A STUDY OF THE VEGETATIVE AND REPRODUCTIVE MORPHOLOGY OF GRASSLANDS HUIA WHITE CLOVER (*Trifolium repens* L.) WITH EMPHASIS ON THE EFFECTS OF DEFOLIATION AND PARAQUAT ON SEED YIELD AND QUALITY

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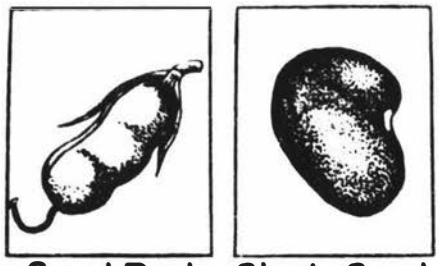
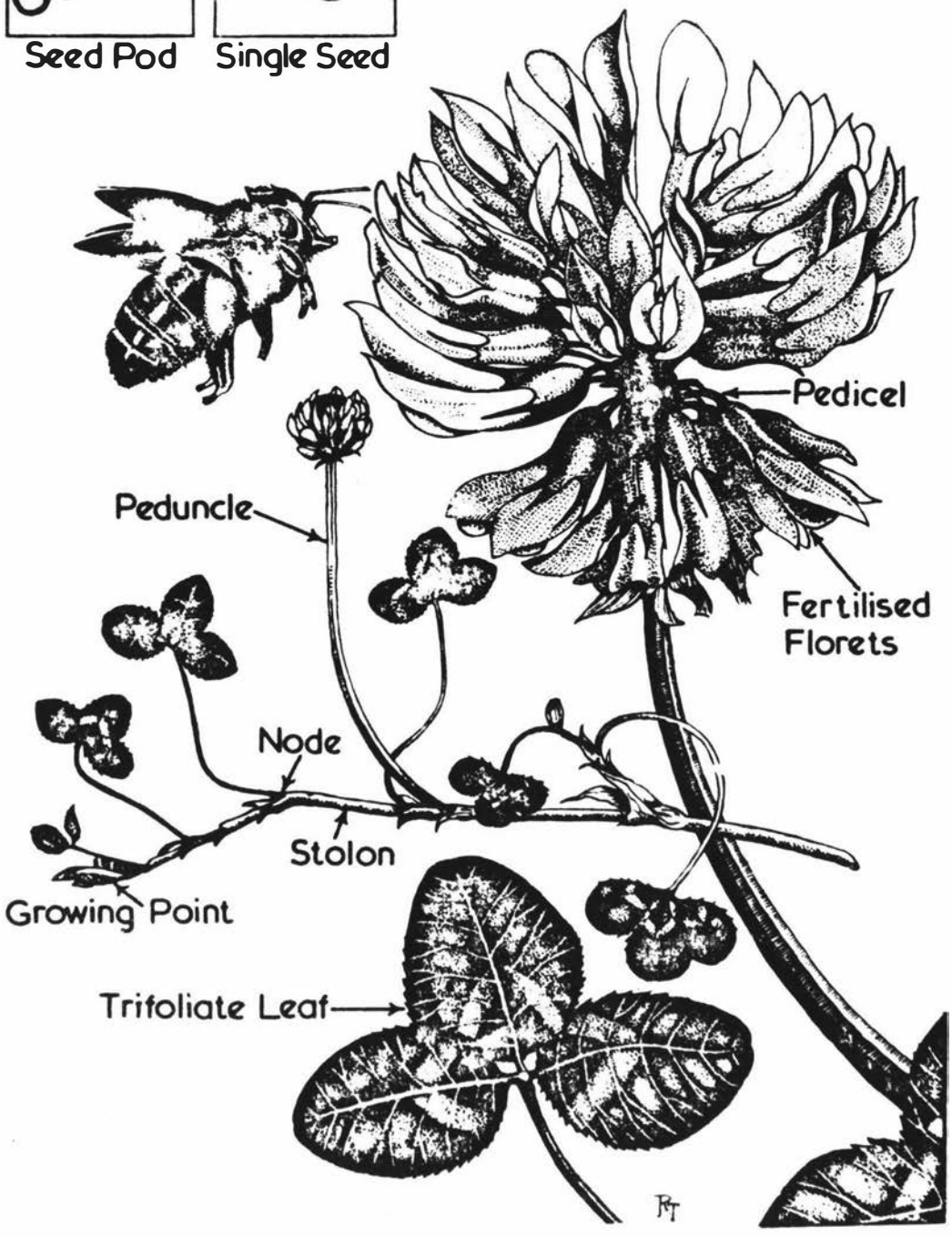
Depicting vegetative and floral parts of white clover plant.



Seed Pod Single Seed



ABSTRACT

The present study examined the effect of management systems, particularly closing date and paraquat application, on the vegetative and reproductive morphology of'Grasslands Huia' white clover (Trifolium repens L.) over two successive years in a field mixed (grass and clover) sward situation. More detailed studies involving two different genotypes of 'Grasslands Huia' white clover grown in monoculture were also carried out to examine the effects of cutting and paraquat application.

The mixed sward studies clearly showed that November closing dates resulted in highest seed yields. In grass/clover swards the closing of crops in November accompanied by paraquat spraying to remove grass competition either in mid October or at closing, enhanced seed yield. Later grazing and spraying was deleterious to seed yield unless climatic conditions allowed continued vegetative growth into December. In this latter case spraying in November and closing in December gave high seed yields. Treatments involving closing in September and October and spraying 30 days before, at closing or 30 days after closing, always gave less seed yield than November treatments sprayed 30 days before or at closing time.
Closing time and spraying time had a marked effect on seed yield components. The most consistent and major effect of closing and spraying treatments in the two mixed sward experiments was on inflorescence numbers. Other components such as seed set, seed weight, and floret numbers were not consistently influenced by management but did vary according to environmental conditions.

Studies on the effects of cuttings and paraquat application on plant structure and on seeding potential and yield of 'Grasslands Huia' white clover clearly showed that there is a partitioning between vegetative growth and reproductive development. The vegetative process was characterised by a high percentage of the nodes on main stolons forming lateral stolons in the winter and early spring. Reproductive development in late spring and summer showed that approximately 80% of inflorescences were formed on main stolons. Highest inflorescence numbers were produced from nodes formed in October and November, although floral initiation started during late winter (August). The two genotypes used in this study showed considerable variation in relation to reproductive development. Differences of 47% in inflorescence numbers, 25% in seeds per floret and 13% in seed weight were observed between genotypes.

The effects of defoliation by cutting involved a reduction in stolon elongation and a general increase in lateral stolon production, particularly when terminal buds were also removed. Both light and heavy cutting treatments resulted in seed yields which were 70 to 90 kg/ha less than the 657 kg/ha produced by uncut plants. The effect of paraquat application was also detrimental to seed yield, mainly through a direct effect on white clover morphology. Although paraquat reduced the amount of lateral branching on the main stolon the destruction of stolon tissue by the herbicide reduced plant recovery and resulted in a seed yield of only 392 kg/ha compared with 657 kg/ha from unsprayed plants. This effect was most pronounced in pure swards of white clover and was less obvious when the grass component of a mixed sward provided some protection for the white clover by reducing the extent of direct contact with the herbicide.

Some potential areas for future research and the potential for white clover seed production in Colombia are also discussed.

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INTRODUCTION

White clover has a world wide distribution. It is agreed that the plant is indigenous to all the countries of Europe and many parts of Asia and Africa (Daday 1958). The species also became naturalized in Mongolia, China, Japan, and as European man colonized other temperate regions of the world it became distributed throughout the United States, Canada, and South America (Brougham *et al* 1978). The species also occurs wild in high altitude areas near the equator such as Colombia, and Hawaii (Thomas 1982a).

As one of the most important pasture plants, white clover is necessary in the sward for two reasons. First, and most importantly in the farming system, is its ability to fix atmospheric nitrogen through symbiosis with root nodule *Rhizobium* bacteria (Martin 1960). In New Zealand, for instance, white clover is capable of fixing up to 500 kgN ha/annum (Sears 1945) in a mixed sward. This nitrogen becomes immediately available for clover growth, and ultimately available for associated grass growth through the excretion of nitrogenous compounds from nodules of clover, the decay of clover root nodules and aerial plant parts or through fertility recycling in the urine and faeces of grazing animals (Butler *et al* 1959). Secondly, white clover has advantages over grass in terms of quality for animal feeding. In general, it has less fibre than grasses, and a higher ratio of soluble and insoluble carbohydrate as well as higher protein content (Minson *et al* 1964). These factors result in more milk production and animal growth on clover dominant pasture than grasses dominant ones (Johns 1966). In addition, white clover has roughly double the mineral content of grasses, especially magnesium and calcium, both of which are necessary for grazing animals. It also has a high level of digestibility (Raymond and Spedding 1965) which is retained over the growing season. In this respect it is superior to grasses or other legumes such as red clover or lucerne.

The most common method of establishing white clover for seed involves sowing a simple mixture of 22 kg/ha of ryegrass and 3 kg/ha of clover with the aim of harvesting both for seed in successive years (Scott 1973). Usually, ryegrass is taken in the first year and white clover in the second year after sowing (Shillito 1974). For the success of this method in terms of white clover seed production the levels of nutrients, the ryegrass cultivar in the sward and the intensity of grazing should be kept in mind to obtain high seed yield. Besides these factors, the time of the last grazing, or cutting, and/or dessication with paraquat as a means of reducing or eliminating competition from ryegrass should be carefully controlled. In general, farmers close their swards early in order to ensure enough vegetative bulk to facilitate harvesting of the crop. However, research by the Grasslands Division of the Department of Scientific and Industrial Research in New Zealand has shown that, with adequate soil moisture, deferring closing to mid November gives highest seed yields (Clifford 1980).

The seed production capacity of white clover is the combined result of a number of morphological and structural changes which are strongly influenced by seedcrop management practices. In New Zealand, white clover seed production is complicated by the dual purpose use of the white clover plant involving the production of herbage for animal feeding and the production of seed in the same farming year. The different crop management systems used by farmers in New Zealand are capable of effecting vast differences in seed yields gained depending on the system chosen. Such variable seed yields suggest the need for more knowledge of plant reaction to management, particularly grazing management, and the spraying with herbicide for grass control, on both vegetative and reproductive structure and development of plants.

The development of the inflorescence in white clover is usually determined by responses to local conditions of temperature and photoperiod, and knowledge of these environmental responses is necessary to manage the paddock correctly for seed production. Management should be based on a knowledge of the time of inflorescence initiation and of management practices which ensure maximum seed yields.

The present study was divided in three parts. The purpose of the first part (first two year experiments) was to determine, in a mixed sward, the effect of the time of cessation of grazing (closing date) and paraquat spraying on:

a) the botanical composition of the sward
b) the vegetative growth of white clover
c) the development of inflorescences and the effects of seed yield components on the resultant yield of seed.

In the second study, carried out on white clover alone, more emphasis was placed on the vegetative and reproductive pattern of growth and development in two different genotypes.

Finally, in the last experiment, the effect of different intensities of defoliation on white clover reproductive development and seed yield was investigated. In particular three aspects of defoliation which were studied:

a) the effect of the severity of defoliation on white clover seed production under field conditions
b) a comparison of the responses of paraquat spraying and cutting treatments on white clover
c) the reaction of two different genotypes to different defoliation treatments.

CHAPTER I

REVIEW OF LITERATURE

The amount of information on white clover is voluminous. For this reason, this review concentrates on aspects considered to be relevant to the present study. In addition to a general description of the plant, particular emphasis is given to vegetative and reproductive structure and development and to seed crop management practices, including defoliation and paraquat effects, on white clover seed yield.

A - General Description of White Clover

White clover (*Trifolium repens* L.) is a legume which belongs to the sub-family Papilionoideae of the family Fabaceae. It is an excellent pasture legume, growing wild or cultivated in all temperate climates where there is enough moisture. Its ability to draw on and fix nitrogen from the air makes it an ideal plant for use in almost all pasture-improvement programmes (Jolly 1957). In addition to the stimulus which it can give to the grasses in the sward, white clover itself makes a valuable and direct contribution to the total production of the pasture (Bean 1978). It stops growing when hot and dry weather sets in, but usually revives when conditions become favourable (Turner and Begg 1978). It is preferably used for grazing but in some cases is cut for hay or silage (Whyte *et al* 1953).

There are many cultivars of white clover. These are classified into three main groups according to leaf size (Langer 1973).

1) Small Leaved Types

These are very prostrate and have been selected from cultivars existing in old pastures (e.g. 'Kent wild clover' and 'S-184'). They are very hardy, but remain small even when under favourable growing conditions. Thus they do not stand up to grass competition when nitrogen fertilizer is used or when conservation cuts are taken. Their real value is in tightly grazed swards, conditions

of low fertility and at high altitudes (Langer 1973).

2) Medium Leaved Types

This is the most important type used in New Zealand (e.g. 'Grasslands Huia'). However, leaf size is not a consistent characteristic in the field, because if these cultivars are used in a sward that is regularly and tightly grazed, leaf size will become quite small (Davies and Levy 1931, Corkill 1952).

3) Large Leaved Types

'Ladino' clover is the best known cultivar with large leaves, although it is not used widely in New Zealand (Leffel and Gibson 1955).

From these three general types of white clover the most important in New Zealand is 'Grasslands Huia'. This cultivar is the highest producing legume which is available for use where grazing is continuous, and where annual rainfall is 640 to 760 mm per year, with no prolonged (3 weeks or more) summer soil moisture deficit (Langer 1973). The cultivar 'Grasslands Huia' was known as New Zealand white clover prior to 1964. Developed from ecotypes found in the country by the D.S.I.R., this cultivar is the keystone on which pastoral production in New Zealand is based (Jolly 1957). For this reason a background of the characteristics of this cultivar is worth considering in some detail.

Leaves in white clover are characteristically trifoliate (Gibson and Hollowell 1966). Leaflets are elliptical to broadly ovate or ovate to almost heart shaped. The leaflet margin varies from entire to serrated. Leaflets are pinnately veined, with prominent midribs on the lower surface and glabrous, and always have a mark usually seen as a white to light green crescent appearing as an inverted V on the upper surface (Crowder 1960). The amount and distribution of red pigment (anthocyanin) in the leaflets range from a total absence to an almost complete coverage of the leaf (Thomas 1985).

In white clover, the fruit is a small oblong pod with slight constrictions between seeds and usually containing two or three seeds per pod (Clifford 1980). Thousand-seed weight is 0.60 - 0.65 g (Clifford 1979).

B - Vegetative Morphology and Growth

White clover is a stoloniferous legume which behaves more like an annual than a perennial (Hollowell 1966) under U.S. conditions. In white clover plants it is possible to recognize two stages of growth, vegetative growth providing the basic structure from which reproductive growth develops.

Vegetative growth can be divided into two systems (Chow 1966). the primary system which includes the growth and development of the tap-root, primary stem and main stolons, and the secondary system which includes the adventitious roots, axillary buds, and lateral buds, branches and stolons (Chow 1966, Thomas 1985).

The primary stem of white clover is short and contains a number of internodes. Its axillary buds give rise to stolons six or eight weeks after germination (Leffel and Gibson 1975), and elongation of the primary stem stops, or is limited, after stolon growth begins (Chow 1966).

Stolons develop radially from the primary stem to a maximum number of ten, even though it theoretically has the capacity to produce an infinite number of them (Chow 1966). These stolons constitute the main stolons which later produce a secondary stolon system which has the capacity to lead an independant existence after the first system has rotted and died (Chow 1966).

1 - Stolon Structure and Growth

White clover has a procumbent solid stolon which is the basic structure of the plant (Williams 1983). It consists of a series of internodes (larger than those of the primary stem) separated by nodes which form as a result of growth at the apical bud (Williams 1983). Each node bears a leaf, two root primordia and an axillary bud (during vegetative growth) which can grow into a lateral stolon (Daday 1958, Crowder 1960, Leffel and Gibson 1975). Stolons vary greatly in size depending on the cultivar. Stolons can grow indefinitely at their tips, achieving physical independance when the main stolon decays (Erith 1924, Beinhart *et al* 1963, Harvey 1970).

Any particular part of the stolon does not appear to survive longer than one year (Beinhart *et al* 1963). McCree and Throughton (1966), working with 'Grasslands Huia' white clover, found that stolons began to die after three months under controlled environmental conditions. Normally rooting occurs at the nodes which causes the development of branches from the axillary buds. In this way an old white clover plant consists of several stolons of various origins and of different developmental and chronological ages. This can create a problem when attempting to identify the different kinds of stolons on one plant. To overcome this problem Thomas (1985) suggested that each plant should be divided into a series of categories, i.e. main stolon, axillary bud, lateral branch, and lateral stolon. The definitions adopted by Thomas (1985) are presented below and are shown diagramatically in Figure 1.

Main Stolon: A stolon which bears (or has produced) at least 8 leaves with unfolded leaflets along its main axis and which possesses adventitious roots at one or more nodes or at its base.

Axillary bud: A vegetative shoot in a leaf axil which has produced fewer than two leaves with unfolded leaflets.

Lateral Branch: Any vegetative axillary outgrowth which has produced two or three leaves with an unfolded leaflet. The apical bud of such a branch contains an active meristem.

Lateral Stolon: A lateral branch growing from a main stolon, or from another lateral stolon, which has produced 3 or 4 leaves with unfolded leaflets and has a stem which, from its base to the node of attachment of its youngest leaf with unfolded leaflets is at least twice as long as the stipules of its subtending leaf or has produced 5-7 leaves with unfolded leaflets or has produced more than 7 leaves with unfolded leaflets but has developed no adventitious root system.

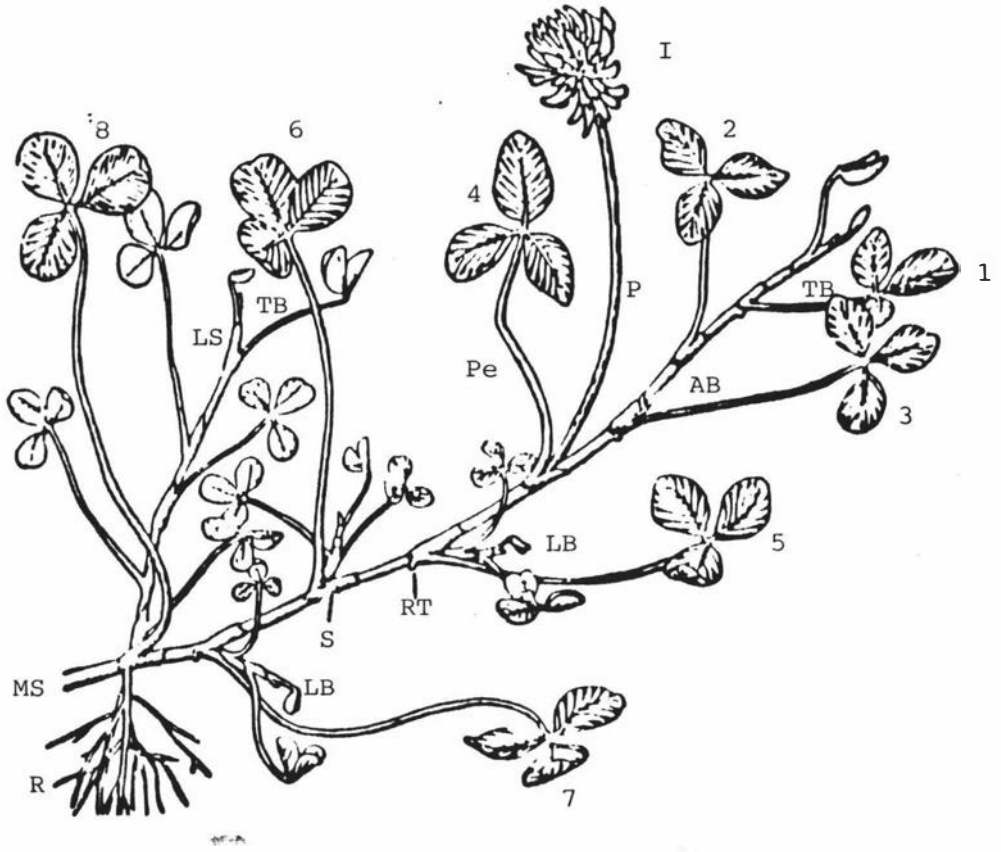

Figure 1: Vegetative and floral parts of a white clover plant

MS = main stolon, LS = Lateral stolon, LB = Lateral branch Pe = Petiole, P = Peduncle, I = Inflorescence, AB = Axillary bud, S = Stipules, RT = Root tip, TB = Terminal bud, R = Root 1 to 8 = node number with unfolded leaves (Thomas 1985)

According to Thomas (1985), a main stolon always has, by virtue of its root system, the capacity to be self-supporting for mineral nutrients and water immediately after it has been severed from its parent stolon, while a lateral stolon does not necessarily have this capacity.

Stolon growth results from the activity of an apical bud consisting of several young leaves enclosed in a stipular sheath of the youngest visible leaf (Thomas 1980a). Its elongation depends on temperature and light intensity providing other factors are not limiting (Widdup 1980). Mitchell (1956, a,b) and Beinhart *et al* (1963) showed that the optimum temperature for stolon growth in white clover is 20°C - 28°C with little stolon elongation below 5°C. Thomas (1980a) has also indicated that the increase in stolon length was smaller during the winter in comparison with spring and summer, when the temperature increased.

Light intensity mainly influences the size of the stolon and shows a strong interaction with temperature. Thus, Mitchell (1956a,b) obtained a different reaction on stem elongation when he compared the effect of shading at two different temperatures (22°C and 12°C). While stem elongation was reduced by shading to about 20% of that recorded under full daylight intensity at 22°C; at 12°C some shaded stolons grew longer than under full light conditions. Widdup (1980) also concluded that longer stolons generally were associated with less light intensity in the canopy at a given temperature.

2 - Branching

Different rates of branching in white clover have been detected between and within cultivars. Thus, Widdup (1980) determined that 'Kent', 'Huia', and 'Russian' clovers produced more branches than 'Spanish' and 'Ladino' clovers in cool and cold environments. He indicated that under controlled environment conditions, there is an inverse relationship between stolon growth and stolon number. These factors are controlled genetically and are strongly modified by environmental conditions, especially by temperature. Similar results were obtained by Williams and Hoglund (1978), when they compared Spanish and New Zealand white clovers. Beinhart *et al* (1963) and Gibson and Hollowell (1966) also state that these differences between cultivars may be genetic in origin.

An inverse linear relationship has been established between temperature and branching frequency in white clover (Beinhart 1963). At 10°C, branches emerged from 71% of all nodes, while at 30°C only 14% of nodes developed branches. Mitchell (1956b) obtained similar results with New Zealand white clovers when he found that branching was stimulated by both short days and low temperatures. Beinhart *et al* (1963) also noticed that in spaced plants of 'Ladino' white clover branching frequency was greatest in the spring and early summer but decreased later in the summer and again increased in the autumn as a result of the development of tertiary branches. Decreased summer branching did not appear to be caused by limited carbohydrate supply because branching increased in autumn during a period characterised by a decline in sugar

concentration. These changes in branching could be explained as a result of an increase in flowering during the summer. This is due to competition with branches for places of flower formation, since the development of a flower head and a branch cannot occur at the same node (Thomas 1980a). In fact, the development of flower heads during the summer provides a new sink source which might be expected to be undergoing rapid cell division and to have high metabolic rates which may inhibit branch formation (Harvey 1970).

Another environmental factor which affects the rate of branching is light intensity (Beinhart 1963). High light intensities increase branching and together with low temperatures produce maximum development of new stolons (Beinhart *et al* 1963). Thus, in a clover plant growing at low temperatures, any practice which allows a deeper penetration of light into the canopy will promote the development of new stolons. This effect has been also clearly shown by Brougham (1958) and by Davidson and Birth (1972) in work with subterranean clover. Haggar *et al* (1963), working with ryegrass and white clover swards, encouraged vigorous stolon branching after close defoliation. However, once the canopy closed as a result of regrowth, shading between plants resulted in reduction of light intensity which in turn reduced stolon formation. After complete shading, several of the newly initiated stolons died out. This effect continued to occur while new stolons and their leaves were shaded (Hunt 1968).

3 - Leaf and Petiole Growth

Leaves of white clover are located behind the apex of any stolon where they are enclosed in the stipular sheath of the youngest visible leaf. Each apical bud normally contains 6 or 7 leaf primordia giving approximately equal rates of leaf initiation and emergence (Thomas 1980a). In this way each time a new leaf primordium forms at the apical meristem, the oldest leaf within the apical bud emerges through its enclosing stipular sheath to become visible as the youngest leaf. Thus, growth occurs first in the leaf blades, then petioles, and finally the stem internodes (Mitchell 1955).

The rate of initiation of new leaf primordia at the apical meristem is mainly dependent on temperature and to a lesser extent on day length, but it remains constant within each genotype (Thomas 1980a,b). On the other hand, petiole elongation and rate of leaf unfolding is determined by the light intensity through the clover canopy (Brougham 1958, 1962).

Temperature affects the process of cell division in the leaves, so that an increase of 6°C in temperature from winter to spring results in an eight fold increase in the rate of cellular activity (Mitchell 1956b). Similar results have been obtained by Thomas (1980a) in natural environments with white clover. He found that leaves were initiated at a maximum rate of about two per week under summer growing conditions but in winter the rate dropped to about one every two or three weeks, with the rate of leaf emergence parallelling these changes.

Studies made by several authors (Mitchell and Lucanus 1960, Brougham 1962, Beinhart *et al* 1963, Carlson 1966 and Widdup 1980) have also shown the strong effect of temperature on leaf development. Thus, for any particular stolon, at optimum temperatures (20°C - 25°C), the rate of leaf appearance is about 1.5 leaves per week while at temperatures of 5°C - 8°C this rate slows to 0.3 - 0.6 leaves per week. Under moisture stress and high temperature conditions the rate of leaf appearance falls from 1.4 to 0.6 leaves per week (Beinhart *et al* 1963).

4 - Root Growth

White clover develops a primary taproot which may grow to a depth of one metre or more but which dies before or during the second year of growth of the plant (Westbrooks and Tesar 1955). This taproot development occurs in addition to the adventitious root developed in the branches. In this way, perenniality of clover eventually depends on the adventitious root system from the nodes of stolons and on the proportion and rate of axillary buds developing into stolons rather than into flowers (Gibson 1957). Nodules are present on the roots of the clover as a result of the symbiosis between the host plant and the soil bacterium *Rhizobium trifoli* (Leffel and Gibson 1975).

C - Reproductive Morphology and Growth

1 - Inflorescence Structure and Growth

In white clover, each inflorescence is a roundish raceme bearing closely crowded florets, each of which is borne on a short pedicel. The number of florets per head ranges from 50 to 100 or more with the oldest at the base and the youngest at the apex (Metcalfe and Elkin 1980). Florets are predominately white, sometimes with a pinkish hue (Gibson and Hollowel 1966). Each floret is a complete flower with a calyx of five sepals, a corolla of five petals (2 wings, 2 keels and 1 standard), 10 diadelphus stamens and a carpel (Leffel and Gibson 1975).

Flowering heads are produced on peduncles somewhat longer than the petiole of leaves and it takes about one week for all florets to develop (Leffel and Gibson 1975). According to Dessureaux (1951) in 'Ladino' clover the number of ovules per floret ranges from 2.4 to 6.4 and number of seeds from 0.5 to 4.3 with an average of 2.4. Rambert (1977) found that two to four ovules begin development along the placental ridge, which may produce two to four seeds. Thomas (1981a,b) pointed out that, in 'Grasslands Huia' white clover, some florets may produce up to six ovules and the rate at which these ovules develop into seeds is dependant on environmental conditions. The development of the inflorescence in white clover follows a series of specific steps and may have a range of requirements, even within a variety, in terms of daylength, temperature, and light intensity (Cooper 1960, Bean 1978, Thomas 1980a,b). A knowledge of environmental responses is important for the prediction of flowering behaviour as well as seed yields in different climates and latitudes.

Inflorescences in white clover form at the stolon apex. In vegetative plants the youngest axillary bud appears in the axil of the third oldest leaf primordium at the apex of a stolon. With the transition to the reproductive state, a bud forms in the axil of the youngest leaf primordium (Thomas 1980a). This precocious axillary bud then grows into a seedhead primordium which usually emerges from its stipular sheath a few days after the emergence of its subtending leaf (Thomas

1961b, 1980a). The subsequent behaviour of the stolon tip according to Thomas (1980a) varies with the genotype of the plant and with environmental conditions. He states that in some plants precocious axillary buds form with every leaf primordium initiated once the reproductive state is reached. However, more often, the formation of precocious axillary buds at one node is followed by the formation of two or three leaf primordia without buds. This sequence may be repeated for three or more cycles. In all cases, flowers form only in a lateral, axillary position and the apical meristem of the stolon always remains vegetative (Thomas 1980a). The first sign of the onset of flowering is the formation of double ridges. No elongation of the apical dome is detectable before such a stage is reached (Thomas 1961b).

It could be expected that on any stolon at the time of transition to the reproductive phase, every vegetative axillary bud at each node of the stolon has the potential to produce an axillary bud at the time of initiation of its next leaf primordium. However, this does not happen. The initiation of inflorescence primordia occurs only at the terminal apical meristem on main stolons and on elongating lateral branches (Thomas 1980a,b). It seems that a correlation exists within the plant, which prevents axillary buds within the apical bud and at the nodes immediately basal to it from forming inflorescence primordia. Even the youngest axillary buds initiated at the stolon apex just before its transition to the reproductive state always remain vegetative (Booysen and Laude 1964a).

2 - Factors which Affect Inflorescence Development

Cooper (1960) catalogues white clover as a long day plant with no winter chilling requirement. However, later studies of flowering behaviour with New Zealand white clover have shown it to be a short-long day plant at temperatures between 15°C and 25°C (Thomas 1961a). When transferred from cold short days to warm long days (21°C) each stolon on plants of 'Grasslands Huia' white clover immediately initiates one inflorescence primordium in the axil of the next leaf primordium formed. This reproductive node is commonly followed by the formation of two or three vegetative nodes before a second inflorescence primordium

is initiated. A third (and frequently final) inflorescence is then initiated following the production of a further two or three non-flowering nodes (Thomas 1961a). The pattern of flowering produced is thus most frequently a sequence of three flower heads each of which is separated from the next by two or three non-flowering nodes.

Under New Zealand conditions the time of flower initiation and emergence in different clones of 'Grasslands Huia' white clover indicates that plants initiate inflorescences in the cool and short days of winter, starting in May or June. Two clones (A and C) behaved as short-long day plants, initiating inflorescences in November or December in response to long days, but tending to stop when days were longest (Thomas 1979). Norris (1984) found that most of his cultivars initiated flowers under 12, 14 or 16 h daylengths. These correspond to natural daylengths found in southern Britain in mid-March, mid-April and mid-May, respectively. Those clones of 'Grasslands Huia' that initiate inflorescences in warm and long days do so for approximately 3 weeks and then initiation stops. Exposure of these non-initiated plants to a period of short days enables them to initiate inflorescences again when transferred back into long days. For this reason they have been termed short-long day plants (Thomas 1961a). A similar cessation of inflorescence initiation in long days has been shown for plants growing in the field in arctic Norway and in southern England (Thomas 1980b, 1981a).

In other work, Thomas (1982) studied the flowering behaviour of 9 lines of white clover from different latitudes, growing under natural conditions in Palmerston North. The results obtained in this comparative investigation showed that all lines exhibited a direct response to low temperature, initiating inflorescences in cool short days, whereas in most lines no inflorescence initiation occurred at the same daylength in warm conditions.

Based on these distinctions, Thomas (1982) divided white clover into 2 broad groups, which correlated with their latitude of origin. The first group consisted of plants originating from higher latitudes, which were characterised by their late initiation of inflorescences

in direct response to cool conditions and strong initiation in natural long days and continuous light. The second group consisted of plants with a low latitude origin, such as a 'Spanish' line, and c.v. 'Louisiana', which initiated inflorescences much earlier that did plants in the high latitude group. Low temperature pretreatment tended to strongly promote the initiation of inflorescences in subsequent warm short days within this group. In addition, the plants tended to initiate poorly in natural long days and continuous light with the exception of 'Louisiana', which initiated strongly in November.

'Grasslands Huia' has been catalogued by Thomas (1982) as an intermediate type between these two groups, because it initiates inflorescences earlier in the autumn (typical of the high latitude group) but does so poorly when transferred from natural long days to continuous light. However, it does initiate in response to natural long days in November.

Some authors have found that white clover plants under long days require a minimum night temperature to flower. Thus, Roberts and Struckmeyer (1938) reported that white clover flowered in long days when a minimum night temperature of 13°C to 18°C prevailed, but did not flower if night temperatures were 21°C or 24°C. Later, Britten (1960) in Hawaii, found that some clones of white clover under natural daylength remained vegetative at a continuous temperature of 23°C, but they flowered if they were grown at a 23°C day temperature and 8°C night temperature.

Light intensity has also been shown to be an important factor affecting the amount of flowering of white clover. However, few studies have been done in this area. Zaleski (1970), working with 'S-100' white clover under controlled conditions, indicated that one of the most important factors determining the initiation of floral primordia and inflorescence formation is the amount of light available at the stolon level. He does not explain how the light intensity may affect flower initiation or the number of inflorescences formed. However, it has been suggested that plants growing under a canopy receive mainly far-red light, which causes more stem elongation, but less branching. Therefore, the plant has less potential sites for flower development (Salisbury and Ross 1978).

D - Effect of Flowering on Vegetative Growth

Thomas (196ac,d) has shown that there is no reduction in leaf size associated with the production of flower heads. On the contrary, a slight increase in the rate of leaf growth has been recorded with the beginning of flower initiation. Booysen and Laude (1964b) have indicated that the basal internodes of first formed flowers are longer than the average and those produced immediately apically tend to be shorter. The nature of internodal response to inflorescence initiation suggests a gibberellin mechanism, as since gibberellic acid is known to promote stem elongation and is generally considered to occur in highest concentration in young rapidly growing tissues such as differentiating and developing reproductive buds (Leopold and Kreidemann 1975).

The only clear effect of flower production on white clover growth is a reduction in the number of branches because a flower head and a branch cannot both develop in the same axillary bud. Thus, each node can produce a branch or a flower head but never both (Zaleski 1970). In this way, a plant in which many nodes do not initiate flower heads has in theory much more capacity for regrowth from the younger regions of its stolon than those with more flower heads. Gibson (1957) confirmed these results, when he found that persistence of 'Ladino' clover was related to flowering intensity and duration of inflorescence initiation. Based on his results, more intense flowering clovers showed lower persistance than weaker ones. Similarly, strains that stopped inflorescence initiation earlier in the growing season persisted better than strains which continued to initiate flowers freely right into the hot and dry period.

E - Seed Structure and Development

The seed of white clover, as in other leguminous plants, is essentially devoid of endosperm and consists of a seed coat that surrounds a large, well developed embryo (Gunn 1981). The seed is small, approximately 1540 per gram, and is heart shaped or oval (Langer 1973). The seed coat is marked with an oval hilum or seed scar (Leffel and Gibson 1975).

At one end of the hilum is the micropyle, a tiny hole formed by the integuments during seed development. The tip of the hypocotyl-radicle axis is located below the micropyle. At the other end of the hilum is the raphe, a small groove extending to the chalaza, the point at which the integuments were attached to the ovule proper. When the funiculus separates from the seeds, the hilum shows the central scar which is formed by parenchyma tissue (Gunn 1981).

The seed coat has three distinct layers: epidermis, hypodermis and an inner parenchyma layer (Figure 2) (Polhill 1976, Gunn 1981). The seed coat may be impermeable causing hard seed, which may pass intact through the digestive tract of the grazing animal, thus allowing reseeding and spread of the species (Leffel and Gibson 1975). The embryo consists of two cotyledons, a plumule with two primary leaves and a radicle.

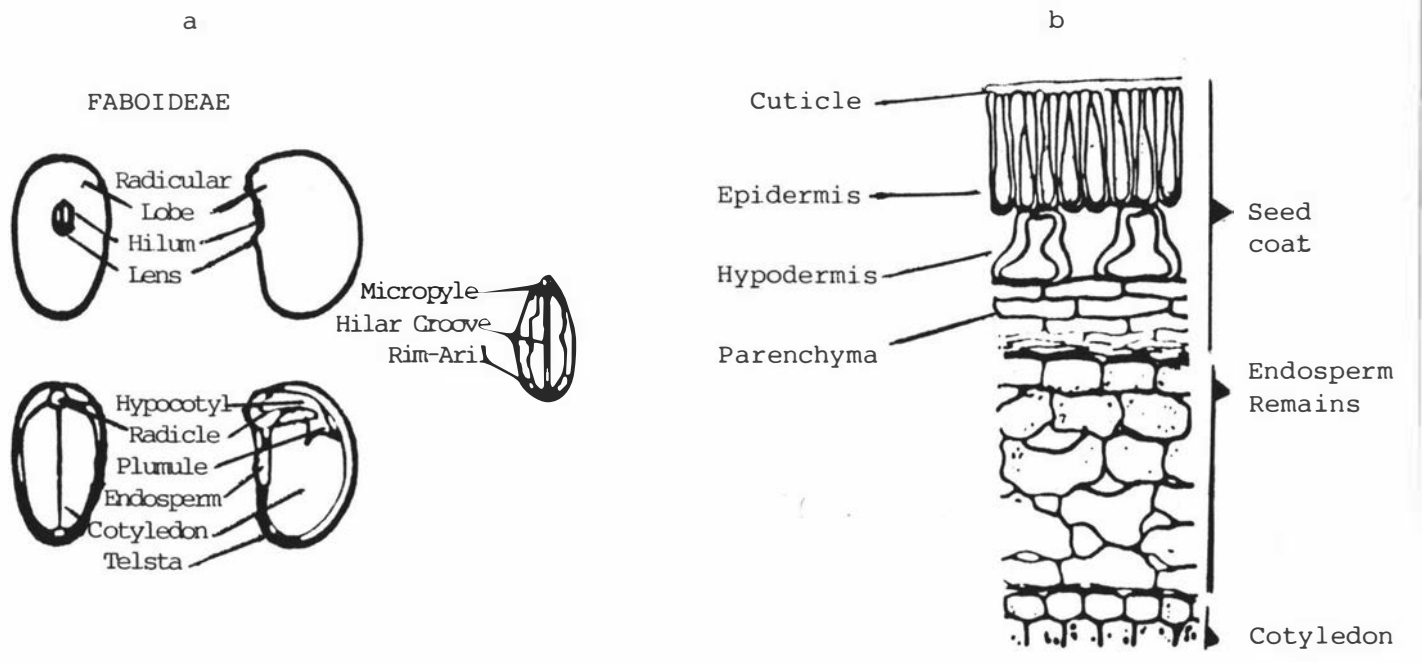

Figure 2: a) External and internal morphology of a legume seed
b) Anatomic features of a legume seed coat (Gunn 1981)

White clover seeds vary in colour and are usually yellow, green, sometimes reddish and become brownish or black with age or weathering (Scott 1973). Seed development commences soon after fertilization when the fertilized egg is surrounded by a cell wall. Then follows a series of cell divisions resulting in the formation of a mass of cells, which develop ultimately into the rudimentary plant, or embryo, of the mature seed. This seed in turn develops into the mature plant after germination (Bhatnagar and Johri 1972).

Various physiological, biochemical, and morphological changes accompany this development which finishes with the attainment of maximum seed dry weight. This point has been called 'physiological maturity'. At this stage the seed also exhibits maximum vigour and viability (Andrews 1966, Popinigis 1977).

In several studies on seed development in both grasses and legumes (Hyde 1950, Hyde *et al* 1959, Griffith *et al* 1967, Hill 1971, Popinigis 1977, and Win Pe 1978) most attention has been paid to two main phases of seed development viz. the relationship of moisture content to dry weight and the development of viable seed from the time of anthesis to seed maturity. Hyde (1950) considered that three stages can be commonly recognized in the development of white clover seed after pollination.

1 - The Growth Stage

This stage occupies a period of 10 days after pollination and during this time there is a rapid increase in seed weight. The growth rate of the ovule is logarithmic and is presumably determined by the rate of cell division in the embryo and seed coat. The seed moisture content is high and constant at approximately 79% of the fresh weight. The seed during this first period is non-viable.

2 - The Stage of Food Reserve Accumulation

This second stage takes up a period of 10 to 14 days following the first stage or 20 to 24 days after pollination. It is characterized by a constant rate of growth and is presumably determined by the rate

at which food reserves can be transferred from the parent plant to the seed. The dry weight of the seed during this stage increases about three times, reaching a maximum at the end of the period. The actual amount of water in the seed decreases slightly, but seed moisture content changes from 79% to 63%. At the end of this stage the seed is structurally complete and attains total viability and vigour.

3 - The Ripening Stage

This stage lasts three to seven days from the completion of the second stage. During this period the seed dries out rapidly and shrinks in size. The dry weight changes very little, but the fresh weight falls to less than half as the moisture content decreases from 63% to 10%, the seed finally reaching equilibrium with the relative humidity of the surrounding environment. The time required for this stage is strongly dependant on weather conditions in the field (Delouche 1980). The main modifications which occur during seed maturity include variation in seed moisture content, seed size, seed weight, seed colour, hard seededness, and seed biochemical composition.

In general, the rate and extent of moisture content change varies with species and stage of seed development (Hyde 1950, Hyde *et al* 1959, Hill and Watkin 1975, Copeland 1976, and Popinigis 1977), but is little affected by temperature (Austin 1972).

According to Popinigis (1977) the moisture content in the seed is a very important parameter for determining seed maturity and correct harvest timing. Thus, Hill and Watkin (1975), working with different grass species, presented a 'drying curve', which used moisture content and percentage of viable seed yield indicators to forecast the time of harvesting to attain maximum viable seed. Delouche (cited by Popinigis 1977) indicates that it is necessary to know the number of days required from anthesis to the attainment of equilibrium moisture content in different crops in order to develop a programme for determining correct time of harvest and the use of harvesting machinery.

However, in white clover crops it is difficult to determine physiological maturity precisely because the plants have an indeterminate type of flowering resulting in a wide range of seed maturity within the crop (Zaleski 1970).

F - Seed Yield Components and Seed Quality

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 florets per head; number of flower heads per unit area or per plant; number of seeds setting per floret, and seed weight (Zaleski 1961, 1964, 1970, Haggar *et al* 1963, Van Bogaert 1977, Clifford 1977, 1979, 1980, Thomas 1980a, 1981a,b,c, 1982, Norris 1984). These components all differ in their relative contribution to total seed yield and change with the genetic variability within the species, as well as with the environmental conditions (Zaleski 1961, 1970, Thomas 1980a, 1981a,b,c, 1982). 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 40.3% and 58.6% 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 independant of inflorescence number. Zaleski (1961, 1970), Gaspar *et al* (1981) and Haggar *et al* (1963) in determined white clover correlation coefficients between seed yield and associated characters and found that seed yield was positively correlated with seed set, heads per branch, branches per plant and seed weight and negatively correlated with flowering date (early-flowering resulted in less seed production). In this context, the number of florets per head was of relatively little significance (Zaleski 1961). Seed set was determined by the number of ovules per floret, fertility of pollen and embryo sac and pollination (Thomas 1980a). These factors can all be affected by climatic conditions (Win Pe 1978).

From an agronomic point of view, seed size and seed weight are important because they are closely associated with seed viability and seed vigour. Several authors have investigated this relationship. For example, Erickson (1946) reported a definite relationship between seed size and germination in the soil. He found with lucerne seeds that the level of emergence of the smallest seed was only one tenth that of the largest seed. However, Delouche (1980), working with crimson clover (*Trifolium incarnatum*) found that the emergence percentage of seedlings from intermediate sized seed was higher than from the largest or smallest seeds. Other authors have found that seed size has little effect on germination. Thus, Black (1956), in work with subterranean clover, reported that germination percentage was uniform amongst different seed size groups. Vaugham and Delouche (1968) working with white clover, red clover, and crimson clover found no consistent relationship between seed size and viability. Similar results were found by Gaspar *et al* (1981) in the same species. According to them each species reacted somewhat differently and there was also considerable variation within individual seed lots. However, they found that intermediate to large sized seeds were higher in germination than small seeds and also noted that an increase in seed size was accompanied by an increase in seedling vigour. These observations corroborate data presented by other authors. Black (1956) Beveridge and Wilsie (1959) Vaugham and Delouche (1968) further stated that heavy seeds maintained viability longer than light seeds. Vaugham and Delouche (1968) found similar results for white clover seeds and other legumes. Hyde (1950) indicated that the weight of white clover seedlings was directly proportional to initial seed weight. In more recent experiments with white clover and other legumes, Gaspar *et al* (1981) also found that the proportion of abnormal seedlings was not affected by seed size and that a negative correlation existed between the number of dead seeds and seed weight.

