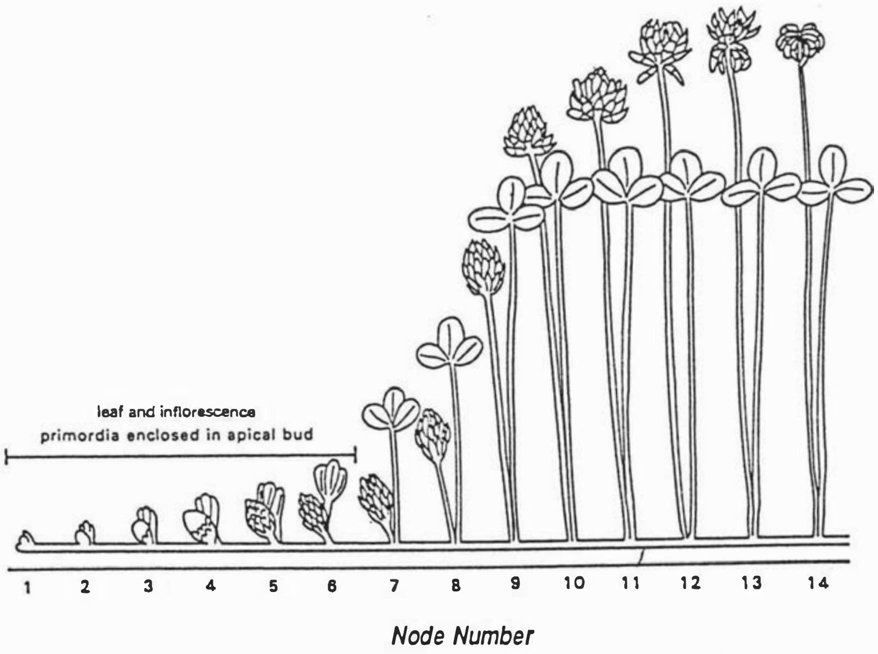

FIGURE 4.1 : Diagrammatic representation (not to scale) of stages of inflorescence and leaf development in white clover relative to distance from the stolon apex. Nodes from youngest to oldest are numbered 1 - 14. The youngest leaf with unfolded leaflets occurred at node 7, younger nodes are enclosed in apical bud (Thomas, 1981).

4.2 MATERIALS AND METHODS

4.2.1 Plant material

Data were collected in two separate experiments, in both of which ramets of clone A of "Grasslands Huia" white clover were used. Clone A had previously been the subject of study in controlled environment conditions (Thomas, 1961a) when it was referred as New Zealand Government Stock. The plants for study were grown from stolon tip cuttings taken from stock plants and grown in a mixture of sand and peat in 1000 cm3 plastic pots as described in chapter 3. These established plants were maintained for a month in a glasshouse before they were transferred to the growth room. During summer the maximum day/ minimum night temperatures in the glasshouse were generally in the range of 28-33^{o} C/ 15-17^{o} C while during winter they were in the range of 20-23^{o} C/13-15^{o} C. These plants received about 85% of daylight.

4.2.2 Experimental procedure

Both experiments were conducted in a controlled environment growth room. Temperatures in the growth room averaged 23 $\pm 1^{o}$ C. The light source used, a combination of 15- Watt incandescent lamps (Philips, 15W, clear) and fluorescent bulbs (Philips, TLD 58W/33,white), gave an intensity of approximately 10000 lux at leaf surfaces.

Experiment 1: The established plants in the glasshouse were brought into the controlled growth room on 7th July 1987. They were artificially induced to initiate inflorescence by exposing them to continuous light for three weeks. Three stolon apical buds were selected at random, dissected and examined microscopically to determine the stage of inflorescence development (node position) and to ensure that inflorescence initiation had occurred before the plants for study were subjected to various light regimes. Using the numbering system described previously by Thomas, (1961b), the node bearing the youngest leaf primordium in the apical bud is designated as node 1 (N1), and the youngest leaf with unfolded leaflets occurred at node 7 (N7) (see Fig. 4.1).

Plate 4.1 : *Plants growing in different light intensities in the controlled environment growth room (Experiment 1 & 2). The photograph showing four light intensity treatments (from left 2800 , 4500, 6500 lux & full light (10,000 lux).*

After this preliminary treatment, plants were grown under five different light intensities ranging from 2000 to 10000 lux as shown in plate 4.1. This was achieved by using one to three layers of shade cloth. Twelve plants were used for each treatment. The result of each treatment was recorded by measuring the length of floral organs on oldest florets from the inflorescence base at different stages of inflorescence development (inflorescences at node 7 to node 11). Five florets from each of two flower heads for each stage of development per treatment were collected for measurements.

Experiment 2: Light intensity treatments were started on 12 Jan 1988, with light intensities ranging from 2000 to 10000 lux. Investigations were made to observe the influence of light intensity on the ovule number in each carpel, the ovule fertility and the average seed number per floret. Ten flower heads were used for each treatment. When all the lower florets in an inflorescence had reached anthesis, three florets were removed from each flower head to determine the ovule number and the ovule fertility. The remaining florets were used for seed set observations.

4.2.3 Measurements

4.2.3.1 Number of ovules per floret

Twenty florets from the base of ten inflorescences showing white corolla colour in their oldest florets (inflorescence situated at node 11)) were collected for all treatments. Ovaries were dissected out of the florets and fixed in Formalin/acetic/alcohol (FAA). Later, ovules were dissected out of these ovaries. The number of ovules in a carpel and the length and width of each ovule were recorded.

4.2.3.2 Ovule fertility

Ten florets from ten inflorescences showing corolla colour in their oldest florets (N11) were collected for all treatments and fixed in FAA. A stain-clearing technique described in chapter 3 was used to observe the cytoplasmic state of the embryo sac.

To prepare slides, ovules were dissected out of stained and cleared ovaries and mounted in methyl salicylate. They were covered with 22x50 mm wide coverslips and examined using Nomarski interference optics.

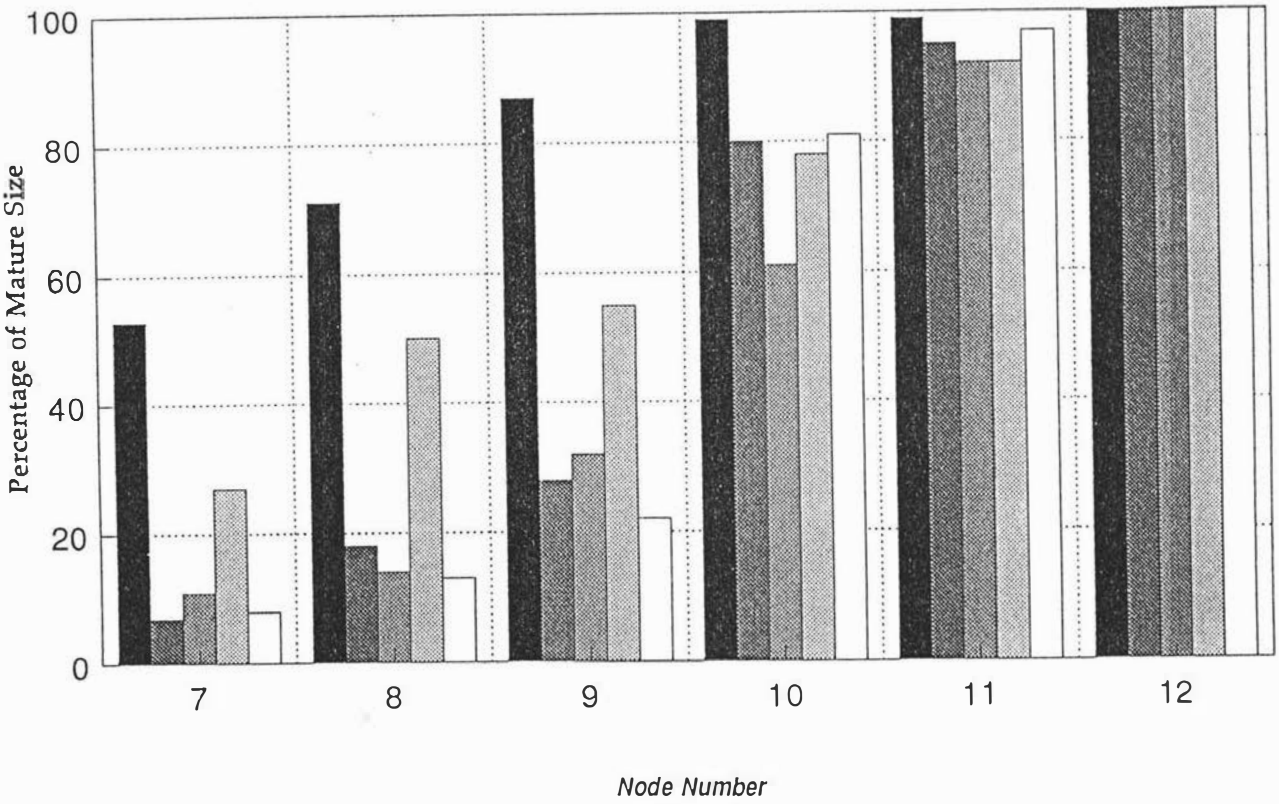

FIGURE 4.2 : The size of floral organs (expressed as % of maximum size attained at maturity) in the oldest florets of inflorescences present at successive nodes of stolons of plants growing at 10,000 lux light intensity in the controlled environment growth room.

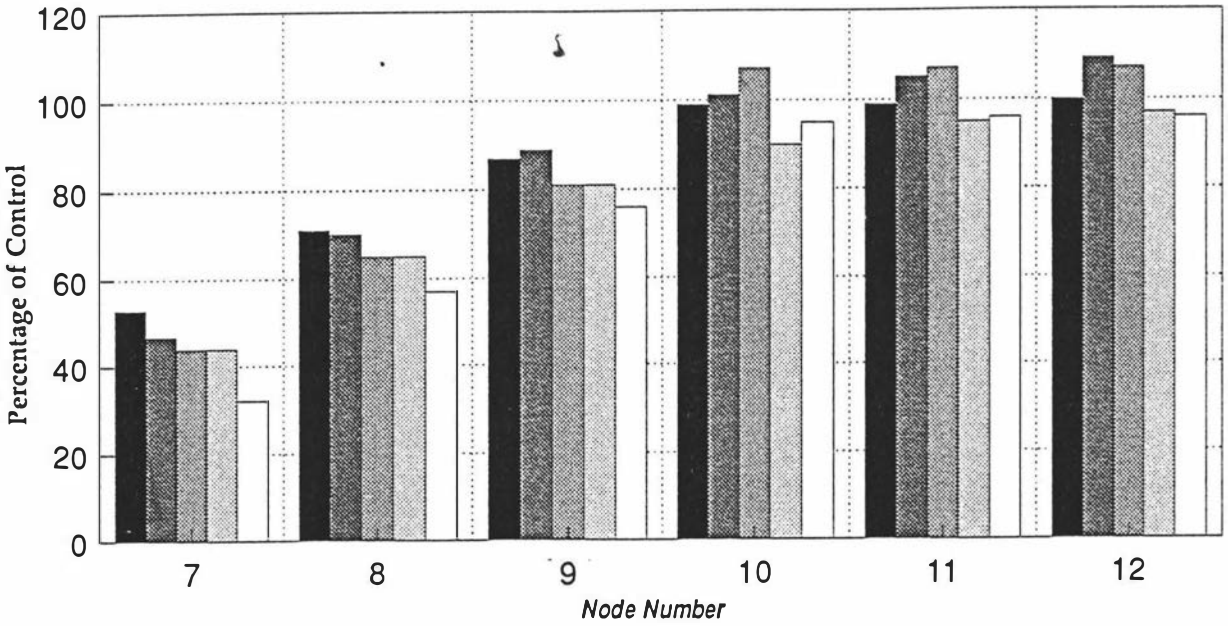

FIGURE 4.3A : The effect of light intensity on sepal growth. Sepal size (expressed as % of control at 10,000 lux) was measured in the oldest florets of inflorescences at successive nodes of stolons of plants growing in five different light intensities (10,000, 6,000, 4,500, 2,800, 2,000 lux) in a controlled environment growth room. See appendix 4A for statistical variability (Standard errors).

■ 10,000 lux ▩ 6,000 lux ▨ 4,500 lux
░ 2,800 lux □ 2,000 lux

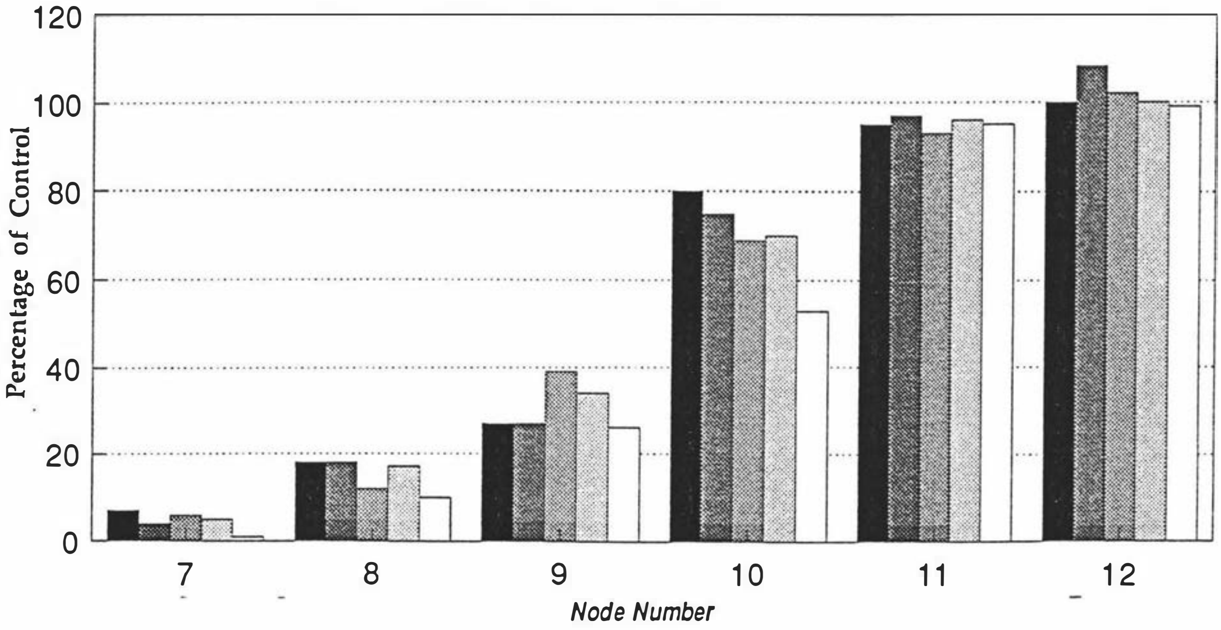

FIGURE 4.3B: The effect of light intensity on petal growth. Petal size (expressed as % of control at 10,000 lux) was measured in the oldest florets of inflorescences at successive nodes of stolons of plants growing in five different light intensities (10,000, 6,000, 4,500, 2,800, 2,000 lux) in a controlled environment growth room. See appendix 4B for statistical variability (Standard errors).

■ 10,000 lux
▨ 6,000 lux
▨ 4,500 lux
▨ 2,800 lux
□ 2,000 lux

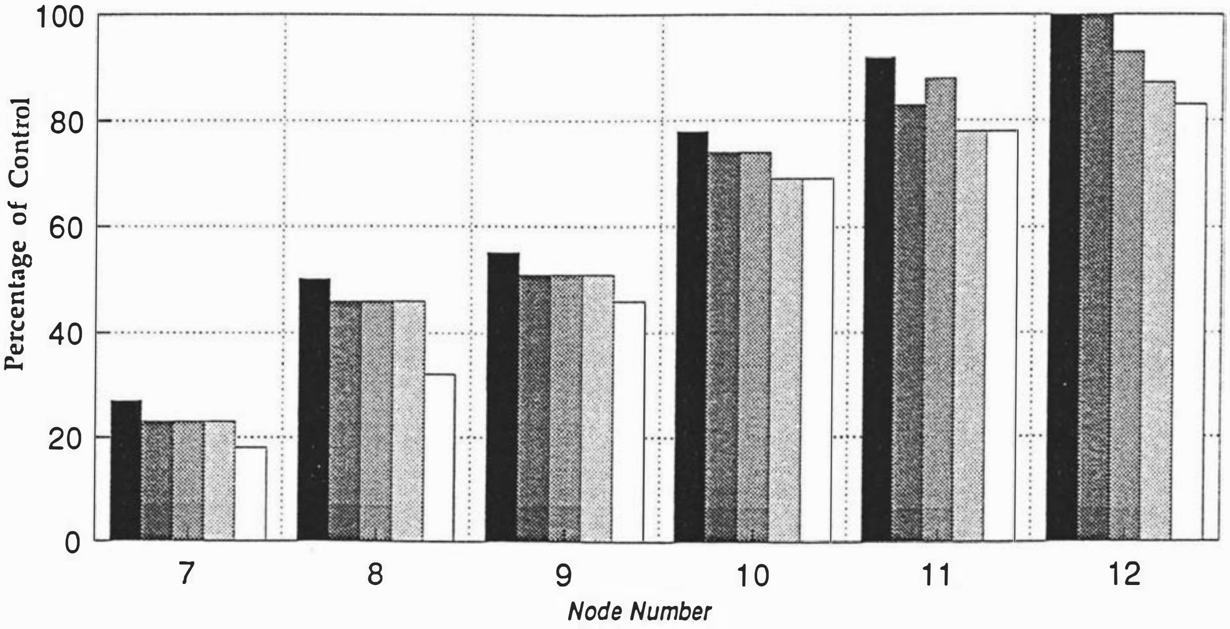

FIGURE 4.3C: The effect of light intensity on ovary growth. Ovary size (expressed as % of control at 10,000 lux) was measured in the oldest florets of inflorescences at successive nodes of stolons of plants growing in five different light intensities (10,000, 6,000, 4,500, 2,800, 2,000 lux) in a controlled environment growth room. See appendix 4D for statistical variability (Standard errors).

■ 10,000 lux
▩ 6,000 lux
▨ 4,500 lux
░ 2,800 lux
□ 2,000 lux

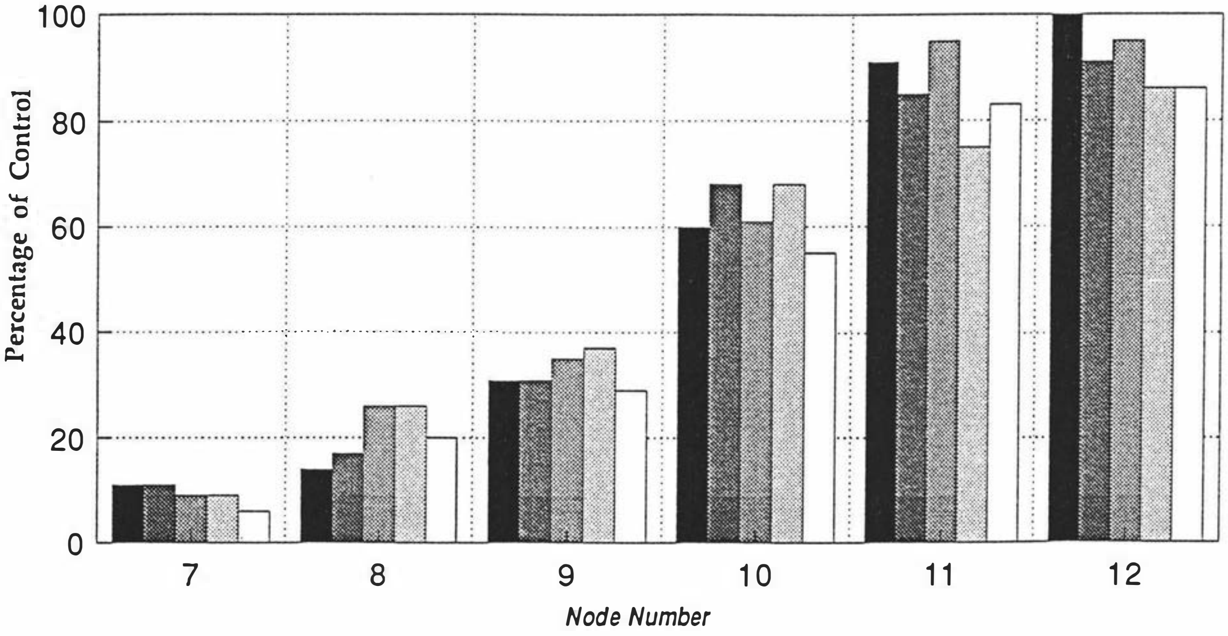

FIGURE 4.3D: The effect of light intensity on filament growth. Filament size (expressed as % of control at 10,000 lux) was measured in the oldest florets of inflorescences at successive nodes of stolons of plants growing in five different light intensities (10,000, 6,000, 4,500, 2,800, 2,000 lux) in a controlled environment growth room. See appendix 4C for statistical variability (Standard errors).

■ 10,000 lux
▩ 6,000 lux
▨ 4,500 lux
▒ 2,800 lux
□ 2,000 lux

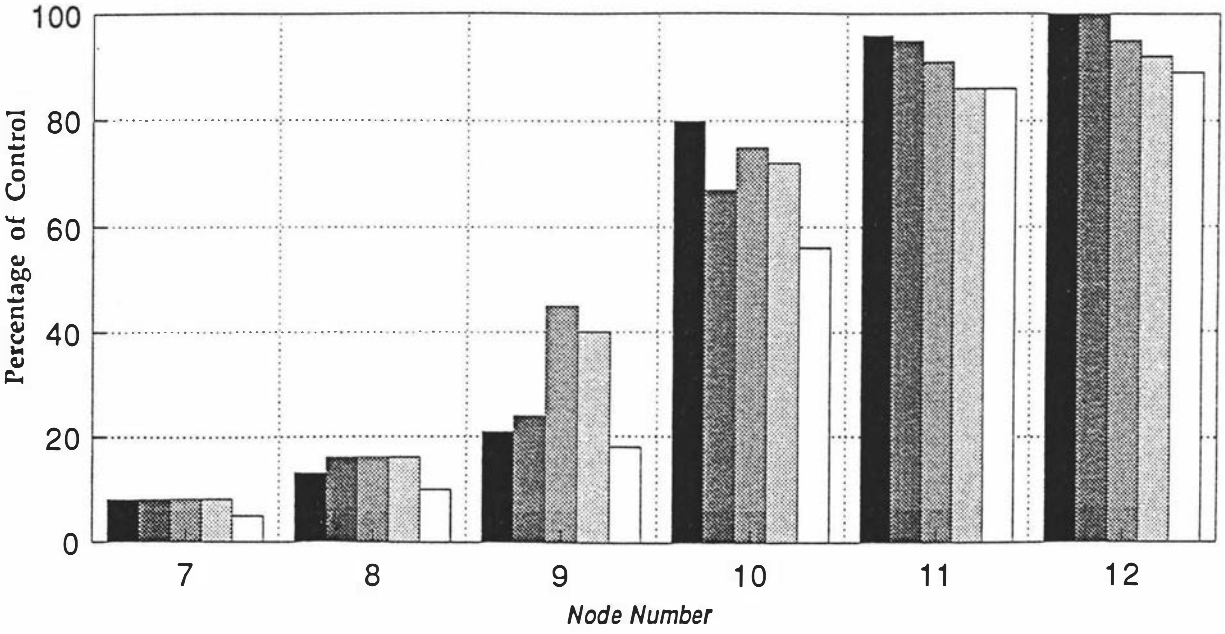

FIGURE 4.3E: The effect of light intensity on style growth. Style size (expressed as % of control at 10,000 lux) was measured in the oldest florets of inflorescences at successive nodes of stolons of plants growing in five different light intensities (10,000, 6,000, 4,500, 2,800, 2,000 lux) in a controlled environment growth room. See appendix 4E for statistical variability (Standard errors).

4.2.3.3 Seed number

When the flower heads on the plants in the growth room were fully developed, with all the florets showing white corollas, the plants were transferred into a prolifically flowering field plot of white clover where they were cross pollinated by natural means. The mean February temperatures were 22.8/13° C (maximum/minimum). 10-15 days after pollination, the flower heads were collected and stored at 4° C. For each treatment, the number of florets per inflorescence was counted. Thirty florets were then taken from each flower head and the mean number of seeds per floret recorded.

4.3 RESULTS

4.3.1 Floral development

The results obtained show that the most rapid growth of different floral organs occurs at different stages of inflorescence development. In the lower most florets on inflorescences of control plants growing at 10000 lux, 71% of the growth of sepals and 50% of the growth of ovaries occur by the time inflorescences are at node 7 to node 8 (N7-N8). The petals, filaments and styles seem to grow at later stages of inflorescence development (N10-N11). The growth of ovaries appears to be diauxic, two phases of high growth rate being separated by a slower phase. The slower phase occurs as the inflorescence moves from N8 to N9 (Fig.4.2). Similar trends were observed in all other light intensity treatments (Fig.4.3c). The effect of light intensity on the development of floral organs appears to vary from organ to organ (Fig.4.3). In the plants grown in intensities of 6000-2800 lux, the sepals and petals showed an etiolation response at later stages of their development (N9-N12) and the plants grown at 6000 and 4500 lux had longer sepals and petals than plants grown in 10000 lux (Fig.4.3a&b). The styles and filaments showed an etiolation response at early stages of their development (N7-N10). The final length of the styles, filaments and ovaries was less at lower light levels (Fig.3). It is clear from Fig.3 that ovary development is more sensitive to light compared to other floral organs.

TABLE 4.1: Effect of light intensity on average ovule number, size, and percentage of "fertile" ovules (Experiment 2).

Ovules were considered to be "fertile" when they consisted of healthy embryo sacs. Healthy embryo sacs contained four cells and four or five nuclei: two synergid cells, an egg cell, and a cell with either two polar nuclei or a single polar fusion nucleus. Ovules lacking fully developed embryo sacs were considered to be infertile.

Light Intensity (Lux)	Ovule Number per carpel (± SE)	Ovule Size		Percent of "fertile" Ovules
		Length (mm) (± SE)	Width (mm) (± SE)	
10000	5.6 ± 0.16	0.47 ± 0.0071	0.33 ± 0.0057	63
6000	5.5 ± 0.17	0.45 ± 0.0072	0.32 ± 0.0066	45
4500	5.3 ± 0.15	0.44 ± 0.0071	0.29 ± 0.0053	42
2800	5.0 ± 0.13	0.44 ± 0.0072	0.29 ± 0.0041	24
2000	5.2 ± 0.13	0.41 ± 0.0065	0.29 ± 0.0044	17

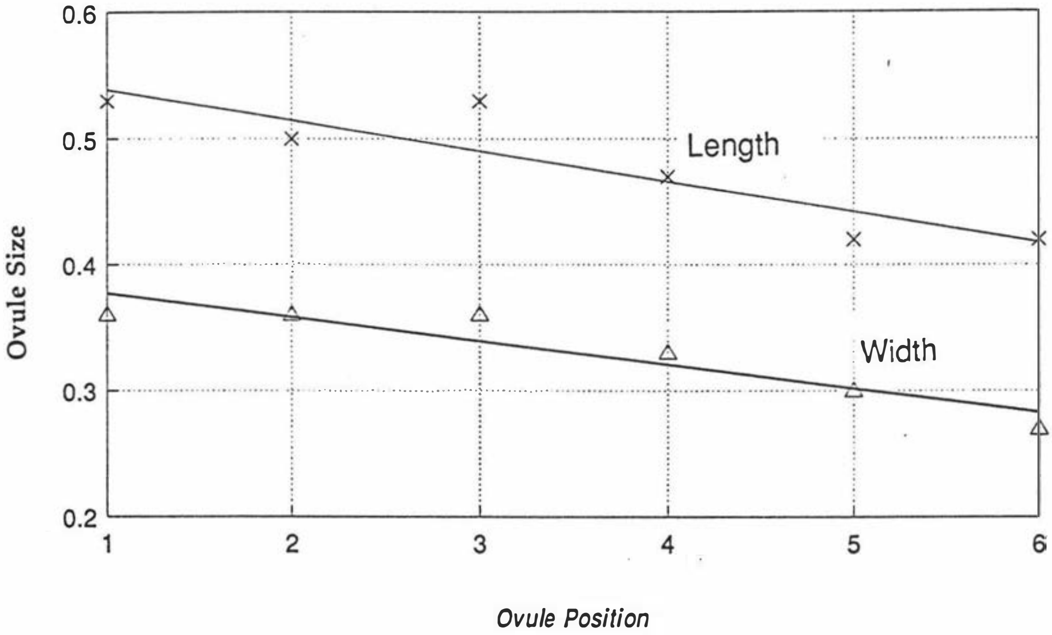

FIGURE 4.4 : The relationship between ovule size and position within the carpel. The mean lengths and widths (mm) are plotted for each of the six positions within the carpels of the oldest florets in inflorescences at node 11 of plants growing at 10,000 lux light intensity in a controlled environment growth room. The proximal (basal) ovules in a carpel are numbered 1 and distal ovules 6. See appendix 2 for statistical variability (Standard errors).

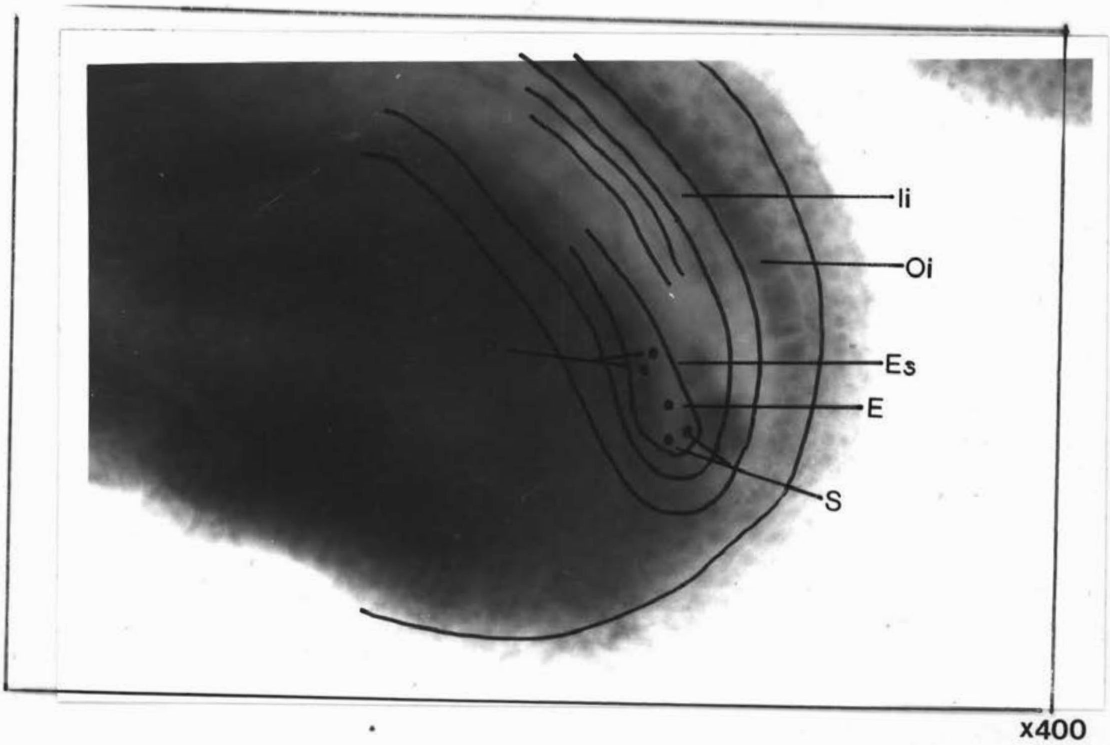

Plate 4.2 : *A fertile ovule consisting of an outer and an inner integument, nucellus, and an embryo sac. Embryo sac with four cells and five nuclei; two synergid cells, an egg cell, and two polar nuclei.*

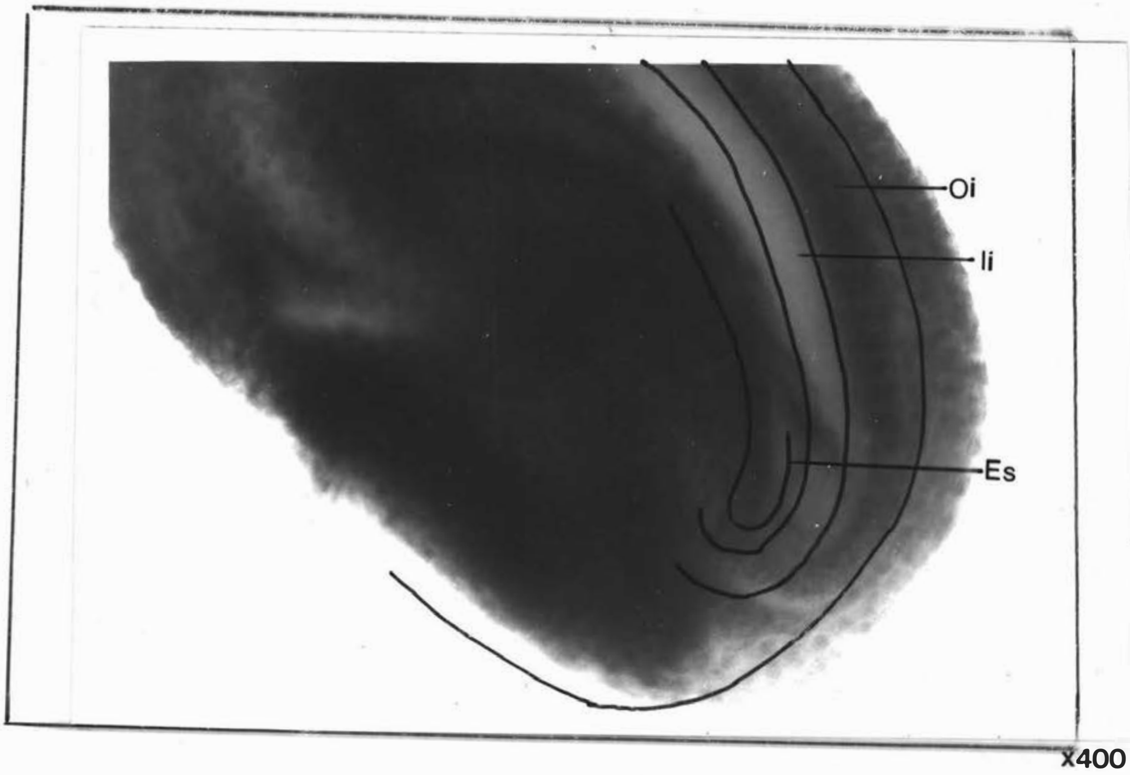

Plate 4.3 : *A sterile ovule consisting of an outer and an Inner integument, nucellus, and an embryo sac which Is rather shrunken with no visible nuclei.*

Outer integument; Ii= Inner integument; P= Polar nuclei; S= Synergid cells; E= Egg cell; Es= Embryo sac.

