SEED PRODUCTION IN BIRDSFOOT TREFOIL (<u>Lotus corniculatus</u> L.)

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A B S T R A C T

This thesis reports the results of three years' research on seed production of birdsfoot trefoil (Lotus corniculatus L.). Previous work by other researchers has suggested that a protracted flowering period is a major factor limiting seed production in this legume. The research programme was begun in 1986 with an investigation of the cause(s) of this protracted flowering period. By carefully examining the growth and flowering behaviour of tagged shoots formed at different times of the year, it was identified that the protracted flowering pattern in this plant is caused by the continuous production of shoots with the capacity to flower. The continuous development of new shoots replacing older flowering shoots was the main cause of the long flowering period. Results of the first year's research also showed that seed yield is primarily determined by the number of inflorescences produced. In turn, inflorescence numbers are primarily dependent on the number of shoots available at the time of flowering. It was suggested by the results that manipulation of the number and branching of shoots should be an important management strategy for improving seed production in this plant.

The second stage of the study involved a number of hand removal and cutting treatments designed to investigate the possible effects of shoot removal on plant growth and seed production. Hand removal of crown shoots affected branching behaviour in this plant. Removal of young crown shoots at different growth stages resulted in a range of plant structural forms which influenced seed production potential. Cutting treatments, the major effect of which was to remove a section

of shoot from the top, showed no obvious beneficial effects on seed yield in birdsfoot trefoil.

In the third stage studies were extended to investigate a more practical situation in which plant growth regulating chemicals (paclobutrazol (PP333), ethofumesate (Nortron) and fatty alcohols (Fatol Super)) were used to manipulate shoot growth and development. Research at this stage involved both widely spaced individual plants and plants grown in a sward to simulate commercially practised situations. Results showed that PP333 is a promising pre-peak flowering shoot manipulator which acts by promoting branching. Nearly a 40% increase in seed yield was consistently obtainedin two years' experiments when this chemical were applied during the time from the pre-bud stage to the early flowering stage. Nortron showed its value in sward situations when applied at early plant development stage. However, Nortron was of limited value when it was used as a post-peak flowering shoot manipulator because of its injurious effect on plant growth and flowering. Fatol Super showed some potential as a post-peak flowering shoot manipulator, particularly when applied to spaced plants, but was less effective in sward situations. This reduces its value for use in the narrow row or sward seed production systems currently practiced in New Zealand for birdsfoot trefoil.

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GENERAL INTRODUCTION

Lotus corniculatus L., commonly known as birdsfoot trefoil, is a legume introduced into New Zealand about one hundred years ago (Thomson, 1922). It has shown promise as a pasture species in areas where environmental conditions are considered to be too harsh for the good performance of other pasture legumes, such as white clover, red clover and lucerne (Hunt and Wagner, 1963). Under these conditions, a number of advantages have been noted for Lotus corniculatus as a forage crop. Lotus corniculatus produces well in areas where soils are infertile, acid, dry or poorly drained (Robinson, 1934; Chevrette et al., 1960;   ; Charlton et al., 1978; Morton, 1981; Scott and Charlton, 1983). Although it is often claimed to be a poor-land crop, Lotus corniculatus also grows well in fertile soils and under good environmental conditions. It responds well to fertilizer application, and therefore could be a highly productive legume in fertile pasture situations (Varney, 1958; Hughes, 1962; Hunt and Wagner, 1963). Once established, it provides highly productive and long-lived permanant pasture for hay, forage and silage. Its high late season forage productivity allows it to make a substantial contribution for the even supply of animal feed available during the late summer and autumn (MacDonald, 1946; Davis and Bell, 1957; Suckling, 1960; Davis, 1969; Charlton, 1973; Greenwood and Sheath, 1981). Lotus corniculatus compares favourably in feed value with other common legumes (MacDonald, 1946; Suckling, 1960; Smith, 1964; Charlton, 1971; John and Lancashire, 1981), and its non-bloating nature is a unique and valuable character which makes it superior to

other common legumes in some circumstances (Marten and Jordan, 1979).
Lotus corniculatus possesses a notable nitrogen-fixation ability _
which makes it a valuable pasture species, particularly when grown in
a mixture with grasses (Brophy et al., 1987).

In New Zealand, because of the generally favourable climate, clovers
play a dominant role as pasture legumes in most farming systems. The
amount of research on _Lotus corniculatus_ has until recently occupied
only a marginal place in the country's pasture science research
programme. However, with the need to develop productive pasture on
land areas traditionally regarded as poor, the advantages of _Lotus
corniculatus_ as a forage legume have become better recognized.

The difficulty of achieving a high seed yield in _Lotus corniculatus_
is frequently quoted as a major factor limiting its more widespread
use in pasture systems. A number of aspects have been identified
which may severely limit the seed production of this plant (McGraw
and Beusilinck, 1983). The indeterminate growth habit of the plant
results in an extended flowering period which may make it difficult
to harvest the crop with maximum seed recovery (Beusilinck and
McGraw, 1988). Also, pod dehiscence often leads to a heavy loss of
seed at harvesting. It has been suggested that low assimilate
distribution to reproductive parts might be important in limiting the
potential for high seed yield in this plant (MacDonald, 1946; Seaney
and Henson, 1970; McGraw and Beusilinck, 1983; McGraw et al., 1986).
As a result of these factors commercial seed yields in _Lotus
corniculatus_ are often low and unreliable. Seaney and Henson (1970)
have stated that 50-175 kg/ha is a commonly harvested commercial seed
yield, while 200-575 kg/ha is among the highest yield which can only

be obtained occasionally.

The current research programme commenced as a result of the need for a better understanding of this plant and the need for the development of appropriate management strategies for better seed production. The pod dehiscence character and the low assimilate contribution to reproductive growth in this plant have already been explored by previous researchers (Buckovic, 1952; Anderson, 1955; Metcalfe et al., 1957; McGraw and Beuselinck, 1983). It was therefore decided that the emphasis of the current research should be on the long flowering period of this plant, with particular interest in the contribution of different shoot age groups to the long flowering pattern. The study also examined possible management systems which could be used to overcome the protracted flowering sequence by shoot manipulation strategies. While the use of Lotus corniculatus is at present restricted to certain agronomic situations, it is possible that more widespread use could be achieved in the future as a result of a better understanding of its reproductive characters and success in achieving a sufficient and reliable seed supply through better seed crop management techniques.

The present study was divided into four parts, each emphasising one particular aspect. Part one (Chapter 3) involves a series of field experiments designed to provide basic information on the growth and development of the plant, mainly its shoot system, and its relationship with the flowering pattern. Particular attention has been directed towards the contribution made by different shoot age groups to the duration and intensity of flowering. Part two (Chapter 4) deals with flower and seed development and the contribution of

individual seed yield components to total seed yield. In this part some seed characters, such as hardseededness and dormancy behaviour, are also investigated. Based on the information obtained in the first two parts, part three (Chapter 5) comprises a series of experiments involving hand removal of shoots from different age groups, and cutting treatments. In this part particular emphasis has been placed on investigating the effects of physical shoot manipulation of plant growth in order to provide detailed information on the possibility of using shoot manipulation to improve seed yield in birdsfoot trefoil. In part four, which includes Chapters 5 and 6, treatments were extended to a more practical situation using plant growth regulating chemicals to manipulate both pre- and post- peak flowering shoots in favour of improved seed yield.

Plate 1.1. A view of the <u>Lotus corniculatus</u> crop at
peak flowering (6 January 1988)

CHAPTER I: LITERATURE REVIEW

Before starting this review, it seems helpful to give some brief definitions of some terms used in this work which may otherwise cause confusion. The term 'birdsfoot trefoil', although mostly used for the species Lotus corniculatus L., is also used in the literature to describe other species of the genus Lotus that are of agricultural importance, including Lotus uliginosus Schk. syn. Lotus pedunculatus Cav., Lotus angustissimus L., and Lotus hispidus Desf (MacDonald, 1946). The species Lotus corniculatus L. also has several synonyms which could lead to its confusion with other species from time to time, e.g., Lotus, birdsfoot trefoil, broadleaved birdsfoot trefoil and trefoil. The work described here is concentrated on the 'true' birdsfoot trefoil, Lotus corniculatus L.

1.1. Origin and distribution

Lotus corniculatus L. (birdsfoot trefoil) is a perennial legume which is thought initially to have come from the Mediterranean basin, since the greatest diversity of wild plants and distribution of the species was found to occur there (Seaney and Henson, 1970). According to Robinson (1934), the first description of this plant was in English in Gerard's Herbal in 1597, while Ellis' (1774) report on the use of birdsfoot trefoil as a feed for cattle in England could be regarded as the earliest use of this species in agriculture. By the time of Robinson's review (1934), birdsfoot trefoil had been widely found in Europe (with the exception of Finland), as well as in Russia, Africa, India, and greater Asia. In MacDonald's later review (1946), extended

areas of use included Tibet, Australia, New Zealand and North and South America.

The occurrence of Lotus corniculatus in New Zealand dates back to about 1864, according to Thomson (1922). Levy (1918) appeared to be the first person to describe this plant with consideration of its possible agricultural use in this country. Since then the number of cultivars introduced has increased steadily. By 1978 there had been more then 200 cultivars of Lotus corniculatus available for evaluation (Charlton et al., 1978), including 64 from Europe, 31 from the Mediterrannean Region, 25 from South America, 23 from U.S.S.R. and 21 from North America.

Despite its long history, its widespread distribution and numerous published reports, as reviewed by Robinson (1934), MacDonald (1946) and Seaney and Henson (1970), great interest in Lotus corniculatus did not begin until 1900 or later. The failure to use this species earlier in its early history is perhaps, at least partially, attributable to the extreme difficulty in harvesting substantial quantities of seed (Boitel, 1893, cited by Robinson, 1934).

1.2, Agronomic value

The use of Lotus corniculatus in agriculture dates back beyond its actual culture and recognition as a valuble crop plant. Many pioneer agriculturalists have mentioned the agricultural merit of Lotus corniculatus in their papers (e.g. Worlidge, 1681, cited by MacDonald, 1946). According to Robinson (1934), Ellis (1774) is

credited as the first person to give a useful account of its agriculture value. In 1774, he wrote: 'I can affirm it for truth, that the Tyne grass (wild vetch) and the Lady-finger grass (birdsfoot trefoil) are the two best sorts of natural meadow grasses that are, for feeding and fattening the conies, deer and race-horses, or, indeed, for feeding and fattening any other sort of cattle that will eat them, whether given to them as grass or hay.' Anderson (1777, cited by MacDonald, 1946) also described and discussed the agricultural value of birdsfoot trefoil, and emphasised its comparative palatability with many other native plants. Martyn (1779, cited by MacDonald, 1946) stated that birdsfoot trefoil 'is of quality equal, if not superior, to most of the trefoils, and might doubtless be cultivated to good advantage alone.' Later, Morton (1855, cited by MacDonald, 1946) considered birdsfoot trefoil to be as nutritious as clover. Robinson (1934) stated that, by 1810, seed of birdsfoot trefoil had become an article of commerce, being listed for sale by a seedsman in London, and indicating recognition of the merit of birdsfoot trefoil by the farming public. Sinclair (1826, cited by MacDonald, 1946), estimated that the plant would produce at full bloom about 10209 pounds per acre (apparently, green material). This may be the earliest quantitive measurement of birdsfoot trefoil as an agricultural plant. Later, many researchers (cited by MacDonald, 1946), e.g. Lawson (1843), Low (1847), Lecoq (1862), Vianne (1870), Vilmorin-Andrieux (1892), also recognized the value of this species. It should be specially pointed out that among the pioneer workers, Stebler and Schroeter (cited by MacDonald, 1946), in 1889, presented a comprehensive discussion of birdsfoot trefoil, including its description, adaption, culture, production, nutritive value, and use, which served as a turning point in interest and

studies on birdsfoot trefoil. The beginning of the twentieth century, as pointed out by Seaney and Henson (1970) in their review, heralded a real expansion in trefoil culture.

Lotus corniculatus is a nutritious herbage plant both for grazing and hay. With the development of modern analytical techniques, more accurate quantitive evaluations were made which confirmed its high quality as a pasture species. The chemical composition of birdsfoot trefoil, in terms of crude protein, readily fermentable carbohydrate and various mineral elements, which are usually used as the indicators of feeding value of a plant, is similar, or sometimes higher than, that of the commonly used high quality pasture legumes, such as clovers, lucerne and sainfoin (MacDonald, 1946; Suckling, 1960; Smith, 1964; Charlton, 1971, 1973; Van Soest, 1975; John and Lancashire, 1981).

Lotus corniculatus has also been shown to be a valuable forage crop since its protein content remains high until very late in the growing season (MacDonald, 1946; Suckling, 1960). This is possibly related to its long flowering period and the fact that its reproductive growth occupies very little of the total growth period. According to John and Lancashire (1981), the late season's stemmy growth of birdsfoot trefoil is more palatable to livestock than lucerne.

The non-bloating character of lotus species has advantages in ruminant animal farming. It has been well established that legumes have a higher feed value in many circumstances than grasses (Ulyatt, 1970). However, the occurrence of bloat following grazing of legumes such as red and white clover and lucerne has discouraged the more

widespread use of these legumes as important protein sources for livestock. The non-bloating character of lotus species makes them satisfactory alternative legumes in pastures. Marten and Jordan (1979) showed that substitution of a pure stand of Lotus corniculatus for one-third of a lucerne-grass pasture system gave a 23% increase in daily weight gains in lambs.

It has been suggested that a disadvantage of birdsfoot trefoil as a forage plant is its high lignin content which affects its animal digestibility (John and Lancashire, 1981). However, Henson and Schoth (1962) found that although livestock found lotus herbage somewhat less acceptable than other common legumes, they readily consumed it once they became accustomed to it.

Depending on pasture type (e.g. climate, soil fertility and moisture conditions, etc.) and management methods, the performance of birdsfoot trefoil as a pasture plant differs greatly. Generally, it is not as productive as clovers and lucerne in many situations. However, there are plenty of situations in which birdsfoot trefoil has outyielded these legumes where the environment is less favourable for the good performance of legume species requiring high fertility and moisture such as white and red clovers and lucerne (Hunt and Wagner, 1963; Taylor et al., 1973; Lambert et al., 1974). MacDonald (1946) has also shown that birdsfoot trefoil produced comparable amounts of herbage both for hay and for grazing in a series of comparative experiments involving clovers and lucerne. Similarly, Charlton's work (1971) in Scotland has shown that in both spaced and sward situations most of the birdsfoot trefoil cultivars tested performed satisfactorily compared with white and red clovers under

the same experimental conditons. Suckling (1960) compared birdsfoot trefoil with five white clovers, two red clovers and several other legumes which were all commonly used in pastures in New Zealand. The results showed that birdsfoot trefoil outyielded all species except red clover.

The inferior production by birdsfoot trefoil compared to other legumes under favorable environmental conditions is largely attributed to its slow establishment and therefore less competitive nature. Although the herbage yields produced in established lotus stands can be higher than that produced by other legumes at the same stage, its slow early growth often decreases the chances of its playing an important role in a mixed pasture with more aggressive species. (MacDonald, 1946; Davis and Bell, 1957; Peacock and Wilsie, 1960; Suckling, 1960; Parson and Davis, 1961; Scholl and Brunk, 1962; Buxton and Wedin, 1970; Taylor et al., 1973; Greenwood and Sheath, 1981). Davies' experiment at Aberystwyth (1969) also showed that although the growth of lotus was comparable with clovers, poor establishment behaviour hindered its possible role as a promising plant for hill land pastures in Wales.

It is essential to choose the most suitable management methods for birdsfoot trefoil based pastures. Since birdsfoot trefoil is sensitive to continuous grazing, this system leaves less chance for recovery growth and will eventually eliminate the species from a pasture. Davis and Bell (1957) and Van Keuren et al., (1969) noted that birdsfoot trefoil could be retained in a pasture and could produce satisfactorily with a rotational grazing system of about two weeks grazing and four weeks rest. Henson and Schoth (1962)

considered that the use of a continuous grazing system should only be recommended for cattle grazing on semierect 'Empire' types of birdsfoot trefoil, and by maintaining a continuously high residue. Davis and Bell (1957), Smith and Nelson (1967), Van Keuren and Davis (1968) and Van Keuren et al. (1969) have all highlighted the positive persistence and productivity responses of lotus not subjected to continuous grazing.

Mixtures of birdsfoot trefoil with non-aggressive grass species have shown better performance than birdsfoot trefoil alone. MacDonald's experiments (1946) clearly shown this in pastures used both for hay and for grazing. He considered that the most suitable companion grasses were those that produced an open sward such as Phleum pratense, Dactylis glomerata and Poa pratensis. Parsons and Davis (1961) found that a late rather than an early season D. glomerata cultivar was less competitive and hence more compatible with lotus. Under strong competition birdsfoot trefoil was eventually eliminated from pasture (Chevrette et al., 1960).

Depending on the growth habit involved, the response to defoliation differs between varieties of birdsfoot trefoil. Semi-erect 'Empire' types are more persistent and will produce better under intensive defoliation than the erect 'Viking' type. The former are therefore recommended for grazing, while the latter type is encouraged for hay production (Seaney, 1975).

Despite the extensive overseas use of birdsfoot trefoil for hay, it has not been generally recommended for this purpose in New Zealand. Scott and Charlton (1983) stated that 'there is little doubt that

other species, particularly lucerne, will generally be more
productive for hay production in New Zealand. Thus birdsfoot trefoil
is likely to have a role as a grazing species in the less fertile
drier regions, as does Maku lotus in the less fertile wetter regions'

Lotus corniculatus is becoming more important in New Zealand
agriculture as a forage legume for use under conditions where soil
fertility is moderate to low, particularly in dry situations
(Charlton et al., 1978; Scott and Charlton, 1983).

Seasonal production of Lotus corniculatus suggests its major role is
as a feed supply for late season production. As mentioned earlier,
Lotus corniculatus is less productive during the early growing season
(spring). However, its later season growth, particularly under dry
conditions which often occurs in the New Zealand summer, overyields
other legumes. The herbage quality of this later production is also
better than in some other legumes such as Grasslands Maku lotus
(Lotus pedunculatus Cav. syn. Lotus uliginosus Schk). Charlton (1971)
found that digistibility of birdsfoot trefoil was significantly
higher than that of Lotus pedunculatus, both in the vegetative and
flowering stages of growth. Scott and Charlton (1983) mentioned that
the later stemmy growth of Lotus corniculatus was eaten more readily
than that of lucerne.

Among the other advantages of birdsfoot trefoil as a pasture plant is
its persistence, a character which has been recognized for some time.
In Canada, Chevrette et al., (1960) found that birdsfoot trefoil was
more persistant than lucerne in long term leys on imperfectly drained
soils. It also persisted longer than Ladino or alsike clovers on the

two soil type on which these crops were compared. Scott and Charlton (1983) stated that birdsfoot trefoil was the most persistent legume and often the only remaining productive legume when grazing is lax, and after fertility had been depleted. In an evaluation trial involving clovers, lucerne, lotus and other commonly used legumes, Scott (1985) showed that lotus species were superior to other species tested in terms of persistence, with <u>Lotus corniculatus</u> being superior to other lotus species.

Birdsfoot trefoil has often been referred to as a drought resistant poor land plant (Charlton <u>et al</u>., 1978; Greenwood and Sheath, 1981; Scott and Charlton, 1983). Its response to soil moisture stress in the aspects of growth, reproduction, and mortality was better than white clover, but not as good as lucerne (Foulds, 1978).

While most reports claim that birdsfoot trefoil is drought resistant (Charlton et al., 1978; Scott and Charlton, 1983), there are some reports stating that it will also perform satisfactorily on imperfectly drained soils (Robinson, 1934; Chevrette <u>et al</u>., 1960). Morton (1981) reported birdsfoot trefoil tolerated acid and poorly drained Pakihi coastal soils better than white clover ⁻ .. These features will assist birdsfoot trefoil gain a recognized place among commonly used legumes.

Birdsfoot trefoil has usually been considered as a poor-land forage crop because it performs better in such situations than other legumes. However, it is erroneous to conclude that birdsfoot trefoil does not respond to better soil conditions. Various reports have shown that with liming and fertilization, the production of birdsfoot

trefoil could be increased significantly (Varney, 1958; Hughes, 1962; Hunt and Wagner, 1963).

1.3. Description of the plant

1.3.1. Below ground parts

The root system of birdsfoot trefoil consists primarily of a strong taproot with numerous branches. The long, tapering and usually forked primary root is surmounted by a dense crown from which the shoot system emerges. Depending on the growth stage, the dimensions of the root system vary, but a fully developed root system could well exceed 1 metre in depth and 0.75 metre in width (MacDonald, 1946). This is a much larger root system than that occurs in red clover. The root system of birdsfoot trefoil is not as deep as that of lucerne, but is more extensive in distribution. Considering that roots are the main point of water uptake, the differences in the root systems of the three legumes may explain why birdsfoot trefoil and lucerne are more resistant to water stress than clover (Charlton et al., 1978; Faulds, 1978; Scott and Charlton, 1983).

A major difference between Lotus corniculatus and Lotus pedunculatus is that the former seldom possesses rhizomes while the latter is frequently rhizomatous (Sheath, 1981). Despite this, Wasson and Barnett (1971) observed that the old woody stems of birdsfoot trefoil are capable of producing some rhizomatous shoots, although in most common situations the plant is non-rhizomatous.

1.3.2. Above-ground parts

There are considerable variations in the above-ground morphology of Lotus corniculatus, in terms of size, shape and growth habit, all of which are influenced by genotype and environment. The stem of birdsfoot trefoil is usually green and square in cross section in its upper regions despite being round at the base. Under optimal environmental conditions, stems may reach 1 metre in length (MacDonald, 1946).

Most morphological variations occur in the growth habit and branching behaviour. The stems may be prostrate, ascending, or erect. According to MacDonald (1946), in the more prostrate type the stems arising from the seedling, or later from the buds on the crown of the established plant, lie on the ground without reaching any considerable height. In general, the internodes of this plant are short, with some elongation towards the middle or near the apex of the stem during the season of most rapid growth. Based on their growth habit, two distinct types of birdsfoot trefoil were recognised (Seaney and Henson, 1970), --- the erect 'European' type (cultivar 'Viking' as a typical representative) and semierect 'Empire' type. The 'European' type is considered more suitable for use as hay because of its upright growth habit and less persistence and productivity under grazing pressure. The semierect 'Empire' type is hardy, late maturing, with many branched leafy stems and is considered more suitable for grazing (Seaney, 1975). However, it should be borne in mind that the growth habit of the shoots varies widely among the numerous cultivars of birdsfoot trefoil, which include many intermediate forms in terms of shoot morphology.

Apart from variations in genotype, environmental factors (particularly photoperiod) greatly influence the growth habit of birdsfoot trefoil. Rhykerd et al., (1959b) reported that in short days or low light intensities the leaf:stem ratio tends to be lower than in plants grown under long days. McKee (1962) stated that plants grown under short days had a prostrate, rosette type of growth compared to the more erect form of plants grown under long days. In a series of studies using controlled environmental conditions for variety identifications, Nittler and Kenny (1965) found that plants of the 'Viking' type were easily distinguished from plants of the 'Empire' type by their response to photoperiod in terms of shoot length and number.

The branching of birdsfoot trefoil also varies greatly. MacDonald (1946) showed that this plant was dominated by the shoots arising from the crown area. Although many small lateral branches appeared from leaf axils of the crown shoots, these lateral branches were much smaller than the main shoots. Similarly, the plants in Hughes and Heath's paper (1949) also show that birdsfoot trefoil is comprised mainly of stems from the crown and very few small lateral structures from leaf axils. Smith (1962), Nelson and Smith (1968a, b) found that in early spring there was a basal flush of shoots available for growth but that subsequent regrowth occurred from axillary buds on residual stubble. They mentioned that branches arose mainly from the upper axillary region of the shoots rather than the basal or lower branches.

The leaves of birdsfoot trefoil are borne alternately along opposite sides of the stem. Each leaf consists of 5 leaflets, 3 attached to the terminal end of the petiole and 2 at the base which could be easily mistaken for stipules (Heyn, 1976). The true stipules in birdsfoot trefoil are small projections at the base of the leaf. Leaflets are typically obovate, although the shape may vary from rounded to oblanceolate between varieties (MacDonald, 1946; Seaney and Henson, 1970).

1.4. Reproductive structures

1.4.1. Flowers

The inflorescence of birdsfoot trefoil is a typical umbel consisting of varying numbers (usually 4-8) of florets attached by short pedicels to a long peduncle. Each floret consists of a calyx with 5 united sepals and a typical papilionaceous legume corolla with 5 petals. The reproductive parts of the flower are typical of the structures in the Leguminosae with 9 fused plus one separate stamens as male structures and one simple pistil as female structure (MacDonald, 1946; Seaney and Henson, 1970). Petal colour may vary from a light to dark yellow, and may be tinged with faint orange or red stripes (Jones and Crowford, 1977; Abbott, 1981). Colour at the keel tip can be yellow, brown,or red at different stages (Buzzell and Wilsie, 1963).