Colour changes during seed development are an indication of maturity and may be helpful in determining the proper time for harvesting the crop. In grasses, for instance, caryopses without green pigmentation are considered mature, although in some grasses physiological maturity is obtained when the seed coat is still green. In legumes, changes

from green to yellow seed coat colour have been found to be a good visual indication of seed maturity (Jolly 1957, and Scott 1973). Vaugham and Delouche (1968) and Hyde *et al* (1959) reported that colour changes in white clover seeds commence about 18 days after pollination and that yellow seeds give better germination than green or brown seeds. Similar results were obtained by West and Harris (1963) in white clover who suggest that changes in seed coat colour from yellow to red denotes a loss of viability of seeds. Another seed characteristic which is related to seed maturity is hard seededness. This is the condition of the seed coat of ripe seed which prevents the uptake of water and gases and inhibits germination. According to Hyde (1950) hard seeds first appear in seed samples collected 12 days after flowering and the proportion increases with advancing age at harvest, until on the 25th day 96% of viable seeds are hard. These results indicate that most clover seeds which ripen on the plant develop a hard seeded condition and that this plays an important role in the natural regeneration of white clover in the field.

Under commercial seed production conditions, the hard seed percentage changes with environmental conditions (Zaleski 1970). In general, the drier the atmosphere in which the seeds ripen the greater the amount of hard seed produced. Thus, seed ripened at a relative humidity of 70% all acquired the ability to germinate within 5 months, while those ripened at 30% relative humdity still showed 97% hard seededness after 18 months (Hyde 1950). Therefore, it is possible that white clover seeds which ripen in humid weather or beneath dense foliage will germinate faster, whereas seeds ripening under dry conditions may remain dormant for longer periods due to hard seededness.

According to Hyde (1950), there are three important aspects of seed quality which may be affected by the stage of seed development. These include viability, seedling vigour and storage life. Hyde *et al* (1959) found that seeds of different species initially acquire the ability to germinate at the beginning of the food accumulation stage. However, full germination capacity may not be achieved until the attainment of seed maturity. Hyde *et al* (1959) also pointed out that the lack of seedling vigour from immature seeds was due to inadequate food

reserves at the early develoment stage. Another important aspect of quality in seeds is the ability to retain germination capacity during storage. Hyde (1950) found in white clover that seeds harvested at different stages of maturity and stored under more adverse conditions (36°C and 70% relative humidity) than those normally encountered in a seed store began to decline in germinating capacity after only one month. The rate of decline was greater in extremely immature seed, and was progressively slower with samples of increasing age at harvest. From all points of view mature seed proved to be better than immature seed. This, of course, is not surprising because it has been shown that seed viability and vigour both increase to their highest level when the seed is fully mature and at maximum dry weight. Seed harvested while still immature has not reached maximum dry weight. As a result, it lacks an adequate supply of food reserves and hence has a shortened storage life.

G - Seed Crop Management

1 - Seed Production

White clover needs favourable climatic conditions for seed production which includes a dry period during the latter part of January and first days of February to facilitate harvesting and adequate moisture during autumn, winter and spring to promote the necessary vegetative growth (Calder 1941, Leitch 1949, Jolly 1957 and Scott 1973). Most suitable soil types can vary but medium silty loams where irrigation is available are likely to be the most suitable for seed production (Scott 1973). Leitch (1949) states that in some seasons heavy soils can give excellent crops, but if rain falls in December or January seed yields decrease significantly due to the excessive vegetative growth of grasses and clover, which prevents the formation of inflorescences (Zaleski 1970). On lighter soils good yields can be obtained in seasons when crops on heavier soils are ruined because of too much moisture.

Seed production is also influenced by the indeterminate nature of the plant's reproductive habit, which is under both genetic and environmental control.

Several agricultural techniques have been developed for successful white clover seed production (Haggar 1961, Zaleski 1964, Scott 1973, Clifford 1979, 1980). Generally these highlight the importance of proper establishment, management and harvesting techniques. Some of the most important recommendations are discussed in this section.

There are three main methods used to establish white clover for seed in New Zealand (Scott 1973). The common practice in the first method is to sow a cereal (usually wheat or barley) in late autumn-winter and establish the white clover at 3 kg per ha the following spring. However, white clover often fails to establish, and this is clearly related to competition between the cover crop and the clover. Crop species, particularly cereals, have larger seeds, higher growth rates, taller shoots and deeper root systems than white clover and are therefore able to compete more successfully than clover for soil nutrients (except nitrogen) and moisture. Because of their greater stature they also shade the clover and reduce its growth (Scott 1973, Bean 1978).

The second method involves sowing white clover alone as a specialist seedcrop. The legume is rather slow to establish and may easily become invaded by weeds, as well as providing very litte autumn or winter grazing (Scott 1973, Bean 1978). However, seed yields are often higher where clover is sown alone than in a mixture with perennial ryegrass. The latter method can reduce white clover seed yields by between 35 and 44% (Zaleski 1964).

The third method involves probably the most common techniques for establishing white clover for seed production, i.e. the sowing of a simple mixture of ryegrass and white clover with the aim of harvesting both species for seed in successive years (Jolly 1957, Scott 1973, Bean 1978). In the first year the management of the pasture is directed towards ryegrass seed production, while in the next season or two the area is managed to secure a white clover seed crop (Flay 1943). Under this system it is necessary during the first year to provide a more lenient type of grazing in early spring to promote ryegrass growth and to keep the white clover in check. Although on most occasions the relatively limited amount of white clover in the sward in the first harvest does not present a problem (Calder 1941, Scott 1973, Bean 1978).

In the second harvest year the area is managed to suit the clover, involving much heavier grazing in winter and spring to keep the ryegrass in check and to encourage the clover to dominate (Calder 1941, Jolly 1957, Scott 1973). The seed mixture of ryegrass and white clover may be either broadcast or drilled, depending on climate and situation, the drill tending to be used where conditions are dry at the time of sowing. Normally, white clover sown in autumn does not produce a great deal of seed in the first year unless the weather is exceptional. The best crop is usually obtained at the second harvest (Scott 1973). From the point of view of establishment either spring or early autumn sowing is satisfactory (Calder 1941).

The management of white clover seedcrops aims at encouraging vigorous plant growth and strong root development during autumn, winter and spring. Foliage growth is kept under control by defoliation until the flower heads begin to appear. At this stage the plants expend their energy on seed rather than leaf production (Harvey 1970, Scott 1973). The common management system that has been used in New Zealand for many years consists of the control of growth of the sward in the autumn by even grazing to about 1.25 - 2.5 cm. Later, the growth is allowed to recover, and then grazed again, leaving about 1.25 - 2.5 cm of growth to go into the winter. If some growth occurs during winter the area can be grazed leniently by cattle or sheep. In spring the area should be grazed closely during the flush of growth and kept reasonably short until the middle or end of October or even into November in some seasons, depending on weather conditions. Generally, the area should be closed for a seed crop when the first flower heads begin to appear (Calder 1941, Smith 1942, Scott 1973). In more recent studies Clifford (1977, 1979), working with 'Grasslands Huia' and 'Grasslands Pitau' white clover stands on a moisture retentive silt loam soil in Canterbury, found that over two seasons mid-November closing resulted in highest seed yield. Closing later than this markedly reduced seed yield.

It has also been suggested that spraying the sward with herbicide can check grasses more efficiently than grazing or cutting, thus producing better domination by the clover. Several herbicides have been evaluated during different seasons of the year. For instance, dalapon

(2,2 - dichloro propionic acid) and asulam (methyl, 4-amino phenyl sulphonyl carbamate) favour white clover domination by controlling grasses, but extensive damage to clover plants can occur when they are applied on a short pasture where the clover is not protected by the grass canopy (Murtagh 1977, Haggar and Elliot 1978). In New Zealand and the United Kingdom carbetamide (D-N-ethyl-2-(phenylcarbamoyloxy) propionamide) and propryzamide (3,5-dichloro-N-(1,1-dimethylpropynyl benzamide) have been used as grass suppressant herbicides during the winter or early spring without damage to the white clover (Wasmuth and Miles 1972, Soper and Hutchinson 1974, Haggar and Oswald 1975, Haggar and Squires 1979, Haggar and Bastian 1980). Paraquat (1,1' - dimethyl-4,4' - bipyridylium ion) is a common herbicide that has been advocated for selective control of grasses in white clover seed crops and for inducing legume dominance in pasture swards (Blood 1962, Sharp 1968, Zaleski 1970, Clifford 1980). Scott (1973) suggests the application of paraquat in late September or early October. He recommends grazing the sward until mid-September followed by spraying with paraquat in early October. He does not recommend application later than this time because paraquat may damage or depress white clover regrowth and subsequent flowering. He also found that paraquat application under cool conditions reduces the recovery of clover plants and may seriously affect seed yields.

Soil type has a strong influence on the length of time in the spring during which the crop can be grazed. Seedcrops grown on land with a good supply of moisture and nutrients can be closed or sprayed later than those on light dry soils. In general, the recommendation is to close the area early rather than too late (Jolly 1957, Scott 1973, Clifford 1980).

Seed ripeness in white clover is usually defined by farmers in terms of seed colour and consistency. White clover crops are ready for harvest when seeds are hard and bright yellow in colour with 80% of inflorescences having turned brown (Zaleski 1970, Scott 1973). However, the optimum time to cut clover for seed is more difficult to determine since flowering takes place over a long period and is dependant on weather conditions. In New Zealand, peak flowering normally occurs

between the second week of December and the first week of January and seed harvesting is usually done at the end of January or in February (Clifford 1980). The ideal weather for harvesting is hot and dry without heavy winds to scatter the cut material. Rain during harvesting can seriously reduce seed yield and decreases the quality of the seed. Approximately 15 - 21 days of dry weather before threshing the seed would be ideal for the harvesting of white clover seed (Jolly 1957).

In New Zealand both swath - and direct methods can be used for harvesting white clover seedcrops. In swath harvesting the crop is cut with a mower, the swath allowed to dry and a side rake or hay rake used to draw the crop into windrows. After natural drying, the crop is picked up and threshed. In direct harvesting, the processes of cutting and combining are performed together at a later stage of crop maturity (Scott 1973). In recent years chemical sprays with paraquat or diquat which dessicate the foliar parts have been widely used. This has greatly facilitated the harvesting of white clover (Leonard 1964, Logan and Arnst 1974). Seed germination is not affected as a result of such dessication (Deakins and Osmrod 1956).

Another important practice to increase white clover seed production is by placement of beehives in the seed crop. White clover is a self sterile plant requiring cross pollination before it can set seed (Chapman and Carter 1976). Pollination is more straight forward than in red clover, as the tripping mechanism is simple to operate and the corolla tube short so that it may be visited by honey or bumble bees, both being generally successful in achieving pollination (Win Pe 1978). As a result of a survey Haggar *et al* (1963) found that beehives in close proximity to a field of white clover could increase seed set and seed yield by 20 - 40%. They recommend one beehive for every 1.2 - 1.6 ha of crop, but New Zealand results suggest that one beehive per 3.2 ha is ample (Palmer *et al* 1962). In general, placement of beehives in the vicinity of seedcrops improves seed yield as a result of better pollination and seed setting.

2 - Effect of Defoliation on Vegetative Growth, Reproductive Growth and Seed Production

Defoliation is considered to be a disturbance to normal plant development and is defined as a removal of any part of the plant (Harris 1978). The amount of defoliation may be considered in terms of frequency, intensity and timing. 'Frequency' can be defined as the time interval between successive defoliations; 'intensity' as the proportion removed at a particular defoliation; and 'timing' as the number of defoliations carried out in relation to the developmental phases of plants and the season of the year (Harris 1978).

Defoliation may occur as a result of grazing, cutting, herbicides or some environmental factors, e.g. hail, wind, etc. However, only the first three will be discussed. Grazing is made by animals, generally cattle or sheep and is characterized by a spatial heterogenity of removal in both vertical and horizontal dimensions. In general, animals graze from the top to bottom of the sward and in patches, which leads to unevenness in the severity of removal both within and between plants. In addition selectivity results in the preferential consumption of young lamina before other plant parts (Watkin and Clements 1978). Cutting and spraying differ from grazing in the greater uniformity of removal or destruction of plant material on a single occasion (Rolston and Chu 1976). Another difference between grazing and cutting or spraying is that grazing causes more prolific branching as a result of apex removal (Harris 1978).

A number of workers have reported decreases in dry matter with more frequent and intensive defoliations (Alcock 1964). In fact, defoliation induces a carbon shortage in plants since the removal of transpiring photosynthetic tissue impairs assimilate production. This causes a redistribution of energy and nutrient sources in plants and affects root activity (Davidson and Birch 1972). At the same time, the removal of terminal buds in various developmental stages creates a new pattern of bud dominance (Tainton and Booysen 1963) which affects vegetative growth, reproduction and plant survival of white clover as well as sward structure (Tainton 1970, Harris 1978, Chapman 1983). This

suggests that the terminal bud exerts an inhibitory influence on some lateral buds preventing their development. The reason for this pattern of growth has been explained by early work in the field of apical dominance, as a result of an inadequate supply of assimilates to the buds (Loeb cited by Phillips 1969, Goebel cited by Pascoe 1973). Later, Phillips (1969) stated that indole acetic acid (IAA) formed in the apex was involved in preventing nutrients from reaching the axillary buds, possibly by inhibiting vascular connections. Regrowth following grazing in white clover is characterized by an increase in the development of axillary meristematic zones followed by an increase in the number of branches and leaves per unit area of the sward (Brougham 1966). In the same way, the regrowth of clover after cutting or spraying is followed by an increase in the number of lateral buds and branches, which leads to an increase in the number of leaves (Davidson 1969, Rolston and Chu 1976).

In general, close grazing by sheep in a ryegrass and white clover sward encourages vigorous stolon branching in the clover (Haggar *et al* 1963), but once the canopy closes during regrowth, shading results in a reduction of light, which in turn decreases new stolon formation (Zaleski 1970). After complete light interception the death of new initiated stolons starts and continues as new stolons and their leaves become progressively shaded (Hunt 1968). Defoliation of the clover plant affects not only branch numbers but also branch growth. For example, Mitchell (1956a) in work on white clover seedlings found poor stolon elongation with defoliated plants. This effect was greater with an increase in temperature.

The influence of defoliation on leaf growth has been studied extensively for many years. In a pure white clover sward, defoliation caused a decrease in leaf development and the ceiling leaf area index (L.A.I.) occurred twenty days later than in undefoliated swards (Brougham 1958). Similar results were obtained by Mitchell (1956a) in white clover under a controlled environment and at a temperature of 22°C. He found that defoliation retarded the weekly rate of leaf appearance from 1.3 to 1 per week. When defoliation was stopped each succeeding leaf increased in area at a rate similar to that of non-defoliated seedlings. The

pattern of leaf growth after defoliation was explained by Carlson (1966) as an effect of growth regulating substances, which may influence metabolite utilization after defoliation or maintain low levels of endogenous activities, which may decrease the production of metabolites by the developing leaves. Petiole length and weight are also severely reduced by defoliation (Mitchell 1956a, Brougham 1958, Carlson 1966) although they gradually increase during regrowth. However, Pascoe (1973) found significant differences in petiole length and weight depending on the frequency and intensity of defoliation.

The general effect of defoliation on root growth of white clover is to reduce root elongation as well as root weight (Mitchell 1956b, Butler *et al* 1959, Evans 1973). It has also been reported that severe defoliation causes a significant decrease in nodule number and changes in nodule colour (Butler *et al* 1959, Chu 1971). In young white clover plants Evans (1973) observed a decrease in the relative rate of nitrogen assimilation of roots compared with non-defoliated plants.

In view of the prostrate growth form of white clover it might be expected that the effect of defoliation would be mainly to remove leaf with little damage to shoot apices. This is in contrast to the effect on more upright species in which the damage to shoot apices may be considerable. Thus, Haggar *et al* (1963) showed that continuous grazing by sheep during spring increased the number of inflorescences and final seed yield in white clover. Clifford (1980) also found that, with adequate soil moisture, frequent grazing until mid-November gave the best balance between flower head number and seed yield, as well as the maximum concentration of heads in comparison with earlier closing dates. He also observed that earlier closing resulted in loss of early formed heads through deterioration under the canopy, while later closing resulted in a much larger percentage of heads supported by short stalks which were subsequently lost at harvest. Clifford (1979) in other experiments pointed out that defoliation after mid-November caused drastic reductions in head numbers and no improvements could be obtained by irrigation. These investigations also showed that the greater rate of inflorescence production was probably due to a greater degree of branching in defoliated swards. This resulted in a higher number of

shoots apices being available for inflorescence production. In the same way it was shown that defoliation during advanced flowering sharply reduced inflorescence density and consequently seed yields. Similar results were found by Rossiter (1961, 1972), Collins and Aitken (1970), Hagon (1973), and Collins (1978), which clearly confirm that frequent defoliation enhances branching and inflorescence number in subterranean clover. However, it is possible that defoliation also causes a faster rate of leaf and inflorescence production on individual branches. This effect has been reported by Rossiter (1972) for infrequently defoliated swards.

In white clover, more recent work by Thomas (1981b) has indicated that late defoliation (e.g. mid-November) delayed flowering and inflorescence formation. He also reported that defoliation had a stimulatory effect on inflorescence initiation in plants that had stopped initiation in long days. This effect has been previously observed in 'Grasslands Huia' white clover grown in controlled environments (Thomas 1979).

Zaleski (1961, 1970) studied the effects of defoliation on seed production in a pure stand of white clover and reported that the total number of inflorescences formed under different cutting treatments appeared to be affected by the season more than by any other character. In wet seasons the lowest number of heads were produced in comparison with dry seasons. Wet weather conditions resulted in lush vegetative growth and sprouting of seed, which caused an extremely low yield of seed of poor quality. Zaleski's experiments pointed out that the highest numbers of inflorescences were obtained in late defoliated treatments, and the lowest numbers in undefoliated treatments. He also found that the main effect of defoliation was on the number of flower heads produced. Similar results have been obtained in New Zealand by Clifford (1977, 1979, 1980) using 'Grasslands Huia' white clover. He found a greater amount of inflorescences and seed set when the last defoliation was made during mid-November. Earlier closings resulted in loss of early formed heads, while late closings resulted in inflorescences being lost by removal during defoliation.

H - Paraquat Properties

As paraquat was the herbicide applied in these studies a brief review of its properties will be described. Paraquat is normally employed as a dichloride salt and is the herbicide with most wide-spread use in agriculture (Harper and Harvey 1978). It is a bipyridylium non-selective weed killer with rapid dessicant action (Blood 1962). Although transported to a limited extent in the xylem (Baldwin 1963, Slade and Bell 1966) it is not metabolically degraded by plants (Akhavein and Linscott 1968). Irreversible adsorption of the herbicide on soil colloids and clay minerals ensures that residues in the soil are not available for root uptake. However, some uptake can occur in peat and other soils where paraquat is weakly adsorbed to organic components and strong adsorption on inorganic soil components occurs more slowly (Burns and Audus 1970).

Perennial plants with stolons or underground organs, although susceptible to top-kill by paraquat, generally regrow from the base (Brian *et al* 1958). This can be attributed not only to the inherently lower toxicity of this compound in non-photosynthetic tissues but also to its very limited movement out of the foliage (Baldwin 1963, Summers 1980). This lack of translocation would appear to be an important factor for white clover tolerance and recovery from paraquat application.

CHAPTER II

EFFECTS OF CLOSING DATE AND TIMING OF PARAQUAT APPLICATION ON VEGETATIVE AND REPRODUCTIVE GROWTH OF HUIA IN MIXED SWARDS

A - FIELD EXPERIMENT 1982 - 1983

1 - Introduction

In species with a determinate growth pattern the onset of reproductive growth generally marks the cessation of active vegetative growth. In white clover, however, the plant's indeterminate growth habit results in active vegetative growth continuing during reproductive development. This partitioning of vegetative and reproductive growth complicates management decisions for clover seed production. The aim of the present field experiment was to study the effects of different closing dates and the timing of paraquat application on the vegetative and reproductive growth of 'Grasslands Huia' white clover growing in a mixed sward with ryegrass.

2 - Materials and Methods

a - Experimental site and field procedures

This experiment was conducted in 1982/83 on the Pasture and Crop Research Unit, Massey University. The soil type was a Tokomaru silt loam, of moderate fertility (Appendix 1). Details of the prevailing temperature, precipitation, wind speed and soil moisture content are presented in Appendices 2 and 3.

The sward was a 'Grasslands Huia' white clover: 'Grasslands Ruanui' perennial ryegrass association in its second year from sowing, with a balance of approximately 20% white clover and 80% grasses. Prior to the commencement of the experiment, the field had been grazed with sheep whenever the herbage reached a height of 10 - 20 cm. The residual herbage following grazing was approximately 1.5 - 2.5 cm in height. The last preparatory grazing was completed on 1 August

1982. The experimental area had received a spring application of 200 kg/ha of 30% potassic superphosphate prior to the beginning of the experiment.

The experiment utilised a split-plot design with the main plots for grazing dates, subplots for time of paraquat application, and three replications. The areas per replicate, mainplot, and subplot were: 384 m^2, 96 m^2, and 24 m^2 (8 x 3 m^2), respectively (Figure 3).

Grazing started in mid-August, and continued at fortnightly intervals involving two or three days at each grazing time until the required dates of closing were reached. The closing dates chosen were 15 September, 15 October, 15 November and 15 December. After each grazing, all remaining herbage was cut with a rotary mower to a height of 2.5 cm and the cut material removed from the plots.

Three times of paraquat application were compared: viz. 30 days before closing, at closing, and 30 days after closing. The last grazing in treatments designated 'at closing' in fact involved closing 7 days before spray application on 15 October, 15 November or 15 December. This was necessary to allow sufficient regrowth after grazing to provide sufficient green material for improved paraquat effectiveness. This gave a total of 16 treatments (4 closing dates x 3 paraquat applications plus a control plot without herbicide) with three replicates which gave a total of 48 plots (Table 1). Paraquat[1] was applied at the rate of 300 g ai per hectare plus 0.5% of surfacant[2] with water at a volume equivalent to 250 l/ha. Spraying was carried out using a knapsack sprayer with one TJ.8003 fan nozzle at a pressure of approximately 1.3 kg/cm^2 (Plate 1). Two beehives were placed at each side of the field experiment.

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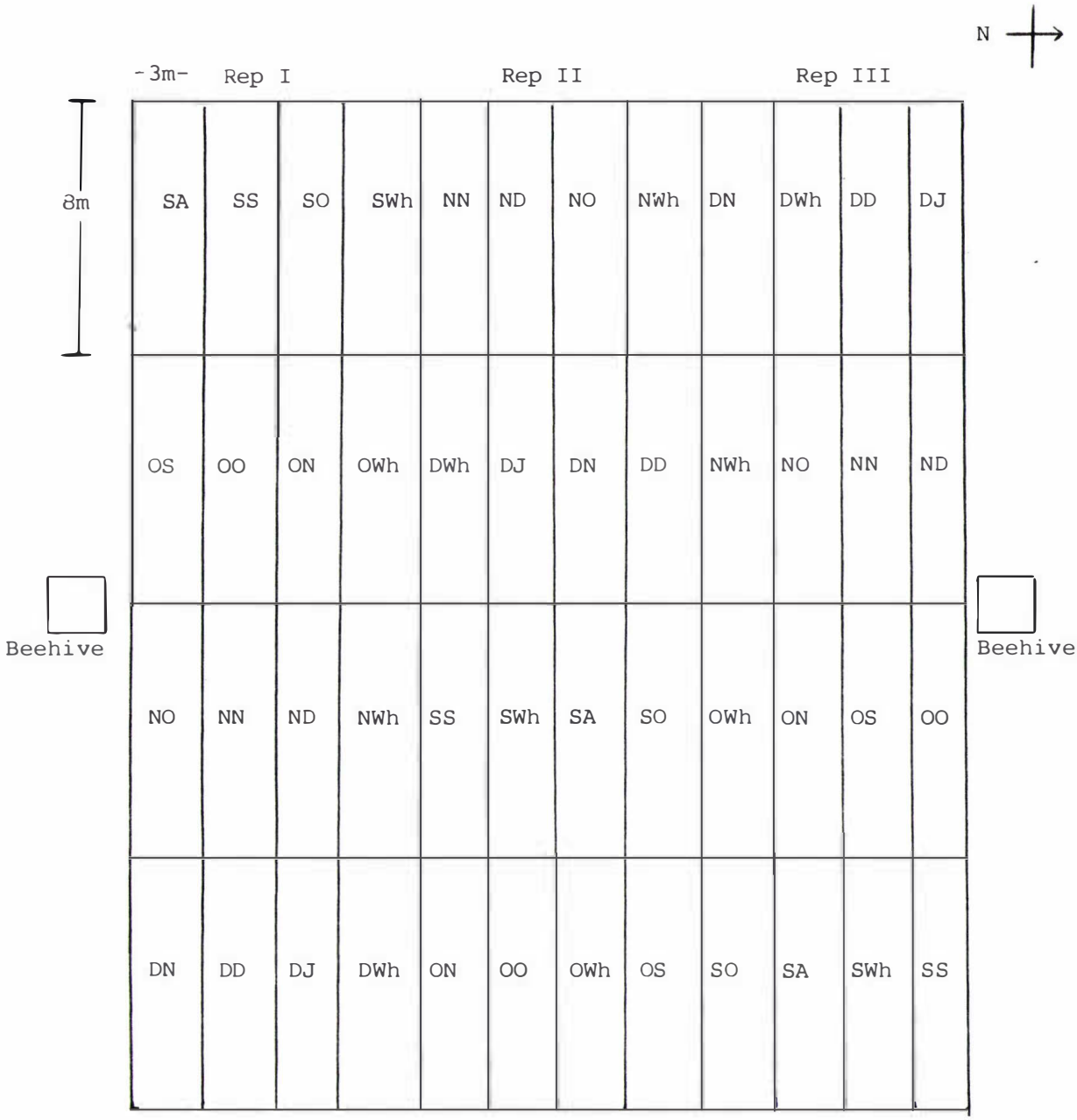
1 200 g/l a.i. of bipyridylium present at dichloride salt in the form of aqueous concentrate.

2 'Shell Super Spread' containing 30% active alkylphenol ethylene oxide condensate, in a liquid nonionic form.

Plate 1: Knapsack sprayer used for paraquat application



Closing Time

September	(S)
October	(O)
November	(N)
December	(D)

Spraying Time

August	(A)
September	(S)
October	(O)
November	(N)
December	(D)
Without herbicide	(Wh)

Figure 3: Trial Layout. Field experiment 82/83

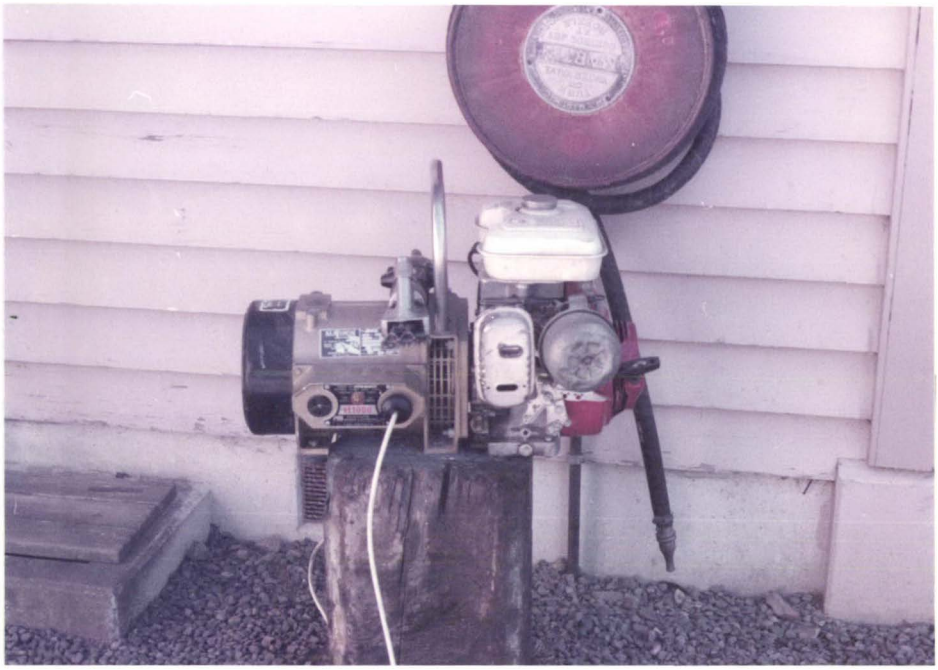


Plate 2: Portable shearing machine used in cutting treatments

During the flowering period, all treatments were sprayed with malathion to control insects, particularly *Aphis* spp., which causes severe damage to buds, flowers and pods. To avoid bee death the entrance to each hive was blocked with rolled newspaper immediately prior to spraying in the evening. The newspaper was removed 12 hours after spraying.

Table 1: Treatments used and their identification 1982/83

Closing Date	Time of paraquat application	Treatment Identification
15 September	30 days before closing	S - A
	At closing	S - S
	30 days after closing	S - O
	Without herbicide	S - Wh
15 October	30 days before closing	O - S
	At closing	O - O
	30 days after closing	O - N
	Without herbicide	O - Wh
15 November	30 days before closing	N - O
	At closing	N - N
	30 days after closing	N - D
	Without herbicide	N - Wh
15 December	30 days before closing	D - N
	At closing	D - D
	30 days after closing	D - J
	Without herbicide	D - Wh

b - Plant measurements and statistical analysis

At 15 day intervals after the completion of each treatment two quadrats (0.25 m^2) were cut to ground level using a 'Sunbeam' portable shearing machine (Plate 2). Sampling was continued until two weeks before seed harvest. The samples were weighed and subsamples (200 g) taken for botanical analysis. Herbage was dried at 80°C in an air oven to constant dry weight (approximately 24 h). The effect of

treatments on sward composition and white clover content was determined by dissecting the herbage subsamples into four components i.e. white clover, grass, dead material and weeds. Each component was calculated on a dry matter per unit area basis. The leaf area of the clover as also measured, using an automatic leaf area meter (Hayashi Denko, Model AA M-7).

Every 30 days after the completion of each treatment, two quadrats (10 x 25 cm) were dug from the field. The soil was removed by washing and the stolons were separated from the rest of the vegetative material. Stolon samples were weighed, and subsampled (200 g) to determine the number of nodes and terminal buds. The stolon subsample was used to estimate the number of nodes/dm^2, and the number of terminal buds/dm^2.

The number of white inflorescences was recorded in two quadrats (0.25 m^2) per plot every five days. These values allowed the peak flowering date of the plots to be determined.

Seed yield was determined by harvesting four quadrats (0.25 m^2) per plot at 25, 30, 35 and 40 days after peak flowering. At each seed harvest date, seed yield per unit area and seed yield components were measured. These included the total number of inflorescences per unit area, number of florets per inflorescence, number of seeds per inflorescence, number of seeds per floret and 1000 seed weight. 1000 seed weight was determined by counting and weighing two lots of 100 seeds at each harvest time. The number of seeds per inflorescence and seeds per floret were calculated from the seed yield and seed yield components according to the following formula:

$$\text{seeds set/floret} = \frac{1000 \times SY}{SW \times NI \times NF}$$

Where SY = Seed yield in g/m^2

SW = 1000 seed weight in g

NI = Total number of inflorescences at each harvest

NF = Total number of florets at each harvest

In order to determine the germination percentage, four replicates of 50 seeds from each treatment were germinated on top of paper and covered with a flat plastic lid. They were kept in a cabinet at a constant temperature of 20°C under continuous light. Seedling counts were made at 4 and 7 days according to the ISTA Rules (1976). Hard seeds remaining at the end of the test were scarified, germinated and finally the percentage of each seed or seedling category was calculated i.e normal and abnormal seedlings, and hard, fresh ungerminated and dead seeds.

Data for most characters were analysed according to the procedure of a split-plot design (Mead and Curnow 1983) and also by regression analysis (Draper and Smith 1981) using Genstat (Alvey *et al* 1981).

3 - Results

a - Effect of treatments on dry weight of grass and dead matter

Tables 2 and 3 show the changes in dry weight of the grass and dead matter components in swards depending on the time of closing and spraying. Because between replicate variation was extremely high (C.V. % up to 120%) no statistical analysis was performed for these variables. Nevertheless, some interesting trends were observed. All plots sprayed with paraquat at 0.3 kg ai./ha showed a reduced level of grasses between 70% and 100%, compared to the control plots (without herbicide). In general, greater grass control was obtained in sprayed plots which were closed in November or December. This suggests paraquat is an important and effective management tool by allowing more space for white clover to grow. Treatments without herbicide showed lower grass dry weights at the later closing times. S - Wh plot showed the highest dry weight of grass, followed by O - Wh and N - Wh plots and the lowest grass dry weight was present in D - Wh plots. In this way, grass competition in plots without paraquat was more severe in September and October than in November and December. Treatments sprayed 30 days after closing showed higher grass dry weight than those sprayed at closing time or 30 days before closing (with the exception of O - N treatment). In general, in the

Treatments	Date of Sampling day-month									
	1-10	15-10	1-11	15-11	1-12	15-12	1-1	15-1	1-2	15-2
S - A	8.27	3.89	4.56	26.63	1.26	0.00	8.67	0.32		
S - S	1.09	5.04	2.87	6.76	28.19	4.00	26.00	16.48		
S - O			58.61	52.46	42.66	93.65	97.00			
S - Wh	50.35	112.08	198.02	282.39	460.65	639.63	412.67			
O - S			3.66	26.71	51.95	0.43	9.00	20.25		
O - O			4.15	15.39	72.07	74.56	13.00	14.03	2.88	19.36
O - N					0.74	2.45	1.67	0.96	19.40	
O - Wh			41.24	174.52	288.90	311.57	443.33			
N - O					0.87	0.00	16.00	4.21	2.08	4.21
N - N					0.00	0.00	1.67	4.00	0.53	
N - D							18.67	19.93	28.40	15.09
N - Wh					19.25	62.56	130.33	144.48		
D - N							5.00	2.00	6.00	3.00
D - D							4.00	9.00	0.00	
D - J									0.00	0.00
D - Wh							62.00	88.00	90.00	

Table 2: Effect of closing time and paraquat treatments on grass dry weight (g/m^2) 1982 - 1983

Treatments	Date of Sampling day - month 1-10	15-10	1-11	15-11	1-12	15-12	1-1	15-1	1-2	15-2
S - A	0.0	1.1	1.9	14.4	25.9	15.8	8.7	27.5		
S - S	17.9	1.8	0.7	7.4	27.2	46.7	14.7	24.1		
S - O			81.5	37.7	13.8	30.3	19.0	40.4	29.5	72.1
S - Wh	0.1	0.2	0.9	28.3	52.2	78.4	90.7			
O - S			3.5	3.4	12.1	26.8	17.0	35.8		
O - O			42.6	10.1	8.8	10.0	14.0	64.1	25.3	51.8
O - N					81.1	47.1	10.7	37.7	30.2	
O - Wh			2.3	3.4	14.6	23.6	72.7			
N - O					8.3	2.0	8.0	15.1	27.3	54.1
N - N					7.9	7.5	8.3	14.1	14.7	
N - D							81.7	77.2	24.6	45.1
N - Wh					2.4	4.6	8.7	43.4		
D - N							4.0	18.1	12.8	44.6
D - D							37.3	5.3	14.9	21.1
D - J									96.2	101.9
D - Wh							4.3	38.9	25.3	

Table 3: Effect of closing time and paraquat treatments on dead matter dry weight (g/m^2) 1982 - 1983

first treatments (S - O, N - D and D - J), grasses continued growing following the last grazing until paraquat application, 30 days after closing. In other treatments, grasses were controlled before (30 days) or at the same time sheep were removed from the field causing less competition to the clover. Paraquat was less effective in S - O, N - D and D - J treatments due to the denser canopy formed by the grasses, which reduced the level of control of these species.

Total dead matter dry weight levels were generally low in most closing time and spraying treatments. The exception occurred in plots which were not sprayed until 30 days after closing (S - O, O - N, N - D, and D - J), where the dry weight of dead material tended to be higher than in other treatments. In all plots with zero paraquat, the amount of dry weight of dead material was low 15 days after closing, increasing later due to the death of the bottom leaves of the species present in the sward.

b - Effect of treatments on white clover dry weight

The results presented in Table 4 show the changes in total clover dry weight, in g/m^2 at 2 weekly intervals. The values are replicate means.

The growth of clover comprises two phases in all treatments, except for D - N, D - J, and the unsprayed control treatments. In plots closed in September, white clover matter accumulation increased over the first 10 - 12 weeks. One probable explanation of this increase is that immediately following the destruction of plant tissue by paraquat application, there was a strong reduction in ryegrass density. The decreased competition on the white clover, occurred through the creation of more space for its growth and development. As growth increased, the percentage of ground covered by the clover plant also increased, so that by the 10th or 12th week it is likely that maximum absorption of light energy was obtained. Subsequently, growth rate entered a second phase characterised by reduced dry matter accumulation. It is possible that the decomposition of plant tissues in the lower layers of the canopy could have influenced clover dry weight, although

Treatments	Date of Sampling day - month									
	1 - 10	15 - 10	1 - 11	15 - 11	1 - 12	15 - 12	1 - 1	15 - 1	1 - 2	15 - 12
S - A	78±8.0	112±12.0	206±25.7	221±27.6	256±15.3	267±30.9	257±36.8	192±29.9		
S - S	25±5.2	67± 9.3	120±21.7	175±26.5	186±26.7	282±46.1	237±34.9			
S - O			27± 4.5	109±17.2	137±19.5	184±29.1	207±28.0			
S - Wh	34±4.0	39± 8.5	87± 3.5	59± 6.4	49±11.0	48±11.0	54±12.0	56±17.0		
O - S			57± 5.1	125±17.9	172±30.4	277±46.7	295±46.0	319±33.0		
O - O			18± 2.1	58± 6.1	117±21.4	186±19.7	226±44.5	278±42.6	149±26.6	78±10.4
O - N					23± 8.6	145±16.8	192±15.5	230±39.3	197±23.8	
O - Wh			17± 4.2	39± 8.6	44± 8.8	106±12.1	117±16.9			
N - O						105±18.4	199±22.8	267±47.8	206±34.2	140±30.5
N - N						107±14.4	144±25.9	215±35.0	199±23.4	
N - D							92±17.4	149±21.7	189±22.9	109±15.7
N - Wh						49± 6.7	94±14.0	149±21.3		
D - N							114±24.9	251±20.1	195±26.1	190±21.9
D - D							54± 8.0	98±19.2	164±16.6	142±34.6
D - J									82±12.7	94±25.5
D - Wh							50± 6.5	91±20.2	97±20.1	

Table 4: Effect of closing time and paraquat treatments on white clover dry weight (g/m^2) (± standard errors) 1982 - 1983

other factors such as a limiting supply of plant nutrients or water in the soil could have also affected dry matter production. In fact, soil analysis of the experimental area indicated low amounts of calcium, magnesium, and potassium, although other elements such as iron and manganese were considerably above the desired level (Appendix 1). The soil moisture content (Appendix 3) taken every 15 days in each plot also showed a decrease of 5% or 6% between December 15th and January 15th for plots closed in September and sprayed in August or September (S - A and S - S). In the plots closed in September and sprayed in October (S - O) a decrease in clover dry weight did not occur during the experimental period. This may have reflected the increased mulch of dead material produced by the ryegrass after the application of paraquat, which may have reduced surface water evaporation. This effect could have been important despite the fact that soil moisture content was similar in all treatments in December and January.

Plots closed in September without herbicide application (S - Wh) showed a slight rise in clover dry weight for four weeks, followed by a more rapid increase in weight during the next two weeks. After this period, white clover dry weight decreased and later remained more or less constant until the plots were harvested in January. A comparison of the amount of growth in the control plots closed in September (S - Wh) and the remaining three treatments closed in the same month indicated that the control of grasses following spraying with paraquat in a mixed grass: clover sward induces more prolific regrowth and development of the clover. Similarly, plots closed in October, November or December also showed two phases of regrowth. The first phase was characterised by a rapid increase in clover dry weight. This was followed by a second growth phase in which there was generally a reduction in clover weight. The S - O and O - S treatments, (treatment closed in September and sprayed in October and closed in October but sprayed in September) were the only ones which continued to show a rise in dry weight during the experiment, with the exception of O - Wh treatment. It is also important to note that most of these treatments reached their maximum dry weight by January 15th, with the possible exception of the O - S, N - D, D - D and D - J treatments. The soil moisture content data shows that low availability of water by 1 February could have limited

further clover dry matter production during the second phase of growth (Appendix 3). Unsprayed plots closed in October, November or December (O - Wh, N - Wh, D - Wh) showed a continuous increase in clover dry weight with time.

c - Effect of treatments on white clover leaf area index

The effects of different treatments on leaf area index (LAI) are presented in Table 5. For S - A, S - S, O - S and O - O treatments (plots closed in September and sprayed in August or September and plots closed in October and sprayed in September or October) the LAI of white clover 60 days after the last grazing reached ceiling values of approximately 4.5, 4.1, 3.4 and 3.6, respectively. Thereafter, until the end of the experiment, only small changes occurred, with the exception of O - O treatment, which showed a strong reduction in this variable in February 15th. However, the LAI of the S - O treatment (plots closed in September and sprayed in October) reached a maximum of 2.4 ninety days after paraquat application. This difference could be due to the shading produced by the mulch of dead grass left after paraquat application. This mulch is likely to have intercepted almost all the incident light energy. The same trend can be observed in the unsprayed plots closed in September (S - Wh), where the first reaction of the clover plant was to increase its LAI to a peak value of 1.8. Thereafter, competition between the clover plant and the companion grasses caused a reduction in leaf area index.

The plants in the N - O plot (treatment closed in November and sprayed in October) also obtained maximum LAI 6 weeks after closing. This point may not have been reached in the N - N treatment before measurement was discontinued. The data suggest that maximum leaf area index is reached later when the last grazing or spraying is delayed. However, plots grazed or sprayed in December apparently did not have time to subsequently reach a maximum LAI value. Thus, plants in D - N and D - D treatments showed a rapid rise in LAI during the first 30 days, but only a slight changer after this time.

Treatments	Date of Sampling day - month						
	1 - 10	15 - 10	1 - 11	15 - 11	15 - 12	15 - 1	15 - 2
S - A	1.9±0.70	2.7±0.40	4.4±0.30	4.5±0.45	3.9±0.30	3.9±0.35	
S - S	0.7±0.15	1.7±0.25	2.9±0.35	4.1±0.45	4.0±0.86	4.0±0.52	
S - O			0.8±0.29	1.1±0.10	2.0±0.20	2.4±0.36	
S - Wh	1.0±0.15	1.3±0.21	1.8±0.29	0.9±0.15	0.3±0.06		
O - S			1.6±0.10	2.5±0.32	3.4±0.38	3.3±0.46	
O - O			0.6±0.05	1.3±0.06	3.6±0.38	3.5±0.41	0.8±0.15
O - N					3.3±0.39	3.2±0.31	
O - Wh			0.5±0.06	0.9±0.10	1.3±0.20		
N - O					2.1±0.31	3.4±0.45	1.8±0.30
N - N					1.9±0.21	3.8±0.45	
N - D						1.6±0.31	1.4±0.25
N - Wh					1.0±0.20	1.1±0.20	
D - N						2.4±0.31	2.4±0.36
D - D						1.0±0.25	1.3±0.21
D - J							1.3±0.25
D - Wh						1.2±0.26	

Table 5: Effect of closing time and paraquat treatments on white clover LAI (± standard errors) 1982 - 1983

The LAI of the O - O, N - O and N - D treatments was very low by February 15th. This decrease in LAI may be attributed to the low available water supply during February, when the moisture content in the soil fell to between 7% and 9%. The results also indicate that plants in D - N and D - D treatments show a rapid rise in LAI during the first 30 days, but only a slight change subsequently.

A comparison of the treatments involving different closing dates and spraying times showed that treatments receiving more grazings and which were closed later in the season tended to have a lower LAI than plants receiving fewer grazings and closed earlier. Thus, S - A and S - S treatments showed the highest values of LAI, followed by treatments closed and sprayed in October where spraying coincided with, or preceded, a November closing.

d - Effect of treatments on number of nodes and terminal buds

Monthly records of the number of nodes and terminal buds per dm^2 are shown in Tables 6 and 7. No major differences occurred between treatments sprayed with paraquat at all closing dates. However, there was a tendency for treatments closed in September to form fewer nodes fifteen days after closing, compared to similar treatments involving later closing dates. This difference might occur because treatments closed in September and sprayed with herbicide produced denser canopies than treatments closed later (October, November and December). This might cause a higher level of decay in branches under the canopy as a result of lower light intensity at the level of the stolons, and therefore, fewer nodes/unit area. The number of terminal buds per dm^2, was initially high in all treatments before falling in January and February. This reduction during the summer might be a reaction to prevailing high temperatures and low soil moisture contents. It is also likely to be associated with the shading effects of increasing dry weight and LAI previously described. Terminal buds are perhaps poorly placed in the competition for light, moisture and nutrients under these conditions. Fewer terminal buds were present in February in all treatments.