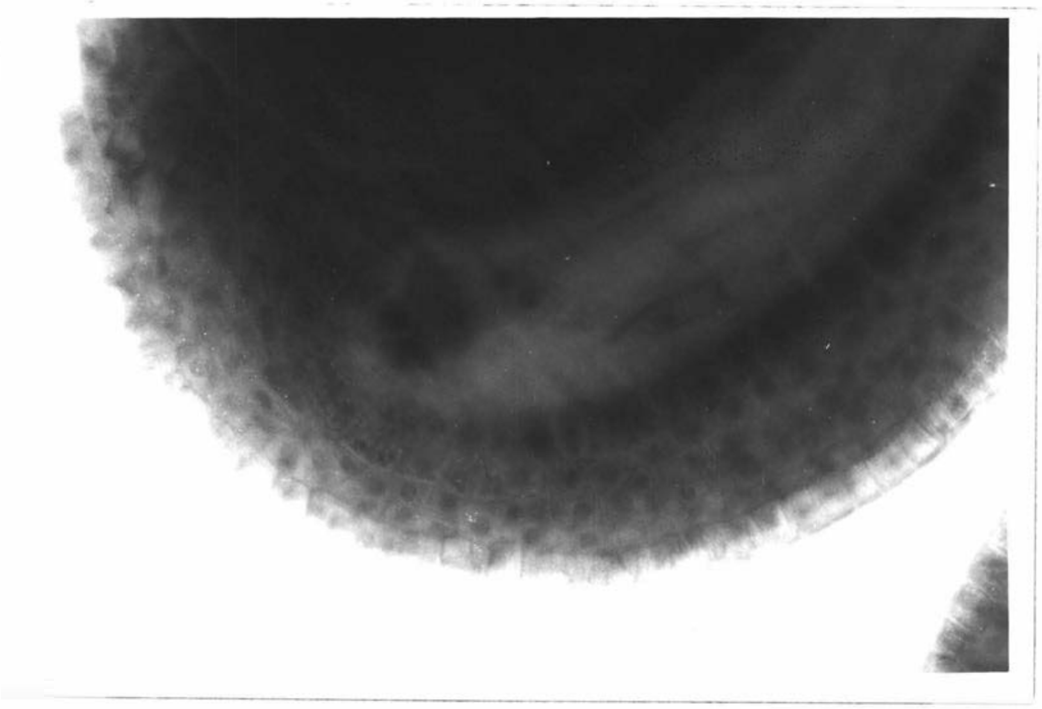

Plate 4.4A : Enlarged version of Plate 4.2 showing polar nuclei more clearly.

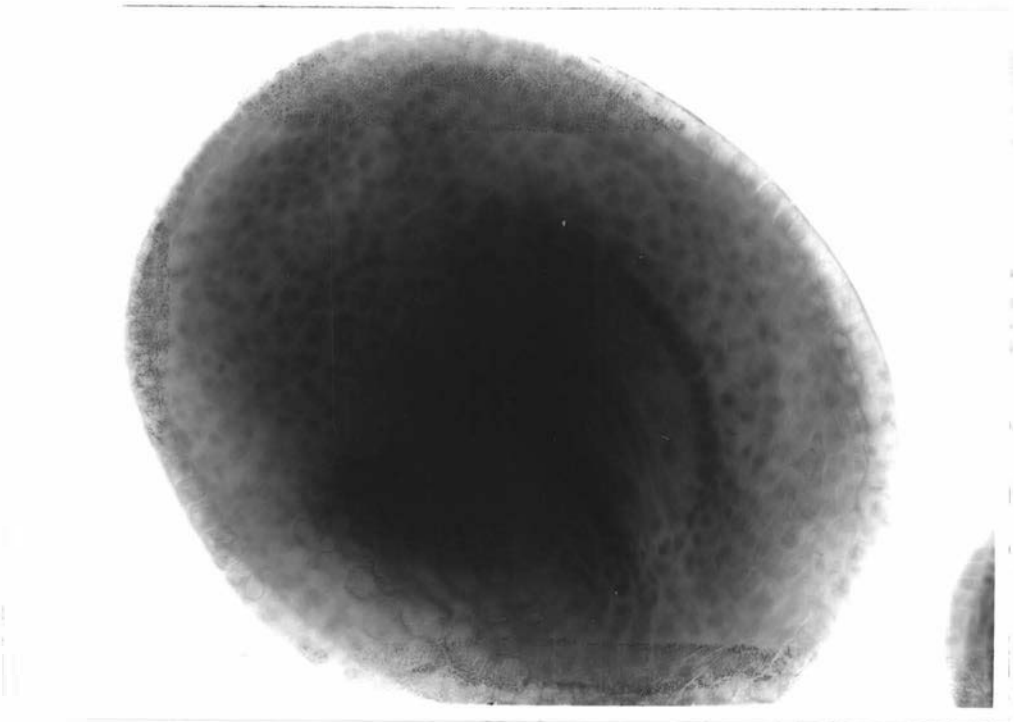

Plate 4.4B : Immature ovule from a mature carpel.

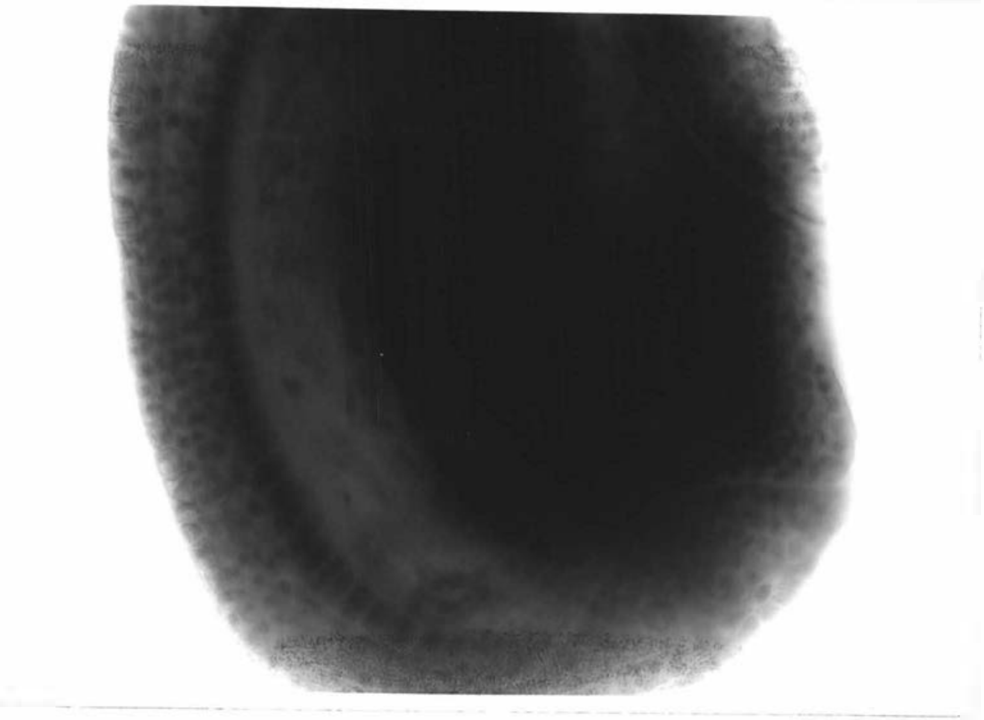

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Plate 4.4C : Ovule containing an embryo sac with no visible nuclei.

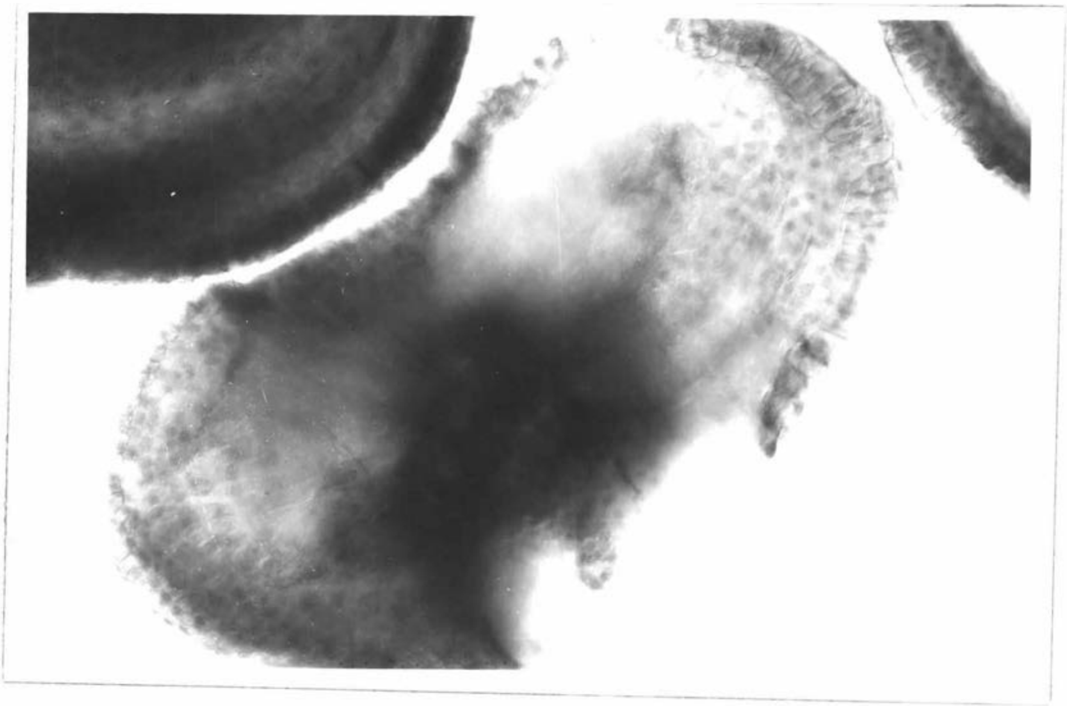

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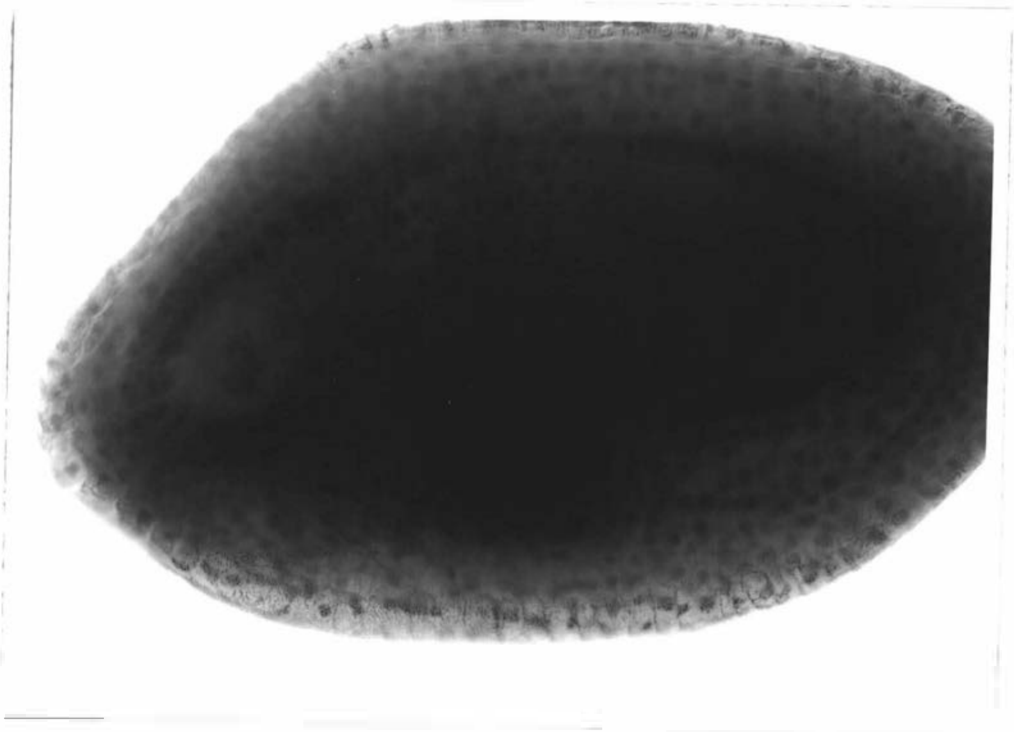
Plate 4.4D : Shrivelled ovule. Soft tissues damaged by extracting from carpel.



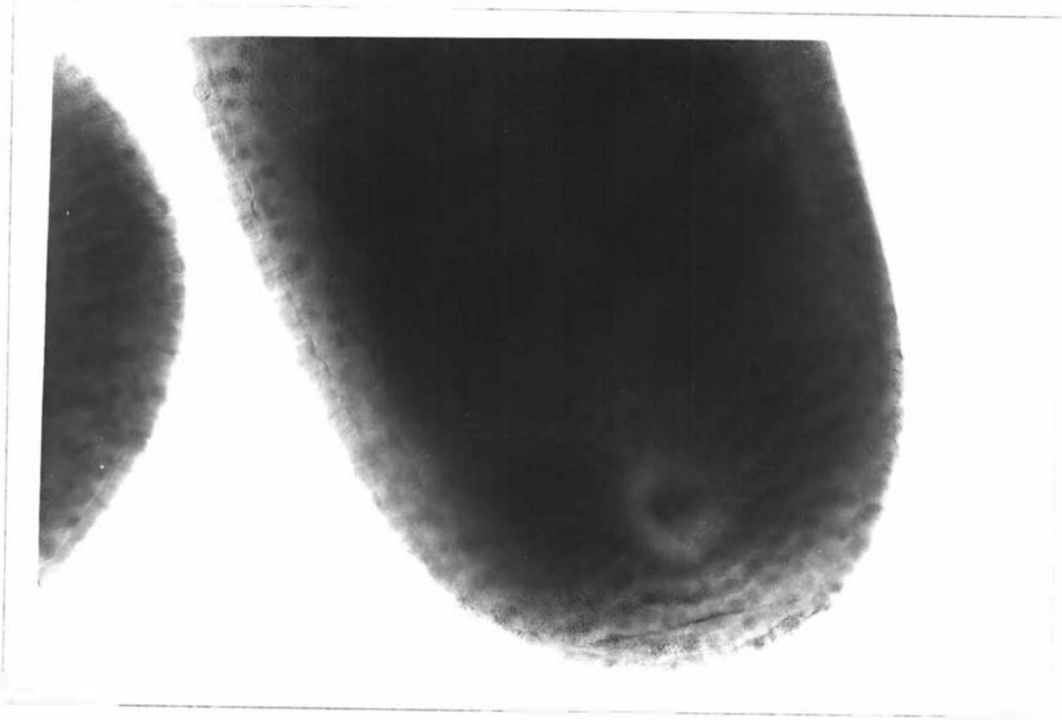


Plate 4.4E : Ovules in wrong orientation with micropyle facing upwards. When ovules are viewed from this angle it is not possible to see the embryo sac contents clearly.

4.3.2 Ovule number

Light intensity had no significant effect on the ovule number (Table 4.1), but the size of the ovules within the carpel was affected by light intensity. The ovules of plants grown under high light levels (10000 lux) were found to be slightly larger than those plants grown under low light intensities (Table 4.1). When the lengths and widths of the fully grown but unfertilized ovules were compared at the proximal and distal ends of the carpels, the proximal ones were found to be slightly larger (Fig.4.4). Similar trends were observed in all light regimes (Appendix 2).

4.3.3 Ovule fertility

The size and cytoplasmic state of the embryo sac and the presence or absence of polar, egg, and synergid nuclei therein were recorded. These observations were made to record the percent of fertile ovules in a carpel under different light conditions. In general, the normal embryo sac developing from the megaspore intensely absorbs nucellus tissue, so that by the time of the formation of a mature embryo sac the nucellus is completely expended and the embryo lies in the cavity within the integuments. The development of the female gametophyte of ***Trifolium repens*** is of this type. The fertile ovules consist of an outer and an inner integument, nucellus, and an embryo sac. Embryo sacs of a fertile ovule contained four cells and four or five nuclei; two synergid cells, an egg cell, and a cell with either two polar nuclei or a single polar fusion nucleus. The egg apparatus and the polar nuclei are concentrated on the micropylar end of the embryo sac and in close contact with one another (Plate 4.2). There was no significant morphological difference between the fertile and sterile ovules, but the cytological observation showed that the embryo sacs were not fully developed. In sterile ovules, the embryo sacs were not completely grown, mostly being small and rather shrunken with no or few visible nuclei (Plate 4.3&4.4). Light appears to have a strong impact on embryo sac development. The low light intensities significantly decreased the percentage of fertile ovules (Table 4.1). A correlation test indicated a positive correlation between light intensity and the percentage of fertile ovules (r= 0.969; R-squared=0.939).

TABLE 4.2: Effect of light intensity on seed yield components (Experiment 2).

Standard errors are given in parentheses. Values followed by the same letter in the same row (i.e. within a treatment) are not significantly different at 5% level. In 2000 lux treatment the fully developed florets were only 10-12 florets and were used to record the % of fertile ovules.

Light Intensity (Lux)	*No of fully developed florets per head*	*Number of Seeds per carpel*	*Percent of ovules setting seed in fully developed florets*	*Number of Seeds per head*
10000	*55.1a*	*3.56a*	*63a*	*194.3a*
	(2.30)	*(0.044)*		*(8.65)*
6000	*47.9b*	*3.05b*	*55b*	*145.1b*
	(1.46)	*(0.130)*		*(5.73)*
4500	*42.9c*	*2.54c*	*48c*	*108.0c*
	(1.79)	*(0.114)*		*(5.21)*
2800	*25.7d*	*1.60d*	*31d*	*41.1d*
	(4.60)	*(0.267)*		*(12.82)*

4.3.4 Seed yield components

As expected, lower light intensities led to floret abortion and thereby reduced the number of florets per flower head which reached anthesis. Most striking, however, was the effect of reduced light intensity on the percentage of ovules setting seeds (Table 4.2). As with the percentage of fertile ovules in a carpel, the seed number per floret was higher in high light intensities. Although no count was made of the total number of seeds per inflorescence, this can be estimated by multiplying the average number of seeds per floret by the average number of florets per inflorescence. The result of such calculation is shown in Table 4.2. It is clear that the total number of seeds per inflorescence was higher in high light intensity treatments (Table 4.2).

4.4 DISCUSSION:

After inflorescence emergence, which occurs at the node bearing the youngest leaf with unfolded leaflets (N7), very rapid peduncle elongation was closely followed in the oldest florets by cessation of sepal elongation and rapid elongation of petals, filaments and style (Fig.4.2). As a result, petals protruded beyond the sepals to show their white colour and the stage of anthesis was reached. These results are consistent with those of Thomas (1987). The growth curve of the ovary was expected to be a sigmoid, but surprisingly it was diauxic. Three phases of ovary growth can be distinguished. In the first phase the growth is exponential. In the second phase, lasting only about 4-5 days (as the inflorescence moves from node 8 to node 9), growth in length is much less rapid than in the first phase. In the third phase, growth is initially about as rapid as in the first, but gradually declines to zero. The graphs previously published by Thomas (1987) show similar phases in ovary growth. It appears that the onset of the slower phase in growth in ovary length may be the result of the development of ovules within the carpel, the ovule having filled the space available within the carpel. Thomas (1987) has reported that when the inflorescence moves from node 8 to node 9, rapid development of the ovules takes place. Some of the changes happening in the ovules when the inflorescence moves from node 8 to node 9 are as follows: inner integuments extend to the tip of the nucellus and almost enclose it; later, chiefly through pressure of the growing parenchyma of the outer integument adjoining the funicle, the nucellus and embryo sac becomes strongly curved and the ovules finally assume their campylotropous form. An examination of the literature shows that diauxic growth occurs in soybean seeds (Carr and Skene, 1961).

The observed effects of light intensity on floret development in the present investigation might result both from a direct effect of light and possible indirect effect from a reduction in photosynthates. The etiolation response of filaments, sepals and petals is a good indicator for a direct effect of light. The decrease in the final length of the styles, filaments and ovaries at lower light intensities suggests that photosynthates may be limiting when plants are grown in low light intensities. To separate direct and indirect effects of light, experiments were undertaken to study the effect of low light intensity on floral organs by shading individual flower heads on plants which were otherwise fully illuminated.

The lower light intensities led to floret abortion and thereby reduced the number of florets per flower head which reached anthesis. This result is consistent with the Zaleski's (1970) observations. He found that insufficient light led to a complete or partial abortion of inflorescences, even under suitable temperature and daylength. He also found a highly significant positive correlation between light intensity and the number of inflorescences formed, indicating the importance of high light intensity for inflorescence formation.

Light intensity also significantly affected the ovule size. Though the length and width of an ovule is greatly influenced by the position of an ovule within the carpel, unpublished X-ray photographs showing the number of ovules forming seeds in individual florets in white clover clearly demonstrate that abortion of ovules is random and is not confined to proximal or distal ends of a carpel. This suggests that the ovule size has no effect on seed set. Most striking, however, was the effect of reduced light intensity on the percentage of ovules setting seed and the percentage of fertile ovules (Table 4.1 & 4.2). The inverse correlation between the two very strongly suggests that direct cause of low seed set was ovule sterility (r=0.979;R-squared=0.957).

The main factor determining seed production capacity in white clover, or any other plant, is the number of fertile ovules produced per plant (Thomas, 1961a). The results obtained show that light has a strong influence on ovule fertility and suggest that low seed set at low light intensities was caused by ovule sterility. The results also show that when plants are grown in low light intensities, their flower heads produced 26-79% fewer seeds per inflorescence than those of plants grown in high light intensity.

This reduction was brought about by an increase in the number of florets aborting and by a higher proportion of ovules being sterile.

In field conditions, the whole white clover plant does not receive such low light intensities as they did in this investigation. The young flower heads, however, develop at light intensities which vary with the structure of the foliage canopy from very high (perhaps about 50% of full light) in an open canopy, to a level as low as about 5% on a dense canopy (Brougham, 1958). Therefore, further research has to be done to understand the effect of shading individual young flower heads on ovule fertility and seed set in plants that are otherwise grown at a high light intensity. Such experiments are described in chapter 5.

Glasshouse Experiments.

CHAPTER 5

INFLUENCE OF SHADE ON INFLORESCENCE DEVELOPMENT AND SEED YIELD

5.1 INTRODUCTION

It is known that low light intensity leads to total abortion of developing flower heads (Zaleski, 1970) and that the number of seeds per floret in a "good" (warm, dry, sunny) summer is usually up to 50% higher than in a "bad" (cool, rainy, dull) summer (Romero, 1985). In earlier studies (Chapter 4) it was found that low light intensities (2000-6000 lux) led to floret abortion and thereby reduced the number of florets per flower head which reached anthesis. Low light intensities also significantly reduced the percentage of fertile ovules and the percentage of ovules which set seeds. The positive correlation between the percentage of fertile ovules and the percentage of ovules which set seeds strongly suggested that the direct cause of low seed set was ovule sterility. The light intensities in which the plants were grown were well (2-10%) below those experienced by plants growing in field conditions.

Young flower heads, though, develop in the field at light intensities which vary with the structure of the foliage canopy; intensities received by young flower heads can be high in open canopies but they can be as low as 5% of incoming radiation in dense canopies (Brougham, 1958 & Appendix 5 give data on the effect of clover canopy on PAR photon flux beneath them). In the field the young flower heads, young leaves and shoot tips were not only subjected to low irradiance but also subjected to different light quality. Scott et al. (1968) analysed transmitted and reflected radiation in a pure stand of white clover. The leaves in the canopy absorbed most of the visible radiation, while light of longer wavelengths were transmitted. Under clear conditions 40% and 33% of incident radiation at 800 nm and 1000 nm, respectively reached ground level.

In this Chapter, three separate experiments were conducted to determine the effect of shading individual flower heads on ovule sterility and seed set in plants which otherwise received high light intensities.

Experiment 1: Newly emerged individual flower heads were shaded on plants which were otherwise fully illuminated.

Experiment 2: The young inflorescences and shoot tips were shaded on plants which were otherwise fully illuminated by using a neutral shade (1% of incoming radiation).

Experiment 3: To simulate field conditions in the glasshouse, the young inflorescences and shoot tips were subjected to filtered light which had a quantity and spectral quality equivalent to canopy shade.

5.2 MATERIALS AND METHODS

5.2.1 Plant material

Clonal material of clone A of "Grasslands Huia" white clover (Thomas, 1979) was used. Plants were propagated and maintained as described in chapter 3.

5.2.2 Experimental procedure

5.2.2.1 Experiment 1

In the late summer of 1987/88 tests were conducted to study the effect of shading individual young flower heads on plants which were otherwise fully illuminated. This was achieved by covering newly emerged flower heads (i.e. when they were situated at node 8 and their peduncles were about 20 mm long) with paper tubes of varying thicknesses to create environments of different light intensities. Flower heads were shaded for six days only, by which time they had grown up to just below the levels of their subtending mature leaf blades. Light intensities given ranged from 1 to 100% of incident light. Seed set and ovule fertility observations were made only in those flower heads subjected to lowest light intensity treatment (1% of control) and in 100% of incident light (control). In this experiment, 10 flower heads on separate plants were used for each treatment. The glasshouse maximum daytime temperatures at these time were generally in the range 28-33° C and minimum night temperatures about 15-17°C.

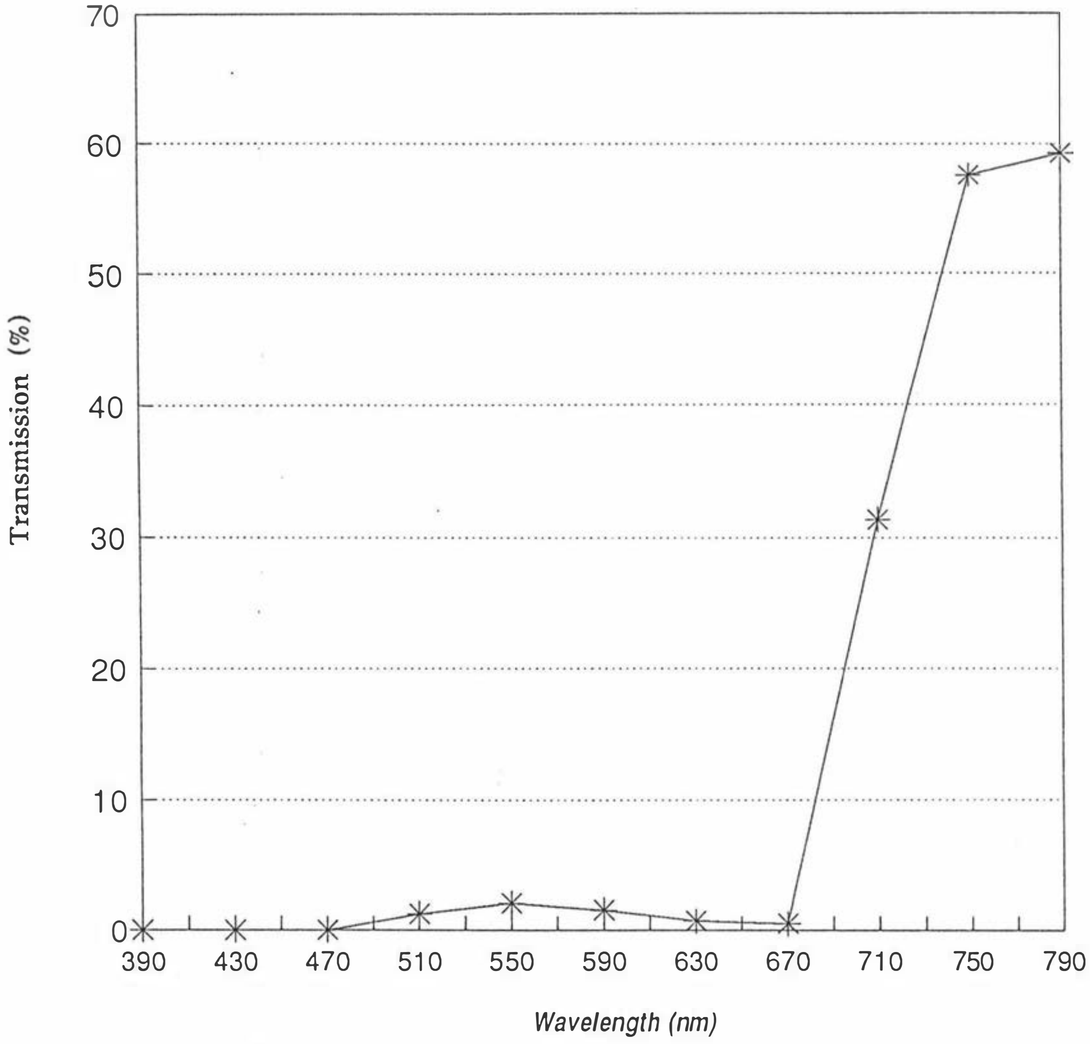


FIGURE 5.1 : Percentage transmission of different wavelengths of light by the cellophane filters used in Experiment 3.

Plate 5.1 : The wooden box used in experiments 2 & 3 to shade the stolon tips and inflorescences being studied. Mother plants are outside the box with one stolon tip from each projecting into the box which was then covered with a sheet of perspex or perspex plus aluminium foil. A fan at left hand end drew air through the box; box temperature was recorded by a maximum and minimum thermometer at the right hand end.

5.2.2.2 Experiment 2

Experiment 2 was undertaken in the summer of 1988/89 in an attempt to identify the stage of inflorescence development most sensitive to low light intensities. Four stages of inflorescence development between node 6 to node 9 were exposed to low light. Stage of inflorescence development was relative to distance from stolon apex (flower head position with respect to stolon apex). In the present investigation, the inflorescences at node 6, 7, 8, and 9 were designated as E, N1, N2, and N3 respectively. Experiments for each stage of development were done at different times of the season (i.e November 1988-February 1989). Plants were in full light except stolon tips and inflorescences which were shaded as described below. The main stolon tip, consisting of all the leaves less than 2 cm long, and the inflorescence being studied, were inserted through a hole in a wooden box. The top of the wooden box was either closed with clear perspex or perspex plus aluminium foil. The aluminium foil was arranged in such a way that the stolon tip and inflorescence were exposed to low light intensity (1% of control i.e ambient light) for six days. The wooden box was fitted with a rotary fan to circulate the air within it. The experimental system is illustrated in Plate 5.1. Temperatures inside the box were measured by a maximum and minimum thermometer and the average temperature was 28 ± 2^{o} C. Twenty flower heads on separate plants were used for each treatment.

5.2.2.3 Experiment 3

The experimental procedure was similar to that of Experiment 2 except coloured cellophane film (2 sheets of red and 2 sheets of green) was used as a filter instead of aluminium foil. This was to simulate light filtered through a leaf canopy. About 2% of visible light was transmitted by the filters. The spectral quality of transmitted light by the filters is shown in Fig.5.1.

5.2.3 Measurements

5.2.3.1 Floral development

The effect of each treatment was recorded by measuring the length of the floral organs - sepals, petals, stamens,ovaries, and styles on the oldest florets, i.e. those at the base of an inflorescence, when the inflorescence had reached node 11 from the stolon apex. Ten to twenty florets were collected from four inflorescences for each treatment.

5.2.3.2 Ovule fertility

One hundred florets consisting of 60 apical florets and 40 basal florets per treatment, were collected from 20 inflorescences and fixed in formalin/acetic/acid. The fixed ovaries were dissected out, stained with Mayer's haemalum, a positive stain for chromatin and nuclei, and optically cleared with methyl salicylate as described in Chapter 3. The cytoplasmic state of embryo sacs and the presence or absence of polar, egg, and synergid nuclei of stained ovules were examined using Nomarski interference microscopy. Bright field Kohler illumination and a light green filter were used for viewing. The embryo sacs lacking nuclei were classified as sterile as described in Chapter 4.

5.2.3.3 Pollen fertility

Pollen fertility was assessed by examination of a sample of microspores from each floret mounted on a glass slide and stained with Snow's alcoholic carmine as described in Chapter.3. Approximately 100 grains were counted from each floret and the number with or without cytoplasm was recorded. Pollen grains devoid of contents were classed as sterile and those containing cytoplasm as fertile (Plate 5.2). At least two florets from each of five flower heads were used for each treatment.