1.4.2. Pods

Several pods are borne at almost right angles to the tip of the peduncle, giving the appearance of a bird's foot. Pods are long, cylindrical, and brown to almost black at maturity. After pollination, pods develop rapidly and reach maximum size in about three weeks, but it takes another 1-3 weeks, depending on weather conditions, for the pods to mature (Anderson, 1955; Wiggans et al., 1956; Winch, 1958; Seaney and Henson, 1970; Beuselinck and McGraw, 1988). Winch (1958) mentioned average pod dimensions in the cultivar Viking of 25 mm (length) by 3 mm (diameter). Birdsfoot trefoil has 20-70 ovules per ovary, the average number being about 45 (Giles, 1949; Bubar, 1958). However, only about 40% of these ovules develop into mature seeds. Average pod number per inflorescence varies considerably in different environmental conditions and in different flowering seasons. The number of pods per inflorescence could be as low as 2 in the late flowering season (Beuselinck and McGraw, 1988). Seeds are attached to the ventral suture of the pod (Seaney and Henson, 1970). Pods readily dehisce at maturity, creating severe problems during seed production and harvesting in this species (Kelly, 1988). In a study of the morphological factors associated with pod dehiscence, Buckovic (1952) suggested that the differential loss of moisture from exocarp and mesocarp tissue resulted in tension between fibre layers which causes separation and twisting of the two valves of the pod. Pods dehisce at 40-60% of their original moisture content. Metcalfe et al. (1957) indicated that relative humidity indirectly influenced pod dehiscence by equilibrating moisture content of the pod with low levels of humidity. Around 10% moisture content, or 30% relative humidity seemed to be the 'trigger' for pod

dehiscence. Rapid drying, resulting in rapid loss of moisture, encourages a high incidence of dehiscence, while pods which are dried slowly do not dehisce even though the percentage of pod moisture is low (Buckovic, 1952).

1.4.3. Seeds

The seeds of birdsfoot trefoil are small. Measurements quoted by Seaney and Henson (1970) showed the average dimensions of seed of 'Viking' to be 1.4 mm long 1.2 mm wide, and 0.9 mm thick. 1000-seed weights vary considerably in different reports, ranging from 0.92g to 1.57g (MacDonald, 1946; Robinson, 1949; Anderson, 1955; Albrechtsen et al., 1966; Beuselinck and McGraw, 1988). There is also a considerable variation in seed colour in birdsfoot trefoil, ranging from olive green, to brown, and sometimes almost black (Seaney and Henson, 1970).

Hard seeds are present in a large percentage in mature seeds, especially when the crop is hand-harvested. In New York, Brown (1955) found that hand harvested seed contained up to 90% hard seed, while the amount of hard seed fell to about 40% when seed was machine harvested. Percentage of hard seed is also influenced by handling methods after harvest. MacDonald (1946) reported that a crop cut and cured in the field for a period of 10 days had an average germination of only 7%. However, in a similar area, seeds obtained from a crop cut at the same time, wilted, cooked over night, stacked for 18 hours to sweat, and then dried, gave an average germination of 81%. It is not clear whether the first treatment promoted hard seed development, or the second treatment softened hard seed. In the natural habitat

Taylor et al. (1973) reported that 45-54% of Lotus corniculatus seeds occurring on or near the soil surface were hard seeds.

1.5. Growth and development of the plant

1.5.1. Vegetative growth

The most suitable temperature for birdsfoot trefoil seed germination is about 20^0C. Germination is delayed below 15^0C, and both delayed and reduced by temperatures above 30^0C (Woods and MacDonald, 1971). Best field emergence is achieved when seeds are placed in a firm, smooth soil bed, at a depth of 0.5-1.0 cm (Stickler and Wassom, 1963). Field emergence of birdsfoot trefoil is also affected by soil moisture conditions, germination being delayed by a soil moisture stress of 4.5 atm and completely inhibited by a soil moisture stress of 11 atm (Woods and MacDonald, 1965,1971).

The seedling growth rate of birdsfoot trefoil is slow compared with other commonly used forage legumes such as lucerne and red clover (McKee, 1962; Smith, 1962; Nelson and Smith, 1968a, b, 1969; Buxton and Wedin, 1970; Seaney, 1975; McKersie and Tomes, 1982). This factor can directly affect its slow establishment and hence subsequent lack of competitive ability in a mixed pasture situation. Studies comparing the establishment of birdsfoot trefoil with or without a companion crop demonstrate that companion crops are often detrimental to seedling growth and persistance (Winch, 1958; Chevrette et al., 1960; Scholl and Brunk, 1962; Wakefield and Skaland, 1965). However, in practice, mixture with less aggressive grasses is recommended for

better performance of the pasture or for higher seed yields (MacDonald, 1946; Anderson and Metcalfe, 1957).

The regrowth of birdsfoot trefoil is also low compared with lucerne and red clover (Smith, 1962; Smith and Nelson, 1967). This feature is possibly related to its lower root reserves. Unlike lucerne in which there is at least one root reserve accumulation period during the most vigourous growing season, lotus maintains a very low root reserve level throughout the growing season until the approach of the cold season (Smith, 1962; Heichel et al., 1985). The low root reserves force the plant to rely on current photosynthesis from residual green tissues for recovery growth. This explains the plant's slow recovery growth rate and its low tolerance to heavy grazing (Smith and Soberalske, 1975).

Despite the slow establishment and slow recovery growth of birdsfoot trefoil, its late season growth rate is in many cases as good as, or even better than, other common forage legumes such as white clover and lucerne (MacDonald, 1946; Davis and Bell, 1957; Suckling, 1960; Davies, 1969; Charlton, 1973; Greenwood and Sheath, 1981).

1.5.2. Reproductive growth

Reproductive growth commences in response to daylengths exceeding 14 hours in early summer (Joffe, 1958; McKee, 1962, 1963; Nittler and Kenny, 1964, 1965). Flowering occurs first at the lower nodes (or lower lateral branches), then progresses up the stem on branches as the stem continues to elongate from the tip. Flowering and seed

setting lasts from early summer until late summer or early autumn when unfavourably shortening photoperiods prevent further flower production. After pollination, pods develop rapidly during the first 20 days, then continue their development at a slower rate till the pod maturity stage is reached at about 30 days after pollination (Anderson, 1955).

1.5.3. Factors influencing growth and development

1.5.3.1. Environmental factors

Among the environmental factors, photoperiod, temperature and soil moisture status are the most commonly used indicators of the adaptation and agronomic value of a plant to specific situations.

Birdsfoot trefoil is a typical quantitative longday plant with a minimum 14-14.5 hours daylength requirement and saturation at about 16 hours daylength for flowering. Joffe's experiment (1958) showed the quantitative response of this plant to photoperiod in that birdsfoot trefoil required 16 or more hours day length for maximum flowering response. At a day length of 15 hours, the number, rate and degree of floral primodium development was restricted. At 14 hours, blooming was sparse. McKee (1963) confirmed Joffe's findings by showing that 14-14.5 hours daylength is a critical daylength for the plant to flower, but flowering is profuse only at 16 hours or longer daylengths. By 1964, the response of birdsfoot trefoil to daylength had been well recognized so that Nitter and Kenny (1964, 1965) were able to use it as an aid for species identification.

Birdsfoot trefoil is relatively insensitive to temperature for flowering. By using different combinations of temperature and photoperiod, Joffe (1958), Nitter and Kenny (1964) and Long et al.(1989) have shown that either constant temperatures (13-30^{0}C) or alternating temperature regimes (27/13^{0}C-30/20^{0}C, day/night) had little effect on plant flowering.

1.5.3.2. Internal factors

The internal factors governing a plant's growth and development mainly involve the plant's assimilation capacity and the allocation of assimilate to different parts.

Studies on assimilation characters of birdsfoot trefoil have been carried out by a number of researchers including Rhykerd et al. (1959a, b), Shibles and MacDonald (1962), Smith (1962), Cooper (1966, 1967), Smith and Nelson (1967), Nelson and Smith (1968a, b, 1969), and Greub and Wedin (1971a, b). Its assimilation rate was reported to be comparable with that of other commonly used legumes such as white clover and lucerne. In general, birdsfoot trefoil has a lower photosynthetic rate than lucerne, however a higher NAR (Net Assimilation Rate) achieved from a higher carbon dioxide fixation rate in lucerne was often offset by a larger leaf area ratio which resulted in a higher RGR (Relative Growth Rate) in birdsfoot trefoil. The assimilate partitioning between roots and aboveground parts in birdsfoot trefoil was studied in detail by Smith (1962), Smith and Nelson (1967), and Nelson and Smith (1968a, b, 1969). Carbohydrate reserves in roots follow a cyclic pattern of utilization and storage, root reserves being very low after the initial spring growth and

during the entire growing season until cool season conditions cause growth to decease. During the growing season, the root reserves represent only about 15% of the total available carbohydrate of the plant. In a more recent study, Heichel et al. (1985) stated that in birdsfoot trefoil, less than 10% of assimilate was distributed into the roots and crown. Since the regrowth of the plants after defoliation depends either on root reserves or on the assimilation ability of the residual green parts of the plant, the low partitioning of assimilate to root may be a partial explanation of the reason why birdsfoot trefoil has a slow recovery growth after cutting or heavy grazing.

The partitioning of assimilate among the aboveground parts of the plant was carefully examined by McGraw and Beuselinck (1983) and McGraw et al., (1986). It was found that the stems, including leaves, account for most of the dry matter (DM) accumulation throughout the period from late vegetative growth until pod dehiscence. The seed at the pod dehiscence stage accounts for only 4% - 10% of the distribution of aerial DM. Other elements, such as phosphorus and nitrogen, were found to be present in higher proportions in the seed. An important point made in the paper by McGraw et al. (1986) was that during seed filling, the fractional accumulation of DM and various elements remained relatively stable in stems, but markedly decreased in leaves. Such results indicate that seed development in birdsfoot trefoil relies more on the current supply of assimilate from leaves than on the redistribution of the food reserves from vegetative stocks as occurs in some other plants such as maize (Bunting, 1975; Pain, 1978).

Floral abortion is a commonly recorded phenomenon in <u>Lotus</u> <u>corniculatus</u>, particularly at the flower bud stage (Giles, 1949; Bubar, 1958; Joffe, 1958; Seaney and Henson, 1970; Stephenson, 1984; Stephenson and Winsor, 1985). Incomplete self-incompatibility and self-sterility are major causes of abortion in this species (Silow, 1931; Seaney, 1964; Dobrofsky and Grant, 1980a,b). However, even when each flower is outcrossed a large proportion of florets still produce aborted pods (Stephenson, 1984). Joffe (1958) investigated the effects of possible causal factors, including boron treatment, photoperiod, and temperature, on the abortion of the flower buds. None of the factors tested were found to satisfactorily explain the cause of bud abortion. In a more recent study Stephenson (1984) considered that the availability of maternal resource (assimilate supply) played a key role in regulating the abortion of inflorescences.

Abortion has also been reported to occur frequently at other stages of reproductive development. Seaney and Henson (1970) in their review of seed production of <u>Lotus corniculatus</u> mentioned that only 40% of the 20-70 ovules in an ovary develop into mature seeds. Stephenson (1984) reported that only one of every three flowers produced a mature fruit and that three of every five which initiated fruits aborted. He also dismissed the possibility of abortion being caused by pollination failure using controlled pollination experiments. He suggested that it was a lack of assimilate supply, rather than a pollination failure which limited the reproductive output in this species.

1.6. Seed production

1.6.1. Pollination

Lotus corniculatus is primarily cross-pollinated (Tome and Johnson, 1945), but crosses between florets of an individual plant were reported to be as effective as crosses between plants (MacDonald, 1946). Insects of the Hymenoptera, particularly the large bumblebee, are considered to be 'the only effective pollinators' (Knuth, 1908). Bader and Anderson (1962b) found that pollen-collecting bees are more efficient than nectar-collectors. The number of seeds set per pod increases with the number of visits to the floret by the bee and the time spent by each bee at the floret (Morse, 1956). Kubisova-Kropalova and Nedbalova (1978) found that the pollination rate by honey bees is high, with 3-6 flowers pollinated per bee per minute. Morse (1956) found that a population of one bee per 0.9 M^{-2} was sufficient for all flowers to be pollinated.

The self-sterile character of birdsfoot trefoil has been known for a long time. As far back as 1931 it was found found that in the absence of insects only 8.7-30.3 seeds were observed per 100 flowers (Silow, 1931). Even when flowers were artificially self-pollinated, the seed yield only increased to a maximum of 416 seeds per 100 flowers which is less than one-tenth the seeds formed as a result of cross-pollination. Seaney (1964) obtained similar indications of self-incompatibility, observing only rare seed formation in self-pollinated flowers. The exact mechanism of self-incompatibility in birdsfoot trefoil is still unclear at the present time, although many researchers have identified that both morphological and

physiological barriers are involved (Tome and Johnson, 1945; Giles, 1949; Brandenburg, 1961; Seaney, 1962; Miri and Bubar, 1965).

1.6.2, Seed development

Detailed studies on seed development have been carried out by Anderson (1955), Wiggans et al. (1956), Winch (1958) and Long et al. (1989). After pollination, pods develop rapidly during the early growth stage and reached maximum length in about three weeks. Another one week or so was needed for the pods to attain morphological maturity when the colour of the pod changes from green to light brown, and finally dark brown. Seeds attained high germinability when the colour of the pods is still light brown. The rate of pod development is also influenced by weather conditions. Anderson (1955), in Iowa, found that 24-47 days were needed for the formation of mature seeds from pollination whilst Winch (1958), in New York, found that the same stage of development required 26-38 days. It is worth noting the finding by Wiggans et al. (1956) that seeds mature 7-10 days before pod dehiscence, indicating a time allowance for harvesting seed without significant losses caused by pod dehiscence.

1.6.3, Seed yield and yield components

Seed yield in a legume is comprised of a series of yield components which in turn are determined by a combination of plant and environmental factors. Adams (1975) described a general yield component structure for legumes involving pods/plant, seeds/pod and weight/seed. In Lotus, because of the special character of the inflorecence, most researchers emphasise the number of inflorecences

as a more important yield component then the number of pods. The inflorescences (umbels)/plant, pods/inflorescence, seeds/pod and weight/seed are commonly quoted as the main yield components in this species (Albrechtsen et al, 1966; Drogomir, 1981; Mos, 1983; Stephenson, 1984; McGraw et al., 1986). Among these four yield components, number of inflorecences is the most flexible yield component, being strongly influenced by environmental and genetic factors. The response of these yield components to plant population density showed a declining order of importance from inflorescence number, to pod number and seed number. Seed weight was the least sensitive component to plant population density (Pankiw et al., 1977; McGraw et al., 1986).

Seed yields in Lotus corniculatus vary greatly in different situations. In Seaney and Henson's review (1970) 50-175kg per hectare was the commonly harvested commercial seed yield. Highest yields of 200-575kg can only be obtained occasionally. With more appropriate management techniques and more understanding of the plant, the seed yield in this species has the potential to be greatly increased. Seed yields of 308-603 kg/ha were reported in experimental trials in United States of America and in Canada (Pankiw et al., 1977; McGraw, 1981). Seaney (1975) estimated that under optimum conditions in North America, potential seed yields of Lotus corniculatus ranged from 500 to 1000kg per hectare, while Turkington and Franko (1980) in Canada quoted a potential seed yield of 675-1125 kg per hectare. However, because of the problems of indeterminate flowering behaviour and pod dehiscence, actual harvested yields are often much lower than potential yield. It should be particularly pointed out that, despite the relatively long history of seed production in this species, high

yields are still not consistently and reliably obtained. Year-to-year variations in seed set and harvest recovery of seeds often cause wide differences in average seed yields and very low yields (below 50kg/ha) can be commonly encountered (Anderson and Metcalfe, 1957).

In New Zealand there are no published papers containing information on seed yields of Lotus corniculatus. Most yield data have come from personal communication with seed farmers and concerned scientists. Seed yields have been around 200-350kg/ha in farmer situations and about 500kg/ha in research trials. Yearly variation are common, mainly due to unsettled weather conditions (Personal communication, Hare).

1.6.4, Main obstacles for seed production and seed yield

There appear to be three major obstacles which severely limit seed production in birdsfoot trefoil. Firstly, its indeterminate flowering habit results in plants flowering over an extended period (McGraw and Beuselinck, 1983, Kelly, 1988). During flowering young flower buds, blooming flowers, young pods, and mature pods ready to dehisce can be present simultaneously on an individual plant. This makes it extremely difficult to determine the correct time to harvest the crop for the recovery of maximum seed yield. Secondly, the pod dehiscent nature of this plant worsens the situation of seed production in birdsfoot trefoil, particularly in dry environments, because of the heavy loss of seed from shattering at harvesting (Buckovic, 1952; Metcalfe et al., 1957). It is practically impossible to recover all potential seeds because a certain portion of seeds is unavoidably lost either due to many immature pods being harvested or due to seeds

being lost through pod dehiscence. Thirdly, the assimilate partitioning rate to reproductive growth is very low in birdsfoot trefoil. At its highest point, which is during the seed filling stage, only about 10% of assimilate is translocated to seeds (McGraw and Beuselinck, 1983; McGraw et al., 1986). This character obviously is not favour for high production of seed.

1.6.5, Management for seed production

Due to its slow establishment and non-aggressive growth, establishment of a satisfactory lotus plant population is of prime importance in order to achieve high seed yields. Seed fields should be carefully prepared before seed is sown (Stickler and Wasson, 1963), and after seedling emergence attention should be particularly paid to the need for strict weed control (MacDodald, 1946; Parsons and Davis, 1964; Scholl and Brunk, 1962; Wyse and McGraw, 1987).

The subsequent management strategy after successful establishment and weed control in birdsfoot trefoil mainly aims at overcoming the main obstacles to seed production, i.e., the extended flowering period and pod dehiscence. In order to obtain a more uniform and contracted flowering pattern, several cutting or clipping treatments have been investigated (Anderson and Metcalfe, 1957; Bader and Anderson, 1962a; Winch, 1958). However, none of these have been successful in achieving a higher seed yield compared with uncut controls in all circumstances. Winch's work (1958) in New York showed that while early clipping of plants delayed seed maturity and evened the time of maturity of the crop, it did not necessarily result in higher seed yields. Later clipping in his study significantly lowered seed yields

compared with uncut plants. Similar results were obtained by
Anderson and Metcalfe (1957) and by Bader and Anderson (1962a), who
showed that clipping at any time during the spring and early summer
resulted in decreased seed yields, caused by the removal of early
growth. Seaney (1975) considered that clipping could be used to vary
timing of seed harvest, but pointed out that the practice should not
be generally recommended or used by seed growers, presumbly because
of the likelihood of reduced seed yields.

Regarding the problem of pod dehiscence, frequent observation of
crops and precise harvest timing are highly recommended (Seaney
1975). Anderson (1955) reported that for maximum seed yield, the best
time to harvest was when most pods are light green to light brown.
MacDonald and Winch (1957) suggested that harvest should begin when
70-80% of the pods are light brown to brown in colour. Any delay in
cutting crops after pods are completely mature results in significant
seed loss from pod dehiscence and seed shattering (Anderson, 1955;
Winch, 1958). The practice of carefully observing pod colour changes
and cutting the crop in the early morning when the relative humidity
is high is recommended in a similar crop, e.g., Lotus pedunculatus
(Grasslands Maku Lotus) and has proved successful in reducing the pod
dehiscence problem in this species (Lancashire and Gomez, 1980; Hare
and Lucas, 1984).

With increasingly wide use of chemicals in agricultural practice,
there are also several options of using plant growth regulators (or
growth regulator-like chemicals) to manipulate the structure of the
plant for improved seed production. Chemicals such as Alar
(Daminozide), CCC (Chlormequat) and PP333 (Paclobutrazol) have been

successfully used in seed production of several pasture species. These chemicals have been shown to improve seed yields directly by increasing flower numbers and potential seed yields or indirectly by altering fertile shoot (tiller) production and flowering pattern to facilitate recovery of maximum seed yield (Hampton and Hebblethwaite, 1985a,b; Marshall and Hides, 1986,1987). The use of chemicals such as herbicides, insecticides and pre-harvesting desiccants have been shown to be beneficial in enhancing birdsfoot trefoil seed production. However, the use of plant growth regulatory chemicals is still not fully explored and the few results obtained are still in some doubt and cannot be used to make general recommendations for improving seed production in this plant. For example, Clifford and Hare (1987) claimed that growth regulators such as Alar and PP333 greatly increase seed yield of Lotus pedunculatus in New Zealand, whilst White et al (1987) in U.S.A., who screened 12 chemicals in the greenhouse tests and applied 4 of them including PP333 in field trials, found that none of them were promising for use in enhancing birdsfoot trefoil seed production.

It is common that birdsfoot is grown alone for better seed production, because of its low competivity with other species. However, there was report that growing birdsfoot trefoil with a companion grass could improve seed yield by preventing lodging, which substantially increased recoverable seed yield (Anderson and Metcalfe, 1957).

Seeding rate, and subsequently plant population density, appeared to have little effect on seed yield in birdsfoot trefoil. McGraw and Beuselinck (1987) demonstrated that a wide range of seeding rates

from 0.6 to 9kg/ha (established plant population densities from 17 to 109 plants m^{-2}) resulted in similar seed yields in four years of a five year experiment. Only in the first year did the lowest seeding rate (0.6kg/ha or 17 plants m^{-2}) outyield the highest seeding rate (9kg/ha or 109 plants m^{-2}). The experiment showed that although first year crop exhibited a wide range of plant population densities as a result of different sowing rates, plant densities in following years tended to be evened, because some plants in the high population plots died due to severe inter plant competition while plant numbers in the low population plots increased due to reseeding from seeds shed in previous years. They further recommended use of a low seeding rate for seed production as long as a 15-20 plants m^{-2} population density could be achieved.

Plants grown in wide rows (50cm row spacing) were shown to be capable of producing more seed than plants grown in narrow rows (15cm row spacing) (Bratu et al., 1981). However, work by Pankiw et al. (1977) showed that high seeding rate and close row spacing had the advantage of enabling the crop to supress weeds from emergence and growth which could severely reduce seed yield of Lotus corniculatus. Work by McGraw et al. (1986) showed that the optimum plant population density required for seed production was lower than that required for forage production in birdsfoot trefoil, a plant population density of 19 plants per square metre was able to achieve 90% of the highest seed yield level whilst 26.5 plants per square meter was required to reach the same level of forage yield.

Among other common agronomic practices, insect and weed control, trace element application, and the use of chemical defoliants for

easy harvesting (and thereby reducing seed loss) have all proved to be beneficial for seed production in birdsfoot trefoil if used properly (Wiggans et al., 1956; MacCollom, 1958; Ridgway and Gyrisco, 1959; Bader and Anderson, 1962a; Panait et al., 1979; Wyse and McGraw, 1987).

CHAPTER 2 : CONTRIBUTION OF VEGETATIVE GROWTH TO REPRODUCTIVE GROWTH

2.1. Introduction

Birdsfoot trefoil has an indeterminate flowering habit which often results in a long and sporadic flowering pattern. This, together with its pod dehiscent behaviour, makes it difficult to select the optimum time for seed harvesting (Seaney and Henson, 1970). New flowers, young pods and ripe pods are often present simultaneously on plants at harvest. As a result, a considerable portion of the crop's potential seed yield tends to be lost, either due to high levels of immature seed or pod dehiscence at harvest (Seaney and Henson, 1970; McGraw et al, 1986). To improve the harvestable seed yield in Lotus corniculatus, knowledge of the cause or causes of the protracted flowering pattern is needed. While the protracted flowering of this plant has been mentioned by previous researchers (MacDonald, 1946; Joffe, 1958; Seaney and Henson, 1970; McGraw and Beuselinck, 1983; McGraw et al., 1986), there do not appear to be any detailed studies on this aspect of its agronomic performance.

In an indeterminate plant, a long flowering period may be caused by any one of a number of factors, including: (1), the sequential development of different shoot orders (i.e., main shoots vs. lateral branches); (2), the delayed development of flowers along stems in which there are several vegetative nodes between successive flowers (such as in white clover) (Thomas, 1987) and (3), the sequential development of shoots originating at different times which may also be responsible for creating a long flowering period. In this age

hierarchy, early shoots are often the first to flower because of their earliness of formation (and often larger size) whilst flowering in late formed shoots tends to be delayed, resulting in a long total flowering duration. Apart from the effects of any one of these causes, it is also possible that the extended flowering period may be caused by a combination of some or all of these factors. The present study examined the relative contribution of different shoot age groups, in terms of both numbers and reproductive capacity, to the inflorescence population in Lotus corniculatus.

2.2. Materials and methods

The Lotus corniculatus seed used in this study was an unreleased selection (code number S2078), supplied by the Grasslands Division of the Department of Scientific and Industrial Research (DSIR). The experiment was carried out from May 1986 to February 1987 at Massey University, Palmerston North, New Zealand (40^0S, 175^0E). Seeds were sown in 15 cms row directly into a cultivated field of Tokomaru silt Loam (aeric fragiaqualf, gleyed yellow-grey earth) loam on 26 March at a seeding rate equivalent to 1.7kg/ha and emerged about one month later. A basal application of 350kg/ha of 30% potassic superphosphate was applied immediately prior to sowing. The plant population density was 44 plants m^{-2} at the end of the experiment (mid February, 1987).