Treatments	Date of Sampling day - month 1 - 10	1 - 11	1 - 12	1 - 1	1 - 2
S - A	390 ± 79.6	539 ± 123.0	581 ± 49.3	594 ± 127.7	
S - S	392 ± 68.5	531 ± 150.0	555 ± 122.5	585 ± 88.1	
S - O		416 ± 71.5	646 ± 114.5	639 ± 121.0	208 ± 44.1
S - Wh	299 ± 75.0	411 ± 77.5	299 ± 41.0	104 ± 25.0	
O - S		645 ± 66.1	698 ± 71.6	695 ± 81.1	
O - O		505 ± 114.9	509 ± 78.7	434 ± 77.3	376 ± 52.5
O - N			563 ± 74.9	559 ± 93.1	593 ± 63.5
O - Wh		422 ± 100.7	440 ± 77.0		
N - O			643 ± 146.5	672 ± 120.7	655 ± 95.0
N - N			533 ± 101.6	674 ± 94.9	682 ± 112.5
N - D				447 ± 61.9	474 ± 76.2
N - Wh			421 ± 65.1	526 ± 87.3	
D - N				672 ± 127.1	572 ± 86.5
D - D				587 ± 85.2	423 ± 70.4
D - J					415 ± 76.2
D - Wh				516 ± 92.6	459 ± 78.1

Table 6: Effect of closing time and paraquat treatments on the number of nodes/dm^2 (± standard errors) 1982 - 1983

Treatments	Date of Sampling day - month 1 - 10	1 - 11	1 - 12	1 - 1	1 - 2
S - A	68 ± 7.8	85 ± 13.5	75 ± 15.3	52 ± 15.3	
S - S	52 ± 12.5	83 ± 14.7	73 ± 14.6	35 ± 8.0	
S - O		38 ± 7.4	87 ± 6.2	57 ± 7.7	9 ± 1.5
S - Wh	39 ± 7.6	39 ± 8.6	18 ± 5.5	7 ± 2.1	
O - S		102 ± 25.9	106 ± 22.6	87 ± 16.2	
O - O		54 ± 8.6	86 ± 16.2	49 ± 8.5	20 ± 2.5
O - N			67 ± 12.6	53 ± 8.5	23 ± 2.1
O - Wh		57 ± 8.9	57 ± 7.7	17 ± 2.5	
N - O			94 ± 25.3	91 ± 27.8	47 ± 6.2
N - N			82 ± 14.5	102 ± 23.8	54 ± 14.7
N - D				72 ± 12.5	37 ± 19.1
N - Wh			57 ± 12.1	64 ± 8.5	
D - N				101 ± 21.5	40 ± 3.7
D - D				107 ± 22.5	44 ± 8.5
D - J					33 ± 2.8
D - Wh				89 ± 13.5	28 ± 3.1

Table 7: Effect of closing time and paraquat treatments on the number of terminal buds/dm^2 (± standard errors) 1982 - 1983

e - Effect of treatments on the date of peak flowering

The progress of flowering was observed to determine the time of peak flowering in the field (Table 8). These observations were necessary to assess the date on which maximum flowers were present in the crop. In most treatments it was found that a delay in closing time delayed peak flowering, while paraquat treatments did not have the same effect. Within late closing dates, paraquat application had less effect on the date of peak flowering. In fact, with December closing, peak flowering occurred on the same date in all paraquat treatments, while with September closing there was a difference of 29 days among them. It was also observed that weather conditions (especially rain) caused major variation in the flowering behaviour of white clover plants causing the production of several peaks.

f - Effect of treatments on seed yield and yield components

The effects of time of closing and paraquat application on seed yield per unit area (kg/ha) are shown in Table 9. Because of the similarity of response in each treatment across four harvesting dates (25, 30, 35 and 40 days after peak flowering), only the results taken 30 days after peak flowering are specifically discussed. Closing time was responsible for major differences in the seed yield values obtained. Significant differences ($P < 0.01$) occurred between treatments closed in September and October compared with plots closed in November and December.

The differences in seed yield between paraquat sprayed treatments were relatively small, (a maximum of 30 kg/ha) but the control plots (without herbicide) gave extremely low seed yields at all harvesting dates (45 kg/ha in comparison with paraquat application at closing time which gave a seed yield of 141 kg/ha.)

The interaction between grazing time and spraying time for all harvest dates was highly significant. Highest seed yields were secured from S - O, O - O, N - O, N - N, N - D, D - N and D - D treatments. Paraquat sprayed plots closed in September showed that the S - O treatment (closed in September and sprayed in October), gave a

Treatments	Date day-month		Year	Treatment	Date day-month		Year
S - A	27	12	82	N - O	25	1	83
S - S	31	12	82	N - N	15	1	83
S - O	25	1	83	N - D	25	1	83
S - Wh	25	11	82	N - Wh	27	12	82
O - S	27	12	82	D - N	25	2	83
O - O	25	1	83	D - D	25	2	83
O - N	10	1	83	D - J	25	2	83
O - Wh	29	11	82	D - Wh	20	1	83

Table 8: Effect of closing time and paraquat treatments on the date of peak flowering 1982 - 1983

HARVESTING

Closing Time	Days after peak flowering 25	30	35	40
Mid-September	86.5	78.1	78.8	66.1
Mid-October	67.1	73.1	67.8	70.7
Mid-November	146.2	142.4	146.2	123.4
Mid-December	135.8	137.9	127.4	106.8
Significance	**	**	**	**
LSD 5%	39.2	22.3	34.1	29.6
1%	59.2	33.7	51.6	44.9
S.E.D. ±	16.0	9.1	13.9	12.1

Paraquat Treatments	Days after peak flowering 25	30	35	40
30 days before closing	140.3	132.8	132.5	107.3
At closing	137.1	141.2	146.0	119.2
30 days after closing	119.2	112.2	107.4	99.3
Without herbicide	29.0	45.4	53.2	41.2
Significance	**	**	**	**
LSD 5%	25.8	15.6	25.4	21.7
1%	35.1	21.2	34.6	29.5
S.E.D. ±	12.6	7.5	12.4	10.5

Significant Interactions

Treatments				
S - A	88.7	82.0	87.8	76.5
S - S	101.6	89.5	89.2	79.9
S - O	155.7	140.0	136.8	107.0
S - Wh	0.2	0.7	1.2	0.9
N - O	227.5	185.6	223.3	148.1
N - N	141.5	156.7	139.1	158.7
N - D	179.6	175.2	162.7	150.0
N - Wh	36.0	49.2	59.7	37.1
Significance	**	**	*	**
LSD(a) 5%	51.7	36.0	50.9	49.2
1%	70.0	52.0	69.2	71.1
S.E.D.(a) ±	27.0	16.0	25.5	21.9
LSD(b) 5%	60.8	31.2	57.4	43.4
1%	87.8	43.9	83.0	59.0
S.E.D.(b) ±	25.1	15.1	24.7	21.1

O - S	35.5	49.4	47.8	63.5
O - O	150.4	154.3	137.8	115.8
O - N	78.5	85.0	78.3	98.1
O - Wh	4.1	0.5	7.3	5.3
D - N	249.5	202.1	171.2	141.3
D - D	154.8	174.5	217.7	122.4
D - J	63.2	48.7	51.9	42.2
D - Wh	75.9	126.2	160.5	121.4

(a) When comparing means with different levels of main-plots

(b) When comparing means with the same levels of main-plot

Table 9: Effect of closing date and time of paraquat application on seed yield per unit area (kg/ha) at each harvest 1982 - 1983

significantly higher ($P < 0.01$) seed yield than the other two treatments closed in September and sprayed in August or September, (S - A and S - S). It was also interesting that seed yield from S - O plots was similar to other high yielding treatments which were sprayed later (except D - N). Similarly, comparisons between treatments closed in October showed that O - O, (closed in October and sprayed in October), was the highest seed yielding treatment with an advantage in yield of 67% and 45% over the other two treatments closed in October and sprayed in September or November (O - S and O - N).

Considering the timing of paraquat application in November closed treatments, highest seed yields were obtained in N - O, (closed in November and sprayed in October), followed by N - D and N - N (closed in November and sprayed in November or December) treatments. These latter two treatments were similar.

Paraquat treatments associated with a December closing time showed that the D - N and D - D (closed in December and sprayed in November or December) treatments had a considerably higher seed yield than the D - J (closed in December and sprayed in January) treatment and the unsprayed control plot (D - Wh). In general, all plots that were not sprayed with paraquat (S - Wh, O - Wh, and N - Wh) gave significantly low seed yields. The one exception was the D - Wh treatment (plots grazed until December but without any paraquat application), where a moderate yield of 126 kg/ha was obtained.

In general, late grazing to December resulted in less competition by grasses and therefore reasonable white clover seed yields were obtained. November application of paraquat gave the highest yield, but late closing, particularly in December still gave high seed yields provided paraquat application was applied at or before closing.

Within treatments involving paraquat application and grazing a wide variation in seed yield occurred (49 to 202 kg/ha). Although the poorest yields were very low by commercial standards the best treatments produced yields which are considered to be commercially acceptable, falling within the average range of 120-190 kg/ha quoted by Hampton and Scott (1983).

This tendency towards low seed yields in all treatments could be partly attributed to heavy rain and strong winds during the main flowering period. Such conditions would be likely to inhibit pollination and were observed to increase sprouting of seeds in the inflorescence. Paraquat sprayed treatments closed in September and October subsequently produced excessive vegetative recovery growth. In addition, heavy rain during December resulted in the majority of inflorescences (mainly brown flower heads) becoming lodged and a large proportion of seeds sprouted in the inflorescence as a result of the wet conditions.

It was also interesting to note that the lowest seed yield was obtained at the harvest made 40 days after peak flowering compared with the rest of the harvesting times (25, 30 and 35 days after peak flowering). This might occur because the peduncle of the inflorescence, when mature, is brittle and the inflorescences might shed under the canopy and decay or seed may sprout in the head.

Since major differences in seed yield occurred as a result of both grazing and spraying treatments, further investigation was carried out to determine the effect of these treatments on the components which influenced seed yield. Five seed yield components, inflorescence numbers/m^2, number of florets per inflorescence, number of seeds per floret, 1000 seed weight, and number of seeds per unit area were examined.

Inflorescence numbers/m^2 at harvest

The mean number of total inflorescences at the time of harvest is shown in Table 10. The number of inflorescences at all harvest dates was considerably affected by spraying time, but not by the time of grazing. The control plots (without paraquat), where a relatively low clover dry weight and low LAI had been obtained, produced fewer (205 - 275) inflorescences/m^2. Conversely, in plots where paraquat was applied 30 days before closing or at closing, the number of inflorescences/m^2 was significantly ($P < 0.01$) higher than in other treatments (462 - 717). Plots sprayed 30 days after the last grazing (S - O, O - N, N - D and D - J) were significantly different from

HARVESTING

Closing Time	Days after peak flowering 25	30	35	40
Mid-September	455	355	298	295
Mid-October	409	353	293	304
Mid-November	631	561	572	493
Mid-December	585	496	468	338
Significance	N.S.	N.S.	N.S.	N.S.
LSD 5%				
1%				
S.E.D. ±	69.8	59.3	62.0	70.2

Paraquat Treatments	Days after peak flowering 25	30	35	40
30 days before closing	717	576	500	462
At closing	616	547	484	445
30 days after closing	473	397	384	318
Without herbicide	275	245	268	205
Significance	**	**	**	**
LSD 5%	75.4	41.0	74.2	48.0
1%	102.5	55.7	100.8	65.2
S.E.D. ±	36.6	19.9	35.9	23.4

Significant Interactions

Treatments				
S - A	685	496	391	404
S - S	636	488	353	430
S - O	492	426	432	329
S - Wh	7	13	16	15
N - O	756	642	664	533
N - N	677	592	531	554
N - D	683	629	664	528
N - Wh	410	382	430	354
Significance	**	**	**	**
LSD(a) 5%	212.0	154.0	198.0	182.0
1%	306.2	222.0	286.0	263.0
S.E.D. ±	94.2	68.6	87.8	81.0
LSD(b) 5%	150.6	82.4	148.0	96.2
1%	204.7	112.0	201.0	130.8
S.E.D. ±	73.1	39.8	71.7	46.7

O - S	542	479	377	468
O - O	461	450	408	353
O - N	589	443	347	363
O - Wh	42	41	40	34
D - N	883	688	567	441
D - D	691	661	643	441
D - J	128	91	93	53
D - Wh	640	545	568	416

(a) When comparing means with different levels of mainplots

(b) When comparing means with same levels of mainplots

Table 10: Effect of closing date and time of paraquat application on the total number of inflorescences/m^2 present at each harvest 1982 - 1983

the unsprayed control treatment and produced an intermediate number of inflorescences/m^2 compared with earlier sprayed or unsprayed treatments.

The interactions for total number of inflorescences/m^2 were significant at each harvesting time. An examination of results obtained during the second harvest (30 days after peak flowering) indicated that control plots showed a significant reduction in inflorescence numbers, which was greater with early closing. The main exception occurred in the control plots closed in December. The D - J treatment also gave very low numbers of inflorescences/m^2 (91 - 128) because some of them were removed during the last grazing and others were destroyed by the application of paraquat.

Florets per inflorescence and seed per floret

Ten white inflorescences taken five days after peak flowering were selected at random from each treatment and the mean number of florets counted. The results in Table 11 show that neither closing time nor spraying treatments had any significant effect on the number of florets per inflorescence. Only the unsprayed control plots showed a significant reduction in floret numbers per inflorescence compared with the rest of the treatments. No interactions were significant.

Analysis of variance of mean seed set per floret (number of seeds per floret) (Table 12) indicates that closing time, spraying treatment and closing x spraying treatment interactions were all highly significant. Plots closed in December gave the highest seed set/floret (1.720), while early closing (September) resulted in florets with lowest seed set (0.608). In general, late closing tended to result in more seeds being formed per floret.

At each harvest time, spraying also influenced the number of seeds set per floret. Control plots (no paraquat) and plots sprayed either 30 days before closing or at closing did not differ significantly from each other. However, plants from these treatments produced fewer seeds/floret than plants in plots sprayed 30 days after closing time.

Table 11: Effect of closing date and time of paraquat application on the number of florets per inflorescence at peak flowering 1982 - 1983.

Closing time			
Mid - September	Mid - October	Mid - November	Mid - December
54	47	54	50
Significance	N.S.		
S.E.D. ±	2.2		

Paraquat treatments			
30 days before closing	At closing	30 days after closing	Without herbicide
55	55	52	44
Significance	**		
LSD 5%	4.24		
1%	5.77		
S.E.D. ±	2.1		

The closing time x spraying interactions indicated significant responses between treatments. Generally, plants closed and sprayed in November or December had a significantly higher seed set in comparison to plants closed in September or October (with the exception of S - O and O - O treatments). Both of these treatments were delayed in their peak flowering to 25 January when the conditions were apparently more favourable for pollination and seed set.

The present study showed a minimum seed set of 0.1 seeds/floret with a mean value of approximately 1 seed per floret. These values are much lower than the 2 seeds per floret obtained by Clifford (1979). This was probably a reflection of the strong winds and excessive rainfall during the flowering period. It is likely that these

HARVESTING

Closing Time	Days after peak flowering 25	30	35	40
Mid-September	.608	.676	.662	.672
Mid-October	.693	.831	.940	.933
Mid-November	.824	.859	.870	.815
Mid-December	1.100	1.307	1.427	1.720
Significance	*	**	**	**
LSD 5%	.245	.144	.158	.381
1%	N.S.	.218	.240	.577
S.E.D. ±	.1001	.0587	.0647	.1556

Paraquat Treatments	Days after peak flowering 25	30	35	40
30 days before closing	.070	.724	1.009	.756
At closing	.852	.917	1.039	.936
30 days after closing	1.206	1.315	1.328	1.712
Without herbicide	.532	.717	.852	.733
Significance	**	**	**	**
LSD 5%	.178	.159	.187	.419
1%	.242	.216	.255	.569
S.E.D. ±	.0865	.0771	.0910	.2035

Significant Interactions

Treatments				
S - A	.620	.603	1.543	.703
S - S	.663	.703	.913	.633
S - O	1.043	1.107	1.120	1.007
S - Wh	.107	.290	.390	.343
N - O	.950	.937	1.007	.833
N - N	.937	.927	.960	1.047
N - D	.980	.937	.773	.930
N - Wh	.430	.637	.743	.450
Significance	**	**	**	**
LSD(a) 5%	.405	.328	.383	.867
1%	.586	.474	.554	1.252
S.E.D. ±	.1802	.1458	.1704	.3852
LSD(b) 5%	.356	.318	.375	.838
1%	.484	.408	.509	1.139
S.E.D. ±	.1730	.1543	.1821	.4069

O - S	.337	.460	.550	.573
O - O	1.067	1.183	1.203	1.140
O - N	.660	.863	1.043	1.167
O - Wh	.710	.817	.963	.857
D - N	.893	.897	.937	.913
D - D	.740	.857	1.080	.937
D - J	2.140	2.353	2.377	3.747
D - Wh	.627	1.123	1.313	1.283

(a) When comparing means with different levels of mainplots

(b) When comparing means with the same levels of mainplots

Table 12: Effect of closing date and time of paraquat application on the seed number per floret present at each harvest 1982 - 1983

conditions encouraged vegetative growth of the clover and discouraged effective pollination and seed set. During this year wind-run was high throughout the experiment until February and rainfall in December and January (period of maximum flowering in white clover) was also high with 92 mm more precipitation than the twenty-year average (Appendix 2).

Seed Weight

The results of 1000 seed weight measurements show that closing time and spraying time significantly affected seed weight (Table 13). The 1000 seed weight from November and December closings was higher than from the September closing at all harvest dates. Significant differences also occurred between September and October closings at the first harvest 25 days after peak flowering. October and November closings were only significantly different at the second harvest. Clover plots which had been sprayed with paraquat ultimately produced heavier seeds than treatments without herbicide. The differences within paraquat treatments were highly significant at all four harvest dates.

The interactions of closing time and spraying time were not significantly different for the harvest made 35 days after peak flowering but were highly significant ($P = 0.01$) at other harvest times. Eight treatments (S - O, O - O, N - O, N - N, N - D, D - N, D - D and D - J), produced seeds with a 1000 seed weight of more than 5.0g; with lightest seeds occurring in inflorescences from the S - Wh treatments. The remaining treatments formed seed with 'intermediate' seed weights. However, even the heaviest seeds were lighter than the 1000 seed weight of 0.6 g described by Clifford (1979) in his experiments with this species. A comparison of treatments at any one closing time suggested that grass competition had a major effect on the production of lighter white clover seed.

HARVESTING

Closing Time	Days after peak flowering 25	30	35	40
Mid-September	.3482	.4286	.4176	.4599
Mid-October	.4290	.4628	.4747	.4847
Mid-November	.4632	.5071	.5134	.5402
Mid-December	.5082	.5258	.5322	.5481
Significance	**	**	**	**
LSD 5%	.0408	.0225	.0551	.0396
1%	.0618	.0341	.0834	.0601
S.E.D. ±	.166	.091	.225	.162

Paraquat Treatments	Days after peak flowering 25	30	35	40
30 days before closing	.4490	.4927	.5038	.5267
At closing	.4950	.5152	.5198	.5346
30 days after closing	.4849	.5068	.5070	.5305
Without herbicide	.3198	.4097	.4073	.4412
Significance	**	**	**	**
LSD 5%	.0531	.0214	.0620	.0342
1%	.0722	.0291	.0842	.0464
S.E.D. ±	.258	.104	.301	.166

Significant Interactions

Treatments			
S - A	.3473	.4268	.4786
S - S	.4196	.4595	.5207
S - O	.5166	.5088	.5471
S - Wh	.1093	.3196	.2930
N - O	.5324	.5533	.5765
N - N	.4472	.5048	.5262
N - D	.4910	.5446	.5693
N - Wh	.3822	.4256	.4888
Significance	**	**	**
LSD(a) 5%	.1075	.0456	.0742
1%	.1553	.0659	.1072
S.E.D. ±	478.2	202.89	330.3
LSD(b) 5%	.1065	.0430	.0683
1%	.1447	.0585	.0929
S.E.D. ±	.517	.208	.332

O - S	.3893	.4401	.4643
O - O	.5699	.5402	.5379
O - N	.4319	.4738	.4958
O - Wh	.3248	.3973	.4409
D - N	.5271	.5507	.4268
D - D	.5430	.5563	.4594
D - J	.5000	.4999	.5088
D - Wh	.4628	.4962	.3196

(a) When comparing means with different levels of mainplots

(b) When comparing means with the same levels of mainplots

Table 13: Effect of closing date and time of paraquat application on 1000 seed weight (g) at each harvest 1982 - 1983

g - Effect of treatments on seed quality

The quality of seeds in terms of percentage of normal seedlings, abnormal seedlings, hard seed, dead seed and viable seed content was examined. Results are presented in Figures 4 and 5, Appendices 4, 5, 6, 7 and 8.

Percentage of normal seedlings

Closing time had no significant effect on the germination percentage of seed, when clover was harvested 25 days after peak flowering. Seed from clover harvested at 30 - 35 and 40 days after peak flowering showed highly significant differences with the highest percentage of normal seedlings in treatments closed in November or December. The germination capacity of seed in the last two closing dates generally decreased, partly due to an increase in hard seed content. Even though some differences in normal seedling percentage occurred in different treatments and at different harvest times the overall percentage was low, with the maximum values up to 18%, but generally in the 10 - 13% range.

The effect of paraquat treatments on the percentage of normal seedlings was significant in the second harvest (30 days after peak flowering). In the second harvest, seed from plots without paraquat application gave a higher percentage of normal seedlings while in the third harvest, plots sprayed 30 days after closing showed the highest values. Interactions between closing date and paraquat treatments also showed highly significant differences and these differences changed among treatments.

Percentage of abnormal seedlings

Despite some significant differences between closing dates and spraying time the level of seedling abnormality was low, reaching a maximum 13% but generally with the range 2 - 8%. Harvest time had little effect on abnormal seedling levels, suggesting that even early harvested seed (25 days after peak flowering) did not produce more

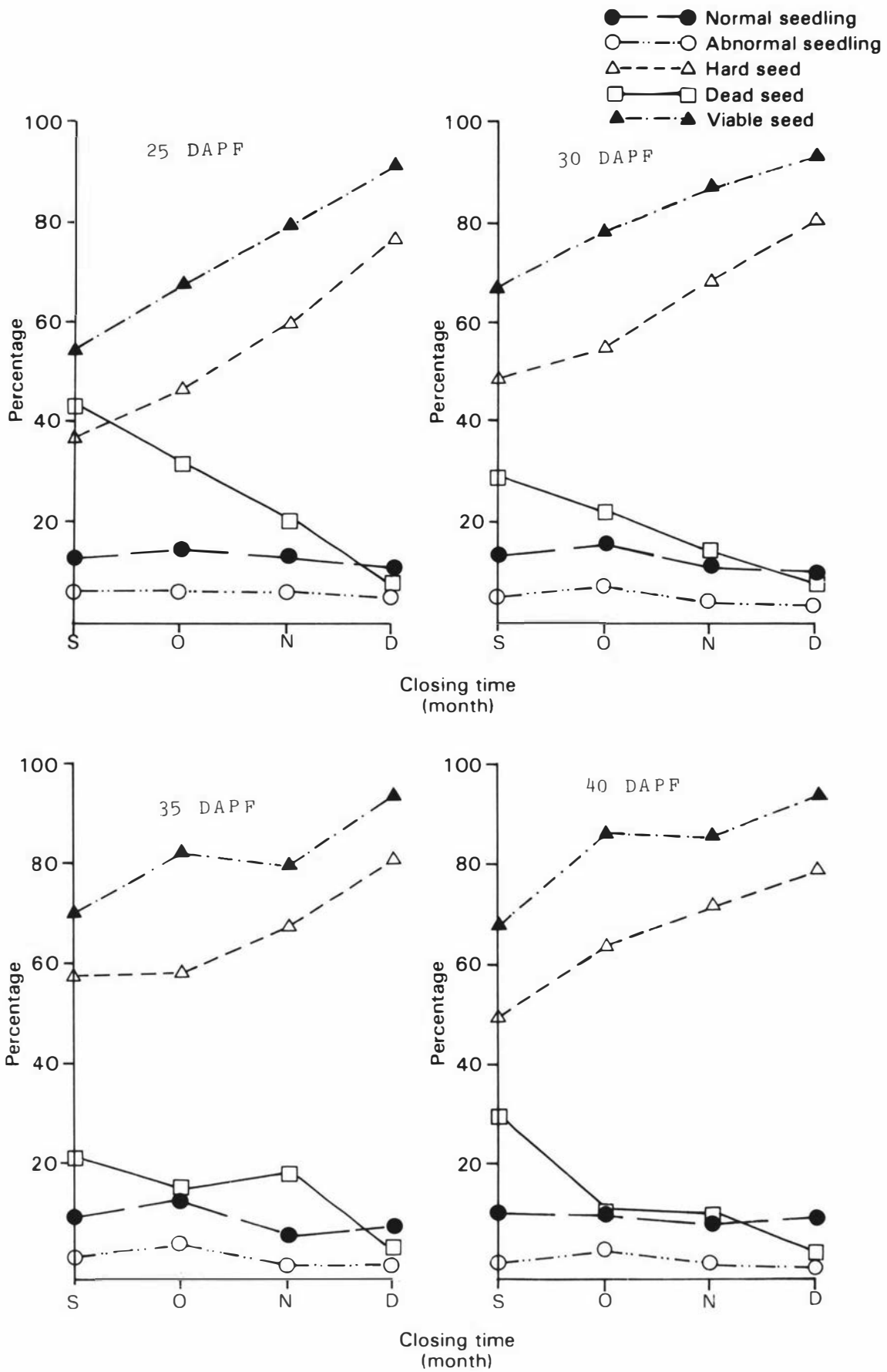


Figure 4 Effect of closing time on the percentage of normal seedlings, abnormal seedlings, hard seeds, dead seed and viable seed at each harvest.

DAPF = Days after peak flowering

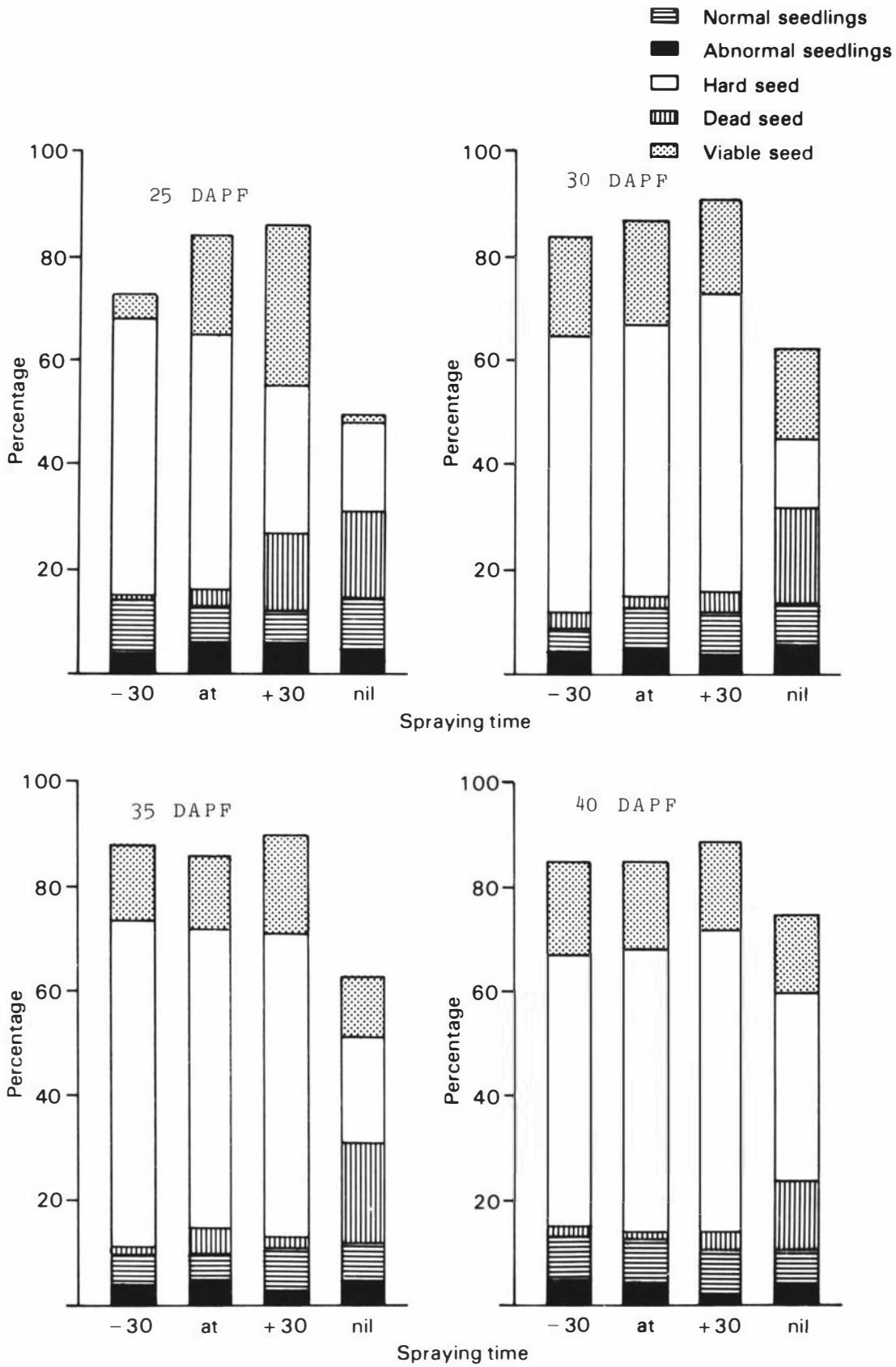


Figure 5 Effect of time of paraquat application on the percentage of normal seedlings, abnormal seedlings, hard seed, dead seed and viable seed at each harvest.

DAPF = Days after peak flowering

abnormal seedlings as a result of seed damage during seed extraction or due to lack of seed maturity.

Percentage of hard seeds

The level of hard seededness rose to high levels in harvests made 35 or 40 days after peak flowering (up to 81%). Again, closing time had strong effect on hard seed in all harvests with the highest percentages from November or December closing dates, and lowest values from September or October closing dates.

It was also noticed that the level of hard seededness in paraquat treatments was higher than in unsprayed plots. It seems possible that the high relative humidity under the canopy formed in unsprayed treatments prevented dehydration of clover seed and therefore delayed or decreased the percentage of hard seed formed. These results also suggest increases in hard seededness with increase in seed age, presumably as a result of dehydration.

Percentage of dead seed

September closing resulted in the highest percentage of dead seeds followed by October and November closings. The lowest dead seed percentage was obtained from the December closing. Highest dead seed values tended to occur in the earliest harvest date 25 days after peak flowering.

The percentages of dead seed in paraquat sprayed treatments were variable. There did not appear to be any consistent trend. However, the results do show that paraquat application did not increase the percentage of dead seeds. Significantly higher dead seed values occurred in treatments which had not been sprayed. Again, it is possible that the microclimate within the canopy in unsprayed plots was responsible for higher seed mortality, particularly 25 days after peak flowering. This was presumably due to encouragement of seed sprouting in the inflorescence, especially in early closed treatments.

Subsequently this problem was less severe as a result of increasing seed maturity, perhaps accompanied by higher percentages of hard seed.

Percentage of viable seed

Seed viability values were obtained by summation of the percentages of living seed (normal and abnormal seedlings and hard seed content). Both closing date and spraying treatment had an effect on seed viability (Figures 4 and 5). Treatments closed in September produced seeds with the lowest viability while treatments closed in October, November or December showed improved levels of viable seed. Generally, higher levels of seed viability were obtained in seed samples from late closings and where paraquat had been applied. The effect of paraquat was highly significant compared with the unsprayed control.

The maximum level of viable seed in each treatment is very high. This is mainly due to the contribution made by hard seed levels to the total. Apart from the unsprayed plots where viability levels were low (49 - 75%), treatments show maximum viable seed levels of more than 80%, with many treatments over 90%.

h - Relationship between seed yield and yield components

Although this field experiment examined the reaction of vegetative and reproductive characters to defoliation and spraying, an attempt was also made to determine the effect of these characters on ultimate seed yield. Work by Clifford (1979, 1985) has suggested that vegetative characters such as leaf area index have a strong influence on seed yield. It was also thought that the number of nodes and terminal buds could also have a strong effect on reproductive development and therefore on seed yield. In the present experiment, however, only poor and non significant relationships (r^2 = less than 0.25), were established between vegetative characters and seed yield. However, some strong correlations were found between various seed components and seed yield (Table 14).

Table 14: Correlation coefficients between seed yield (kg/ha) and yield components.

Character	Correlation Coefficient r value
Number of total inflorescences/m^2	+ 0.85**
Number of florets/inflorescence	+ 0.62*
Seed number/floret	+ 0.16 N.S.
1000 seed weight (g)	+ 0.86**

* Significant at 5% level

** Significant at 1% level

N.S. No Significance

The correlation coefficients were obtained for seed yield from seed harvested 30 days after peak flowering, and from some of the seed yield components of white clover. It appears that the main factors affecting yield in this experiment were the total number of inflorescences at harvest, the number of florets per inflorescence and 1000 seed weight. Of these, the major components of yield were the number of inflorescences/m^2 and 1000 seed weight. Both were significantly and positively correlated with seed yield. When the relationship between seed yield and number of florets was examined a significant correlation coefficient was also obtained. However, no significant correlation was found between seed yield and the number of seeds/florets (seed set). It appears that any cultural practices which result in greater numbers of inflorescences and heavier seed would be important in promoting high seed yield.

B - FIELD EXPERIMENT 1983 - 1984

1 - Introduction

The results of the field experiment carried out in the previous year had shown that the number of inflorescences, number of florets/ inflorescence and seed weight all had a major influence on seed yield. Therefore it was thought to be important to examine the changes that occurred in these seed yield components, as well as the role of specific management systems on stolon development and the proportion of nodes that became reproductive.

2 - Materials and Methods

a - Experimental site and field procedure

This second field experiment was carried out on the Pasture and Crop Research Unit, Massey University using a two year old mixed "Grasslands Huia" white clover and "Grasslands Ruanui" perennial ryegrass sward. The botanical composition of the experimental area at the beginning of the experiment was 24% white clover, 73% ryegrass, and 3% dead matter. The percentage of weed present was negligible (trace %). The field was managed in the same way as in the previous year. Prior to the commencement of the experiment, the field had been grazed with sheep whenever the herbage reached a height of 10 - 20 cm. The last preparatory grazing was completed on September 1st 1983. The soil type and its fertility was similar to the experimental site used in the previous year (Appendix 1). Details of the prevailing temperature, precipitation, wind speed and soil moisture content are presented in Appendices 9 and 10.

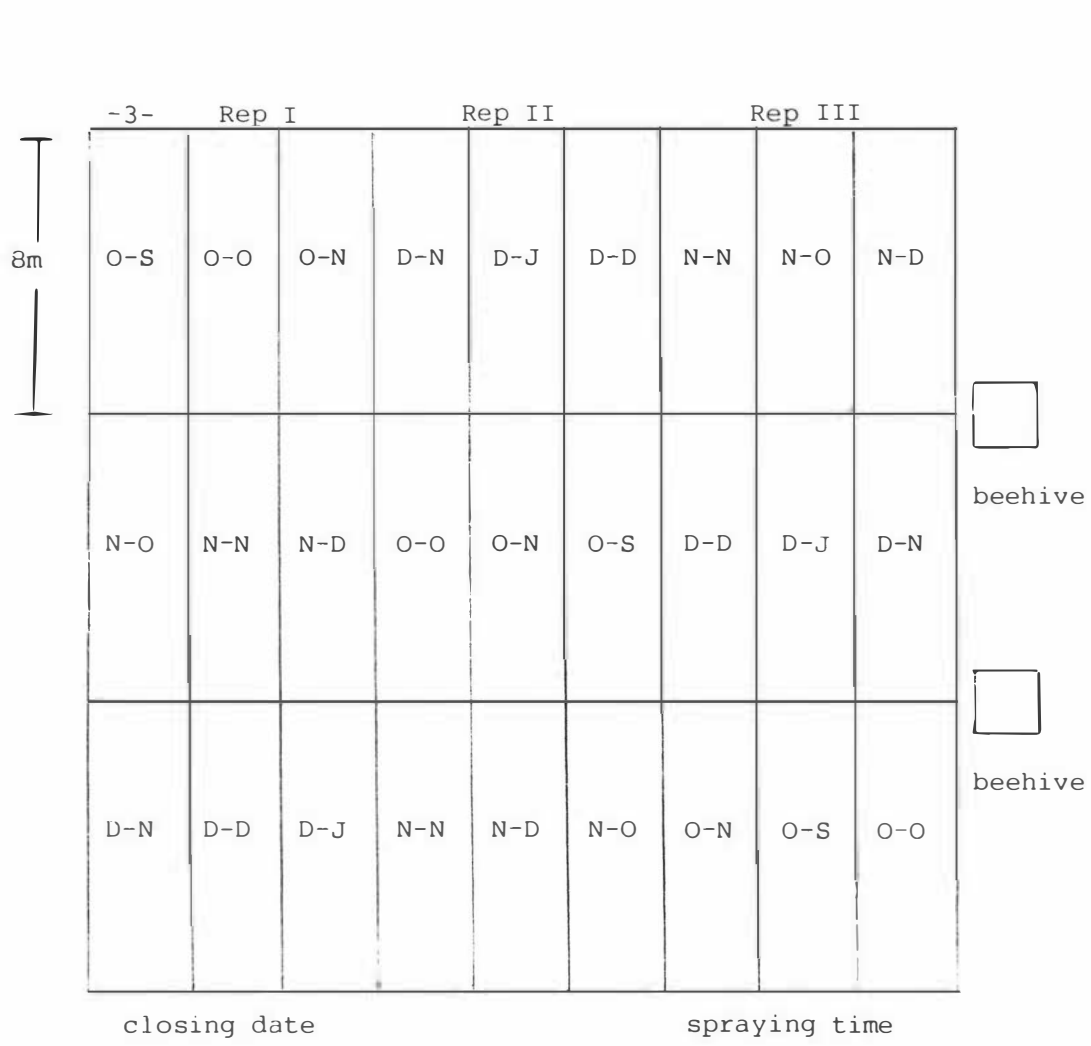
The experiment utilised the same split plot experimental design as the 1982/83 experiment with the main plots for grazing date, subplots for spraying time, and three replications. The size of the plots was the same as in the previous experiment (24 m^2) (Figure 6). In this second study the previously used September closing date and unsprayed treatments were omitted because of their poor performance.

Closing dates were 15 October, 15 November and 15 December and times of paraquat application were 30 days before closing or 30 days after closing. The third spraying treatment, designated 'at closing' in fact involved closing 7 days before spray application on 15 October, 15 November or 15 December. This was necessary to allow regrowth after grazing to provide sufficient green material for improved paraquat effectiveness. The timing of three paraquat spraying treatments were therefore, in fact, 30 days before closing, 7 days after closing or 30 days after closing. This gave a total of 9 treatments (3 closing dates x 3 times of paraquat applications) with three replications which gave 27 plots (Table 15 and Figure 6). Paraquat plus surfactant was applied at the same rate as in the previous experiment.

Table 15: Treatments used and their identification

Closing Date	Time of paraquat application	Treatment Identification
15 october	30 days before closing	O - S
	At closing	O - O
	30 days after closing	O - N
15 November	30 days before closing	N - O
	At closing	N - N
	30 days after closing	N - D
15 December	30 days before closing	D - N
	At closing	D - D
	30 days after closing	D - J

Herbage dry matter sampling, stolon sampling, and seed germination tests were carried out in the same way as previously described. Seed yield was determined by harvesting four quadrats (0.25 m^2) per plot 30 days after peak flowering.



mid - October (O)
mid - November (N)
mid - December (D)

September (S)
October (O)
November (N)
December (D)
January (J)

Figure 6: Trial layout. Field Experiment 1983/84

b- Plant measurements and statistical analysis

The effect of the treatments was measured using techniques described in the previous experiment. Sward composition and white clover content were determined by dissecting herbage samples into white clover, grasses, weeds and dead matter. Leaf and leaflet area of clover plants was also measured, as well as the number of nodes/dm^2, the number of terminal buds/dm^2. Some lateral stolon observations were recorded, ten lateral stolons per plot (30 per treatment) being marked after closing or paraquat application. Steel staples covered with coloured plastic tubing were placed over selected stolons at a position one node behind the first unfolded leaf from the apex. Subsequently, counts were made weekly of the length of the stolon, the number of inflorescences and the branches on each stolon.

In this study the number of inflorescences was recorded in two quadrats (0.25 m^2) every fifteen days from November 1st until harvest. In order to determine peak flowering date the number of white inflorescences was counted in the same way as in the previous investigation. Seed yield and seed yield components were also measured.

Data collected in this experiment was analysed in the same way as in the previous experiment by the use of Genstat (Alvey *et al* 1977).

3 - Results

a - Effect of treatments on grass and dead matter dry weight

Although the main emphasis in this experiment was on the yield of clover seed, brief reference was made to the effect of the treatments on grass and dead matter dry weight, since they had been shown in the previous experiment to influence reproductive development and seed production in white clover. Because between-replicate variation was very high (c.v. up to 120%) no statistical analysis was carried out. Nevertheless some interesting trends were observed. All spraying treatments appeared to give good control of grass as shown by the reduction in grass dry weight (Table 16). In general, plots sprayed with paraquat

Treatments	Date of Sampling day - month							
	1 - 11	15 - 11	1 - 12	15 - 12	1 - 1	15 - 1	1 - 2	15 - 2
O - S	22	41	20	16	10	5		
O - O	5	11	23	16	12	45		
O - N			1	2	13	7	14	
N - O			9	16	23	54	58	45
N - N			1	0	3	4	7	3
N - D					0	0	5	2
D - N					1	14	13	13
D - D					0	0	0	12
D - J							5	3

Table 16: Effect of closing date and time of paraquat application on grass dry weight (g/m^2) 1983 - 1984

in November or December resulted in better control of grass than plots sprayed in October. This might be explained in terms of seasonal growth patterns of clover and grass. For the last two months of the year the rate of ryegrass growth normally declines, whilst for white clover it remains constant (Brougham 1958). Therefore application of paraquat during this time of the year tends to give better control of grasses than October application, since in October the grass plants are still exhibiting active vegetative growth, conferring improved recovery from the effect of the herbicide.

In 1983, dead matter dry weight (Table 17) showed the same trend as in the previous experiment. Spraying with paraquat had a considerable effect on dead material dry weight with higher values being recorded in plots sprayed 30 days after closing. This dead material produced a thick mulch which presumably decreased light penetration to the stolon and caused a subsequent reduction in the flowering of the white clover plant.

b - Effect of treatments on white clover dry weight

The effect of closing time, spraying treatment and the interactions of both variables on white clover vegetative characters were studied.

Dry weight of white clover in all treatments (Table 18) followed a curve with two distinct phases, except in D - D and D - J treatments (closed in December and sprayed in December or January). Treatments closed in October or November showed, over the first 1.5 to 2 months a rapid increase in dry matter, followed by a period of 2 to 4 weeks when dry matter yield remained relatively constant or decreased. A similar trend was observed in D - N plots, but with 1.5 months in the first phase and 2 weeks in the second phase. In the rest of the treatments closed in December the period between the date of closing or spraying and harvest was too short to allow the clover plants to exhibit both phases of growth.

Treatments	Date of Sampling day - month							
	1 - 11	15 - 11	1 - 12	15 - 12	1 - 1	15 - 1	1 - 2	15 - 2
O - S	0	0	2	13	92	188		
O - O	37	0	6	5	62	90		
O - N			147	383	116	96	158	
N - O			0	1	12	31	66	32
N - N			62	37	40	10	35	37
N - D					201	143	86	35
D - N					10	19	11	9
D - D					79	87	32	45
D - J							170	87

Table 17: Effect of closing date and time of paraquat application on dead matter dry weight (g/m^2) 1983 - 1984

Treatments	Date of Sampling day - month 1 - 11	15 - 11	1 - 12	15 - 12	1 - 1	15 - 1	1 - 2	15 - 2
O - S	38±9.0	68±11.5	113±12.0	223±25.3	358±37.0	301±43.5		
O - O	28±7.6	52±15.5	84±15.8	121±16.1	231±27.8	237±36.8		
O - N			30±11.0	37±10.6	159±17.0	206±24.0	205±27.8	
N - O			23± 3.2	54±13.5	141±20.4	217±22.0	213±25.8	218±25.3
N - N			7± 1.1	18± 2.0	113±14.7	203±26.0	212±23.4	217±24.4
N - D					22± 2.5	53±16.8	61±14.9	134±14.0
D - N					69±16.0	149±13.2	164±19.5	163±15.0
D - D					40± 8.0	44±11.7	52±14.3	102±16.5
D - J							29± 3.2	35± 2.1

Table 18: Effect of closing date and time of paraquat application on white clover dry weight g/m^2 (± standard errors) 1983 - 1984

In this experiment clover dry weight was considerably affected by the time of spraying treatment. In plots closed in October and November highest clover dry weight was obtained from plots sprayed with paraquat 30 days before closing. The level of clover dry weight was intermediate in plots sprayed at closing and was lowest in treatments sprayed 30 days after closing. The dry weight of white clover was higher in plots closed in October than in plots closed in November or December. Similarly, D - N, D - D, and D - J treatments gave less clover dry weight than the equivalent treatments in November (N - O, N - N, and N - D). Earlier closing and/or earlier spraying, apparently allowed clover to produce more recovery dry weight which later could affect the reproductive character of the plants.

c - Effect of treatments on white clover leaf area index and leaflet area

Leaf area index (LAI) showed a marked response to closing date (Table 19). Treatments closed in October reached their ceiling LAI by 1 January. LAI then declined in response to drier soil conditions. All other treatments showed a reduction in leaf area during January but later recovered in response to enhanced soil moisture levels during February. In the present experiment the O - S treatment produced a maximum LAI of 3.86, which is higher than the critical leaf area index of 3.5 for 'Grasslands Huia' white clover determined by Brougham (1958). This critical leaf area index is defined as the LAI at which 95% of the incident light energy is intercepted. At this stage the number of leaves which die is equal to the number of new leaves being formed (Brougham 1958).