The staining technique described above could lead to an under estimation of sterility, as the presence of cell contents does not necessarily indicate fertility. For this reason in experiment 2, pollen germination tests were conducted in addition to staining pollen grains. Pollen tube growth was studied ***in vitro*** on liquid media (Plate 5.4). Florets were collected from plants in experiment 2 (both control and treated). A standard germination media was used consisting of 25 ml of 100% (W/V) sucrose, 10 ml of 0.01M calcium nitrate, 10 ml of 0.01M magnesium sulphate, 10 ml of 0.01M boric acid, 10 ml of 0.01 M potassium nitrate and 35 ml of water. A drop of medium was placed on a micro concavity slide and a piece of visking tubing placed over the solution. The visking tube was used to simulate the natural environment. For instance, it separates pollen grains from the liquid media thus keeping them partially dry and

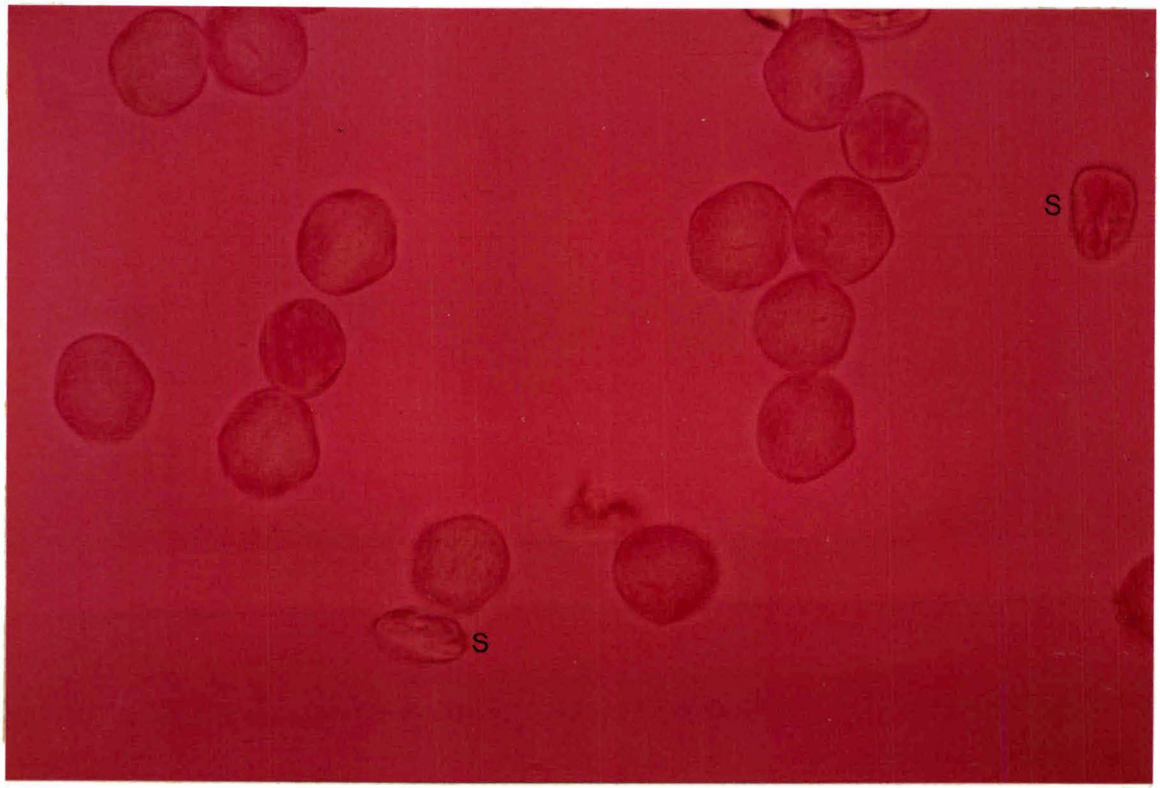


Plate 5.2 : Pollen grains stained with Snow's alcoholic carmine. Pollen grains containing cytoplasm were classed as fertile and those devoid of contents or shrivelled (S) as sterile.

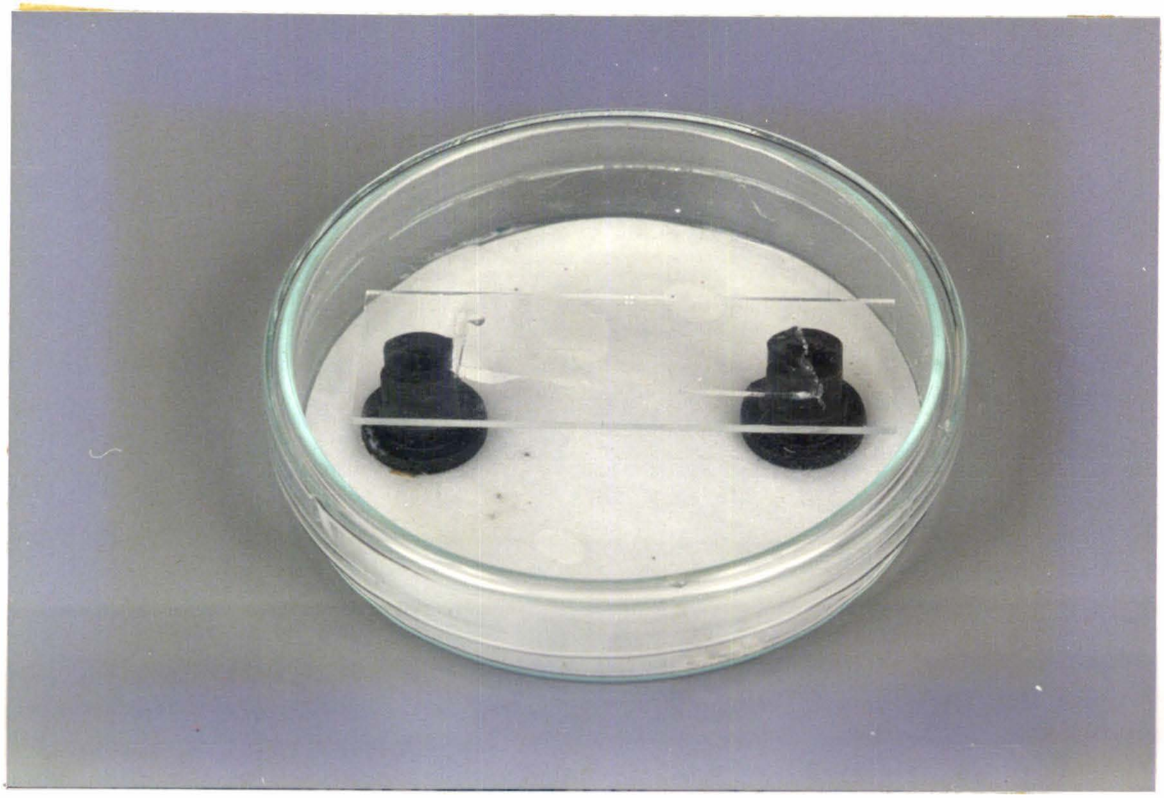
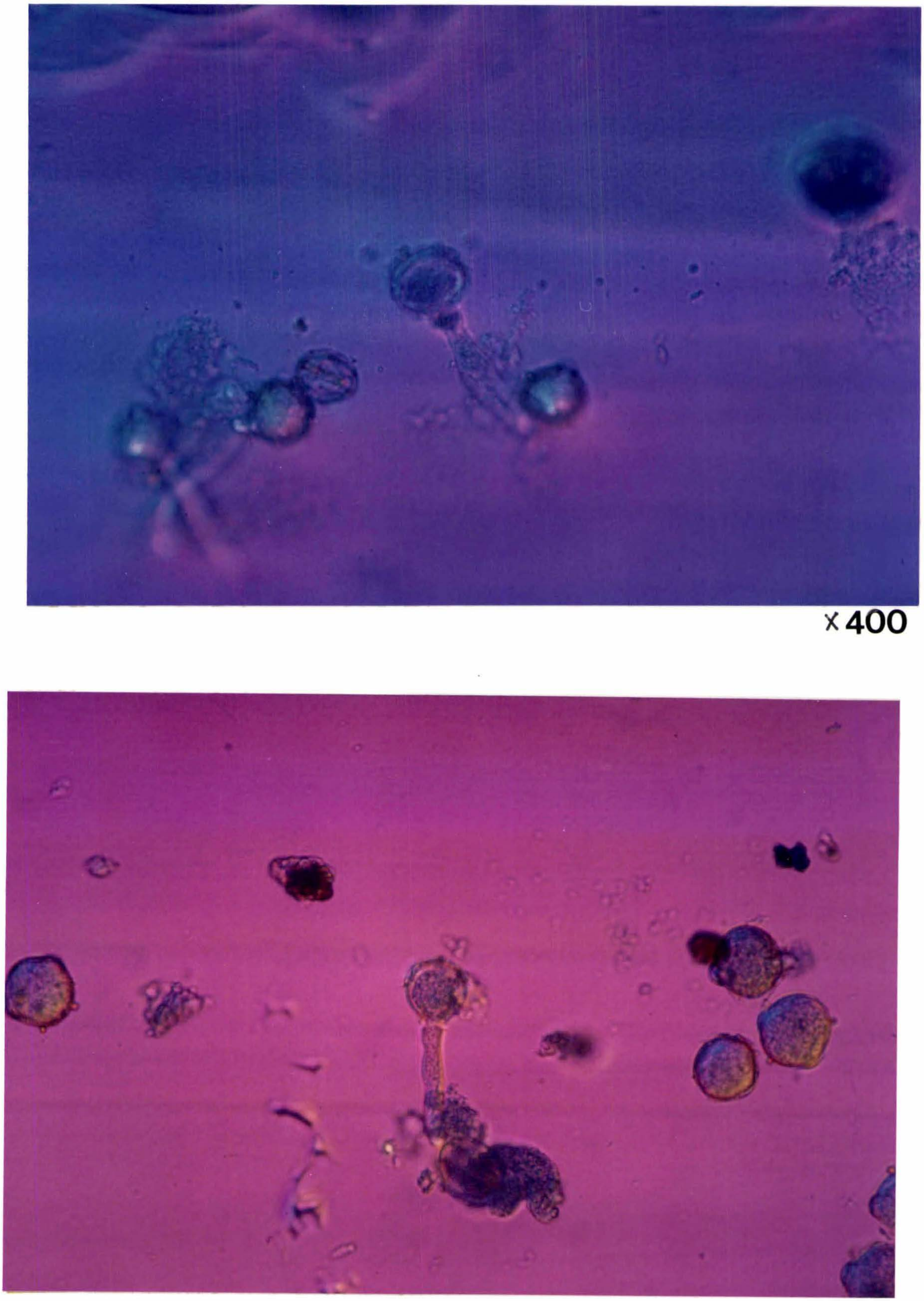


Plate 5.3 : ***Physical set up of the apparatus used for measurement of pollen grain germination showing a micro concavity slide placed in a petri dish with a water-saturated filter paper in the bottom.***



×400

×400

Plate 5.4 : Pollen tube growth in vitro on liquid media. 4 pollen tubes in upper and one in lower.

also it acts like a stigmatic surface. Pollen was dispersed over the visking tubing by tapping an inverted floret over it. Two florets from each of two flower heads were used for each treatment. The slide was placed in a petri dish with a water- saturated filter paper in the bottom. The petri dish was covered and incubated for 12 hours at $25^{o}C$ in darkness. The physical set up of the apparatus used to measure the pollen germination is shown in Plate 5.3.

5.2.3.4 Seed number per floret

When the flower heads were fully developed, with all florets showing white corollas, the plants were transferred into a prolifically flowering field plot of white clover where they were naturally cross pollinated (see Plate 5.5). Ten days after pollination, the inflorescences were collected and stored at 4^{o} C. For each treatment, the number of florets per inflorescence was counted. The mean number of seeds per floret were recorded from 400 pods sampled both from 200 apical florets and 200 basal florets of 20 inflorescences per treatment.

5.3 RESULTS:

5.3.1 Experiment 1: Shading young flower heads with paper tubes.

5.3.1.1 Floral development

Shading individual young flower heads on fully illuminated plants, decreased the length of both sepals and ovaries (Fig.5.2). Reducing the light intensity reaching the flower heads to 1% reduced the length of the ovaries by 17% and of sepals by 8%. Low light intensity had no significant effect on the length of petals, filaments and styles.

5.3.1.2 Seed yield components

Results showed that light intensity had no significant effect on ovule number, but strongly influenced the number of embryo sacs which developed normally (Table 5.1). Flower heads which developed in 1% light produced only 64% of fertile ovules

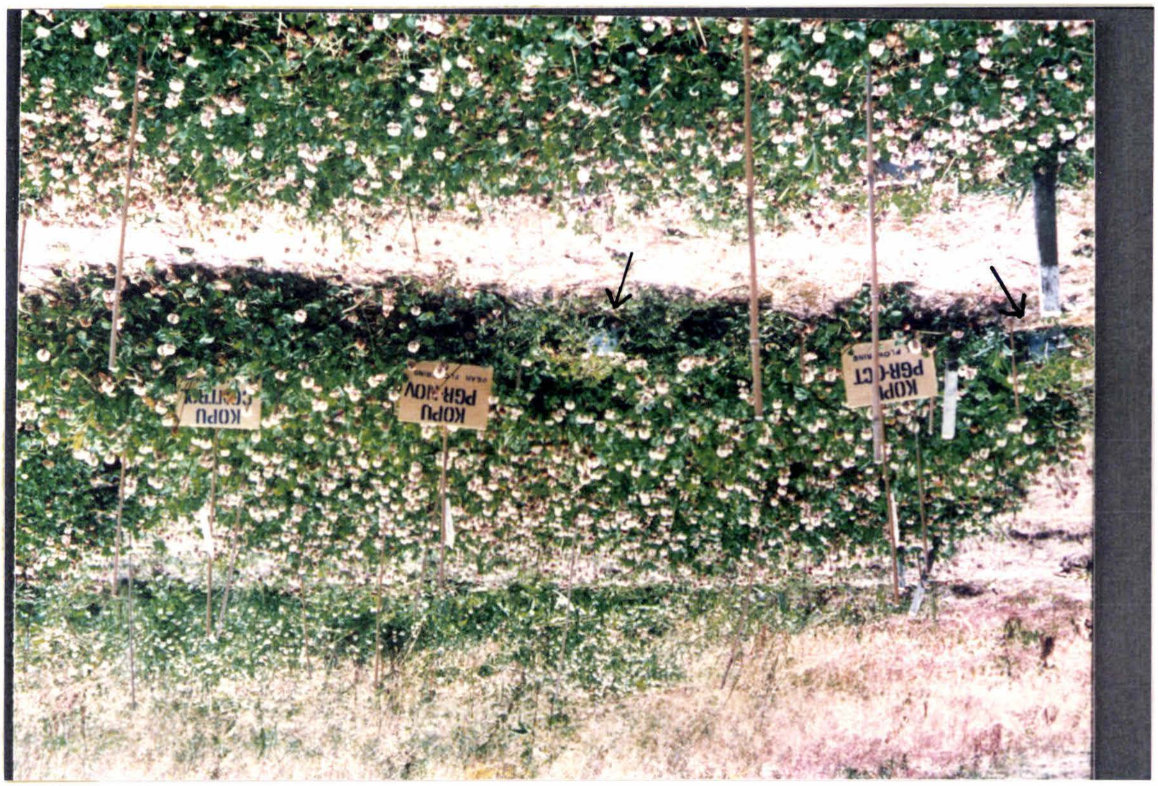


Plate 5.5: *Plants transferred from the glasshouse into a prolifically flowering field plot of white clover where they were cross pollinated by natural means. Transferred plants are indicated by arrows.*

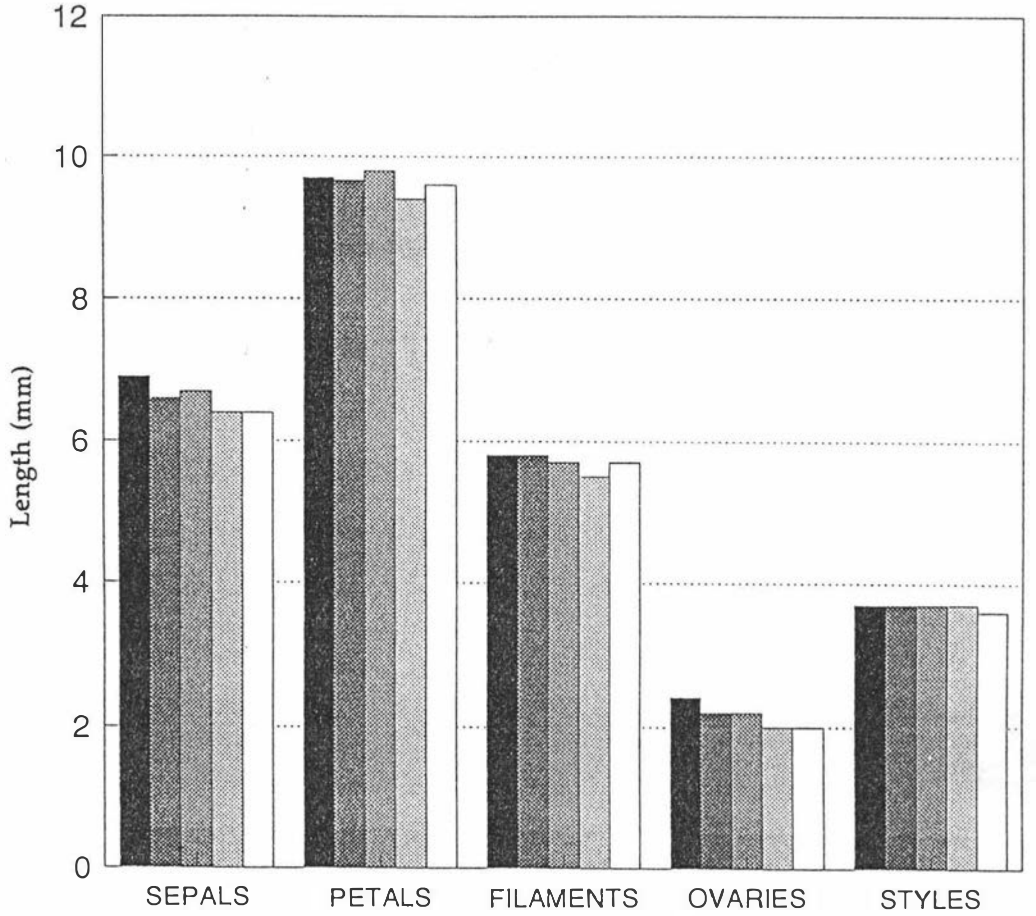


FIGURE 5.2 : The effect of low light on flower head development. The vertical bars represent the mean lengths of floral organs in the oldest florets in inflorescences which were grown in five different light intensities. Florets were harvested for measurement when flower heads reached node 11. Each value is the mean of 10 replicates. The range of standard errors was 0.02 - 0.06 for sepals, 0.04 - 0.05 for petals, 0.02 - 0.05 for filaments, 0.015 - 0.021 for ovaries and 0.021 - 0.034 for styles.

■	100% light	▩	46% light	▨	28% light
░	15% light	□	1% light		

TABLE 5.1: Effect of low light on flower head development in glasshouse grown plants (Experiment 1)

The newly emerged inflorescences (with peduncles 15-20 mm long) were covered with paper tubes (1% of control), while the rest of the plant in full light. Measurements of florets from apical and basal region of flower heads were recorded separately. Values followed by the same letter between treatments (in the same column) are not significantly different at 5% level).

Treatment	Ovule number per carpel			% of fertile ovules			% of ovules setting seeds			Number of fully developed florets per head (± SE)
	apical (± SE)	basal (± SE)	mean	apical	basal	mean	apical	basal	mean	
Control	4.90 ± 0.07	5.10 ± 0.14	5.0a	80a	82a	81a	72a	71a	71.5a	48 ± 1.4a
Shade	4.83 ± 0.08	5.1 ± 0.14	4.97a	59b	69b	64b	58b	66b	62.0b	47 ± 1.5a

TABLE 5.2: Effect of low light on pollen fertility (Experiment 2).

The stolon tips and inflorescences being studied were exposed to full light or continuous low light. (1% of control). Flower head position (stage of inflorescence development) was relative to distance from the stolon apex. Values at different nodes recorded at different times reflect inflorescence development. Values followed by the same letter in each pair of treatments are not significantly different at 5% level.

Flower head Position	*Pollen fertility (%)*			
	(A) Staining test		*Pollen germination test*	
	Full Light	*Low Light*	*Full Light*	*Low Light*
Emergence	*80.5a*	*79.3a*	*75.0c*	*70.5c*
Node 7	*85.5a*	*80.4a*	*81.0c*	*73.5c*
Node 8	*92.0a*	*81.0b*	*82.5c*	*74.8d*
Node 9	*89.6a*	*86.4a*	*84.5c*	*82.3c*

TABLE 5.3: Effect of low light on ovule number, % of fertile ovules, and % of ovules setting seed. (Experiment 2).

The stolon tips and inflorescences being studied were exposed to full light or continuous low light (1% of control). Flower head position (stage of inflorescence development) was relative to distance from the stolon apex. Values at different nodes recorded at different times reflect inflorescence development. Values followed by the same letter in each pair of treatments are not significantly different at 5% level.

Flower head position	*Ovule Number*		*% of Fertile Ovules*		*% of Ovules Setting Seed*	
	Full Light	*Low Light*	*Full Light*	*Low Light*	*Full Light*	*Low Light*
Emergence	*5.20a*	*5.42a*	*71.5a*	*62.0a*	*69.8a*	*57.5a*
Node 7	*5.26a*	*5.22a*	*66.4a*	*63.8a*	*64.6a*	*66.3a*
Node 8	*5.40a*	*5.23a*	*68.4a*	*57.5b*	*65.0a*	*50.8b*
Node 9	*5.24a*	*5.35a*	*67.9a*	*64.0a*	*64.5a*	*60.0a*

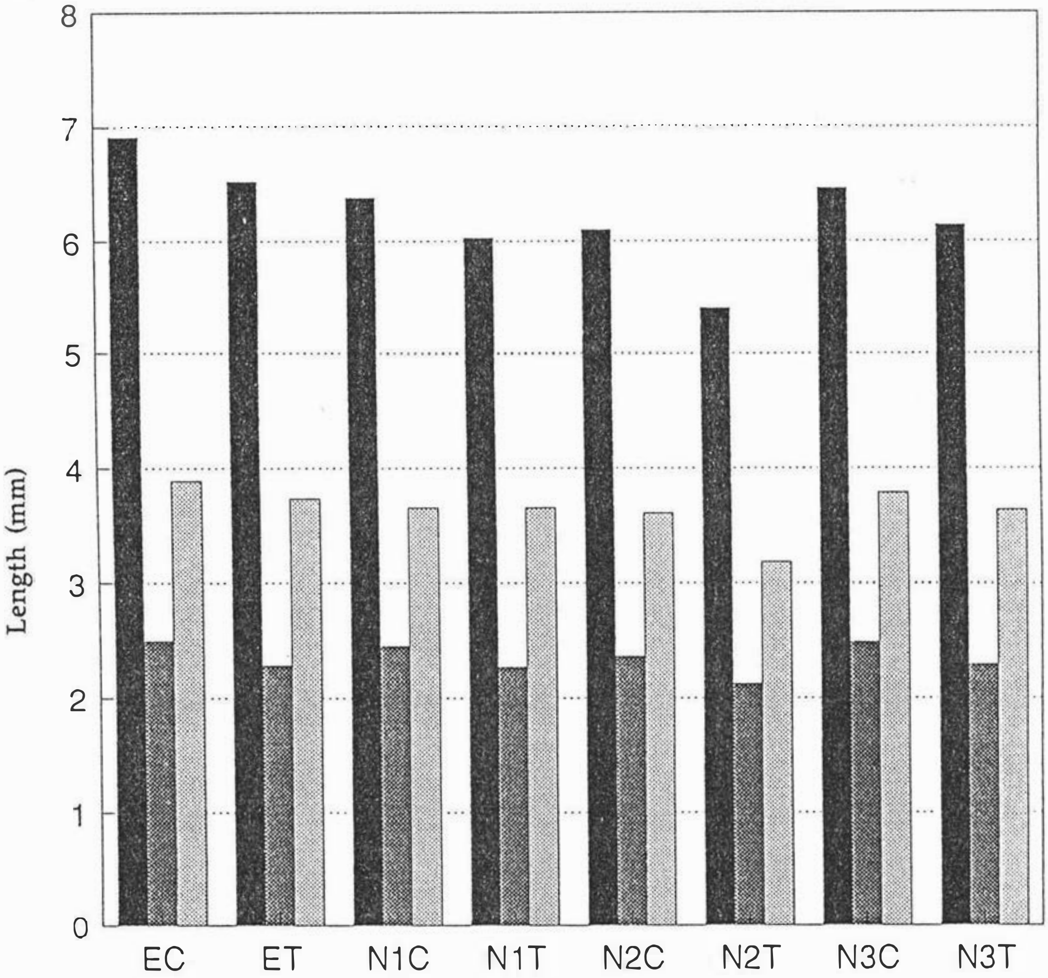


FIGURE 5.3 : *The effect of low light on flower head development (Experiment 2). The vertical bars represent the mean lengths of floral organs in the oldest florets of inflorescences which were grown in full light (C) and in shade (T). Data are shown for inflorescence at nodes 6, 7, 8, and 9 (designated as E, N1, N2, and N3 respectively). The values are the mean of 20 replicates. The range of standard errors was 0.047 - 0.12 for sepals, 0.02 - 0.04 for ovaries and 0.03 - 0.07 for styles.*

■ SEPALS ▩ OVARIES ▨ STYLES

compared with 81% in full light. The apical florets on an inflorescence had about 2-10% fewer fertile ovules than those which developed lower down. Shading the developing flower heads had no significant effect on the number of florets per inflorescence.

The ovules in flower heads which developed in full light were found to be slightly larger than those developed in low light intensities. When the lengths and widths of fully grown but, unfertilized, ovules were compared at the proximal and distal ends of the carpels, the proximal ones were found to be slightly larger (Appendix 4). Similar trends were observed in flower heads developed in all light regimes.

The number of seeds per floret was highest in the flower heads developed in full light and lowest in those which had been shaded. Shading the flower heads reduced the seed number per floret by 9.5% in comparison with the flower heads developed in full light. There was a close relationship between the percentage of ovule fertility and the percentage of ovules setting seed: 81% and 71.5% respectively in the flower heads developed in full light; 64% and 62% respectively in the flower heads developed in 1% light (Table 5.1).

5.3.2 Experiment 2: The effect on floral development and seed yield caused by shading inflorescences at different stages of development.

5.3.2.1 Floral development

Irrespective of the stage of inflorescence development tested, exposing the flower heads to low light affected the lengths of sepals and ovaries. In contrast, the stylar length was not affected when the flower head was exposed to low light at node 7 (N1) but length was reduced in all other stages of development. Shading the flower head decreased the ovary length which is consistent with previous results (Fig.5.3). A slight effect on pollen fertility was observed, but the effect was significant only when shade was applied to the flower head at node 8 (N2) (Table 5.2). A similar trend was observed in the pollen germination test. The pollen fertility levels as determined by germination test were low when compared with results obtained from staining technique (P=0.026), suggesting that the staining technique has led to an under estimation of sterility. Although the differences between the two techniques are significant, they were small i.e. 3-9%. The staining technique being the easier and quicker technique , this technique was used to assess the pollen fertility for further experiments.

TABLE 5.4: Effect of low light on number of fertile ovules per carpel (ExperimentS2 & 3).

The stolon tips and inflorescences being studied were exposed to full light or continuous low light/light simulating that filtered through a foliage canopy (1-2% of control). Flower head position (stage of inflorescence development) was relative to distance from the stolon apex. Values at different nodes recorded at different times reflect inflorescence development. Measurements of florets from apical and basal region of flower heads were recorded separately. Standard errors are given in parentheses.

Flower head Position	Experiment 2				Experiment 3			
	Full Light		Low Light		Full Light		Filtered Light	
	Apical	Basal	Apical	Basal	Apical	Basal	Apical	Basal
Emergence	3.28 (0.12)	4.15 (0.15)	2.82 (0.18)	3.91 (0.22)	3.23 (0.12)	3.68 (0.16)	3.04 (0.17)	3.45 (0.14)
Node 7	3.13 (0.10)	3.85 (0.10)	2.89 (0.15)	3.78 (0.27)	3.16 (0.12)	3.98 (0.16)	2.82 (0.19)	3.53 (0.19)
Node 8	3.31 (0.10)	4.08 (0.13)	2.82 (0.11)	3.20 (0.18)	2.96 (0.12)	4.00 (0.12)	2.44 (0.16)	2.98 (0.11)
Node 9	3.12 (0.17)	4.00 (0.21)	2.97 (0.13)	3.88 (0.24)	3.21 (0.09)	3.98 (0.07)	2.69 (0.14)	2.90 (0.15)

TABLE 5.5: *Effect of low light on seed number per carpel (Experiments 2 & 3).*

The stolon tips and inflorescences being studied were exposed to full light or continuous low light/light simulating that filtered through a foliage canopy (1-2% of control). Flower head position (stage of inflorescence development) was relative to distance from the stolon apex. Values at different nodes recorded at different times reflect inflorescence development. Measurements of florets from apical and basal region of flower heads were recorded separately. Standard errors are given in parentheses.

Flower head Position	Experiment 2				Experiment 3			
	Full Light		Low Light		Full Light		Filtered Light	
	Apical	Basal	Apical	Basal	Apical	Basal	Apical	Basal
Emergence (E)	3.17 (0.09)	4.09 (0.09)	2.41 (0.16)	3.82 (0.09)	2.87 (0.11)	3.38 (0.10)	1.99 (0.19)	3.41 (0.17)
Node 7 (N1)	2.99 (0.11)	3.82 (0.12)	2.97 (0.12)	3.95 (0.11)	3.09 (0.11)	4.07 (0.09)	2.45 (0.11)	3.73 (0.29)
Node 8 (N2)	2.76 (0.10)	4.27 (0.09)	2.78 (0.18)	2.99 (0.20)	2.84 (0.09)	4.02 (0.07)	2.06 (0.21)	3.04 (0.29)
Node 9 (N3)	2.84 (0.09)	3.92 (0.12)	2.67 (0.19)	3.76 (0.15)	2.72 (0.10)	3.79 (0.11)	2.21 (0.19)	2.96 (0.21)

TABLE 5.6: Effect of neutral shade on seed yield components (Experiment 2).

The stolon tips and inflorescences being studied were exposed to full light or continuous low light (1% of control). Flower head position (stage of inflorescence development) was relative to distance from the stolon apex. Values at different nodes recorded at different times reflect inflorescence development. Values followed by the same letter in each pair of treatments are not significantly different at 5% level. Percentage reductions of seed number per head between treatments are given in parentheses. 20 flower heads were used for each treatment.

Flower head position	*No. of aborted flower heads*		*Number of fully developed florets per head*		*Seed number per fully developed floret*		*Seed number per head*	
	Full Light	*Low Light*	*Full Light*	*Low Light*	*Full Light*	*Low Light*	*Full Light*	*Low Light*
Emergence	*0*	*2*	*56.9± 0.84a*	*50.0 ± 3.9a*	*3.63a*	*3.12b*	*207*	*156 (25)*
Node 7	*0*	*1*	*53.1± 0.96a*	*48.6 ± 3.2a*	*3.40a*	*3.46a*	*181*	*168 (7)*
Node 8	*0*	*7*	*50.2± 0.79a*	*22.8 ± 4.8b*	*3.51a*	*2.88b*	*176*	*66 (63)*
Node 9	*0*	*0*	*53.2± 1.2a*	*45.2 ± 2.4b*	*3.38a*	*3.21a*	*179*	*147 (8)*

5.3.2.2 Seed yield components

The effect of flower head shading on the average percentage of fertile ovules per floret is shown in Table 5.3. The degree of ovule sterility was greatest when the flower heads were shaded at node 8 (N2). This resulted in 43% of ovules being sterile when shade was applied at node 8 (N2) compared with a) 38% at the emergence stage (E), b) 39% at N1, c) 36% at N3. The apical florets on an inflorescence which developed either in full light or low light, had an average of 21% fewer fertile ovules than those developed lower down (i.e.basal florets) (Table 5.4). Most striking, however, was that even in full light only 69% of the ovules formed in a flower head were fertile and capable of setting seeds (Table 5.3).

Shading the flower head led to a complete or partial abortion of some buds and inflorescences. The degree of abortion was greatest when shade was applied to the flower head at N2 . This resulted in 35% abortion of inflorescences when shade was applied at N2 compared with 10% at emergence stage (E) and 5% at N1 (Table 5.6). No effect was observed when shade was applied at N3. Partial abortion of inflorescences (floret abortion) was observed when shade was applied at later stages of inflorescence development (N2&N3). The degree of floret abortion was greatest when flower heads were exposed to low light at node 8, when 55% of florets aborted compared with 15% at node 9 (Table 5.6).

Shading the flower head decreased the mean number of seeds per floret, but the effect was observed only at certain stages of inflorescence development (Table 5.5), namely, at a very early stage of inflorescence development (E) or at position N2 . An average of 26% fewer seeds were set in the upper florets than in the lower florets within a flower head; this parallels the results obtained for ovule fertility. Once again there was a close relationship between the level of ovule fertility and the percentage of ovules setting seed (Table 5.3). Although no count was made of the total number of seeds per inflorescence, this was estimated by multiplying the average number of seeds per floret by the average number of florets per inflorescence. The results of such calculations are shown in Table 5.6. Averaging the results of all developmental stages showed that the flower heads developed in low light produced 28% fewer seeds than those developed in full light.

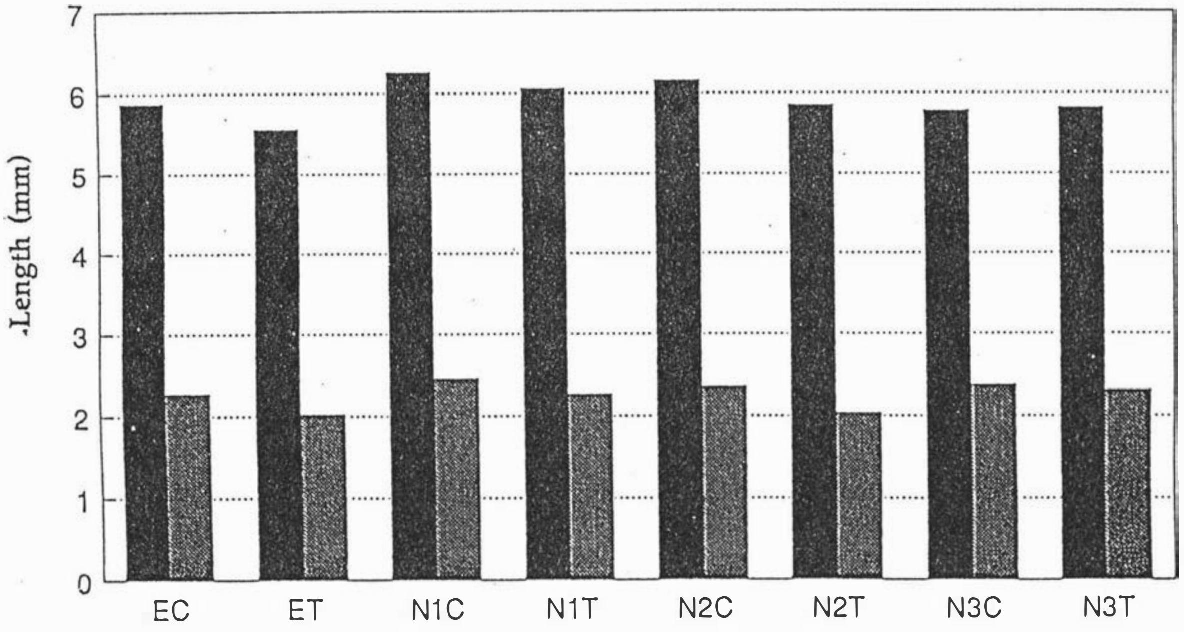


FIGURE 5.4 : The effect of simulated foliage canopy shade on flower head development. The vertical bars represent the mean lengths of floral organs in the oldest florets of inflorescences which were in full light (C) or shade light simulating that filtered through a foliage canopy (T). Data are shown for inflorescences at nodes 6, 7, 8, and 9 (designated as E, N1, N2, and N3 respectively). The values are the mean of 20 replicates. The range of standard errors was 0.08 - 0.21 for sepals and 0.026 - 0.043 for ovaries.