Plant growth was measured by the monthly dry matter (DM) increase of plant above-ground parts from 20 randomly sampled plants during the experimental period. Leaf status was assessed by counting the number of leaves per shoot and by measuring leaf size and proportion of different sized leaves on shoots bearing 10 to 20 nodes on 2 December

1986. The reason for choosing shoots of this size was that most flowering shoots were in this category and because a shoot stopped increasing node numbers and internode extensions when it flowered.

Starting on 10 May, 27 plants were randomly selected from three 35 m^2 plots. All new shoots formed in each month were identified by tagging with coloured plastic rings to allow later examination of shoot population composition and flowering behaviour in these different aged shoot groups (Plate 2.1). A new shoot was tagged when it had at least one fully unfolded leaf. Shoot age in this paper refers to the month in which the shoot was tagged (i.e., a shoot tagged in September is called a September shoot). Shoot population composition was examined on 15 November (pre-flowering stage), 15 December 1986 (pre-peak flowering stage) and 15 January 1987 (post-peak flowering). At each time, the number of shoots in different age groups was recorded.

The number of inflorescences in full bloom produced by groups of different aged shoots was recorded every 5 days (with the exception of first two countings) during the reproductive stage from 28 November 1986 to 21 February 1987 and the total number of inflorescences per shoot was counted when the shoot ceased to produce more flowers. It was observed that 2-4 days were needed for a new inflorescence (an inflorescence with only one yellow floret open) to reach full bloom (all florets open) and another 2-4 days were needed for these florets to wither. Therefore, a 5-day inflorescence counting interval avoided repeat or missed countings. In order to investigate flower-carrying capacity in different shoot age groups, the position of the first inflorescence to form, the number of

inflorescences per shoot and the number of florets per inflorescence were also recorded.

Numbers of inflorescences were also recorded on a unit area basis. All inflorescences in seven one-square meter permanent quadrats were recorded at five day intervals as described above, from the beginning of November 1986 to mid February 1987.

A further 30 plants were tagged monthly from 20 May for the measurement of shoot growth and development in terms of increase in internode length and node number (Plate 2.2). Monthly development of node numbers was recorded by examining the number of nodes between two different coloured rings tagged on successive months. Monthly growth in internode length was determined by measuring the length between two successive rings more than one month after tagging with the second ring.

2.3. Results

2.3.1. Shoot system

Birdsfoot trefoil has an aerial shoot system which mainly comprises of shoots arising directly from the crown (primary shoots) and a few shoots arising from nodes immediately above the crown on the primary shoots. These two types of shoots behave similarly in terms of size, growth and flowering (Plate 2.3) so that they were termed as main or crown shoots in this study. The middle section of each shoot usually bears no branches. Although a few tiny lateral branches are occasionally observed immediately below the first inflorescence,

Plate 2.1. <u>Lotus corniculatus</u> plant showing tagging
to identify months of shoot production

Plate 2.2. <u>Lotus corniculatus</u> plant showing monthly
tagging method for assessing changes in
node number and shoot length

these lateral shoots behave rather like peduncles as they bear only one or two leaves. They are extremely small compared with the main shoots, although they are still capable of occassionally producing inflorescences. Late formed shoots bore no lateral structures. The 'typical' plant from this experiment illustrated in Plate 2.3 clearly shows that in Lotus corniculatus there are no lateral structures which substantially influence flowering pattern, and that the lengthy flowering pattern is determined almost entirely by the behaviour of the main shoot population.

Observation from a clipping trial also supports this situation (Table 2.1). Twelve plants were clipped on 7 October. Two months after clipping, the components of the shoot population were examined. Immediately after clipping a flush of small lateral shoots emerged from most of the nodes on the clipped main shoots. However, these lateral structures stopped further development, and gradually wilted and died. Despite some intra plant variation, the results clearly show the importance of main shoots compared with lateral branches, with 80% of shoots being main shoots from the crown and only 20% being small lateral branches on 15 December. This result further reflects the minimal involvement of lateral branches as important contributors to the flowering pattern.

Table 2.1: Effects of Clipping on Shoot Development

	No. shoots Clipped (7 October)	No. of newly emerged shoots on:			
		15 November		15 December	
		main	lateral	main	lateral
Total shoots	84	99	67	118	31
Shoots per Plant	7.0	8.3	5.6	9.8	2.6
S.E.	0.44	0.89	2.0	1.2	0.78

Lotus corniculatus L.

Plate 2.3. <u>Lotus corniculatus</u> L. plant showing:
(1), shoot system mainly comprises shoots
from the crown area and immediately above
the crown
2), flower buds, blooming flowers, young
pods, mature pods and shattered pods
existing on the plant simultaneously

2.3.2. Plant general growth

The above-ground growth of birdsfoot trefoil follows a typical sigmoid curve in which vigorous vegetative growth starts about mid August when temperature rises and lasts until mid January of the next year when weather conditions became unfavorable for plant growth (Figure 2.1 \ Partial growth of different plant parts shows that ₍and Appendix 2). shoot growth is the main contributor to the increase of DM (particularly during the late growing season). Reproductive structures (including young flowers, green pods and mature pods) represent less than 10% of the aerial DM until very late in the season.

It was observed that flowering actually terminated the vegetative development of individual shoots. When a shoot bore inflorescences, its apex gradually stopped further development. The shoot tip turned yellowish, wilted and subsequently died. This character is more like the nature of a determinate flowering pattern. However, Lotus corniculatus could not be classified as being a determinate plant since new shoots continue to emerge and flower throughout the reproductive season. More importantly from the point of view of morphogenesis, flower buds initiate in the leaf axils instead of at the terminal shoot apex. Such a character botanically defines Lotus corniculatus as an indeterminate flowering plant.
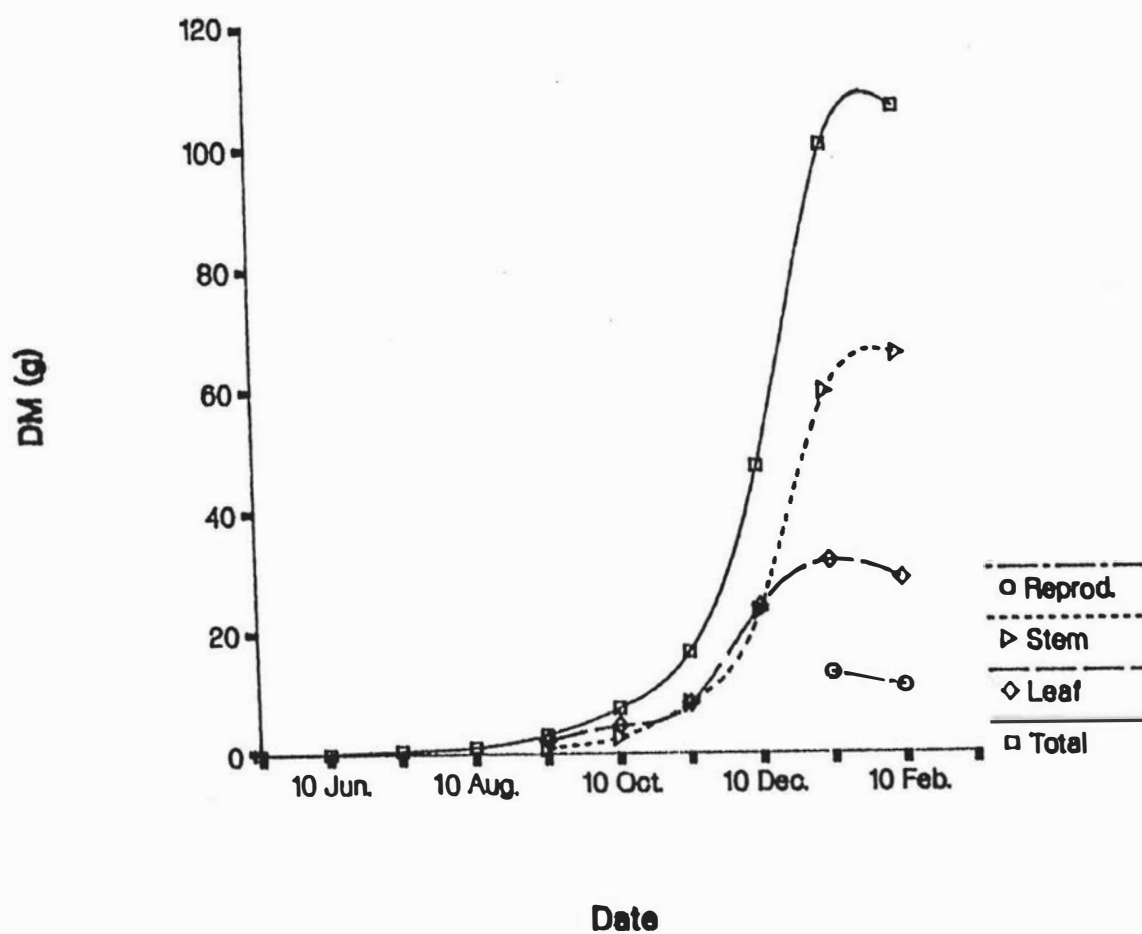
Figure 2.1. General plant growth showing above-ground
dry matter changes with time

2.3.3. Shoot dynamics and tissue turnover

The development of the plant occurred in a 'continuous replacement' manner. Old shoots tended to die soon after new shoots emerged and grew vigorously (Tables 2.2 and 2.3). Shoot growth rate (measured by increments in node numbers and internode length) was greatly affected by shoot age, as shown by the fact that in the same month, new shoots grew faster than old ones. Weather conditions at different times also affected shoot growth rate, which was much greater in the summer than in the winter and spring. The final size of shoots, as measured by total node number and final shoot length, was not solely determined by shoot age. New shoots had a faster growth rate, and often eventually became larger (longer) than older shoots even though the latter may have had a longer growth duration. The life span of shoots from different age groups also varied. Early shoots (tagged in July and August) often disappeared from the shoot population due to their slow growth and subsequent replacement by new shoots. Late shoots (tagged in December and January) had fewer nodes and shorter internodes at the final measurement (20 February) because these two shoot age groups (particularly January shoots) were still in an active growing stage and had not reached their final determinant size (Tables 2.2 and 2.3).

The 'continuous replacement' of older shoots by newer shoots resulted in the shoot population at most times being mainly composed of 'young' shoots (Table 2.4). At the first observation (15 November), all May and June shoots had already died. The number of older surviving shoots declined both in number and as a percentage of the total shoot population at different observation times, with new

Table 2.2: Node development in different shoot age groups

SHOOT AGE GROUP	NUMBER OF NODES DEVELOPED IN THE PERIODS OF:								TOTAL NO.OF NODES
	20/6-20/7	21/7-20/8	21/8-20/9	21/9-20/10	21/10-20/11	21/11-20/12	21/12-20/1	21/1-20/2	
JUN. SHOOT	1.6	1.8	3.4	3.0	1.6	0	-	-	11.4
JUL. SHOOT	-	2.0	3.6	4.4	1.4	0	-	-	11.4
AUG. SHOOT	-	-	3.8	5.0	3.7	3.8	1.2	0	17.5
SEP. SHOOT	-	-	-	5.9	6.3	7.6	0.9	0	20.7
OCT. SHOOT	-	-	-	-	7.8	8.5	1.9	0	18.2
NOV. SHOOT	-	-	-	-	-	11.3	4.3	0	15.6
DEC. SHOOT	-	-	-	-	-	-	9.2	2.7	11.9
JAN. SHOOT	-	-	-	-	-	-	-	5.2	5.2

N.B. 1) 20/6, 21/7,etc.represent 20 June, 21 July, etc. respectively.
2) Dec. and Jan. shoots were still increasing in node number at final observation date (20 February).
3) -: No data available because shoots had not been formed or had subsequently died.

Table 2.3: Monthly increase in internode length
in different shoot age groups (cm)

SHOOT AGE GROUP	INTERNODE LENGTH EXTENSION IN THE PERIODS OF:						FINAL LENGTH (cm)
	21/7-20/8	21/8-20/9	21/9-20/10	21/10-20/11	21/11-20/12	21/12-20/1	
JUL. SHOOT	0.76	0.90	1.1	0.50	0	-	10.3
AUG. SHOOT	-	0.93	1.44	1.57	1.58	0	22.54
SEP. SHOOT	-	-	1.28	2.42	2.63	0	42.79
OCT. SHOOT	-	-	-	2.34	3.05	1.28	46.61
NOV. SHOOT	-	-	-	-	2.11	1.73	31.28
DEC. SHOOT	-	-	-	-	-	1.70	15.64

N.B. 1) 21/7, 20/8,etc. represent 21 July,20 August, etc.
2) Nov. Dec. shoots were still increasing in internode length at final observation date (20 February).
3) final length was the sum of the products of internode length and node number in each month
4) -: Data not available because shoots had not been formed or had subsequently died.

shoots providing the greatest proportion of the shoot population. The three observation dates in Table 2.4 represent three different growth stages --- onset of flowering, pre-peak flowering and post-peak flowering, respectively. At the latter two stages (15 December and 15 January) the newest shoots (November and December) represented more than one third and nearly two thirds of the shoot population respectively. The oldest surviving shoots (July) had almost disappeared, representing only 0.3% of the total shoot population. August shoots provided only 4.4% of the shoot population at the final observation on 15 January.

Leaf status in different sized shoots (representing different ages) bearing ten to twenty nodes showed that the number of leaves per shoot is not a 1:1 linear function of the number of nodes per shoot so that old shoots (shoots with large node numbers) did not necessarily have more leaves than younger ones (Figure 2.2). The death of old leaves on the lower region of a shoot particularly reduced the number of leaves on 'older' shoots, resulting in old shoots bearing similar number of leaves to those of more vigorous young shoots despite their greater number of nodes per shoot.

Because it was the old leaves at the lower section of a shoot that died, the proportions of different sized leaves on a shoot also remained similar in all shoots measured. The results in Table 2.5 clearly showed this situation in that the percentage of different sized leaves in shoots with different node numbers (from 10 to 20) remains not significantly different when checked using a Chi-Square examination.

Table 2.4: Shoot survival, number of shoots per plant and components
of the shoot population at different times

| | SHOOT AGE GROUPS | | | | | | | | SHOOTS |
	JUL.	AUG.	SEP.	OCT.	NOV.	DEC.	JAN.	TOTAL	/PLANT
NO. OF SHOOTS TAGGED	42	65	122	126	132	450	159	1096	-
ON 15 NOV.									
no. of shoots present	29	56	102	96	-	-	-	283	9.8
% survival	69	86	84	76	-	-	-	-	-
% in total shoots population	10.3	19.8	36.0	33.9	-	-	-	100	-
ON 15 DEC.									
no. of shoots present	14	38	68	84	103	-	-	307	11.4
% survival	33	59	58	67	78	-	-	-	-
% in total shoots population	4.6	12.4	22.0	27.4	33.6	-	-	100	-
ON 15 JAN.									
no. of shoots present	2	30	56	74	83	438	-	683	25.3
% survival	4.8	46	46	59	63	97	-	-	-
% in total shoots population	0.3	4.4	8.2	10.8	12.2	64.1	-	100	-

N.B. -; data not available because shoots had not been formed.

Table 2.5: Numbers and percentages of different sized leaves
on different sized shoots

| Leaf size- | Shoot size (no. of nodes per shoot) | | | | | | Average (%) |
	10-13 nodes (6 shoots)		14-17 nodes (8 shoots)		18-20 nodes (8 shoots)		
Small leaf	4	11%	16	17%	11	15%	14%
Medium leaf	22	56%	58	60%	45	60%	57%
Large leaf	13	33%	32	33%	19	25%	29%
Total	39	100%	96	100%	75	100%	100%

N.B.: Leaves were grouped into small, medium and large
leaves according to their leaf areas
Leaf areas; small leaf = 0.441 cm^2/leaf
medium leaf = 0.757 cm^2/leaf
large leaf = 1.115 cm^2/leaf
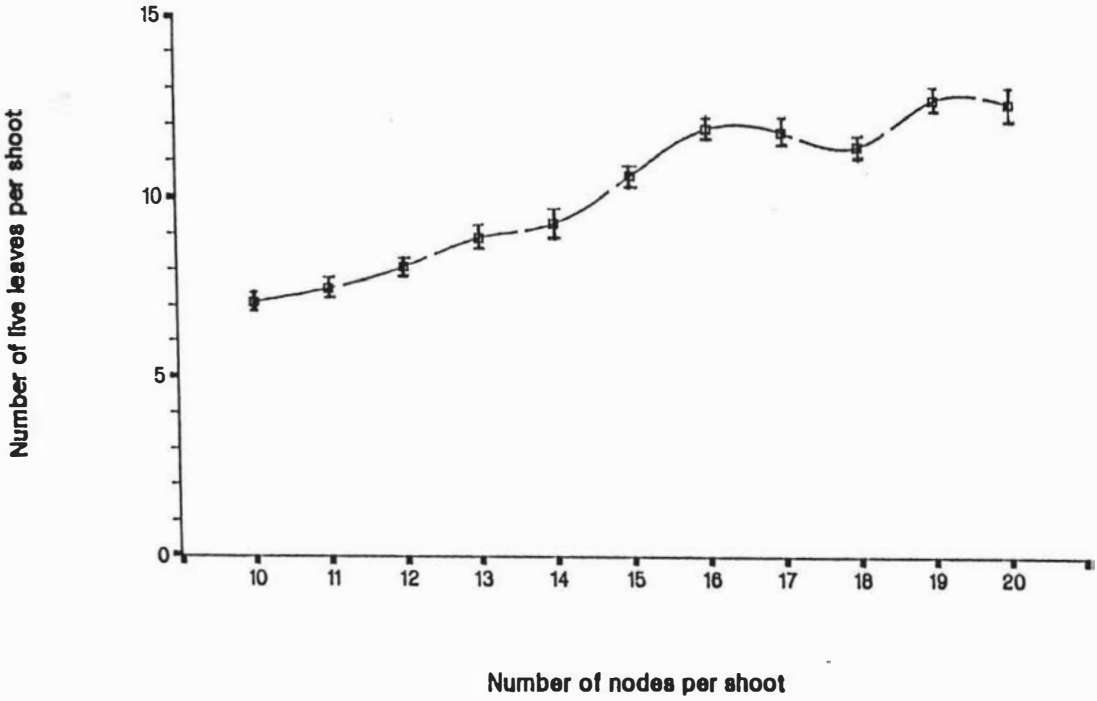
Figure 2.2. Relationship between leaf numbers and node numbers per shoot

(Vertical bars are standard errors of means)

2.3.4. Flower-carrying ability of different shoot age groups

Older shoots bear flowers at higher nodes while younger shoots commence flowering at lower nodes (Table 2.6). This can be attributed to the effects of both shoot age and shoot size, as well as environmental conditions for flowering. (Appendices 1 and 2). Early shoots, initiated before the climate becomes favorable for flowering, have already developed to a certain size (in terms of node numbers) before becoming fertile. Late shoots, originating under favorable flower-induction conditions, become fertile while still comparatively young so bear fewer nodes at flowering. Nevertheless, the number of flowers produced per shoot does not vary greatly in different shoot age groups until December or January. Similarly, the number of florets per inflorescence shows relatively small changes in all shoot age groups except for January shoots where a decline in floret number occurred, presumably because of the onset of unfavorable weather conditions for flower initiation.

Table 2.6: First flower appearance position and relative flower bearing ability of shoots from different age groups

SHOOT GROUPS	AUG. SHOOT	SEP. SHOOT	OCT. SHOOT	NOV. SHOOT	DEC. SHOOT	JAN. SHOOT	L.S.D. (0.05)
first flowering node	−	20th	16th	14th	10th	9th	1.85
no. of inflorescences per shoot	3.1	3.4	3.2	3.2	2.6	2.0	1.6
no. of florets per inflorescence	−	5.7	5.9	5.9	5.2	4.4	1.3
no. of shoots per plant (x) [1]	1.1	2.1	2.7	3.1	16.2	5.8	−
no. of inflorescences per plant (y) [2]	2.7	4.6	12.0	10.3	25.9	6.9	−
regression equation		$y = 92.6 + 1.35x$			$r = 0.83$		

N.B.   1, average of 27 tagged plants on 15 January, 1987.
       2, average of 27 tagged plants, total inflorescences produced.

Although shoots produced an average of about three inflorescences (Table 2.6), a fourth and even a fifth inflorescence is occasionally formed. It was observed that the floret numbers produced by inflorescences at differnt positions along a shoot was fairly consistent, only the last inflorescence (the fourth or fifth inflorescence from the bottom) showing reduced floret numbers (Table 2.7).

Table 2.7: Number of florets per inflorescence at different positions along a shoot

Inflor. position	First inflor.	Second inflor.	Third inflor.	Fourth inflor.	Fifth inflor.
Means	5.95	6.25	5.95	5.50	4.75
S.E.	0.17	0.19	0.34	0.36	0.53

Early inflorescences bear more florets than late inflorescences. Within the same shoot age group there is a tendency for early inflorescences to produce more florets than late inflorescences. However, this situation is not the case between shoot age groups, since at the same date of observation, inflorescences in early shoot age groups did not neccessarily bear more florets than inflorescences in late shoot age groups (Table 2.8).

Table 2.8: Number of florets per inflorescence in different shoot age groups at different times

Observation date	Aug. shoot	Sep. shoot	Oct. shoot	Nov. shoot	Dec. shoot	Jan. shoot
13 Dec.	6.0	6.4	6.0	-	-	-
28 Dec.	5.6	5.3	5.8	5.9	5.5	-
12 Jan.	-	-	-	-	4.8	4.9
27 Jan.	-	-	-	-	4.0	4.0
L.S.D.(0.05)	0.14	0.36	0.15	0.16	0.16	0.27

N.B. -: data not available because shoots were infertile or had already ceased flowering at time of obversation.

2.3.5. Contribution of different shoot groups to flowering pattern

The number of inflorescences changed dramatically with time, showing a sharp flowering peak on 2 January and a smaller subsequent peak about 3-4 weeks later (Figure 2.3). The total flowering period extended over a period of approximately three months from the end of November to the end of February. Flowering patterns obtained by counts of inflorescence numbers on tagged plants and in quadrats are almost identical except for a difference in the magnitude of the two curves. This implies that the tagging operation did not disrupt the plant's 'normal' flowering pattern. The total flowering period can be conveniently divided into three parts to describe the contributions made by early, intermediate and late flowers. Flowers formed during a period of about 20 days (from 18 December to 7 January) contributed over 70% of the total inflorescence population.

Early (pre 18 December) and later (post 7 January) flowers together contributed less than 30% of the flower population. This shows that although birdsfoot trefoil has a very protracted flowering period, most inflorescence numbers are concentrated into a comparatively short period of 3-4 weeks.