The experimental data suggests that meristematic activity increases after grazing and/or paraquat application and that this results in the production of a large bulk of leaves. Such a change in meristematic activity after defoliation could be associated with an increase in the nutrition of apical growing points allowing the production of new leaves, and to higher light penetration following the destruction or removal of the existing canopy, which promotes stolon growth and the subsequent formation of new leaves.

Treatments	Date of Sampling day - month							
	1 - 11	15 - 11	1 - 12	15 - 12	1 - 1	15 - 1	1 - 2	15 - 2
O - S	0.74±0.03	1.41±0.16	1.85±0.14	3.31±0.45	3.86±0.69	2.37±0.26		
O - O	0.71±0.05	1.52±0.17	1.54±0.17	2.03±0.18	3.18±0.64	2.07±0.21		
O - N			0.63±0.15	0.59±0.04	2.26±0.58	1.93±0.43	1.03±0.10	
N - O			0.40±0.05	0.77±0.04	2.07±0.29	2.29±0.61	2.14±0.37	2.96±0.72
N - N			0.11±0.02	0.82±0.02	1.82±0.16	1.49±0.21	1.76±0.20	2.58±0.40
N - D					0.49±0.07	0.44±0.05	0.58±0.09	1.88±0.22
D - N					1.20±0.14	0.92±0.19	1.64±0.16	2.17±0.24
D - D					0.77±0.08	0.39±0.12	0.42±0.09	1.26±0.36
D - J							0.24±0.05	0.53±0.03

Table 19: Effect of closing date and time of paraquat application on leaf area index (± standard errors) 1983 - 1984

Table 20 shows the development of leaflet area at successive intervals in each treatment. These results follow the same pattern previously described for LAI, showing marked changes according to the treatment, as well as to seasonal variation in the environment. For instance, treatments closed in October produced plants with a greater leaflet area than those closed in November or December. Similarly, paraquat caused strong variation in leaflet area, with maximum values for those plots sprayed 30 days before closing.

d - Effect of treatments on the number of nodes, terminal buds, and stolon length

In this experiment, results for the number of nodes/dm^2 and terminal buds/dm^2 (Tables 21 and 22) showed the follocorny trends. Paraquat spraying 30 days before closing caused a general decline in stolon tip numbers with time irrespective of closing date. Spraying 30 days after closing caused a general increase in stolon tip numbers with time irrespective of closing date. Finally, paraquat spraying at closing caused peak stolon numbers to be progressively later with a delay in closing date.

Observations on stolon elongation from the apex of marked stolons indicated that elongation rates (Table 23) were high in all treatments. This is not surprising since stem elongation in white clover has been shown to be very high in late spring and summer when temperatures approach the optimum for stolon elongation of 20 - 25°C (Chapman 1983).

Maximum stolon elongation rates of 15.5, 19.3 and 20.0 mm per week were obtained for treatments closed in October; 16.5, 15.0, and 14.5 mm/week for treatments closed in November and 17.0, 14.5 and 10.0 mm/week for treatments closed in December. These results are comparable to rates measured by Chapman (1983) in a grazed hill pasture under rotational sheep grazing. It seems that the main differences in stolon elongation rate are due more to temperature and soil moisture content than to the treatment itself. Higher stolon elongation rates in some treatments may be due to the effect of the mulch left by the grasses after paraquat application in reducing light penetration to

Treatments	Date of Sampling Day - Month 1 - 11	15 - 11	1 - 12	15 - 12	1 - 1	15 - 1	1 - 2	15 - 2
O - S	32 ± 3.6	57 ± 12.6	91 ± 11.7	125 ± 24.7	152 ± 22.5	85 ± 12.8		
O - O	8 ± 1.4	54 ± 12.5	56 ± 11.1	65 ± 12.6	73 ± 12.5	56 ± 14.2		
O - N			28 ± 2.0	63 ± 15.3	72 ± 13.2	50 ± 9.2	54 ± 13.5	
N - O			32 ± 2.0	32 ± 12.1	56 ± 13.2	42 ± 11.4	47 ± 4.9	53 ± 12.8
N - N			25 ± 3.1	37 ± 11.1	39 ± 11.0	39 ± 11.0	43 ± 11.4	54 ± 12.1
N - D					49 ± 12.8	31 ± 12.3	31 ± 4.2	32 ± 11.9
D - N					54 ± 14.2	26 ± 2.1	30 ± 4.2	52 ± 12.1
D - D					35 ± 13.5	23 ± 11.4	22 ± 13.5	38 ± 11.4
D - J							19 ± 2.1	26 ± 3.5

Table 20: Effect of closing date and time of paraquat application on leaflet area mm^2 (± standard errors) 1983 - 1984

Treatments	Date of Sampling day - month 1 - 11	15 - 11	1 - 12	15 - 12	1 - 1	15 - 1	1 - 2	15 - 2
O - S	360±75.4	422±76.9	512±71.5	390±62.1	353±35.1	343±53.5		
O - O	374±60.5	432±75.3	549±73.6	454±71.0	392±49.1	244±26.8		
O - N			198±26.1	219±29.6	291±33.5	347±30.2	436±78.0	
N - O			460±74.7	619±88.1	452±45.4	427±48.7	389±75.2	513±79.7
N - N			296±33.0	323±36.8	474±56.6	369±38.1	348±73.1	397±60.8
N - D					287±33.5	261±40.3	486±72.7	481±70.6
D - N					536±56.4	402±35.4	498±79.7	516±81.4
D - D					182±17.5	427±59.5	556±87.9	647±86.0
D - J							432±73.9	435±68.6

Table 21: Effect of closing date and time of paraquat application on the number of nodes/dm^2 (± standard errors) 1983 - 1984

Treatments	Date of Sampling day - month							
	1 - 11	15 - 11	1 - 12	15 - 12	1 - 1	15 - 1	1 - 2	15 - 2
O - S	80 ± 15.1	90 ± 22.3	78 ± 17.5	57 ± 5.9	41 ± 3.6	44 ± 5.5		
O - O	37 ± 14.8	39 ± 12.2	82 ± 15.6	74 ± 8.7	51 ± 5.8	39 ± 5.8		
O - N			23 ± 4.9	32 ± 4.6	34 ± 4.9	80 ± 9.9	66 ± 12.3	
N - O			112 ± 11.2	92 ± 12.2	78 ± 10.2	49 ± 6.3	39 ± 11.5	68 ± 6.0
N - N			40 ± 7.7	65 ± 11.5	97 ± 17.3	63 ± 16.6	43 ± 8.0	73 ± 10.6
N - D					48 ± 8.6	57 ± 9.7	84 ± 17.6	83 ± 12.1
D - N					126 ± 16.0	96 ± 14.5	73 ± 10.2	75 ± 9.0
D - D					32 ± 9.9	77 ± 10.8	77 ± 11.9	98 ± 18.7
D - J							75 ± 16.3	74 ± 11.9

Table 22: Effect of closing date and time of paraquat application on the number of terminal buds/dm^2 (± standard errors) 1983 - 1984

Treatments	Date of Sampling Day - Month							
	1 - 11	15 - 11	1 - 12	15 - 12	1 - 1	15 - 1	1 - 2	15 - 2
O - S	37 ± 8.0	68 ± 13.0	95 ± 15.5	130 ± 29.6	160 ± 20.0	172 ± 22.5		
O - O	27 ± 8.0	52 ± 12.5	82 ± 12.5	120 ± 16.0	159 ± 23.6	169 ± 23.6		
O - N			28 ± 4.0	64 ± 12.5	108 ± 14.1	146 ± 26.6	166 ± 16.6	196 ± 26.6
N - O			33 ± 7.0	62 ± 14.1	98 ± 8.0	117 ± 18.7	128 ± 21.6	151 ± 22.4
N - N			20 ± 3.3	40 ± 8.3	78 ± 16.2	108 ± 15.5	118 ± 25.5	151 ± 23.0
N - D					20 ± 2.0	38 ± 8.0	64 ± 18.5	93 ± 17.5
D - N					26 ± 4.0	58 ± 8.7	92 ± 15.5	121 ± 24.5
D - D					22 ± 4.1	35 ± 2.5	56 ± 8.5	78 ± 14.7
D - J							25 ± 6.4	45 ± 8.8

Table 23: Effect of closing date and time of paraquat application on the length (mm) of a stolon (± standard errors). Average of 30 main stolons per treatments 1983 - 1984

the stolons. Such a situation has been shown to increase stolon growth rate (Salisbury and Ross 1978).

e - Effect of treatments on branch production per stolon

Records of stolon growth (Table 24) showed that side branch production was affected by closing date. An analysis of the treatments between closing dates indicated that those treatments closed in October had more lateral branches than treatments closed in November. Similarly, these treatments caused main stolons to form more lateral branches than on plants closed in December. The effect of the time of paraquat application was most clearly seen when plants were sprayed 30 days after November or December closing. By these two treatments (N-D and D-J) the number of branches on the stolon was reduced.

It was observed that in some treatments, some main stolons were more active in the formation of branches than others, as indicated by the standard error values. These differences between stolons in axillary bud activity may have been caused by subtle changes in temperature, moisture, light or soil fertility between microsites or may have been genetic in origin.

f - Relationship between vegetative characters

The various vegetative components of white clover examined in this experiment exhibited strong and positive correlations (Table 25). In all cases highly significant correlations existed between the length of the stolon and clover dry weight except with a December closing. Clearly, clover dry weight reflects the general level of meristematic activity in the plant, but is not necessarily a reliable indicator or leaf area since LAI was not well correlated with dry matter yield in some treatments.

Treatments	Date of Sampling Day - Month							
	1 - 11	15 - 11	1 - 12	15 - 12	1 - 1	15 - 1	1 - 2	15 - 2
O - S	2.1 ± 0.23	2.3 ± 0.35	2.4 ± 0.24	2.4 ± 0.45	2.9 ± 0.64	2.7 ± 0.29		
O - O	1.8 ± 0.27	2.0 ± 0.27	2.3 ± 0.21	2.4 ± 0.30	2.6 ± 0.41	2.6 ± 0.23		
O - N			1.2 ± 0.44	1.8 ± 0.16	2.5 ± 0.32	2.7 ± 0.29	2.4 ± 0.24	
N - O			1.3 ± 0.50	2.2 ± 0.34	2.0 ± 0.49	2.0 ± 0.49	1.3 ± 0.58	
N - N			1.2 ± 0.10	2.1 ± 0.44	2.5 ± 0.32	2.4 ± 0.52	2.4 ± 0.52	
N - D					0.7 ± 0.10	1.3 ± 0.58	1.1 ± 0.92	1.7 ± 0.92
D - N					1.0 ± 0.20	1.6 ± 0.32	1.6 ± 0.76	1.8 ± 0.68
D - D					0.9 ± 0.19	0.9 ± 0.29	1.0 ± 0.66	1.9 ± 0.38
D - J							1.0 ± 0.39	1.5 ± 0.32

Table 24: Effect of closing date and time of paraquat application on the number of branches per stolon (± standard error). Average of 30 main stolons per treatment 1983 - 1984

Table 25: Effect of closing date and time of paraquat application on the strength of association between clover dry weight (g/m^2) and some vegetative characters measured every 15 days after the completion of each treatment until harvest 1983 - 1984

Treatments	LAI	Correlation Coefficients: Length of stolon (mm)	Correlation Coefficients: No. branches/ stolon
O - S	+0.96**	+0.86*	+0.89*
O - O	+0.85*	+0.97**	+0.92*
O - N	+0.70	+0.96**	+0.79
N - O	+0.94**	+0.96**	+0.14
N - N	+0.84*	+0.96**	+0.77
N - D	+0.80	+0.95*	+0.95*
D - N	+0.46	+0.85*	+0.98*
D - D	+0.48	+0.90*	+0.99**

* Significant at 5% level

** Significant at 1% level

An analysis of the relationship between clover dry weight and LAI in each treatment showed a positive and significant correlation coefficient in the O - S, O - O, N - O and N - N treatments. The lack of relationship between these two variables in the O - N and N - D treatments can be explained by the shading resulting from the dense mulch of dead ryegrass present after the application of paraquat. In treatments closed in December the lack of a relationship was probably influenced by adverse environmental conditions, such as high temperature and low soil moisture, which occurred in January after the last grazing or spraying and affected plant recovery.

Within all treatments there was a strong, positive and significant correlation coefficient between clover dry weight and stolon length, ranging from 0.85 to 0.97.

The relationship between branching and clover dry weight in the O - N, N - O, and N - N treatments was comparatively poor. In the latter two treatments this may be due to increased inflorescence initiation promoted by a reduction in shading at the level of the stolon at this time of the year. This reproductive development competes for sites with branch development. In the case of the O - N treatment, however, the poor correlation may be explained by the shading caused by the dead grass, which inhibits branch formation without reducing clover dry weight. In general, under a canopy, plants tend to form longer stems, petioles and larger leaves, which compensate for loss in the weight of the plant (Pascoe 1973). In the remaining treatments, significant correlations were found between branching and clover dry weight.

These results, together with those obtained from the previous experiment, indicate that both closing and spraying treatments have an important impact in determining the pattern of vegetative growth of white clover.

g - Effect of treatments on the number of inflorescences

Two 25 x 25 cm^2 quadrat counts were taken at random in each plot every fifteen days from November and the total number of inflorescences recorded. The results obtained were converted to inflorescences/m^2 and are presented in Table 26.

In all plots inflorescences were first visible around October 15th. Major treatment effects occurred since some inflorescences were removed by sheep grazing and some were dessicated by paraquat. However, after the completion of each treatment the general reaction of the clover plant was to increase inflorescence production. This reached a maximum in mid January for October closing; mid February for November closing and end of February for December closing. Paraquat application had

Treatments	Date of Sampling Day - Month 1 - 11	15 - 11	1 - 12	15 - 12	1 - 1	15 - 1	1 - 2	15 - 2
O - S	30 ± 2.8	80 ± 7.4	144 ± 16.0	368 ± 16.0	464 ± 56.2	608 ± 80.5	H	
O - O	10 ± 1.5	26 ± 2.8	48 ± 5.3	256 ± 48.8	448 ± 46.3	576 ± 90.8	H	
O - N			0	64 ± 9.2	112 ± 17.3	320 ± 46.9	128 ± 16.4	H
N - O			10 ± 1.2	112 ± 16.0	128 ± 19.3	620 ± 60.5	560 ± 83.2	796 ± 87.3
N - N			26 ± 2.90	96 ± 21.9	128 ± 15.4	592 ± 61.3	500 ± 63.3	672 ± 83.4
N - D					16 ± 1.8	240 ± 26.5	272 ± 33.3	384 ± 32.0
D - N					32 ± 2.7	384 ± 45.2	528 ± 66.4	480 ± 96.8
D - D					16 ± 1.5	192 ± 24.7	240 ± 22.7	240 ± 33.7
D - J								80 ± 16.7

H = Data not available as seed already harvested

Table 26: Effect of closing date and time of paraquat applciation on the total number of inflorescences/m^2 (± standard errors). Average of four quadrats of 25 x 25 cm per plot 1983 - 1984

No major effect on inflorescence production except where it was delayed until 30 days after closing because the mulch of grasses left after spraying paraquat did not allow light penetration at the stolon level, which inhibited inflorescence initiation.

h - Relationship between inflorescences/m^2 and vegetative characters

The number of inflorescences/m^2 showed highly significant correlations with several of the vegetative characters measured from November 1st on each stolon (Table 27). Clover dry weight showed significant correlation coefficients in the N - O, N - N, and D - N treatments. The length of stolons was also positively related in the O - S, O - O, N - O, and N - N treatments, but no such relationships were found in other treatments. Similarly, in O - S and O - O treatments the number of branches was positively correlated with inflorescence numbers per m^2. All other correlations, including LAI and LLA, were not significant.

Table 27: Correlation coefficient for each treatment between inflorescence numbers/m^2 emerged at different times of the year and several vegetative characters of white clover plants 1983 - 1984.

Treatments	Clover dry weight (g/m^2)	LAI	LLA † (cm^2)	Length of stolon (mm)	Number branches per stolon
O - S	+0.80	+0.75	+0.66	+0.97**	+0.88*
O - O	+0.82	+0.75	+0.54	+0.96**	+0.87*
O - N	+0.77	+0.64	+0.19	+0.73	+0.80
N - O	+0.91*	+0.80	+0.50	+0.94**	+0.28
N - N	+0.94**	+0.76	+0.79	+0.94**	+0.66
N - D	+0.84	+0.69	-0.69	+0.80	+0.84
D - N	+0.99**	+0.51	-0.53	+0.86*	+0.80
D - D	+0.55	+0.01	-0.48	+0.81	+0.58

* Significant at 5% level

** Significant at 1% level

† = LLA Leaflet area

The positive relationship between clover dry weight and inflorescence number in some treatments indicates that leaves are the most important organ in supplying the nutrients needed for growth and development of the inflorescences.

No relationship was found between inflorescence numbers and any of the variables measured in the O - N, N - D and D - D treatments. The difference in response between the latter two treatments and the D - N treatment may be explained by the time of paraquat application. In general, paraquat at 0.36 kg a.i./ha caused extensive withering of leaves and emerged inflorescences in white clover plants (Plates 3 and 4). However, in treatments where spraying was delayed until December, inflorescences were generally unable to recover from the damage caused by the herbicide, and some stolons also showed extensive damage. In contrast, grazing involves the removal of only some leaves and stolons of the plant. This is generally not a total removal of the green material and enough leaves, terminal buds, and inflorescences remain following defoliation to allow a faster plant recovery. This stresses the difference between the non selective damage to white clover inflorescences caused by paraquat compared to the more selective effects of sheep grazing in December.

The positive and significant correlation coefficients obtained in this experiment between the number of inflorescences, and the length of the main stolon in some of the treatments closed in October and November are supported by similar results obtained by Knight (1953), Zaleski (1961), and Thomas (1980a). They indicate that under long days, high temperatures lead to maximum stolon elongation, as well as to rapid node emergence and therefore to maximum inflorescence emergence. However, treatments closed or sprayed in December did not show this effect on the number of inflorescences/m^2 since the last grazing or paraquat application resulted in a greater number of inflorescence being removed or killed in the field. Inflorescence initiation during this time of the year also decreases (Thomas 1982c), with a consequent reduction in the number of inflorescences formed, and therefore in seed yield.

Plate 3: Damage caused by paraquat in white clover leaves one week after spraying

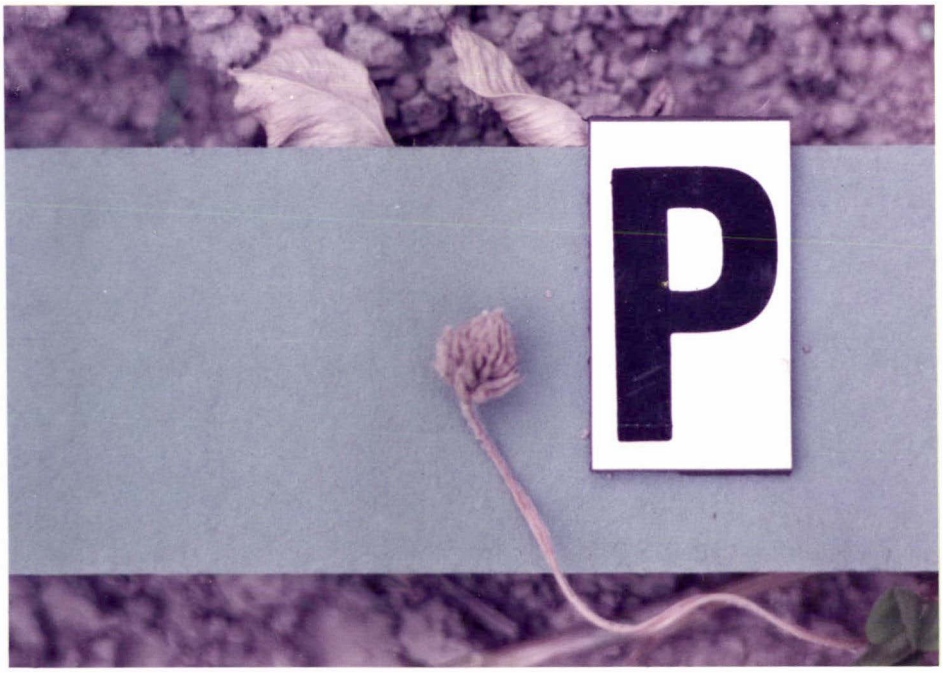


Plate 4: Damage caused by paraquat application to white clover inflorescence one week after spraying.

Only very slight changes in the number of new branches formed occurred after the completion of each treatment. This suggests that most of the branches responsible for the increase in inflorescence numbers are formed during autumn, winter and spring when the temperature is more suitable for their development.

i - Effect of treatments on the date of peak flowering

As mentioned before, white flowers started appearing in all plots from mid October. From this time, the number of inflorescences changed according to the treatment (Table 28). Treatments closed in October showed earlier peak flowering than plots closed in November or December. In fact, peak flowering in the early-closed treatments (O - S, O - O, and O - N) occurred during the last week of December and the first four days of January, while treatments closed in November and December did not reach peak flowering until mid to late January. The main effect of delayed closing was to reduce the differences in peak flowering in different treatments. The effect of paraquat application appeared to be minimal.

Table 28: Effect of closing date and time of paraquat application on the date of peak flowering 1983 - 1984

	Date of peak flowering		
	Day	Month	Year
O - S	23	12	83
O - O	27	12	83
O - N	4	1	84
N - O	14	1	84
N - N	19	1	84
N - D	19	1	84
D - N	25	1	84
D - D	19	1	84
D - J	21	1	84

j - Effect of treatments on seed yield and yield components

As in the previous experiment, closing and spraying times were responsible for differences in seed yield (Table 29). In this year highest seed yield was obtained from plots closed in November with intermediate yields from plots closed in December and lowest seed yield from October treatments. However, the difference between October and December closings failed to reach significance.

Table 29: Effect of closing date and time of paraquat application on seed yield (kg/ha) 1983 - 1984

Closing time		Paraquat treatments	
Mid - October	117	30 days before closing	302
Mid - November	408	At closing	246
Mid - December	149	30 days after closing	127
Significance	**		**
LSD 5%	75.3	LSD 5%	33.6
1%	124.6	1%	47.1
S.E.D. ±	27.1	S.E.D. ±	15.4

Interactions

Treatments

O-S	O-O	O-N	N-O	N-N	N-D	D-N	D-D	D-J
128	119	105	457	511	257	321	108	20.3

LSD(a) 5%	88.1		LSD(b) 5%	57.9
1%	138.0		1%	81.4
S.E.D.(a) ±	34.7		S.E.D.(b) ±	26.6

(a) When comparing means at different levels of mainplots

(b) When comparing means at the same levels of mainplots

The differences in seed yield between times of paraquat application indicated that highest seed yield was obtained at all closing times in treatments sprayed with paraquat 30 days before closing. This effect was intermediate for plots sprayed at closing and was lowest ($P = 0.01$) in plots sprayed 30 days after closing. The significant interactions of closing and spraying treatments showed that some closing times responded differently to spraying treatments. For example, the N-N and N-O treatments gave significantly higher seed yields ($P = 0.01$) than other treatments. No significant differences were found between spraying treatments closed in October. However, in November and December closings, spraying 30 days before closing or at closing tended to give higher seed yield than plots sprayed 30 days after closing. The results indicate that the best combination of closing and paraquat application, without adversely affecting seed yields, was a November closing with spraying in either mid October or mid November. Beyond this time, grazing or herbicide application is likely to reduce seed yields. Delaying either closing or spraying until December was particularly injurious. Of these two factors, however, delayed spraying was more damaging than delayed closing. The best treatments in this experiment (N-N and N-O) produced seed yields of 457 to 511 kg/ha. This was considerably higher than the yields from the same treatments (156 to 227 kg/ha) in 1982/83.

The seed production capacity of white clover represents the cumulative expression of four principal components: number of inflorescences/ unit area; number of florets/inflorescence; number of seeds per floret; and 1000 seed weight (Clifford 1979; Thomas 1980a). These components also differ in their relative contribution to total seed yield and can change with the type of management of the sward, as well as with environmental conditions (Zaleski 1970). An analysis of these components was made to determine their contribution to white clover seed yield.

Total number of inflorescences/m^2 at harvest

The number of inflorescences/m^2 at harvest was considerably affected by spraying time and to a lesser extent by the date of closing (Table 30). November closed plots had the highest number of inflorescences

and December closed plots the lowest. As mentioned before, spraying treatments had a considerable effect on the total number of inflorescences. The highest number of inflorescences were present in treatments sprayed 30 days before closing in all three closing times and significant differences were found between them. The interactions were also significant and again, N - O and N - N treatments showed the highest number of total inflorescences.

Table 30: Effect of closing date and time of paraquat application on the number of inflorescences/m^2 at harvest 1983-1984

Closing time		Paraquat treatments	
Mid - October	598	30 days before closing	835
Mid - November	799	At closing	642
Mid - December	441	30 days after closing	362
Significance	*	Significance	**
LSD 5%	200.4	LSD 5%	165.5
1%	N.S.	1%	117.94
S.E.D. ±	72.1	S.E.D. ±	54.1

Interactions

Treatments

O-S	O-O	O-N	N-O	N-N	N-D	D-N	D-D	D-J
721	637	437	963	931	503	821	358	145

LSD(a)	5%	258.3	LSD(b)	5%	204.5
	1%	N.S.		1%	N.S.
S.E.D.(a) ±		105.2	S.E.D.(b) ±		93.8

(a) When comparing means at different levels of mainplots

(b) When comparing means at the same levels of mainplots

Floret numbers per inflorescence at harvest

Ten ripe inflorescences were selected at random from each plot at harvest and the means of the number of florets/inflorescence were used for statistical analysis. Results are presented in Table 31. Closing date appeared to have some effect on the number of florets per inflorescence. However, the results showed a tendency for highest numbers of florets in November closed plots (47) and lowest numbers following a December closing (37). Similar differences were obtained when October and November closings were compared with October closing showing the lower number of florets per inflorescence (41).

Table 31: Effect of closing date and time of paraquat application on the number of florets per inflorescence at harvest 1983 - 1984

Closing time		Paraquat treatments	
Mid - October	41.2	30 days before closing	46.7
Mid - November	47.6	At closing	46.0
Mid - December	37.8	30 days after closing	33.9
Significance	*	Significance	**
LSD 5%	4.95	LSD 5%	4.51
1%	N.S.	1%	6.33
S.E.D. ±	1.78	S.E.D. ±	2.07

Interactions

Treatments

O-S	O-O	O-N	N-O	N-N	N-D	D-N	D-D	D-J
42.3	46.3	35.0	49.3	53.6	39.7	48.3	38.3	27.0

LSD(a)	5%	7.88	LSD(b)	5%	7.82
	1%	N.S.		1%	N.S.
S.E.D.(a) ±		3.43	S.E.D.(b) ±		3.59

(a) When comparing means at different levels of mainplots

(b) When comparing means at the same levels of mainplots

In each season time of paraquat spraying exercised a great influence on the number of florets/inflorescence. Plots sprayed 30 days before closing or at closing did not differ significantly from each other, but both produced inflorescences bearing more florets (46) than plots sprayed 30 days after closing (34). The interactions were significant and showed that N-O, and N-N treatment combinations gave the highest number of florets/inflorescence (49 - 54). Treatments sprayed 30 days after closing, generally formed fewer florets/inflorescence. Delaying grazing or spraying until December caused the removal of a proportion of the first inflorescences, and those inflorescences formed in the sprayed aftermath tended to have lower floret numbers (27 to 38).

Individual inflorescences varied greatly in their floret numbers (range 20 to 75) but the mean range of floret numbers across all treatments was only 35 to 55 florets per inflorescence. These deviations clearly show the relative insensitivity of floret number to closing and spraying treatments, but that this yield component was more highly variable between inflorescences within the same treatment (Appendix 11).

Effect of treatments on seeds/floret

The data presented in Table 32 shows that the number of seeds/floret was greatest with November (2.75) or December (2.25) closings. This result was consistent with data obtained in the previous experiment. Neither the spraying treatments nor the interactions (closing x spraying) presented significant differences in the number of seeds/floret.

Table 32: Effect of closing date and time of paraquat application on the number of seed per floret at harvest 1983 - 1984

Closing time		Paraquat treatments	
Mid - October	1.28	30 days before closing	1.96
Mid - November	2.75	At closing	2.11
Mid - December	2.25	30 days after closing	2.21
Significance	**	Significance	N.S.
LSD 5%	0.401	LSD 5%	N.S.
1%	0.663	1%	N.S.
S.E.D. ±	0.14	S.E.D. ±	0.17

The low seed set in October (1.28) closed treatments might be due to the greater growth of the leaf petioles in comparison to the peduncles during flowering. This could hinder access by bees to the inflorescences. However, it is also possible that in these treatments weather conditions during flowering decreasing seed set by a direct effect on bee activity. It is interesting that mean seed numbers per floret in this experiment exceeded 2. This is similar to values obtained by Clifford (1980) but is double the value obtained in the previous experiment. The highest value of 3.17 seeds/floret occurred in the N-N treatment.

Effect of treatments on 1000 seed weight and seed numbers/m^2

Using bulked samples of seed for each closing time and spraying treatments, four seed weight counts of 200 seeds each were made (Table 33). Neither closing time nor paraquat treatment had a significant effect on 1000 seed weight.

Table 33: Effect of closing date and time of paraquat application on 1000 seed weight (g) 1983-1984

Closing time		Paraquat treatments	
Mid - October	0.559	30 days before closing	0.597
Mid - November	0.533	At closing	0.524
Mid - December	0.545	30 days after closing	0.515
Significance	N.S.	Significance	N.S.
LSD 5%	N.S.	LSD 5%	N.S.
1%	N.S.	1%	N.S.
S.E.D. ±	0.021	S.E.D. ±	0.029

These results are contrary to those obtained by Huxley *et al* (1979), who suggested that seed weight in white clover makes an important contribution to seed yield. However, Zaleski (1961) did not find significant differences in seed weight in some of his experiments.

The present experiment shows that 1000 seed weight across all treatments ranged from 0.46 to 0.64 (28% difference). The previous experiment, however, produced, seed weights ranging from 0.34 to 0.56 across different closing and spraying treatments. This represents a difference of approximately 40%.

k - Effect of treatments on seed quality

Neither closing time nor spraying treatment appeared to have much effect on the percentage of normal seedlings (Table 34). It is interesting, however, that normal seedling percentages were very low (less than 25% in all treatments and commonly under 10%). There was also a suggestion that treatments sprayed before closing gave higher normal seedling percentages, particularly when this spraying treatment was combined with an October closing.

Table 34: Effect of closing date and time of paraquat application on normal seedling percentage 1983 - 1984

Closing time		Paraquat treatments	
Mid - October	14.1	30 days before closing	13.4
Mid - November	11.3	At closing	10.7
Mid - December	6.1	30 days after closing	7.5
Significance	N.S.	Significance	*
LSD 5%	N.S.	LSD 5%	3.52
1%	N.S.	1%	N.S.
S.E.D. ±	2.43	S.E.D. ±	1.62

Interactions

Treatments

O-S	O-O	O-N	N-O	N-N	N-D	D-N	D-D	D-J
24.3	12.3	5.7	11.7	13.8	8.5	4.2	5.8	8.3

Significance	**			
LSD(a) 5%	8.29	LSD(b) 5%	6.10
1%	12.89	1%	8.57
S.E.D.(a) ±	3.33	S.E.D.(b) ±	2.79

(a) When comparing means at different levels of mainplots

Seedling abnormalities, were generally few in number with a maximum of only 3.2% (Table 35). The main types of abnormalities were seedlings in which the primary root was stunted or short and without hairs, or glassy or short hypocotyls.

A significant relationship was detected between closing time and the level of hard seededness. This was seen as a lower level of hard seed formation in early closed (October) treatments. Spraying also had a strong and highly significant influence (Table 36). The highest percentage of hard seed was found in treatments sprayed with paraquat 30 days after closing (except in the D-J treatment) and the lowest in plots sprayed at closing or 30 days before closing. The levels of hard seed were very high in all cases (84 to 95%), except in the earliest closed and sprayed treatment (O-S).

Dead seed results (Table 37) showed that the O-S treatment had a significantly higher percentage of dead seeds compared with the rest of the treatments. This particular treatment also showed the highest percentage of abnormal seedlings, and the lowest percentage of hard seeds. This effect might be due to the dense canopy formed in the O-S treatment, which might increase the degree of deterioration of the seed prior to harvest. In addition, the seeds formed in this treatment would have been exposed to the field environment longest before harvest. All of these factors could have affected seed quality. The rest of the treatments did not show any statistical differences. Apparently higher seed mortality was associated with early closing and spraying.

Table 35: Effect of closing date and time of paraquat application on abnormal seedling percentage 1983 - 1984

Closing time		Paraquat treatments	
Mid - October	2.2	30 days before closing	1.4
Mid - November	0.5	At closing	1.4
Mid - December	0.8	30 days after closing	0.7
Significance	N.S.	Significance	N.S.
S.E.D. ±	0.24	S.E.D. ±	0.37

Table 36: Effect of closing date and time of paraquat application on hard seed percentage 1983 - 1984

Closing time		Paraquat treatments	
Mid - October	81.1	30 days before closing	83.1
Mid - November	87.7	At closing	86.4
Mid - December	92.0	30 days after closing	91.2
Significance	*	Significance	**
LSD 5%	5.50	LSD 5%	3.23
1%	N.S.	1%	4.53
S.E.D. ±	1.97	S.E.D. ±	1.48

Interactions

Treatments

O-S	O-O	O-N	N-O	N-N	N-D	D-N	D-D	D-J
67.2	83.8	92.2	87.3	84.8	91.0	94.8	90.7	90.5

Significance	**				
LSD(a) 5%	7.08		LSD(b) 5%		5.58
1%	10.89		1%		7.83
S.E.D.(a) ±	2.88		S.E.D.(b) ±		2.56

(a) When comparing means at different levels of mainplots

(b) When comparing means at the same levels of mainplots

Table 37: Effect of closing date and time of paraquat application on dead seed percentage 1983 - 1984

Closing time		Paraquat treatments	
Mid - October	2.6	30 days before closing	2.1
Mid - November	0.4	At closing	1.5
Mid - December	1.1	30 days after closing	0.6
Significance	**	Significance	**
LSD 5%	1.21	LSD 5%	1.08
1%	2.01	1%	1.51
S.E.D. ±	0.44	S.E.D. ±	0.49

Interactions

Treatments

O-S	O-O	O-N	N-O	N-N	N-D	D-N	D-D	D-J
5.3	1.7	0.8	0.7	0.5	0.2	0.3	2.3	0.7

Significance	**				
LSD(a) 5%	1.93		LSD(b) 5%		1.87
1%	2.87			1%	2.63
S.E.D.(a) ±	0.82		S.E.D.(b) ±		0.86

(a) When comparing means at a different levels of mainplots

(b) When comparing means at the same levels of mainplots

1 - Relationship between seed yield and seed yield components

Table 38 lists the correlation coefficients for the relationship between seed yield and some of the seed yield components measured in the field in 1983-1984. The total number of inflorescences produced gave high correlation coefficients with seed yield at all closing times and in all spraying treatments except when spray application was carried out 30 days before closing. These varied from +0.72 to +0.96. It was estimated that 62% to 92% of the total variation in seed yield is explained by the estimated linear regression of inflorescence numbers.

Table 38: Correlation coefficients between seed yield and yield components from plots closed at different dates and sprayed with paraquat

Closing time	Correlation coefficient		
	Inflorescence (m^2)	Florets/inflorescence	No of seed (m^2)
Mid - October	+0.72*	+0.06	+0.48
Mid - November	+0.95**	+0.93**	+0.75*
Mid - December	+0.96**	+0.86**	+0.86**
Paraquat treatments			
30 days before closing	+0.69	+0.58	+0.94**
At closing	+0.86**	+0.81**	+0.92**
30 days after closing	+0.86**	+0.75*	+0.84**

* = Significant at 5% level

** = Significant at 1% level

More productive treatments were also characterised by the production of inflorescences bearing relatively high numbers of florets. Highly significant correlation coefficients, from +0.93 to +0.86 for November and December closings and from +0.81 to +0.75 for spraying treatments were recorded. The exception was again the treatment where spray application was carried out 30 days before closing. The results show generally, that inflorescences with more florets tend to produce more seed.

Highly significiant correlation coefficients were obtained between seeds/inflorescence and seed yield for November and December closing dates and between the different times of paraquat application. This response indicates the importance of paraquat as a management tool to increase seed set and seed yield. Again, the early closed treatment (October) produced fewer seeds per floret, reinforcing this treatment's generally poor performance.

C - DISCUSSION

Paraquat spraying was particularly effective in controlling grass in a mixed grass:clover sward. The reduced competition from grass growth following spraying was reflected in higher seed yields, particularly when spraying was carried out at closing or 30 days before closing and where spraying was associated with early closing date. The suppression or retardation of white clover plant recovery in plots sprayed 30 days after closing had a deleterious effect. The dead mulch created by this spraying treatment caused a serious reduction in both the vegetative and reproductive growth of white clover. Plants which were not sprayed showed severe competition by grasses on white clover growth and development, particularly following closing in September or October. Other authors (Blood 1962, Sharp 1968, Zaleski 1970 and Clifford 1980) have also shown that spraying a mixed sward with paraquat can check grasses, thus producing better domination by the clover. These investigations showed that swards applied with

the herbicide produced higher seed yield than those unsprayed. Scott (1973) suggested the application of paraquat in late September or early October. In fact, he recommended grazing the sward until mid-September followed by spraying with paraquat in early October. He does not recommend application later than this time because paraquat may damage or depress white clover regrowth and subsequent flowering. However, soil type has a strong influence on the length of time in the spring during which the crop can be grazed. Seed crops grown on heavy or medium soils with a good supply of moisture and nutrients can be closed or sprayed later than those on light, dry soils (Jolly 1957, Scott 1973).

Peak flowering was delayed by late closing, although delaying closing until December resulted in a reduction in the variation in peak flowering date between spraying treatments. These results are not in agreement with those previously obtained by Rossiter (1972) and Hagon (1973) in subterranean clover, which showed that cutting prior to inflorescence initiation delayed this event but did not alter the time of peak flowering compared with an undefoliated control. However, in white clover, more recent work by Thomas (1981b) has indicated that late defoliation (e.g. mid-November) delayed flowering and inflorescence formation. These responses are not surprising because defoliation causes a brief cessation of growth as well as a reduction in storage food until photosynthesis is reestablished at a level that meets the growth requirements of the plant (Harris 1978). Hence, it is to be expected that defoliation causes an initial reduction in stolon elongation and therefore a delay in the date of peak flowering.

The two years of the field experiments were quite different climatically. The first year (1982/3) was wet, cool and windy compared with the second year. These differences were reflected in the number of seeds formed per floret (seed set). Following the generally less favourable climate during pollination in 1982/83 seed set was low (approximately 1 seed/floret). This response is not surprising because white clover needs favourable climate conditions for seed set, which includes a dry period, low wind speed and clear days during flowering

(Win Pe 1978). Furthermore, if rain falls during the maximum flowering period (November or December in Palmerston North) seed set may also be decreased by excessive vegetative growth of grass and clover, which prevents access by the pollinator to the florets (Clifford 1980). In the next year experiment (1983/84) more suitable conditions during pollination (November, December & January) were reflected in a seed set of approximately 2 (and up to 3.5) seeds per floret. These second year results compare favourably with values recorded by Clifford (1979). Nevertheless, even the highest values obtained are a poor result considering that generally 6 ovules are present per floret (Thomas 1981c). This suggests that even under apparently good climatic conditions for pollination and seed set, considerable wastage occurs between potential and final seed numbers per floret.

The results of the first field experiment showed a good correlation between vegetative dry weight of clover, stolon length and LAI. As explained before, all these characters are depressed after defoliation. Similar results have been reported by Knight (1953), Mitchell (1956a,b) Brougham (1958), Pascoe (1973) and Clifford (1985) in white clover. However, there was a generally poor correlation between clover dry weight and branching numbers, particularly in the highest seed yielding treatment (N-N). It is likely that the lack of relationship between those characters is due to the reduction of branching in the summer as a direct effect of temperature (Beinhart 1963), while the number of leaves tends to increase during this season (Thomas 1980a).

Branch production was affected by closing date, being encouraged by early closing. This character, however, seemed not to be closely associated with high seed yield. There was a tendency, however, for side branching to be lower in late (N-D and D-J) treatments. Similar results were found in white clover by Beinhart (1963). According to them, branching frequency was greatest in the spring, and lowest in the summer. These changes could be explained as a result of an increase of flowering during the summer (Thomas 1980a, Clifford 1985), because it is impossible for the formation of an inflorescence and a branch to occur at the same node. In fact, the development of inflorescences provides a new sink source which might be expected

to be undergoing rapid cell division and to have high metabolic rates which may inhibit branching formation (Harvey 1970). High seed yields were obtained in both years from plots closed in November. In the first year plots closed in November and sprayed in October were highest yielding, while in the second year November closed plots sprayed in either October or November were best (Figure 7). However, delaying closing and spraying until December reduced seed yield, with late spraying being more damaging than late closing. The situation in the first year, however, suggested that closing could be delayed until December provided climatic conditions encouraged white clover growth into the summer. In this year the treatment sprayed in November and closed in December (D-N) also produced high seed yields. Inflorescence numbers were also affected by late closing or spraying. Generally, the major effect was seen following spraying with paraquat 30 days before closing or at closing. By comparison the effect of closing date was relatively minor. The above results are corroborated by the work of Haggar *et al* (1963) in white clover, who found that continuous grazing by sheep during spring increased the number of inflorescences per unit area and final seed yield. Clifford (1980) also found that, with adequate soil moisture, frequent grazing until mid November gave the best seed yield, as well as the maximum concentration of inflorescences in comparison with earlier closing dates. In other experiments, Clifford (1979) also showed that defoliation after mid-November caused a dramatic reduction in inflorescence numbers and seed yield.

The number of florets per inflorescence was found to be relatively insensitive to the timing of closing or spraying. However, unsprayed treatments invariably resulted in low floret numbers per inflorescence. This presumably reflected the competitive effect of grass component on reproductive development. Similar results were obtained by Zaleski (1964) who found that one of the main effects of defoliation were on the number of florets produced per inflorescence. Undefoliated clover had lower numbers of florets while the difference between early and late defoliation was not significant.