TABLE 5.7: Effect of filtered light on pollen fertility, % of fertile ovules, and % of ovules setting seed (Experiment 3).

The stolon tips and inflorescences being studied were exposed to full light or continuous low light simulating that filtered through a foliage canopy (2% of control). Flower head position (stage of inflorescence development) was relative to distance from the stolon apex. Values at different nodes recorded at different times reflect inflorescence development. Values followed by the same letter in each pair of treatment are not significantly different at 5% level.

Flower Head Position	Pollen Fertility		Ovule Fertility		Seed Set	
	Full Light	Filtered Light	Full Light	Filtered Light	Full Light	Filtered Light
Emergence	91.8a	89.2a	68.8a	64.0b	62.3a	54.8b
Node 7	86.2a	83.6a	67.9a	62.8b	68.1a	58.6b
Node 8	86.0a	78.8b	67.6a	51.1b	66.6a	45.7b
Node 9	85.1a	81.5b	69.8a	54.5b	63.2a	50.1b

TABLE 5.8: Effect of filtered light on seed yield components (Experiment 3).

The stolon tips and inflorescences being studied were exposed to full light or continuous low light/light simulating that filtered through a foliage canopy (2% of control). Flower head position (stage of inflorescence development) was relative to distance from the stolon apex. Values at different nodes recorded at different times reflect inflorescence development. Values followed by the same letter in each pair of treatments are not significantly different at 5% level. Percentage reduction of seed number per head between treatments are given in parentheses.

Flower head position	*No. of aborted flower heads*		*Number of fully developed florets per head*		*Seed number per fully developed floret*		*Seed number per head*	
	Full Light	*Shade*	*Full Light*	*Shade*	*Full Light*	*Shade*	*Light*	*Shade*
Emergence	*0*	*0*	*53.4a*	*48.0a*	*3.13a*	*2.70b*	*167*	*130 (22)*
Node 7	*0*	*1*	*49.5a*	*45.4a*	*3.58a*	*3.09b*	*177*	*140 (21)*
Node 8	*0*	*2*	*52.1a*	*39.8b*	*3.43a*	*2.55b*	*179*	*102 (43)*
Node 9	*0*	*1*	*48.4a*	*38.7b*	*3.26a*	*2.58b*	*158*	*100 (37)*

5.3.3 Experiment 3: The effect on floral development and seed yield, caused by exposing inflorescences, at different stages of development, to low R:FR light.

5.3.3.1 Floral development

As in experiments 1 & 2 the sepals and ovaries were shorter in those inflorescences subjected to light simulating that filtered through a foliage canopy (Fig.5.4). The strong effect of filtered light on ovary length is consistent with previous results (Table 5.3). A slight effect on pollen fertility was observed, which became significant only at later stages of inflorescence development (N2&N3) (Table 5.7).

5.3.3.2 Seed yield components

Table 5.7 shows the effect of filtered light on the average percentage of fertile ovules per carpel. Irrespective of the stage of flower development, filtered light influenced ovule fertility. The degree of ovule sterility was greatest when shade was applied at N2. This resulted in 49% of ovules being sterile when shade was applied at N2 compared with a) 36% at emergence stage, b) 37% at N1, c) 46% at N3 position. As in the previous observations (Table 5.3), about 30% of ovules failed to develop into fertile ovules even in full light (Table 5.7). The apical florets on an inflorescence had an average of 17% fewer fertile ovules than those developed further down the inflorescence (Table 5.4).

Filtered light led to complete or partial abortion of some buds and inflorescences (Table 5.8). The degree of inflorescence abortion was greatest (10% abortion) when filtered light was applied to the flower head at N2. This compares with 5% abortion at N1 and N3 position. As with the low light treatment, filtered light decreased the floret number per flower head when applied at later stages of inflorescence development (Table 5.8). Once again the degree of floret abortion was greatest (24%) when flower heads were exposed to filtered light at node 8 (N2), compared with 20% at node 9 (N3).

Irrespective of the stage of inflorescence development, filtered light had a significant effect on the mean number of seeds set per floret. As shown in Table 5.5 filtered light led to 29% less seeds being set in the upper florets than in the lower florets within a inflorescence. The calculated seed number per head is shown in Table 5.8. Averaging

TABLE 5.9: Observed and expected frequency from the binomial distribution of pods with 0 to 5 seeds

Seed Number per carpel	Expected frequency (E)	Observed frequency (O)	$\frac{(O-E)^2}{E}$
0	2.7	0.0	2.7
1	26.8	19	2.27
2	114.3	111	0.078
3	243.2	242	0.004
4	258.3	280	1.8
5	109.7	103	0.33
Total	755	755	7.18

Chi-square = $(O-E)^2$ /E

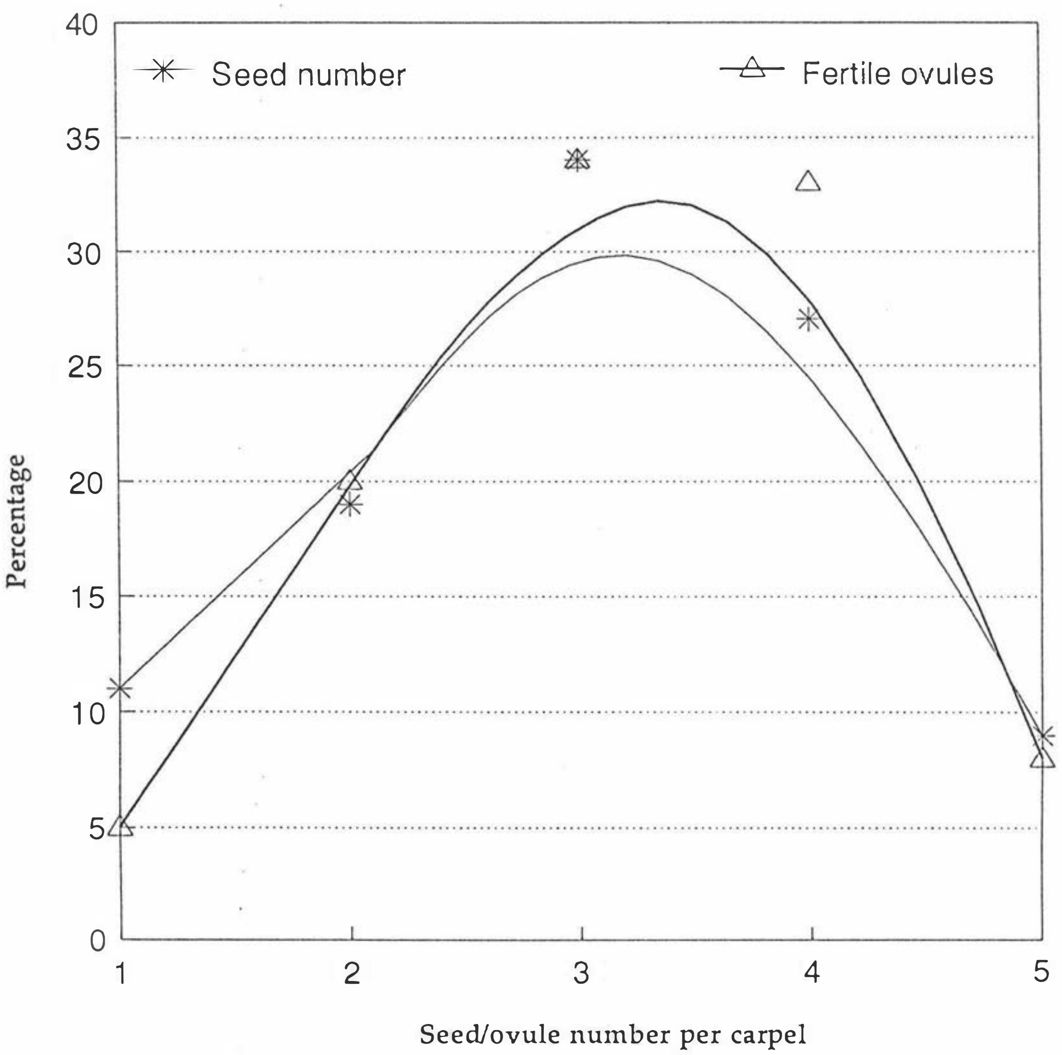


FIGURE 5.5 : The frequency distribution of fertile ovules per carpel and the number of seeds per pod of 100 fully developed florets selected randomly from the flower heads developed in full light.

the results of all developmental stages shows that flower heads developed in filtered light produced 30% fewer seeds than those developed in full light.

5.3.4 Fitting the binomial distribution and testing the goodness-of-fit

755 pods from the flower heads developed in full light in experiments 2 and 3, were examined individually. The frequency distribution of the pods in relation to the number of seeds they contained was recorded. Each pod contained from 1 to 5 seeds. To test the hypothesis that seed set pattern was random, the expected frequency of pods with 0 to 5 seeds from the binomial distribution was calculated. To do this, firstly, the probability (P) of a seed being set was determined:.

$$P = \frac{\text{Total number of seed set}}{\text{Potential number of seeds}} = \frac{2602}{755 \text{x} 5} = 0.68$$

The expected probability and frequencies for each class (number of seeds per pod i.e 0 to 5) were then calculated using the standard formula. Chi-squared values were then calculated using the formula:

$$\text{Chi-squared} = \frac{(O-E)^2}{E}$$

where O= Observed and E= Expected. The calculated values are shown in Table 5.9. The 5% significance level of chi-squared for 4 degrees of freedom is 9.491 and the value of 7.18 for the chi-squared statistics give no reason to doubt that the binomial model fits the observed values.

5.3.5 Correlation test

100 florets and pods were selected at random from the flower heads developed in full light. The number of seeds or fertile embryo sacs per carpel was recorded. The frequency distribution of fertile ovules per carpel and the number of seeds per pod are shown in Fig.5.5. A correlation test between ovule fertility and seed set showed a positive correlation (R=0.955), suggesting that the direct cause of low seed set was ovule sterility.

5.4 DISCUSSION:

Irrespective of the stages of inflorescence development at which shading was applied (i.e node 6 to node 9), a decrease in ovary length was observed. The present results agree with the observations made in earlier experiments (Chapter 4). Low light had a slight negative effect on pollen fertility but only at later stages of inflorescence development. These coincide with the period from formation of the pollen mother cells to pollen grain formation within the anther (Thomas, 1987), suggesting that shading may somehow affect the normal formation of pollen grains, perhaps by interfering with meiosis.

The most striking observation was that even in full light, only 70% of ovules formed in a flower head have fertile ovules, although the morphological appearances of the ovules are normal. Cytological observation showed that the embryo sacs are not fully developed. In some cases there was no sign of polar nuclei, egg and synergids and in others the whole embryo sac appeared shrivelled. The cause of early abortion of ovules is not known but it could be argued that it is due to resource limitation imposed by the mother plant against the interest of individual offspring as discussed in chapter 11 (Willson and Burley, 1983).

Treatments given in this investigation had no effect on the ovule number per floret, but strongly influenced the number of embryo sacs which developed normally. The treatments would be expected to have had no effect on the number of ovules per carpel, because observations by Thomas (1987) suggest that ovule initiation would have taken place when the inflorescences were at node 5 and in the present investigation all the treatments were given when the inflorescences were at node 6 or later.

Shading the young flower head alone (neutral shade or filtered light) for 6 days increased ovule sterility (Table 4). The degree of ovule sterility was greatest when shade (both neutral shade and filtered light) was applied to the flower head at node 9 (N2). This particular stage of inflorescence development coincided with the slower phase of ovary growth which separated periods of higher growth rates (Chapter 4). The growth curve of the ovary was diauxic. Three phases of ovary growth can be distinguished. In the first phase the growth is exponential. In the second phase, lasting only about 4-5 days (as the inflorescence moves from node 8 to node 9), growth length is much less rapid than in the first phase. In the third phase, growth is initially about as rapid as in the first, but gradually declined to zero. The slower phase in growth in ovary length may be the result of the development of ovules within the carpel as described in Chapter 4. Carapetian and Rupert (1989) have compared the development of safflower (***Carthamus tinctorius*** L.) ovules and gametophytes in fertile and genetically sterile genotypes. They found no significant morphological differences in the development of the ovules between the two genotypes, although, major differences occurred in the development of the gametophyte. In sterile plants, there was a delay in initiation of meiosis which was arrested at metaphase 1. Embryo sacs did not form in sterile florets except for rare observations of uninucleate embryo sac which began to degenerate before anthesis. Though white clover and safflower belong to different families, it is possible that treatments given to white clover in the present investigation could have been interfering with meiosis in the same way as reported to safflower and so account for ovules being devoid of embryo sacs.

The results in the present investigation showed that shading the inflorescence at later stages of development (N2&N3) led to floret abortion, thereby reducing the number of florets per flower head which reached anthesis. Roberts (1979) showed a 50% increase in seed yield in white clover at 60 cm drill spacing compared to a broadcast sowing. This was attributed to a higher number of seeds per inflorescence as well as an increase in inflorescence production. Mohamed (1981) had compared the seed yield components in white clover such as the size of inflorescence (floret number) and the seed set per floret between plants established as single individuals with 90 cm spacing and in swards. He reported that number of seeds per inflorescence was reduced by 28.4% under sward conditions. There was an 18% reduction in the size of inflorescences (floret number per head) and an 11.8% reduction in the seed set per floret. Mohamed suggested that the decrease in seed yield in clover growing in swards may be a consequence of increased inter and/or intra-plant competition. The present results suggest that the decrease in floret number per flower head and low seed set per floret could be due to low light intensity at the level of the developing flower head in the sward conditions compared with similar plants established at 90 cm spacing. Light intensities have been shown to be an important factor affecting flower head production and other components of seed yield of white clover. The results of the present study suggest that major effects of light on seed production are the enhancement of floret fertility and the decrease in floret abortion.

Efficiency of pollination is also important for high seed yield (Mohamed, 1981). This is shown by the report of Haggar and Holmes (1963) that when beehives located in close proximity to fields could increase seed yield by 20-40% in white clover. In the present investigation, 32% (P=0.32) of mature ovules failed to develop into seeds. This might be due to genetic inviability (internal control) or other external factors such as the limitation of pollination preventing all genetically viable ovules to develop into seeds. If seed abortion was due to genetic inviability then the probability of ovules setting seed may be the same for each ovule in each carpel. Therefore the binomial model should fit the data. Alternatively, if a single external factor such as pollination was limiting, then in a good season such as that experienced in the summer of 1988/89 one would expect a greater proportion of pods to have the maximum number of seeds and thus to deviate from normal distribution. The results obtained clearly show that the binomial model fits the observed values and suggest that an internal control such as genetic inviability is likely the cause for seed abortion. The observation of a random pattern of seed set amongst ovules within a carpel and a positive correlation between the ovule fertility and the seed set strongly suggests that pollination was probably not the limiting factor in this instance and that the main limiting factor was the degree of sterility of unfertilized ovules.

Pechan (1988) observed that in ***Brassica napus*** the major factor limiting the number of seeds per pod was the failure of fertilization. Sufficient pollen germination on a stigma did not guarantee full seed set and even when pollen tubes were present at the micropylar regions, ovules were not penetrated. He suggested that there may be a 'barrier' between the pollen tubes and ovules which could prevent fertilization. It is possible that a chemical is produced by the ovules and emitted through the micropyle which facilitates pollen tube entry into the ovule. The precise origin of such a chemical is uncertain but it might originate in the synergids (Rosen, 1975). Wilms (1981) has shown that in ***Spinacia*** immature ovules cannot be penetrated by pollen tubes but in mature ovules, subsequent to pollination, synergids release substances which dissolve the middle lamellae of the nucellus in the micropylar region, thus allowing pollen tubes to penetrate the ovule. Pechan's (1988) observation that in the normal process of ovule fertilization in ***B. napus***, one of the two synergids degenerates but in unfertilized ovules both remain intact, strengthens the suggestion that synergids may be important in the fertilization process. Foster (1966) reported that in white clover the low seed number per pod was not due to lack of pollination, but the observed low seed number was due to number of seeds that shrivelled before reaching maturity. In the present investigation, the absence of a normal embryo sac in an ovule must be the cause of failure of fertilization and this in turn may be limiting the number of seeds per pod.

In the present work, it was observed that flowers in the basal whorl produced more seeds per pod than those more distal. This could be due to variation in size of vascular bundles present in pedicels of upper and lower florets depending on the size of the pedicel or due to competitive hierarchy among florets based on their sequence of development and also their sink strength as discussed in chapter 11.

The neutral shade and filtered light treatments reduced the seed yield per flower head by 28 and 30% respectively. The close correlation between the percentage of apparently normal embryo sacs and the percentage of ovules setting seed strongly suggests that the reduction in seed number per pod was brought about by an increase in ovule sterility. The reduction in seed number was also contributed to an increase in the number of florets aborting in the flower heads developing under shade. Row orientation effects on seed yield in field grown bush beans (Kaul and Kasperbauer, 1988) have been demonstrated to be due to subtle differences in the R:FR ratio which acts via the phytochrome system to regulate partitioning of photoassimilates within the plant. The low R:FR ratio light (i.e filtered light treatment) on the developing inflorescence in the present investigation might have a similar effect.

It is often recommended that a white clover seed crop needs to be managed as spaced plants rather than in swards for high seed yield (Clifford, 1980). The results in the present study agree with the above recommendation and also explain why low seed yield is obtained in swards.

Field Experiments.

CHAPTER 6

FIELD EXPERIMENT 1988/1989- CLONAL MATERIAL OF "GRASSLANDS HUIA"

6.1 INTRODUCTION

The two factors most strongly influencing seed yield in white clover are the number of flower heads per unit area (Zaleski, 1961) and adequate pollination of these by bees (van Bogaert, 1977). Under normal summer conditions, only about 50% of ovules develop into seeds. Although Thomas (1981) found the mean number of ovules per floret in "Grasslands Huia" was 5.5, Clifford (1979) found the average number of seeds per floret to be only 2.2. The reasons for poor seed set are not known although such knowledge would be highly desirable as a basis for the development of improved management practices for seed production.

A possible clue to the cause of this low seed production is the observation that low light intensity leads to total abortion of developing flower heads (Zaleski, 1964) and the number of seeds per floret in a "good" (warm, dry, sunny) summer is usually up to 50% higher than in a "bad" (cool, rainy, dull) summer (Romero, 1985). The light intensities found by Zaleski to be most effective in causing abortion (1000-3000 lux) are below the intensity of natural radiation falling on the foliage of white clover growing in the field, even in the dullest summers. But light intensity beneath the foliage canopy of a white clover seed crop is often as low as this even at midday when incoming radiation is most intense (Brougham, 1958 and Appendix 5). In a dense canopy, the flower heads spend several days in this environment immediately following their emergence from the apical buds of stolons. Could it be that the low light intensities beneath the white clover canopies result in partial abortion of developing flower heads?

The effect of light intensity on flower head development and floret fertility were studied in earlier experiments (Chapters 4 & 5) in controlled environment and in glasshouse conditions. It was found that in full light most florets developed fully, but about 20% of their ovules were sterile. In densely shaded conditions, however, not only did many flower heads abort completely, but, in those that did not do so, between 40 and 80% of ovules were sterile.

TABLE 6.1: Basic weather data for Palmerston North: Mean values for each month over the experimental period 1988-1989.

Recorded at DSIR Grasslands Meteorology Station (approximately 2km from experimental plot).

Month	*Temperature in °C*				*Rainfall (mm)*		*Sunshine (Hours)*	
	Maximum		*Minimum*		*88/89*	*89/90*	*88/89*	*89/90*
	88/89	*89/90*	*88/89*	*89/90*				
August	13.8	14.2	5.7	5.3	93.8	53.1	140.5	124.1
September	15.6	16.5	9.4	12.4	144.0	25.3	67.9	150.9
October	17.1	18.0	10.2	9.6	98.0	123.1	137.6	129.9
November	19.8	20.0	10.9	12.0	63.3	23.0	181.5	191.5
December	22.9	20.1	13.4	11.3	57.0	58.8	224.9	157.8
January	24.1	23.0	15.2	13.0	92.4	104.2	223.1	200.9
February	23.1	24.9	12.9	15.2	75.3	17.5	192.5	223.2

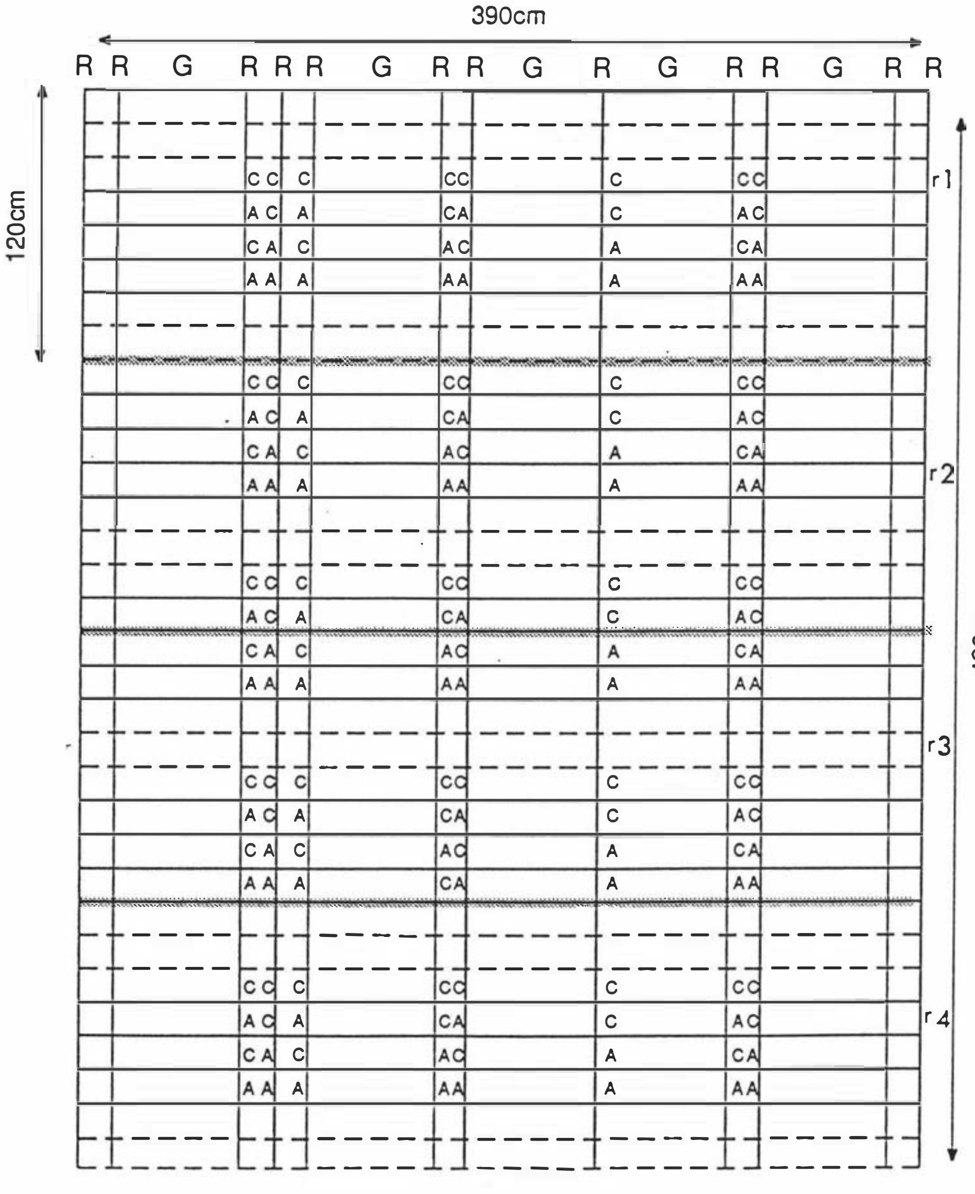


FIGURE 6.1 : Trial layout. Field experiment 1988-89.
R-R (row) spacing = 15 cm.
Spacing within row = 15 cm.
Light well (G) = 60 cm.
(·····) = Buffer of sown clover seed.
(-----) = Clone A or clone C.
r1-r4 = Replicates 1 to 4.

Realizing the significance of these observations for seed production practice, field experiments were therefore set up in Palmerston North during the summers of 1988/89 and 1989/1990 to determine to what extent and under what growing conditions flower head development and seed yield are influenced by canopy density and overcast weather conditions in plants of "Grasslands Huia" and "Grasslands Pitau".

6.2 MATERIALS AND METHODS

6.2.1 Experimental site and field procedure

The experiment was carried out in a paddock previously used for a summer forage crop at the Seed Technology Centre, Massey University, using plants of "Grasslands Huia" white clover. The soil type was a Tokomaru silt loam of moderate fertility. Details of prevailing temperature, precipitation and sunshine hours are presented in Table 6.1. The land was ploughed, harrowed and rolled on 22 & 23 May 1988.

Clonal material of clones A&C of "Grasslands Huia" white clover (Thomas, 1979) was used. Tip cuttings were grown in a 1:1 mixture of sand and peat in 1000 cm^3 plastic pots and maintained for one month in a glasshouse as described in Chapter 3 and for two months outside before being planted out in the field at two densities on 4 & 5 August 1988.

The herbicide Paraquat was applied to all the plots by hand spraying between rows of plants at the rate of 3 litres/hectare on 9 September 1988. 30% potassic superphosphate was applied at the rate of 150 kg/hectare on 28 October 1988.

The trial was laid out in a randomized complete block design. Four experimental treatments were each replicated four times (see Fig.6.1). Ramets were planted in rows. Clones A and C were planted alternately to ensure an adequate source of foreign pollen for cross pollination. Within the rows all plants were spaced 15 cm apart, but inter-row spacing differed between the treatments. The four experimental treatments were:

A) Open canopy: Inter-row spacing of 60 cm; unshaded.

B) Open canopy with pre-fertilization shade (Pre.f.Shade): Inter-row spacing of 60 cm. Overcast weather conditions were simulated by artificially shading plants from

Plate 6.1 : Plants being shaded artificially before pollination to simulate overcast weather conditions.

December 1988 to 16 January 1989 with neutral shade cloth as shown in Plate 6.1. these plants received only 45% of incoming radiation. After removing the shade, 25 flower heads in which corollas of the oldest florets were just showing white were tagged per replicate.

C) Dense canopy: Inter-row spacing of 15 cm; unshaded.

D) Open canopy with post-fertilization shade (Post.f.Shade): Inter-row spacing of 60 cm. 25 randomly selected flower heads per replicate were tagged on 16 January 1989 just before anthesis. After they had been pollinated (as judged by the degree of reflexion of their florets), the plants were artificially shaded with cloth from 27 January to 15 February 1989 so that they received only 45% of incoming radiation.

6.2.2 Measurements

On 16 January 1989, 25 flower heads per replicate were tagged in each treatment.

6.2.2.1 Ovule fertility

On 20 January 1989, 100 florets were collected at random from tagged flower heads and fixed in formalin/acetic/alcohol. The fixed ovaries were dissected and stained with Mayer's haemalum, a positive stain for chromatin and nuclei, and optically cleared with methyl salicylate as described in Chapter 3. The cytoplasmic state of embryo sacs and presence or absence of polar, egg and synergid nuclei of stained ovules were examined using Nomarski interference microscopy. Embryo sacs lacking nuclei were classified as sterile.

6.2.2.2 Pollen fertility

On 20 January 1989, 10 florets were sampled from five flower heads selected randomly per treatment to assess the pollen fertility. Pollen fertility was assessed by examination of a sample of microspores from each floret mounted on a glass slide and stained with Snow's alcoholic carmine. Approximately 100 grains were counted for each floret and the number with or without cytoplasm was recorded. Pollen grains devoid of contents were classed as sterile and those containing cytoplasm as fertile (Plate 5.2).

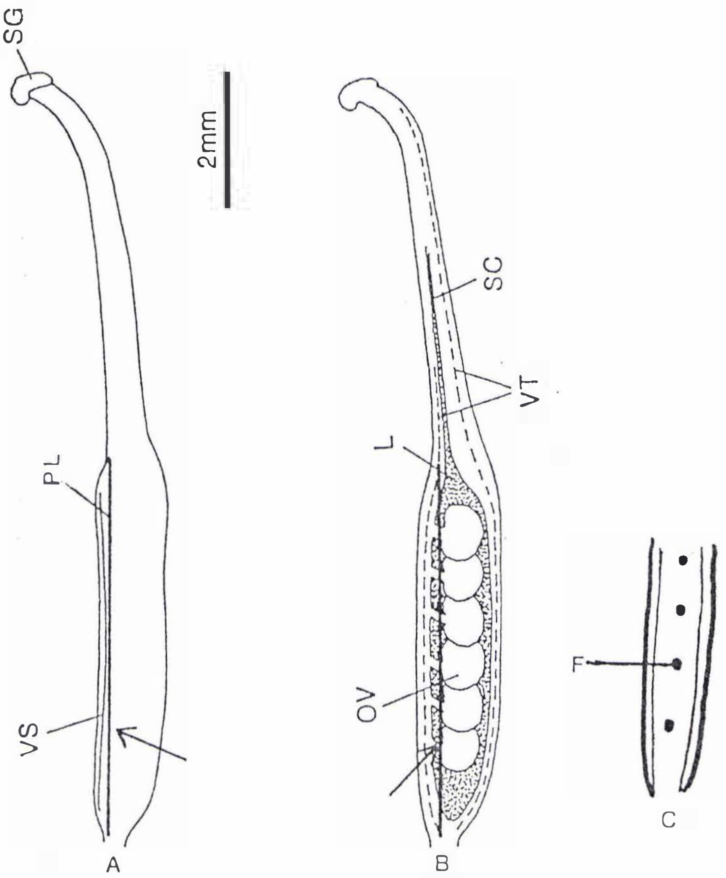


FIGURE 6.2 : Carpel structure. (A) Intact carpel (B) Diagrammatic representation of half a carpel (C) The carpel placenta viewed from inside the ovary after removal of ovules. The placental region of the carpel (PL ; indicated by arrows) was dissected out to assess the pollen load (number of pollen tubes per ovary). VS = ventral suture; SC = stylar canal; PL = placenta; OV = ovules; VT = vascular trace; L = loculus and F = funicle.

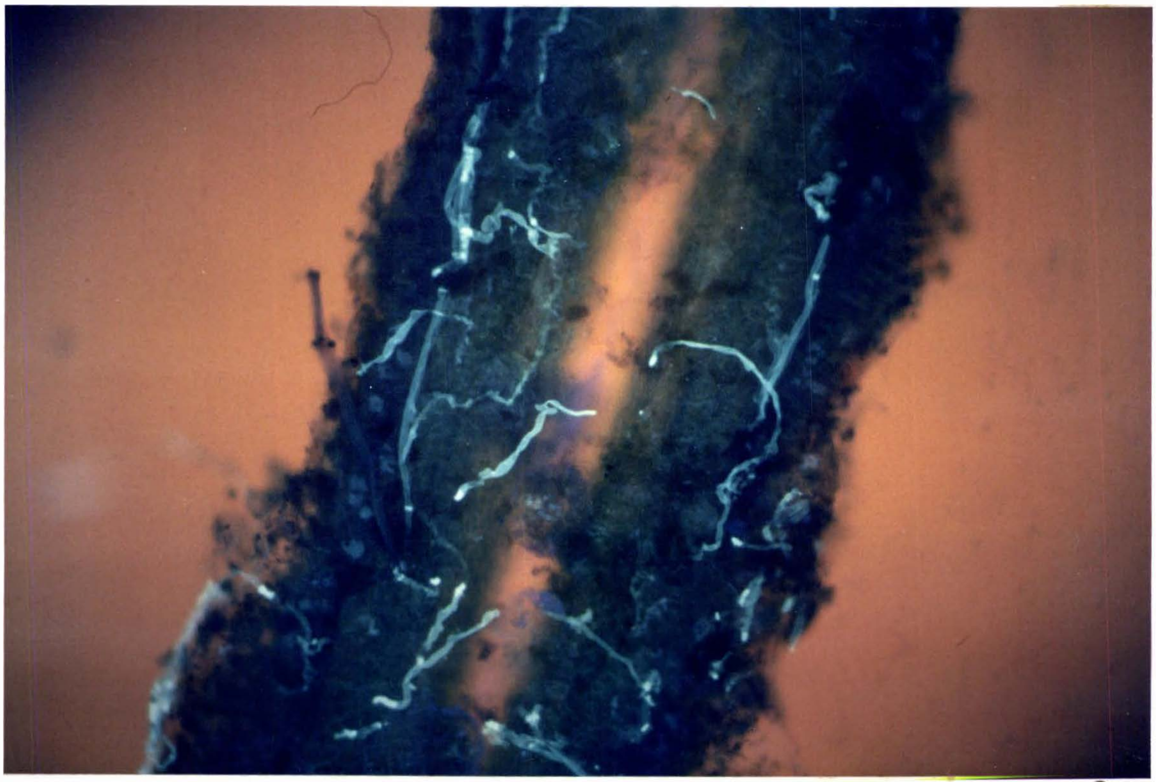


Plate 6.2 : Carpel placenta viewed from inside the ovary after removal of ovules. Pollen tubes growing on the surface of the placenta show callose plugs, which are present at regular intervals and fluoresce yellow to yellow/green when stained with aniline blue and examined microscopically with direct illumination from an ultra violet lamp with an emission wavelength of approximately 356 nm.