All shoots from different age groups (August to February) are important in their contribution to the long flowering period (Figure 2.4). Although flowering in each of the seven shoot age groups did not last long (often less than one month), the cumulative effect of
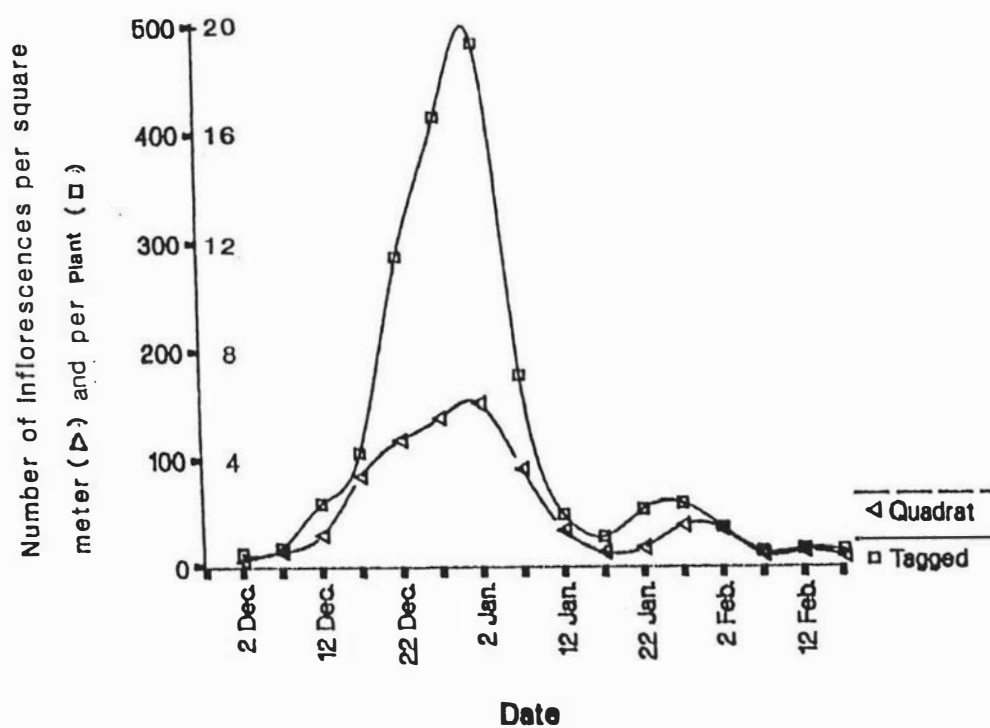
Figure 2.3. Flowering pattern on tagged plants and on plants in quadrats
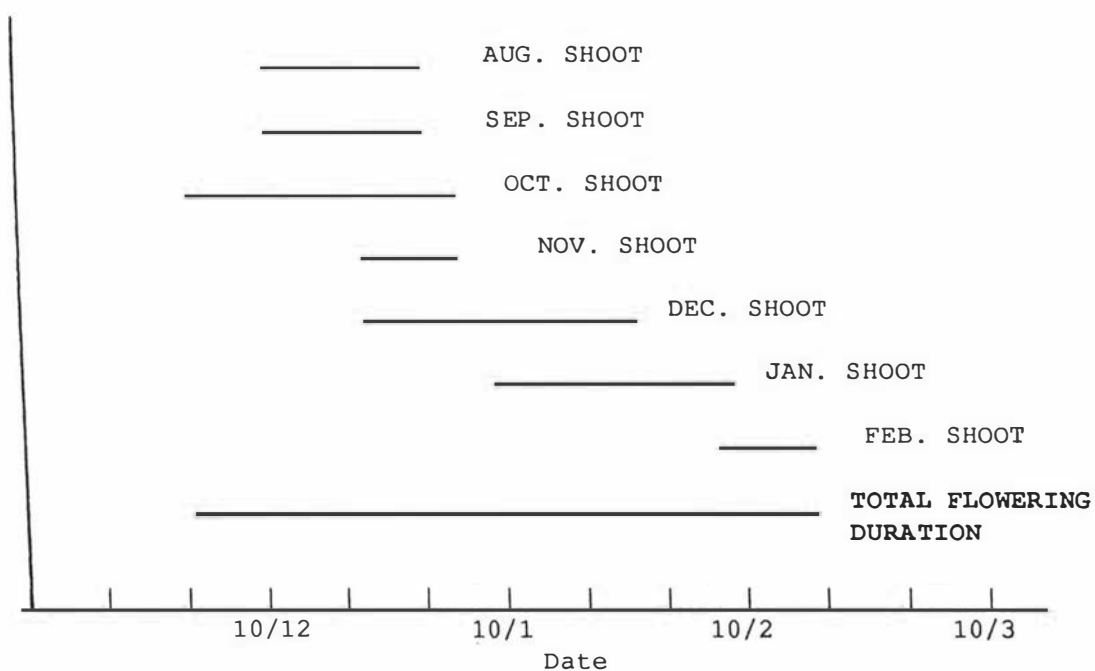
FIGURE 2.4: Flowering duration in each shoot age group and
total flowering duration of the plants, showing an
accumulative effect of different shoot age groups to
the lengthy flowering period of Lotus corniculatus.

all flowering shoots resulted in a lengthy total flowering period. However, shoots from different age groups varied greatly in their contributions to the flower populations (Table 2.9). Although all seven shoot age groups (August to February) made some contribution to the flowering pattern, only three groups were major contributors to total flower number. October, November and December shoots contributed more than 75% of the total inflorescence population, and these shoots contributed nearly 90% of the total inflorescences present during a period of approximately 25 days of most intense flowering from 20 December to 11 January. August, September, January and February shoots made relatively minor contributions to flower numbers, but were responsible for greatly extending the total flowering period.

Table 2.9. Contribution of different shoot age groups to total inflorescence population

Date	Month of Shoot Origin							Sum	%
	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.		
29 Nov.	0	1	5	0	0	0	0	6	0
13 Dec.	16	17	23	2	0	0	0	58	3%
23 Dec.	28	44	121	86	8	0	0	279	18%
28 Dec.	14	37	84	103	177	0	0	315	20%
2 Jan.	11	22	80	81	289	0	0	483	31%
7 Jan.	4	4	11	6	140	13	0	178	11%
12 Jan.	0	0	0	0	33	16	0	49	3%
17 Jan.	0	0	0	0	10	18	0	28	2%
22 Jan.	0	0	0	0	13	41	0	54	3%
27 Jan.	0	0	0	0	16	43	0	59	4%
1 Feb	0	0	0	0	8	29	0	37	2%
6 Feb.	0	0	0	0	2	13	0	15	1%
11 Feb.	0	0	0	0	2	8	7	17	1%
16 Feb.	0	0	0	0	1	4	11	16	1%
21 Feb.	0	0	0	0	0	2	8	10	0
Total	73	125	324	278	699	187	26	1584	
%	4%	7%	19%	16%	41%	11%	2%		100%

N.B. data from 27 tagged plants

2.4. Discussion

The description of plant structure in this study has emphasized the overwhelming dominance of main shoots arising directly from the crown, and to a lesser extent the smaller number of shoots arising from nodes immediately above the crown, as contributors to reproductive growth. This effect is reasonably similar to that pictured by MacDonald (1946) although he did show more small, but non-flowering lateral branches arising further up main shoots. MacDonald's description (1946) and the structure shown in Plate 2.2 of this study both support the suggestion that lateral structures make no substantial contribution to the long flowering period. The plant structure found in this study contrasts with the description by Nelson and Smith (1968a,b) in which they mentioned that branches arose mainly from the upper axilliary regions.

The present results on dry matter (DM) distribution between reproductive growth and vegetative growth of the aerial parts of the plant agree with results by previous researchers (McGraw and Beuselinck, 1983; McGraw et al., 1986). These workers emphasised low assimilate partitioning to reproductive growth as one important factor limiting the seed production of Lotus corniculatus. Although the slow down of DM accumulation in vegetative parts was accompanied by the commencement of reproductive growth, it is unlikely that there is a preferential translocation of assimilate from vegetative growth to reproductive growth, as shown by the fact that both vegetative and reproductive growth slowed down during the final stage of plant growth. It is likely that such a slowdown is mainly caused by slower assimilation rate caused by the onset of unfavourable environmental

conditions at the end of the growing season. In addition, the accumulation of assimilate in roots late in the season may be another factor reducing DM accumulation in the above-ground partS. This explanation is supported by results of Smith (1962), Nelson and Smith (1968b, 1969) and Heichel et al. (1985) who have reported that late autumn was the only season in which birdsfoot trefoil actively accumulated root reserves.

The present study has highlighted the lengthy (three months) flowering period in Lotus corniculatus L. Experiments conducted by Joffe (1958) and Mckee (1963) have shown that Lotus corniculatus is a quantitative longday plant, requiring a 14-14.5 hour minimum light period for flowering. In Palmerston North, the period from the beginning of November to the beginning of February meets this daylength requirement for flower-induction (Gerlach, 1974). This suggested flower-induction period agrees well with the observed flowering pattern shown in Figure 2.3 where flowering began in mid November and finished in mid February.

The death of old shoots when new shoots emerged results in a continuous replacement shoot succession. Under favorable flowering induction weather conditions new shoots become reproductive very quickly after emergence. Each shoot age group is therefore responsible for a particular part of the total flowering pattern. Older (early emerged) shoots contribute to the early part of the flowering sequence while new replacement shoots are responsible for supporting later flowering. Although flowering duration in each shoot age group is relatively short (often less than one month), the cumulative effect of all flowering shoots is sufficient to support an

extended flowering period. Because this is a continuous process, the flowering period in <u>Lotus corniculatus</u> L. appears to last as long as climatic conditions permit. The results on plant flowering pattern show that more than 70% of inflorescences appear within a period of less than one month, resulting in a contracted peak of flowering within a protracted total flowering period. Although seven monthly shoot age groups contribute to the total flowering period, only three (October, November and December shoots) are substantial contributors to the total flower population. The profuse flowering of shoots from these three monthly groups reinforces the importance of shoots formed at these times as direct contributors to seed yield in this plant. The low inflorescence numbers during the early stages of flowering simply reflect the lack of shoots available at that time. The subsequent onset of unfavorable light conditions (less than 14 hours photoperiod) in early February diminishes flowering potential, resulting in low inflorescence numbers in late formed shoot populations (January and February), despite the fact that there are plenty of shoots available for flowering during that time.

The relative flower bearing ability in terms of number of inflorescences per shoot and number of florets per inflorescence in different shoot age groups does not vary greatly compared with the extreme variation in the number of inflorescences produced within different shoot age groups. The similarity of leaf status in shoots of different size (representing different age) is strong support for the similarity of flower bearing ability in different shoot age groups. It is important to note that, regardless of the difference in shoot age, most flowering shoots bear a similar leaf number per shoot. If it is considered that the reproductive growth of birdsfoot

trefoil is mainly supported by current photosynthesis instead of drawing reserves from existing vegetative stalk (McGraw et al., 1986), the similar flower bearing ability in all flowering shoots may be well explained by the fact that similar leaf numbers are borne on all flowering shoots. The decline in flower bearing ability on late shoots (December and particularly January) is likely to be influenced by the onset of suboptimum or inappropriate weather conditions, particularly daylength. However, the depression in the number of inflorescences in mid January and the decline in flower bearing ability in December shoots may be influenced by a short drought period in late December and early January during this study.

Virtually no sterile shoots were present during the period of most profuse flowering in this study, a result also reported by McKee (1963) and Nitter and Kenny (1964). It is therefore reasonable to suggest that the number of shoots represents the number of fertile shoots in each shoot age group. Table 2.6 also presents a regression equation of inflorescence number on shoot number. Despite the fact that January shoots had not yet fully expressed their flowering ability since such shoots were too young for flowering at the time of recording, the trend obtained from the regression clearly shows that the number of shoots present at flowering is the most important single factor determining the flowering pattern. The good fit of the regression of the number of inflorescences on the number of shoots (r=83.3%, Table 2.6) supports this point.

CHAPTER 3: REPRODUCTIVE DEVELOPMENT AND YIELD COMPONENTS STUDY

3.1. Introduction

Among the research on the seed production of birdsfoot trefoil, a number of studies have been conducted on its seed development sequence and seed yield components (Anderson, 1955; Winch, 1958; Albrechtsen et al., 1966; Mos, 1983; Stephenson, 1984 and McGraw et al., 1986). It is generally agreed that the number of inflorescences (or umbels) is the most important seed yield determinant in various situations (Albrechtsen, 1966; Mos, 1983; Stephenson, 1984 and McGraw et al., 1986). The number of pods per inflorescence in some situations also has a role to play in determining final seed yield (Stephenson, 1984). However, previous research does not include information on the changing pattern of each seed yield component during the extended flowering period of this plant. Since harvest timing is an important factor affecting the recovery of seeds from the crop (due to its indeterminate growth habit and pod shattering behaviour at maturity), it is pertinent to know the changing pattern of each yield component, and particularly, to clearly define the role of inflorescence numbers on ultimate seed yield. It was hoped that such a definition would assist in determining the most reliable index for deciding best harvest time. The primary objective of this study was to investigate the changing pattern of each seed yield component during the flowering of <u>Lotus corniculatus</u> L. and to identify the most important yield determinants.

3.2. Materials and Methods

The experiment was carried out in the same field area described in Chapter 2. Flower development was monitored by carefully observing the morphological and colour changes on tagged flowers. Colour photographs were also taken regularly during the observation period.

The first part of this study involved identifying inflorescences formed at different times by tagging their peduncles with different coloured plastic rings at 10 day intervals from 12 December 1986 to 31 January 1987. This tagged population of approximately 2,000 inflorescences was used to determine the time required for the development of flowers originating at different times and to examine the changing pattern of each seed yield component.

Inflorescence numbers were recorded at 5 day intervals within eight $1m^2$ permanent quadrats. The five day interval was decided after observing the morphological and colour changes in flowers and ensured that each inflorescence was not counted twice or missed.

Numbers of pods per inflorescence was recorded every 10 days from 20 randomly sampled umbels on tagged inflorescences. Number of seeds per pod was recorded from 100 pods sampled from tagged inflorescences also at 10-day intervals. Seed weight was recorded on a 1000-seed weight basis at seed moisture contents within the 6.8-7.5% range in air-dried seed lots which had been harvested at different times.

The second part of the study involved investigation of the seed development sequence, which was measured by assessing changes in seed

viability, germinability and hardseededness using samples collected at different times after flowering. On 18 January 1987, about 300 fully blooming flowers were tagged with fine plastic rings. From 18 days after tagging (days after flowering, DAF), 40 pods were collected every four days until 42 DAF from previously tagged inflorescences. This allowed seven samples of pods of different maturity levels (DAF) to be harvested. Seeds were removed from pods from half of each sample immediately after collection, and their germinability tested. Germination tests were carried out according to the International Seed Testing Association (ISTA) Rules (1985) with four replicates of 50 seeds. Viable seeds were referred to as the sum of normal seedlings, abnormal seedlings, fresh ungerminated seeds and hard seeds. The remaining pods from each sample were kept in paper envelopes under ambient conditions for air drying. On 17 March all air dried pods were threshed and the seeds were germinated. Seed moisture contents were measured according to the method prescribed in the International Seed Testing Association Rules (1985).

Numbers of visible floret primordia and number of florets per inflorescence were also recorded to assess the importance of reproductive abortion at different floret or pod development stages.

On 8 May 1987, seeds from hand harvested mature pods were tested for hard seed content and several methods were employed to examine their efficiency in breaking hardseededness. These methods included boiling water immersion for 10 or 20 minutes prior to germination test and seed coat chipping with a scalpel to ensure seedcoat rupture.

3.3. Results

3.3.1. Flower and pod development

Floral development was measured according to morphological and colour changes. It was observed that for an 'average' inflorescence with six florets, it took about ten days for the visible floral primordia to reach the anthesis, and another 2-4 days for all florets to complete blooming. Florets remained at this full bloom stage for only one or two days (the term 'flowering' used in this context refers to this stage) and then the petals gradually wilted. The colour of petals changed from bright yellow at blooming to dark-yellow at wilting. Plate 3.1 shows the changing floral sequence with day 0 as first floret blooming and day 3 as the floret wilting stage (inflorescences used in this study were tagged within these three days). Four or five days after flowering, young pods were visible emerging from the cover of the keel petals. Pods developed rapidly to a maximum size at about 20 days after flowering (DAF). Initial pod colour was green, gradually changing to purple green at about 20 DAF and finally to light brown at the ripe stage 32 DAF.

3.3.2. Changes in seed yield components

The changes in different yield components with time are presented in Figure 3.1. Inflorescence numbers showed a dramatically changing pattern throughout the three month flowering period. A sharp peak of flowering occurred at the beginning of January and the number of inflorescences then dropped rapidly. Number of pods per inflorescence remained reasonably constant during the peak flowering period, but
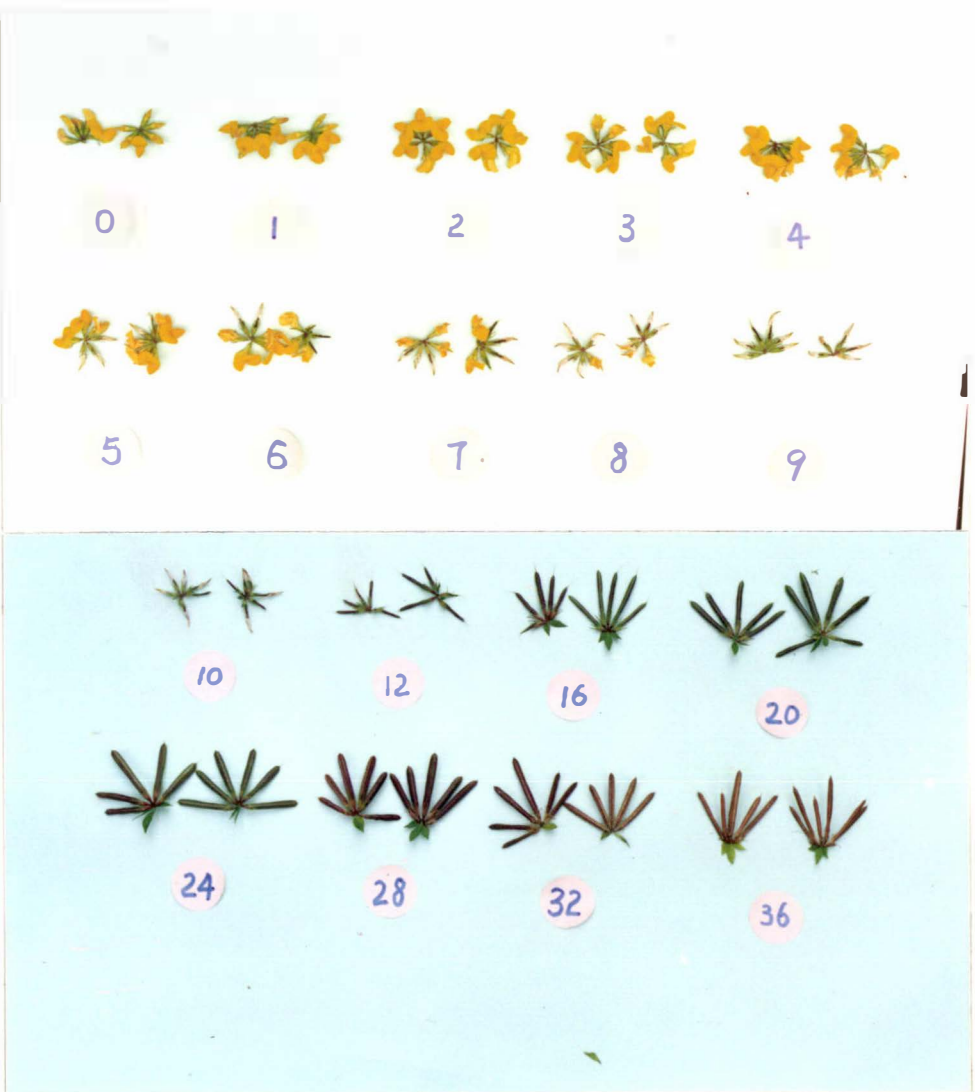
Plate 3.1. Flower and pod development sequence

fell by approximately 50% in the late flowering season (early February). This effect was also reflected in a similar pattern of change in floret numbers per inflorescence (Figure 3.2). Number of seeds per pod and 1000-seed weight remained relatively constant with only minor fluctuations during the entire flowering period. By regression analysis it was found that the number of inflorescences per unit area was the only single factor determining final seed yield (r=0.97 at p=0.01). The calculated final seed yield, based on the data in Figure 3.1 (mean seed moisture content 7.3%) was about 560kg/ha.

3.3.3. Abortion at different stages of flower development

Floral abortion was observed at different stages of plant reproductive growth (Plate 3.2(a-c)). Plate 3.2a shows unopened inflorescences with one or more small floret buds aborted. Plate 3.2b shows a later stage of abortion with one or more open florets aborted. Plate 3.2c shows nearly mature umbels with one or more pods aborted. Entire floral bud abortion and seed abortion in a pod were also occasionally observed in this study.

In order to compare abortion levels during different stages of floret and pod development, the number of visible floret buds, number of blooming florets, and number of pods per inflorescence (umbel) were recorded and compared (Figure 3.2). The base scale (time axis) was adjusted according to time differences during the developmental sequence of the three components (about 10 days from visible buds to blooming flowers and 20 days from blooming flowers to nearly mature pods). It can be seen that during the time from visible floral bud
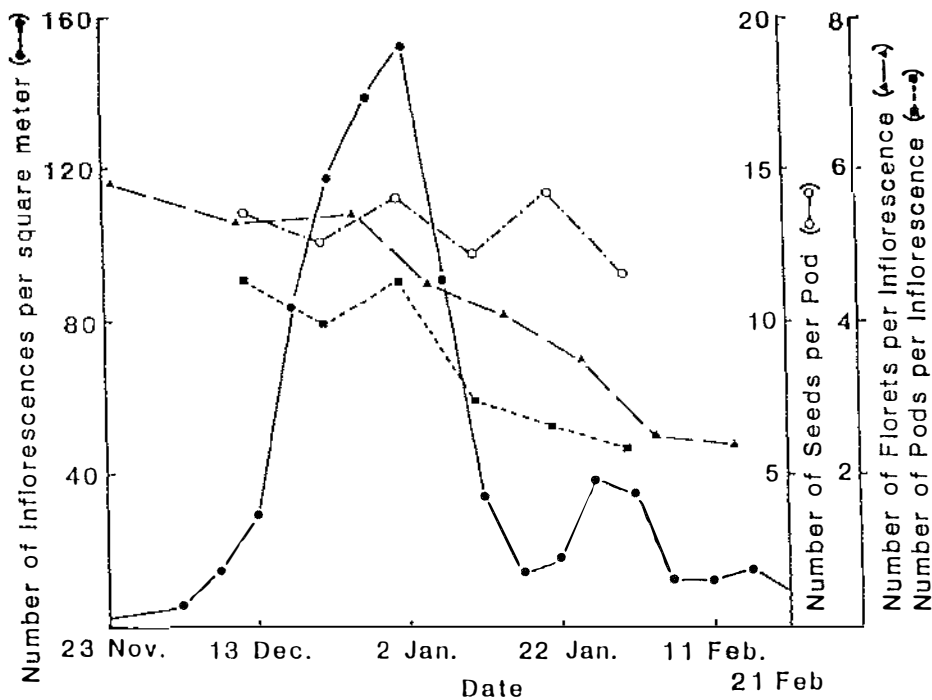
Figure 3.1: Changes in seed yield components
during the period of flowering
in _Lotus corniculatus_.
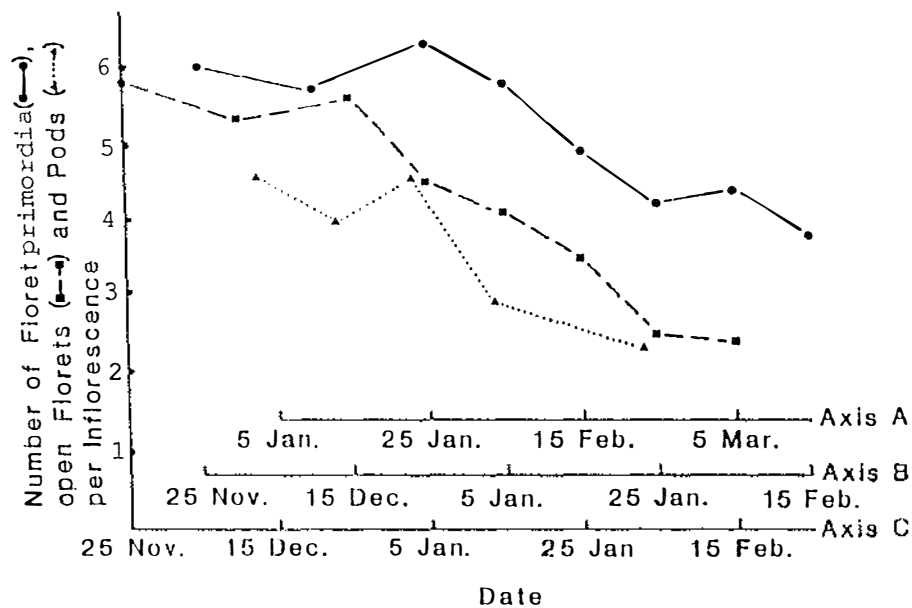
Figure 3.2: Number of flower buds, open
Florets and Pods per Inflorescences
in _Lotus corniculatus_ at different times.