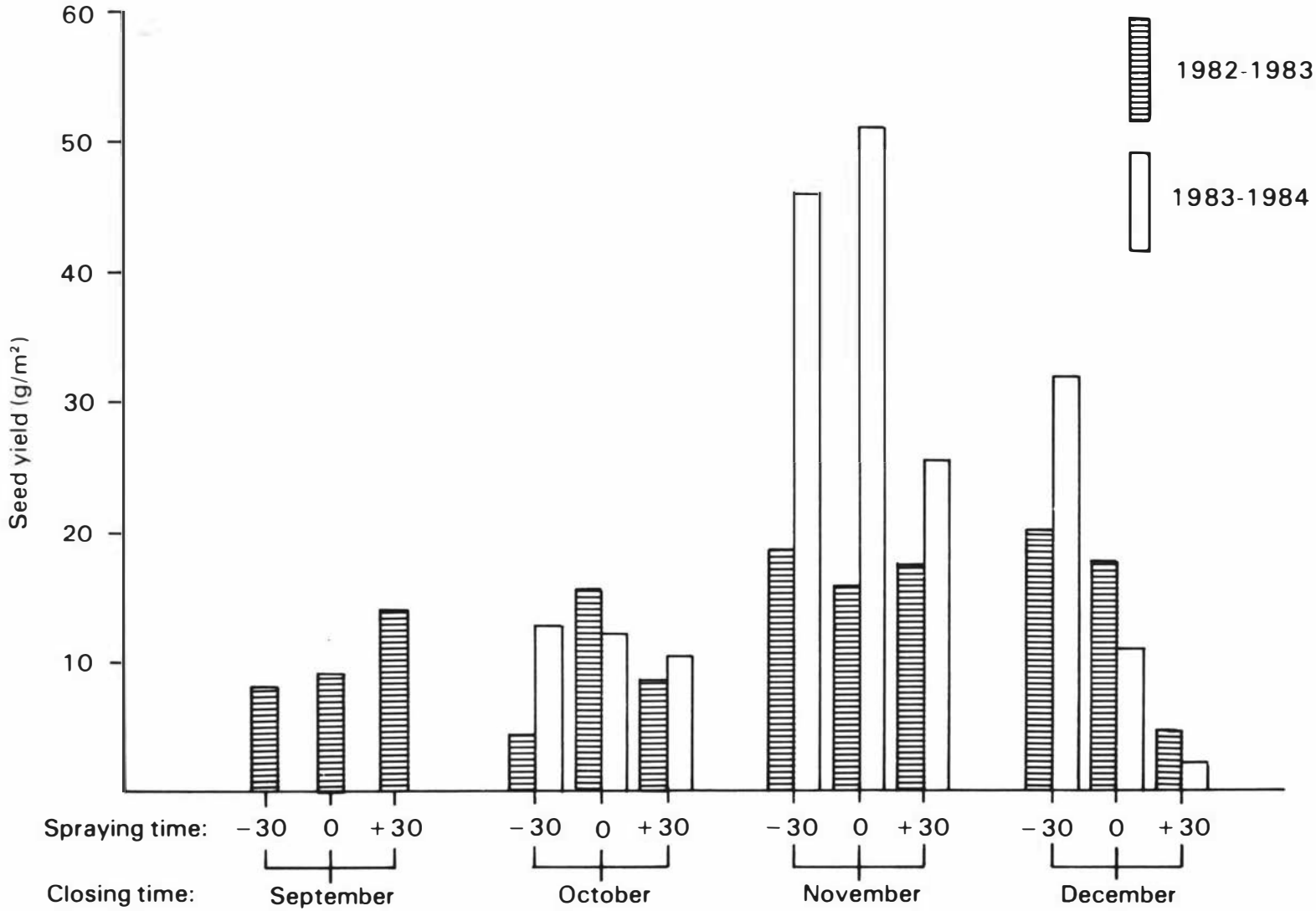


Figure 7 Effect of closing time and paraquat treatments on seed yield per unit area (g/m²). Two year experiments.

There appeared to be a complementary or compensatory effect between seed set and seed weight. In the first year, seed weight was an important component of seed yield but seed numbers per head were comparatively unimportant. In the second year the reverse trend occurred. Although seed weight was not generally affected by treatments, values for this component were approximately 25% higher than in the first year. The first year results were characterised by low seed set and high seed weight. The second year results were more affected by high seed set but lower seed weight. Gaspar *et al* (1981) reported the same trend in white clover and pointed out that there is a tendency for the clover plant to compensate for low numbers of seeds/floret by increasing seed weight and vice-versa.

The components of seed quality were variable between treatments. The general trend however, was for all seed samples to exhibit low levels of normal seedlings, abnormal seedlings and dead seed, and high levels of hard seed. There was also a tendency for unsprayed treatments and seed harvested early (25 days after peak flowering) to show poorest seed quality. One characteristic which is related to seed maturity in white clover is hard seededness. According to Hyde (1950) hard seed levels increase with seed age at harvest, until on the 25th day 96% of the viable seeds are hard. However, under commercial seed production conditions the hard seed percentage changes with environmental conditions (Zaleski 1970). In general, the drier the atmosphere in which the seeds ripen the greater the amount of hard seed produced. Thus, seed ripened at a relative humidity of 70% all acquired the ability to germinate within 5 months, while those ripened at 30% relative humidity still showed 97% hard seededness after 18 months storage (Hyde 1950). Therefore, it is possible that white clover seeds which ripen in humid weather or beneath dense foliage will show reduced and shallower hard seededness, whereas seeds ripening under dry conditions may remain dormant for a longer period.

One interesting feature of these field experiments was the unexpectedly poor relationship established between vegetative characters such as node numbers, number of terminal buds and lateral branch development

and seed yield. This was particularly surprising, since many other workers have shown the importance of a strong vegetative structure to provide high numbers of sites for inflorescence development (Beinhart *et al* 1963, Zaleski 1970, Thomas 1980a).

CHAPTER III

STUDY OF THE GROWTH PATTERN OF HUIA AND THE EFFECT OF CUTTING AND PARAQUAT ON ITS REPRODUCTIVE DEVELOPMENT AND SEED YIELD

A - THE IMPORTANCE OF PLANT STRUCTURE ON SEEDING POTENTIAL AND YIELD

1 - Introduction

The potential seed yield of a plant is determined by its vegetative growth and development; by the profuseness of its inflorescence production; by the efficacy of the pollinating agent; and by the completeness of seed formation and development. The primary developmental factors influencing potential seed yield are those controlling the initiation of the inflorescence and its growth. In conditions suitable for initiation the seed production capacity is determined by the number of inflorescence formed, the number which develop to anthesis, the number of ovules in each floret, and the fertility of pollen and embryo sacs (Zaleski 1964, Thomas 1961 a,b,d, 1981a, c). Subsequently, seed yield is a function of effective fertilisation (seed set), and seed development to maturity.

A considerable number of investigations have been carried out into the environmental factors affecting pollination of inflorescences by bees (Bohart 1960, Palmer *et al* 1962, Haggar *et al* 1963, Chapman and Carter 1976, and Win Pe 1978) and the role of inflorescence development as a factor setting the initial limits on possible seed yield (Zaleski 1964, 1970, Clifford 1979, 1980, and Thomas 1980a, 1981a, b, c, 1982). In addition, it has been asserted that because several different genotypes may exist within a small area there may be considerable variation in plant morphology and flowering behaviour (Knight 1953, Beinhart *et al* 1963, and Burdon 1980), as well as in the response of individual plants to the environment.

The present investigation was undertaken to observe changes in 'Grasslands Huia' white clover growth in a field at Palmerston North in relation to its vegetative and reproductive pattern of growth and development, and to compare the responses of two different genotypes.

This work was also carried out to try to clarify some of the results obtained in the previous two field experiments (Chapter II).

2 - Materials and Methods

a - Experimental site and field procedures

Two white clover plants grown from 'Grasslands Huia' breeder's seed, line C3085, were obtained from an established field at Grasslands Division, DSIR Palmerston North in May 1984. These plants were transferred to the glasshouse and were designated as genotype I and genotype II. Cuttings comprising 4 to 6 emerged nodes from each plant were taken at the end of May and were held in an unheated glasshouse at the Seed Technology Centre for approximately 4 weeks. During this time they became well rooted. The plants were grown in 30 x 40 x 6 cm^3 plastic germination trays in a 5:2:3 mixture of sand, soil and peat. Daily glasshouse temperature averaged 18°C maximum and 13°C minimum during May, and 14°C maximum and 10°C minimum during June.

On July 1st, 50 plants of similar size from each genotype were transplanted to the field at Massey University using a square planting of 60 cm. The soil type was a Tokomaru silt loam with similar characteristics to those of previous experiments (Appendix 1). The 1984/85 year was characterised by generally low rainfall except in January, warmer conditions (+1.4 to 1.9°C), and low wind speeds during flowering in November, December and January. Climatic conditions during the experiment are compared with the 20-year average in Appendix 12.

Weeds were removed by hand every 15 days from the experimental site during the growing season. The plants were also sprayed with malathion at 1 kg a.i./ha and phorate at 2 kg a.i./ha to control aphis and stem weevil, respectively.

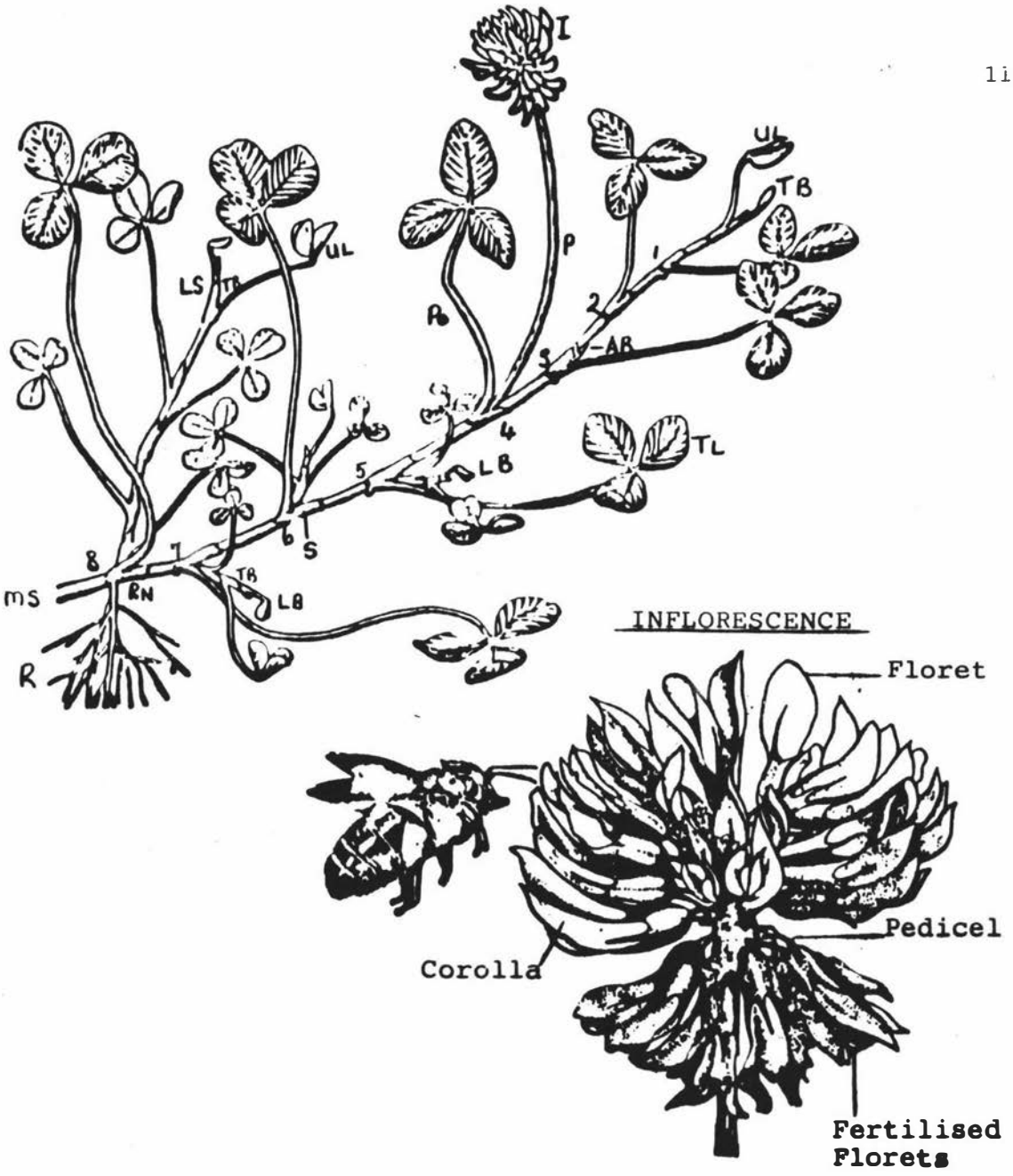


Figure 8: Vegetative and floral organs of a white clover plant (Bean 1978 and Thomas 1985)

Definitions:

MS = Main stolon (stolon with at least 8 emerged nodes)
LS = Lateral stolon (stolon with more than 4 nodes without root system)
LB = Lateral branch (vegetative outgrowth with 2 or 3 unfolded leaves)
Pe = Petiole (leaf stalk)
S = Stipule (appendage at the base of leaves)
TL = Trifoliate leaf (leaf with three leaflets)
1.2.3. ... nodes = node number
AB = Axillary bud (vegetative shoot in leaf axil with 2 unfolded leaves)
TB = Terminal bud (terminal with 6 or 7 unemerged nodes and an apical meristem)
I = Inflorescence (Roundish capitulum bearing florets)
P = Peduncle (inflorescence stalk)
F = Floret (complete flower with calyx, corolla, stamens, and one carpel)
RN = Rooted node (node with adventitious root system)
R = Root
UL = Unfolded leaf

b - Plant measurements

The diagram and key shown in Figure 8 is used to describe the various vegetative and reproductive structures of the white clover plant referred to in this study. In the field 50 main stolons of each genotype were marked on 1 July using coloured staples placed immediately behind the node bearing the youngest unfolded leaf. This process was repeated at monthly intervals until seed harvest. Each new marking was carried out using different coloured staples and was made on the original main stolons.

The following vegetative measurements were taken each month from marked stolons:

- Number of nodes emerging from the terminal bud of each main stolon (N)
- Increase in length of main stolons
- Number of lateral stolons formed on main stolon (L)
- Number of lateral branches formed on main stolon (B)
- Number of axillary buds on main stolon (X). This data was calculated by subtracting the total number of nodes (N) from nodes with lateral stolons (L), plus nodes with lateral branches (B), plus nodes with inflorescences (I) according with the formula: $X = N - (L + B + I)$.
- Number of rooted nodes formed on a main stolon.

In addition, every 15 days from August 1st, the number of unexpanded leaves and leaf primordia present in the terminal apex was determined by microscopic examination of 10 dissected stolon apices from each genotype at a magnification of 40 x.

The following reproductive measurements were taken:-

- Inflorescence initiation was determined every 15 days by dissection of 10 stolon apices and examination of them under a microscope at a magnification of x 40. Scanning electron micrographs were

taken at different stages of inflorescence development.

Twenty stolon apices were selected at each observation date and the number of nodes bearing inflorescences and their position relative to the apical meristematic dome was recorded. Estimated times of inflorescence initiation were calculated as described by Thomas (1979). He has shown that from counts of the number of nodes emerging per month, the number of days needed to form a node can be calculated for each month. Data from dissections can be used to determine in which node of the terminal bud the inflorescence is located in relation to the apex. Therefore, the date of flower initiation can be estimated from the formula:

$$t = s - y\,x$$

Where t = date of the inflorescence initiation
s = date of sampling of the terminal bud
y = number of days needed to form a node
x = the node in the terminal bud where the inflorescence is located at the time of the dissection

In a sample of 10 marked inflorescences, the pattern of inflorescence development from emergence to harvest was also observed. Photographs illustrating the sequence of development were taken.

- The number of emerged inflorescences present per month on a main stolon and on lateral stolons was determined by counting the number of inflorescences present at harvest between the staples used to mark stolons as described earlier.

- Peak flowering was determined by counting the number of white inflorescences present per week in two 50 x 50 cm^2 quadrats.

- The length of peduncles on ten inflorescences in which the corolla of the floret had turned brown was measured. The length of the petioles on the subtending leaf and the number of florets on the inflorescence was also measured in each genotype every 15 days from October to February.

- The number of ovules/ovary for each genotype was counted in ten white florets taken at random from 10 inflorescences every 15 days from October to February.

- The number of fertilized ovules per ovary in each genotype was determined at 2 weekly intervals, using 100 florets taken at random from 10 inflorescences in which both the corolla and peduncles were brown, and fully formed seed could be rubbed out in the hand. As the method initially used for counting fertilized ovules was very tedious and slow, a system was developed which employed x-ray photography. The selected florets were placed on a polaroid film type 55 and then transferred to the x-ray equipment (Hewlett Packard. Model 43804N) and exposed at 35 KVP and 3MA for one minute.

- The total number of inflorescences present in two 50 x 50 cm^2 quadrats was recorded 30 days after the peak flowering.

- The number of florets/inflorescence at harvest was determined by counting the florets on 10 inflorescences at harvest time.

- The number of seeds/floret was calculated from 1000 seed weight (SW), seed yield (SY), number of florets per inflorescence (NF), and the number of inflorescences (NI)/m^2, according to the formula used in previous experiments (Chapter 2).

- 1000 seed weight was determined by the method prescribed in the International Rules for Seed Testing (ISTA 1976).

- Seed yield was obtained from the total number of inflorescences previously calculated. These inflorescences were threshed using different sized sieves and the inert matter was removed by means of a vertical column aspirator (Burrows 1836-4 laboratory blower).

Standard errors were calculated at each sampling date.

3 - Results

a - Vegetative growth and development

The relationship between the rate of node appearance and time of the year is shown in Figure 9 and Table 39 for the two genotypes used. In general, the rate of node appearance increased from an average of about 2 or 3 per month between July and September to about 5.5 per month in October and a maximum of 7.7 to 8.2 nodes in January. There were relatively small differences between the two genotypes in this regard. Based on the stolon length data (Figure 10 and Appendix 13) the rate of elongation was very small during the spring months, August to September. Subsequently, stolon elongation showed a marked increase from October to December, followed by a cessation of growth in January. Of particular interest was the increasing difference between genotypes in the rate of stolon elongation during November and December. The stolons of genotype II had stopped extending by November.

The number of lateral stolons was recorded at the time of harvest on February 1st (Figure 11 and Table 39). According to these results the number of lateral stolons produced per month from July to October did not change drastically during the season. From October to January, however, there was a constant decrease in the number of lateral stolons until the end of January when very few new lateral stolons were observed to develop. The response of each of the two genotypes was very similar throughout the experiment. The percentage of nodes that bore lateral stolons in each month $\left(\frac{\text{Number of lateral stolons}}{\text{number of nodes}} \times 100\right)$ was also calculated using data from Appendix 14. It was possible to determine from dissected samples taken in February that 77% to 99% of nodes formed between July and September formed lateral stolons.

The percentages decreased from 44% to almost 0% from October to January, respectively. A similar trend was found in each genotype.

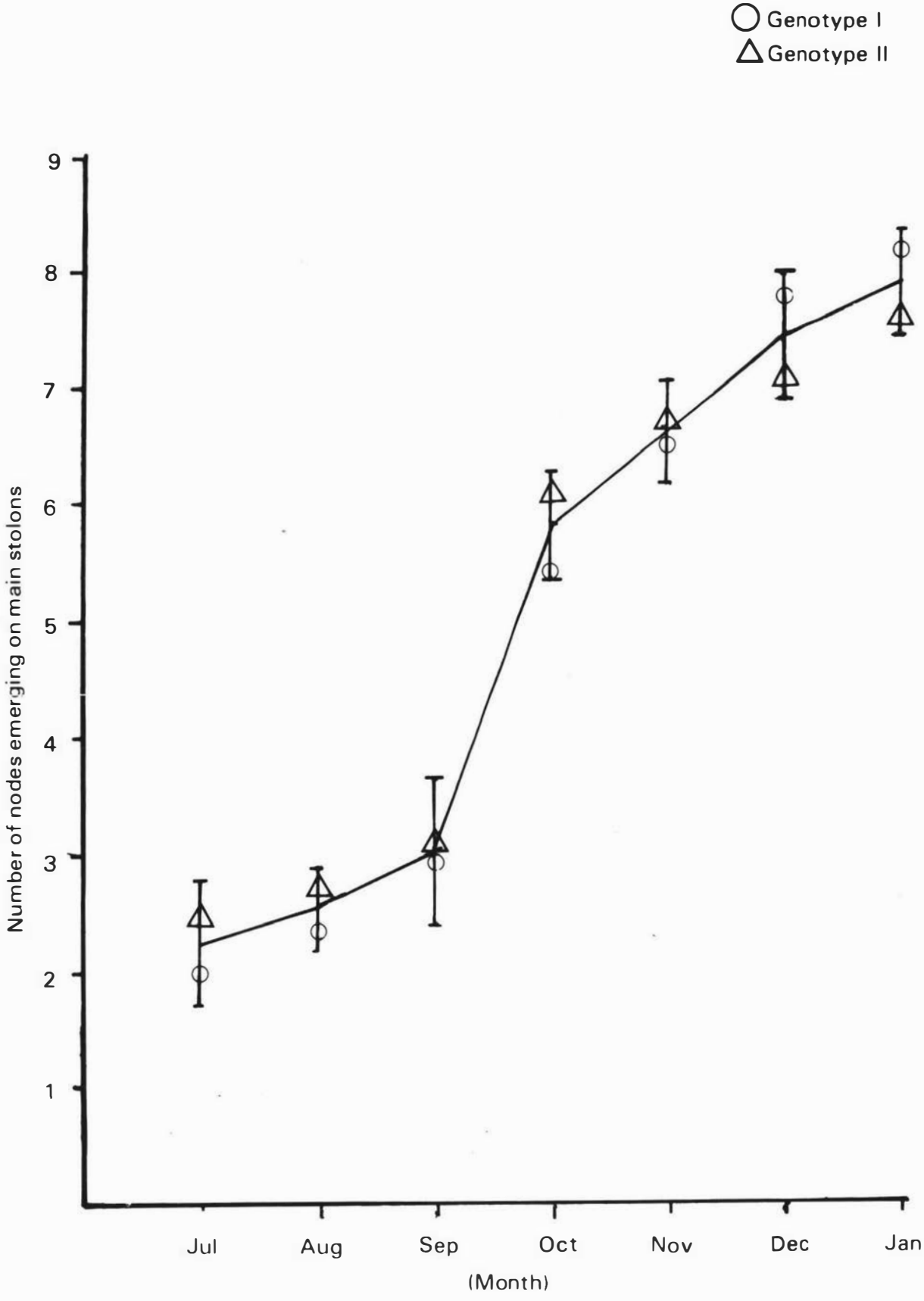


Figure 9 Number of nodes emerging from the terminal bud on main stolons per month.

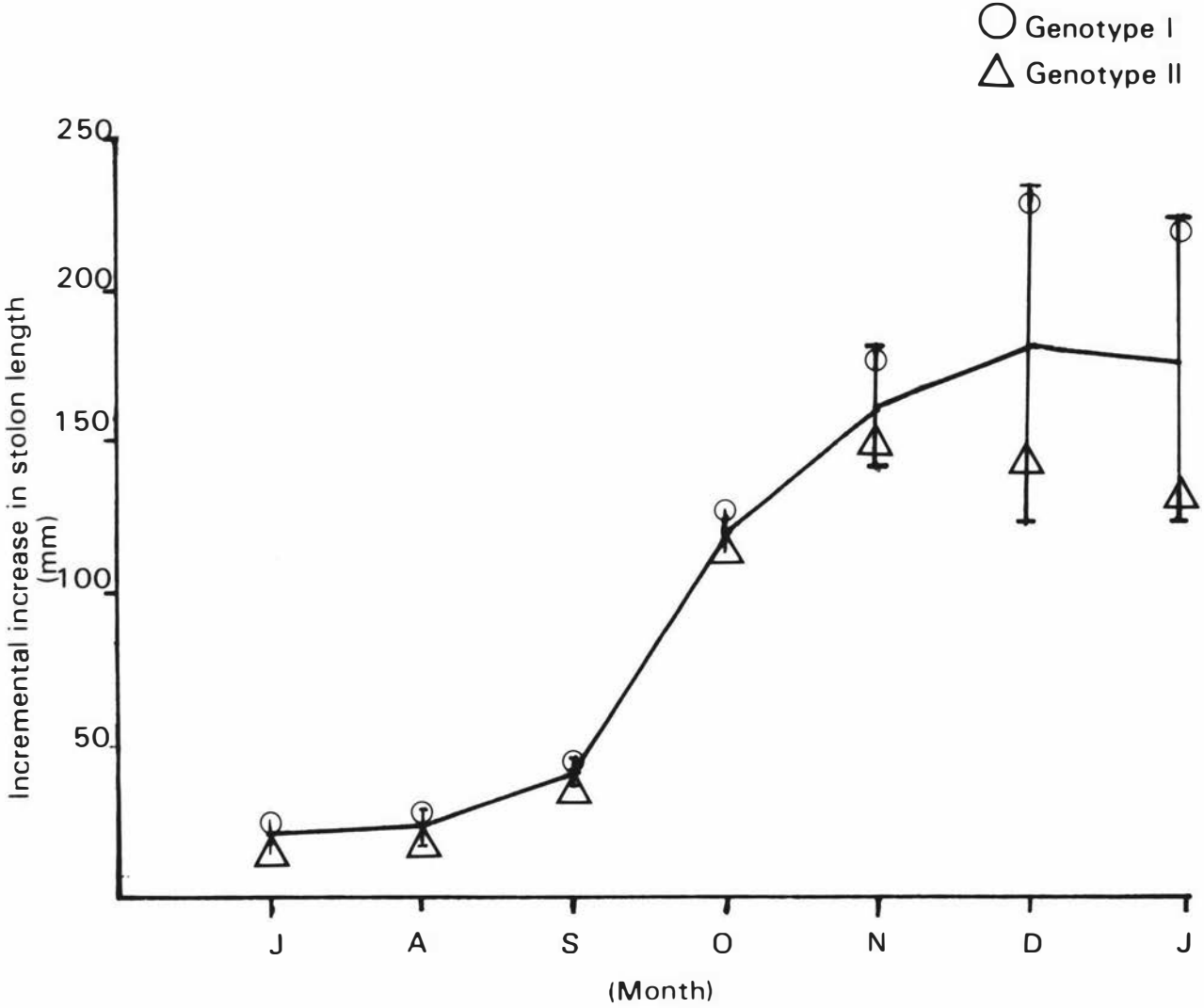


Figure 10 Monthly incremental length increase in main stolons (July to January).

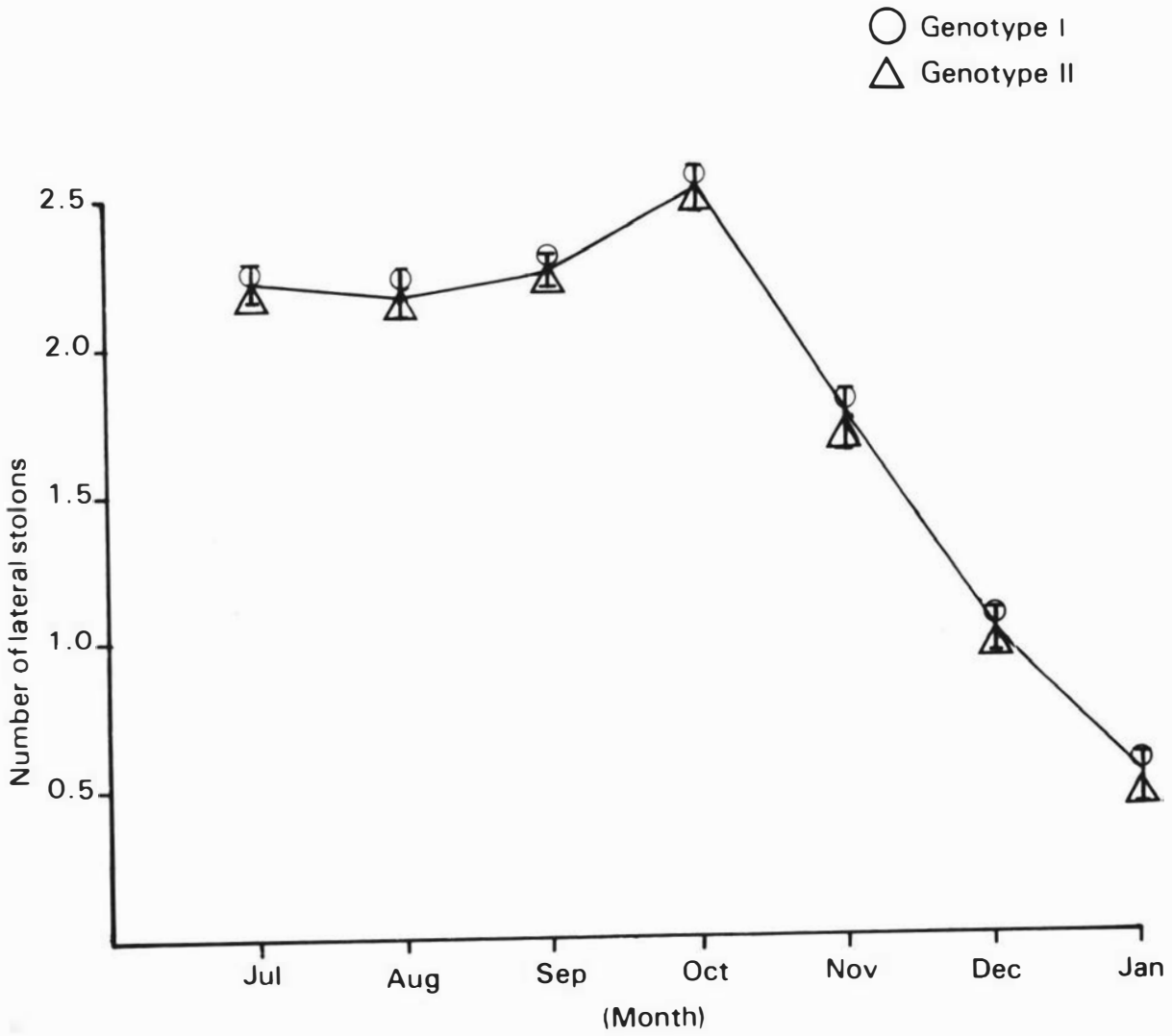


Figure 11 Total number of lateral stolons present in February at nodes which emerged from terminal buds on main stolons from July to January.

Month	Number of nodes on main stolon	Number of lateral stolons on main stolon	Number of lateral branches on main stolon	Number of axillary buds on main stolon	Number of inflorescences on main stolon
			AVERAGE		
July	2.25 ± 0.50	2.23 ± 0.20	0.00 ± 0.00	0.14 ± 0.20	0.00 ± 0.00
Aug	2.55 ± 0.31	2.19 ± 0.15	0.00 ± 0.00	0.36 ± 0.32	0.00 ± 0.00
Sept	3.04 ± 0.61	2.25 ± 0.11	0.38 ± 0.06	0.03 ± 0.04	0.38 ± 0.12
Oct	5.81 ± 0.45	2.53 ± 0.14	1.07 ± 0.12	1.20 ± 0.36	1.00 ± 0.17
Nov	6.61 ± 0.37	1.75 ± 0.19	1.14 ± 0.06	1.96 ± 0.12	1.76 ± 0.18
Dec	7.46 ± 0.48	1.04 ± 0.09	1.74 ± 0.11	3.13 ± 0.55	1.55 ± 0.46
Jan	7.92 ± 0.46	0.51 ± 0.10	2.00 ± 0.13	4.89 ± 0.51	0.54 ± 0.20
			GENOTYPE I		
July	2.00 ± 0.14	2.25 ± 0.18	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Aug	2.35 ± 0.12	2.28 ± 0.11	0.00 ± 0.00	0.13 ± 0.06	0.00 ± 0.00
Sept	2.96 ± 0.41	2.27 ± 0.14	0.39 ± 0.06	0.06 ± 0.04	0.27 ± 0.06
Oct	5.45 ± 0.32	2.56 ± 0.37	1.12 ± 0.13	0.88 ± 0.10	0.89 ± 0.06
Nov	6.53 ± 0.25	1.80 ± 0.28	1.13 ± 0.17	1.97 ± 0.13	1.63 ± 0.17
Dec	7.80 ± 0.28	1.08 ± 0.10	1.82 ± 0.22	3.67 ± 0.41	1.22 ± 0.11
Jan	8.19 ± 0.28	0.53 ± 0.08	2.03 ± 0.12	5.15 ± 0.63	0.44 ± 0.08
			GENOTYPE II		
Jul	2.50 ± 0.35	2.21 ± 0.30	0.00 ± 0.00	0.29 ± 0.08	0.00 ± 0.00
Aug	2.75 ± 0.35	2.16 ± 0.23	0.00 ± 0.00	0.59 ± 0.10	0.00 ± 0.00
Sept	3.12 ± 0.25	2.25 ± 0.35	0.37 ± 0.09	0.00 ± 0.00	0.50 ± 0.08
Oct	6.16 ± 0.60	2.50 ± 0.28	1.02 ± 0.13	1.52 ± 0.20	1.12 ± 0.18
Nov	6.70 ± 0.56	1.70 ± 0.16	1.18 ± 0.15	1.92 ± 0.16	1.89 ± 0.20
Dec	7.14 ± 0.44	1.00 ± 0.26	1.66 ± 0.22	2.60 ± 0.33	1.88 ± 0.14
Jan	7.65 ± 0.51	0.49 ± 0.12	2.08 ± 0.17	4.47 ± 0.62	0.64 ± 0.18

Table 39: Total number of nodes, lateral stolons, lateral branches, axillary buds; and inflorescences (± standard errors) present in February at nodes which emerged from terimal buds of main stolons during each month from July to January

Figure 12 (Appendix 14) shows the number of lateral stolons of different sizes formed by the end of the experiment (February). Lateral stolons with the most nodes (more than 11) were those originating from nodes which emerged in July, August and September. The lateral stolons formed at nodes in October were intermediate in node numbers (8 to 11) and those with the smallest number of nodes (4 to 7) originated on lateral stolons emerging in November and December. In January few nodes produced lateral stolons in both genotypes. None of these produced more than 7 nodes. These differences are likely to be very important in determining which nodes have the opportunity to produce inflorescences. However, the results also suggest that it is not only important to encourage a high percentage of new stolon formation but also to ensure the production of stolons with large numbers of nodes which can later make a significant contribution to inflorescence numbers. If each node exists as a potential site for the formation of an inflorescence then stolons carrying a high number of nodes present higher possibilities for reproductive growth. The number of lateral branches (Figure 13 and Table 39) rapidly increased from September to January with only minor differences between genotypes. The results in February indicated that during the late winter and spring (August to September) most of the nodes formed produced a lateral stolon (Table 39). Very few of these nodes remained as axillary buds or developed inflorescences. However, during the late spring and summer (October to January) the number of nodes forming lateral branches or axillary buds increased. The number of lateral branches and axillary buds both reached a maximum in January. It was particularly interesting that 61% of nodes had not developed a vegetative branch or inflorescence, but remained as axillary buds when examined in January. Inflorescence numbers reached a peak from nodes formed in November.

The percentage of nodes bearing adventitious roots on main stolons ($\frac{\text{number of rooted nodes}}{\text{number of total nodes}} \times 100$) followed the same pattern as that described for the percentage of lateral stolons present on main stolons per month. Observations in February showed that the percentage of rooted nodes decreased from 91% in July to 28% in January (Figure 14). Both genotypes showed a similar and consistant trend in relation to the percentage of rooted nodes (Appendix 15).

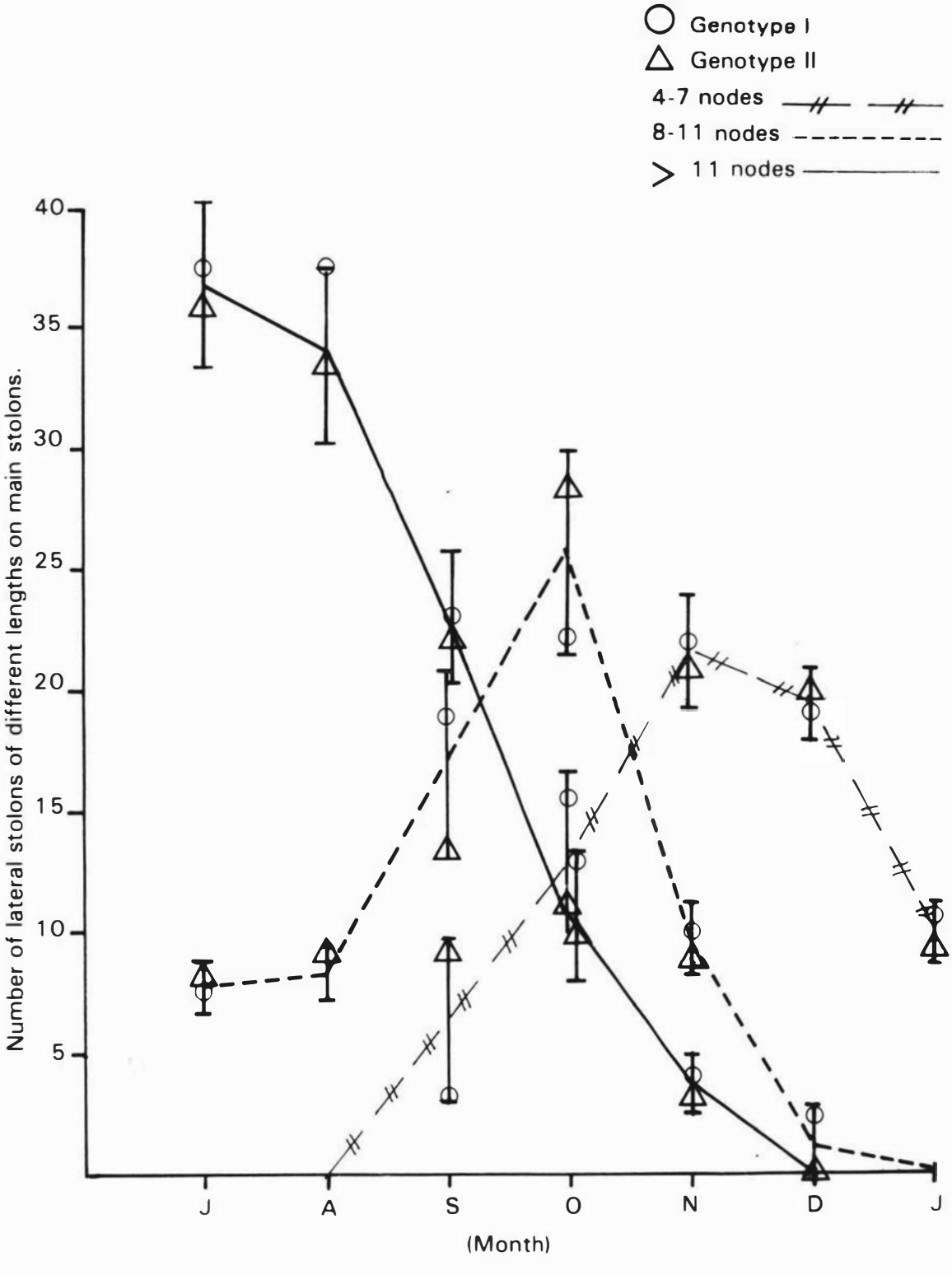


Figure 12 Number of lateral stolons of different lengths present in February at nodes which emerged from terminal bud of twenty main stolons from July to January.

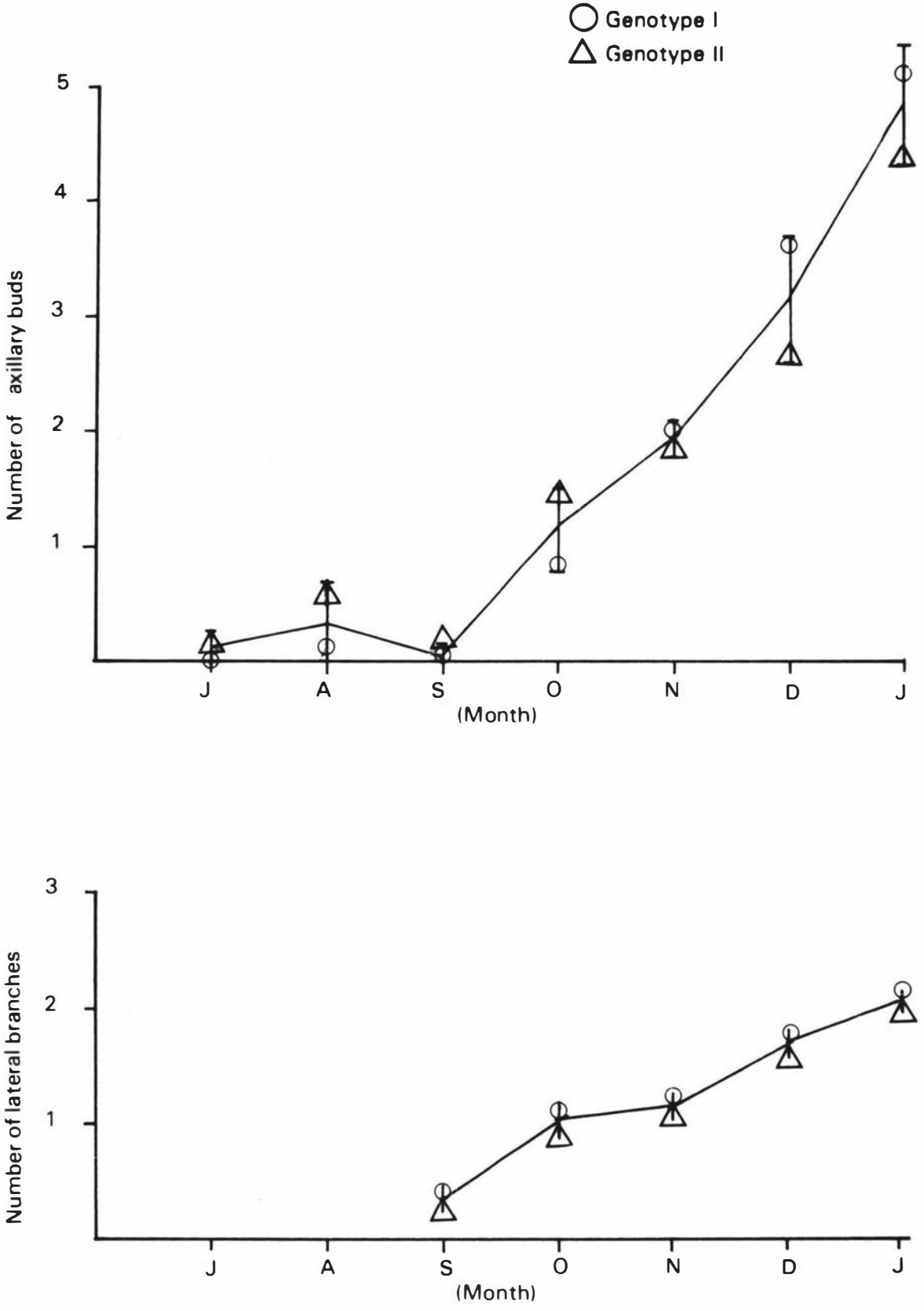


Figure 13 Number of lateral branches and axillary buds present in February at nodes which emerged from terminal buds of main stolons from July to January.

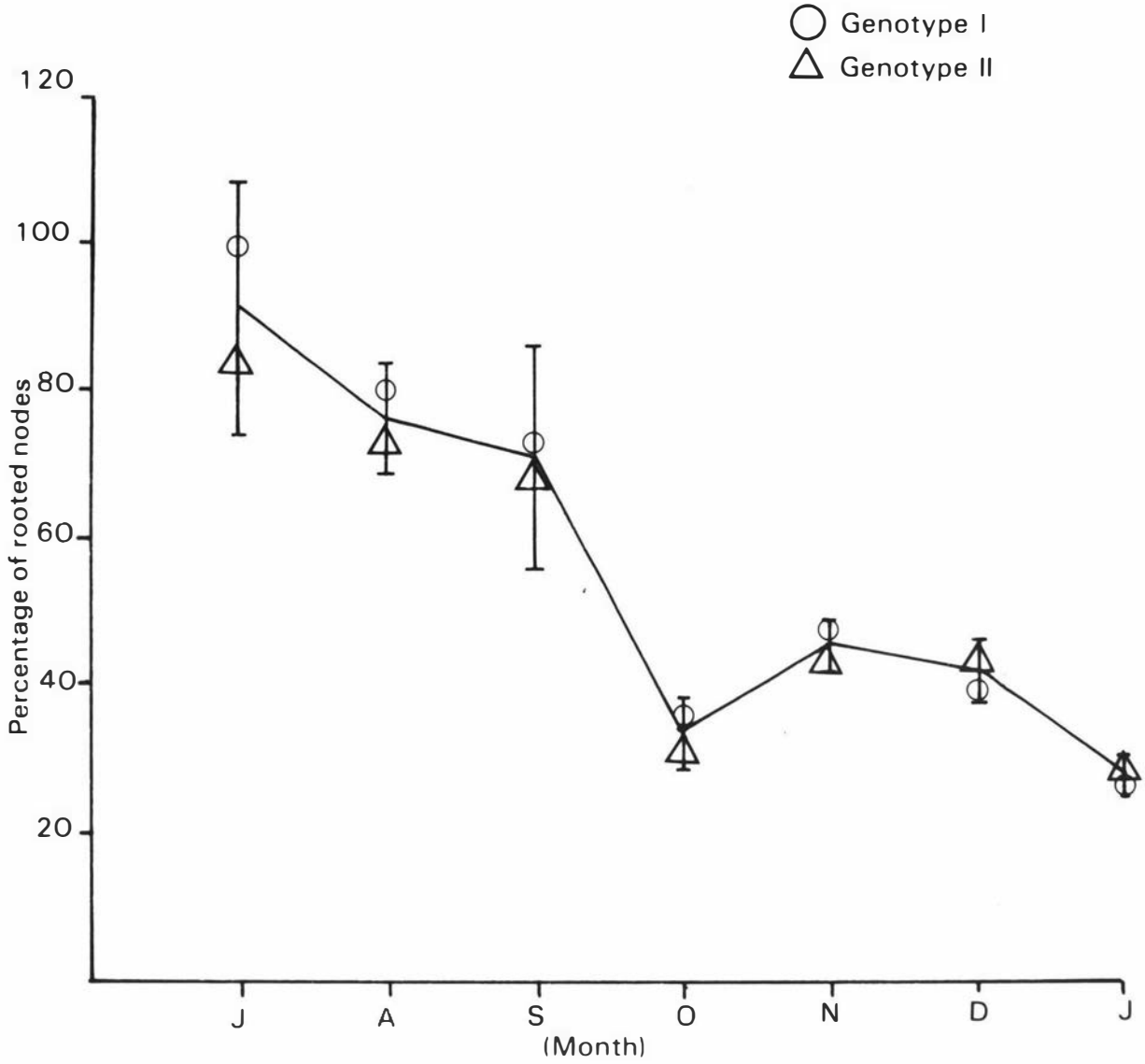


Figure 14 Percentage of rooted nodes present in February at nodes which emerged from terminal buds of main stolons from July to January.

b - The morphology of inflorescence initiation and development

One of the most noticeable differences observed in the two previous field experiments was the number of inflorescences produced in each treatment. For that reason, in this investigation a detailed study was made of the changes occurring during reproductive development.