Plate 6.3 : X-ray photograph showing number of ovules forming seeds in carpels of upper florets collected from flower heads developed in open (A) and dense canopies (B).

6.2.2.3 Pollen load

Twenty five florets which had just been pollinated were selected randomly from the experimental plots on 16, 21, and 29 January 1989 to assess the pollen load (pollen tubes per ovary). This was done by counting the number of pollen tubes present in the placental region of the carpel. The placenta region of the ovary was dissected out as shown in Fig.6.2 and stained in water soluble aniline blue dissolved in 0.1 M $K_3 PO_4$ for 5-10 minutes. The stained placenta was mounted in a few drops of aniline blue stain and examined under the microscope. This method requires a microscope with direct illumination from an ultra violet lamp with an emission wavelength of approximately 356 nm. In suitable preparations, the callose plugs, which are present in the pollen tube at regular intervals, fluoresce a yellow to yellow/green colour (Plate 6.2).

Tagged flower heads were harvested from clone C plants on 15 February and the following measurements made:

1) Number of florets per inflorescence: 10 inflorescences were sampled per treatment.

2) Number of seeds per pod: Two harvests were made to assess the seed number per floret. The first harvest was carried out 10 days after pollination and the second one at the time of final harvest (15 February). In the first harvest, 10 flower heads were selected randomly per treatment and stored at 4^{o} C until required for counting. At the time of counting, 200 pods per treatment were sampled both from apical (100) and basal (100) florets of 10 inflorescences. The seed number per pod was counted manually. In the second harvest, 200 pods per treatment were sampled both from 100 apical and 100 basal florets of 25 inflorescences. To count the number of seeds per pod, the pods were placed on Polaroid photographic paper and X-rayed using a Faxitron Hewlett-Packard X-ray machine (25 KVA, 1 min exposure time). Typical results are shown in Plate 6.3.

3) Seed weight: using the bulked samples of seed for each treatment, four weight counts of 100 seeds each were made. The 1000-seed weight was standardized to 10% moisture content.

TABLE 6.2: Effect of treatments on ovule number per carpel, % of ovules setting seed, and % of fertile ovules per carpel.

*Treatments were a) open canopy: inter-row spacing of 60 cm; b) open canopy with pre-fertilization shade: inter-row spacing of 60 cm and plants artificially shaded before fertilization (45% of incoming radiation); c) dense canopy: inter-row spacing was 15 cm; d) open canopy with post fertilization shade: inter-row spacing 60 cm and plants artificially shaded after fertilization (45% of incoming radiation). The intra-row spacing for all treatments were 15 cm. *= not recorded. Correlation values between seed set and fertile ovules are given in parentheses.*

Treatment	*Ovule Number per ovary (± SE)*	*Pollen fertility (%)*	*Fertile Ovules (%)*	*Ovules setting seed(%)*
Open canopy	*4.52± 0.09*	*95± 0.9*	*71 (0.966)*	*67.6*
Open/pre.f.Shade	*4.53± 0.05*	*92± 1.8*	*63 (0.903)*	*56.2*
Dense canopy	*4.31± 0.04*	*84± 2.0*	*54 (0.931)*	*49.3*
Open/post.f.Shade	*4.52± 0.09*	*--**	*--**	*49.1*

TABLE 6.3: Effect of treatments on Seed yield components.

*Treatments were a) open canopy: inter-row spacing of 60 cm; b) open canopy with pre-fertilization shade: inter-row spacing of 60 cm and plants artificially shaded before fertilization (45% of incoming radiation); c) dense canopy: inter-row spacing 15 cm; d) open canopy with post fertilization shade: inter-row spacing 60 cm and plants artificially shaded after fertilization (45% of incoming radiation). The intra-row spacing for all treatments were 15 cm. Values followed by the same letter between treatments (in same column) are not significantly different at 5% level. += 10% moisture content; *= not recorded . DAP= days after pollination.*

Treatment	*Number of fully developed florets (± SE)*	*Seed Number per fully developed floret*		*Seed number per head*	*1000 Seed Weight^{+} (mg)*	*Seed Weight per head (mg)*
		10 DAP (± SE)	*30 DAP (± SE)*			
Open canopy	*103 ± 3.9a*	*3.39 ± 0.17a*	*3.06 ± 0.07a*	*315*	*0.573 ± 0.022a*	*180.5*
Open/pre.f. Shade	*94 ± 3.6a*	*2.61 ± 0.21b*	*2.55 ± 0.13b*	*240*	*0.588 ± 0.012a*	*141.1*
Dense canopy	*90 ± 2.1b*	*2.31 ± 0.12b*	*2.13 ± 0.08b*	*192*	*0.476 ± 0.012b*	*91.4*
Open/post. f.Shade	*--**	*3.39 ± 0.17a*	*2.22 ± 0.06b*	*228*	*0.530 ± 0.003a*	*120.8*

6.3 RESULTS

6.3.1 Pollen fertility

Table 6.2 shows the effect of the treatments on the average percentage of fertile pollen in the anthers. The degree of pollen abortion was greatest in the flower heads developed in dense canopies, 16% of pollen grains being sterile in dense canopies compared with only 5% in open canopies. Those plants which were artificially shaded had only 8% sterile pollen.

6.3.2 Ovule fertility

Treatments given had no significant effect on the number of ovules per floret, but strongly influenced the number of embryo sacs which developed normally (Table 6.2). Flower heads which developed in dense canopies produced only 54% of normal embryo sacs compared with 71% in open canopies. Shading of plants before pollination reduced the ovule fertility to 63%. There was a close correlation between the level of embryo sac fertility and the percentage of ovules setting seed: 71% and 68% respectively in the open canopy, 54% and 49% in the dense canopy and 63% and 56% in the pre-fertilisation shaded open canopy.

6.3.3 Number of florets per head

The effect of treatments on the number of florets that developed fully on a flower head is shown in Table 6.3. The flower heads developed in an unshaded open canopy had the highest number of florets per inflorescence. In dense canopies and in plants which were artificially shaded before pollination, there was a reduction in the floret number between 8.7 and 12.6% (Table 6.4). The effect of post-fertilisation shade was not recorded.

6.3.4 Number of seeds

The effect of treatments on the number of seeds set per floret and flower head showed similar trends (Table 6.3). The number of seeds was highest in the open canopy and lowest in the dense canopy. In the dense canopy the number of seeds per floret was

TABLE 6.4: Percentage reduction in various seed yield components.

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*Values are calculated for each treatment with respect to the control treatment (open canopy). See caption of Table 6.3 for further details. *= not recorded.*

Treatment	*Floret number*	*Seed number*	*Seed number/head*	*Seed weight per head (mg)*
Open/pre.f.Shade	*8.7*	*16.7*	*23.8*	*21.6*
Dense canopy	*12.6*	*30.3*	*39.0*	*49.3*
Open/post.f.Shade	*---**	*27.4*	*27.6*	*33.5*

TABLE 6.5: Observed and expected frequency from the binomial distribution of pods with 0 to 5 seeds.

Seed Number per carpel	*Expected frequency (E)*	*Observed frequency (O)*	*$(O-E)^2/E$*
0	*1.0*	*0.0*	*1.00*
1	*7.1*	*11.0*	*1.38*
2	*22.1*	*19.0*	*0.50*
3	*34.5*	*34.0*	*0.007*
4	*26.9*	*27.0*	*0.0004*
5	*8.4*	*9.0*	*0.04*
Total	*100*	*100*	*2.93*

Chi-square = $(O-E)^2/E$

reduced by 30.4% in comparison with the open canopy. Pre-fertilisation shading of open canopy plants reduced the seed number by 16.6%, and post-fertilisation shading reduced it by 27.5%. Although no count was made of the total number of seeds per inflorescence, this was estimated by multiplying the average seed number per floret by the average floret number per inflorescence. The results of such calculations (Table 6.4) show, once again, that the total number of seeds per head was highest in the open canopy and most heavily reduced in the dense canopy (by 39%). Artificially shading the plants before or after pollination also resulted in reduction of the number of seeds per flower head by 24 and 28% respectively (Table 6.4). From 10 days after pollination to the final stage of seed development (seed maturation), there was a very small proportion of the total number of seeds lost (Table 6.3).

6.3.5 Seed weight

The results obtained show the open canopy seeds to have been heaviest while those from the dense canopy were lightest. Artificially shading the plants before or after pollination had no significant effect on 1000-seed weight.

6.3.6 Fitting the binomial distribution and testing the goodness-of-fit

The observed values and the expected frequency of pods with 0 to 5 seed from binomial distribution was calculated as described in chapter 5. The observed and expected frequencies for each class (0 to 5 seeds) are shown in Table 6.5. Chi-squared values were then calculated using the formula (see Chpater 5). The 5% significance level of chi-squared for 4 degrees of freedom is 9.41 and the value of 2.93 for the chi-squared statistics gives us no doubt that binomial model fits these data. If pollination was the limiting factor, then in a good season such as that experienced in the summer of 1988/89, one would expect a greater proportion of pods to have a maximum number of seeds and thus deviate from a normal distribution. The results obtained clearly show that the binomial model fits the observed values and suggest that genetic inviability of ovules is likely to be the cause for seed abortion as described in chapter 5.

TABLE 6.6: Effect of treatment on pollen load

Values followed by the same letter between treatments are not significantly different at 5% level.

Treatment	*% carpels with >10 pollen tubes*
Open canopy	*92a*
Dense canopy	*88a*

6.3.6 Pollen load

The number of pollen tubes present in the placenta region of the carpel was counted and the carpels were classed into two groups; carpels containing more than 10 pollen tubes and less than 10 pollen tubes. The results obtained (Table 6.6) show that in both dense and open canopies, 88-92% of carpels had more than 10 pollen tubes, suggesting that the florets had more than sufficient foreign pollen to fertilize the ovules and pollination was not limiting.

6.4 DISCUSSION

The main underlying factor determining seed production capacity in white clover, or any other plant, is the number of fertile ovules produced per plant. Provided that the ovules show a high level of fertility, the seed-producing ability of a plant will be strongly affected by the number of ovules formed (Thomas, 1961a). The number of ovules formed on a white clover plant is determined by the number of flower heads, the number of florets per head, and the number of ovules per floret. Until now, in commercial practice, the most significant component of seed yield has been shown to be the number of flower heads produced per unit area per unit time, i.e, flower density (Zaleski, 1961), but all components of seed yield must have an effect. Given high head density, seed yield can be strongly influenced by the number of florets per head (Clifford, 1979; Van Bockstaele and Rijckaert, 1988). Likewise, given both high head density and a large number of florets per head, seed yield should theoretically be affected by the number of seeds per floret (Thomas, 1981).

The present investigation clearly shows that the level of apparent fertility of pollen formed in both dense and open canopies was very high. Although the average percentage of abortion in dense canopies was higher than in open canopies, probably pollen sterility was not high enough to reduce the seed set under conditions of thorough pollination. The results suggest that the pollen fertility was little affected by light. The above results are consistent with the observations of Thomas (1961a).

Low light intensity, brought about by either high canopy density or artificial shading, increased ovule sterility (Table 2). The flower heads developed in dense canopy produced slightly fewer ovules per carpel compared to those developed in an open canopy. Flower heads developing in dense canopies would have experienced shade during the stage of ovule initiation. These produced 39% fewer seeds per head than those formed in an open canopy (Table 6.3). The close correlation between the percentage of apparently normal embryo sacs and the percentage of ovules setting seed strongly suggests that this reduction was largely brought about by an increase in ovule sterility, perhaps as a result of inadequate photosynthate leading to competition either between the ovules in a floret or between the florets in a flower head. The results obtained show that only a very small proportion of total number of seeds lost beyond 10 days after pollination. These results are similar to those obtained by Robbie (1988). Robbie reported that the majority of seed abortion occurred within a five to eight day period following floret maturity and pollination. Beyond this period, occasional seed abortion was observed at all stages of seed development. The reduction in seed number was also contributed to by an increase in the number of florets aborting in the dense canopy, again possibly as a result of limited availability of photosynthate.

Roberts (1979) showed a 50% increase in seed yield of varieties at 60 cm drill spacing compared to a broadcast sowing. This was attributed to increased inflorescence production and a higher number of seeds per inflorescence. Mohamed (1981) compared the seed yield components between plants established as single individuals with 90 cm spacing and in swards. In swards, the floret number was reduced by 17% compared with similar plants established at 90 cm spacing. Similarly, he noted that the seed set per floret was reduced by 13% under sward conditions. He suggested that the decrease in seed yield per inflorescence in swards may be a consequence of increased inter-intra plant competition in sward. But this may equally have resulted from low light intensities at stolon levels in the sward compared with similar plants established at 90 cm spacing. Bourdot and Butler (1981) found that the seed yield of white clover declined with increasing density of yarrow to almost zero at 500 yarrow flower stems per square metre and this was caused mainly by a reduction in the number of seed heads and the number of seeds per head. They suggested that poor pollination at high yarrow densities could account for the reduced number of seeds per head but this may equally have resulted from competition for light, for the yarrow stems reached 1 m in height shading the lower-statured clover.

Efficiency of pollination is also important for high seed yield per inflorescence (van Bogaert, 1977). In the past, most often low seed set has been attributed to poor pollination. The variation in number of seeds present in a carpel (i.e some carpels had all ovules set seeds and in some all of them aborted) as seen in Plate 6.3 also suggests that pollination could be limiting. But the results obtained in the present investigation, showing that a high percentage of carpels have sufficient pollen tubes to fertilize ovules, strongly suggest that poor pollination could not account for the reduced number of seeds per head. Smith et al. (1990) studied the effect of pollen load on seed set in alfalfa (***Medicago sativa*** **L.**) and found that only 46% of the ovules in ovaries formed seeds even with heavy pollen loads (71 pollen tubes/ovary). Sayers and Murphy (1966) also showed that the pollen tube number within the ovary was not related to seed set. In the present investigation, a positive correlation between the percentage of fertile ovules and the percentage of ovules setting seeds and random seed set pattern within a carpel strengthens the theory that seed yield was not limited by pollination.

CHAPTER 7

FIELD EXPERIMENT 1989/1990- CULTIVAR "GRASSLANDS PITAU"

7.1 INTRODUCTION

In the first field trial, clonal material of "Grasslands Huia" was used to minimize genetic variation. In contrast, commercial seed producers use high yielding cultivars with high genetic variation. Another field trial was therefore set up to compare the response of a genetically variable crop of "Grasslands Pitau" in similar experimental conditions.

7.2 MATERIALS AND METHODS:

7.2.1 Experimental site and field procedure:

The experiment was carried out at the Pasture and Crop Research Unit, Massey University, using a two-year-old "Grasslands Pitau" white clover crop growing on Tokomaru silt loam. Table 6.1 shows the prevailing temperature, precipitation, and sunshine hours. Before the start of the experiment, the crop had been grazed by sheep whenever the herbage reached a height of 10-20 cm. The last preparatory grazing was completed on 26 August 1989. The experimental area received a spring application of 250kg/hectare of 30% potassic superphosphate on 9 October 1988.

The trial was laid out in a randomized complete block design and all treatments replicated 6 times. The area per replicate was $2m^2$(Fig.7.1). All the plots were defoliated on 30 October 1989 using the herbicide Paraquat at the rate of 3 litres/hectare. Four experimental treatments were given:

A **Open canopy:** The plots were manually defoliated for a second time on 15 December to leave residual herbage of about 3-4 cm high.

B **Open canopy with pre-fertilization shade (Pre.f.Shade):** The plots were manually defoliated for a second time as in treatment A. Overcast weather conditions were simulated by artificially shading plants from 16 December 1989 to 15 January 1990 with neutral shade cloth as shown in Plate 6.1 These plants received only 45% of incoming radiation. After removing the shade, about 16 flower heads in which the corollas of the oldest florets were just showing white were tagged per replicate.

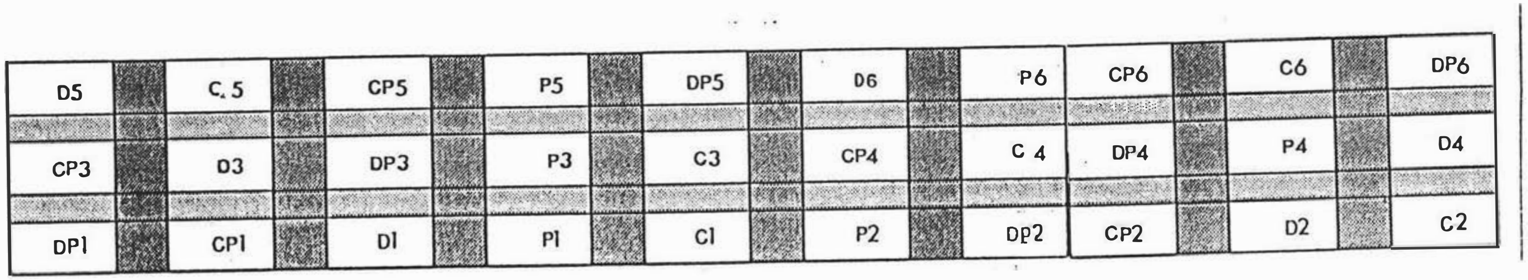

FIGURE 7.1 : Trial layout. Field Experiment 1989-90

1 - 6 = Replicates 1 to 6 (Blocks).
C = Open canopy.
P = Pre-fertilization shade.
CP = Open-Post fertilization shade.
D = Dense canopy.
DP = Dense Post-fertilization shade.
▨ *Path.*

C **Dense canopy:** The plots received no second defoliation treatment.

D **Open canopy post-fertilization shade (Post.f.Shade):** The plots were manually defoliated for a second time as in treatment A. On 15 January 1990 about 16 randomly selected flower heads per replicate were tagged just before anthesis. After these had been pollinated (as judged by the degree of reflexion of their florets), they were shaded with shade cloth as shown in Plate 6.1 until 15 February so that they received only 45% of incoming radiation.

E **Dense canopy post-fertilization shade:** As in treatment C, the plots received no second defoliation treatment. On 15 January, about 16 randomly selected flower heads per replicate were tagged just before anthesis. After these had been pollinated, they were shaded with shade cloth until 15 February so that they received only 45% of incoming radiation.

7.2.2 Measurements

On 15 January, about 16 flower heads per replicate were tagged in all treatments. Ten randomly selected flower heads were harvested from open canopy plots to count the ovule number per floret. On 15 February, the tagged flower heads were harvested and the following measurements were made:

1 Number of florets per inflorescence: 96 inflorescences were sampled per treatment.

2. Number of seeds per pod: 1200 pods per treatment were sampled both from the apical (600) and the basal (600) florets of 96 inflorescences. To count the number of seeds per pod, the pods were placed on Polaroid photographic paper and X-rayed using a Faxitron Hewlett-Packard X-ray machine (25 KVA, 1 min exposure time).

3. Seed weight: Using the bulked samples of seed for each treatment, four weight counts of 100 seeds each were made. The 1000-seed weight was standardized to 10% moisture content.

TABLE 7.1: Effect of treatments on seed yield components.

Treatments were a) open canopy: plots were defoliated twice b) open canopy with pre-fertilization shade: plots were defoliated twice and plants artificially shaded before fertilization (45% of incoming radiation); c) dense canopy: plots were defoliated once; d) open canopy with post fertilization shade: plots were defoliated twice and plants artificially shaded after fertilization (45% of incoming radiation); e) dense canopy with post fertilization shade: plots were defoliated once and plants artificially shaded after fertilization. Values followed by the same letter between treatments (in same column) are not significantly different at 5% level. += 10% moisture content.

Treatment	*Number of fully developed florets (± SE)*	*Seed Number per Floret*			*Seed number per head*	*1000 Seed Weight+ (mg)*
		apical	*basal*	*mean (±SE)*		
Open canopy	*57.8 ± 1.17a*	*2.67a*	*3.74a*	*3.21 ± 0.12a*	*186 ± 6.16a*	*0.635 ± 0.026a*
Open/pre.f. Shade	*46.0 ± 2.65b*	*2.36a*	*3.20b*	*2.78 ± 0.14b*	*128 ± 8.72b*	*0.597 ± 0.008a*
Dense canopy	*44.5 ± 2.19b*	*2.10b*	*3.15b*	*2.63 ± 0.11b*	*116 ± 3.11b*	*0.599 ± 0.030a*
Open/post. f.Shade	*52.8 ± 2.37a*	*2.27b*	*3.05b*	*2.66 ± 0.09b*	*140 ± 11.27b*	*0.577 ± 0.019a*
Dense/post. f.Shade	*48.8 ± 1.23b*	*2.29a*	*3.39b*	*2.85 ± 0.14b*	*140 ± 5.43b*	*0.608 ± 0.012a*

TABLE 7.2: Percentage reduction in various seed yield components.

Values are calculated for each treatment with respect to the control treatment (open canopy). See caption of Table 7.1 for further details.

Treatment	*Floret Number*	*Seed Number per floret*	*Seed Number per head*	*Seed Weight per head (mg)*
Open/pre. f.Shade	*20.4*	*13.4*	*31.1*	*35.3*
Dense canopy	*23.0*	*18.0*	*37.3*	*41.2*
Open/post. f.Shade	*8.6*	*17.1*	*24.8*	*31.6*
Dense/post. f.Shade	*16.6*	*11.2*	*24.5*	*27.3*

7.3 RESULTS

7.3.1 Number of florets per head

The results obtained show that the flower heads developed in an unshaded open canopy had the highest number of florets per head (Table 7.1). In dense canopies and in plants which were artificially shaded before pollination, there was a reduction in floret number by 23%. The post-fertilization shading of dense canopy plants also led to a statistically significant (16%) reduction in the number of fully developed florets. Flower heads which developed in an open canopy and which then were shaded after fertilization had a slightly reduced floret number per head but the reduction was not statistically significant (Table 7.1).

7.3.2 Number of ovules per floret

The average ovule number per floret was 5.54.

7.3.3 Number of seeds

The effect of treatments on the number of seeds set per floret and flower head are shown in Table 7.1. The number of seeds per pod was highest in the open canopy and lowest in the dense canopy. In the dense canopy the number of seeds per floret was reduced by 18% in comparison with the open canopy. Pre-fertilization shading of open canopy plants reduced the seed number by 13.4%, and post-fertilization shading reduced it by 17.1%. Post-fertilization shading of dense canopy plants reduced seed number by 11.2% (Table 7.2). There was a tendency for the apical florets on a head to possess fewer seeds than those developing lower down (basal) (Table 7.1). Although no count was made of the total number of seeds per inflorescence, this was estimated by multiplying the average seed number per floret by the average floret number per inflorescence. The results of such calculations (Table 7.1) show that the total number of seeds per head was highest in the open canopy and most heavily reduced in the dense canopy (by 37%). Artificially shading the open canopy plants before or after pollination also resulted in a reduction of the number of seeds per flower head by 31 and 25% respectively. The post-fertilization shading of dense canopy plants also reduced the seed number by 25% (Table 7.2).

7.3.4 Seed weight

Table 7.1 shows that treatments given in this experiment had no significant effect on the 1000-seed weight.

7.4 DISCUSSION

The results obtained in the present experiment show that the flower heads developed in a dense canopy produced 37% fewer seeds per head than those formed in an open canopy (Table 7.2). The reduction in seed number was contributed to by an increase in the number of florets aborting in a dense canopy. The flower heads developing under shade before pollination either by canopy shade or by artificially shading the plants (simulated overcast weather) produced between 20-23% fewer florets per inflorescence than those developed in an open canopy. Flower heads which developed in an open canopy and those which were shaded after fertilization had a slightly reduced (8.6%) floret number. The above results suggest that low light levels during the early stages of inflorescence development probably increased the number of florets aborting, possibly as a result of limited availability of photosynthate.

The reduction in seed set per floret observed due to treatments given in this investigation (Table 7.1) could be the result of (a) inadequate photosynthate leading to post-fertilisation competition either between ovules in a floret or between florets in a flower head or (b) an increase in ovule sterility as observed in the earlier field experiment. In the present experiment, only 58% ovules set seed in open canopies, 50% in pre-fertilization shaded open canopies, and 47% in dense canopies. The close correlation between the percentage of apparently normal embryo sacs and the percentage of ovules setting seeds in the earlier field and glasshouse experiments with "Grasslands Huia" clones strongly suggests that the reduction in seed set in "Grasslands Pitau" was also brought about by an increase in ovule sterility.

The results obtained in present and in earlier field experiment clearly show that when plants were artificially shaded before or after pollination there was a 24-31% reduction in seed number per head. This suggests that overcast weather during early stages of inflorescence development or during seed maturation could lead to about 30% reduction in seed yield per flower head, possibly due to shortage of available photosynthate.

The most striking observation was that post-fertilization shading of dense canopy plants produced 16% more seeds per head than those flower heads which developed in a dense canopy. This result was quite unexpected and is difficult to explain. One possibility is that artificially shading the plants with shade cloth changed the micro-climatic conditions of the plants, perhaps by increasing moisture retention. Clifford (1986) found that, in clover, the yield per inflorescence declined by 34% in later flowering of an unirrigated crop as moisture stress increased. However, in contrast, later flowering of an irrigated crop gave the highest yield per inflorescence. He found that the increase in the yield per inflorescence was contributed to by 4% increase in seed weight and by a 27% reduction in ovule abortion. The weather data (Table 6.1) also clearly show that in February 1990 the average temperatures were high with low rainfall, and the plants developed in artificial shade might have retained more seeds as moisture stress was reduced. Because it was a dry and hot summer, precautions were taken to harvest the tagged flower heads before pods dehisced and shed seed.

Defoliation prior to flowering has been reported to have beneficial effects on flower production and other components of seed yield of white clover. According to Zaleski (1970), flower head initiation is enhanced as a result of more light reaching the stolon level. In the writer's opinion, enhancement of flower initiation as a result of more light reaching stolon level is unlikely because observations by Thomas (1987) suggest that the photoperiodic stimulus is perceived by the leaves. He also reported that there is no evidence that stolons were also perceptive. The increased number of flower heads observed by Zaleski and other workers could be the result of reduction in abortion of very young inflorescences. Results of the present investigation have showed (Chapter 5) that shading individual flower heads led to 5-35% abortion of developing inflorescences. This suggests that the major advantage of the practice of defoliation at the time of closing for seed production might be the enhancement of floret fertility; and that decreased seed yield in duller, wetter summers is probably, at least in part, attributable to increased ovule sterility and floret abortion in the dense canopies formed under these conditions. This must be a major reason for delaying "closure" for seed production when weather conditions favour rapid and lush growth.

SECTION B

Influence of low light on the growth and sink activity of young flower heads and peduncles.

INTRODUCTION

Seed yield of white clover tends to be correlated with climatic conditions, being higher in warmer, sunnier, drier summers than in cooler, duller, wetter ones, and it is often suggested that this is the result of lower activity of bees as pollinators in cooler, duller conditions (Van Bogaert, 1977; Romero, 1985).

However factors other than bee activity might be important in duller summers. Low light intensities, for instance, are known to affect flower head development adversely. Thomas (1961, 1987) and Zaleski (1964) found that when plants were grown in warm short photoperiods or low light intensities many of their flower heads aborted either completely or partially. Partial abortion reduced the number of functional florets per head. The light intensities found by these authors to be most effective in causing abortion (1000-3000 lux) are below the intensity of natural radiation falling on the foliage of white clover growing in the field, even in the dullest summers. But light intensities beneath the foliage canopy of a white clover seed crop are often as low as this even at midday when incoming radiation is most intense (Brougham, 1958 and Appendix 5 give data on the effect of a clover canopy on PAR photon flux beneath it).

Flower heads of white clover emerge from the stolon apex in the axil of the youngest leaf. At this stage all their florets have been initiated, the oldest being about one quarter of final size and the youngest much smaller, and they are borne on very short peduncles (Thomas, 1981). Over the next few days the flower heads continue to grow and are gradually raised above the stolon by elongation of their peduncles. In a dense canopy this post-emergence growth takes place for several days in heavy shade before the flower heads are raised above the foliage.

Developing inflorescences of white clover contain chlorophyll and under laboratory conditions ***in situ*** photosynthesis can contribute as much as 70% of their carbon requirement (Pasumarty, 1987). In field conditions the contribution of developing inflorescences to their own carbon economy is probably less because they are generally heavily shaded. In other species (eg. soybean), however, there is evidence that shading of organs reduces their ability to draw assimilates from source leaves (Heindl and Brun, 1983). It is thus possible that shading may also affect sink activity of white clover inflorescences.

The present investigation was undertaken to determine the extent to which the growth and sink activity of young flower heads and peduncles is influenced by the shaded conditions that exist within dense white clover canopies.

CHAPTER 8

GENERAL MATERIALS AND METHODS

8.1 GROWTH STUDIES

8.1.1 Plant material

Clonal material (clone A) of "Grasslands Huia" white clover was used. The propagation and maintenance of plants were similar to those described in chapter 3.

8.1.2 Method

A linear voltage displacement transducer (LVDT) was used to measure the peduncle elongation rates of inflorescences in shade and light.

The transducer incorporated an integral oscillator/demodulator so that both input and output signals were d.c. As the peduncle elongated, a mV output, proportional to the displacement, was produced. The small changes in output signals were recorded on a chart recorder with a range of 1 mV to 100 V. The resolution of the instrument used was adequate to accurately measure small changes in displacement (e.g. of 0.1mm). The transducer was capable of measuring displacement of several centimetres without re-setting armature. There was a linear relationship between the displacement armature and the output signal. A 6- Volt battery was used as a source of power.

The LVDT was attached by means of cotton thread to the peduncles immediately below their flower head bases. The perspex cover protected the LVDT and the pulley system to reduce the effect of air currents on the linkage between the pulley and the inflorescence.

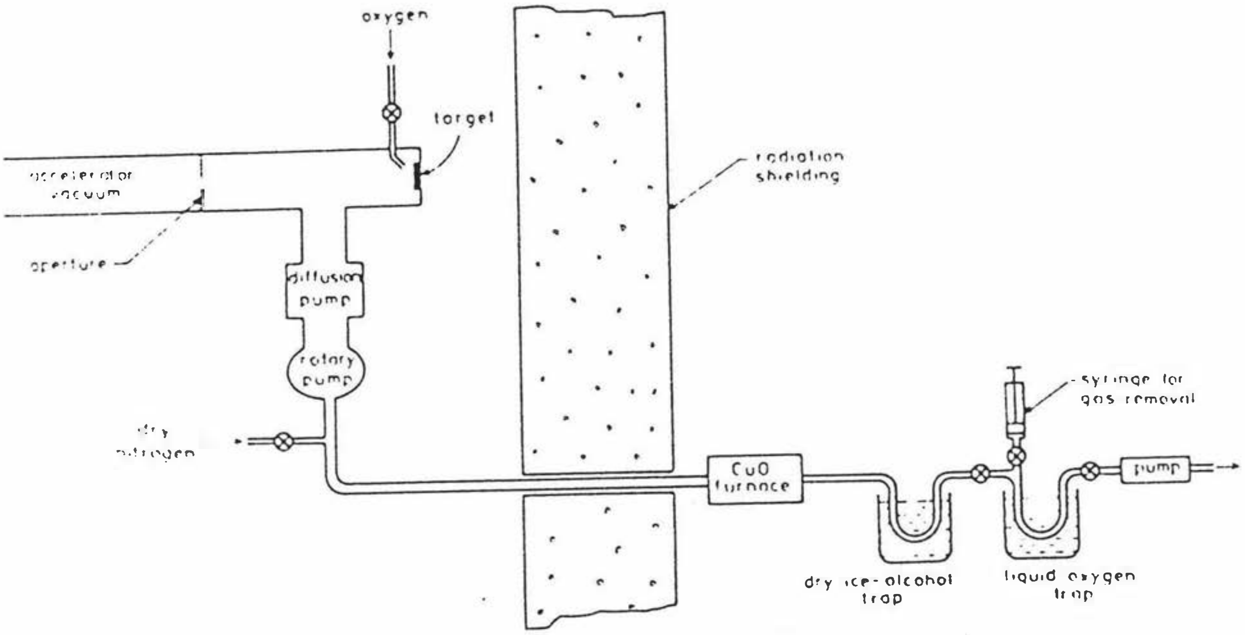

FIGURE 8.1 : $^{11}CO_2$ production line and gas recovery system (McCallum et al. 1981).

8.2 TRANSLOCATION STUDIES

8.2.1 Plant Material

Clonal material of clone A of "Grasslands Huia" was grown in commercial potting mixture, watered via a capillary mat, within an environmentally controlled cabinet maintained at 16 h photoperiod of 600 μmoles m^{-2} sec^{-1}(PAR), with a relative humidity of about 50% and day/night temperature of 25/18°C. All tracer experiments were carried out in a cabinet maintained under the same conditions.

8.2.2 Method

Carbon-11, a short-lived isotope, was used to determine the influence of shade on sink activity of young flower heads and peduncles. Carbon-11 has a half-life of 20.4 min, so it must be produced very near to the site of use. This required having access to some form of nuclear particle accelerator. For phloem translocation studies the isotope was produced as $^{11}CO_2$. The labelled CO_2 was supplied to an illuminated leaf and the movement of the labelled sucrose produced by photosynthesis was then followed by placing radiation detectors at suitable places.

Short-lived isotopes, because of the nature of their emissions, allow *in vivo* measurements. The use of a short-lived isotope with *in vivo* measurements allowed many experiments to be carried out on the same plant. This technique overcame difficulties of variability between plants which are associated with a destructive technique such as ^{14}C labelling, and thereby allowed observation of the dynamic behaviour of single plants.