N.B: Axes A,B,C, are used to show the time sequence of pod,
floret bud and open floret formation respectively.
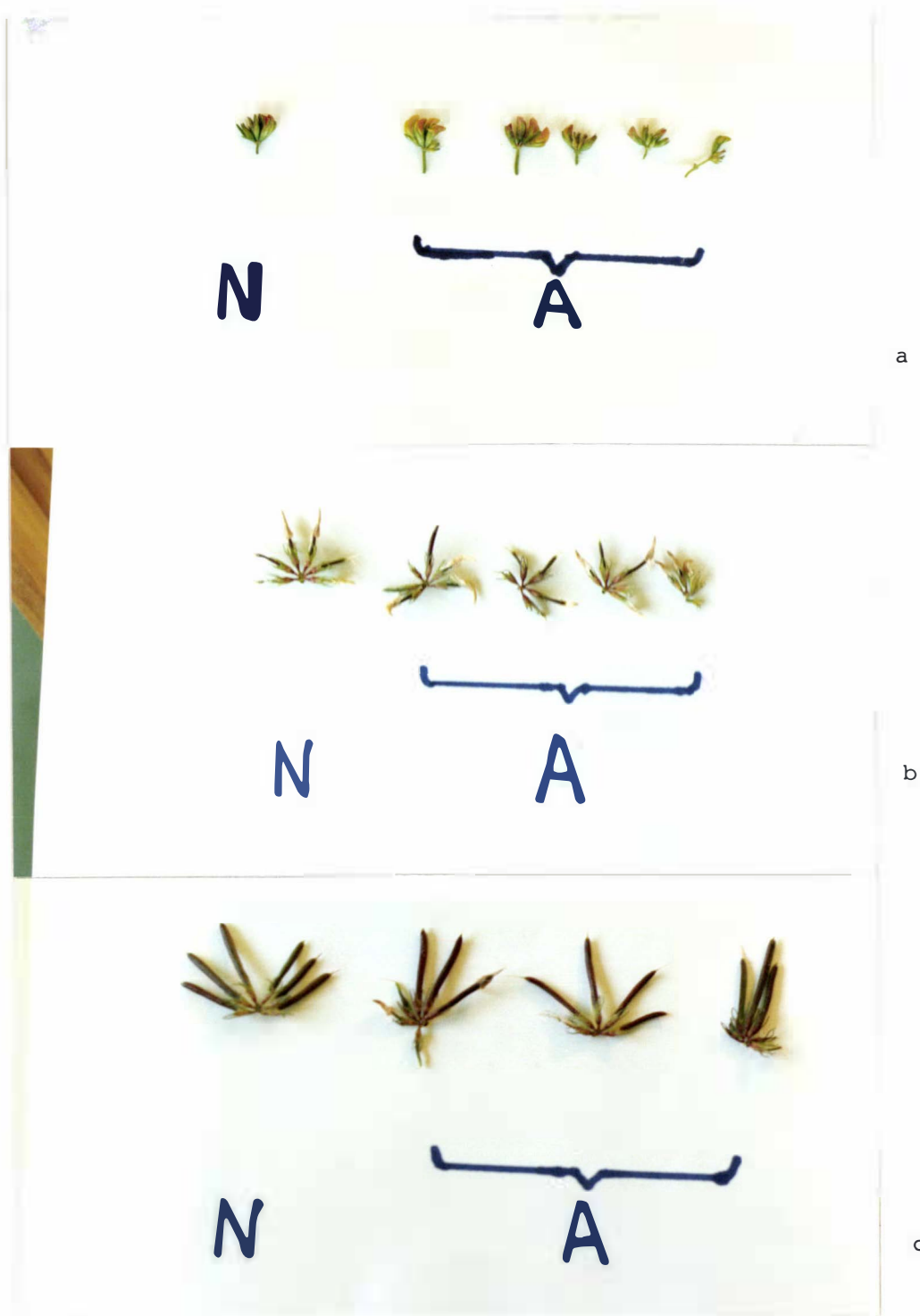
Plate 3.2. Reproductive abortion at different stages
[N: Normal inflorescenc; A: Abnormal
inflorescence with one or more florets
(pods) aborted]

formation (representing potential pod-bearing ability) to the production of nearly mature pods (representing the actual pod carrying ability) there was a relatively consistent one pod loss in yield potential regardless of time course. This effect is either due to the failure of floral buds to develop into blooming florets or to the failure of blooming florets to form pods. It is interesting that in the early flowering season, this one pod loss was mainly due to the abortion of floral buds while in the late flowering season the loss was mainly due to the failure of florets to form pods successfully.

3.3.4. Seed development and the quality of seeds harvested at different times

The time required for seed development was recorded for each group of tagged inflorescences. The results of laboratory germination tests on freshly harvested seeds are presented in Table 3.1A. By 18 DAF about half of the seeds had gained viability. The percentage of viable seeds increased steadily to a maximum of approximately 95% at 34 DAF. About half of the freshly harvested seeds of less than 30 DAF showed viability when tested for germination immediately after removal from the plant. However, these seeds did not retain this ability following air drying (Table 3.1B). After air drying, only seed lots which had been removed from plants more than 30 DAF were capable of retaining a high percentage of viability.

Dried seed began to retain viability approximately 22 DAF and reached maximum viability 12 days later. Germinability, as shown by the production of normal seedlings, commenced at 26 DAF and reached a

peak at about 30 DAF. As a result of increase in hard seeds,
germinability dropped from 34 DAF.

Table 3.1: Germination results in freshly harvested seeds
and seeds after air drying

| DAF | GERMINATION TEST RESULTS (%) | | | | | TOTAL |
	Normal seedling	Abnormal seedling	Hard seed	Fresh ungerm.	Dead seed	VIABLE SEED(%)
A, Freshly harvested seeds						
18	0	0	0	42	58	42
22	0	0	0	49	51	49
26	2	8	0	51	39	61
30	32	13	0	40	15	85
34	40	15	0	40	5	95
38	36	9	0	52	3	97
42	39	5	0	54	2	98
B, Seeds after air drying						
18	0	0	0	0	100	0
22	0	0	0	8	92	8
26	5	12	0	13	70	30
30	28	18	16	14	25	75
34	23	7	62	4	4	96
38	2	3	85	0	10	90
42	6	0	92	0	2	98

Seeds of early flowers usually required less time to reach maturity
(based on pod colour change) (Table 3.2). Seed quality, in terms of
viability and hardseededness, was similar among seeds produced at
different times (Table 3.2). The percentages of viable seed remained
high in all seed lots. Only the final two lots showed a small drop in
viability, but still retained acceptably high levels (95% viable
seeds). All seed lots contained a high percentage of hard seeds.
Since germination tests were conducted on the 26th of March,
approximately one month after the late seed lots were collected, the
lower levels of hard seeds in late lots may have reflected the effect
of incomplete dehydration prior to testing.

Table 3.2: Quality of seeds harvested at different times

HARVEST DATE	DAF	GERMINATION TEST RESULTS (%)					TOTAL VIABLE SEED(%)
		Norm. seedl.	Abnorm. seedl.	Hard seed	Fresh ungerm.	Dead seed	
15 JAN.	32	1	0	99	0	0	100
20 JAN.	–	1	0	98	0	1	99
26 JAN.	35	10	1	89	0	0	100
31 JAN.	–	5	0	95	0	0	100
6 FEB.	37	4	0	95	0	1	99
11 FEB.	–	1	0	86	0	1	99
16 FEB.	40	12	0	86	0	2	98
22 FEB.	–	10	0	85	0	5	95
3 MAR.	40	11	1	83	0	5	95

3.3.5. Seed dormancy and hardseed development

Table 3.1 also shows the development of primary dormancy and hardseededness in birdsfoot trefoil seed. Freshly harvested seeds contained no hard seeds. However, about half of the seeds were dormant and were recorded in germination tests as imbibed but non-germinated seeds under the fresh ungerminated seed category. The percentage of dormant seeds remained relatively constant from 18 DAF to 42 DAF. The major increase in percentage of viable seeds came from an increase in the number of normal seedlings. The drying process had a profound influence on this seed property in that dormancy had almost completely disappeared, with increasing levels of hardseededness developing progressively in air dried seed lots removed from plants more than 30 DAF. The seed sample at 42 DAF contained 92% hard seeds. At the same time percentage of normal seedlings fell to a very low level.

Boiling water scarification seemed inefficient in breaking hardseededness (Table 3.3). Ten minutes in boiling water was not sufficient to soften seedcoats, while twenty minutes immersion was too severe for the seeds since 14% of the seeds were killed whilst

60% of the seeds still remained hard. Hand chipping completely removed the hardseededness. However, an small increase in abnormal seedlings was brought about by this operation.

Table 3.3: Hardseededness and methods of breaking hard seeds

Treatments	GERMINATION TEST RESULTS (%)				
	Normal seedling	Abnormal seedling	Hard seed	Fresh ungerm.	Dead seed
Boiling water 10 minutes	14	3	81	0	2
Boiling water 20 minutes	15	11	60	0	14
Hand chipping	86	11	0	0	3
Control	4	0	95	0	1

3.4. Discussion

The protracted flowering pattern and subsequent pod dehiscence which occurs in birdsfoot trefoil has been identified by many researchers as the major obstacle to obtaining high seed yield (Buckovic, 1952; Anderson, 1955; MacDonald and Winch, 1957; Metcalfe et al., 1957; Seaney and Henson, 1970; McGraw and Beuselinck, 1983). In seed production practice these aspects make the precise determination of the correct time to harvest the crop an important management decision to achieve maximum harvestable seed. It seems logical that more information on seed yield components could assist in determining a suitable index for deciding correct harvest time. The present study provides information on changes in yield components during the three month flowering period.

The results presented clearly show that number of inflorescences has the greatest influence on seed yield in <u>Lotus corniculatus</u> L. since the greatest fluctuation occurred in this component during the whole flowering period. Other components showed comparatively smaller changes, and fluctuated to a lesser extent. Regression analysis confirmed that the number of inflorescences was the only factor having a significant correlation with final seed yield (p=0.01). The high correlation coefficient between these two parameters (R=0.97) indicated the strong dependence of seed yield on the number of inflorescences. These results agree with those obtained by previous researchers (Albrechtsen <u>et</u> <u>al</u>., 1966, Mos, 1983; Stephenson, 1984; McGraw <u>et</u> <u>al</u>., 1986). The current work, and the results obtained by these workers, cover a wide range of situations, including different genotypes, several years with an identical genotype, different management strategies, and different climatic locations. Despite these differences all of these studies conclude that the number of inflorescences is the most important single seed yield component in <u>Lotus corniculatus</u> L. This strongly suggests that the number of inflorescences could well be used as a guideline for deciding peak flowering time and consequently deciding correct harvest timing. Approximately 30 to 40 days are required for blooming flowers to develop into mature pods ready for harvesting. It is recommended that highest recovery of the seed in the field could be achieved by harvesting the crop about 35 days after maximum inflorescence numbers are observed. A similar recommendation was made by Hare and Lucas (1984) for <u>Lotus pedunculatus</u> Cav. (Grasslands Maku lotus).

The seed yield component values reported in this study are comparable with other reports for this species (MacDonald, 1946; Seaney and Henson, 1970; Bratu et al., 1981). The calculated seed yield (560kg/ha) based on the values obtained in this study is also comparable with other reports (Seaney and Henson, 1970; McGraw and Beuselinck, 1983; White et al., 1987), but is considerably higher than commercially harvested seed yields in New Zealand (about 200-350kg/ha) (pers. comm. with growers). The calculated yield was considered to be the potential seed yield in the present study. This potential yield assumes that all inflorescences are available for harvesting at one time. Such a situation is unlikely because of the indeterminate flowering habit and pod shattering behaviour of this plant. It seems likely that in practice, only half about of the seeds produced are actually recoverable during a once-over machine harvesting operation. A more realistic calculation would seem to be to only include those inflorescences produced in a period about 20-25 days during the period of most intense flowering from 20 December 1986 to 10 January 1987. Using this value the calculated seed yield is about 420kg/ha. Such a seed yield is considered to be obtainable in practice if seed losses can be minimized during the harvesting and cleaning processes.

Reproductive abortion seems to be a common phenomenon in Lotus corniculatus, particularly at the flower bud stage as shown by many workers (Giles, 1949; Bubar, 1958; Joffe, 1958; Seaney and Henson, 1970; Stephenson, 1984). Incomplete self-incompatibility and self-sterility is one major cause of abortion in this species (Silow, 1931; Seaney, 1964; Dobrofsky and Grant, 1980). However, even when each flower is outcrossed, a proportion of florets still fails to

develop pods (Stephenson, 1984). Although originally explainable by Darwin's theory of evolution (Stephenson and Winsor, 1985), the exact mechanism of bud abortion is largely unclear. Several factors have been reported to cause reproductive abortion in a wide range of plant species. These include photosynthetic activity (Johnston et al., 1969; Schou et al,. 1978), nutrient deficiencies (Gauch and Dugger, 1954), competition between vegetative and reproductive growth and within reproductive structures (Thrower, 1962; Biomquist and Kurst, 1971; Kollman et al., 1974). Gauch and Dugger (1954) suggested that boron deficiency is a particularly important cause of abortion in flowers and fruits in various plants. However, Joffe's experiment (1958) disagrees with this suggestion in Lotus corniculatus. After examining a large proportion of aborted buds present under both suitable and suboptimum photoperiods, temperature regimes and with and without boron, Joffe concluded that none of these factors caused bud abortion. At other stages of floret development, abortion has also been reported to occur frequently. Seaney and Henson (1970) in their review of seed production in Lotus corniculatus mention that only 40% of the 20-70 ovules in an ovary develop into mature seeds. Comparable figures were obtained in the present study (11-14 seeds per pod) (Figure 3.1). Stephenson (1984) also reported that in this species only one in every three flowers produces a mature fruit and three of every five initiated fruits abort. He also dismissed the possibility of abortion being caused by pollination failure (using controlled pollination) and concluded that it occurred as a result of lack of assimilate supply. The present results support his conclusion since, if external factors such as unfavourable photoperiod, temperature, or pollination problems were responsible for the level of abortion, abortion rate would likely to have been more highly

variable during the lengthy flowering period. The reasonably consistent 'one pod' loss found during the whole flowering period in the present study more strongly implicates the role of internal regulating mechanisms. It is likely the availability of assimilate supply acts as a key factor limiting reproductive output. The precise regulating mechanism(s) involved, however, are still not clear from the current study and more research is needed to identify the cause(s) of this problem.

Seeds of Lotus corniculatus substantially gain viability about 30 DAF, which is similar to the time reported for Lotus pedunculatus Cav. in New Zealand (Hare and Lucas, 1984). At this stage the seeds retain high viability and resistance to drying. 30 DAF seems an important turning point in seed development. With freshly harvested seeds high levels of normal seedlings are produced about this time. Also, a transient, but relatively high percentage of abnormal seedlings are present at this stage. This suggests the production of intermediate types of seeds developed at the interface between non-viability and viability. The consequent reduction in percentage of abnormal seedlings indicated that seeds had successfully completed their development, and occurred in either normal seedling or fresh ungerminated seed categories. The development responses of seeds after air drying showed a similar trend in the percentage of viable seed. Hardseededness began to develop at 30 DAF. From this time the increase in the percentage of hard seeds was accompanied by a rapid decline in normal seedling numbers.

The results obtained in this study agree well with results by Anderson (1955) who also found that about 30 days was needed for

Lotus corniculatus seed to complete its development from pollination
to maturity. Similar results were obtained by Winch (1958); Seaney and
Henson (1970); Beuselinck and McGraw (1988). However some variation was
found in the time required to complete seed development in flowers of
different times of origin, within the range from 30 to 40 DAF .
Early-formed flowers required fewer days (32 days) than late flowers
(42 days) to complete their full development. Such results agree
well with the results by Witchwoot (1987) on another Lotus species
(Lotus pedunculatus Cav. cv. Grassland Maku) grown in the same
experimental field, but is the reverse of those obtained by
Beuselinck and McGraw (1988) who suggest that fewer days are needed
by late flowers to complete seed development than by early flowers.
Such an opposite effect might be explained by differences in the
effect of temperature on reproductive growth. In Beuselinck and
Mcgraw's experiment, flowers used were formed prior to the highest
temperature period. As a result, late flowers had an advantage by
receiving higher temperatures during pod development than the early
flowers. In the present study, however, flowers used were formed at
or after the period of highest temperature (Appendix 2). In this case
early flowers had a temperature advantage compared to late flowers.
Under higher temperatures flowers and pods presumably develop faster
and therefore need fewer days to complete their development.

The patterns of seed viability and hardseed development observed in
the present study are similar to the patterns described in Grasslands
Maku lotus (Lotus pedunculatus Cav.) (Hare and Lucas, 1984) and other
forage legumes such as red and white clover (Hyde et al., 1959),
apart from some differences in the time of onset and duration of
each seed development stage. Lotus corniculatus seed reached maximum

viability about 34 DAF with pod colour changing from dark brown to
light brown at this stage. It is suggested that pod colour could be
used as an important guide for deciding harvest timing. In this
regard, MacDonald (1946) has suggested that harvesting should be
carried out when 70-80 of pods turn light brown.

The seed chipping results clearly show that seedcoat impermeability
is the main cause of hardseededness. Seedcoat impermeability develops
around 30 DAF as shown by the increasing percentage of hard seeds in
different seed lots. Differences in hard seed levels between
different aged seed lots were not thought to have been caused by
differences in their moisture content (range 6.9-7.1% among seed
lots). It seems more likely that differences in hard seed levels
were directly associated with the degree of completion of seed coat
structural development depending on seed age when removed from the
plant. This suggestion is supported by work by Watson (1948) who
showed that the development of deposits of suberin on the tips of
Malpighian cells acted as a major barrier to seedcoat permeability in
Lotus corniculatus.

The reported percentage of hard seeds formed in Lotus corniculatus
varies widely, ranging from 19% (MacDonald, 1946) to more than 90%
(Brown, 1955). Harvesting methods and post-harvesting handling have
both been shown to influence the percentage of hard seeds. It has
been shown that the percentage of hard seeds is much higher when hand
harvested than when they are machine harvested (MacDonald, 1946;
Brown, 1955). The present study suggests that seed maturity is a
major factor affecting the occurrence of hard seeds. It therefore
seems likely that other factors such as harvesting method and timing

may influence levels of hard seed indirectly by affecting the relative proportion of seeds with different levels of maturity, or by causing mechanical damage to seedcoat during harvesting. The present study has shown that there is difference of a few days between the occurrence of highest seed viability and highest hardseededness.

No research appears to have reported on seed dormancy (other than hardseededness) in Lotus corniculatus. The present study, however, has shown that a short period of primary dormancy does exist during seed development in this species. While the exact dormancy mechanism is not clear it may possibly be imposed by some kind of inhibitor in the seed or be simply a so-called 'Acquisition of desiccation tolerance' post-maturation seed phenomenon as suggested by Kermode and Bewley (1986) which occurs in many cereals. The chipping of seed to remove hard seed dormancy prior to a germination test clearly allows any other dormancy mechanism to be exposed. Comparison of germination of unhardened seed and chipped seed (Table 3.1) shows that the primary dormancy exhibited in freshly harvested Lotus corniculatus seed is short-lived and disappears soon after seeds are dried.

The current study on the changing pattern of each seed yield component shows that inflorescence number is the most variable yield component during the long flowering period. The present result, supported by results by previous researchers, suggests that this seed yield component, because of its flexibility, may be amenable to improvement by management strategies which aim to increase the number of inflorescences and consequently increase seed yield in birdsfoot trefoil. In the previous chapter it was shown that the number of

inflorescences was closely dependent on the number of shoots at flowering. This again suggests that manipulation of shoot numbers is a promising approach for improving seed yield in this plant.

The abortion of reproductive structures at various developmental stages suggests there might be a shortage of assimilate supply for reproductive development. This is likely to be a result of competition between reproductive growth and vegetative growth. Such a hypothesis agrees with the previous suggestion that one way to improve seed production in this plant might be to reduce this competition by removal of late formed shoots.

CHAPTER 4: SHOOT MANIPULATION TO IMPROVE SEED PRODUCTION

4.1. Introduction

Previous researchers have shown that seed yield in Lotus corniculatus was mainly determined by the number of inflorescences (Albrechtsen et al., 1966, Mos, 1983; Stephenson, 1984; McGraw et al., 1986). Results in Chapter 3 of the present study on seed yield components agree with these previous findings, showing that the number of inflorescences is the most important seed yield component determining final seed yield in Lotus corniculatus. Results in Chapter 2 of this study have clearly shown that number of inflorescences is mainly determined by number of shoots rather than by other factors such as the flower-carrying ability of individual shoots. These results suggest that manipulation of shoot number therefore seems likely to be an important aspect for improving seed production in Lotus corniculatus.

Certainly, a high shoot population at flowering seems a prerequisite for a high seed yield because of the strong reliance of number of inflorescences on number of shoots. The results in the previous Chapters clearly show the important role that October, November and particularly December (late spring to mid summer) shoots play as direct contributors to seed yield. However, there are perhaps risks in simply concluding that shoots originating during these three months are more important than shoots formed in the autumn, winter and early spring in influencing seed yield. Early shoots, although they show a less important direct contribution to flowering and

therefore seed yield, may have an important role in supporting the development and growth of later shoots (November and December shoots in this study). Particularly, spring shoots (August, September and October shoots) may have a precursory influence on the flush of November and December shoots. Since all shoot age groups from September to December have a similar flower producing ability on individual shoots, it is presumed that a high seed yield can be achieved as long as a high shoot population is attained at peak flowering time regardless of shoot age.

Late formed shoots (January and February), although they contribute a considerable portion of the total shoot population, make a negligible contribution to flowering in the current year because they emerge too late for flowering. Instead, late shoots may have a detrimental effect on seed yield simply because they grow simultaneously with the development of flowers (or pods) on early shoots. Competition between reproductive growth in flowering shoots and vegetative growth in late formed young shoots is likely to occur and possibly this competition is important in adversely affecting final seed yield. In addition, late formed vegetative shoots may increase seed crop lodging problems which can severely affect seed yield in Lotus corniculatus (MacDonald, 1946; Anderson and Metcalfe, 1957). January and February shoots may therefore be considered to be undesirable for seed production in this species. This suggests that one management strategy which could be used to improve seed production would be to suppress, instead of encouraging, late shoot growth and development. A systematic approach to improve seed production might combine the effect of manipulating shoot status from two directions. Early in the pre-flowering season it may be

important to encourage the shoot population to attain high shoot numbers. Subsequently, at around peak flowering, management might be imposed to try to prevent late shoot emergence or to suppress late shoot development to allow more assimilate to be translocated to the reproductive organs already formed on middle season shoots (October to December) resulting in a high and contracted period of seed production.

Manipulation of the 'continuous replacement' shoot development pattern carries with it the risk of running out of shoot stocks for flowering if early shoots die (as happened naturally in this study) and late shoots are prevented or suppressed. However, such a situation is unlikely to occur if the continuous emergence of new shoots occurs at the expense of the death of old shoots. The prevention or suppression of late shoot formation may improve the longevity and possibly the reproductive capacity of older shoots. Such an assumption might also suggest the possibility that the flower-carrying ability (number of inflorescences per shoot or number of florets per inflorescence) of early shoots could be enhanced.

In this study a series of hand removal treatments were designed to examine the effects of removing shoots at different times on plant growth and on final seed yield in Lotus corniculatus. Since cutting is likely to be the most convenient shoot manipulating method in practice, a number of cutting treatments were also included in this study with special interest in the effects of timing and intensity of cutting on seed production.

4.2. Materials and Methods

This experiment was carried out mainly during 1987/88 in an experimental field adjacent to the 1986/7 experimental area at the Seed Technology Center (STC). The total area was about 600m^2, divided into 6 equal sized plots. Seeds were directly handsown into soil on 9 May 1987 with 80cm inter-row and 60cm intra-row space. Each row contained 11 plants including two border plants. Herbicides were applied on 11 May (pre-emergence, 'Roundup' (Glyphosate) at 2kg a.i./ha) and 29 July (inter-row post-emergence, 'Paraquat' (Gramoxone) at 1% rate) for weed control.

On 20th of each month from August to November 1987, newly emerged crown shoots were tagged with different coloured plastic rings on each of 27 plants. A further 9 plants were also tagged at the same time, and any newly emerged crown shoots arising after tagging were removed by hand. Numbers of tagged shoots on these plants were examined monthly to assess the effect of post tagging shoot removal on shoot survival and shoot branching behaviour.

A further four hand removal treatments were carried out at different plant development stages to examine their effects on flowering, seed yield and yield components. The timing of these four treatments were; 30 October (pre-bud stage, Hand 1), 20 November (bud stage, Hand 2), 10 December (early flowering, hand 3) and 25 December (pre-peak flowering, Hand 4). Peak flowering occurred on 5 January. In these treatments newly emerged crown shoots were removed by hand at the date of treatment and checked regularly afterwards to ensure only those shoots emerged before treatment remained. All treatments were

replicated three times, each with nine plants.

Six cutting treatments were carried out at three different times (28 October, 12 November and 30 November) with two cutting levels (to 1/3 and 2/3 plant height). Experimental details are presented in Table 4.1.

Table 4.1: Experimental Details:

Treatment	Times of Application			
Hand removal(87/88)	30 Oct.	20 Nov.	10 Dec.	25 Dec.
Hand removal(88/89)	8 Dec.	23 Dec.	3 Jan.	13 Jan.
Cutting(L) treatment[1]	28 Oct.	12 Nov.	30 Nov.	--
Cutting(H) treatment[2]	28 Oct.	12 Nov.	30 Nov.	--

N.B.: 1, Cutting L treatment (Cut L 1-3): plants cut to 1/3 original height.
2, Cutting H treatment (Cut H 1-3): plants cut to 2/3 original height.

Inflorescence numbers and floret numbers were recorded in the same way as described previously. Pod numbers per umbel and seed numbers per pod were recorded after plants were hand harvested in early February and seed mass (1000-seed weight) was measured following ambient air drying of seeds at room temperature for approximately one month. Seed yields were expressed at mean seed moisture content of 10.3% for hand removal treatments and 11.6% for cutting treatments.

On 25 December 1987 five plants were selected for hand removal of flowers. These plants were kept in a 'non-flowering' state by hand removal of any flowers on them. At the end of the experiment (11 February), numbers of shoots per plant in these treated plants were recorded and compared with those in intact control plants.

Three cutting treatments were also carried out on a sward crop located in DSIR's Aorangi experimental field 11 km from the STC site on a Kairanga silt loam (Holocene siliceous sandy alluvium soil). Details of the experimental site and management are given in Table 5.1 page 110. Plants were cut to 1/2 of original height at each of the different date (27 October, 12 and 27 November). Inflorescence numbers, floret numbers and final seed yields were recorded.