The sequence and rate of inflorescence development was also observed in terminal buds formed some months prior to harvest and photographs were taken using a scanning electron microscope to illustrate the sequence of reproductive growth. Different phases of terminal bud development were selected to provide a sequence of developmental stages showing distinct morphological characteristics. Based on Figure 15 published by Thomas (1981c), the following stages in the development of the apical meristem were selected.

Vegetative primordia:

This stage is characterized by the presence in the apex of a dome covered by 6 or 7 leaf primordia. Dissection of this apical bud by successively removing leaves reveals the structure shown in Plate 5. In the process of growth, each time a new leaf primordium forms at the apical meristem, the oldest leaf enclosed within the apical bud breaks through its stipular sheath to become visible as the youngest emerged leaf.

Reproductive primordia:

Inflorescence initiation

Inflorescence initiation occurs at the stolon apex. With the transition to the reproductive state a bud is first observed in the axil of the youngest leaf primordium (Plate 6).

Floret initiation

As the precocious axillary bud continues to grow, florets are differentiated from a series of small bulges formed over its surface (Plate 7). According to Thomas (Figure 15) the inflorescence normally initiates florets when it is at the 3rd or 4th node from the apical meristem.

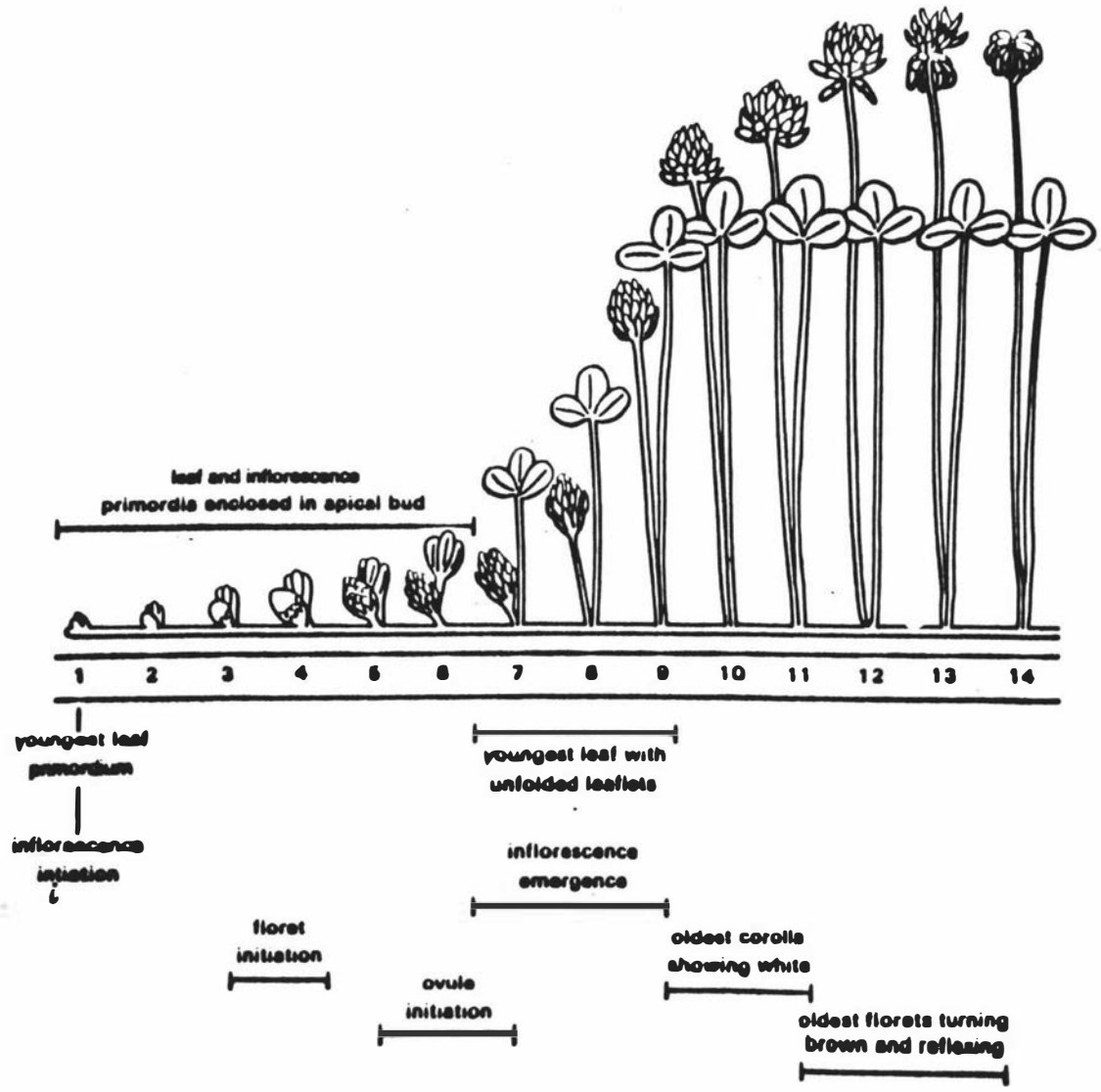


Figure 15: Diagrammatic representation of stages of inflorescence and node development in white clover relative to distance from the stolon apex. (Thomas 1981b)

Plate 5: Scanning electron micrograph showing the morphological appearance of a leaf primordium (x 550)

AM = Apical meristem; LL = Leaflet; LP = Leaf primordium

Plate 6: Scanning electron micrograph showing flower initiation (x 430)
IP = Inflorescence primordium; LP = Leaf primordium; AM = Apical meristem

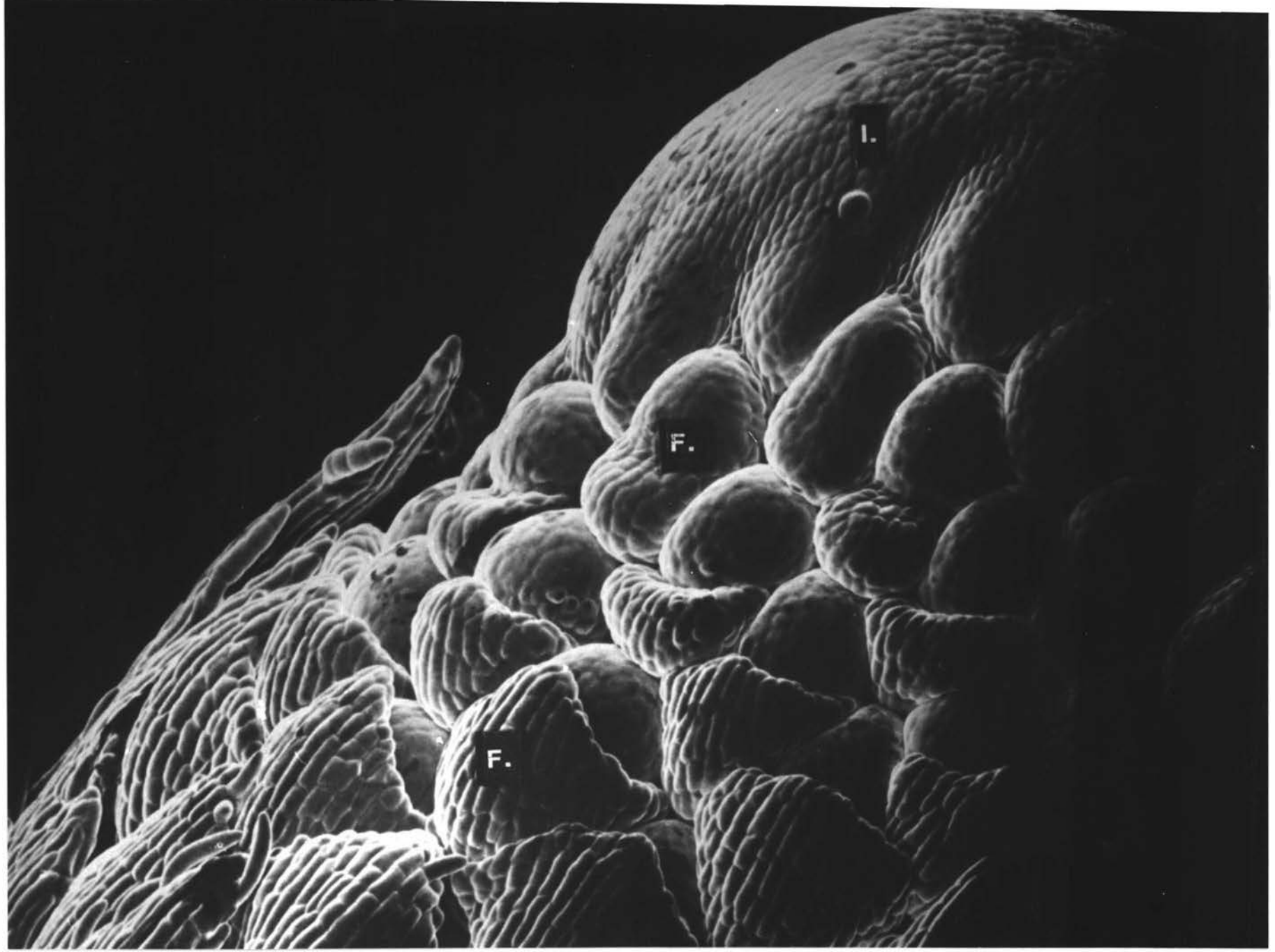


Plate 7: Scanning electron micrograph showing floret initiation (x 300)
I = inflorescence; F = floret initiation

Ovule initiation

After floret initiation starts it may continue until inflorescence emergence. According to Thomas (1981a) ovule initiation occurs when the inflorescence is forming in the fifth node from the apex. Most ovules are completely differentiated at the time of inflorescence emergence (Figure 15).

Inflorescence emergence

In the present investigation an inflorescence was considered to have emerged when it had completely developed from the surrounding stipular sheath. Generally, this occurred at the sixth or seventh node from the apical meristem (Figure 15), a few days after the emergence of its subtending leaf (Plate 8).

Inflorescence changes after emergence

After an inflorescence emerges from its stipular sheath the lowest florets begin to show a white corolla (Plate 9 stage 2) when the inflorescence has reached the 10th or 11th node from the apical meristem. This occurs two or three nodes after flower emergence. Full white corolla development is eventually followed by a change to brown and the florets reflex. These last changes occur approximately at the 12th or 13th node from the apex (Plate 9 stages 4 and 5).

As the rate of node emergence during the experiment was very similar in both genotypes it is possible to estimate when each stage of inflorescence development occurred, relative to the sampling date. In this study close observation of ten marked inflorescences per genotype was made from their emergence on November 15th until seed formation. Based on these observations it was found that between 15 and 20 days were necessary for florets to become white with another 12 to 15 days for them to reflex and approximately 7 more days to turn completely brown.

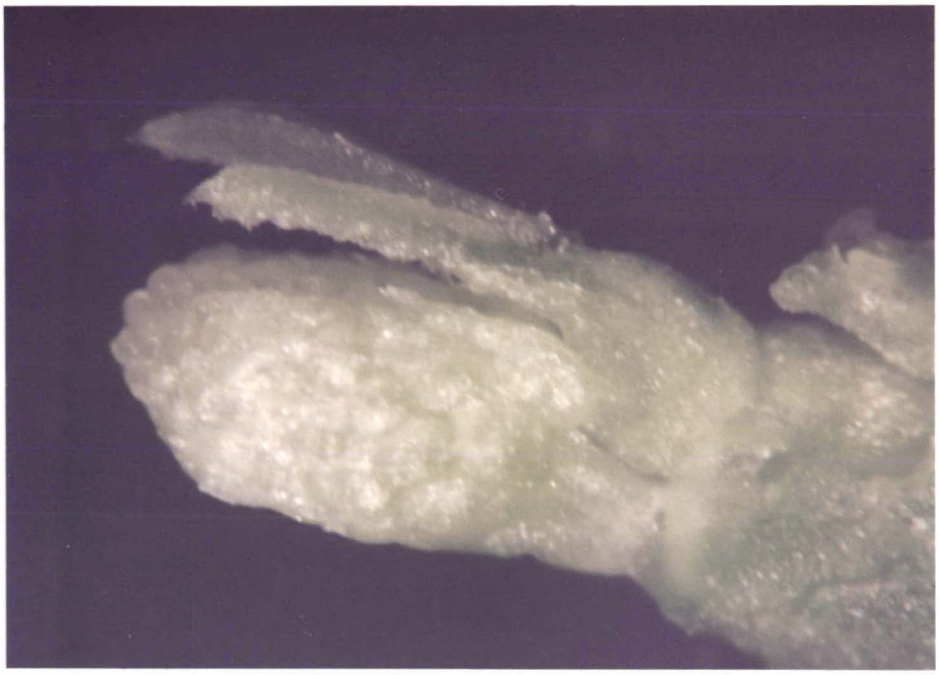


Plate 8: Stage of growth of an inflorescence at early emergence (x 60)

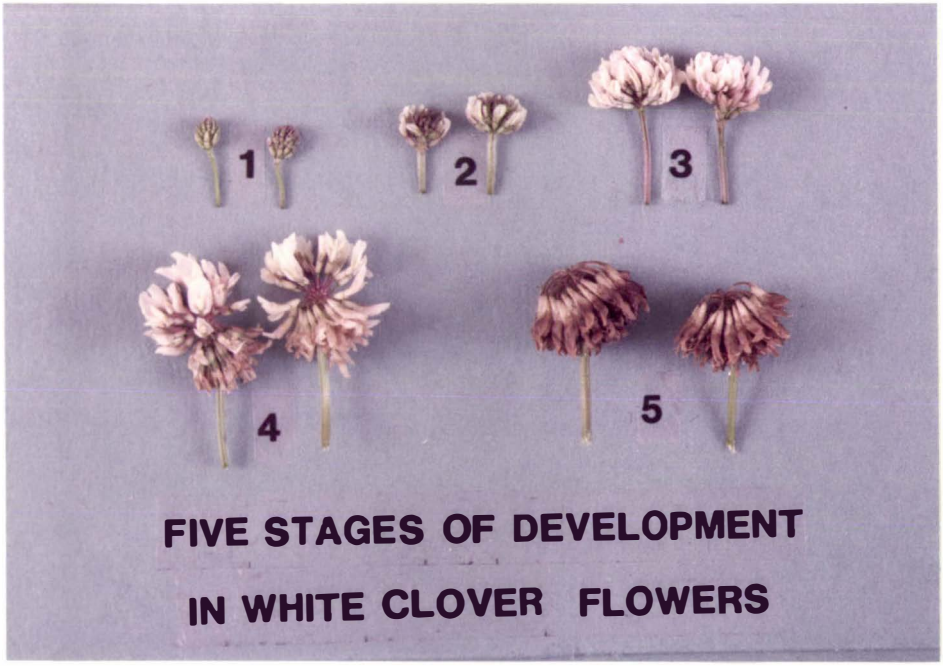


Plate 9: Stages of development of inflorescences after emergence

Time of inflorescence initiation

The estimated time of inflorescence initiation was determined in samples of 10 apices dissected every 15 days in each genotype. Results are presented in the Figure 16 and Appendix 16. It was observed that white clover plants started to initiate inflorescences in late winter (August 15th). Initiation then increased during the spring until October 15th when it reached maximum intensity, before falling again in early December. Dissections made at the end of January showed that inflorescence initiation had stopped.

The big difference between the two genotypes was in the more intense and earlier initiation observed in genotype II. Genotype I tended to have significantly less initiation, but this process continued for longer than in genotype II.

A comparison of the time of inflorescence initiation in these two genotypes, month by month, compared with the 'Grasslands Huia' clones A, B, and C used by Thomas (1979, 1981a, b, 1982), shows that genotype I behaves very much as clone C, while genotype II behaves similarly to clone A. Both genotypes initiate inflorescences in November and December in response to long days but tend to stop in mid summer.

Number of inflorescences present in each terminal bud

The number of inflorescences per terminal bud at each sampling date is shown in Figure 17 (Appendix 17). In both genotypes the maximum number of inflorescences formed in the terminal bud was found from dissections carried out from October 1st to December 15th. This reached a peak in samples examined on October 15th. Some differences in flowering intensity were determined between genotypes. Genotype II showed about 25% more inflorescences in its terminal buds throughout the experimental period than genotype I.

Rate of inflorescence appearance

The number of inflorescences emerged on each plant or per unit area has been previously shown to be the main component of yield (Chapter II).

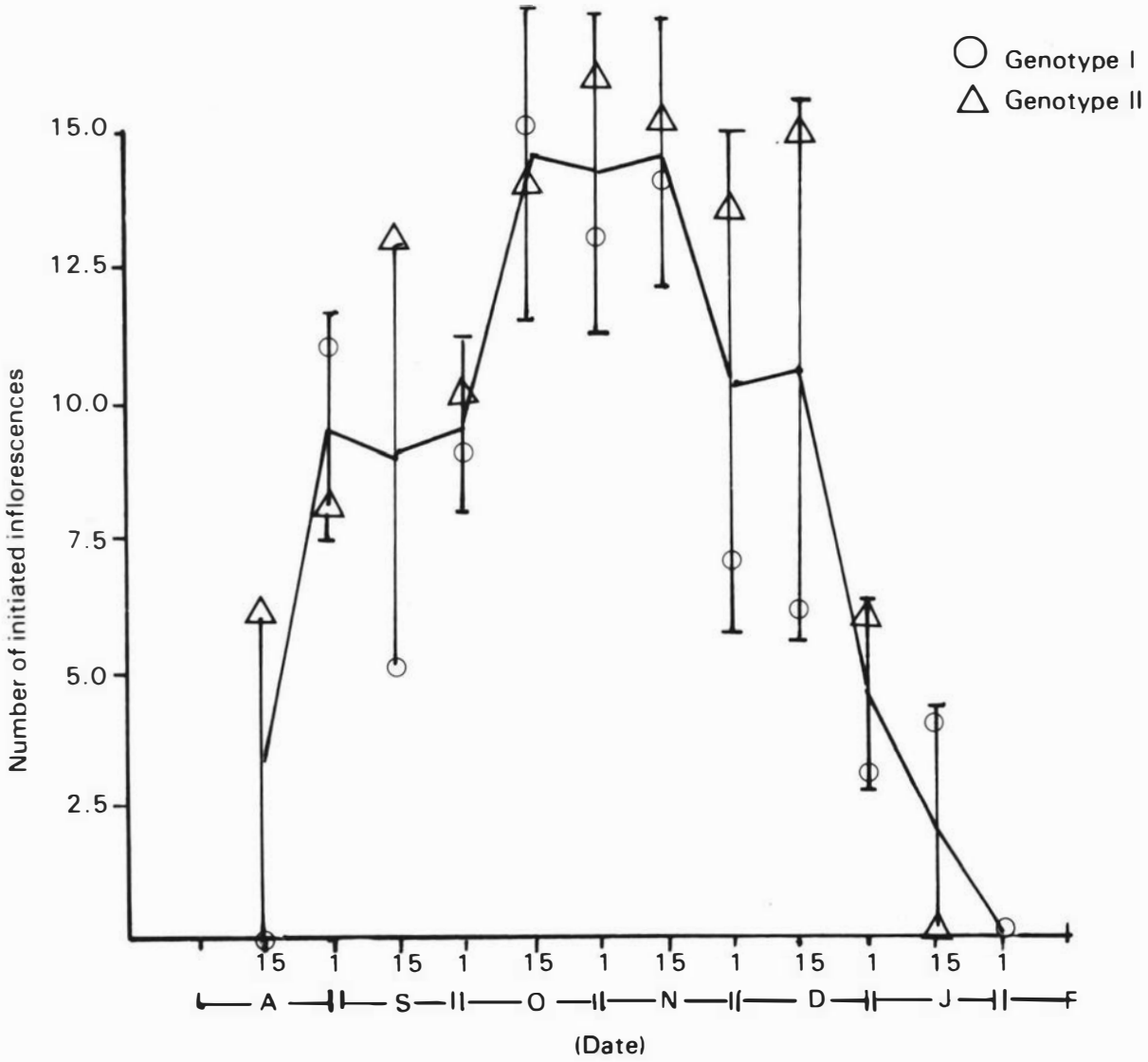


Figure 16 Estimated time of inflorescence initiation in 10 terminal buds on main stolon from July to February.

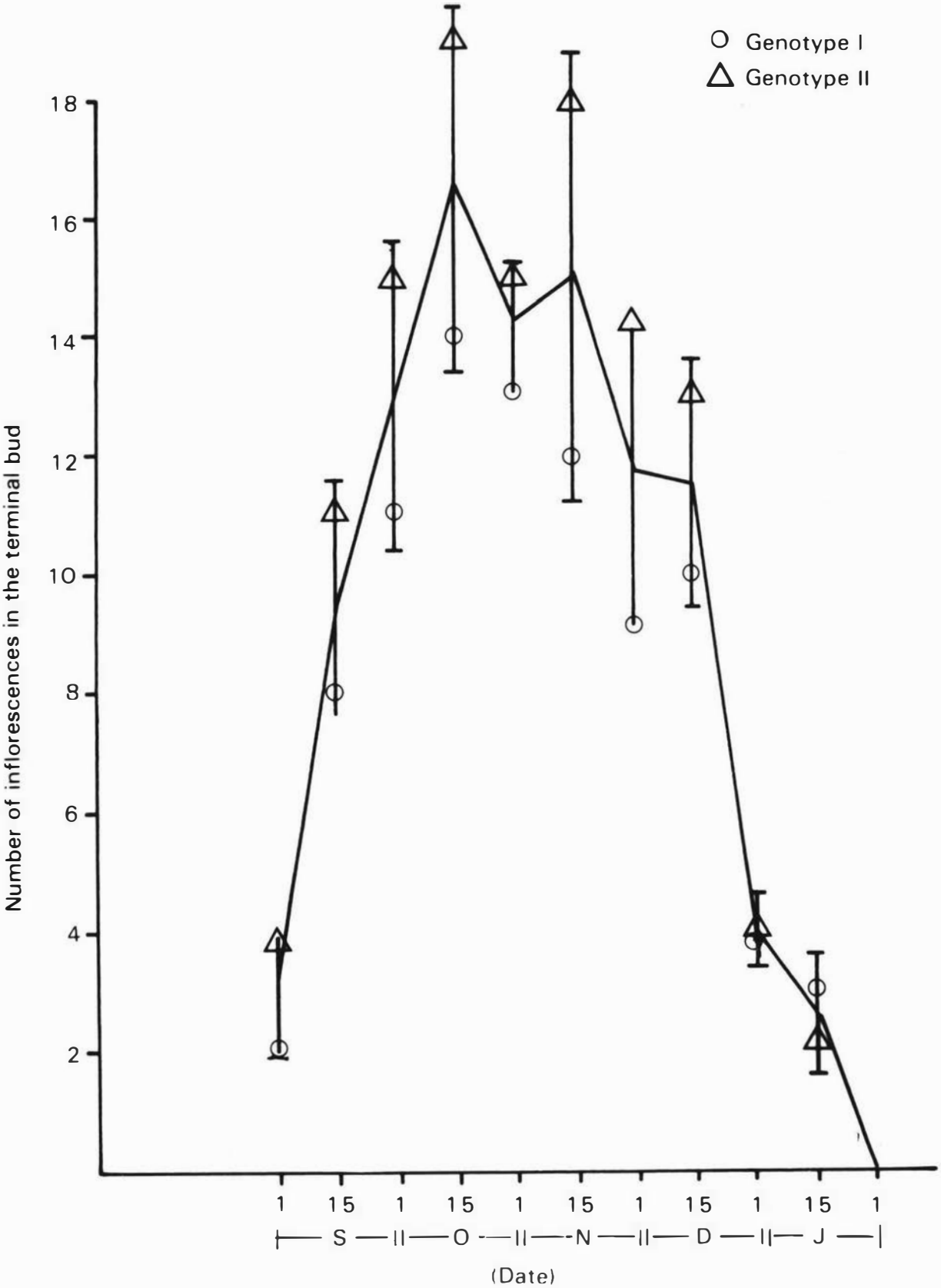


Figure 17 Number of inflorescences present in ten terminal buds on main stolons from July to February.

In the present study, the number of inflorescences produced on main stolons or on lateral stolons is shown in Figure 18. These results indicate that maximum inflorescence density occurs during November and December. This was almost certainly the result of high levels of inflorescence initiation in October and November. In general, 80% of these inflorescences were formed on main stolons while only 20% were formed on lateral stolons (Figure 18). The emergence of inflorescences occurred during long days and warm temperatures. The results also stress the low reproductive potential of nodes formed on lateral stolons, particularly during the winter. This suggests that management systems which discourage side branching of main stolons particularly during the summer are likely to result in plants with higher seeding potential as a direct result of improved flowering on main stolons. Differences between genotypes were observed at all sampling dates in relation to the number of emerging inflorescences. Genotype I always showed less flowering intensity (Plate 10). The total number of inflorescences produced by genotype II at all sampling dates was more than 50% higher than in genotype I.

Peak flowering

Based on the average of the number of white inflorescences (Figure 19, Appendix 19), peak flowering was found to occur on about January 1st with some variation between genotype I (January 7th) and genotype II (January 1st). This difference in the time of peak flowering between genotypes was probably due to the slower inflorescence development observed in genotype I as a consequence of its denser canopy (Plate 10). This possibly suggests that differences in peak flowering date between genotypes may ocur because of the modification in light intensity under the canopy. It was interesting that flowering in both genotypes commenced at the same time (21 October) and continued with only minor differences until 26 November. Subsequently, however, major differences occurred. At peak flowering genotype I produced 60 white inflorescences per square metre compared with 120 for genotype II.

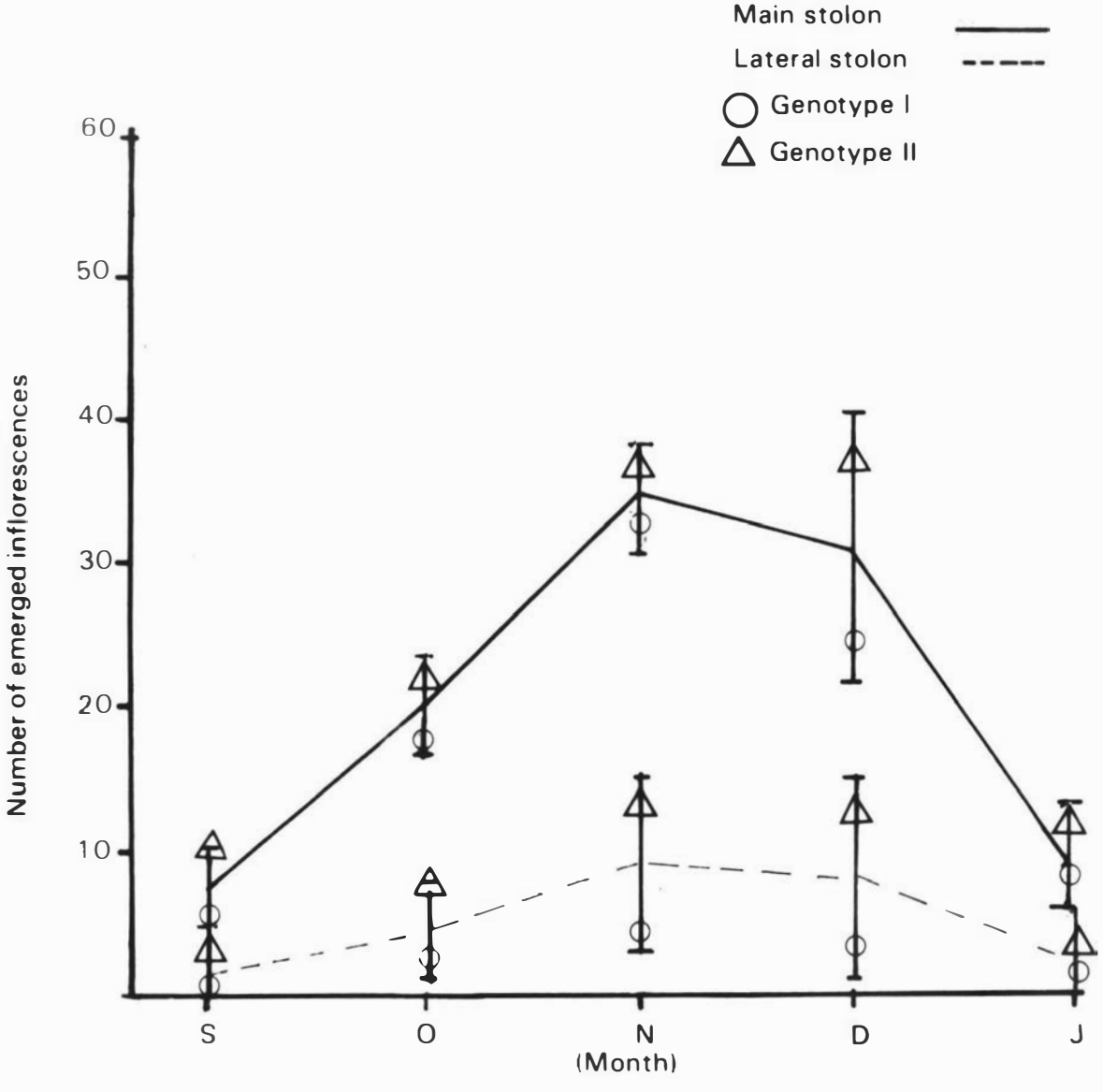


Figure 18 Number of inflorescences emerged on main and lateral stolons from September to January.

Plate 10: Differences in flowering density between the two genotypes in December 1984. Left genotype I, Right genotype II.

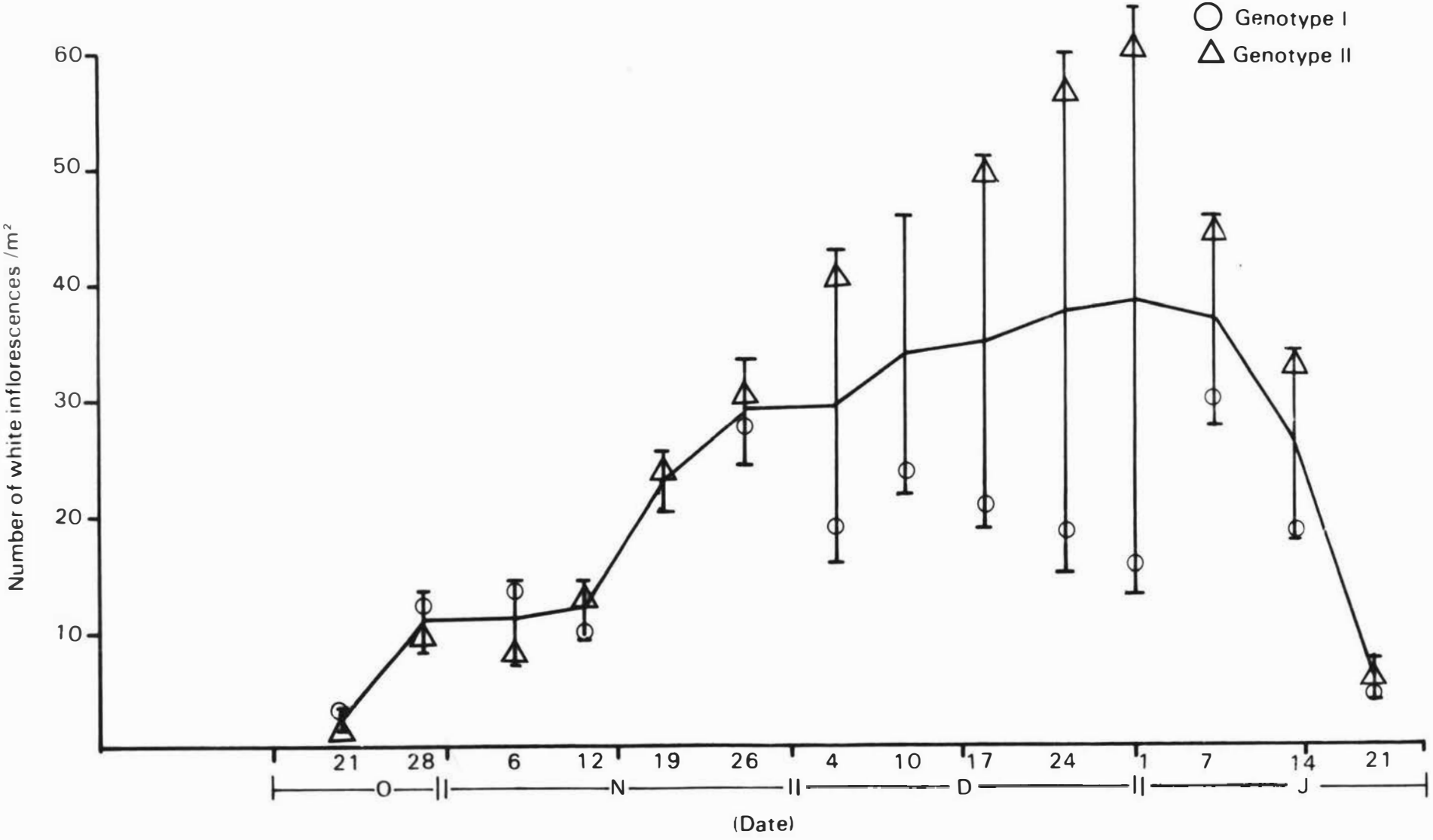


Figure 19 Number of white inflorescences present per unit area (0.25m²).

Petiole and Peduncle Length

Besides being influenced by the number of inflorescences produced per plant, seed yield is affected to some extent by the position of inflorescences in relation to the general level of foliage. When inflorescences fail to rise above the leaf canopy, the chances of pollination are reduced. Similarly, under wet conditions, many inflorescences tend to rot even after pollination or fertilization and seeds can often be found which have begun to sprout in the heads under the canopy. This suggests that the relative lengths of the peduncle and petiole can have a major influence on both seed yield and quality.

The average length of fully elongated peduncles and petioles or their subtending leaves are shown in Table 40 (Appendix 20). There seemed to be no consistent relationship between petiole and peduncle length with time from November to February unlike the reduction situation previously described by Thomas (1981c). Peduncles were over 70% longer than petioles on January 1st and up to 95% longer by February 1st. Plants from genotype I produced longer petioles and peduncles than plants from genotype II (Appendix 20). The peduncles in genotype I also tended to show downward bending which often appeared to prevent some inflorescences from rising above the leaf canopy.

Floret numbers per inflorescence

The number of florets per inflorescence (Table 40) was determined in samples taken every 15 days from 10 inflorescences located at node 12 on average (Figure 15) and which were in the stage 3 of development (Plate 9). From November to February the number of florets per inflorescence varied considerably. In general the number of florets decreased during this period from 86 to 64. This trend is supported by work by Thomas (1981c) which has shown that more florets are initiated on inflorescences formed under short days and low temperatures. This parameter also varied between genotypes, particularly in samples taken after December 1st (Appendix 20). Genotype I had fewer florets per inflorescence than genotype II at each sampling date.

Date of Sampling day-month	Petiole legnth m.m.	Peduncle length m.m.	Floret number in white flower heads	No. of ovules per ovary in white florets	No. of seeds formed per pod	% of ovule fertilized
1 Nov	87.9 ± 10.75	173.9 ± 12.6	85.8 ± 5.8	6.02 ± 0.03		
15 Nov	77.9 ± 5.35	174.8 ± 12.1	85.2 ± 2.4	6.00 ± 0.02	4.47 ± 0.26	74
1 Dec	77.5 ± 6.5	155.2 ± 6.5	88.6 ± 12.1	6.02 ± 0.05	4.45 ± 0.51	74
15 Dec	100.2 ± 16.72	192.3 ± 15.0	80.2 ± 6.8	5.95 ± 0.12	4.06 ± 0.35	68
1 Jan	107.8 ± 22.75	185.0 ± 24.3	72.0 ± 4.2	6.00 ± 0.00	3.35 ± 0.27	55
15 Jan	78.0 ± 15.08	157.1 ± 19.6	74.9 ± 7.7	5.65 ± 0.38	3.11 ± 0.06	55
1 Feb	119.1 ± 28.87	231.2 ± 50.7	63.7 ± 5.9	5.20 ± 0.34	2.47 ± 0.21	47

Table 40: Changes in petiole and peduncle length, inflorescence size, number of ovules per floret and number of seeds per pod (± standard errors) during different time of the year. Average values of the two genotypes 1984 - 1985

Plate 11: Ovules present in an unfertilised floret (x 60)

Number of ovules per ovary

The number of ovules present in the ovary of unfertilized florets was very consistent. Most florets bore an average of 6 ovules (range 5 to 7) (Plate 11). The variation in this character was small in both genotypes and at different sampling times with a difference of not more than one ovule between samples taken in November and February (Table 40 and Appendix 20).

Number of seeds produced per pod

The change in the number of seeds formed per pod from November to February (Table 40) was remarkable, decreasing by 45% from 4.47 to 2.47. X-ray photographs showed that in some florets all ovules aborted, while in others all 5 ovules formed seed (Plate 12). These effects could not be explained by lack of pollination because a large number of florets were found to contain several seeds. There was no clear pattern in the position of aborted ovules in the ovaries examined. It is likely that more investigation is necessary to determine the cause of this variation as well as factors which affect it.

c - Seed yield and seed yield components

Table 41 shows the seed yield and yield components at harvest for each genotype (inflorescence numbers/m^2, number of florets/inflorescence, number of seeds/floret and 1000 seed weight), which directly affect seed production in white clover.

Table 41: Variation in yield components and seed yield (± standard errors) between two 'Grasslands Huia' genotypes 1984-1985

	Genotype I	Genotype II
Number of inflorescences/m^2	454 ± 31.30	856 ± 69.60
Number of florets at harvest	58.8 ± 4.07	80.0 ± 7.03
Number of seeds per floret	2.31 ± 0.18	2.01 ± 0.10
1000 seed weight (g)	0.54 ± 0.05	0.62 ± 0.04
Seed yield kg/ha	464 ± 36.26	850 ± 52.89

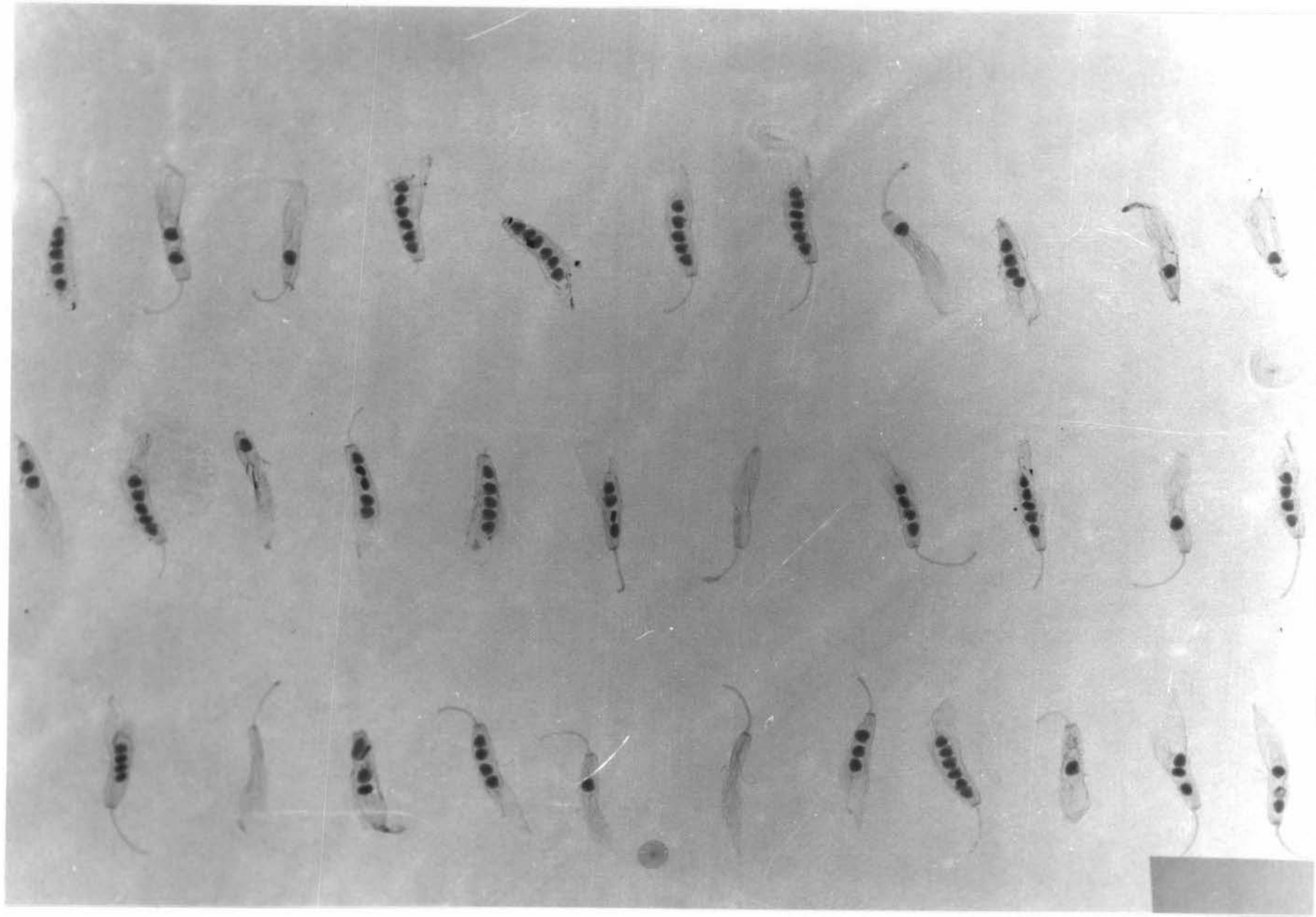


Plate 12: X-ray photograph showing the number of ovules forming seeds in individual florets

A big variation in seed yield was found between genotypes. Genotype I gave 45% less yield than genotype II. Such major differences in seed yield warranted further observation to try to determine the respective contribution of those components which influenced seed yield. Inflorescence numbers per unit area at harvest had a major influence on seed yield. Genotype I showed 47% fewer inflorescences compared to genotype II. This result was not surprising because genotype II had showed a higher intensity of flower initiation and inflorescence density throughout the investigation. The size of inflorescences at harvest (number of florets/inflorescence) was also a major component affecting seed yield. On average, inflorescences produced 26% fewer florets/inflorescence in genotype I than in genotype II. This difference could be genetic in origin or alternatively be due to the time of initiation of the florets as affected by daylength and temperature.

The third important component of seed yield was the number of seeds per floret. As the number of ovules per floret was similar between genotypes a big difference in this character was not expected. In fact, seed set was similar between genotypes. However, there was a tendency for genotype I (2.31) to have more seeds/florets than genotype II (2.01). It is possible that in genotype I the lower inflorescence numbers/m^2 was compensated for by better seed set per floret.

The final component which might be expected to influence seed yield was seed weight. Seeds from genotype I were 13% lighter than seeds from genotype II.

The results for different yield components clearly emphasise the dominating influence of inflorescence numbers on seed yield. However, other components such as number of florets at harvest and to a lesser extent seed weight also contributed to seed yield. Seed numbers per floret were not of major importance in determining ultimate seed production. However, there is strong potential for improvement of this component since seed numbers per floret of 2.0 to 2.3 were low compared with the 6 - 7 ovules consistently produced in unfertilised florets.

4 - Discussion

The results suggest there is a partitioning between vegetative growth and reproductive development in white clover. The vegetative process was characterised by the high percentage of nodes on main stolons which formed lateral stolons in the winter and early spring, and the high proportion of nodes which developed only axillary buds in December and January. These vegetative characteristics ensured plant perenniality. Reproductive development was conditioned by the fact that over 80% of inflorescences were formed on main stolons and only 20% on lateral stolons. This emphasises the role of lateral stolons in ensuring continued vegetative growth and the important role main stolons play in inflorescence production and seed yield. Peak inflorescence production occurred from nodes formed in October and November. This suggests that crop management systems which promote high numbers of nodes on main stolons; discourage lateral stolon development in October, November and December; encourage high levels of inflorescence numbers and which enhance seed yield components such as floret numbers and seed weight will have a major bearing on the production of high seed yields. In addition, favourable climatic conditions, including low rainfall, and warm and calm conditions during flowering, as experienced in the present experiment, could be expected to improve pollinator activity and 'seed set'.