8.2.2.1 $^{11}CO_2$ Production

The labelled CO_2 was produced in Institute of Nuclear Science at DSIR. An enriched boron-10 disc, 15 mm diameter, 3 mm thick, was bombarded with 2.0 Me V deuterons in an accelerator beam line (see Fig.8.1). The 30 μA beam was well focused to produce the "hot" area needed to evolve ^{11}C. A small flow of oxygen was passed across the target; this acted as a reactant to capture the ^{11}C atoms to form ^{11}CO and also as a sweep gas to remove the product. A 6 inch diffusion pump was used to

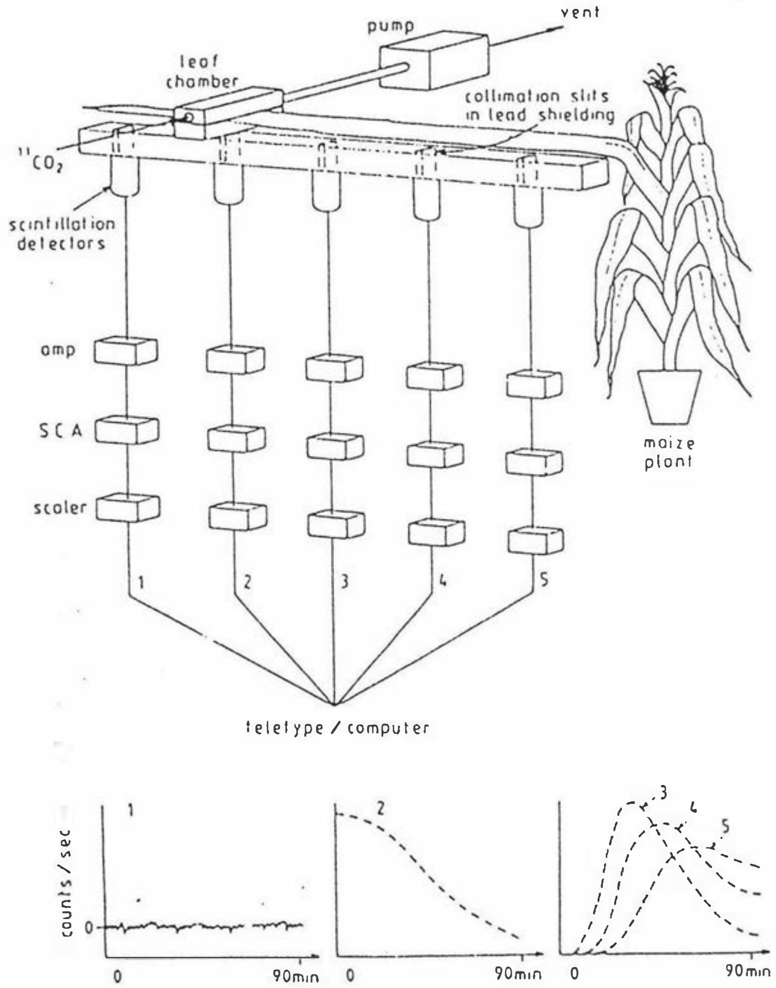

FIGURE 8.2 : Schematic diagram showing how a linear configuration of scintillation detectors is used to observe phloem translocation of ^{11}C-photosynthate along a maize leaf. Representative results are shown for each detector. AMP, amplifier; SCA, single channel analyzer (McCallum et al. 1981).

isolate the target area from the accelerator vacuum. The exhaust gases from this pump were drawn to a recovery area (10 metres away) where they were passed through a furnace containing CuO wire at 600 ^{o}C to oxidize the ^{11}CO. A small flow of dry nitrogen inserted at the high pressure side of the target pumping system and a small local pump was used to assist the flow of gases through the system. After passing through a dry ice-alcohol moisture trap, a liquid oxygen trap was used to selectively remove $^{11}CO_2$ from other gases which included N_2, CO, and O_2. The $^{11}CO_2$ was recovered from the trap when it was isolated from the system (More and Troughton, 1973; McCallum et al. 1981).

8.2.2.2 Data handling

A computer system which can provide on-line data acquisition and display facilities is essential for this technique. The equipment developed at Physics and Engineering Laboratories (DSIR) for the carbon-11 studies were used. This system, illustrated in Fig.8.2, consisted of up to six 50 mm diameter 50 mm NaI (Tl) detector-photomultiplier assemblies shielded by lead, each with a standard combination of preamplifier, amplifier and single channel analyser. The analysers were set to record the 511 Ke V γ-radiation arising from positron annihilation. Pulses from the analyser were fed to scalers and counted for equal time intervals. At the end of each counting period, the scaler outputs were recorded on a teletype and also transferred directly to the disc of a computer. In the course of an experiment, all data collected could be displayed in graphical form on a VDU located in the laboratory. The displays were updated as new data acquired.

CHAPTER 9

INFLUENCE OF SHADE ON PEDUNCLE ELONGATION

9.1 INTRODUCTION

White clover has horizontally placed leaf blades borne at the top of thin erect petioles. When matured, adjacent leaves are sufficiently close to one another to form a distinct canopy. Flower heads of white clover emerge from the stolon apices in the axils of the youngest leaf. The developing inflorescences continue to grow and are gradually raised above the foliage canopy by elongation of their peduncles. In the dense canopy, this post-emergence growth takes place for several days in shade before the inflorescences are raised above the foliage. The present investigation was undertaken to determine to which extent the growth of the peduncles is influenced by the shaded conditions that exist within the dense clover canopies.

9.2 METHOD

Peduncle elongation studies were performed in a laboratory using incandescent "flood lamps" (250 W,GEC, Canada) as a source of light. A flowing water filter system was placed above the plants to reduce heating, and a continuous flow of air over the plants maintained them at a reasonably constant temperature of 23±2°C. The light intensity at the level of the pot surfaces was 700 μmoles $m^{-2}sec^{-1}$ (PAR) (see plate 9.1). The plants were growing in the glasshouse before the treatments. The propagation and maintenance of plants were described in chapter 3.

Inflorescences selected for measurement were situated at 8 nodes from the stem apical meristem (at node 8: Thomas (1981)) and had peduncles about 15 mm long. A linear voltage displacement transducer (LVDT) was used to measure the peduncle elongation rates of inflorescences in shade and light. These were measured over a 24 hr period in a way similar to that described by Harding and Sheehy (1980), the LVDT being attached by means of cotton thread to the peduncle immediately below the flower head base. The small changes in output signals were recorded on a chart recorder (Fig.9.1). Mean lengths and elongation rates were then determined over hourly intervals for 4-5 replicates in each experimental treatment. Inflorescences were shaded by placing them in an opaque plastic tube (see Fig.9.2) which reduced the light intensity to 1-2% of incident light.

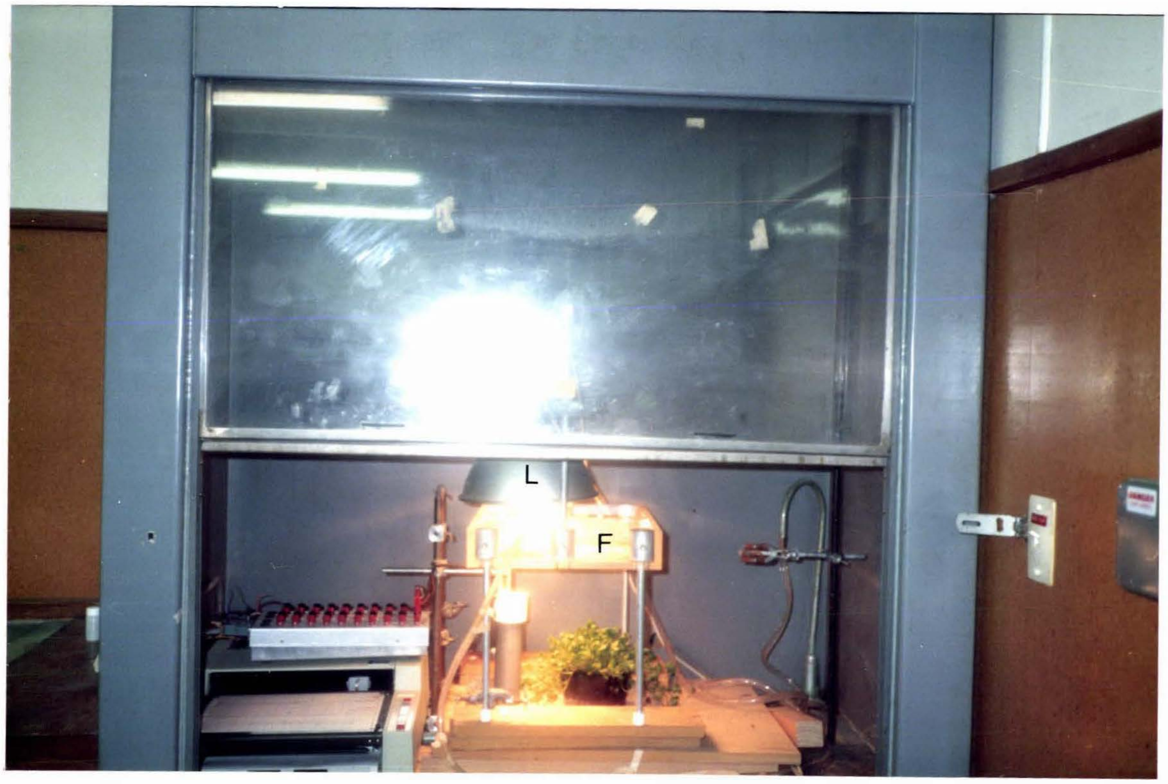

Plate 9.1 : *Physical set up of the apparatus used to measure the peduncle growth of an inflorescence in shade. The elongation studies were performed in a laboratory using incandescent "flood lamps" as a source of light (L). A flowing water filter system (F) was placed above the plant to reduce heating.*

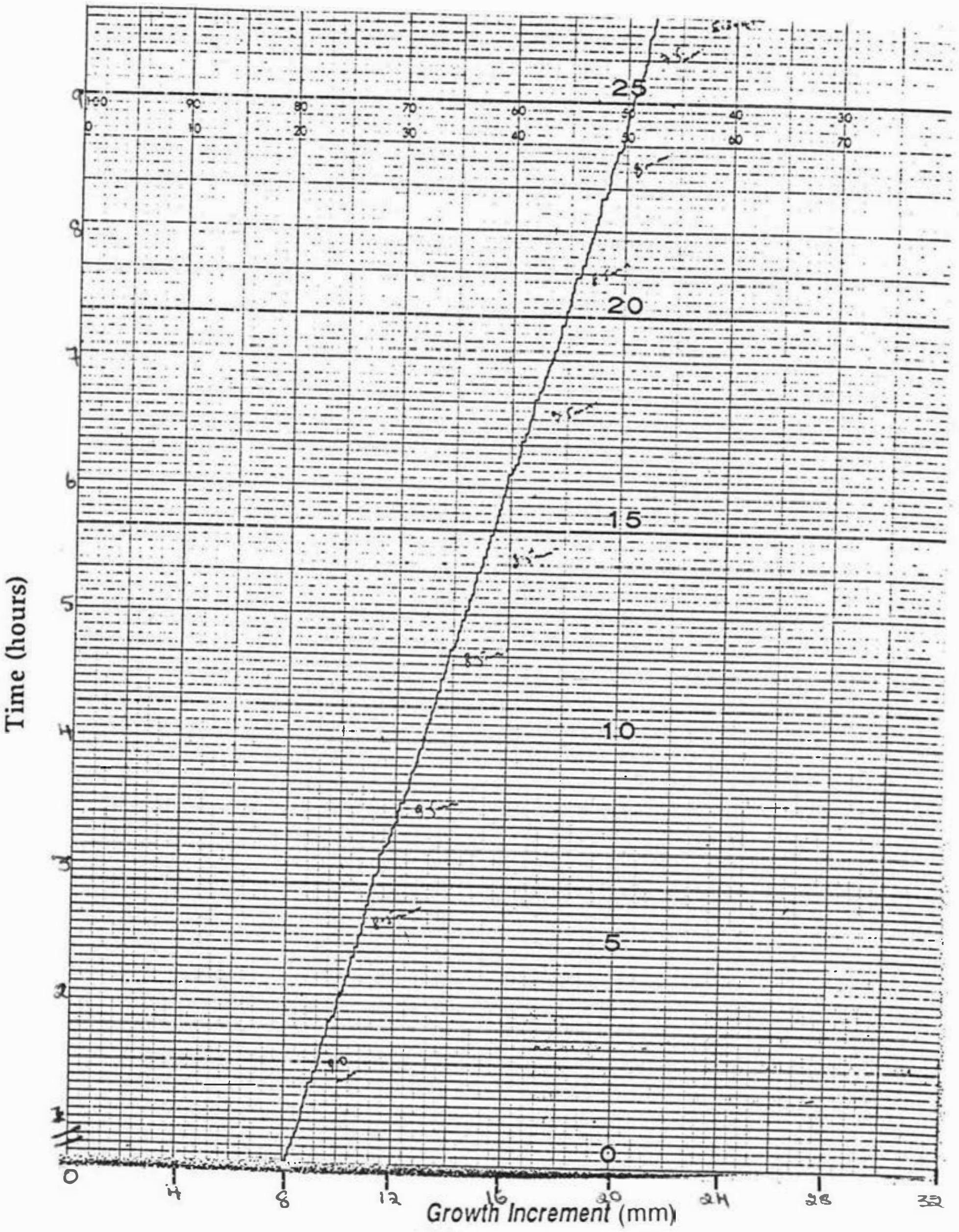

FIGURE 9.1 : Observations of peduncle growth over a 10-hour period. Two and half divisions on the chart recorder represent 1 mm growth. The speed of the chart reorder was 15mm per hour.

Four experimental treatments were applied.

In **A** the whole plant received full light;
in **B** the whole plant was in total darkness;
in the next two treatments, the whole plant except the inflorescence being studied was exposed to full light.
In **C** the inflorescence was shaded continuously;
and in **D** the inflorescence was shaded for 14 hours and then given 5 hours of full light after which shade was reimposed. A cold fibre optic light source was used to supply light to the inflorescences between the two shade treatments.

9.3 RESULTS

When the whole plant was in full light (Treatment A) the rate of peduncle elongation was approximately 0.53 mm/hr (Table 9.1) and when it was in darkness peduncle elongation was increased by about 29% as shown in Fig.9.3. By shading the inflorescence alone, leaving the rest of the plant in full light (Treatment C), peduncle elongation was increased markedly by 58%. Figure 9.3 shows the mean lengths and elongation rates of peduncles of an inflorescence in light (Treatment A) and shade (Treatment C).

Figures 9.4 a & b show the cumulative growth and the hourly increment in length of peduncles over a 24 hour period in Treatment D. At time "0", inflorescences were shaded for 14 hours before being illuminated for 5 hours and then returned to shade for 5 hours. When inflorescences were first shaded, the peduncle elongation became reasonably constant after a delay of 2 to 3 hours. The apparent immediate response to the change from shade to light and back again is misleading as results are from points representing hourly means. On removing the shade, the elongation rate did not begin to decrease until 25-30 minutes after the change. The rate did not return to the original higher level until 30-35 minutes after reimposing shade.

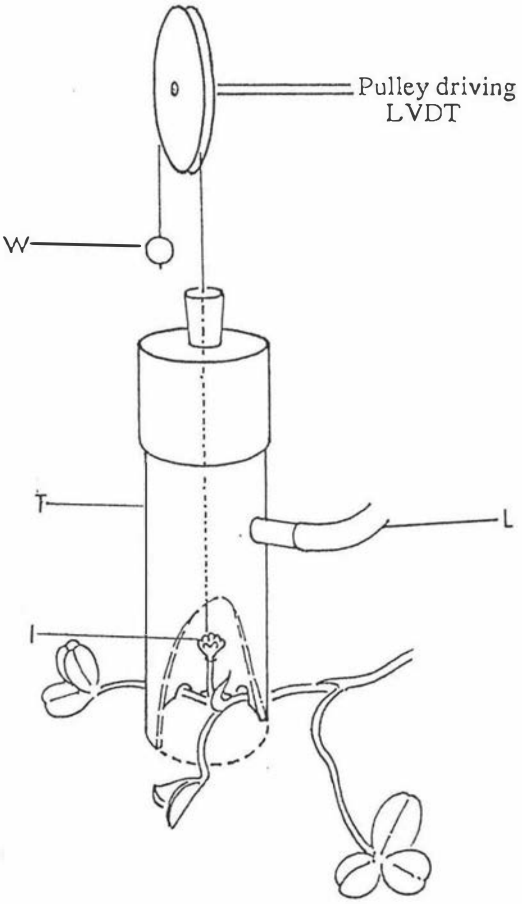

FIGURE 9.2 : Schematic diagram showing the system used for measuring peduncle growth rate of a clover plant. A thin cotton thread was attached to the peduncle and activated a linear voltage displacement transducer while a constant tension was maintained in the thread by a weight (W). A plastic tube (T) was used to shade the inflorescence (I). A fibre optic light source (L) was used to supply light between the two periods of shade in Treatment D.

TABLE 9.1: Effect of light level on peduncle elongation rate.

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*A plastic tube was used to shade the inflorescence alone, while the rest of the plant was illuminated. Shade 1 refers to shade treatment for 14 hours followed by 5 hours in light. Shade 2 is the subsequent shading after this light treatment. Values followed by the same letter in each pair of treatments are not significantly different at 5% level. * using paired t-test there was a significant difference between shade 1 and light, between shade 2 and light, but not between shade 1 and shade 2 (P=0.05). The values are the means over the each full period of dark or light.*

Treatment		*Growth Rate mm/hour (± SE)*	*No. of Replicates*
Whole plant			
A	*Light*	*0.53 ± 0.03a*	*5*
B	*Dark*	*0.75 ± 0.04b*	*5*
Inflorescence			
C	*Shade*	*1.25± 0.09b*	*5*
D	*Shade 1*	*1.57± 0.17*a*	*4*
	Light	*1.17± 0.13b*	*4*
	Shade 2	*1.69± 0.17a*	*4*

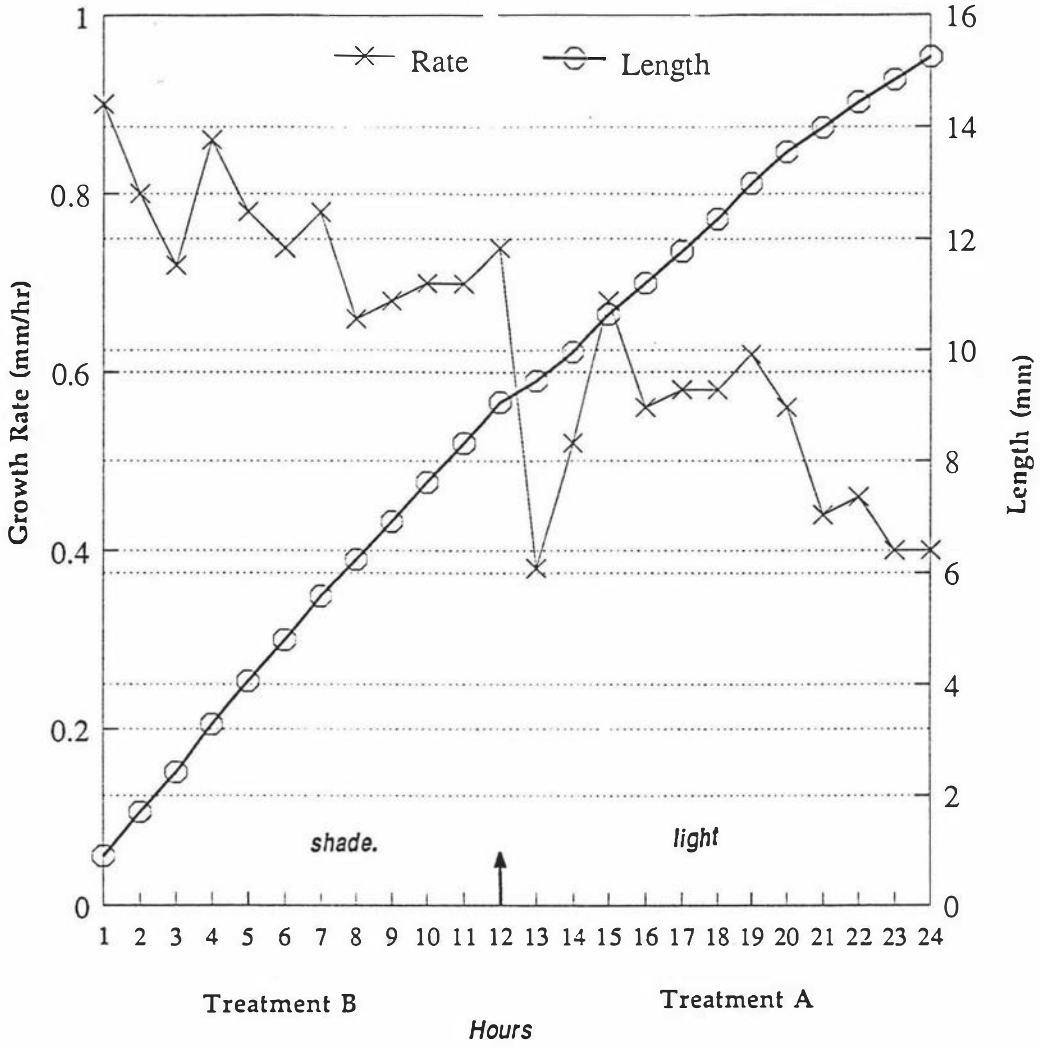


FIGURE 9.3 : Peduncle elongation of inflorescences when the whole plants were in full light and total darkness. a) Cumulative growth of peduncles over a 24 - hour period. At time 0 the whole plants were in total darkness for 12 hours, then they were illuminated for 12 hours. b) Incremental data derived from (a). Values shown are the mean lengths and elongation rates of 5 replicates at hourly intervals.

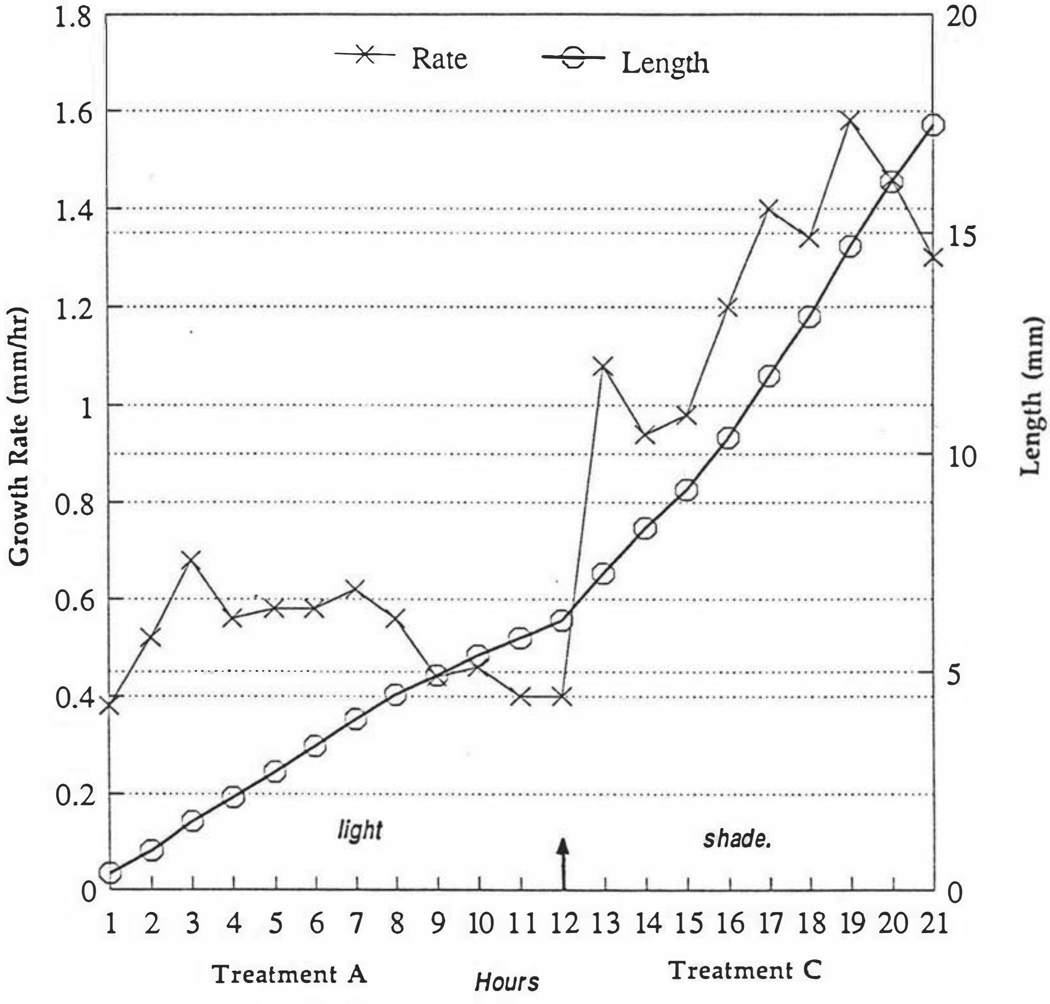


FIGURE 9.4 : Peduncle elongation of inflorescences in light and shade. a) Cumulative growth of peduncles over a 21 - hour period. At time 0 the whole plants were in full light for 12 hours, then inflorescences alone were shaded for 9 hours. b) Incremental data derived from (a). Values shown are the mean lengths and elongation rates of 5 replicates at hourly intervals.

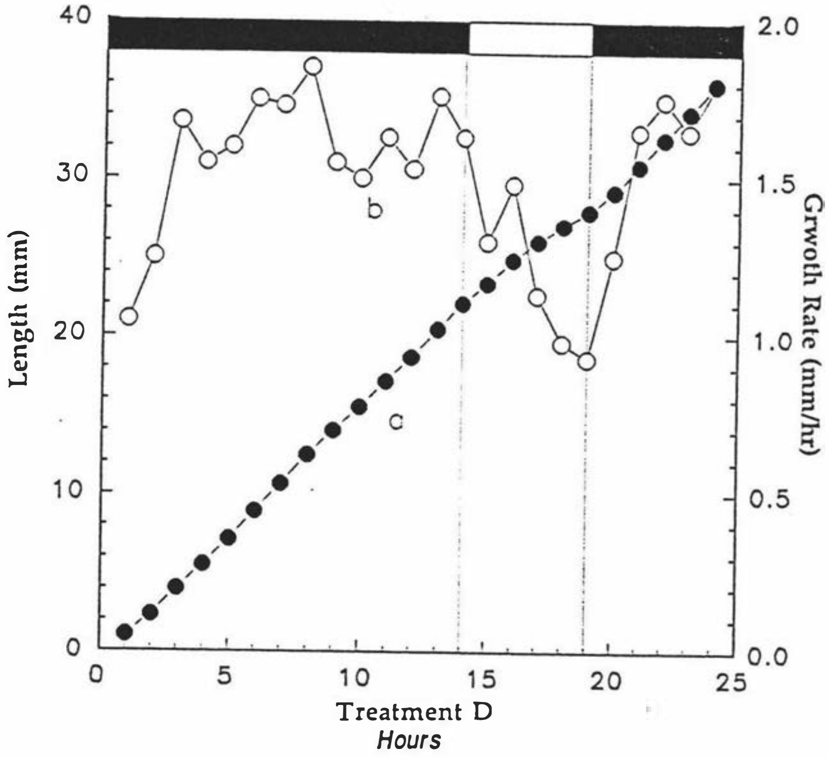


FIGURE 9.5 : Peduncle elongation of inflorescences in shade and light. a) Cumulative growth of peduncles over a 24 - hour period. At time 0 inflorescences were shaded for 14 hours, then illuminated for 5 hours, before being returned to 5 hours shade. b) Incremental data derived from (a). Values shown are the mean lengths and elongation rates of 4 replicates at hourly intervals.

9.4 DISCUSSION:

The level of light falling on a clover inflorescence has a strong effect on the peduncle elongation rate. Shading the inflorescence alone, while the rest of the plant in full light, peduncle elongation markedly increased. This is interpreted as representing the onset of a partial etiolation response. The most common morphogenic effects of seedling growth in complete darkness are the extreme elongation growth of the internodes and suppression of leaf development (Smith, 1974). In general, Smith (1974) had suggested that phytochrome was the photoreceptor for the stimulation of internode elongation in etiolated seedlings. Similarly phytochrome might be the photoreceptor responsible for the marked increase in peduncle elongation observed in the present investigation when individual inflorescences were shaded on plants which were otherwise fully illuminated. When the whole plant was in total darkness or when the inflorescence alone was shaded, leaving the rest of the plant in full light, peduncle elongation rate increased suggesting that light-dependent processes are occurring in the inflorescence rather in the stem or leaves. Peduncle elongation rate was higher when the plants but not the inflorescences were in full light compared to those plants (including inflorescences) in total darkness. This result suggests that supply of photoassimilates is limiting when the whole plant is in total darkness.

CHAPTER 10

INFLUENCE OF SHADE ON THE SINK ACTIVITY OF YOUNG FLOWER HEADS AND PEDUNCLES

10.1 INTRODUCTION

Inflorescences of white clover emerge from the stolon apex in the axil of the youngest leaf. The developing flower heads spend several days in canopy shade before the flower heads are raised above the foliage canopy. In other species (e.g soybean), however, there is evidence that the shading of organs reduces their ability to draw assimilates from the source leaves. It is thus possible that shading may also affect the sink activity of white clover inflorescences. The present investigation was undertaken to determine the extent to which the sink activity of young flower heads and peduncles is influenced by the shaded conditions that exist within dense clover canopies.

10.2 METHOD:

For each experimental treatment, a plant was arranged among lead with a single stolon extended out and the developing inflorescence held within a glass tube through which air was continuously drawn (Plate 10.1). This ensured that the inflorescence was exposed to the ambient light level. The inflorescence was shaded with heavy paper (similar to that used to store photographic film) which reduced the light to below 2% of ambient. This also facilitated the accurate placement of lead to collimate the 511 keV g-rays produced by carbon-11 such that the detector was sensitive to label within the flower head (Hd) alone. The air drawn over the inflorescence was passed through a caustic carbon dioxide trap placed against a radiation detector. Another detector was positioned to be sensitive to label within the entire inflorescence (In). The leaf subtending the inflorescence of interest was sealed into a perspex chamber through which air was continuously pumped. A third radiation detector was positioned to sense label within the whole plant (Pl) (Fig.10.1). Relative sensitivities of the radiation detectors were measured by successively placing a small piece of labelled leaf within the field of view of each detector at a position occupied by the experimental plant. In this way differences in sensitivities introduced by geometrical and detector differences were accounted for.

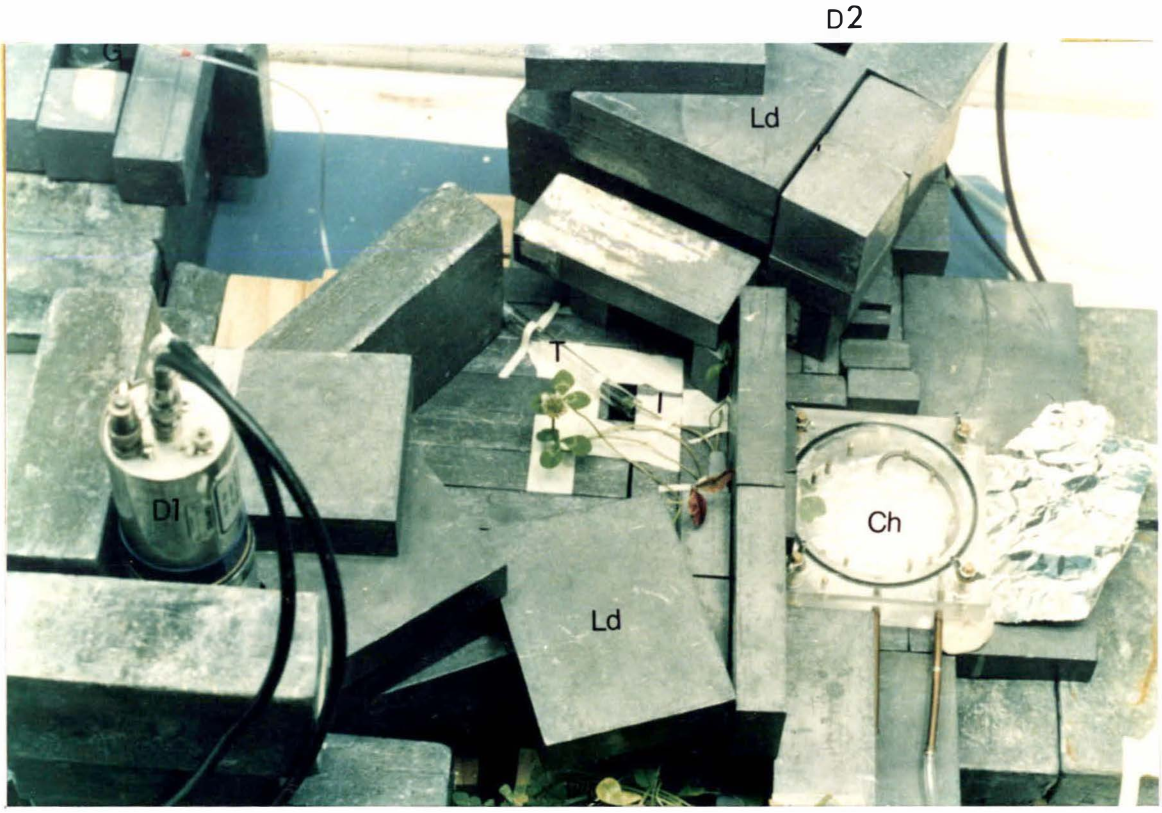


Plate 10.1 : *One stolon from the mother plant (Pl) visible at the bottom of the photograph was laid between blocks of lead and arranged in a central chamber. One leaf lamina from this region was enclosed within a leaf chamber (Ch) where labelling with $^{11}CO_2$ took place. A developing inflorescence (I) was held within a glass tube (T) through which air was continuously drawn by a pump (G). Three detectors sensed ^{11}C localization within the plant. One (D1) recorded total ^{11}C mobilised from the fed leaf; another (D2) is focused on head and peduncle of a young inflorescence (I); and a third detector (below the flower head and not visible in the photograph) was focused on the flower head only.*

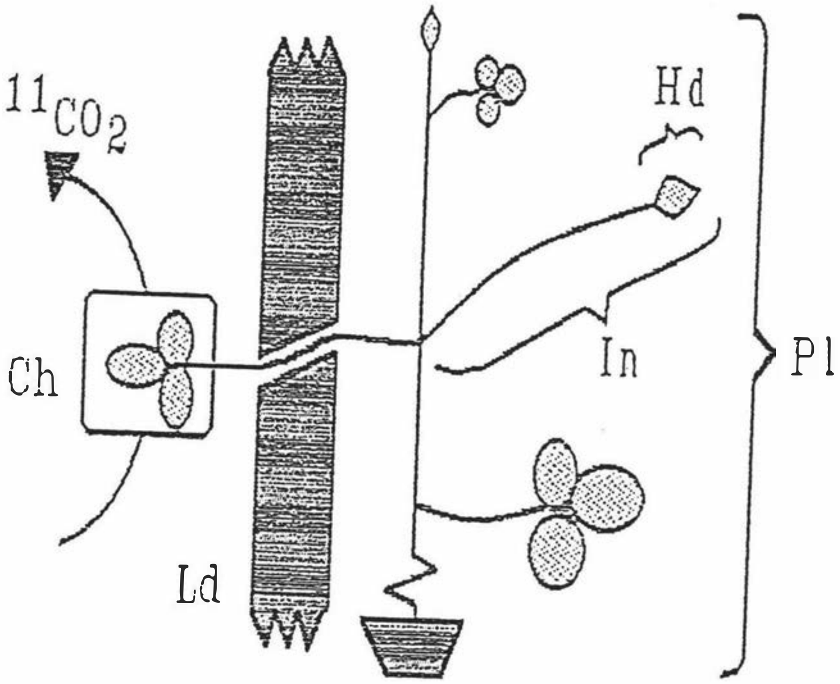


FIGURE 10.1 : Schematic diagram showing a clover plant with one load leaf within a leaf chamber (Ch) where labelling with $^{11}CO_2$ took place. The subtending inflorescence was placed to allow scintillation detectors to be sensitive to label within the entire inflorescence (In), developing flower head (Hd) and the entire plant (Pl) minus the load leaf. A barrier of lead (Ld) was used to shield these detectors from the strongly radioactive leaf chamber. Computer drawn by Dr. P.E.H. Minchin.