In 1988-89 four hand removal treatments were carried out at the STC site on 45 by 50cm spaced plants. Seeds were sown in early May 1988. The timing of the first two treatments overlapped with the previous year's last two treatments (8 December, early flowering and 23 December, pre-peak flowering). The last two treatments were carried out on 3 January (peak flowering) and 13 January (post-peak flowering) (Table 4.1). Plants were harvested on 2 February and seed yield was recorded.

4.3. Results

4.3.1. Effects of shoot manipulation on shoot growth and development

Results in Table 4.2 show the effects of removing newly emerged crown shoots on the survival of already existing older shoots. Removal of new shoots resulted in almost all of the old shoots surviving to flowering (20 December represents pre-peak flowering). In intact control plants, which were allowed to express normal 'continued replacement' growth, old shoots tended to die before flowering (refer Chapter 2). Unlike intact control plants (plate 2.3), the shoot removal treatment greatly changed the plant's branching behaviour,

the few old shoots present forming many well developed lateral

branches (Plate 4.1).

Table 4.2: Effects of New Shoot Removal on the Survival of Old Shoots

Tag date	Treatment	No of shoots tagged/plant	No. of tagged shoots remaining				% Survival
			20 Sep.	20 Oct.	20 Nov.	20 Dec.	
20 Aug.	control[1]	2.81	2.66	1.37	1.07	0.30	10.7%
	removal[2]	3.11	3.11	3.11	3.11	3.11	100.0%
20 Sep.	control	3.59	3.59	3.22	3.04	2.00	55.6%
	removal	5.56	5.56	5.44	5.25	5.25	94.4%
20 Oct.	control	8.81		8.81	8.37	7.33	83.2%
	removal	12.22			12.22	12.22	100.0%
20 Nov.	control	19.33			19.33	18.56	96.0%
	removal	28.67				28.67	100.0%

N.B. 1. Control: tagged plants without removing newly emerged shoots afterwards. Numbers are averages of 27 plants.
2. Removal: tagged plants with all subsequent newly emerged shoots removed. Numbers are averages of 9 plants.

4.3.2. Effect of hand removing treatment on flowering pattern

In 1987-88 the four hand removal treatments showed different effects

on the number of inflorescences produced and the time of peak

flowering. Of these four treatments, three increased total

infloresence numbers and the intensity of peak flowering (Figure

4.1). The first treatment (30 December) also advanced peak flowering

by approximately 15 days. Hand 3 (10 December) is the treatment which

decreased the total number of inflorescences and infloresence numbers

at peak flowering. The different results from the four treatments

reflect the plant's response to the timing of shoot manipulation.

Plate 4.2 (a-e) shows the differences in plant shoot structure

created by the four treatments. It can be seen that the first

treatment (30 October) (Plate 4.2b) reduced plant main shoot numbers

but resulted in larger main shoots. Flower carrying ability of

individual shoots was greatly increased by the existence of many well

Plate 4.1. Plant structure after new shoot removal
(29 November 1987. Top: untreated plant;
Bottom: treated plant)
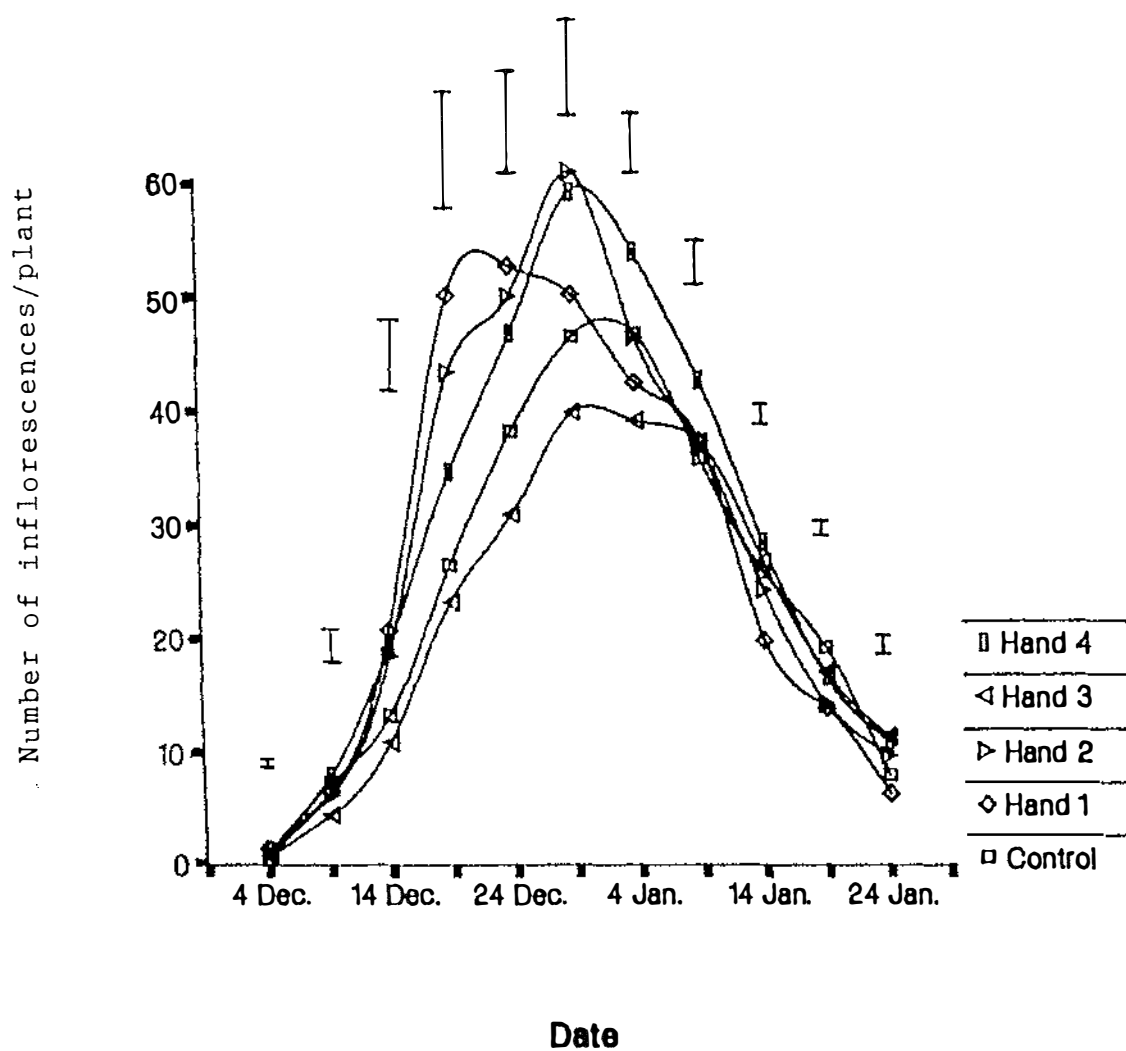
Figure 4.1. Flowering pattern in hand removal
          treatments (STC site, spaced plants)

          (Vertical   bars are least standard
          errors of differences at p=0.05, same
          annotations for all following figures)

a

b

Plate 4.2. (Continued)

c

d

Plate 4.2. (Continued)

e

Plate 4.2(a-e). Plant shoot status in four hand removal
treatments (6 January 1988)

developed lateral shoots capable of flowering. The fewer main shoots present were compensated for by increased flowering ability of individual shoots. As a result, the number of infloresences produced by treated plants was enhanced. However, such an increase was temporary and the pattern of flowering returned to normal after peak flowering as the result of running out of reproductive capacity in these bigger shoots. The Hand 2 treatment (20 November) was carried out 20 days later than the Hand 1 treatment by which stage plants had produced more shoots. As a result, the number of inflorescences produced was greater than in both the Hand 1 treatment and the intact control. The Hand 3 treatment (10 December) had a different effect on the plant. Since the earlier two treatments were carried out before flowering, shoot population at those times contained mainly infertile shoots capable of branching. However, by the time the Hand 3 treatment was carried (at early flowering, 10 December) most existing shoots had became fertile and were incapable of new branch production. At this time the removal of shoots simply reduced the numbers available for flowering and consequently reduced the number of infloresences formed. The Hand 4 treatment, which was carried out at the pre-peak flowering stage (25 December) when a large number of shoots had already been produced, did not enhance shoot branching ability, but still resulted in the production of more inflorescences, possibly by removing competition from the growth of very late vegetative shoots. Because a large shoot population already existed at the time of the Hand 4 treatment, this removal of competition and therefore possible redistribution of assimilate to the existing shoots may have facilitated improved reproductive growth on existing fertile shoots.

4.3.3. Effect of hand removal on seed yield components and seed yield

Other seed yield components (number of florets per inflorescence, number of seeds per pod and seed weight) showed less sensitivity to hand removal treatments than inflorescence numbers. There was an obvious, but short period of increase in floret numbers in response to the first three hand removal treatments. This was not applicable to the fourth treatment, which had not been applied by the time of the first two recordings (Figure 4.2). This increase in floret numbers possibly occurred due to an immediate increase in assimilate supply to flowers by removal of or reduction in the competitive pool of newly emerged shoots. It was noted that in all treatments the number of florets (and pods) per inflorescence was similar at peak flowering (5 January) (Figure 4.3), implying that shoot manipulation had less effect on floret numbers than on inflorescence numbers. Seeds per pod and seed weight components showed little difference among the treatments and between the treatments and the intact control (Figures 4.4, 4.5).

The Hand 2 treatment (20 November, 1987) increased actual harvested seed yield by about 30% as a result of increased inflorescence and floret numbers, but the other treatments showed no significant effects (p=0.05) on seed yield over the intact control (Table 4.3).

Table 4.3: Seed yield in hand removal treatments

| Treat.time (1987/88)	yield (g/plant)	Treat.time (1988/89)	yield (g/plant)
Control	25.3	Control	29.4
30 Oct.	28.1	8 Dec.	26.3
20 Nov.	35.9*	23 Dec.	28.7
10 Dec.	25.3	3 Jan.	28.7
25 Dec.	27.7	13 Jan.	28.4
L.S.D.(0.05)	6.0		NS
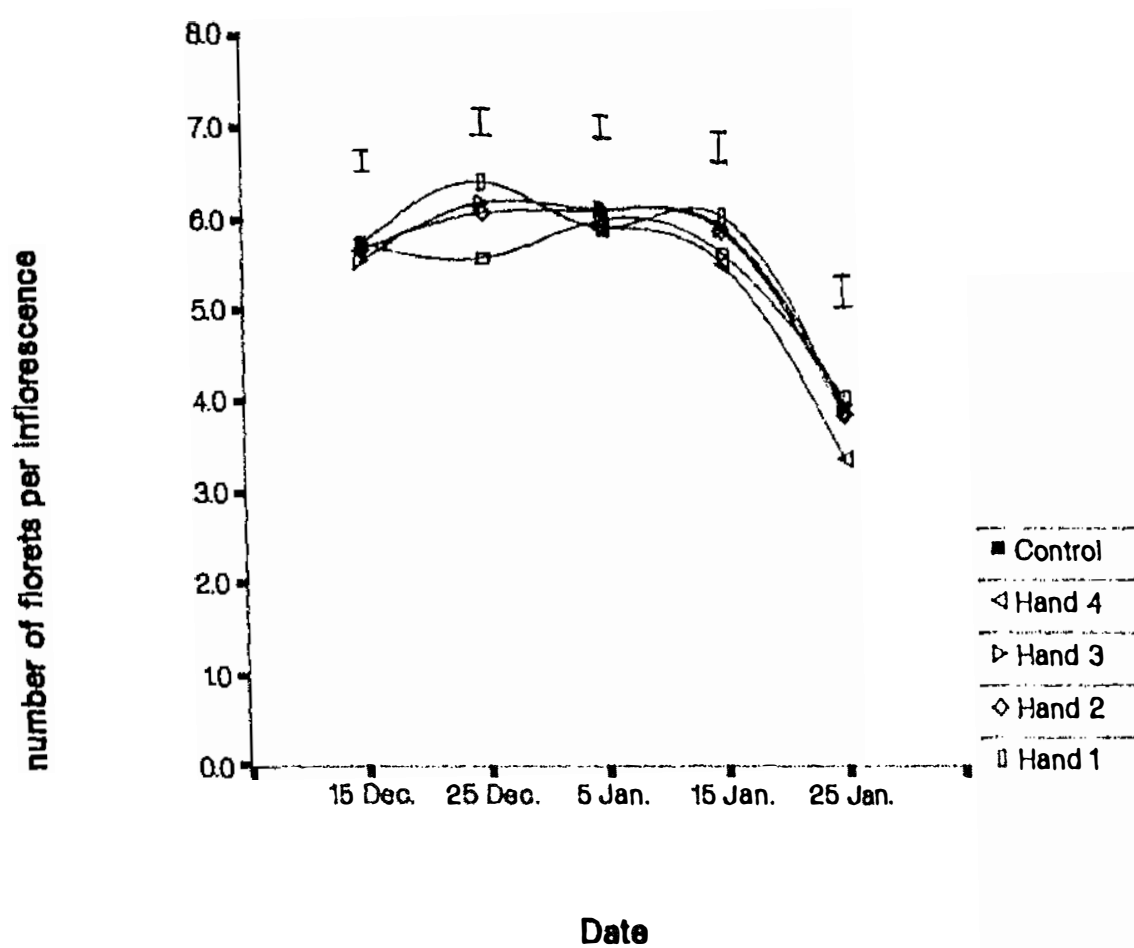
Figure 4.2. Change in floret numbers in hand removal
treatments with time (STC site, 1987/88)
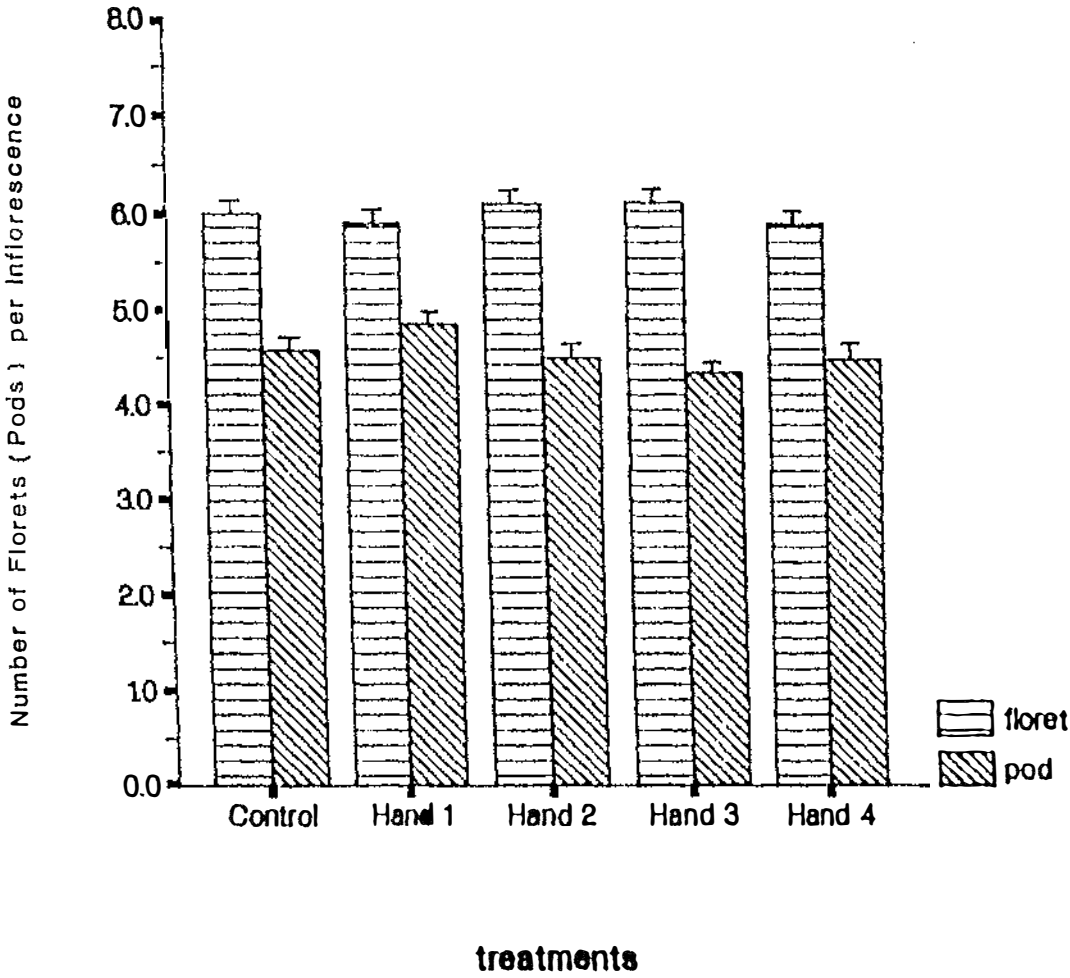
Figure 4.3. Floret numbers and pod numbers in hand
removal treatments (STC site, 1987/88)
(5 January 1988)
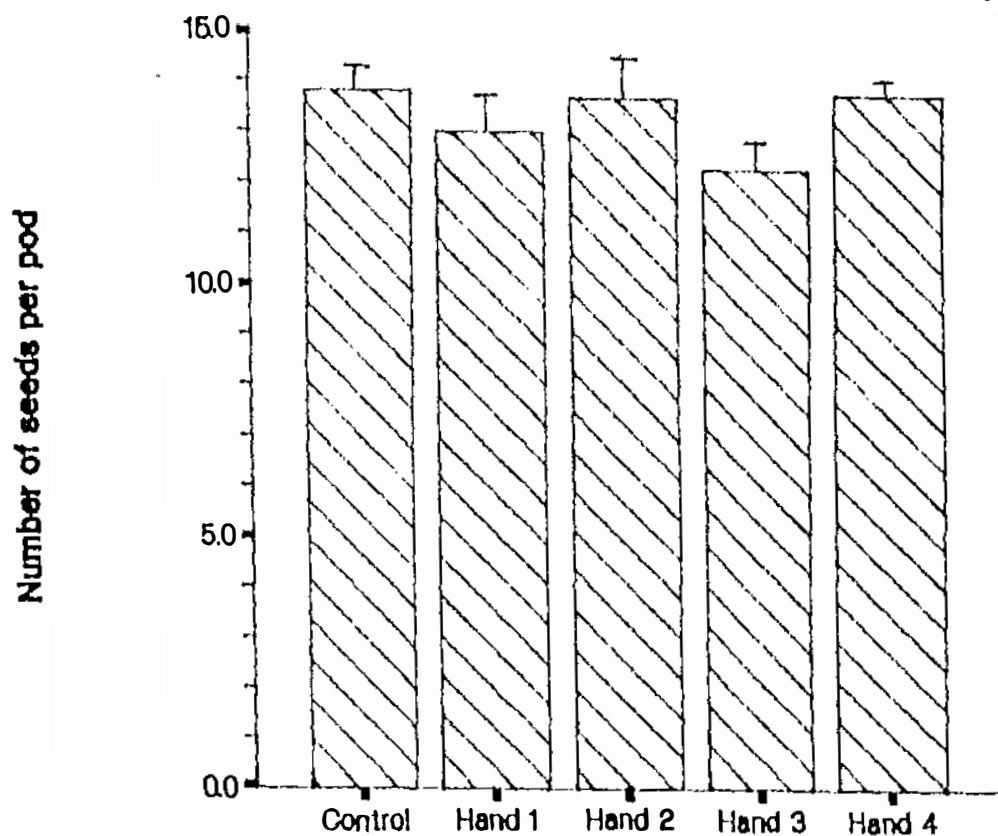
Figure 4.4. Seed numbers per pod in hand removal
treatments (STC site, 1987/88)
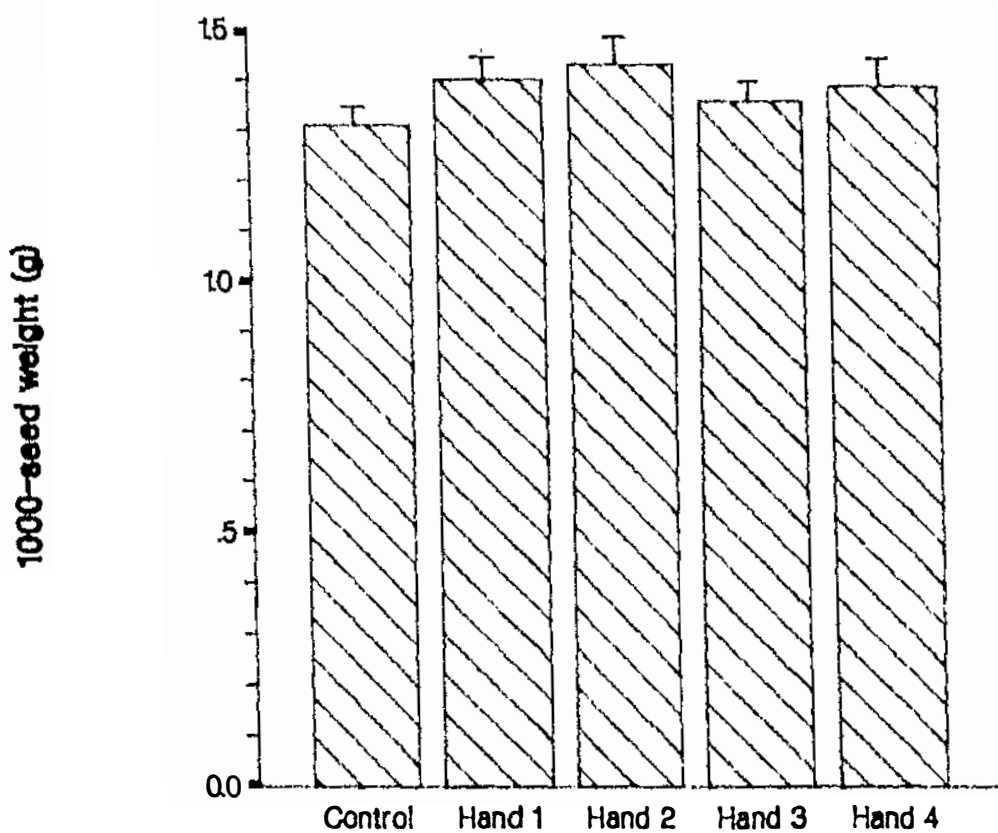
Figure 4.5. 1,000-seed weights in hand removal
treatments (STC site, 1987/88)

Potential and calculated seed yields were estimated by incorporating the data for each seed yield component (inflorescence numbers, pod numbers per inflorescence, seed numbers per pod and seed weight) obtained in this study. Potential seed yields were calculated using all inflorescences produced by plants throughout the entire flowering period from early December to early February. Calculated seed yields were determined by using only the inflorescence numbers which appeared during the 25 day period of most intensive flowering. The three seed yield assessments (Potential, calculated and actual harvested yields) are presented in Figure 4.6.

In 1988-89 only seed yield data were recorded. None of the four hand removal treatments increased or decreased final seed yields (p=0.05) (Table 4.3).

4.3.4. Effect of flower removal on vegetative growth

The effects of flower removal on the plant's vegetative growth in terms of number of shoots produced during a period of fifty days from 25 December 1987 to 11 February 1988 are presented in Table 4.4. Preventing reproductive growth resulted in the number of shoots produced being nearly three times greater than in the control. Flower removal did not change plant growth habit. The shoot population was still predominantly composed of main shoots. There were no obvious visible differences in individual shoot sizes between the flower removal treatment and the intact control.
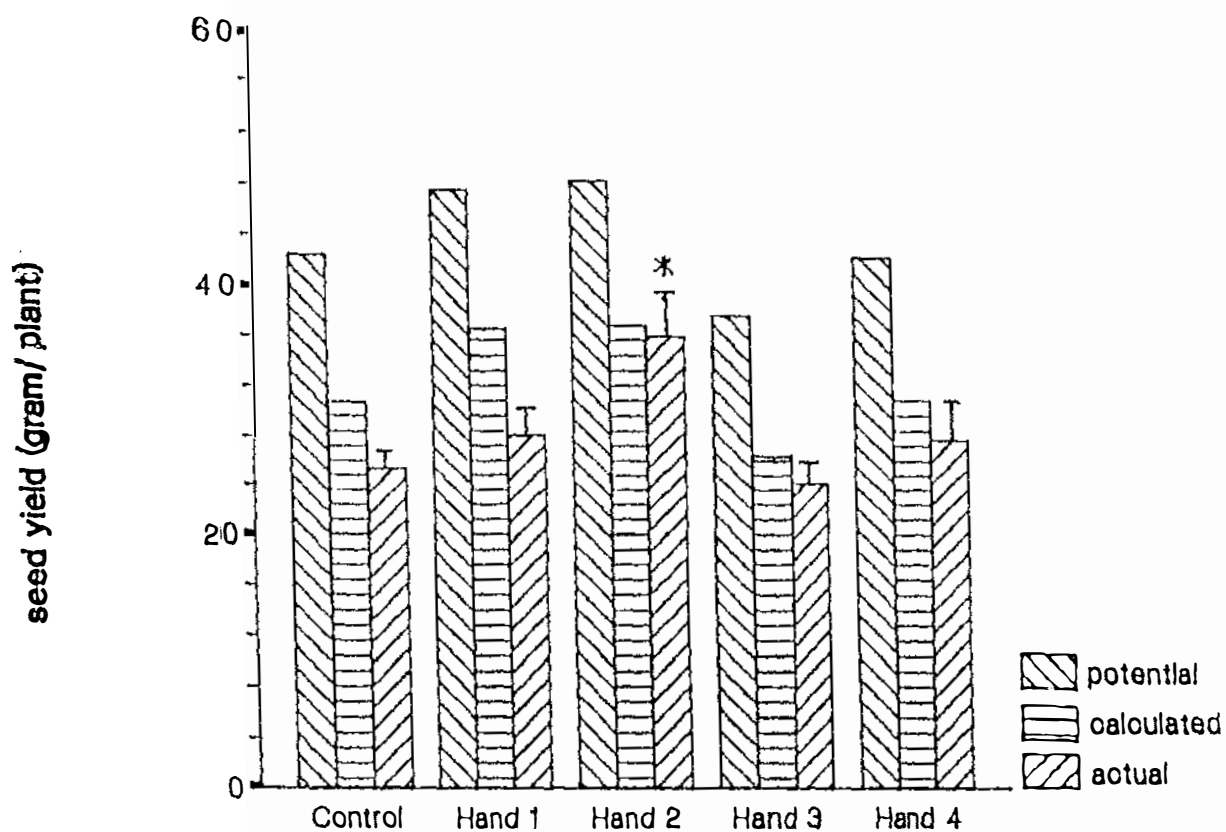
Figure 4.6. Seed yields in hand removal treatments
(STC site, 1987/88)

Table 4.4: Effect of flower removal on vegetative growth

	Control	Flower removing	% difference from Control
No. of shoots before treatment (25 Dec.)	63(3.5)	68(5.5)	+112%
No. of shoots after treatment (11 Feb.)	80(2.5)	118(5.3)	+149%
Increase in shoot numbers and %	17 27%	50 74%	+294%

N.B.: figures in brackets are SEs of the means.