In field and growth room studies, Thomas (1961a, b, d, 1980a) and Beinhart (1963) also observed the partitioning of vegetative and reproductive development in white clover. They indicate that there is an inverse linear relationship between temperature and stolon frequency. In general, stolon frequency (number of nodes with a stolon) was greater in the spring, but decreased later in the summer. These changes occurred as a result of an increase of flowering in the summer (Thomas 1980a). In 'Grasslands Huia' white clover, Thomas (1981a) also showed that peak inflorescence initiation occurred in nodes formed in October and November, although initiation started during the winter. On the other hand, Jolly (1957) and Zaleski (1970) have found that it is necessary to have favourable climatic conditions before there is maximum expression of those yield components which affect seed yield.

It was interesting that while both genotypes showed comparable vegetative development, major differences in the level of floral initiation and the general reproductive response occurred. Genotype II showed an earlier and more intensive floral initiation than genotype I - an effect which was subsequently reflected in more flowering on main and lateral stolons and earlier peak flowering. This variation was presumably due to a different requirement for cool temperatures during the winter in each genotype as shown by Thomas (personal communication). He suggested that both genotypes used in this experiment behaved as short-long day plants in a similar way to his clones A and C (Thomas 1979). Genotype I required a longer cool temperature exposure for maximum inflorescence initiation than genotype II. Eventually these differences were reflected in a difference of 60 white inflorescences/ m^2 and 386 kg/ha in seed yield in favour of genotype II. Certainly the production of over 50% more inflorescences and a total seed yield of 850 kg/ha suggested that genotype II had a much more valuable seed production potential than genotype I. This yield difference in favour of genotype II was a direct reflection of the production of 47% more inflorescences, 26% more florets per inflorescence, and 13% heavier seeds. It was interesting, however, that despite these major differences in reproductive capacity, seed 'set' (seeds per floret) was similar between genotypes.

Although major differences in seed yield occurred between genotypes, the seed production of the plants was consistently poor compared with the potential yield which could be obtained with improved seed set. While the level of seed set (2.0 - 2.3 out of a total of 6 - 7 ovules per ovary) was relatively high by comparison with the previous field experiments (Chapter 2) and with figures given by Dessureaux (1951) and Clifford (1979) it was only 30 - 40% of the potential 'set'. Whether this difference is genetic, environmental, or a reflection of pollination efficiency is not known. It seems possible that ovule or seed abortion is a consequence of inter-ovule competition resulting from fertilized ovules nearest the stigma having a temporary development advantage. Differences in the time of fertilization of as little as one hour have been shown to induce inter-ovule competition and abortion in *Phaseolus vulgaris* (Gates *et al* 1983). However, the physiological causes of this response need to be determined. Atwood

(1943) suggested that abortion of some developing fertilized ovules might arise as a result of competition for nutrients within the inflorescence.

B - EFFECT OF PARAQUAT APPLICATION AND CUTTING INTENSITY ON REPRODUCTIVE GROWTH AND SEED YIELD

In previous experiments it was observed that careful grazing and manipulation of the closing date and paraquat application could result in reduced ryegrass competition on white clover crops and increase clover seed yield. However, the mechanisms by which some of those treatments allowed an increase in seed production was not determined. Haggar *et al* (1963) showed that grazing by sheep in the spring increased the number of white clover inflorescences per unit area. Similarly, the data published by Clifford (1980) and the previous field experiments, indicate that with adequate soil moisture, grazing until mid-November can give the best balance between number of inflorescences and seed yield. These investigations also showed that closing crops later than November caused a drastic reduction in inflorescence numbers due to the removal of the inflorescences by the grazing animal. Beinhart *et al* (1963) indicated that the rate of inflorescence production is caused by the degree of lateral stolon formation in defoliated swards, which resulted in a higher number of shoot apices being available for inflorescence production. Thomas (1981b) observed that defoliation increased the percentage of flowering stolons in plants which had already initiated inflorescences. In his study, there were two types of response to defoliation in white clover: i.e., an increase in the percentage of flowering stolons in already reproductive plants; and a recommencement of inflorescence initiation in plants that had stopped initiation in warm long days. In other experiments, Zaleski (1961) studied the effects of defoliation on seed production in a pure stand of white clover and found that the number of inflorescences formed under different cutting treatments and ultimate seed yield, appeared to be affected by the season more

than by any other character. In wet seasons a lower number of inflorescences were produced than in dry seasons. Wet weather conditions resulted in lush vegetative growth and sprouting of seed, which caused an extremely low yield of seed of poor quality. Zaleski's experiments pointed out that the highest numbers of inflorescences were obtained in late-defoliated treatments, and lowest numbers in undefoliated treatments.

In particular there are three questions related to defoliation which merit investigation. How does the severity of defoliation affect white clover seed production under field conditions? Are the responses to cutting and paraquat application similar? Are the responses to defoliation similar for various clover genotypes, particularly those which differ markedly in flowering intensity? The aim of the present experiment was to investigate these three aspects of defoliation on white clover reproductive development and seed yield.

1 - Materials and Methods

a - Experimental site and field procedures

This investigation was carried out in the same year and in the same field as the previous experiment. The study continued from November 1984 to February 1985. The white clover plants and their management until the establishment of treatments were the same as in the previous experiment. From November 15th 1984, eight treatments using two genotypes with four defoliation treatments (Table 42) were used in a factorial experiment set out in a split-plot design with two replications.

The experimental plots consisted of 12 plants at a 60 x 60 cm^2 spacing. Cutting treatments were carried out using a 'Sunbeam' portable shearing machine and terminal buds were removed by hand, using scissors. Paraquat was applied in the same way as in the previous experiments.

Table 42: Treatments used and their identification

Defoliation Treatment	Procedure
Control	Uncut and without herbicide application
Light cut *	Cut to approximately 2.5 cm height from the soil surface
Heavy cut *	As for the light cut treatments, but also involving the removal of terminal buds from 50% of the main stolons
Paraquat *	Spraying paraquat at an equivalent rate of 0.36 kg a.i./ha plus surfactant at 0.5% of the volume of the solution.

* cuttings and paraquat spraying were made on November 15th.

b - Plant measurements and statistical analysis

In each treatment, plant measurements were done in the same way as in the previous experiment Commencing on 15 November, 50 main stolons were marked with coloured staples at monthly intervals. Staples were placed behind the node bearing the youngest unfolded leaf in each treatment. Different coloured staples were used at each marking time as previously described. At harvest on February 1st, 20 vigorous main stolons of the 50 marked originals were observed and the number of nodes, lateral stolons, lateral branches, rooted nodes, and main stolon elongation was recorded. Seed yield, seed set, number of inflorescences/m^2 and all other seed yield components were also measured. The estimated time of inflorescence initiation was calculated as in the previous experiment, on December 1st, December 15th and January 1st. Ten terminal buds on main stolons were dissected and the number of inflorescences was recorded as well as the node in the terminal bud at which the inflorescence occurred. Thereafter, the time of initiation of each inflorescence at all three sampling times was determined according to the method described by Thomas (1979).

Seed was tested for germination according to the ISTA Rules for Seed Testing (1976). Hard seeds were scarified, germinated and the percentage of each germination category was recorded.

Data collected in this experiment was analysed according to the procedure of a split-plot design following a random model. Plot means were used in the analysis and the least significant difference (LSD) at 5% and 1% levels was calculated to compare differences between treatments. The computer programme used for data analysis was Genstat (Alvey *et al* 1977).

2 - Results

a - Effect of defoliation treatments on white clover vegetative growth and development

Node appearance rate (Table 43) did not show significant differences between genotypes and was highest in late summer (January). A mean of 5 days was needed to form each new node in November compared with only 3.5 days in January. A comparison between the defoliation treatments during November indicated that heavy cutting and paraquat spraying caused a highly significant reduction in node appearance in relation to the rest of the treatments. Paraquat treatments were only significantly different from the control in December and no significant variations were observed among treatments in January. The interactions between genotype and defoliation treatments were not significant at any sampling date, indicating that there were no differences in genotype responses to defoliation.

Table 43: Effect of defoliation treatments on the number of nodes emerging from the apical meristem per month on one main stolon from November to January 1984 - 1985

Genotype	November	December	January
I	5.88	7.84	8.45
II	5.94	7.21	7.68
Significance	N.S.	N.S.	N.S.
LSD 5%			
1%			
S.E.D. ±	0.16	0.16	0.17
Defoliation Treatment			
Control	6.61	7.47	7.92
Light cut	6.30	8.17	8.10
Heavy cut	5.38	7.56	8.28
Paraquat	5.55	6.91	7.97
Significance	**	**	N.S.
LSD 5%	0.44	0.51	
1%	0.67	0.78	
S.E.D. ±	0.18	0.21	0.20

Main stolon elongation increments (Table 44) were higher in genotype I in December and January in comparison to genotype II. This superiority of genotype I may be due to both genetic differences and phenotypic responses to different sward characteristics.

Table 44: Effect of defoliation treatments on the increment in length (mm) of a main stolon during each month from November to January. 1984 - 1985

Genotype	November	December	January
I	143.6	212.0	205.6
II	125.8	142.0	124.2
Significance	N.S.	**	*
LSD 5%		5.0	45.6
1%		25.4	
S.E.D. ±	5.2	0.4	3.6
Defoliation Treatment			
Control	162.2	186.8	176.4
Light cut	164.8	195.0	173.0
Heavy cut	106.8	174.0	170.4
Paraquat	105.0	150.0	160.2
Significance	**	**	**
LSD 5%	22.6	18.6	4.0
1%	34.2	28.0	6.0
S.E.D. ±	9.2	7.6	1.6

Stolon elongation was significantly reduced in November under heavy cutting and paraquat treatments. This slower elongation may, in part, be a consequence of apical meristem removal in the heavy cut treatment (Plate 13). In plots sprayed with paraquat the reduction was due to the direct effect of the herbicide on the plant including terminal buds. In this experiment the characteristic symptom of paraquat treatment was a rapid wilting and dessication of sprayed leaves, petioles, peduncles, and inflorescences. These symptoms indicated that all aerial parts of the clover plant were equally susceptible to the herbicide. In particular, all photsynthetic tissues which were in contact with the herbicide were damaged and the first symptoms were observed as little as 2 hours after the application of the chemical. However, unfolded

Plate 13: Effect of heavy cut (HC) and paraquat (P) treatments on main stolons of white clover 7 days after treatment application.

leaves which were protected by stipules later emerged apparently undamaged and continued to grow (Plate 14). In December and January, paraquat spraying was the only treatment showing a significant reduction in stolon elongation. It was observed, nevertheless, that this reduction was small compared to uncut plots, especially in January. This indicates that white clover plants are capable of regrowth after paraquat application from those tissues which were not in direct contact with the herbicide. It also suggests that white clover plants do not possess a specific physiological or biochemical mechanism of detoxification of the herbicide and that its susceptibility is due to the position of the organ in the canopy at the time of herbicide application. No significant interaction was found in stolon elongation rate between genotypes or defoliation treatments.

Dissections of ten terminal buds (Table 45) indicated that the number of unexpanded leaves per main stolon and leaf primordia were not affected by defoliation treatments, except in the paraquat sprayed treatment for December 1st, which showed a significant reduction. This character remained relatively constant throughout the experiment with an average of 6 - 7 primordia per terminal bud. This constancy in number was not affected by defoliation treatments. Similarly, no differences between genotypes were detected.

The number of lateral stolons (Table 46) was not significantly different between genotypes. However, genotype I tended to form more lateral stolons than genotype II, in December and January. Paraquat caused a significant reduction in the number of lateral stolons in all sampling dates in comparison with heavy cut treatment. Significant differences were also detected in December between light cut and heavy cut plants compared to the control treatment. Increases of 20% and 46% more lateral stolons occurred in light cut and heavy cut treatments respectively, compared to the uncut control at this time. No significant differences were found in the interactions between genotype and defoliation treatments. As observed in the previous experiment the number of lateral stolons produced in all treatments was reduced in late summer by approximately 3 to 4 times compared with the numbers present in November.

Table 45: Effects of defoliation on the number of leaf primordia per terminal bud. Average of 10 terminal buds per sampling date 1984 - 1985

	Date of Sampling (month - day)				
Genotype	Dec 1st	Dec 15th	Jan 1st	Jan 15th	Feb 1st
I	6.65	6.50	6.47	6.53	6.65
II	6.67	6.62	6.47	6.55	6.62
Significance	N.S.	N.S.	N.S.	N.S.	N.S.
LSD 5%					
1%					
S.E.D. ±	0.125	0.025	0.050	0.025	0.125
Defoliation Treatments					
Control	6.70	6.60	6.35	6.45	6.65
Light cut	6.80	6.55	6.60	6.35	6.70
Heavy cut	6.95	6.60	6.55	6.70	6.60
Paraquat	6.20	6.50	6.40	6.65	6.60
Significance	**	N.S.	N.S.	N.S.	N.S.
LSD 5%	0.22				
1%	0.33				
S.E.D. ±	0.089	0.151	0.229	0.098	0.167

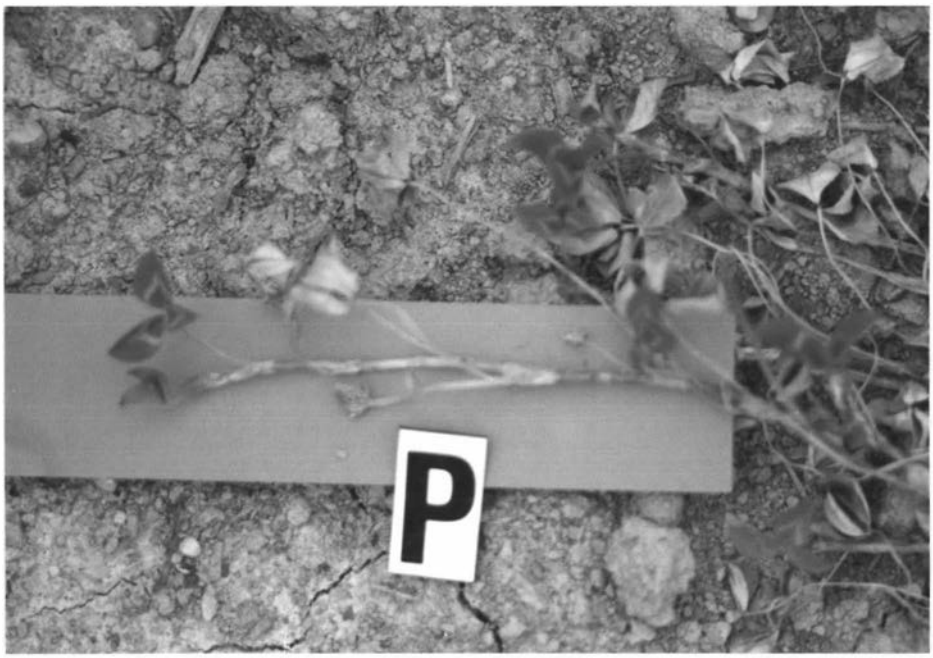


Plate 14: Damage of white clover stolon seven days after paraquat application.

Table 46: Effect of defoliation treatments on the number of lateral stolons present in February at nodes which emerged from terminal buds of main stolons during each month from November to January 1984 - 1985

Genotype	November	December	January
I	1.67	1.37	0.59
II	1.67	1.05	0.35
Significance	N.S.	N.S.	N.S.
LSD 5%			
1%			
S.E.D. ±	0.06	0.04	0.04
Defoliation Treatment			
Control	1.75	1.04	0.51
Light cut	1.88	1.24	0.56
Heavy cut	1.96	1.52	0.54
Paraquat	1.19	1.05	0.27
Significance	**	**	**
LSD 5%	0.39	0.19	0.15
1%	0.59	0.29	0.22
S.E.D. ±	0.16	0.08	0.06

The number of lateral branches (previously defined as side branches with less than 4 nodes) did not differ between genotypes (Table 47). When the effect of defoliation treatments was examined it was found that the control plots generally had fewer lateral branches than the heavy and light cut treatments. The difference between these treatments may be due to the denser canopy formed by uncut and unsprayed plants which inhibited side branching or caused branch decay.

Table 47: Effect of defoliation treatments on the number of lateral branches present in February at nodes which emerged from terminal buds of main stolons during each month from November to January 1984 - 1985.

Genotype	November	December	January
I	1.23	1.84	2.71
II	1.04	1.65	2.33
Significance	N.S.	N.S.	N.S.
S.E.D. ±	0.07	0.05	0.11
Defoliation Treatments			
Control	1.14	1.74	2.05
Light cut	1.18	1.60	2.28
Heavy cut	0.98	1.76	2.74
Paraquat	1.23	1.87	3.01
Significance	N.S.	N.S.	*
LSD 5%			0.44
S.E.D. ±	0.074	0.09	0.18

As the number of rooted nodes did not show a significant genotype x defoliation interaction, only the main effects are presented in Table 48. Genotypes did not differ significantly from each other, but paraquat application did exert a great influence on the number of rooted nodes. Thus, the results show a reduction of 18%, 23% and 13% in the percentage of rooted nodes from November to December in sprayed plants compared to the unsprayed control. Conversely, Rolston and Chu (1976) found no significant differences in the number of rooted nodes between paraquat treated and untreated control plants. This different response may be due to differences in the rate of paraquat, which was higher in this investigation. Also, canopy structure, especially by genotype II, was relatively sparse and many lateral and main stolons were directly dessicated by paraquat with a reduction in the total number of nodes at which roots could form.

Table 48: Effect of defoliation treatments on the number of rooted nodes present in February at nodes which emerged from terminal bud of main stolons during each month from November to January 1984 - 1985.

Genotype	November	December	January
I	3.06	2.92	2.40
II	2.52	3.07	2.33
Significance	N.S.	N.S.	N.S.
S.E.D. ±	0.085	0.092	0.135
Defoliation Treatment			
Control	3.05	3.13	2.44
Light cut	2.92	3.32	2.26
Heavy cut	2.71	3.16	2.64
Paraquat	2.48	2.39	2.13
Significance	**	**	**
LSD 5%	0.22	0.28	0.12
1%	0.33	0.42	0.18
S.E.D. ±	0.090	0.114	0.049

b - Effect of defoliation treatments on white clover reproductive growth and development

Dissections of ten terminal buds per plot every 15 days showed that there were no significant differences in the number of inflorescences formed in the terminal buds between genotypes even though plants of genotype II in December 1st and 15th formed approximately 55% more inflorescences than genotype I (Table 49).

Table 49: Effect of defoliation treatments on the number of inflorescences present in ten terminal buds of main stolons every 15 days from November to January 1984 - 1985.

	Date of Sampling (Month - day)		
Genotype	Dec 1st	Dec 15th	Jan 1st
I	7.50	8.00	5.50
II	13.50	13.50	5.25
Significance	N.S.	N.S.	N.S.
S.E.D. ±	2.00	1.50	0.75
Defoliation Treatment			
Control	11.50	11.50	4.00
Light cut	9.00	11.00	4.00
Heavy cut	9.00	8.00	6.50
Paraquat	12.50	12.50	7.00
Significance	N.S.	N.S.	N.S.
S.E.D. ±	1.53	1.47	1.51

Cutting, irrespective of intensity, caused no differences in the number of inflorescences present in terminal bud apices at any of the sampling dates. However, the total number of inflorescences found in the terminal buds of plants from paraquat sprayed treatments tended to be higher in comparison to cutting treatments. No significant genotype x defoliation interactions were detected.

The estimated time of inflorescence initiation (Table 50) showed significant differences between genotypes for inflorescences initiated before the cutting or spraying treatments were applied in mid November. However, none of the defoliation treatments showed any subsequent effect on the number of inflorescences initiated. There was a trend towards a reduction in inflorescence initiation in cut plots in mid November, and for paraquat spraying to increase inflorescence initiation in early December.

Table 50: Effect of defoliation treatments on the time of inflorescence initiation of ten main stolon apices sampled every 15 days from November to January. Values are the approximate time of initiation of all inflorescences found in the three sampling dates (December 1st, December 15th and January 1st) 1984 - 1985.

	Estimated Date of Inflorescence Initiation			
Genotype	Before the Treatment	November 15 - 30	December 1 - 14	December 15 - 31
I	2.50	5.50	7.75	5.25
II	5.75	10.75	13.25	2.50
Significance	*	N.S.	N.S.	N.S.
LSD 5%	3.17			
S.E.D. ±	0.25	2.25	1.00	0.25
Defoliation Treatment				
Control	3.50	10.50	10.50	2.50
Light cut	3.00	6.50	10.50	4.00
Heavy cut	5.50	5.50	8.00	4.50
Paraquat	4.50	10.00	13.00	4.50
Significance	N.S.	N.S.	N.S.	N.S.
S.E.D. ±	1.45	2.57	2.43	1.34

The number of inflorescences emerged on both main stolons and lateral stolons is shown in Table 51. As expected, the number of inflorescences was highest in genotype II at all sampling dates, showing significant differences for December sampling. Cutting caused a significant reduction in the number of emerged inflorescences in November in comparison with the uncut treatment. However, in December and January, plants which had been cut recovered and bore significantly more inflorescences than the control. Paraquat application in November caused a decrease in the number of inflorescences emerged, through a direct effect of the herbicide on main and lateral stolon mortality. Subsequently, this effect was not apparent in comparison with uncut treatment.

Table 51: Effects of defoliation treatments on the total number of inflorescences emerged on main stolon plus lateral stolons, present in February at nodes which emerged from terminal buds during each month from November to January 1984 - 1985

Genotype	November	December	January
I	0.77	1.13	0.50
II	1.70	3.80	1.34
Significance	N.S.	*	N.S.
LSD 5%		1.27	
S.E.D. ±	0.110	0.100	0.086
Defoliation Treatment			
Control	2.21	1.97	0.57
Light cut	1.26	2.53	0.98
Heavy cut	0.84	3.36	1.57
Paraquat	0.65	2.01	0.56
Significance	**	**	**
LSD 5%	0.19	0.23	0.35
1%	0.29	0.35	0.53
S.E.D. ±	0.08	0.09	0.14

Significant Interaction

Genotype x defoliation	December I	December II	January I	January II
Control	1.39	2.54	0.50	0.63
Light cut	1.06	3.99	0.55	1.41
Heavy cut	1.34	5.38	0.52	2.63
Paraquat	0.75	3.27	0.43	0.68
Significance	**		**	
LSD	5%	1%	5%	1%
(a)	0.38	0.57	0.48	0.73
(b)	0.33	0.50	0.50	0.76
S.E.D. ± (a)	0.154		0.197	
(b)	0.134		0.204	

(a) When comparing means with the different levels of mainplot
(b) When comparing means with the same levels of mainplot

Examination of the genotype x defoliation interactions indicated, once again, that cutting had a stimulating effect which increased inflorescence numbers in the genotype with highest flowering capacity (genotype II). However, this response was not important in genotype I.

Inflorescence numbers formed on main or lateral stolons (Appendices 21 and 22) followed the same trend as the total number of inflorescences and presented the same significant differences between genotype and defoliation treatments. It is important to note that most inflorescences were formed on main stolons rather than on lateral stolons.

A comparison of the number of white inflorescences formed in each genotype showed that genotype II always produced more inflorescences than genotype I (Figure 20). At the same time, genotype II reached peak flowering 14 days earlier in cut plots and 7 days earlier in control plots than genotype I. The paraquat sprayed plots reached peak flowering on the same date in both genotypes.

Variation between defoliation treatments and genotypes in the number of florets/inflorescence, ovules per ovary and seeds per floret were not statistically different, except in genotype II in December 1st and January 1st samplings where the number of florets was significantly higher than in inflorescences of genotype I, (Appendices 23, 24 and 25).

c - Effect of defoliation treatments on white clover yield components and seed yield

Some major variation in yield components was found in previous experiments as a result of different white clover management systems. This final experiment was designed to specifically determine the effect of defoliation and spraying treatments on these components. Four yield components were given particular attention: number of inflorescences/m^2, number of florets/inflorescence, number of seeds/floret and seed weight. All of these characters were recorded at harvest in each plot and the results are presented in Table 52.

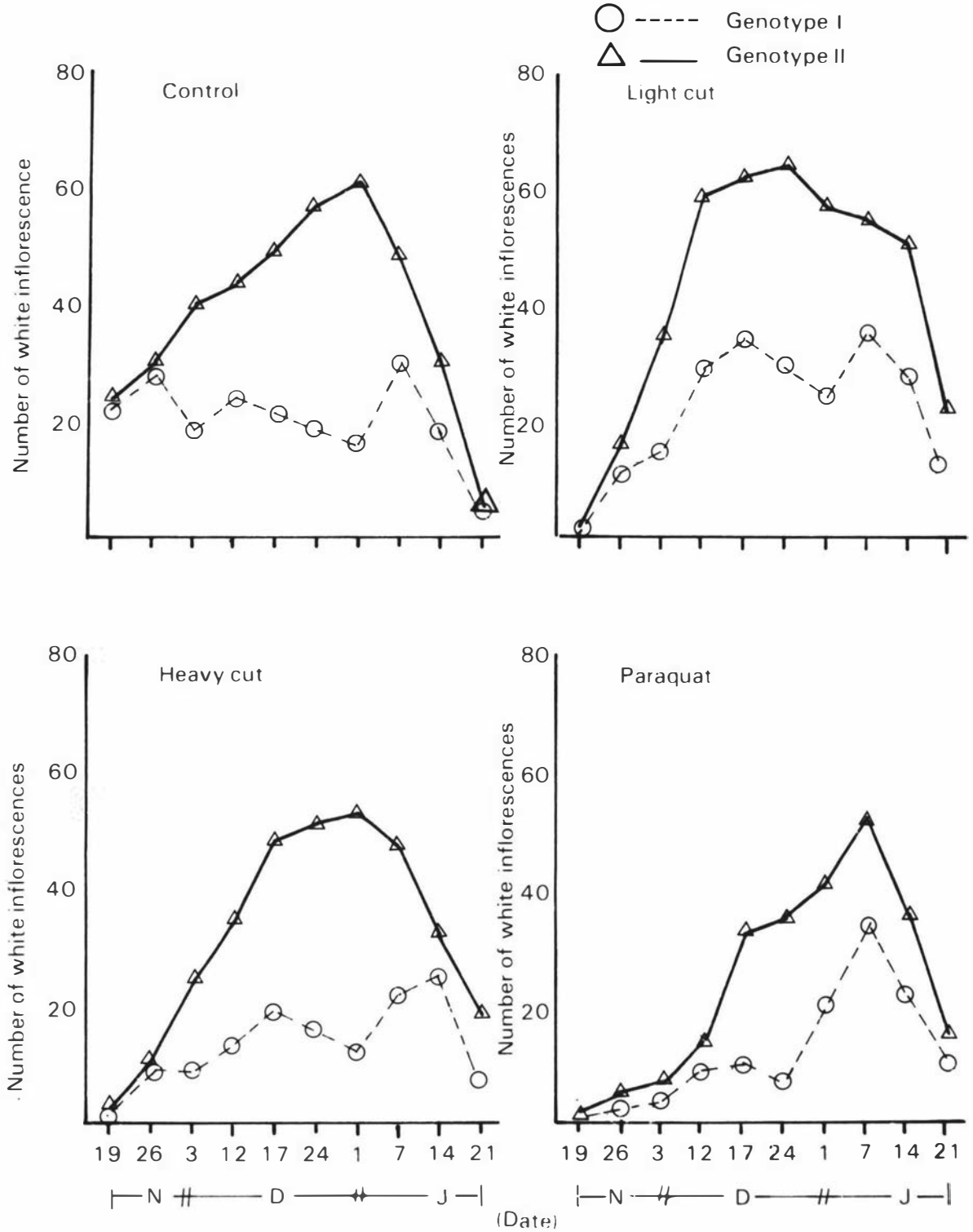


Figure 20 Number of white inflorescences produced in paraquat and different defoliation treatments per unit area (0·25 m²)

Table 52: Effect of defoliation treatments on white clover yield components 1984 - 1985

Genotype	YIELD COMPONENTS: No. of inflorescences per m^2 at harvest	No of florets per head at harvest	No of seeds per floret	1000 seed weight g
I	327.5	61.8	2.60	0.5115
II	888.8	81.8	2.01	0.5881
Significance	**	*	N.S.	*
LSD 5%	54.0	9.5		0.0588
1%	270.7			
S.E.D. ±	4.25	0.75	0.175	0.0046
Defoliation Treatment				
Control	655.0	71.9	2.50	0.5788
Light cut	576.0	72.3	2.24	0.5500
Heavy cut	754.0	73.0	2.23	0.5368
Paraquat	447.5	70.0	2.26	0.5338
Significance	*	N.S.	N.S.	N.S.
LSD 5%	27.6			
S.E.D. ±	11.26	3.07	0.115	0.0186

Significant Interactions

Genotype x Defoliation	No. of Inflorescences/m^2 I	II
Control	454	856
Light cut	296	856
Heavy cut	300	1208
Paraquat	260	635
Significance	*	
LSD (a)	48.34	
(b)	53.33	
S.E.D. ± (a)	14.43	
(b)	15.92	

(a) When comparing means at different levels of mainplots
(b) When comparing means at the same levels of mainplots

The total number of inflorescences present in the heavy cut plots was significantly higher than in other treatments. In contrast, paraquat sprayed plots gave the lowest number of inflorescences. The interactions of genotype defoliation treatments, were also significant indicating that both genotypes responded differently to defoliation. In genotype I, all cuttings and paraquat treatments caused a significant reduction in inflorescence density. This reduction varied between 34% and 43% compared to the uncut and unsprayed control. In genotype II, inflorescence numbers were increased in heavy cut plots and did not show any reduction in light cut plots in comparison to uncut plants. Paraquat application caused a significant destruction of inflorescences. The herbicide had a major effect by decreasing inflorescence formation.

In this experiment, paraquat and cutting had little effect on the number of florets per inflorescence at harvest. However, significant genotype differences were apparent, inflorescences in genotype I producing fewer florets (24%) than inflorescences in genotype II.

Neither the genotype or the defoliation treatments had any obvious effect on the number of seeds formed per floret. It does seem possible that plants tend to compensate for a reduction in the number of inflorescences and florets/inflorescence by increasing the level of seed weight . Seed weight also did not appear to be greatly affected by different defoliation treatments. However, there was a trend for uncut plants to produce heavier seeds than plants from other treatments. Genotypes, however, showed significant differences in 1000 seed weight with genotype II producing heavier seeds.

Both the defoliation treatments and genotypes were responsible for differences in seed yield (Table 53). The differences in yield between uncut plants and defoliated plants were highly significant with uncut plots giving the highest yield (657 kg/ha). Light and heavy cut plants produced an intermediate level of seed yield (567 - 587 kg/ha). Paraquat sprayed plants produced lowest seed yield (392 kg/ha). At the same time, the yield obtained in genotype II plants (822 kg/ha) was 3 times higher than in plants of genotype I. The genotype x

defoliation interaction was also highly significant confirming that the genotypes responded differently to defoliation. In general, cutting treatments in genotype II gave significantly higher seed yields than genotype I. This result was not surprising because both genotypes have been shown to differ in the number of inflorescences formed and therefore in seed yield. Although paraquat plots gave a high frequency of inflorescence initiation they subsequently produced the lowest number of inflorescences and seed yield. It must be stressed that this response occurred as a direct result of the destruction of some stolons and inflorescences by the herbicide. Cutting and paraquat treatments significantly reduced seed yield of genotype I, but only paraquat treatment reduced seed yield of genotype II.

Table 53: Effect of defoliation treatments on white clover seed yield (kg/ha) 1984 - 1985

	SEED YIELD kg/ha	
Genotype	I	II
	279	822
Significance	*	
LSD 5%	414.0	
S.E.D. ±	32.63	

Defoliation x Treatment	Control	Light Cut	Heavy cut	Paraquat
	657	567	587	392
Significance	**			
LSD 5%	20.2			
1%	30.6			
S.E.D. ±	8.25			

Interactions

Genotype x defoliation	I	II
Control	463	850
Light cut	244	889
Heavy cut	243	931
Paraquat	166	618
Significance	**	
LSD	5%	1%
(a)	83.67	126.69
(b)	53.56	81.10
S.E.D. ± (a)	34.15	
(b)	21.86	

(a) When comparing means at different levels of mainplots

d - Effect of defoliation treatments on white clover seed quality

In addition to seed yield component measurements, seed quality in terms of normal seedling percentage (before and after scarification), abnormal seedling percentage and hard seed and dead seed content was also examined. Results are presented in Table 54.

Seed germination percentages obtained in samples tested before and after scarification showed no significant differences between genotypes in the percentage of normal seedlings. However, defoliation did have a significant effect on this character, although the differences in normal seedling percentage between treatments before and after scarification is difficult to understand. Presumably the differential response was caused by variable percentages of hard seed produced in each treatment. Following scarification hard seed content was very low (less than 3%).

No significant differences in the level of abnormal seedlings following scarification occurred in tests of seed from different defoliation treatments. However, it was observed that seeds from heavily cut and paraquat sprayed plants showed the highest proportion of abnormal seedlings. The main types of abnormalities observed included seedlings with stunted or necrotic primary roots, or a decayed, shortened or glassy hypocotyl.

The percentage of dead seeds was low (4 - 8%) and not significantly affected by defoliation treatment or genotype.

3 - Discussion

Paraquat had a major effect on vegetative and reproductive growth of white clover, as found by other workers (Haggar 1961, 1974, Haggar *et al* 1963, and Haggar and Bastian 1980). The spraying of plants in early November had a deleterious effect on the green tissue of the stolons, petioles, peduncles, leaves and inflorescences. This effect was also reported by Brian *et al* (1958), and Haggar and Squires (1979).

	SEED QUALITY				
Genotype	Normal seedlings before scarification (%)	Normal seedlings after scarifiation (%)	Abnormal seedlings after scarification (%)	Hard seeds after scarification (%)	Dead seeds after scarification (%)
I	38.88	79.25	11.56	2.50	6.69
II	10.00	86.13	7.50	1.40	4.94
Significance	N.S.	N.S.	N.S.	N.S.	N.S.
S.E.D. ±	2.37	0.37	2.81	0.56	2.12
Defoliation Treatment					
Control	18.50	83.38	7.63	2.00	5.00
Light cut	22.63	84.75	7.38	1.88	6.00
Heavy cut	28.63	80.38	10.75	1.25	7.63
Paraquat	28.00	80.25	12.38	2.75	4.63
Significance	*	*	N.S.	N.S.	N.S.
LSD 5%	6.71	3.72			
S.E.D. ±	2.74	1.52	2.08	0.99	1.01

Table 54: Effects of defoliation treatments on white clover seed quality 1984 - 1985.

Following spraying, however, regrowth occurred from tissues which had been protected from direct contact with the herbicide, particularly terminal buds. Similar results were reported by Baldwin (1963) and Summers (1980) which indicated that perennial plants with underground organs, although susceptible to top-kill by paraquat, generally regrow from the base. Paraquat subsequently reduced the number of rooted nodes by nearly one quarter and also the formation of lateral stolons as found by Rolston and Chu (1976). Compared with either cut or uncut treatments the paraquat treatment produced a poor seed yield (392 kg/ha) as a consequence of inflorescence destruction. Cutting had a generally deleterous effect on seed yield in genotype I and a promotive effect in genotype II. Such genotype variation has also been reported by Rossiter (1961, 1972) and Thomas (1981b). In general, the quantity of seed produced from plants cut at different intensities was similar ('heavy cut' 587 kg/ha, 'light cut' 567 kg/ha). However these values are well below the 657 kg/ha recorded from uncut plants. This difference is mainly due to the particularly deleterious effect of cutting on seed yield in genotype I.(table 52)

Heavy cutting, in particular, reduced node appearance and stolon elongation (Pascoe 1973). The main effect of cutting was to increase lateral stolon formation particularly in December when 'light cut' and 'heavy cut' plants produced 20% and 46% more lateral stolons than uncut plants respectively. Uncut plants formed few lateral stolons. Similar results have been reported by Mitchell (1956a), Haggar *et al* (1963) and Zaleski (1970). The number of unexpanded leaves and leaf primordia was not affected by cutting but was decreased by paraquat in December as found by Clifford (1980) and Thomas (1982). A consistent number of leaf primordia of 6 or 7 per terminal bud occurred in all cases (Thomas 1982), except where terminal buds had been damaged or destroyed by direct contact with herbicide.

Inflorescence numbers/m^2 was the main determinant of seed yield as also shown by Zaleski (1961, 1970), Clifford (1977, 1979, 1980) and Huxley *et al* (1979). These were borne principally on main stolons, lateral stolons, by comparison, being relatively unproductive inflorescence producers. This suggests that any treatments which

stimulate lateral branching during the flowering period of white clover is likely to result in lower seed yields. This effect was also reported by Thomas (1980a). In the present study, cutting and paraquat application generally increased lateral stolon formation and subsequently produced fewer inflorescences than uncut plots.

The reaction of the two genotypes to cutting was quite different. Genotype I showed a major reduction in inflorescence numbers following cutting or spraying. Genotype II, however, while also showing a reduction in inflorescence numbers following spraying, was much more tolerant of cutting treatments. In fact, heavy cutting stimulated inflorescence production.

The previous experiments have confirmed the number of inflorescences per unit area as the most important determinant of seed yield. They have, however, also clearly shown the important contribution to seed yield made by other yield components, particularly the number of florets per inflorescence and seed weight. The present study confirms the importance of inflorescence numbers/m^2 in determining seed yield. However, none of the defoliation treatments, whether involving cutting or paraquat application resulted in any appreciable differences in floret numbers and seed weight. In contrast, Clifford (1979, 1980) found different responses when white clover was grazed at different times of the year. The results, however, do confirm the general insensitivity of seed quality components - normal and abnormal seedlings, hard and dead seeds - between treatments following scarification as was found by Zaleski (1961).

CHAPTER IV

GENERAL DISCUSSION AND CONCLUSIONS

New Zealand is one of the world leaders in white clover seed production, and its cultivars are recognised internationally as being amongst the best available. 'Grasslands Huia' white clover seed can be regarded as a real success for the New Zealand herbage seed export industry over many years. Since 1972, the amount of clover seed exported has increased from around 2,000 tonnes, to over 4,000 tonnes in 1983. Since 1978, the value of clover seed exports has tripled to NZ$16.4 million in 1983 (Hampton and Scott 1983). This increase is almost entirely due to the increased export of 'Grasslands Huia' white clover.

White clover seed is normally produced from ryegrass/white clover swards, which are closely grazed by sheep before being closed for seed production in late October or early-mid November (Langer 1973, Clifford 1977, 1979, 1980, Leitch 1949, Jolly 1957).

The aim of this chapter is to attempt to bring together the results and findings of the three field studies considered in this thesis. This was thought to be necessary to try to analyse the relationship between overall seed crop management and the results of the field experiments as they relate to various aspects of white clover seed production.

The dual role played by white clover has been emphasised in many investigations. It not only provides herbage for grazing animals but also provides for seed production during the same farming year. However, a conflict exists between these two roles since a high utilization of the herbage capacity of the plant may impair its seed production potential, while the opposite effect is also true. However, research work on this species suggests that with appropriate crop management, such conflict can be partly or completely overcome (Jolly 1957). For this reason, in the present experiments, particular

attention has been paid to the morphological development of the white clover plant as well as to the effects of grazing, cutting and paraquat herbicide application on seed production.

Despite their similar designation as 'Grasslands Huia' white clover, both genotypes showed remarkably different reproductive responses. There was no doubt that plants of genotype I had a much lower reproductive potential than plants of genotype II. This was seen by the production of fewer inflorescences, each bearing fewer florets per inflorescence and lighter seeds in plants of genotype I. By comparison, plants of genotype II produced more inflorescences each bearing nearly 25% more florets and heavier seeds. These differences were ultimately expressed in comparative seed yields of 464 kg/ha in genotype I and 850 kg/ha in genotype II. Seed quality, however, was similar in both genotypes.

This major difference in seed yield between genotypes was entirely a flowering response. This was shown by the fact that vegetative characters such as the numbers of leaf primordia in the terminal bud and the numbers of lateral stolons, rooted nodes, lateral branches and main stolon elongation were all similar between genotypes. The number of inflorescences present in the terminal bud was not statistically different between genotypes, despite an advantage of over 50% in this character in favour of genotype II.

The seed yield of white clover is determined by a series of inter-related factors, including the total number of seeds produced and seed size. The number of seeds are dependent on the number of fertilized ovules, which is determined after the exclusion of ovules which have not been fertilised and ovules in which seeds fail to develop. The number of ovules is the product of the number of florets and the average number of ovules per pod, while the number of florets is determined by the number of inflorescences which are produced, as well as by the size of each inflorescence.

In general, investigations carried out so far have all stressed that the number of inflorescences/m^2 at harvest is the main factor

contributing to seed yield. Since these inflorescences originate mainly from the apices of main stolons the total number of inflorescences produced is closely related to the total length of stolons on the plant. If a high seed yield is desired, it is essential to manage the sward in such a way that main stolon production and growth are promoted before inflorescence formation and that grazing frequency and intensity are controlled to ensure the retention of a certain amount of leaf. The stolons produce the inflorescences, while leaves provide adequate supplies of photosynthethates to the inflorescences and other parts of the plant.

One factor which is very important in the production of seeds in white clover is the morphological development of the meristems of the plant. This includes knowledge of the time of change of the apex from the vegetative to the reproductive condition, its accessibility to stock and the effect of removal of these meristems on the initiation and growth of lateral stolons. A white clover plant has several main and lateral stolons, which form and develop at different times from autumn to early summer (Thomas 1980, 1981, Beinhart 1962, Hoglund and Williams 1984). In general, white clover planted during the winter in the field and harvested for seed in February shows that most of the main stolons and the largest lateral stolons (with more than 8 nodes) are formed during the spring.

Inflorescence initiation, according to Thomas (1981a,b,), starts in the winter and increases with increasing day length. Terminal bud dissections confirm this trend in the present study with inflorescence initiation beginning in August in both genotypes. Only a proportion of main stolons form inflorescences and lateral stolons initiate very few flowers.

Under normal growing conditions the only clear effect of inflorescence production on growth is a reduction in the number of vegetative axillary buds as a result of inflorescence development at axillary bud sites. Each node can thus produce either one vegetative axillary bud or one inflorescence, but never both (Thomas 1981a,b). For that reason it is not surprising that the percentage of nodes bearing lateral

stolons, has been shown to decrease from the spring onwards in an inverse proportion to inflorescence formation.

The difference in inflorescence initiation and flowering intensity between genotypes cannot be explained by the relationship between the production of vegetative axillary buds and reproductive axillary buds as suggested by Gibson(1957), and Thomas (1980). Both genotypes in the present study showed similar vegetative development throughout the experiment. The variation in inflorescence numbers was due chiefly to different requirements for cold temperature during the winter between genotypes, as was found by Thomas (personal communication). It was also determined in the present studies that the largest inflorescences were those which emerged first during the season. These were initiated during August, September and October from stolons developed in the spring. Rossiter (1972), working with subterranean clover, and Zaleski (1970) with white clover also found the same type of results.

One of the most interesting findings in these investigations is the approximately 50% difference in the number of seeds per floret in mature inflorescences formed from plants in the first days of December (4.5 seeds per floret) compared with the situation in January (2.5 seeds/floret). Clifford (1979) seems to indicate the opposite effect, that is, that inflorescences which ripen later produce more seeds per inflorescence than those formed earlier. One possible explanation of this different effect may be related to increased bee activity in the field during the mid-late summer which can result in increased seed set. However, Thomas (1985) in unpublished data, found similar results to those obtained in this thesis. He compared plants that initiated inflorescences in cold or warm conditions during the winter and later were kept under natural conditions during the summer for pollination. Those plants which had received cold pre-treatment showed higher seed set than plants kept under warm temperatures.

The suppression by grasses on white clover in a mixed sward is attributed to two main causes. First, the grass has a depressing effect on white clover inflorescence initiation by reducing light

intensity at the stolon level (Zaleski 1970) and second, the grass decreases the population of white clover by competing with it for light, water and nutrients. There is also a possibility of intra-plant competition existing in white clover plants affecting inflorescence initiation and plant growth.

In the present investigations three types of defoliation were studied. During the first two years of field experiments grazing and paraquat treatments were applied to a mixed sward of ryegrass and white clover at different times during the spring. In the final two experiments, paraquat spraying and two different cutting intensity treatments were done in mid-November on a pure white clover sward and the regrowth of the plants was observed.

The results of the final experiment showed that a heavy cut treatment in which some terminal buds were removed, resulted in a slight initial decrease of growth (elongation) of the main stolon, and a corresponding increase in the number of lateral stolons during the same period. This response was not found in more lightly cut treatments where few, if any, terminal buds were removed. This stresses the dominance of the terminal buds on stolon growth. When the stolon apex was removed there was a removal of apical dominance. This affects the distribution of assimilates and creates a new pattern of bud dominance, promoting new lateral stolon formation. However, the response in this experiment was not as large as expected because only a few terminal buds were removed and because most of the lateral stolons should already have been differentiated when the cutting treatments were applied (mid November). Therefore, during this time, defoliation is more important in its effects on the stimulation of inflorescence initiation than in increasing stolon formation.