The leaf chamber was part of a closed loop system in which gas was circulated at 1500 cm^3 per minute. The dew point was maintained at 10^oC and the carbon dioxide level held at 320 ppm (±5%). The closed loop system used in this experiment was a modification of the closed loop-gas exchange system developed by Geiger and Swanson (1965). A gas-impermeable plastic bag was filled with a $^{11}CO_2/^{12}CO_2$ mix of known specific activity and was held in place under a depth of 15 cm of water (tank made of lead) to give a supply of labelled CO_2 at a constant pressure. The CO_2 concentration within the loop was monitored with an infra-red analyser (Hartman and Brawn AG, Frankfurt, model URA S2). Labelled $^{11}CO2$ was admitted to the circulating gas stream through a solenoid valve from the reservoir held at constant pressure. The duty cycle of the valve was computer controlled according to a requested rate of supply. Every 2 hours all of the label was released at a constant rate over a period of 10 minutes.

Each experiment was performed at least three times. In figures 10.2 and 10.3 the data presented show the response of a single plant in one experiment and is representative of all observations following a given treatment.

All profiles shown in Figs 10.2&10.3 have been corrected for differences in relative sensitivities between radiation detectors, and for dead-time and background. No correction for isotopic decay has been made in these. From the input-output profiles (for instance,Pl-input; In-output) the transfer characteristics of the segment of phloem transport system between these two detectors can be inferred. For a linear time invariant system the most general input-output relationship is:

$$X_K = -a_1 X_{K-1} + b_j U_{K-j}$$

where U_K and X_k are the input and output data; j = delay factor; a_1, b_j = system parameters. The value of j depends on the time delay between input and output, and therefore on the distance from source to sink (assuming a constant rate of flow). Phloem translocation can therefore be described by two parameters, a_1 & b_j, which completely describe the observed dynamics of the tracer movement. A particular model is specified by the number of input and output terms and the value of the model parameters. The process of determining a specific model for given input-output data involves two stages, the first stage to identify the specific model structure (model identification) and the second stage to obtain the best estimates of the model parameters. Model identification and parameter estimation have been carried out using the recursive instrumental variable method of Young(1984).

The instrumental variable method of structural identification is based on a number of test statistics calculated for a small number of different but plausible model structures. For the present investigation, the error variance norm (EVN), or more conveniently the logarithm of this (LEVN), is the only statistical calculation needed. The EVN is defined as the average value of the estimated parameter variance. The procedure for model structure identification is to find the structure which gives a minimum EVN (Young, 1984).

Initial identification of this model assumes that the parameters are constant over the time span of the data set. If the translocation properties are non-linear or varying with time, then the best model parameters (a_1 & b_j) vary with time. This is tested by a simple extension of the recursive parameter estimation algorithm (Norton, 1986). This algorithm in its basic form gives an equal weight to every data point, so at a given time the algorithm in effect has a perfect memory for all the preceding data. By giving the algorithm a decaying memory for earlier data, it is then able to follow the most appropriate current parameter values based on the most recent data (Young, 1984). If the partitioning of photo-assimilate is changing with time, then the model parameters (a_1 & b_j) will also vary, from which variation, the partitioning co-efficient can be followed. The partitioning co-efficient (fraction of tracer entering the sink of interest) is given by:

$$P = b_j / (1-a_1).$$

The analysis method used has been fully described elsewhere (Minchin and Grusak, 1988; Minchin and Thorpe, 1989). Detailed quantitative analysis of these profiles were carried out to obtain the fraction of labelled photosynthates moving between the different regions of the plant and this incorporated corrections for isotope decay. All calculations were performed using the MICROCAPTAIN computer package (Young, 1984).

The partitioning of photoassimilates from the whole plant to the inflorescence is defined as the fraction of mobilised photoassimilates exported from the labelled leaf into the inflorescence. Similarly, inflorescence to flower head partitioning is defined as the fraction of photoassimilate imported into the inflorescence which eventually arrives within the flower head. Carbon-11 is a short-lived isotope, with a half-life of

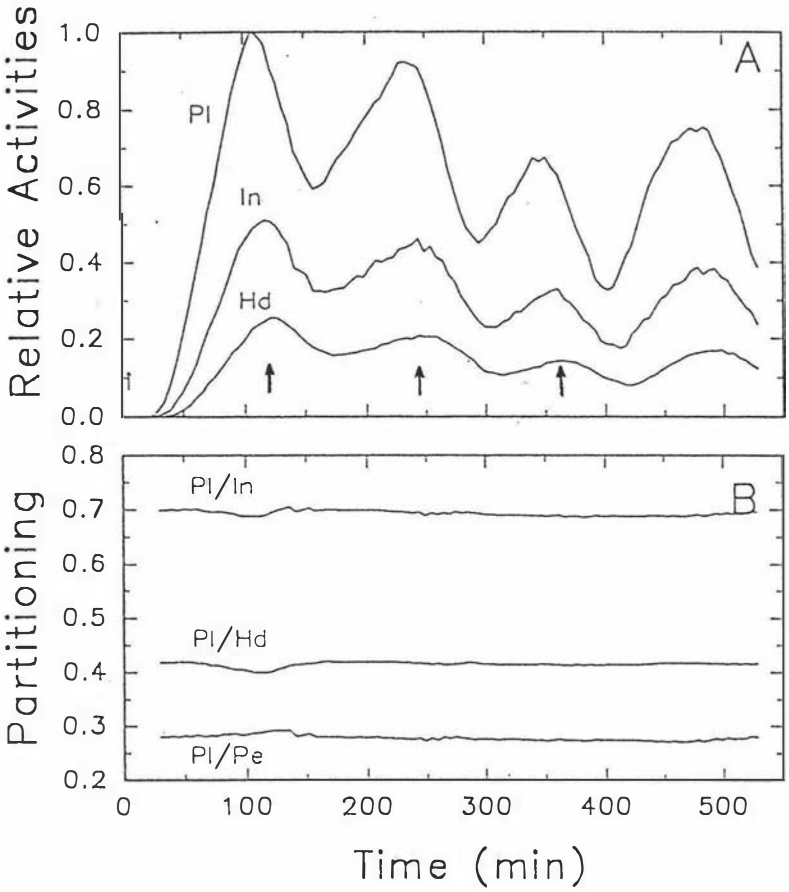


FIGURE 10.2 : Tracer profiles, and derived partitioning coefficients, seen in a clover plant under constant light. Load leaves received a 10-min. pulse of $^{11}CO_2$ at the times indicated by arrows (o, 120, 240 and 360 minutes). a) Tracer profiles observed within the plant (Pl), whole inflorescence (In) and developing flower head (Hd), corrected for background, and differences in detector sensitivities. b) Calculated partitioning coefficients between plant and inflorescence (Pl/In), between plant and flower head (Pl/Hd), and between plant and peduncle (Pl/Pe).

20.4 min, so even though our observations may last up to 500 minutes, the movement of labelled photoassimilate can only give us information on the partitioning of recently fixed photosynthates, that is within a time span short enough so that there is enough isotope remaining to be seen as it moves through the plant.

10.3 RESULTS

Results from a typical control experiment are presented in Fig.10.2A. This shows the tracer activity of mobilised assimilates within the plant (Pl), within the inflorescence consisting of peduncle and flower head (In) and within the flower head alone (Hd). Peaks of activity appeared in the plant (Pl) about 110 mins after the load leaf had received each pulse of $^{11}CO_2$. Slight displacement to the right of the peaks in In and Hd indicates that there was a delay of about 10 min in tracer arriving in the inflorescence from the rest of the plant (Pl), and a further delay of about 10 min between reaching the inflorescence peduncle and reaching its head.

Though the same amount of tracer was supplied to the leaf at each relabelling, the differing heights of the peaks in profile Pl suggest that the export of recently fixed photoassimilates varied during the day. Similar differences are also apparent in the amounts of tracer arriving within the total inflorescence and the flower head alone.

Using the input-output analysis developed for carbon-11 profiles (Minchin and Grusak,1988), the instantaneous fraction of total mobilised assimilates moving into various sinks was calculated. The partitioning coefficient is defined as the fraction of available assimilate from a given source which eventually finds its way into the sink of interest. The fraction of the total mobilised assimilates (recently fixed) within the plant (Pl) which was eventually transported into the inflorescence (In), which is labelled Pl/In in Fig.10.2B, is the partitioning coefficient for recently fixed photoassimilates into the inflorescence. Similarly, the fraction of mobilised label which was eventually transported from the plant into the flower head (Pl/Hd) is the partitioning coefficient for recently fixed photoassimilates into the flower head. Using the tracer profiles Pl, In and Hd, the fraction of total mobilised label which was eventually transported into the peduncle but not subsequently into the head was calculated. This is labelled as Pl/Pe in Fig.10.2B. Figure 10.2B clearly shows that the proportion of assimilates partitioned to the inflorescence (Pl/In) and the partitioning of assimilates within the inflorescence remained constant during the experimental period.

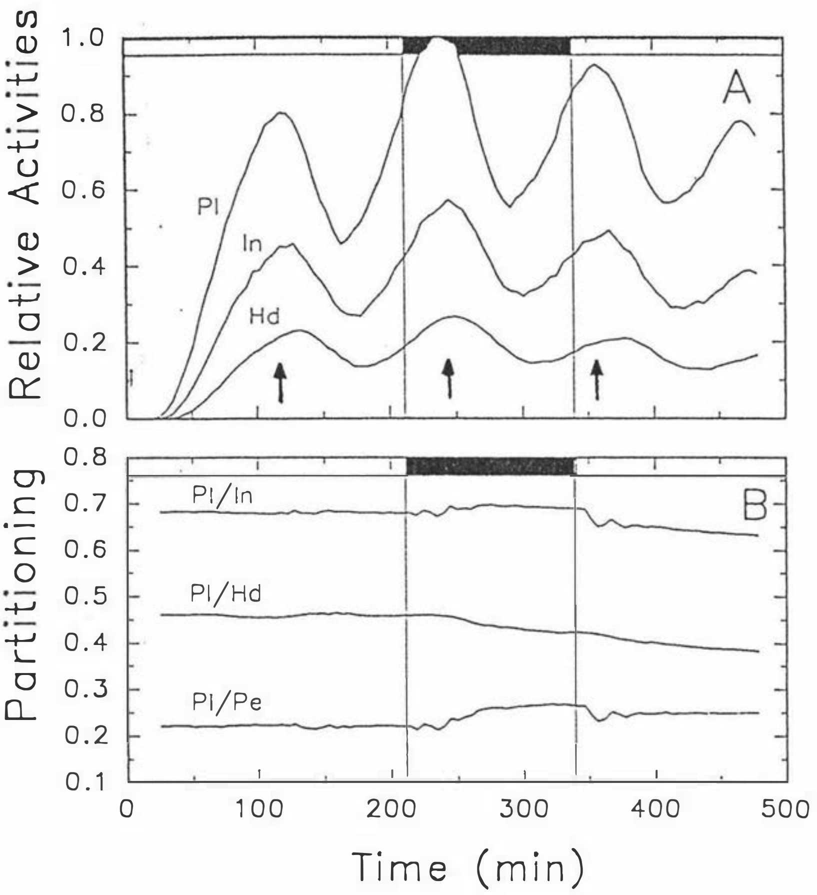


FIGURE 10.3 : Tracer profiles, and derived partitioning coefficients, seen in a clover plant with shade applied to the inflorescence alone at 210 minutes and removed at 340 minutes. See caption of Figure 10.2 for further details.

Figure 10.3 shows similar data, obtained in this case when the inflorescence was shaded for two hours at the time indicated. Figure 10.3B shows that partitioning of photoassimilates from the plant to the inflorescence (Pl/In), remained constant until shade was applied, and increased slightly (2%) after a delay of 30 min. Partitioning from the plant to the head showed a marked decline (8%) on shading, with a delay of 30 min, while that from plant to peduncle showed a large increase (20%) with a delay of about 25 min. Removing the shade resulted in Pl/In falling below its preshade value, while Pl/Hd and Pl/Pe showed a reduction in their rate of change but did not settle to constant values.

10.4 DISCUSSION

The level of light falling on a clover inflorescence clearly has a major effect on the partitioning of photoassimilates within the inflorescence. Shading the inflorescence induces a small increase in partitioning to the inflorescence, but the major response is a marked increase in partitioning to the peduncle, at the expense of the developing flower head.

This finding explains the observation by Thomas (1987) that in certain cases in which developing inflorescences receive insufficient light, the flower heads abort first and the peduncles often continue to grow. Increased partitioning to the peduncle is concomitant with an increase in its elongation rate as seen in chapter 9, this being interpreted as representing the onset of a partial etiolation response. Both measured responses (peduncle elongation rate and partitioning of photosynthates) changed about 20-25 minutes after the change in light intensity was imposed. Whatever perception system is involved, events initiated during this lag period will be of great interest in further studies.

Results in chapter 9 & 10 of the present investigation do not allow a distinction to be made between cause and effect. It is not clear whether the observed increase in peduncle growth rate as observed in chapter 9 causes the observed increase in assimilate partitioning to it or whether it is a result of the increased assimilate partitioning. The effect of light on inflorescence growth could act in two ways: by affecting growth rate, or by affecting sink activity. In the first case light might inhibit the rate of elongation of peduncles, thereby make them weaker sinks or promote the growth of flower heads and so lead them to increase their sink activity. In the second case, light might somehow reduce the sink activity in peduncles and thereby inhibit their growth or enhance the sink activity of flower heads and in turn stimulate their growth as a consequence.

Results reported here may have implications for applied studies for clover management. For flower heads developing under a dense canopy a shortage of photosynthates may result both from a change in partitioning of photoassimilates due to shading (as observed in the present investigation) and, more directly, from a reduction in ***in situ*** photosynthesis (Pasumarty, 1987). Reduced flower head development caused by canopy shade would reduce final seed yield. Obviously, for best seed production, an optimal management strategy would be to grow the crop as spaced plants with an open canopy rather than a denser sward with a closed canopy.

CHAPTER 11

GENERAL DISCUSSION AND CONCLUSIONS

White clover (***Trifolium repens*** L.) is known for its fluctuation in yield of seed. Seed yield is built up from several components which in turn are determined by a combination of plant and environmental factors. The seed production capacity of white clover represents the cumulative expression of four principal components: number of flower heads per unit area, number of florets per head, number of seeds per floret and seed weight. These components all differ in their relative contribution to total seed yield and change with genetic variability within the species. Because it is known that low light intensity leads to total abortion of developing white clover flower heads and that the number of seeds per floret in a "good" (warm,dry,sunny) summer is often up to 50% higher than in a "bad" (cool,rainy,dull) summer, the present study was undertaken to determine the influence of light intensity on flower head development and seed yield components in this species.

Summary of results

From the growth room study (chapter 4), it was clear that the lower light intensities led to floret abortion and thereby reduced the number of florets per flower head which reached anthesis. They also significantly reduced the percentage of fertile ovules and the percentage of ovules which set seeds. The positive correlation between the two strongly suggested that the direct cause of low seed set in this case was ovule sterility.

In this growth room study, however, the light intensities in which plants were grown were well below those normally experienced during the brightest part of the day by plants growing in field conditions. White clover has leaf blades which tend to be horizontally oriented and are borne at the tops of thin erect petioles. At plant maturity, adjacent leaves are sufficiently close to one another to form a closed canopy. Once the canopy closes in a developing sward, the young leaves and inflorescences are subjected to low irradiance of different quality during their development when compared to older leaves and inflorescences (Brougham, 1958). Light intensities beneath the foliage canopy of a white clover seed crop are often as low as one percent of incoming radiation even at midday when light intensity is highest (Appendix 5). Flower heads of white clover emerge from the stolon apices in the axils of the youngest unfolded leaves. At this stage all their florets are initiated, the oldest being about one quarter of their final size and the youngest much smaller; and they are borne on very short peduncles. Over the next few days the flower heads continue to grow and are gradually raised above the stolon by elongation of their peduncles. In a dense canopy this post-emergence growth takes place for several days in heavy shade before the flower heads are raised above the foliage.

Shading the young flower heads alone for six days either by neutral shade or light simulating that passing through a leaf canopy (chapter 5) increased ovule sterility. The degree of ovule sterility was greatest when shade was applied to the inflorescences at node 8 on a stolon. Thomas (1987) reported that when the inflorescence moves from node 8 to node 9 rapid development of ovules takes place. The cause of ovule sterility was not studied. It is possible that shade treatments interfered with meiosis in a way similar to that reported by Carapetian and Rupert (1989) for safflower as discussed in Chapter 5.

The flower heads developed in shade produced 28-30% fewer seeds per head than those formed in full light. This reduction was brought about by an increase in the number of florets aborting, as well as by a decrease in the percentage of ovules setting seeds. The close correlation between the percentage of apparently fertile ovules and the percentage of ovules setting seeds strongly suggests that this reduction was largely brought about by an increase in ovule sterility.

Realizing the importance of light levels falling on developing inflorescences for seed production, field experiments were therefore undertaken to determine to what extent and under what growing conditions flower head development and seed yield are influenced by canopy density and overcast weather conditions. The results obtained in the field study (chapter 6) clearly show that the levels of apparently fertile pollen formed in flower heads developed at both dense and open canopies were very high. These results suggest that pollen fertility is an unlikely cause for low seed set in white clover under favourable conditions for pollination.

Low light intensity, brought about by either high canopy density or artificial shading (chapter 6&7) increased ovule sterility. Flower heads which developed in a dense canopy produced 37 to 39% fewer seeds per head than those formed in an open canopy. The close correlation between the percentage of apparently fertile ovules and

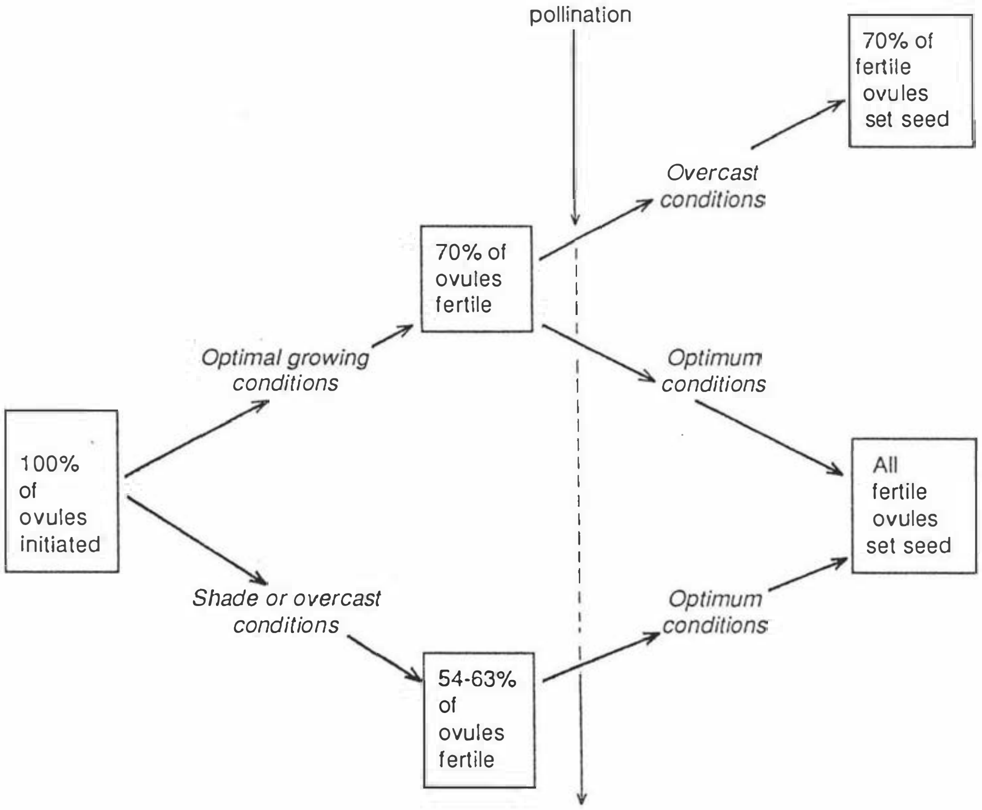


FIGURE 11.1 : Relationships between growing conditions, ovule fertility and post-fertilization abortion.

the percentage of ovules setting seed strongly suggests that this reduction was largely brought about by an increase in ovule sterility. The reduction in seed number was also contributed to by an increase in the number of florets aborting in the dense canopy. In the present study, artificially shaded plants (to simulate overcast weather) before or after pollination produced between 23 to 31% fewer seeds per head than in plants growing with an unshaded canopy, suggesting that overcast weather conditions during early stages of inflorescence development or during the seed maturation period may result in about 30% reduction in seed number per head.

The observed results in the present investigation into white clover are summarized in a diagrammatic form in Fig.11.1.

Compensation

The reduction of seed yield per inflorescence with higher canopy density in plants of "Grasslands Huia" and "Grasslands Pitau" was very similar (39% and 37% respectively). In the former case, some of this reduction was brought about by an increase in the number of florets (13%) aborting but much of it was caused by a higher proportion of ovules being sterile or less seed set (30%). In the latter, it was brought about by a combination of both an increase in floret abortion and a decrease in the percentage of ovules setting seed (23% and 18% respectively). Similar trends were observed when plants were shaded before or after pollination. In "Grasslands Pitau" the increase in floret abortion on an inflorescence was compensated by a decrease in seed abortion per floret. Van Bockstaele and Rijckaert (1988) observed that high seed yields in white clover were achieved by individual cultivars *via* different strategies: cultivar "Lipera" combined high inflorescence number with a small inflorescence size (i.e low number of florets per inflorescence) and cultivar "Lune de Mai" bigger inflorescences were compensated by lower number of heads.

Despite the wide range of factors which can affect different components of crop yield, there is a tendency, within limits, for plants to compensate for losses at one stage of reproductive growth by augmenting other stages. A concept widely accepted by plant breeders was proposed by Grafius (1964), explaining the yield components of barley as a box of XYZ cubic volume. The number of heads per unit area (X), the number of kernels per head (Y), and average kernel weight (Z), multiplied together equals W:- yield. This is a biological concept expressed in geometric form. There is no way in which yield can be changed without changing one or more of the components. On the other hand, changes in X, Y or Z may tend to counterbalance each other giving, in effect, homeostasis for yield. Hence, all changes in the components need not be manifested as change in yield, but all changes in yield must be accompanied by changes in one or more of the components.

Loahasiriwong (1982) reported yield component compensation in the soybean cultivar "Maple Arrow" grown in a controlled climate room. When plants were subjected to water stress at different times, water stress from first flowering through to the beginning of maturity severely reduced yield by reducing the number of pods per plant and, less strongly, the seed weight. If water stress was applied for only part of this period, yields were increased (compared with those under severe stress) either by increased seed weight when pods and seed number decreased due to early water stress, or by increased pod numbers compensating for decreased seed number per pod and seed weight due to later stages of water stress.

Variation in upper and lower florets

There was a tendency for the apical florets on white clover inflorescences to possess fewer fertile ovules and matured seeds than those developing lower down (basal florets). Van Steveninck (1957) similarly showed that in ***Lupinus luteus***, flowers in the basal whorls of inflorescence are more likely to produce more seeds per floret than those more distal. He suggested that these patterns may occur, in part, because flowers in the distal whorls have vascular supplies which are not as well developed at the time of fertilization as those in basal flowers, but many distal flowers will mature into fruits if the basal whorls are removed. Atwood (1940), for instance in white clover, found that the seed number per floret was significantly higher when all but ten florets were removed from flower heads than it was in intact heads. Van Steveninck (1957) in lupins also found that the increase in the number of distal flowers which mature into fruits by removal of basal flowers depends on the pattern of flower removal. If all of the flowers within a vertical column are removed, fewer of the remaining flowers will mature fruits than if an equal number of flowers were removed from whole whorls or in a spiral pattern. Thus, developing fruits seem to affect the flowers above them more than they affect those occupying the same whorl. These fruits may be competing among themselves for resources. Erith (1924) described the anatomy of the peduncle and inflorescence axis of white clover. She reported that peduncles differ considerably in thickness, the number of lobes and the depth of the furrows. The number of lobes varies in different peduncles from five to ten, the greater number being found in the larger peduncles and each vascular bundle being situated near the base of the lobes. She also mentioned that fine vascular branches pass out from inflorescence axes into the pedicels and bracts of the flowers, but she did not describe the anatomy of an individual pedicel or mention any differences in vascular bundle size depending on the size of the pedicel. In the writer's opinion it could be possible that there might be some variation in size of vascular bundles present in pedicels of upper and lower florets depending on the size of the pedicel and that this might affect availability of photosynthates. There is far from complete agreement on the importance of vascular architecture in determining the pattern of assimilate movement (Gifford and Evans, 1981). In general, Watson and Casper (1984) have suggested that the competition may be most intense among flowers lying in common vertical columns, presumably due to constraints on assimilate movement that are imposed by vascular architecture. Furthermore, predominant pathways of translocation can be altered by stress or by changes in the relative strength of sinks. The phenomenon has been particularly well studied in soybean (Egli et al., 1976) where there are rapid changes in translocation patterns following severe manipulation of source-sink relationships. In clover, the basal florets on an inflorescence are fertilized before upper ones are open. The lower ovule fertility and in turn the low seed set per carpel in upper florets may be due to delayed development and consequently reduced ability to compete with more developed seeds on lower florets for available photosynthates.

Photoassimilate partitioning

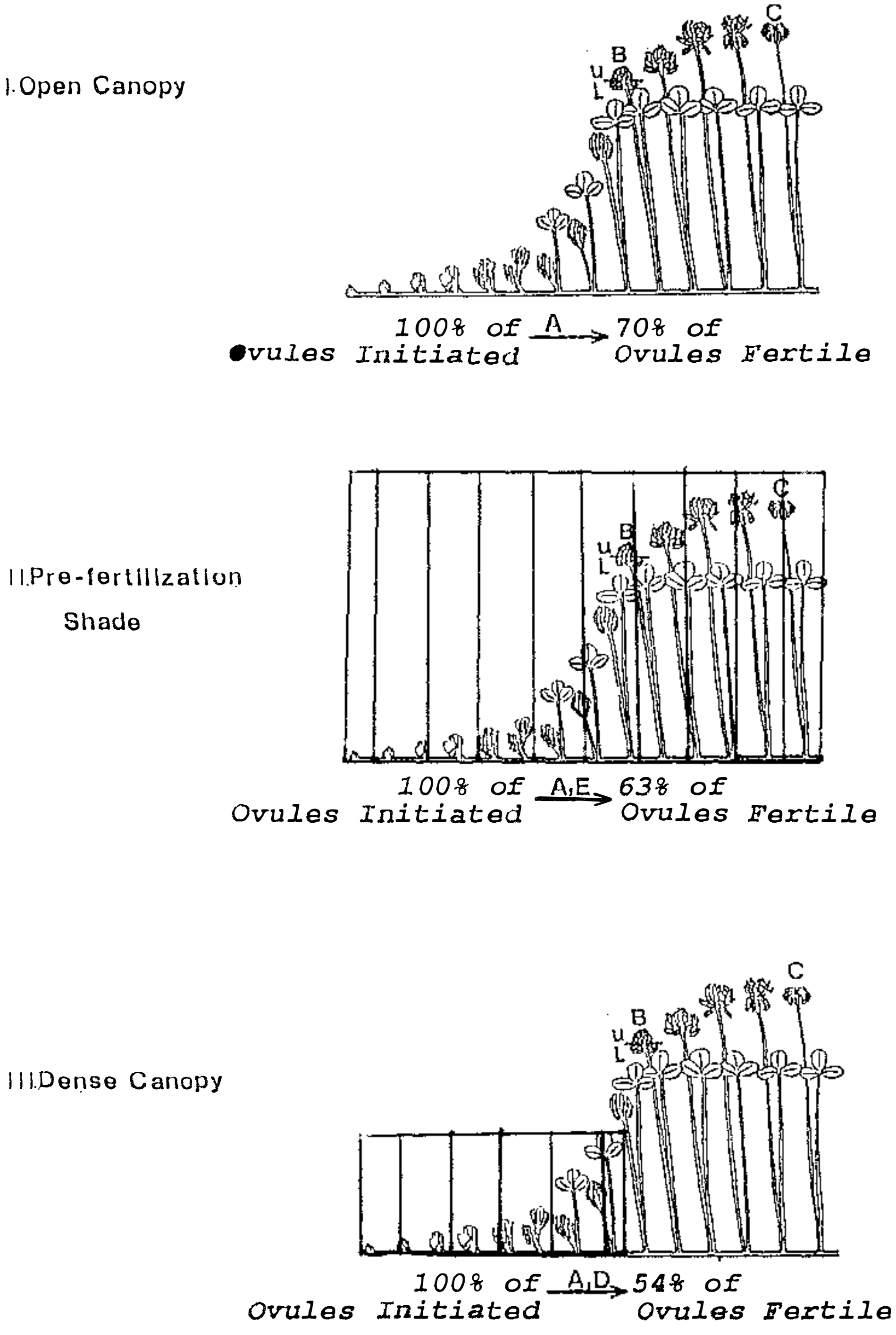
The present results clearly show that there is a direct effect of light intensity on flower head development. Peduncle growth studies (Chapter 9) showed that when individual inflorescences were shaded on plants which were otherwise fully illuminated, there was marked increase in peduncle elongation rate. This is interpreted as representing the onset of a partial etiolation response. In general, Smith (1974) suggested that phytochrome was the photoreceptor for the stimulation of internode elongation in etiolated seedlings. It is possible that phytochrome might be involved in increased peduncle elongation observed in the present investigation when individual inflorescences were shaded.

The ^{11}C translocation studies (Chapter 10) showed the effect of light intensity on sink activity of young flower heads and peduncles. The level of light falling on an inflorescence clearly had a major effect on the partitioning of photoassimilates within it. Shading inflorescences induced small overall increases in partitioning to them, but the major response was a marked increase in partitioning to the peduncles, at the expense of the developing flower heads.

Row orientation effects on seed yield in field grown bush beans and soybean (Kaul and Kasperbauer, 1988; Kasperbauer, 1987) have been demonstrated to be due to subtle differences in the ratio of red to far red light (R:FR) which acts *via* the phytochrome system to regulate partitioning of photosynthates within these plants. The low R:FR ratio within the vicinity of developing inflorescences in a dense white clover canopy (Scott et al. 1968; Solangaarachchi and Harper, 1987) might have a similar effect. Observation of the response of partitioning in the clover system to shade treatment in the present investigation suggests that the phytochrome system within the inflorescence might be regulating the partitioning of photoassimilates within the inflorescence in a similar way.

Causes of ovule sterility

The most striking observation made in this investigation was that even in good growing conditions, an average of only 70% ovules formed in a flower head are fertile and capable of setting seed. The failure of ovular development in general is attributable to several causes. Bawa et al. (1989) have attributed random ovule abortion primarily to genetic load and developmental selection. Lloyd (1980) has speculated that abortion of seeds may allow maternal parents to selectively abort genetically inferior progeny, as the maternal investment is adjusted to match available resources. In field trials of white clover in Poland, Cebrat et al. (1982) observed that an average of 63.5% fewer ovules set seed in higher density machine-sown plots than at 1x1 m spacing. In plants established as single individuals with 1x1 m spacing, only 52% of ovules developed into matured seeds. The 48% of unproductive ovules was attributed to the degeneration of unpollinated ovules (0.8%), degeneration of ovules in spite of sufficient pollination (29.7%), abortion of developing seeds (6.7%) and damage inflicted by insects (11.1%). The 29.7% "degeneration of ovules in spite of sufficient pollination" agrees with the present observation of 29% ovule sterility. The results obtained in the present investigation (chapter 6) show that there was a very



I. Open Canopy

II. Pre-fertilization Shade

III. Dense Canopy

FIGURE 11.2 : Resource limitation model.