4.3.5. Effect of cutting treatments on flowering pattern and seed yield

The time and height of cutting treatments had a major effect by removing a complete 'top' section of 1/3 or 2/3 of the shoot canopy. The results of such operations on seed production at the STC site were not encouraging. None of the six cutting treatments improved the number of inflorescences or seed yield compared with the uncut control. Early cutting (Cuts 1 and 2) had less harmful effect on flowering, but nevertheless still slightly reduced the number of inflorescences present at peak flowering. Late cuttings, however, were particularly detrimental, the number of inflorescences being greatly reduced and peak flowering delayed (Figures 4.7, 4.8). Heavy cutting (Cut L) was more harmful than light cutting (Cut H). At the Aorangi site a similar trend in flowering pattern was found in the three cutting treatments (Figure 4.9).

Despite the fact that final seed yield was not statistically different between the cutting treatments and intact control, there was a suggestion that late cuttings produced less seeds (Appendix 5)
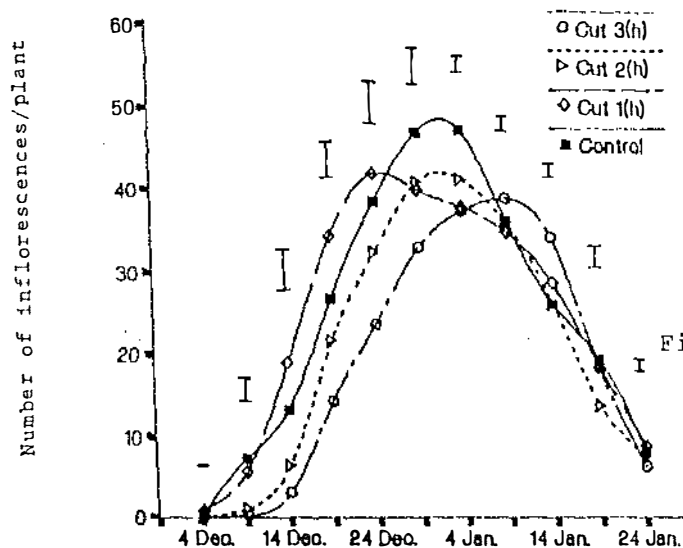
Figure 4.7. Flowering pattern in Cut (H) treatments (STC site, 1987/88)
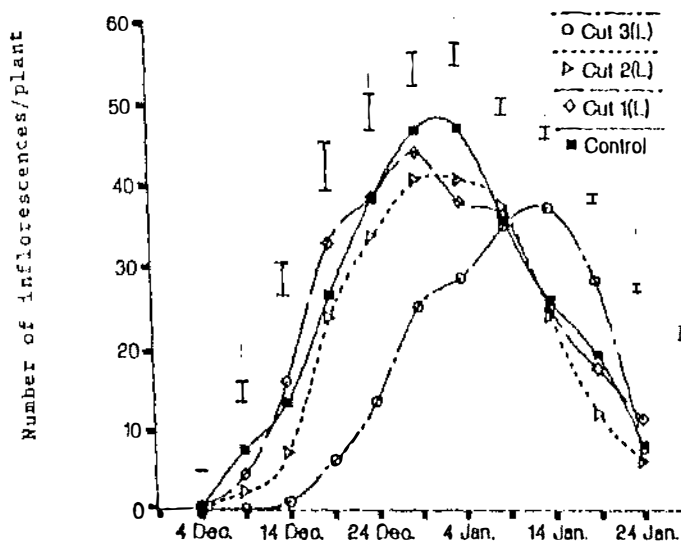
Figure 4.8. Flowering pattern in Cut (L) treatments (STC site, 1987/88)
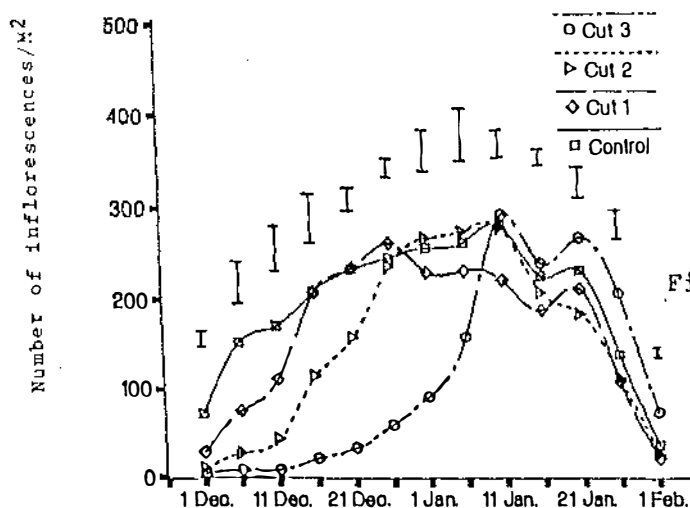
Figure 4.9. Flowering pattern in cutting treatments (Aorangi site, sward plants, 1987/88)

4.4. Discussion:

The idea of young shoot removal comes from the concept of 'source and sink' dual roles of single vegetative structures. In soybean, Thrower (1962) demonstrated that young leaves only imported assimilate (sink activity) without any export of assimilate (source activity) when the leaf area was below 30% of its final mature size. Young shoots are also considered to be net importers of assimilate without contributing to reproductive growth. Young shoots may also have a detrimental role on older shoot longevity in Lotus corniculatus, since old shoots tend to die when new shoots emerge (chapter 2). It is assumed that young shoot removal may not only be beneficial in enhancing reproductive growth on old shoots but may also influence the survival and other behaviour (such as branching) of old shoots.

Results from this study indicate the possibility of shoot manipulation being used as a strategy for improving seed production in birdsfoot trefoil. Shoot status in this species seems readily modifiable in terms of life span, branching and flowering behaviour. The results also reveal some aspects of the plant's internal regulatory mechanisms. Initially the plant shows a predominance of new shoot growth over old shoot maintenance, resulting in a "continuous replacement' system in which old shoots die when the new shoots emerge. The death of old shoots is likely to occur as a result of strong competition by new shoots for assimilate, as suggested by the current results where removal of new shoots greatly extended the life span and sustained the flowering ability of old shoots.

The competition which occurs between vegetative and reproductive growth highlights the principles of a shoot manipulation strategy on seed production in birdsfoot trefoil. That the competitive balance between vegetative and reproductive growth is adjustable is shown by the fact that depressing vegetative growth (removing young shoots) increased the reproductive output of old shoots (flower carrying ability). Conversely, removing flowers from old shoots encourages the emergence of new shoots. Since birdsfoot trefoil has a long flowering period which needs a sustained supply of shoots for flowering, the time at which vegetative growth is suppressed in order to allow increased reproductive growth is a factor which needs to be carefully considered. Too early vegetative shoot depression is unsuitable since it reduces the number of shoots available for flowering. Although the flower carrying ability of these individual shoots can be slightly enhanced by new shoot removal, a shortage of shoots available for flowering negates the effects of enhanced flower carrying ability (the Hand 1 treatment). Removing vegetative competition at the pre-flowering stage to encourage existing old shoots to branch seems to be beneficial for seed production since it can provide more flowering sites (the Hand 2 treatment). However, vegetative shoot removal becomes detrimental if the removal operation is applied when most shoots have already become fertile and therefore incapable of further branching (the Hand 3 treatment).

Another important aspect of the vegetative removal idea is to reduce the competition imposed by very late formed shoots which are unable to flower. Such late shoot removal could be beneficial for seed production in this species. This effect is obviously seen by the increases in floret numbers per inflorescence which occurs following

hand removal treatments. It is perhaps surprising that the late shoot removal treatment (hand 4 treatments in 1987/88 and hand 3 and 4 treatments in 1988/89) did not increase seed yield. This may be due to the incompleteness of removal operations. Possibly, more frequent removal at an earlier stage of shoot development would be more beneficial, since by the time a new shoot is of sufficient size for hand removal it is too late to achieve the proposed competition release effect. It seems more likely that an operation which could entirely prevent the initiation of late buds in order that assimilate can be entirely used for reproductive growth might be more effective. Nevertheless, the obvious increase in floret number following hand removal of shoots supports the principle of the value of shoot manipulation on seed production of Lotus corniculatus.

Cutting does not seem to be a suitable shoot manipulation method for improving seed production in Lotus corniculatus. Because the plant has a dominance of crown shoot growth over lateral shoot growth (Chapter 2), cutting simply removes some old shoots while young shoots continue to be produced from the crown area. Cutting does not promote branching and does not appear to be effective in reducing competition for assimilate by non-fertile late shoots. Early cuttings had less detrimental effects on seed yield, presumbly because they allowed sufficient time for plant growth recovery. Late cutting, however, was more detrimental since the resultant crop had fewer large shoots for inflorescence production at peak flowering. The results of this study are supported by work of previous researchers (Anderson and Metcalfe, 1957; Winch, 1958; Bader and Anderson, 1962) who have also shown that cutting treatments have no obvious beneficial effect on seed production in birdsfoot trefoil. In a

recent study on a similar species (Grasslands Maku Lotus, <u>Lotus</u> <u>pedunculatus</u> Cav.) Hare (1985) demonstrated that cutting is generally detrimental to seed production regardless of the time and severity of defoliation.

The results reported in this chapter support the principle of shoot manipulation for enhancing seed production in <u>Lotus corniculatus</u>. Management methods should aim at encouraging shoot branching and/or reducing competition by late season vegetative growth. The results also show the timing of manipulation of shoots is important in influencing shoot branching behaviour. To encourage crown shoot branching, manipulation needs to be carried out before the shoots becoming fertile. Cutting management, as imposed in this study, seems to be of little value as a management strategy since it does not promote the effects desired for improving seed yield in this plant.

CHAPTER 5: EFFECTS OF PRE-PEAK FLOWERING SHOOT MANIPULATION ON PLANT GROWTH AND SEED PRODUCTION

5.1. Introduction

Results from Chapter 4 have suggested the possibility of a shoot manipulation approach for improving seed production in Lotus corniculatus. However, as effective as the hand removal of shoots may be, such an operation cannot be directly used in practical situations for seed production. As cutting treatments described in the previous chapter generally failed to elicit the same encouraging responses as the hand removal treatments, the present chapter examines the possibility of manipulating shoot population using chemical manipulation methods as a potentially useful alternative approach.

The plant growth regulator PP333 [(2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-1, 2, 4-triazol-1-ylpentan-3-ol, also known as Cultar, paclobutrazol, parlay] has been shown to be successful as a plant regulating chemical for increasing seed yields in several pasture plants. This growth regulator has been shown to alter plant structure by decreasing apical dominance, promoting tillering or branching, increasing assimilate translocation to reproductive tissues, preventing lodging and synchronizing flowering (Hampton and Hebblethwaite, 1985a,b; Marshall and Hides, 1986, 1987). This compound is a soil active, xylem-mobile chemical. It inhibits gibberellin biosynthesis (Shearing and Batch, 1982). Its major effects on plants include shortening the internodes and promoting

lateral branching. It was considered that the branch-promotion effect of this chemical may have some beneficial effect on birdsfoot trefoil seed production by increasing the lateral branches of the crown shoots and thereby enhancing shoot flower carrying ability. The present study investigated the value of PP333 as a pre-peak flowering growth regulator to examine its effect on shoot branching behaviour and seed production in Lotus corniculatus.

5.2. Materials and Methods

Experiments were carried out over two successive growing seasons from March 1987 to March 1989. In 1987-88 plants were grown at two sites. The first site, at the Seed Technology Center (STC), Massey University, involved spaced plants with a row width of 80cm and intra-row spacing of 60cm. The second site, at the Department of Scientific and Industrial Research's experimental farm at Aorangi involved a crop grown in 60cm rows with 64 plants per metre row. In 1988-1989 the experimental field was located at the Seed Technology Center involving spaced (45 by 50cm) plants and sward plants (45cm wide rows, 68 plants per metre row). No fertilizer or irrigation was applied during the experimental period. Management and treatment details are presented in Table 5.1. The three application times in 1987-88 represented the active vegetative growth, pre-flowering bud, and onset of flowering stages. In 1988-89 the chemical was applied at the onset of flowering and the pre-peak flowering stages. All treatments were replicated three times in a Randomized Complete Block Design.

In 1987-88 inflorescence numbers were assessed at five-day intervals from 4 December, 1987 to 24 January, 1988 by counting open flowers on 27 individual plants in the spaced crop and by counting open flowers within a $1m^2$ permanent quadrat for each replicate in the sward crop. Number of florets per inflorescence was assessed at 10 day intervals by counting the number of florets in 30 inflorescences randomly selected from each replicate in the STC site. At the Aorangi site, the number of florets was assessed once on 15 January 1988. Number of pods per inflorescence, number of seeds per pod, thousand-seed weight and seed yield were recorded following hand harvesting five plants at the STC site and harvesting all plants within a $0.6m^2$ quadrat at the Aorangi site. Seed yields were expressed after ambient air drying with mean seed moisture contents being 10.8% and 8.5% for STC site and the Aorangi site samples respectively. In 1988-89 inflorescence numbers, floret numbers and pod numbers (at 10 January) were assessed in the same way as in the 1987-88 season. Seed yields were recorded following hand harvesting all plants within two 0.5 by $0.5m^2$ quadrats for each experimental plot. Harvest Index (HI = seed weight/straw weight) was calculated using recorded seed yields and straw dry weight after drying at 80^0C for two days.

In 1987-88, comparisons were made between untreated control plants and PP333 treated plants in terms of leaf chlorophyll content, photosynthetic activities and general plant growth.

Chlorophyll content was measured by the method described by Holden (1976) using 80% Acetone as the extracting solvent and a PU8600 UV/Vis spectrophotometer for measuring solution optical density (OD).

The results were expressed in mg chlorophyll per gram fresh tissue (mgChl/gFwt)

Comparison of photosynthetic activities was made on five untreated plants and five PP333 treated plants on 2 January 1988. For each of the following measurements, 15 shoots of similar size (about 10 nodes, and bearing one or two open flowers) were used.

(1) Shoots to be used as controls were cut from the crown and kept in moist dark conditions (no photosynthesis). These shoots were termed CD for untreated and PD for PP333 treated plants, respectively.

(2) Shoots on plants in the field were ringed (to cut off phloem translocation by removing skin tissues) immediately above the crown in order that the assimilate accumulated during photosynthesis was retained within these shoots. These plants were left in the field for photosynthesis to take place. After six hours' exposure to sunlight these shoots were taken to the laboratory for measuring leaf dry matter increase compared with the above described controls (CD and PD). These ringed shoots were termed CR and PR for untreated control and PP333 treated plants, respectively.

(3) A further 15 shoots were ringed as previously described and decapitated so that flowers, floral buds and shoot apex with folded leaves were removed. This meant that photosynthate produced by the shoots could not be translocated to other parts of the plant nor be used for new growth (shoot apex and floral).

After 7-8 hours in the field the shoots were taken to the laboratory (CRD and PRD for control and PP333 treated plants, respectively).

(4) In the laboratory, 20 small pieces ($0.146cm^2$ in area) of leaf tissue were taken from each shoot treated as described above, dried at 103^0C for 2 hours, and then weighed. Assimilation rate was expressed in $mgDM/hr.dm^2$.

In this experiment respiratory consumption of assimilate was considered to be neglible and so was omitted from the calculation. Photosynthesis by stem material was assumed to be used for maintaining internode consumption with no net import or export. Under these assumptions the assimilate accumulated by leaf photosynthesis had two sink destinations, either translocated to other parts of the plant or used by new growth of the apex (including floral growth). Therefore, the weight difference between the leaves on the shoots receiving light (and no assimilate being translocated nor used by new growth) (CRD) and leaves on the shoots kept in dark (no photo-synthesis) (CD) represents the total assimilate accumulated by the leaves (TA). If some of the assimilate was allowed to be used by new growth, the remaining portion (the difference between CR and CD) was then considered to be the assimilate retained in the leaves (ARL). The difference between the TA and the ARL is the portion used by the apex for new growth. All the three results can be expressed in the following equations:

(1), Total assimilation (TA):

    TA = CRD - CD (PRD - PD for PP333 treatment)

(2), Assimilate retained in the leaves (ARL):

   ARL = CR - CD (PR - PD for PP333 treatment)

(3), Assimilate translocated from leaves to apex (ATA):

   ATA = (1) - (2) = CRD - CR (PRD - PR for PP333 treatment).

Table 5.1: Experimental details

1987-88	STC (spaced plants)	Aorangi (sward plants)
Soil type:	Tokomaru silt loam	Kairanga silt loam
Replicate size	9 plants	3 by 5m^2 plot
Sowing rate	--	1.05kg/ha
Sowing date	19 April 1987	3 April 1987
Weed control	'Roundup', pre-sowing 'Paraquat', at 29 July	'Trifluralin', pre-sowing '2,4-DB', at 2 May
Grazing:	Nil	Grazed in 23-25 Sep.
PP333 application:		
Date;	30 Oct. 20 Nov. 10 Dec.	16 Oct. 6 Nov. 26 Nov.
Rate;	1kg ai/ha	1kg ai/ha
Harvest date;	2 Feb.	22 Feb.(delay due to rain)"
1988-89	STC (spaced plants)	STC (sward plants)
Replicate size	5 plants	3 by 4m^2 plot
Sowing rate	--	1.5kg/ha
Sowing date	24 March	24 March
Weed control	inter-row cultivation 2,4-DB, at 21 Sep.	same as for spaced plants
Insect control	Maverik, at 10 Dec.	same as for spaced plants
PP333 application:		
Date;	14 Nov. 8 Dec.	14 Nov. 8 Dec.
Rate;	1kg ai/ha	1kg ai/ha
Harvest date;	1 Feb.	1 Feb.

N.B. Treatments are termed as PP 1, PP 2,...... , etc., according
   to the time of application. Smaller numbers denote earlier
   applications

Comparisons on general growth were made on the number of main shoots

per plant and total DM from each of 6 plants from the untreated

control and PP333 treatment at the end of the experiment on 25 March

1988.

The examination of the effect of PP333 in the sward situation at the

Aorangi site involved sampling plants at the pre-peak flowering stage

(20 December). All plants in a 50cm row length were sampled in

untreated and PP 333 (second PP333 application, 6 November) treated plots. Plants parts were separated into categories, i.e., main shoots (MS), leaves on main shoots (MSL), reproductive structures on main shoots (MSR), lateral shoots (LS), leaves on lateral shoots (LSL), reproductive structures on lateral shoots (LSR) and dead material. Material in each category was dried at 80^0C for five days before weighing.

In 1988-89, comparison was made of shoot length between untreated control and PP333 (first PP333 application, 14 November) treated plants. Plants within a half meter length of row were sampled from each replicate and shoots were classified into four groups according to their node number. Thirty shoots from each group were used to measure shoot length.

5.3. Results

5.3.1. Effect on plant growth and development

The major effect of PP333 was to shorten internodes and promote lateral branching in this study (Plate 5.1). PP333 treated plants showed a more compact rosette form with shoot internodes greatly shortened (Table 5.2). Shoot branching ability was obviously enhanced by PP333 treatment, with crown shoots bearing many more lateral branches than untreated plants Table 5.3). However, numbers of crown shoots were reduced following the treatment in spaced plants. Sward plants showed a similar branch enhancing response to PP333 treatment. Although not shown in terms of shoot number, the results for dry matter (DM) distribution among different plant parts in Table 5.4

Plate 5.1. Effects of PP333 treatment II on plant growth
and branching (29 November 1987)

clearly shows enhanced lateral growth by PP333 treatment. Despite the fact that PP333 treated plants produced only half the amount of main shoot (MS) DM compared with untreated control plants, the amounts of lateral shoot (LS) DM produced were almost the same in the control and PP333 treatment. The ratio of lateral shoots to main shoots in PP333 treated plants is almost double that obtained in control plants (Table 5.4).

Table 5.2: Effect of PP333 treatment I on shoot length(cm)
          (STC site, sward plants, 1988-89)

Treatment	Shoot Category			
	6-9 nodes	10-13 nodes	14-17 nodes	18-21 nodes
Control	30.8	46.7	60.9	75.5
PP333	23.1*	26.9*	35.2*	37.0*
Treated as % of control	75%	57%	58%	49%

*: Significantly different with the control at 0.05 level.

Table 5.3: Effect of PP333 treatment II on plant growth and branching
          (STC site, spaced plants, 1987-88)

	Untreated control	PP333 treated	Treated as % of control
Number of crown shoots (19 Dec.)	27.6	12.5*	45%
Number of lateral shoots (19 Dec.)	11.5	33.5*	283%
Number of crown shoots (2 Feb.)	123	73*	60%
Plant DM weight (2 Feb.)	206	126*	61%

*: Significantly different with the control at 0.05 level.

Leaf colour in PP333 treated plants was deeper and darker than in untreated plants. Some photosynthetic characters of PP333 treated plants were measured (Table 5.5). Chlorophyll content in PP333 treated plants was not significantly higher, While photosynthetic tissue (presented as leaf dry matter), and particularly the ratio of leaf/stem, in PP333 treated plants was   enhanced compared to untreated plants (Table 5.4), neither total assimilation rate nor the

partitioning of assimilate to the apex (including shoot apex and reproductive structures) of PP333 treated plants showed superiority over the control plants (Table 5.5). There was a tendency for PP333 treated plants to show a lower total assimilation rate than control plants although the difference was not significant.

Table 5.4: Effect of PP333 on plant dry matter (DM) distribution (Aorangi site ,sward plants, 1987-88)

Sampling Category*	Untreated Control	PP333 treated	Treated as % of control
MS	150.9	74.2*	49%
LS	15.8	15.4	98%
MSL	48.5	45.7	94%
LSL	13.2	19.9*	150%
MSR	5.2	5.1	98%
LSR	2.3	3.5*	152%
DEAD	6.6	2.6*	40%
TOTAL	242.3	166.3*	69%
LS/MS	0.11	0.21	191%
LSL/MSL	0.27	0.44	159%
Leaf DM	61.7	65.6	106%
Leaf/stem	0.37	0.73	197%

N.B. Key for the category:
  MS=main shoots; LS=Lateral shoots; MSL=leaves on main shoots;
  LSL=leaves on lateral shoots; MSR=reproductive structures
  (open flowers and young pods) on main shoots;
  LSR=reproductive structures on lateral shoots;
  DEAD=dead material.
  All weights expressed in grams.
  *: Significantly different with the control at 0.05 level.

Table 5.5: Effect of PP333 on photosynthesis and assimilate distribution (STC site, spaced plants, 1987-88)

	Untreated control	PP333 treated
Chlorophyll content	1.06mg/g.FWt	1.13mg/g.FWt.
Total assimilation	8.43mgDM/hr.dm^2	6.68mgDM/hr.dm^2
Leaf retained assimilate	7.19mgDM/hr.dm^2	5.41mgDM/hr.dm^2
Assimilate to apex	1.24mgDM/hr.dm^2	1.27mgDM/hr.dm^2
% retained by leaf	85.3%	81.0%
% transferred to apex	14.7%	19.0%

N.B.: no significant difference at p=0.05.