According to the results obtained by Beinhart *et al* (1963) and from the final field experiments white clover plants produced most of their main stolons and lateral stolons during autumn and winter, this process being promoted by grazing during the spring. Similar results have been found by Beinhart (1963), Chapman (1983) and Hoglund and Williams (1984). They have shown an inverse linear relationship between season,

temperature and stolon growth and development. On the other hand, defoliation in mid November tended to increase or decrease inflorescence emergence depending on genotype. In these studies, genotype II showed significantly higher numbers of inflorescences emerged on main and lateral stolons in defoliated treatments than unsprayed treatments, while the opposite effect was found in genotype I. This response was the cause of the significant higher seed yields in all the cutting treatments in genotype II. This suggests it could be important to determine the types of clones existing in the sward and to manage the field accordingly. Thomas (1981b) also observed in 'Grasslands Huia' white clover that inflorescence numbers were increased when defoliation (cutting) was carried out in mid-November. The results also show that the apical meristems in heavy flowering plants inhibit lateral stolon inflorescence formation but when the meristem is removed the intensity of inflorescence production increases (Phillips 1969).

It was observed that spraying caused more severe damage to plants in a pure white clover sward than in a mixed sward, destroying parts of main stolons, lateral stolons, leaves and any photosynthetic tissue which came into contact with the herbicide. This damage was least in a mixed sward because a large amount of herbicide was intercepted by the grass which acted as a partial barrier to the paraquat reaching the clover tissue. Herbicide application effectively decreased the number of nodes, stolon elongation rate and the number of rooted nodes on main stolons. Plants were unable to recover from this effect with a resultant reduction in seed yield at harvest.

It was also important to note that paraquat application resulted in a significant increase in inflorescence initiation. Despite this effect, paraquat treatments presented the lowest number of emerged inflorescences, because the herbicide caused a reduction in the number of vegetative and reproductive organs as explained earlier. The increase in inflorescence formation in plants sprayed with paraquat has not been reported before and more physiological studies are necessary to determine the causes of this response.

As far as different defoliation treatments were concerned the heaviest seed and highest seed yield was found in uncut plots. The number of florets per inflorescence was an insensitive parameter to defoliation, and yield components, ovules per ovary and seeds per floret, also showed little response to cutting or paraquat. Similar results were obtained by Zaleski (1970) in his experiments on the effects of different cutting treatments on seed yield in white clover. He reported that the main effect of defoliation was on the number of inflorescences produced per square metre. However, he could find no significant differences in other variables such as florets per inflorescence and seed size. However, Clifford, (1979, 1980), in New Zealand, showed that defoliation later than mid-November caused a drastic reduction in inflorescence numbers, floret numbers and seed weight and therefore, in seed yield.

There were no major differences in the percentage of normal or abnormal seedlings, number of hard seeds and dead seeds between seed samples from defoliation treatments or genotypes as also found by Zaleski (1961, 1970). This suggests that seed quality is generally insensitive to seed crop management practices.

Experiments carried out in 1982 and 1983 indicated that rotational grazing every 2 weeks until November did not reduce either the number of inflorescences produced or seed yield. However, because of the yearly variation in environmental conditions, some differences in the time of onset and rate of inflorescence development occurred. The results strongly suggest that the practice of closing the sward on the same date each year is not advisable if the farmer wants to maximise seed yields. By inspecting the field at regular and frequent intervals during the late spring and recording the time when the first inflorescences emerge, it is possible to determine the best date for closing and spraying the sward.

Good seed yields were obtained when November closing was preceded by paraquat application in mid-October or at closing. In contrast, the application of paraquat 30 days after November closing seriously reduced seed yield. However, in a year when profuse vegetative growth continued until December good seed yield could be obtained by paraquat

spraying in mid-November and delayed closing until mid-December. It is important to note that in years when climatic conditions reduce growth in November or before, poor seed yields were found with D-N and D-D treatments (December closing and paraquat application 30 days before closing or at closing). Similar results were obtained by Clifford (1979, 1980) in New Zealand. His work indicates that, with adequate soil moisture, frequent grazing until mid-November gave the best balance between inflorescence number and seed yield. Earlier or later closing dates resulted in a loss of early inflorescences formed under the canopy or loss of high amounts of inflorescences during grazing by sheep.

In general, these optimum dates for closing and spraying coincide with the time of maximum inflorescence formation of white clover plants. Therefore, another method for determining the optimum time of closing may be to dissect terminal buds under the microscope. When the terminal buds show maximum inflorescence formation that time may be considered as the optimum closing date and paraquat application may then be carried out.

In most of these studies seed harvests were made 30 days after peak flowering when approximately 75% to 90% of inflorescences were brown and mature seed could be rubbed out in the hand. The results of the first experiment showed no large differences in seed quality between seeds obtained at different harvesting times (25 to 40 days after peak flowering). However, there was a tendency for harvests carried out less than 30 days after peak flowering to produce seed of marginally poorer quality.

In New Zealand white clover seed crops are normally cut when about 80% or 90% of the inflorescences are brown (Jolly 1958). This period occurs in January or February when conditions are usually warm and dry. The results during the first field experiment highlight the problems which are likely to occur when wet conditions prevail during the normal harvesting time. Under these conditions growers would not be able to wait until 80% of the inflorescences had turned brown due to the problems of continued vegetative growth of the white clover plants. Scott (1973) observed in wet seasons and recommended the

harvesting of white clover seed crops when 70% of inflorescences were brown. These examples suggest that the practice of harvesting the seed crop 30 days after peak flowering is a haphazard way of trying to consistently maximise clover seed yields. In general, any conditions which promote vegetative growth are likely to increase petiole length and raise the leaf canopy above the inflorescences. This can result in a situation where inflorescences are shaded by the leaf canopy and result in rotting inflorescences and seed sprouting in the inflorescence.

CONCLUSIONS

The results of this study have emphasised the high seeding potential of 'Grasslands Huia' white clover. The highest seed yield of 850 kg/ha obtained is more than five times higher than the average commercial seed yield value of 153 kg/ha quoted by Hampton and Scott (1983) for this cultivar. This clearly shows that 'Grasslands Huia' white clover is a plant which displays considerable genetic, environmental and cultural reaction in relation to both vegetative and reproductive development. As an example, the two genotypes of 'Grasslands Huia' white clover used in this study showed differences of 47% in inflorescence numbers, 25% in seeds per floret and 13% in seed weight, even though the percentage of seed set in each cultivar was similar. Environmental conditions, particularly rainfall, temperature and wind speed, also resulted in major differences in seed numbers per floret in different years, ranging from 0.1 to 4.5. These differences were thought to reflect the effect of climatic conditions on pollinator activity and subsequent seed abortion. Nevertheless, even the highest values were a poor reflection of the potential seed numbers which could have been produced from the consistent 6 to 7 ovules present per floret in this species. Seedcrop management, particularly closing date, paraquat herbicide application and defoliation intensity, also had a major effect on seed yield. This was shown by the alteration in vegetative morphology of the plant by management, particularly in relation to the amount of lateral stolon development on main stolons in the winter and spring and the increase in inflorescence initiation from August to a peak in October and November.

'Grassland Huia' white clover appears to partition its growth between vegetative development to ensure perenniality (a factor reflected in the production of lateral stolons in the winter and early spring) - and seed yield which is determined primarily by the number of inflorescences per unit area and to a lesser extent by floret numbers per inflorescence, seeds per floret and, in some cases, by seed weight. Most of the seed produced by the plant is formed in inflorescences borne on main stolons (80%). Lateral stolons, by comparison have a lower seed production ability but appear to have a major role in vegetative survival and longevity. This vegetative survival role is reinforced by the production of a high percentage of rooted nodes in the winter and early spring (91%) when temperatures are low compared with the summer when temperatures increase. The effects of defoliation by cutting involved a reduction in stolon elongation and a general increase in lateral stolon production, particularly when terminal buds were also removed. Any stimulation of lateral stolon development during floral initiation generally reduced seed yield. Both light and heavy cutting treatments resulted in seed yields which were 70 - 90 kg/ha less than the 657 kg/ha produced by uncut plants. The effect of paraquat application was also detrimental to seed yield, mainly through a direct effect on white clover morphology. Although paraquat reduced the amount of lateral branching on the main stolon the destruction of tissue by the herbicide reduced plant recovery and resulted in a seed yield of only 392 kg/ha compared with 657 kg/ha from unsprayed plants. This effect was most pronounced in pure swards of white clover and less obvious when the grass component of a mixed sward provided some protection of the white clover from direct contact with the herbicide.

Maximum seed yields were obtained from November closing dates. In grass/clover swards the closing of crops in November accompanied by paraquat spraying to remove grass competition either in mid October or at closing enhanced seed yield. In pure swards, uncut and unsprayed plants were most productive. Later grazing and spraying, was deleterious to seed yield unless climatic conditions allowed continued vegetative growth into December. In this case spraying in November and closing in December could also give high seed yields.

The large variation in seed yield obtained in these experiments indicates there are considerable opportunities for increasing the seed yield of white clover. Such increases can be achieved if seed producers could put together a crop management programme which is timed to relate to the stage of growth and development of the white clover plant.

Potential areas of future research:

The results obtained in these studies have made it possible to identify some areas where inadequate or insufficient research data is available. Such areas are obvious targets for continued research on white clover seed production. For example, it seems that most lateral stolons bear few inflorescences while they still remain attached to their main stolons. However, once they become independent of the main stolon their reproductive capacity actually increases. Therefore, it would be interesting to determine the effectiveness of cutting lateral stolons (early in October), perhaps with discs, to improving inflorescence initiation. At the same time it would be interesting to determine the physiological bases of this effect.

Another potentially profitable area of research would be the determination of the causes of variation in seed set per floret and to study the mechanisms controlling this character. If seed set per floret could be increased the production of seed in white clover would be significantly improved in comparison to present commercial seed yields. Since white clover is one of the most important New Zealand seed exports it would also seem to be advantageous to develop a more reliable method to determine the correct time to harvest the crop to ensure the maximisation of seed yields.

Practical application for South American conditions:

In addition to its obvious nutritional value for livestock, white clover has a special value for raising soil fertility and improving soil structure. At the same time, a seed crop of clover provides a break in the rotation of the paddock, and, under correct management,

can give good financial returns. For these reasons, white clover is considered as one of the most important legumes in the temperate zone and certainly shows tremendous potential as a legume for high altitude areas near the Equator. It is hopeful that the results obtained in the present experiments together with several investigations made by other workers in New Zealand could be used to provide the basis for the development of a reliable management system for seed production of white clover in South American countries including Argentina, Uruguay, Chile, Paraguay, Bolivia, Southern areas of Brazil and in high altitude areas of the Andes in Colombia, Ecuador, Peru and Venezuela. For example white clover is found to grow naturally at high elevations in Colombia (from 1,800 to 4,000 metres above sea level), which represent about 5% of the total area of the country. It occurs in cultivated fields and waste lands, along roadways, in lawns, parks, and in natural and improved pastures. In many instances a pure stand of white clover appears after a cultivated crop of small grains, when these are well supplied with phosphate. The plants thrive also in soils with a pH of 5.0 or even less. Furthermore, inflorescences and seed may be developed at all times of the year, although they are more abundant in the dry season (June and July). Some preliminary investigations by Crowder (1960) on collected material have shown that most plants are of the intermediate type and flower profusely. With the short day length (12 to 12½ hours throughout the year) there is a natural selection towards a contracted flowering time.

It is the author's opinion that the results found in the present studies could be used directly in temperate areas of some South American countries with little variation according to the weather conditions of each area. In particular, the results are considered to be important in providing the basis for the development of seed programmes for the production of white clover seed in the high elevation of the Andes.

Year	pH	Calcium	Magnesium	Potassium	Ammonium	Nitrate	Phosphorus A	Phosphorus B	Iron	Manganese
82-83	4.9	900	4.4	53	10	150	5.1	13.4	12	10
83-84	4.5	735	4.7	42	3	150	2.6	5.8	8	10
84-85	5.9	640	3.4	79	2	25	5.1	8.8	30	20

Explanation of Figures: pH ... measured in water, soil: water ratio 1:1. Calcium, Magnesium, Potassium, Ammonia, Nitrate, Iron, Manganese ... parts per million measured in the soil extract determined according to the improved method of Morgan-Venema.

Phosphorus A ... parts per million measured in Sodium Bicarbonate solution of pH 8.5 (half hour Olsen method)

Phosphorus B ... parts per million soluble in diluted Sulphuric Acid (method Beater).

Appendix 1: Soil analysis of the experimental field (M and H Laboratories Limited)

Appendix 2: Basic weather data for Palmerston North; mean values for each month over the experimental period. 1982 - 1983

Month	ELEMENT			
	Wind total run km	Mean temperature °C	Total rainfall mm	Rainfall difference from 20 yr average
Aug	290	9.0	52.7	-40.3
Sep	290	9.9	48.7	-39.3
Oct	361	11.1	80.4	- 4.6
Nov	494	15.1	60.9	-13.1
Dec	396	14.4	160.0	+63.0
Jan	464	15.6	59.7	- 8.3
Feb	299	16.8	29.5	-28.5

Data from DSIR, Palmerston North

Treatments	DATE OF SAMPLING (day - month) 1-10	15-10	1-11	15-11	1-12	15-12	1-1	15-1	1-2	15-2
S - A	30	29	29	22	22	24	19	19		
S - S	29	27	29	21	20	25	20	19		
S - O			29	21	21	23	19	21	12	7
S - Wh	30	29	29	20	21	24	20			
O - S			29	20	21	23	20	20		
O - O			29	21	22	24	21	21	12	7
O - N					21	24	20	22	10	
O - Wh			30	20	22	24	19			
N - O					22	23	19	22	13	8
N - N					21	24	20	19	11	
N - D							20	22	14	8
N - Wh					22	24	19	20		
D - N							19	22	14	9
D - D							20	20	11	6
D - J									12	6
D - Wh							20	20	11	

Appendix 3: Effect of closing time and paraquat treatment on the percent of soil moisture content 1982 - 1983.

HARVESTING

Closing time	Days after peak flowering 25	30	35	40
Mid - September	13.0	18.2	12.3	12.9
Mid - October	15.0	17.8	16.3	16.9
Mid - November	13.8	12.0	8.9	11.1
Mid - December	11.5	7.7	9.7	12.6
Significance	N.S.	**	**	**
LSD 5%	-	2.03	1.37	2.60
1%	-	3.09	2.08	3.93
S.E.D. ±	1.89	0.83	0.56	1.06

Paraquat treatments	Days after peak flowering 25	30	35	40
30 days before closing	13.7	10.5	11.6	13.6
At closing	12.9	14.7	11.1	14.3
30 days after closing	12.2	12.1	13.5	14.2
Without herbicide	14.5	18.2	12.1	11.6
Significance	N.S.	**	N.S.	N.S.
LSD 5%		1.86		
1%		2.53		
S.E.D. ±	1.48	0.90	0.83	1.19

Treatments

S - A	11.2	18.7	8.3	14.6
S - S	14.8	16.7	10.4	21.7
S - O	11.4	18.6	15.3	9.6
S - Wh	14.8	28.9	15.1	5.6
N - O	10.4	5.6	5.5	6.2
N - N	17.1	10.9	12.2	12.6
N - D	11.6	9.3	6.8	11.7
N - Wh	16.1	11.1	11.1	13.9
LSD(a) 5%	-	3.78	3.47	5.22
1%	-	5.46	5.01	7.54
S.E.D.(a) ±	3.19	1.77	1.54	2.32
LSD(b) 5%	-	3.87	3.42	4.90
1%	-	5.26	4.65	6.66
S.E.D.(b) ±	2.97	1.80	1.66	2.38

O - S	17.0	21.9	17.3	25.5
O - O	13.1	14.1	10.1	7.4
O - N	16.3	14.2	24.0	17.9
O - Wh	13.6	20.5	13.9	16.9
D - N	16.1	5.9	15.2	7.8
D - D	6.8	6.3	7.5	15.4
D - J	9.7	6.3	7.9	17.3
D - Wh	13.5	12.1	8.1	9.8

(a) When comparing means at different levels of mainplots

(b) When comparing means at the same levels of mainplots

Appendix 4: Effect of closing time and paraquat treatment on normal seedling percentage 1982 - 1983

HARVESTING

Closing time	Days after peak flowering 25	30	35	40
Mid - September	6.1	6.6	4.6	3.4
Mid - October	6.4	8.5	7.1	6.2
Mid - November	6.3	5.1	3.1	3.2
Mid - December	3.3	2.1	3.1	2.5
Significance	N.S.	**	*	*
LSD 5%	-	0.94	2.17	1.36
1%	-	1.42	-	-
S.E.D. ±	1.20	0.38	0.88	0.55
Treatments				
S - A	5.8	4.3	2.9	3.7
S - S	6.9	7.2	5.3	4.5
S - O	7.8	5.9	4.8	1.4
S - Wh	4.0	8.9	5.3	3.7
N - O	3.0	1.5	0.5	1.3
N - N	12.8	9.7	6.0	5.2
N - D	4.8	2.8	1.7	0.7
N - Wh	4.7	6.5	4.3	5.6
LSD(a) 5%	4.12	3.78	3.44	2.74
1%	5.95	5.46	4.97	3.97
S.E.D.(a) ±	1.83	1.67	1.53	1.22
LSD(b) 5%	3.27	3.87	2.97	2.59
1%	4.45	5.26	4.03	3.53
S.E.D.(b) ±	1.59	1.88	1.44	1.26

Paraquat treatments	Days after peak flowering 25	30	35	40
30 days before closing	4.1	4.7	4.2	4.7
At closing	6.1	5.1	5.1	3.8
30 days after closing	6.4	4.9	3.5	2.4
Without herbicide	5.5	7.5	5.1	4.3
Significance	*	*	N.S.	**
LSD 5%	1.64	1.94	-	1.30
1%	-	-	-	1.76
S.E.D. ±	0.79	0.94	0.72	0.63
O - S	5.7	10.5	10.7	12.5
O - O	2.5	2.3	5.5	3.1
O - N	9.7	9.4	5.0	4.1
O - Wh	7.5	11.8	7.4	4.9
D - N	2.0	2.5	2.8	1.3
D - D	2.1	1.2	3.7	2.4
D - J	3.2	1.8	2.4	3.4
D - Wh	6.0	2.9	3.6	2.8

(a) When comparing means at different levels of mainplots

(b) When comparing means at the same levels of mainplots

Appendix 5: Effect of closing time and paraquat treatment on abnormal seedling percentage 1982 - 1983.

HARVESTING

Closing time	Days after peak flowering 25	30	35	40
Mid - September	37.4	46.1	59.9	51.4
Mid - October	46.2	51.9	59.1	63.8
Mid - November	59.1	68.7	68.5	72.5
Mid - December	76.4	82.1	81.5	79.2
Significance	**	**	**	**
LSD 5%	3.87	2.69	8.92	4.14
1%	5.86	4.08	13.50	6.27
S.E.D. ±	1.58	1.10	3.64	1.69
Treatments				
S - A	67.0	69.7	70.8	47.9
S - S	49.1	43.1	56.2	54.7
S - O	25.6	58.2	62.9	70.6
S - Wh	7.9	13.5	49.5	32.4
N - O	73.7	87.9	89.4	81.5
N - N	47.8	50.9	71.8	56.5
N - D	71.7	79.4	82.6	83.0
N - Wh	43.0	56.7	30.1	69.0
LSD(a) 5%	8.57	7.65	11.79	6.70
1%	12.38	11.05	17.03	9.68
S.E.D.(a) ±	3.81	3.39	5.24	2.98
LSD(b) 5%	8.32	7.64	8.98	5.83
1%	11.20	10.39	12.21	7.92
S.E.D.(b) ±	3.99	3.71	4.35	2.83

Paraquat treatments	Days after peak flowering 25	30	35	40
30 days before closing	68.2	75.9	74.1	66.7
At closing	64.9	64.1	72.1	67.9
30 days after closing	54.6	67.0	71.2	72.5
Without herbicide	54.6	67.0	71.2	72.5
Significance	**	**	**	N.S.
LSD 5%	4.12	3.83	4.49	2.93
1%	5.60	5.21	6.10	3.98
S.E.D. ±	1.99	1.85	2.17	1.41
O - S	55.4	61.1	56.1	51.5
O - O	75.9	72.9	74.2	81.8
O - N	37.5	41.8	53.6	63.3
O - Wh	16.1	32.0	52.3	58.6
D - N	76.7	84.7	80.4	86.1
D - D	86.8	89.7	86.0	78.8
D - J	83.5	88.6	85.8	73.1
D - Wh	58.5	65.5	74.1	78.7

(a) When comparing means at different levels of mainplots

(b) When comparing means at the same levels of mainplots

Appendix 6: Effect of closing time and paraquat treatment on hard seed percentage 1982 - 1983

HARVESTING

Closing time	Days after peak flowering 25	30	35	40	Paraquat treatments	Days after peak flowering 25	30	35	40
Mid - September	43.4	29.1	23.7	31.9	30 days before closing	14.0	8.9	10.8	15.0
Mid - October	32.4	21.8	18.1	13.1	At closing	15.9	15.3	15.5	13.9
Mid - November	20.8	14.1	21.5	13.1	30 days after closing	26.8	15.9	11.2	11.0
Mid - December	7.9	8.0	5.7	5.7	Without herbicide	47.7	32.3	30.9	24.0
Significance	**	**	**	**	Significance	**	**	**	**
LSD 5%	4.31	3.01	6.37	2.62	LSD 5%	3.21	2.80	3.44	2.35
1%	6.53	4.56	9.65	3.97	1%	4.36	3.81	4.67	3.19
S.E.D. ±	1.76	0.89	2.59	1.07	S.E.D. ±	1.56	0.61	1.67	1.14
Treatments									
S - A	16.1	17.2	18.2	33.8	O - S	21.8	6.6	18.6	10.7
S - S	28.9	33.0	29.4	18.4	O - O	8.3	10.6	10.2	7.6
S - O	55.2	17.3	17.0	18.3	O - N	36.5	34.5	17.4	14.6
S - Wh	73.3	48.6	30.9	56.5	O - Wh	62.8	35.7	26.4	19.6
N - O	12.9	4.8	4.6	10.9	D - N	5.2	6.9	1.9	4.8
N - N	22.3	17.5	19.8	25.7	D - D	4.3	2.5	2.8	3.4
N - D	11.9	8.5	8.9	4.6	D - J	3.7	3.3	3.9	6.1
N - Wh	36.2	25.7	58.8	11.5	D - Wh	18.7	19.4	14.2	8.5
LSD(a) 5%	7.24	5.98	8.73	5.06	(a) When comparing means at different levels of mainplots				
1%	10.46	8.65	12.61	7.31					
S.E.D.(a) ±	3.22	1.39	3.88	2.25					
LSD(b) 5%	6.41	5.60	6.86	4.69	(b) When comparing means at the same levels of mainplots				
1%	8.71	7.62	9.32	6.38					
S.E.D.(b) ±	3.11	1.22	3.33	2.28					

Appendix 7: Effect of closing time and paraquat treatment on dead seed percentage 1982 - 1983

HARVESTING

Closing time	Days after peak flowering 25	30	35	40
Mid - September	54.3	66.7	70.9	67.6
Mid - October	67.6	78.1	82.6	86.9
Mid - November	79.2	85.9	80.5	86.3
Mid - December	91.2	91.9	94.3	94.3
Significance	**	**	**	**
LSD 5%	6.36	5.29	10.17	2.69
1%	9.64	8.01	15.40	4.08
S.E.D. ±	2.59	0.88	4.15	1.10
Treatments				
S - A	44.8	82.7	83.0	66.2
S - S	70.8	66.9	71.9	80.8
S - O	83.9	82.8	82.0	81.7
S - Wh	17.7	34.2	46.6	47.1
N - O	88.1	91.5	91.1	89.0
N - N	77.7	82.5	90.0	74.3
N - D	87.1	95.2	95.4	95.5
N - Wh	63.8	74.3	45.5	88.4
LSD(a) 5%	10.86	14.92	20.52	5.04
1%	15.70	21.55	29.64	7.28
S.E.D.(a) ±	4.83	1.94	9.12	2.24
LSD(b) 5%	9.68	14.91	19.32	4.66
1%	13.16	20.27	26.26	6.33
S.E.D.(b) ±	4.70	1.99	9.38	2.26

Paraquat treatments	Days after peak flowering 25	30	35	40
30 days before closing	73.2	84.1	88.2	85.5
At closing	83.9	87.3	86.0	85.3
30 days after closing	86.0	91.1	89.9	89.1
Without herbicide	49.2	63.4	62.9	75.5
Significance	**	**	**	**
LSD 5%	4.84	7.46	9.66	2.32
1%	9.38	10.14	13.13	3.16
S.E.D. ±	2.35	1.00	4.69	1.13
O - S	63.5	65.5	82.6	89.4
O - O	91.7	89.4	89.8	92.3
O - N	78.2	93.3	84.2	85.4
O - Wh	37.2	64.3	73.6	80.4
D - N	96.3	97.7	96.1	95.2
D - D	95.7	97.2	97.2	96.6
D - J	94.8	95.1	98.1	93.2
D - Wh	77.9	80.6	85.8	91.3

(a) When comparing means at different levels of mainplots

(b) When comparing means at the same levels of mainplots

Appendix 8: Effect of closing time and paraquat treatment on viable seed percentage 1982 - 1983

Appendix 9: Basic weather data for Palmerston North; mean values for each month over the experimental period 1983 - 1984

Month	ELEMENT			
	Wind total run km	Temperature °C	Rainfall mm	Rainfall difference from 20 yr average
Sep	343	11.4	116.5	+28.5
Oct	287	13.5	56.9	-28.1
Nov	257	13.8	54.0	-20.0
Dec	351	15.5	67.7	-29.3
Jan	348	16.2	35.3	-32.7
Feb	256	17.1	90.7	+32.7

Data from DSIR, Palmerston North

Treatments	DATE OF SAMPLING day - month							
	1 - 11	15 - 11	1 - 12	15 - 12	1 - 1	15 - 1	1 - 2	15 - 2
O - S	30	28	22	21	16	15		
O - O	28	29	21	23	17	19		
O - N			29	29	20	18	3	
N - O			29	25	16	17	12	28
N - N			28	28	18	19	13	28
N - D					20	21	14	28
D - N					19	20	12	28
D - D					20	22	16	28
D - J							12	28

Appendix 10: Effect of closing time and paraquat treatment on soil moisture content 1983 - 1984

Appendix 11: Variation in number of florets in ten inflorescences at harvest 1983 - 1984

Treatment	Replication	Number of florets/inflorescence									
		1	2	3	4	5	6	7	8	9	10
O - S	I	45	57	55	52	50	61	47	44	45	41
	II	38	45	40	33	46	47	47	52	21	41
	III	30	50	48	38	22	37	26	44	34	27
O - O	I	34	66	42	42	41	70	54	60	39	27
	II	53	45	46	45	59	44	36	49	51	53
	III	64	39	62	56	20	55	47	50	42	21
O - N	I	55	31	31	52	28	72	21	36	20	33
	II	27	32	51	28	43	26	21	24	30	35
	III	53	38	25	44	34	39	42	33	33	29
N - O	I	46	41	43	45	32	47	41	32	37	39
	II	44	44	37	37	43	33	46	40	36	30
	III	45	67	58	50	41	53	48	53	54	42
N - N	I	44	63	64	67	45	67	63	43	63	34
	II	48	57	75	61	44	43	46	37	43	40
	III	60	71	53	55	68	55	58	43	49	61
N - D	I	54	27	44	50	48	44	46	34	40	29
	II	46	42	56	34	34	32	39	34	33	40
	III	30	53	48	57	41	31	32	46	23	32
D - N	I	59	65	64	57	31	46	43	41	45	37
	II	51	52	53	53	54	49	48	41	40	33
	III	50	56	60	58	48	43	44	64	52	34
D - D	I	40	51	46	55	44	50	47	34	23	43
	II	32	35	34	46	50	37	26	40	21	34
	III	51	41	32	44	39	20	60	21	25	30
D - J	I	23	22	20	31	20	30	20	20	12	24
	II	41	45	31	20	21	33	20	25	29	28
	III	34	52	48	56	38	38	49	32	20	27

Appendix 12: Basic weather data for Palmerston North - mean values for each month over the experimental period 1984 - 1985

Month	ELEMENT				
	Wind Total run km	Temperature °C/month max	min	Rainfall mm	Rainfall difference from 20 yr average
Jul	180	13.1	5.6	96.7	+ 7.7
Aug	292	14.3	6.4	46.0	-43.0
Sep	238	15.1	6.9	62.5	-12.5
Oct	374	16.3	8.2	42.0	-46.0
Nov	354	20.1	11.6	79.5	+ 1.0
Dec	305	22.6	13.6	102.0	+ 8.0
Jan	283	23.5	14.3	123.5	+44.5

Data from DSIR Palmerston North

Appendix 13: Increase in length of main stolons from July to January 1984 - 1985

Month	LENGTH (mm) Average	Genotype I	Genotype II
July	20.50± 1.29	21.0±1.41	20.0± 2.83
August	24.33± 3.18	26.2±2.12	22.5± 3.54
September	41.16± 1.62	41.3±2.80	41.0± 1.20
October	120.34± 4.94	123.7±2.00	116.5±10.61
November	162.23±16.29	176.0±7.10	148.6±11.20
December	181.00±55.50	229.0±7.10	144.6± 5.76
January	176.50±50.28	220.0±2.80	132.5± 3.53

Number of nodes	July	August	September	October	November	December	January
			AVERAGE				
4 - 7	0.00±0.00	0.00±0.00	6.40±3.80	13.40±3.11	21.58±2.17	19.60±1.55	10.30±1.61
8 - 11	7.77±0.86	8.25±0.83	15.70±3.90	25.70±3.91	9.67±1.24	1.25±1.58	0.00±0.00
more than 11	36.95±3.32	34.15±3.84	23.07±2.62	11.50±2.77	3.75±0.96	0.00±0.00	0.00±0.00
			GENOTYPE I				
4 - 7	0.00±0.00	0.00±0.00	3.30±1.27	15.60±2.83	22.00±5.65	19.20±5.65	10.60±0.84
8 - 11	7.60±2.62	7.40±1.20	19.00±5.94	22.40±7.64	10.00±2.83	2.50±0.71	0.00±0.00
more than 11	37.50±2.33	38.20±3.11	23.00±4.24	13.20±3.68	4.00±1.41	0.00±0.00	0.00±0.00
			GENOTYPE II				
4 - 7	0.00±0.00	0.00±0.00	9.50±3.53	11.20±3.96	21.10±3.74	20.10±5.30	9.80±1.10
8 - 11	7.90±2.26	9.10±2.05	12.40±2.83	29.00±4.25	9.35±2.90	0.00±0.00	0.00±0.00
more than 11	36.40±5.16	34.10±5.23	23.00±3.41	10.30±3.25	4.50±2.12	0.00±0.00	0.00±0.00

Appendix 14: Number of lateral stolons of different sizes present in February on main stolons from July to January Samples of 20 main stolons. 1984 - 1985

Appendix 15: Percentage of rooted nodes present in February at nodes which emerged from terminal buds of main stolons from July to January 1984 - 1985

	PERCENTAGE OF ROOTED NODES		
Months	Average	Genotype I	Genotype II
July	91.0±16.50	99.8±0.63	83.5±13.30
August	76.0± 7.67	80.0±7.07	73.5± 9.20
September	71.0±14.30	72.5±7.78	69.5±23.30
October	34.0± 5.35	38.5±0.71	29.5± 2.12
November	46.0± 2.58	48.0±1.41	44.0± 1.41
December	42.0± 4.08	39.0±0.71	44.5± 4.95
January	28.2± 2.08	28.5±0.71	28.5± 3.53

Appendix 16: Estimated time of initiation of inflorescences in ten terminal buds on main stolons from July to January 1984 - 1985

Estimated time days - month	NUMBER OF INFLORESCENCES INITIATED		
	Average	Genotype I	Genotype II
1 - 14 Aug	3.0±3.00	0.0±0.00	6.0±0.82
15 - 31 Aug	9.5±2.12	11.0±2.16	8.0±1.82
1 - 14 Sep	9.0±4.40	5.0±1.15	13.0±1.41
15 - 30 Sep	9.5±1.69	9.0±0.20	10.0±1.41
1 - 14 Oct	14.5±2.97	15.0±3.83	14.0±2.31
15 - 31 Oct	14.37±3.23	13.0±2.00	15.8±2.81
1 - 14 Nov	14.50±2.53	14.0±2.20	15.0±2.46
15 - 30 Nov	10.25±4.85	7.0±1.04	13.5±2.08
1 - 14 Dec	10.50±5.04	6.0±0.82	15.0±2.16
15 - 31 Dec	4.5 ±1.77	3.0±0.82	6.0±0.82
1 - 14 Jan	2.0 ±2.20	4.0±0.82	0.0±0.00
15 - 31 Jan	0.0 ±0.00	0.0±0.00	0.0±0.00

Appendix 17: Number of inflorescences present in ten terminal buds of main stolons from July to January 1984 - 1985

Sampling date day - month	NUMBER OF INFLORESCENCE Average	Genotype I	Genotype II
1 - Sep	3.0 ± 1.04	2.0 ± 0.71	4.0 ± 0.71
15 - Sep	9.5 ± 1.96	8.0 ± 0.71	11.0 ± 1.41
1 - Oct	13.0 ± 2.58	11.0 ± 1.41	15.0 ± 1.98
15 - Oct	16.5 ± 3.10	14.0 ± 1.91	19.0 ± 1.91
1 - Nov	14.2 ± 1.15	13.0 ± 1.78	15.5 ± 1.48
15 - Nov	15.0 ± 3.85	12.0 ± 2.83	18.0 ± 1.41
1 - Dec	11.8 ± 2.58	9.0 ± 1.41	14.5 ± 1.91
15 - Dec	11.5 ± 2.04	10.0 ± 0.71	13.0 ± 2.82
1 - Jan	4.0 ± 0.58	4.0 ± 0.71	4.0 ± 0.71
15 - Jan	2.6 ± 1.11	3.0 ± 0.41	2.12 ± 0.71
1 - Feb	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

Appendix 18: Number of inflorescences on main and lateral stolons present in February on nodes which emerged from terminal buds on twenty main stolons from September to January 1984 - 1985

Month	NUMBER INFLORESCENCES EMERGED		
	Main stolon plus lateral stolon	Main stolon	Lateral stolon
		AVERAGE	
Sept	8.90 ± 4.81	7.75 ± 2.45	1.65 ± 0.92
Oct	25.02 ± 6.83	20.15 ± 3.32	4.87 ± 3.51
Nov	44.13 ± 10.04	35.25 ± 3.75	8.88 ± 6.30
Dec	39.33 ± 16.25	31.05 ± 9.30	8.28 ± 6.99
Jan	11.36 ± 1.92	10.76 ± 4.08	2.26 ± 1.50
		GENOTYPE I	
Sept	5.50 ± 2.47	5.50 ± 1.16	0.00 ± 0.00
Oct	20.19 ± 3.43	17.80 ± 1.13	2.39 ± 0.23
Nov	37.03 ± 2.53	32.50 ± 3.39	4.43 ± 0.44
Dec	27.84 ± 6.16	24.50 ± 2.12	3.34 ± 0.33
Jan	10.00 ± 2.81	8.80 ± 1.69	1.20 ± 0.12
		GENOTYPE II	
Sept	12.31 ± 3.07	10.00 ± 1.60	2.31 ± 0.39
Oct	29.85 ± 4.70	22.50 ± 3.53	7.35 ± 0.59
Nov	51.24 ± 5.61	37.90 ± 4.10	13.34 ± 0.65
Dec	50.83 ± 4.59	37.60 ± 2.83	13.23 ± 0.65
Jan	12.72 ± 3.55	12.72 ± 3.55	3.32 ± 0.17

Appendix 19: Number of white inflorescences per unit area

Sampling date day - month	NUMBER OF WHITE INFLORESCENCES Average	Genotype I	Genotype II
21 - 10	2.7 ± 0.50	3.0 ± 0.00	2.5 ± 0.71
28 - 10	11.0 ± 2.58	12.0 ± 2.83	10.0 ± 2.83
6 - 11	11.0 ± 3.59	14.0 ± 1.41	8.0 ± 0.92
12 - 11	12.0 ± 2.58	10.0 ± 1.41	14.0 ± 1.41
19 - 11	23.0 ± 2.39	22.0 ± 2.26	24.0 ± 2.83
26 - 11	29.0 ± 4.24	28.0 ± 4.24	30.0 ± 5.65
4 - 12	29.3 ± 13.59	18.0 ± 2.83	40.5 ± 6.36
10 - 12	34.0 ± 11.92	24.0 ± 1.85	44.0 ± 4.24
17 - 12	35.0 ± 16.43	21.0 ± 2.83	49.0 ± 3.24
24 - 12	37.8 ± 22.39	18.5 ± 0.71	57.0 ± 4.24
1 - 1	38.3 ± 25.32	16.5 ± 1.41	60.0 ± 5.65
7 - 1	37.5 ± 8.85	30.0 ± 2.83	45.0 ± 1.41
14 - 1	25.7 ± 8.42	18.5 ± 0.71	33.0 ± 1.41
21 - 1	5.7 ± 1.25	5.0 ± 1.41	6.5 ± 0.71

Date of sampling day - month	Petiole length mm	Peduncle length mm	Floret number in white flowers	Number of ovules per ovary in white flowers	Number of seed formed per pod in crown flowers	% of ovule fertilization
			GENOTYPE I			
1 - Nov	92.05 ± 11.03	180.7 ± 15.40	84.0 ± 3.72	6.10 ± 0.14	-	-
15 - Nov	79.7 ± 9.53	180.6 ± 14.40	83.6 ± 1.69	5.90 ± 0.08	4.48 ± 0.26	76
1 - Dec	76.2 ± 6.97	153.8 ± 12.90	78.2 ± 0.56	6.00 ± 0.08	4.93 ± 0.21	82
15 - Dec	113.7 ± 14.80	163.8 ± 4.81	79.1 ± 2.12	5.98 ± 0.14	4.16 ± 0.32	70
1 - Jan	125.9 ± 12.80	198.8 ± 11.45	68.5 ± 2.96	6.00 ± 0.14	3.43 ± 0.38	57
15 - Jan	88.1 ± 4.38	173.1 ± 11.35	72.4 ± 2.83	5.80 ± 0.14	3.15 ± 0.04	54
1 - Feb	169.5 ± 12.20	274.0 ± 14.80	60.0 ± 2.48	5.40 ± 0.25	2.65 ± 0.15	49
			GENOTYPE II			
1 - Nov	83.8 ± 10.70	167.1 ± 16.78	87.7 ± 4.15	5.95 ± 0.07	-	-
15 - Nov	76.1 ± 8.34	169.0 ± 9.89	86.7 ± 2.12	6.10 ± 0.08	4.46 ± 0.32	73
1 - Dec	78.9 ± 8.90	156.6 ± 10.36	99.0 ± 4.16	6.05 ± 0.07	3.98 ± 0.23	65
15 - Dec	86.8 ± 9.10	220.9 ± 7.78	81.4 ± 2.13	6.00 ± 0.14	3.96 ± 0.19	66
1 - Jan	89.8 ± 10.70	171.2 ± 9.97	75.5 ± 2.84	6.00 ± 0.14	3.27 ± 0.16	54
15 - Jan	67.9 ± 5.23	141.2 ± 2.83	77.4 ± 2.97	5.50 ± 0.49	3.07 ± 0.09	55
1 - Jan	69.3 ± 8.34	188.0 ± 16.9	67.5 ± 3.23	5.00 ± 0.32	2.29 ± 0.10	45

Appendix 20: Changes in petiole and peduncle length, inflorescence size, number of ovules per ovary and seed numbers per pod in Genotype I and Genotype II 1984 - 1985

Appendix 21: Effect of defoliation on the number of inflorescences per main stolon present in February at nodes which emerged from terminal buds of main stolons from November to January 1984 - 1985

Genotype	November	December	January
I	0.68	1.09	0.49
II	1.04	2.12	0.73
Significance	N.S.	N.S.	N.S.
S.E.D. ±	0.061	0.116	0.048
Defoliation treatment			
Control	1.76	1.55	0.46
Light cut	0.76	1.59	0.66
Heavy cut	0.52	1.99	0.92
Paraquat	0.40	1.28	0.41
Significance	**	**	**
LSD 5%	0.13	0.12	0.21
1%	0.20	0.18	0.32
S.E.D. ±	0.055	0.050	0.087

Significant interaction

Genotype x defoliation	December		January	
	I	II	I	II
Control	1.22	1.88	0.44	0.47
Light cut	1.06	2.14	0.54	0.76
Heavy cut	1.34	2.65	0.52	1.32
Paraquat	0.74	1.80	0.44	0.38
Significance	**		*	
LSD	5%	1%	5%	
(a)	0.32	0.48	0.28	
(b)	0.17	0.26	0.30	
S.E.D. ± (a)	0.130		0.117	
(b)	0.071		0.124	

(a) When comparing means with the different levels of mainplot

(b) When comparing means with the same levels of mainplot

Appendix 22: Effect of defoliation on the number of inflorescences on lateral stolons present in February at nodes which emerged from terminal buds on main stolons from November to January 1984 - 1985

Genotype	November	December	January
I	0.07	0.04	0.02
II	0.66	1.68	0.60
Significance	N.S.	**	*
LSD 5%		0.03	0.09
1%		0.05	
S.E.D. ±	0.078	0.0132	0.0387
Defoliation treatment			
Control	0.44	0.41	0.11
Light cut	0.45	0.94	0.32
Heavy cut	0.33	1.36	0.65
Paraquat	0.25	0.73	0.15
Significance	*	**	**
LSD 5%	0.19	0.14	0.15
1%		0.21	0.22
S.E.D. ±	0.078	0.0583	0.0607

Significant interactions

Genotype x defoliation	November		December		January	
	I	II	I	II	I	II
Control	0.22	0.66	0.17	0.66	0.06	0.17
Light cut	0.06	0.84	0.00	1.87	0.00	0.65
Heavy cut	0.00	0.65	0.00	2.73	0.00	1.30
Paraquat	0.00	0.50	0.00	1.45	0.00	0.29
Significance	**		**		**	
LSD	5%	1%	5%	1%	5%	1%
(a)	0.21	0.32	0.18	0.27	0.20	0.31
(b)	0.11	0.16	0.20	0.30	0.21	0.32
S.E.D. ± (a)	0.087		0.0726		0.084	
(b)	0.044		0.0824		0.086	

(a) When comparing means with different levels of mainplot

(b) When comparing means with the same levels of mainplot

Appendix 23: Effects of defoliation on the number of florets per inflorescence 1984 - 1985

	Date of Sampling (month - day)				
Genotype	Dec 1st	Dec 15th	Jan 1st	Jan 15th	Feb 1st
I	84.7	76.7	66.4	61.8	58.9
II	94.2	88.2	81.8	76.6	65.1
Significance	*	N.S.	**	N.S.	N.S.
LSD 5%	7.62		1.90		
1%			9.56		
S.E.D. ±	0.60	6.27	0.15	3.69	3.75
Defoliation Treatment					
Control	88.6	80.3	78.8	67.9	58.8
Light cut	93.4	79.5	71.9	70.5	65.1
Heavy cut	89.8	86.1	77.3	70.9	62.3
Paraquat	85.9	83.9	73.5	67.6	61.8
Significance	N.S.	N.S.	N.S.	N.S.	N.S.
S.E.D. ±	3.09	3.66	4.27	4.51	4.55

Appendix 24: Effects of defoliation on the number of ovules per ovary 1984 - 1985

	Date of Sampling (month - day)				
Genotype	Dec 1st	Dec 15th	Jan 1st	Jan 15th	Feb 1st
I	6.01	5.85	6.00	5.51	5.21
II	6.02	5.94	6.00	5.50	5.34
Significance	N.S.	N.S.	N.S.	N.S.	N.S.
S.E.D. ±	0.0375	0.0125	0.00	0.0125	0.001
Defoliation Treatment					
Control	6.02	5.95	6.00	5.37	5.07
Light cut	6.00	5.97	6.00	5.45	5.23
Heavy cut	6.02	6.00	6.00	5.70	5.47
Paraquat	6.02	5.65	6.00	5.50	5.27
Significance	N.S.	N.S.	N.S.	N.S.	N.S.
S.E.D. ±	0.0270	0.1150	0.00	0.2490	0.1627

APPENDIX 25: Effects of defoliation on the number of seeds per floret 1984 - 1985

	Date of Sampling (month - day)			
Genotype	Dec 15th	Jan 1st	Jan 15th	Feb 1st
I	4.23	3.40	3.01	2.64
II	3.64	2.90	2.54	2.16
Significance	N.S.	N.S.	N.S.	N.S.
S.E.D. ±	0.087	0.175	0.050	0.150
Defoliation treatment				
Control	4.15	3.35	3.05	2.47
Light cut	3.57	2.80	2.47	2.10
Heavy cut	3.95	3.45	2.95	2.77
Paraquat	4.05	3.00	2.63	2.25
Significance	N.S.	N.S.	N.S.	N.S.
S.E.D. ±	0.363	0.145	0.085	0.179

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