A - Vascular supply.
B - Variation in upper and lower florets.
C - Post fertilization abortion.
D - Direct effect of light - Change in photoassimilate allocation.
E - Indirect effect of light - less photosynthesis.
▥ Shade

small proportion (6-9%) of seeds lost beyond 10 days after pollination. These results are similar to those obtained by Cebrat et al.,(1982) and Robbie (1988).

Resource limitation model (RLM)

In the writer's opinion, the sterility of 30% of the ovules formed indicates limitations in the amount of resources available for the presumably costly process of ovular development. The plant seems to initiate the maximum number of ovules per carpel and then arrest the development of certain ovules randomly. It is probable that the development of the vascular supply to a floret takes place after the initiation of ovules. There might thus be an advantage in initiating an excessive number of ovules per carpel and only arresting their development in relation to the degree of development of the vascular supply (Fig.11.2; Factor A). The increased number of sterile ovules resulting from pre-fertilization shade treatment in the present study supports the resource limitation hypothesis (Fig.11.2; II). Competition for resources within and among carpels of the same inflorescence may be responsible for the increased number of sterile ovules (Fig.11.2; Factor E). The reduction in percentage of fertile ovules in an inflorescence which developed in a dense canopy could also be explained in terms of resource limitation (Fig.11.2; III). The present results showed that light had a direct effect on inflorescence growth and that it also changed the allocation of photoassimilates within the inflorescence. A deprivation of the supply of available resources to the developing ovules would result from competition between the head and its peduncle as the peduncle elongates in response to foliage canopy shade (Fig.11.2; Factor E).

Variation in number of fertile ovules per carpel within an inflorescence may also result from competition for limited resources on a local basis within the inflorescence (Fig. 11.2; Factor B). This could be due to a) flowers have vascular tissue that is not fully developed b) competition among florets presumably due to constraints on assimilate movement that are imposed by vascular architecture c) reduced ability of some florets to compete with more developed florets for available photosynthate i.e there is a competitive hierarchy among florets based on their sequence of development and also their sink strength.

The import of assimilates into an organ may be regulated by the level of endogenous hormones in it. In tomato (***Lycopersicon***) plants growing under adverse light

Kinet (1982) found that the endogenous cytokinin levels in inflorescences were 11-fold lower than in control plants receiving full light whereas the endogenous GA levels were 9-fold higher. A reduction of import was paralleled by the reduction of endogenous cytokinin. Furthermore, the inhibition of flowering in tomato which occurs in adverse light conditions can be reversed by cytokinin application. In this case, flowering is attained by a higher import of dry matter into the inflorescence at the expense of import into the young leaves above the first truss (Kinet et al. 1978). Though it appears that there may be a link between the supply of assimilates and the level of endogenous hormones, it is far from clear what the factors determining the level of endogenous hormones are, and how the hormones facilitate the import of assimilate.

For flower heads developing under dense canopies a shortage of photosynthates may result both from a change in partitioning of photoassimilates due to shading (as observed in the present investigation) and, more directly, from a reduction in ***in situ*** photosynthesis (Pasumarty, 1987). The observed reduction in seed number per inflorescence caused by canopy shade might thus be the result of shortage of photosynthates. Obviously for best seed production an optimal management strategy would be to grow the crop as spaced plants with an open canopy rather than a denser sward with a closed canopy.

Potential areas of future research:

This investigation clearly indicates the need for and the direction of, further study to assist plant breeders.

1) Results from the present study showed that an average of 30% ovules formed in a flower head are sterile and low light intensity brought about by either high canopy density or artificial shading increased ovule sterility. Detailed studies of the developmental stages of the ovules and female gametophyte in fertile and sterile ovules are necessary to find the first significant deviation in gametophyte development in fertile and sterile ovules. Future study might also concentrate on the mode of action of light on ovule fertility.

2) Results from the present study showed that the apical florets on an inflorescence possess fewer fertile ovules than those developing lower down. In the writer's opinion this could be due to variation in size of vascular bundles present in pedicels of upper

this could be due to variation in size of vascular bundles present in pedicels of upper and lower florets depending on the size of the pedicel. Potential area of research would be to study the anatomy of the pedicels of upper and lower florets.

3) A fruitful area of investigation would be to determine the involvement of the phytochrome system within the inflorescence in the regulation of photoassimilates partitioning within it.

4) Screening for cultivars having a high proportion of fertile ovules per head to increase the seed yield per head.

This additional information will provide a firmer basis for developing improved management practices and also for improving seed yield genetically.

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Appendices.

APPENDIX 1: A STAIN-CLEARING TECHNIQUE FOR OBSERVATIONS WITHIN WHOLE OVULES.

The cytological technique used to observe the cytoplasmic state of embryo sacs was a modification of a staining-clearing technique described by Stelly et al. (1985). To achieve clarity, resolution and contrast within the ovules the following combinations of different staining and destaining durations were tried:

1) 12 hours staining and 8, 16, 32 and 48 hours destaining
2) 24 hours staining and 8, 16, 32 and 48 hours destaining
3) 48 hours staining and 8, 16, 32 and 48 hours destaining.

The best stain intensity was achieved by staining the pistils for 24 hours and destaining for 32 hours. Other modifications of the schedule published by Stelly et. at. which were found to be beneficial are shown below.

Stelly et. al. schedule	Modified version
Hydration series:	
25%, 50%, 70%, 95%, 100%, 100% ethanol for 15 minutes each	25%, 50%, 70%, 95%, 100%, 100% ethanol for 15 minutes
100% ethanol for 2-8 hours	100% ethanol for 8 hours.
Clearing:	
Xylene (Xyl) series: 2:1, 1:2 (ethanol: xylene),100% Xyl (3 changes), for 15 minutes each.	Xylene infiltration was omitted and xylene-methy-salicylate series was replaced by an ethanol-MS series.
Methyl salicylate (MS) series: 2:1, 1:2 (Xylene:MS), MS, MS, MS, for 15 minutes each.	Ethanol-MS series: 2:1, 1:2 (ethanol:MS), MS, MS, MS, for 15 minutes each.

APPENDIX 2: EFFECT OF LIGHT INTENSITY ON OVULE LENGTH AND WIDTH AT EACH OF THE FIVE POSITIONS WITHIN CARPELS OF LOWER FLORETS IN A FLOWER HEAD

Plants (Clone A of "Grasslands Huia" were grown in five different light intensities in the controlled environment growth room (Chapter 4; Experiment 2). Standard errors are given in parentheses.

Appendix 2A: Length (mm)

Light Intensity (lux)	Ovule Position					
	1	2	3	4	5	6
10,000	0.53 (0.003)	0.50 (0.012)	0.53 (0.013)	0.47 (0.010)	0.42 (0.012)	0.42 (0.002)
6,000	0.51 (0.009)	0.48 (0.011)	0.47 (0.011)	0.45 (0.011)	0.41 (0.013)	0.35 (0.006)
4,500	0.51 (0.011)	0.45 (0.005)	0.42 (0.011)	0.41 (0.012)	0.39 (0.012)	0.38 (0.011)
2,800	0.47 (0.011)	0.47 (0.015)	0.44 (0.019)	0.42 (0.010)	0.39 (0.011)	0.38 (0.012)
2,000	0.45 (0.018)	0.45 (0.011)	0.42 (0.016)	0.39 (0.011)	0.38 (0.003)	0.38 (0.011)

Appendix 2B: Width (mm)

Light Intensity (lux)	Ovule Position					
	1	2	3	4	5	6
10,000	0.36 (0.011)	0.36 (0.011)	0.36 (0.011)	0.33 (0.013)	0.30 (0.005)	0.27 (0.011)
6,000	0.35 (0.006)	0.33 (0.009)	0.33 (0.011)	0.32 (0.013)	0.27 (0.011)	0.24 (0.006)
4,500	0.32 (0.012)	0.32 (0.012)	0.30 (0.011)	0.29 (0.005)	0.26 (0.015)	0.23 (0.012)
2,800	0.30 (0.011)	0.30 (0.013)	0.30 (0.009)	0.29 (0.011)	0.27 (0.013)	0.26 (0.003)
2,000	0.29 (0.006)	0.30 (0.011)	0.30 (0.013)	0.29 (0.011)	0.27 (0.010)	0.24 (0.008)

1= Proximal (basal) end of the carpel, 6= Distal end of the carpel

APPENDIX 3: SPECTROPHOTOMETER SPECIFICATIONS

A double-beam spectrophotometer (Hitachi model U-2000) was used to determine the quality of light transmitted by the cellophane filters used in experiment 3 in Chapter 5.

Wavelength: The spectrophotometer can scan wavelength ranging from 1100 to 190 nm. The minimum scan width is 10 nm. The reading interval (nm) ranges from 0.1 to 1.0 nm depending on the specified wavelength range.

APPENDIX 4: EFFECT OF LIGHT INTENSITY ON DEVELOPMENT OF FLORAL ORGANS

Plants (clone A of "Grasslands Huia") were grown in five different light intensities in the controlled environment growth room (Chapter 4: Experiment 1). Values are the mean of 10 replicates ± SE.

Appendix 4A: Sepal length (mm)

Light Intensity (Lux)	Node Position					
	Node 7	Node 8	Node 9	Node 10	Node 11	Node 12
10,000	3.0±0.16	4.0±0.20	4.9±0.25	5.6±0.08	5.6±0.08	5.7±0.11
6,000	2.7±0.12	4.0±0.24	5.1±0.14	5.8±0.09	6.0±0.10	6.2±0.08
4,500	2.5±0.08	3.7±0.11	4.7±0.13	6.2±0.18	6.2±0.11	6.2±0.08
2,800	2.5±0.07	3.7±0.10	4.6±0.08	5.2±0.16	5.5±0.07	5.6±0.06
2,000	1.8±0.08	3.2±0.21	4.3±0.20	5.4±0.12	5.5±0.09	5.5±0.13

Appendix 4B: Petal length (mm)

Light Intensity (Lux)	Node Position					
	Node 7	Node 8	Node 9	Node 10	Node 11	Node 12
10,000	0.6±0.06	1.5±0.08	2.3±0.20	6.5±0.15	7.7±0.10	8.1±0.09
6,000	0.3±0.07	1.4±0.22	2.1±0.21	6.0±0.19	7.8±0.16	8.7±0.14
4,500	0.5±0.05	1.0±0.05	3.2±0.11	5.6±0.06	7.5±0.27	8.3±0.05
2,800	0.4±0.06	1.4±0.23	2.8±0.18	5.7±0.04	7.8±0.06	8.1±0.11
2,000	0.1±0.01	0.8±0.18	2.1±0.08	4.3±0.17	7.7±0.10	8.0±0.18

Appendix 4C: Filament length (mm)

Light Intensity (Lux)	Node Position					
	Node 7	Node 8	Node 9	Node 10	Node 11	Node 12
10,000	0.4±0.05	0.5±0.06	1.1±0.09	2.1±0.14	3.2±0.10	3.5±0.06
6,000	0.4±0.05	0.6±0.11	1.1±0.06	2.4±0.08	3.0±0.10	3.2±0.06
4,500	0.3±0.03	0.9±0.06	1.2±0.05	2.1±0.07	3.3±0.13	3.3±0.11
2,800	0.3±0.04	0.9±0.06	1.3±0.09	2.4±0.09	2.6±0.05	3.0±0.09
2000	0.2±0.04	0.7±0.05	1.0±0.04	1.9±0.07	2.9±0.06	3.0±0.09

Appendix 4D: Ovary length (mm)

Light Intensity (Lux)	Node Position					
	Node 7	Node 8	Node 9	Node 10	Node 11	Node 12
10,000	0.6±0.12	1.1±0.09	1.2±0.06	1.7±0.04	2.0±0.04	2.2±0.09
6,000	0.5±0.08	1.0±0.10	1.1±0.04	1.6±0.06	1.8±0.05	2.2±0.04
4,500	0.5±0.05	1.0±0.06	1.1±0.05	1.6±0.07	1.9±0.08	2.0±0.07
2,800	0.5±0.07	1.0±0.03	1.1±0.06	1.5±0.06	1.7±0.05	1.9±0.05
2,000	0.4±0.06	0.7±0.06	1.0±0.05	1.5±0.07	1.7±0.05	1.8±0.04

Appendix 4E: Style length (mm)

Light Intensity (Lux)	Node Position					
	Node 7	Node 8	Node 9	Node 10	Node 11	Node 12
10,000	0.3±0.06	0.5±0.05	0.8±0.07	3.0±0.09	3.6±0.09	3.7±0.09
6,000	0.3±0.06	0.6±0.09	0.9±0.07	2.5±0.07	3.5±0.11	3.7±0.10
4,500	0.3±0.05	0.6±0.05	1.7±0.07	2.8±0.17	3.4±0.13	3.5±0.08
2,800	0.3±0.05	0.6±0.05	1.5±0.06	2.7±0.27	3.2±0.07	3.4±0.09
2,000	0.2±0.03	0.4±0.06	0.7±0.06	2.1±0.13	3.2±0.08	3.3±0.08

APPENDIX 5: EFFECT OF CLOVER CANOPY ON PAR PHOTON FLUX BENEATH IT

White clover has leaf blades borne at the top of thin erect petioles. On average these are oriented horizontally. When mature adjacent leaves are sufficiently close to one another they form a closed canopy. In a stand with a canopy height of 19 cm, Mitchell and Calder (1958) found that 92% of the incident light penetrated to 17.7 cm, 45% to 12 cm and only 5% to 10.1 cm.

The present investigation was undertaken to determine the % of the incident light which reaches the stolon level of the clover canopy and also to determine how the angle of the sun changes the level of light reaching beneath the clover foliage canopy. The experiment was carried out at the Seed Technology Centre, Massey University, using a two-year-old "Grasslands Tahora" white clover crop growing on Tokomaru silt loam.

Three light meters were used, designated as Ag.1, Ag.2 and Bot and calibrated against each other. The comparative readings for the three light meters are shown in Table 1.Appendix 5. The Ag.2 meter was placed above the foliage canopy to measure the intensity of light falling on the foliage of white clover growing in the field, and the other two light meters were placed beneath the clover canopy to measure the light intensities beneath the foliage canopy. The measurements were made on five different days and each day the two light meters were placed in two different locations beneath the canopy. Four out of five days the weather was sunny i.e clear conditions and remaining one day the weather was cloudy i.e overcast weather conditions (i.e 18/1/89). Table 2.Appendix 5 shows the actual incident light intensities falling on the foliage of white clover and the intensities beneath the foliage canopy. The percentage of incident light penetrating the canopy from 7.00 am to 8.00 pm on five different days is shown in Table 3.Appendix 5. Values shown in Fig.1.Appendix 5 are the mean percentage of incident light reaching beneath the clover canopy of 10 observations made on 5 different days. The figure also shows the effect of angle of the sun on the percentage of incident light reaching beneath the canopy. The graph suggests that a higher proportion of incident light reaching a peak of 2% when sun at its highest in the sky. At lower sun angles penetration was below 1%.

Appendix 5: Calibration for the three light meters used.

Table 1.

Replicates	Light Intensity readings		
	Ag.1	Ag.2	Bot
1	480	210	500
2	580	270	610
3	690	300	710
4	320	150	340
5	380	170	400

Note: Bot. meter was taken as standard value.

Ag.2 meter value was multiplied by constant value 2.33

Ag.1 meter value was multiplied by constant value 1.05

Appendix 5: **Light intensity readings** ($\mu E\ M^{-2}\ SEC^{-1}$) beneath the foliage canopy

Table 2 Ag.2 measured incident light. Ag.1 & Ag.2 values are "corrected" values.

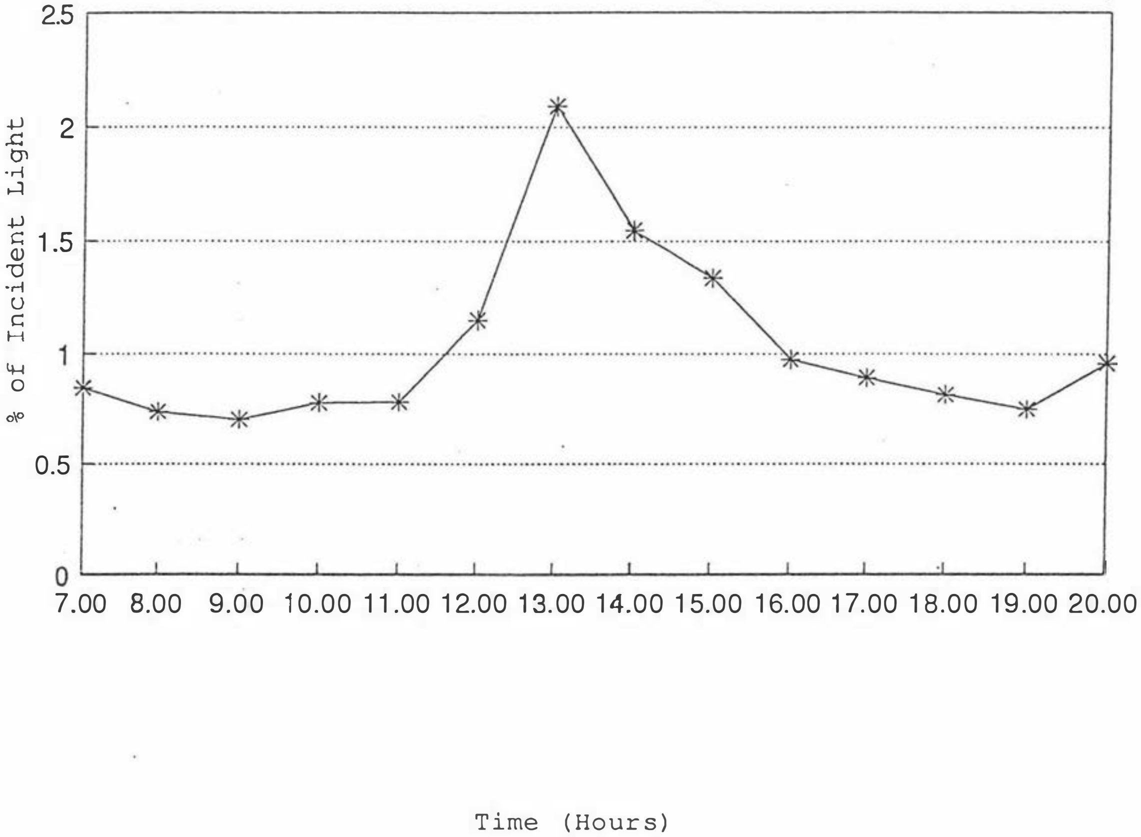
Time	Date														
	12-1-1989			16-1-1989			17-1-1989			18-1-1989			20-1-1989		
	Ag.1	Ag.2	Bot	Ag.1	Ag.2	Bot	Ag.1	Ag.2	Bot	Ag.1	Ag.2	Bot	Ag.1	Ag.2	Bot
7.00	0.2	70	0.1	0.3	280	0.7	0.7	373	0.8	1.3	70	1.8	2.1	135	1.8
8.00	0.7	792	0.4	0.6	885	0.4	1.4	816	1.5	2.9	175	3.4	2.9	172	2.5
9.00	0.7	1118	0.4	1.4	1072	0.4	2.8	1002	2.1	3.5	196	3.8	5.6	385	4.3
10.00	2.9	1445	0.6	2.3	1584	0.8	5.5	1561	2.9	7.0	329	5.2	13.2	1515	13.8
11.00	3.9	1724	0.8	1.6	722	0.7	31.5	1817	3.4	5.9	287	4.9	11.7	1445	11.0
12.00	2.9	1049	0.5	1.9	2027	2.0	29.4	1748	3.7	13.2	643	13.0	16.1	979	33.0
13.00	8.2	2283	1.5	2.4	2167	3.2	63.0	2074	2.7	13.9	615	20.0	9.1	1584	175.0
14.00	4.8	1654	2.5	2.1	909	2.8	15.8	2027	3.7	14.8	643	20.0	11.0	1981	150.0
15.00	4.0	2144	1.3	2.0	2377	2.9	16.1	1608	3.0	15.8	419	12.0	12.9	2120	96.0
16.00	2.5	1025	1.8	0.5	699	1.4	8.5	1118	2.2	16.7	454	14.0	12.3	1817	12.0
17.00	1.1	379	0.5	1.3	1538	18.0	3.0	746	1.5	17.3	489	16.0	6.3	1608	8.0
18.00	1.3	419	0.7	0.4	909	0.5	1.7	676	1.0	1.6	98	5.0	2.8	1398	4.0
19.00	0.5	93	0.2	0.2	350	0.3	1.4	233	0.8	0.5	63	2.0	1.6	280	3.0
20.00	0.4	70	0.2	0.2	47	0.2	0.5	47	0.4	0.4	70	0.6	0.6	60	2.0

Appendix 5: **% of incident light beneath the clover foliage canopy**

Table 3 Ag.1 & Bot measured intensities beneath the canopy and Ag.2 measured incident light.

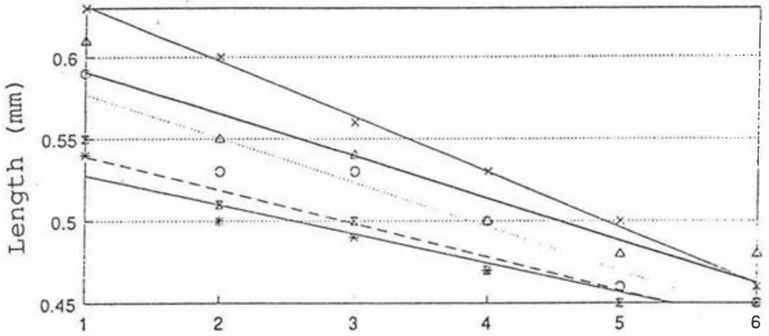
Time	Date														
	12-1-1989			16-1-1989			17-1-1989			18-1-1989			20-1-1989		
	Ag.1	Ag.2	Bot	Ag.1	Ag.2	Bot	Ag.1	Ag.2	Bot	Ag.1	Ag.2	Bot	Ag.1	Ag.2	Bot
7.00	0.30	70	0.14	0.11	280	0.25	0.20	373	0.21	1.80	70	2.57	1.55	135	1.33
8.00	0.09	792	0.05	0.07	885	0.05	0.16	816	0.18	1.68	175	1.94	1.70	172	1.45
9.00	0.07	1118	0.04	0.13	1072	0.04	0.28	1002	0.21	1.76	196	1.94	1.44	385	1.11
10.00	0.20	1445	0.04	0.15	1584	0.05	0.34	1561	0.19	2.13	329	1.58	0.87	1515	0.91
11.00	0.23	1724	0.05	0.22	722	0.10	1.73	1817	0.19	2.05	287	1.70	0.80	1475	0.76
12.00	0.28	1049	0.05	0.09	2027	0.10	1.68	1748	0.21	2.05	643	2.02	1.64	979	3.37
13.00	0.36	2283	0.07	0.11	2167	0.15	3.03	2074	0.13	2.25	615	3.25	0.57	1584	11.0
14.00	0.30	1654	0.15	0.23	909	0.31	0.77	2027	0.18	2.30	643	3.11	0.55	1981	7.57
15.00	0.19	2144	0.06	0.08	2377	0.12	1.00	1608	0.19	3.75	419	2.86	0.60	2120	4.52
	0.24	1025	0.18	0.08	699	0.20	0.76	1118	0.20	3.67	454	3.08	0.67	1817	0.66
17.00	0.30	379	0.13	0.08	1538	0.12	0.41	746	0.20	3.54	489	3.27	0.39	1608	0.49
18.00	0.31	419	0.17	0.05	909	0.05	0.25	676	0.15	1.60	98	5.10	0.20	1398	0.29
19.00	0.57	93	0.22	0.06	350	0.08	0.60	233	0.34	0.84	63	3.17	0.56	280	1.07
20.00	0.60	70	0.29	0.45	47	0.43	1.13	47	0.85	0.60	70	0.85	1.05	60	3.33

APPENDIX 5 : Light intensities beneath foliage canopy in clover. Values shown are the mean of 10 observations made on 5 different days in the month of January 1989 (in Palmerston North, N.Z. ; Latitude 40° 23′ South, Longitude 175° 37′ East).

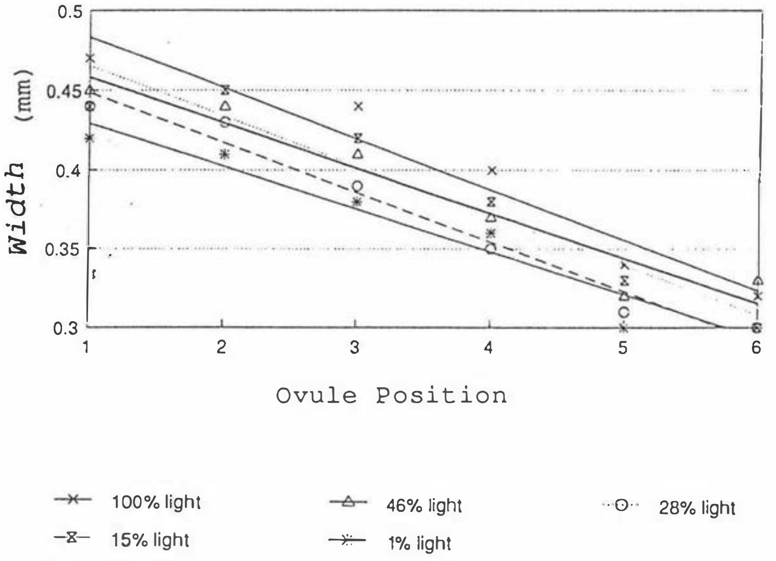


APPENDIX 6 : Influence of low light on ovule size at each of the six positions within carpels of oldest florets in inflorescence which were grown in five different light intensities (Chapter 5 ; *Experiment 1*). Florets were harvested for measurement when flower heads reached node 11. The proximal (basal) ovules in a carpel are numbered 1 and distal ovules 6. Each value is the mean of 10 replicates. The range of standard errors was 0.003 to 0.019 for ovule length and 0.006 to 0.014 for ovule width.

APPENDIX 6A : *Ovule Length*



APPENDIX 6B : *Ovule Width*



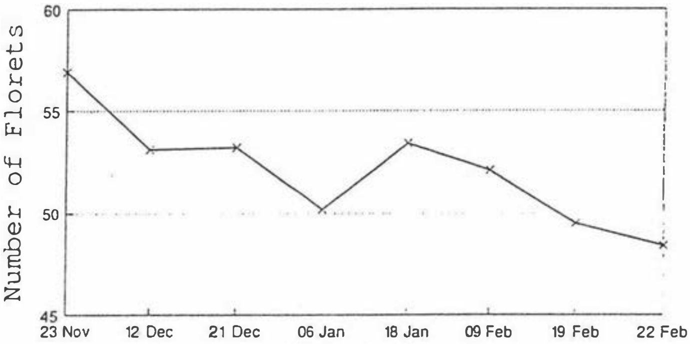
APPENDIX 7: INFLUENCE OF FLORET POSITION IN A FLOWER HEAD ON OVULE NUMBER PER CARPEL.

The plants were grown in a glasshouse. 40 florets were sampled both from 20 apical and 20 basal florets of 20 inflorescences per replicate. The whole plant (including inflorescence) received full light (Chapter 5; Experiment 2 & 3). Values followed by the same letter in each pair of treatments are not significantly different at 5% level. Values are the mean±SE.

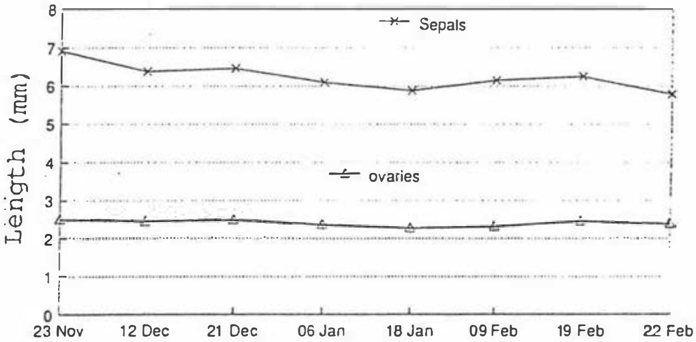
REPLICATES	LOWER FLORETS	UPPER FLORETS
1	5.63±0.130a	4.78±0.085b
2	5.94±0.095a	4.89±0.077b
3	5.70±0.073a	4.85±0.144b
4	5.72±0.070a	4.71±0.060b
5	5.45±0.062a	4.61±0.082b
6	5.68±0.084a	4.80±0.089b
7	5.75±0.116a	4.95±0.096b
8	5.48±0.087a	4.57±0.057b
9	5.41±0.085a	4.47±0.055b
10	5.55±0.062a	4.97±0.116b
11	5.30±0.143a	4.73±0.132b
12	5.43±0.084a	4.87±0.113b
13	5.28±0.069a	4.84±0.058b
14	5.25±0.065a	5.05±0.093b
15	5.23±0.079a	4.52±0.118b
Overall mean	5.52±0.055a	4.77±0.044b

APPENDIX 8 : Changes in inflorescence size, average percentage of fertile pollen and embryo sac, ovule and seed number, and the size of floral organs with time of the year. Clone A of "Grasslands Huia" white clover were used. The plants were growing in glasshouse conditions. The materials for measurements were collected from 23rd November 1988 to 22nd February 1989. The range of standard errors was 0.79 to 1.8 for florets, 0.047 to 0.21 for sepals, 0.567 to 2.3 for pollen fertility, 1.75 to 4.9 for embryo sac fertility, 0.040 to 0.084 for ovule number and 0.068 to 0.097 for seed number.

APPENDIX 8A : *Floret Number*

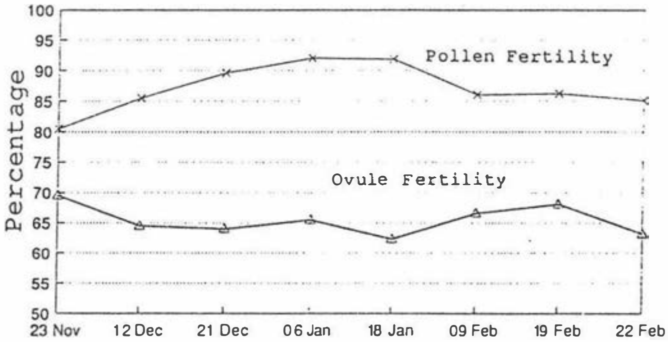


APPENDIX 8B : *Sepal and Ovary Size*

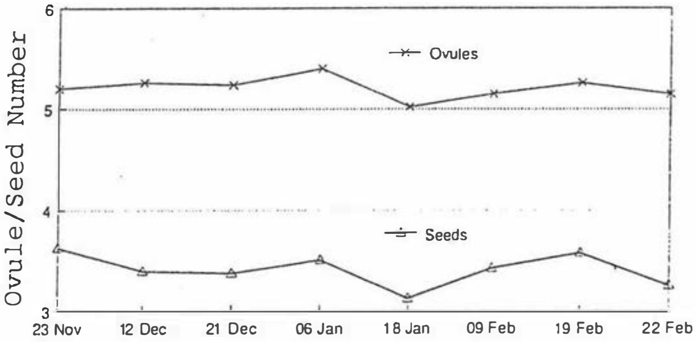


Time of the Year

APPENDIX 8C : *Percentage of Fertile Pollen and Ovule*



APPENDIX 8D : (A) *Ovule Number Per Carpel* (B) *Number of Ovules Setting Seed Per Carpel*



Time of the Year

APPENDIX 9: INFLUENCE OF CANOPY DENSITY AND SIMULATED OVERCAST WEATHER CONDITIONS ON SIZE OF FLORAL ORGANS.

Field experiment 1988/89- clonal material (clone C) of "Grasslands Huia" white clover were used (Chapter 6). Treatments were open canopy: inter-row spacing of 60 cm; open canopy with pre-fertilization shade: inter-row spacing of 60 cm and plants were artificially shaded before fertilization (45% of incoming radiation) to simulate overcast weather conditions; dense canopy: inter-row spacing of 15 cm. All treatments had 15 cm intra-row spacing. Values followed by same letter between treatments (in same column) are not significantly different at 5% level.

Treatment	Sepal length (mm)	Ovary length (mm)	Style length (mm)
Open canopy	5.9±0.072a	2.30±0.026a	3.41±0.048a
Open/Pre.f.shade	5.8±0.090a	2.00±0.054b	3.20±0.057a
Dense canopy	5.7±0.100a	2.16±0.027b	3.35±0.073a

APPENDIX 10: INFLUENCE OF CANOPY DENSITY AND SIMULATED OVERCAST WEATHER CONDITIONS ON PERCENTAGE OF VIABLE SEED, HARD SEED AND DEAD SEED

Appendix 10A: Field experiment 1988/1989 (Chapter 6).

Treatment	Germination (%)	Hard seed (%)	Dead seed (%)
Open canopy	61.3±0.25a	35.0±1.6a	3.75±1.7a
Dense canopy	59.5±2.20a	37.8±3.1a	2.06±1.0a
Open/Pre.f.Shade	75.5±1.90b	22.8±2.4b	1.75±0.48a
Open/Post.f.Shade	77.5±3.90b	20.3±3.7b	2.25±0.48a

Appendix 10B: Field experiment 1989/1990 (Chapter 7).

Treatment	Germination (%)	Hard seed (%)	Dead seed (%)
Open canopy	7.5±1.5a	90.5±2.4a	2.0±1.2a
Dense canopy	6.0±0.8a	90.0±1.8a	4.0±1.4a
Open/Pre.f.Shade	5.0±1.7a	92.5±2.2a	2.5±0.5a
Open/Post.f.Shade	6.0±1.8a	92.0±1.8a	2.0±0.8a
Dense/Post.f.shade	8.0±2.6a	90.0±2.2a	2.0±0.8a

Values followed by the same letter between tratments (in same column) are not significantly different at 5% level.

For plants which were artificially shaded before or after pollination, there was reduction in percentage of hard seed (Appendix 10A). This observed result is not consistent with the result of the other field experiment (Appendix 10B). The difference between two field experiments could have been due to cultivar differences or to variation in growing conditions (i.e weather conditions). The given treatments (pre-and post-fertilization shade) could have lengthened the seed maturation period with the result that in some seeds hardness was not attained at the time of harvest.