5.3.2, Effect on flowering pattern

The effect of PP333 on flowering pattern showed some differences at the two experimental sites (spaced plants and sward situations). At the STC site, spaced plants to which PP333 had been applied showed enhanced branching (Plate 5.1). However the chemical did not allow the production of more inflorescences from lateral branches. Under this wide plant spacing where plants were allowed unrestricted growth, the advantage of improved lateral branch formation promoted by PP333 was counteracted by the larger number of fewer branched main shoots formed on plants in the untreated control. As a result, the number of inflorescences produced in PP333 treated and control plants was similar, despite some differences in the timing of peak flowering between treatments (Figure 5.1). In fact, the first PP333 treatment (30 October) reduced the number of inflorescences when compared with the untreated control.

Different results occurred in sward plants. All of the three PP333 treatments at the Aorangi site greatly increased the number of inflorescences and concentrated flowering pattern (Figure 5.2). Early application (16 October) advanced peak flowering by about 15-20 days. Mid season application (6 November) resulted in a 'normal' but increased flowering pattern. Late application (26 November) resulted in a sudden increase in inflorescence numbers at a late stage. In 1988-89 the first PP333 treatment on 14 November, (which is equivalent to the third treatment used in 1987-88) increased the number of inflorescences but the second application (8 December) failed to do so (Figure 5.3).
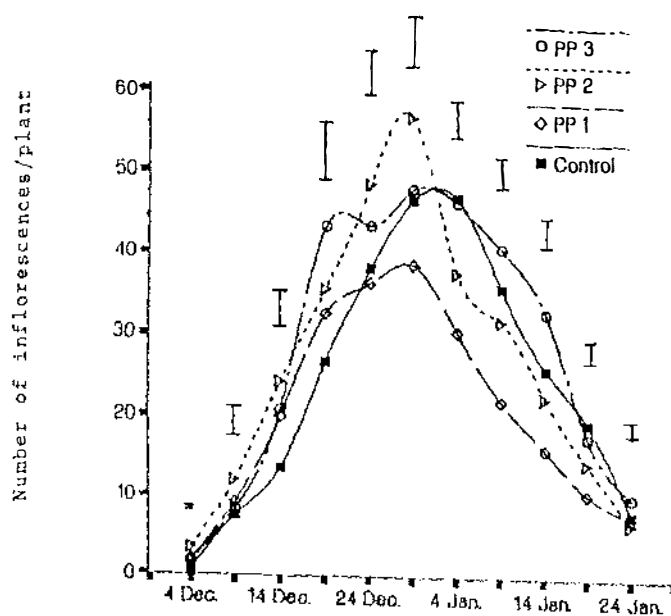
Figure 5.1. Flowering pattern in PP333 treatments (STC site, spaced plants, 1987/88)
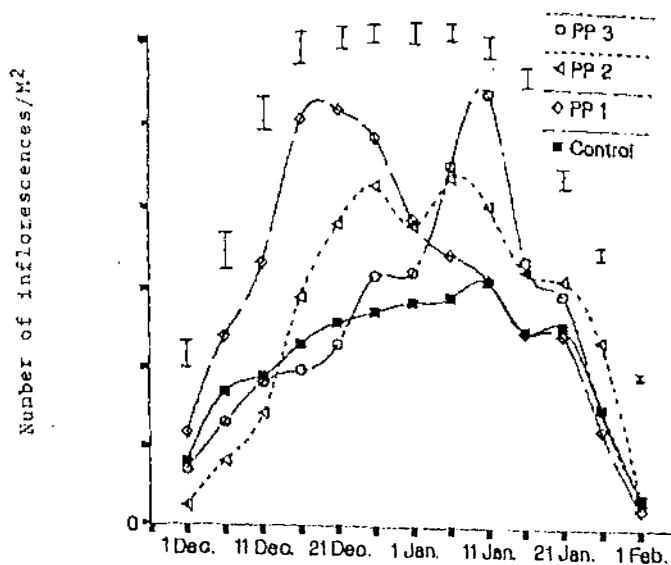
Figure 5.2. Flowering pattern in PP333 treatments (Aorangi site, sward plants, 1987/88)
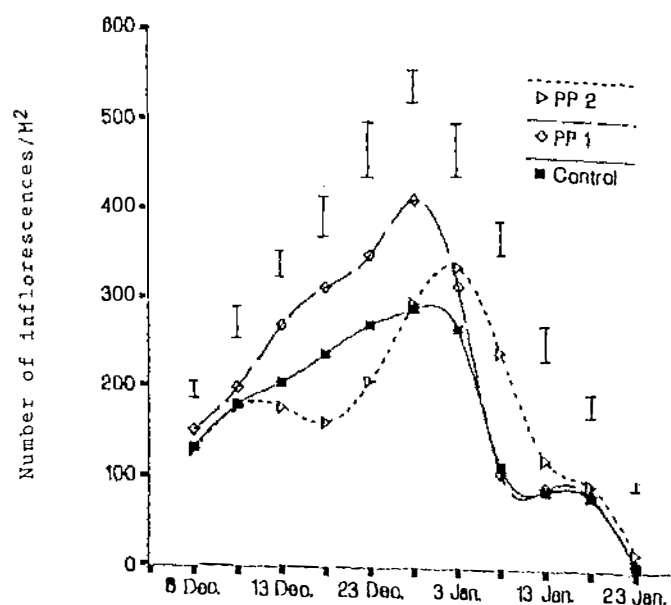
Figure 5.3. Flowering pattern in PP333 treatments (STC site, sward plants, 1988/89)

5.3.3. Effects on seed yield, yield components and seed quality

Number of florets per inflorescence showed little response to PP333 application (Figure 5.4). In 1987/88, changes in floret number in two PP333 applications (20 November and 10 December) and the untreated control were monitored during a forty day period of most intensive flowering from 15 December 1987 to 25 January 1988. Floret numbers in the two PP333 treatments were consistently similar to floret numbers in untreated control plants throughout the period (Figure 5.4). In both the 1987/88 and 1988/89 seasons, the number of florets and number of pods per inflorescence at peak flowering were recorded at the STC site (Table 5.6). It was found that PP333 treatments had no effect on the number of florets per inflorescence. However, the treatments eventually reduced the number of pods per inflorescence produced from an equal number of florets. The other two yield components (seed number per pod and thousand seed weight) showed no significant differences in response to this chemical (Appendix 6).

At the Aorangi site in 1987-88, only number of florets per inflorescence was assessed on 15 December 1987. There was no significant difference between the untreated control and any of the treatments (Table 5.6).

Seed yields showed different responses to the treatments at different sites and with different application times (Table 5.7). In spaced plants none of the PP333 treatments increased seed yield. However, in sward crops, all of the PP333 treatments, except for the second application in 1988-89 season, increased seed yield. This effect was
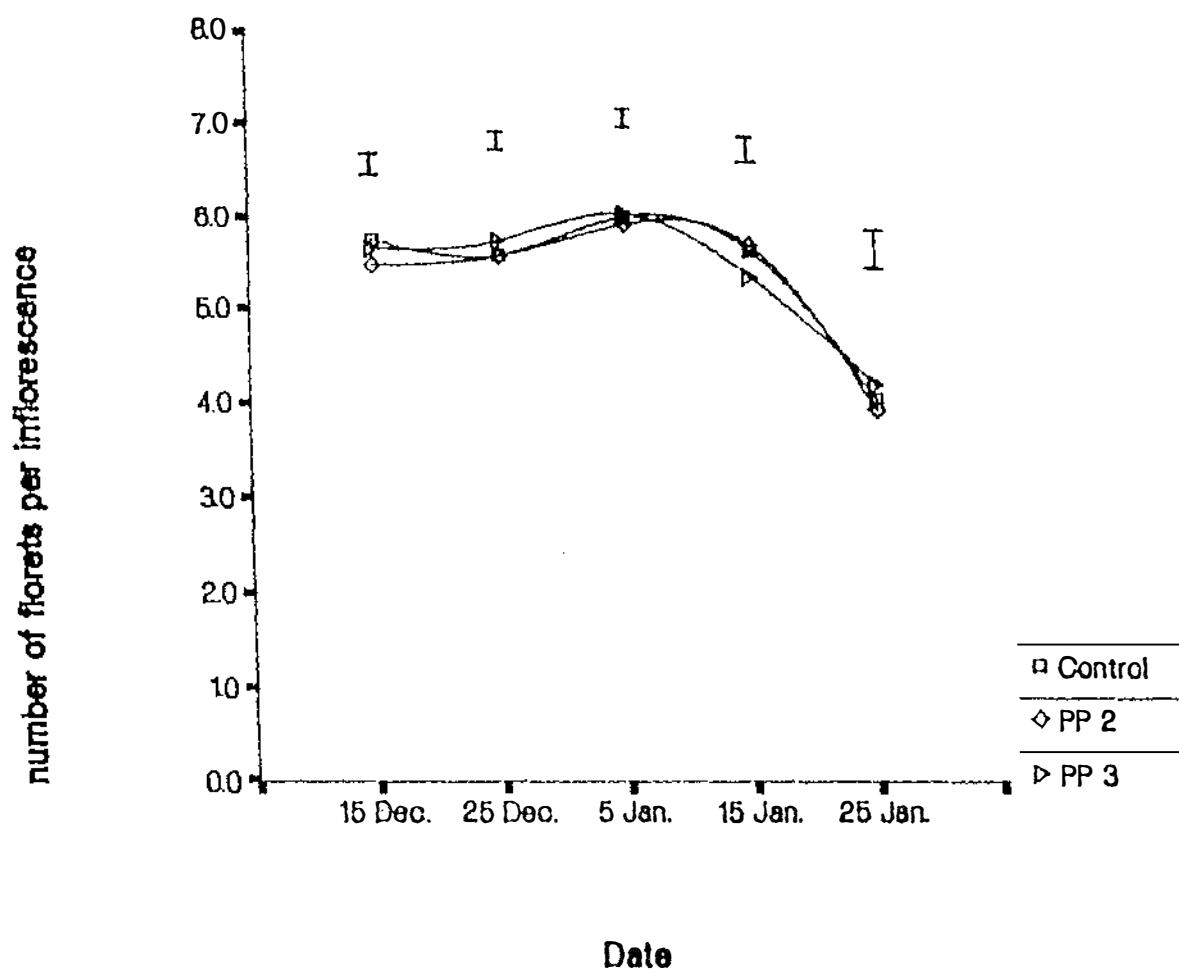
Figure 5.4. Changes in floret numbers in PP333
treatments with time (STC site, 1987/88)

well correlated with the number of inflorescences produced on plants

in different treatments.

Table 5.6: Effect of PP333 on floret numbers and pod numbers

Treatment Time	Spaced plant		Sward crop	
	Floret No.	Pod No.	Floret No.	Pod No.
1987-88	(STC)		(Aorangi)	
Untreated Control	6.0	4.6	5.5	na
Vegetative stage	na	na	5.3	na
Pre-bud Stage	5.9	4.0*	5.4	na
Flower Commencing	6.0	3.9*	5.3	na
L.S.D.(0.05)	NS	0.10	NS	--
1988-89	(STC)		(STC)	
Untreated control	5.5	4.4	5.0	4.5
Flower commencing	5.4	4.1*	5.1	4.0*
Pre-peak Flowering	5.3	3.9*	4.9	3.8*
L.S.D.(0.05)	NS	0.24	NS	0.37

N.B. *; Significantly different from untreated control at P=0.05.
    na; data not available.

Table 5.7: Seed yields and harvest index of PP333 treatments
           in spaced plants (g/plant) and in sward plants (kg/ha)

	1987-88 season				
	Untreated control	Vegetative stage	Pre-bud stage	Flower Commenc.	L.S.D. (0.05)
Spaced plants	25.3	23.8	26.7	24.1	NS
Sward plants	457	584*	632*	610*	107
	1988-89 season				
	Untreated control		Flower commenc.	Pre-peak flowering	
Spaced plants	29.4		na	20.9*	6.4
Sward plants	461		637*	473	128
Harvest Index (HI)	0.064		0.135*	0.105*	0.030

N.B.: *; Significantly different from the Control at P=0.05 level.
      na; data not available

Harvest Index (HI) was also assessed in the 1988-89 sward experiment (Table 5.7). PP333 treatments greatly reduced the straw weight at harvest, and therefore increased harvest indices regardless of whether they increased seed yield or not.

There were no obvious effects of the chemical on seed quality in terms of viability. Seed from all treatments showed high levels of viability (>95%) (Appendix 7).

5.4. Discussion

The effects of PP333 on birdsfoot trefoil were similar to those reported in other plants (Shearing and Batch, 1982; Hampton and Hebblethwaite, 1985a,b; Marshall and Hides, 1986, 1987; Clifford and Hare, 1987), with obvious effects on internode shortening and branch promotion. PP333 treatments did not appear to improve the plant's assimilation efficiency or modify assimilate partitioning in favour of reproductive growth. The overall effect was a retardation of plant growth. It is important to note that PP333 treatment reduced the number of pods per inflorescence from an equal number of florets per inflorescence (Table 5.6). Considering the effects of PP333 on plant photosynthetic character, it seems likely that this may occur as a result of lack of assimilate supply to support reproductive growth, particularly when PP333 treated plants produced many more inflorescences than untreated plants, which eventually resulted in more florets failing to develop into mature pods. Such a result is contrary to results by Hampton and Hebblethwaite (1985a, b) who found that PP333 increased the number of seeds per spikelet in perennial ryegrass.

In sugar beet Jaggard et al. (1982) showed that PP333 increased leaf numbers and leaf thickness. As a result of the thickened leaf, chlorophyll content and photosynthesis expressed in unit leaf area were also increased. However, photosynthesis expressed per leaf and per unit weight of chlorophyll decreased following PP333 treatment. PP333 application also reduced leaf area by 68-78%. Overall, the final yield of sugar beet (both root and total biomass) was reduced by PP333 treatment. Results in the present study generally agree with these results, showing that PP333 has no obviously beneficial effect on the plant's photosynthetic ability.

In this study, measurements on plant photosynthesis characteristics were based on the assumption that leaf photosynthesis was the only source activity which influenced assimilate importation to reproductive parts. Respiratory consumption of assimilate and stem material photosynthesis were considered negligible or omittable since they are supposed to operate in a proportional basis regardless of the different sizes of the plants in untreated control and in PP333 treatments. Experiments using more sophisticated techniques, such as isotope labelling CO_2 for monitoring assimilate translocation, would seem to be desirable for further confirming the results obtained in this study.

Potential seed production in a plant is basically determined by two factors, the sites available for reproductive development and assimilate supply for reproductive growth. The two major effects of PP333 on seed production are somewhat conflicting in birdsfoot trefoil. While the increased branching resulting from PP333 treatment

may provide more sites for flowering (thus increasing inflorescence numbers), the negative effect of PP333 on plant growth may limit assimilate supply for reproductive growth. Therefore, the value of PP333 on seed production may largely depend on the situation in which the crop is grown. In swards where a tightly closed vegetative canopy occurs at flowering, late crown shoot emergence is greatly restricted. In such situations, enhanced shoot branching as a result of PP333 treatment has a great advantage in creating more sites for flowering. The beneficial effect of PP333 in increasing seed yield in such circumstances occurs mainly as a result of increased numbers of lateral branches. In spaced plant situations unrestricted development of crown shoots occurs with fewer lateral branches being formed. The increased lateral branching encouraged by PP333 treatment in sward grown plants does not occur in spaced plants, and therefore PP333 treatments are not advantageous in providing increased inflorescences for enhancing final seed yield.

Results from PP333 applications at different stages of plant development in this study suggest that early application (during active vegetative growth up to the commencement of flowering) is more suitable for enhancing seed yield than later application. This comfirms the results found in the hand removal treatments (chapter 4) which shows that shoot manipulation should be carried out before shoots becoming fertile and incapable of branching. Late application, even though it may promote a larger number of small lateral branches, is not beneficial in increasing the current year's seed production, since the late season photoperiod does not allow these small branches to become fertile. Very early application is also not recommended since it may prevent plants from reaching a reasonable

size for seed production. Similarly, Clifford and Hare (1987) found
that early (September) application of PP333 did not increase final
seed yield in Grasslands Maku lotus (*Lotus pedunculatus*. Cav.).

There are different opinions on the value of PP333 as a plant
regulator for improving seed yield. Increased seed yields following
PP333 application have been reported in a number of pasture plants
including ryegrass (Hampton and Hebblethwaite, 1985a, b), white
clover (Marshall and Hides, 1986, 1987), *Lotus corniculatus* and
Lotus pedunculatus (Clifford and Hare, 1987; Hampton *et al*., 1989).
However, White *et al*. (1987) simply eliminated the use of PP333 in
birdsfoot trefoil by suggesting that PP333 had an injurious effect on
plant growth. Even in cases where increased yield has occurred
following PP333 use, there is little information on the mechanisms
involved, particularly on the physiological effects of this chemical
on the growth and development of plants. The results in this study
show that PP333 does retard plant growth. However, it also
effectively promotes branching and so increased inflorescence
numbers. Particularly in the more normal 'sward' situations under
which *Lotus corniculatus* is commonly grown for commercial seed
production. PP333 appears to show considerate promise for improving
seed yield in this species. This is particularly the case when the
chemical is applied to plants from the pre-bud to the beginning of
flowering stage. Seed yields obtained from such situations were in
excess of 600kg/ha (about 40% over untreated control) which is
considered to be high by commercial seed production standards in New
Zealand.

Bulky vegetative materials accompanying reproductive parts at harvesting often cause problems which reduce seed recovery in pasture species. Wiggans et al. (1956) suggested using desiccants to ease this problem in Lotus corniculatus. Results from this study suggest that PP333 treatment may be another remedy for this problem through its effect of reducing vegetative growth and thereby increasing Harvest Index (HI).

Results from this study support the suggestion that a shoot manipulation approach can be advantageous in improving seed yield and have demonstrated the potential of PP333 as a plant growth regulator for manipulating pre-flowering shoot status in Lotus corniculatus. Although PP333 has some side-effects on plant growth (eg., retardation), its major branch promoting ability seems to be a valuable characteristic for enhancing seed yield in Lotus corniculatus. However, in using this chemical for seed production in this species, care should be taken to consider the timing of application and the plant population situation. Best results from application of this chemical are likely to be achieved in a sward or spaced row situation, the chemical being applied before flowering when the crop has a large population of crown shoots which are capable of responding to the chemical's branch stimulating characteristics.

CHAPTER 6: EFFECT OF POST-PEAK FLOWERING SHOOT MANIPULATION ON SEED PRODUCTION

6.1. Introduction

As discussed in previous chapters, both pre-flowering and post-flowering shoot populations are likely to affect seeding potential and ultimate seed yield in Lotus corniculatus. The presence of a large number of shoots at flowering is obviously important, since the number of fertile shoots and subsequently the number of inflorescences are well correlated with final seed yields. The study reported in chapter 5 has shown the promising effects of PP333 as a pre-peak flowering shoot manipulating chemical on improving seed yield in this plant. Perhaps as important, however, is the potentially negative effect of the continued production of new shoots after peak flowering. This post-flowering 'flush' of new shoots appears to provide an intensely competitive 'sink' for assimilate and may therefore be considered to be 'undesirable' on plants being grown for seed production. It is assumed that depressing such late shoots after peak flowering may be an equally important management approach for enhancing seed yield in Lotus corniculatus. Studies were therefore carried out to investigate the effect of two different chemicals (Nortron and Fatol Super) applied at different times to assess their effectiveness as post-peak flowering shoot manipulating chemicals for enhancing seed production in this species.

Nortron (2-ethoxy-2, 3-dihydro, 3-dimethyl benzofuran-5-yl methane

sulphonate, also known as Ethofumesate, Tramat) is a herbicide which inhibits mitosis in actively growing young tissues. The initiation of new shoot primordia inevitably involves cell division while the growth of older shoots occurs mainly through cell enlargement. This chemical was selected for this experiment in an attempt to prevent late new shoot emergence without harming the growth of already formed older shoots.

Fatol Super (fatty alcohols, primary linear alcohols of chain lengths C_6-C_{12}) is a 'sucker killer' which has been successfully used in tobacco crops for controlling lateral buds (suckers) (Steffens and Selfmann, 1982; O'Connor, 1984). Since this chemical is known to be particularly injurious to young plant tissue with little or no harmful effects on old tissue, it was selected for use in this study in an attempt to destroy late-formed shoots.

6.2. Materials and methods

Experiments were carried out during 1987-88 at the same locations as described in Chapter 5. The first site, at the Seed Technology Centre (STC), Massey University, involved spaced plants with a row width of 80cm and intra-row spacing of 60cm. The second site, at the Department of Scientific and Industrial Research's experimental farm at Aorangi, involved a crop grown in 60cm rows with 64 plants per metre of row. At neither site was fertilizer or irrigation applied during the experimental period. The two chemicals (Nortron and Fatol Super) were applied three to five times at different plant development stages at each site. Management and treatment details are presented in Table 6.1. All treatments at each site were

Table 6.1: Experimental details

	STC (site 1)	Aorangi (site 2)
Soil type:	Tokomaru silt loam	Kairanga silt loam
Replicate size	9 plants	3 by 5m^2 plot.
Sowing rate	--	1.05kg/ha
Sowing date	9 May 1987	3 April 1987
Weed control	'Roundup', pre-sowing 'Paraquat', 29 July	'Trifluralin', pre-sowing '2,4-DB', 2 May
Grazing:	- - Nil	Grazed in 23-25 Sep.
Nortron application		
Date;	25 Oct. 15 Nov. 5 Dec.	27 Oct. 17 Nov. 7 Dec.
Rate;	1% solution 20ml/plant	2.5% solution 300 litres/ha
Fatol application		
Date;	2 Nov. 22 Nov. 12 Dec. 2 Jan. 2 Jan.*	6 Nov. 26 Nov. 21 Dec. 21 Dec.*
Rate;	3% solution 20ml/plant	3% solution 300 litres/ha
Harvest date;	2 Feb. to 9 Feb.	22 Feb.(delay due to rain)

N.B. 1, For each site, treatments are termed Nortron 1, Nortron 2,
...... Fatol 1, Fatol 2, etc., according to the time of
application; smaller numbers denote earlier application.
2, Fatol 4*, 1.5% rate, double volume of application←.

replicated three times in a randomized complete block design.
Inflorescence numbers were assessed at five-day intervals by counting
open flowers on nine plants in each replicate at the STC site (site
1) and by counting open flowers within a 1m^2 permanent quadrat for
each replicate at the Aorangi site (site 2). The number of florets
per inflorescence was assessed at 10 day intervals by counting floret
numbers in 30 inflorescences randomly selected from each replicate at
site 1. At site 2, the number of florets was assessed once at the
post-peak flowering stage on 15 January 1988. Number of pods per
inflorescence, number of seeds per pod, thousand-seed weight and seed
yield were recorded following hand harvesting of 5 spaced plants
(site 1) or following the harvesting of all plants within a 0.6m^2
quadrat (site 2). Seed yields were expressed at mean seed moisture
contents of 10.7% and 8.4% for sites 1 and 2 respectively.

6.3. Results

6.3.1. Effect on plant growth and development

Nortron had a strong inhibitory effect on the emergence of new shoots arising from the crown (Plate 6.1). Nortron-treated plants showed disrupted growth and development, the production of crown shoots being much reduced following application. Already-formed shoots also showed injury symptoms which were readily seen as a twisting or folding of the shoot apices and floral buds. Leaf tissue became slightly yellow and flower corollas occasionally showed a purple-red colouration. As well as inhibiting shoot growth and development, Nortron also had an injurious 'herbicidal' distorting effect on flowers (Plate 6.1).

The effect of Fatol Super was to cause 'burning' symptoms to appear 1-2 days after application (Plate 6.2). Young shoots appeared to be particularly sensitive to the chemical. Leaves on young shoots first wilted, and, finally, the entire shoot etiolated and died. Older shoots were less sensitive to Fatol Super and often showed no or only slightly injurious symptoms. Flowers showed no visible symptoms of injury. This chemical temporarily controlled the emergence and growth of young shoots . However, since Fatol Super is a contact "sucker killer', its effect is rapid but short lived. Several days after treatment new shoots emerged from the crown on plants which had been previously treated. The use of this chemical was effective in killing young vegetative shoots present at the time of application, but was of only limited value in controlling regrowth of new shoots.

Plate 6.1. Effects of Nortron treatment on plant growth
(29 November 1987)

Plate 6.2. Effects of Fatol Super treatment on plant
growth (29 November 1987)

6.3.2. Effect on flowering pattern

At both sites the number of inflorescences produced in Nortron treatments was generally lower than in untreated plants and fluctuated greatly, particularly at the STC site (Figure 6.1). Early treatment (late October) had less harmful effects on flowering, probably because application was more remote from the time of intensive flowering (early January), allowing plants a longer period of recovery growth following injury. Later applications (mid November and early December) showed very obvious effects on flowering, the number of inflorescences dropping significantly following chemical application (Figure 6.1).

At the Aorangi site all three Nortron treatments reduced the number of inflorescences produced during the early flowering season. However, plants in the first two treatments eventually recovered from injury and at peak flowering the number of inflorescences in these two treatments was similar to the untreated control (Figure 6.2)

As already mentioned, Fatol Super treatments had no effective long term effect in controlling the continuous emergence of new shoots, although treatment did not apparently harm reproductive structures on old shoots. Consequently, flowering pattern was not greatly influenced by Fatol Super treatment (particularly the two early treatments). At the STC site treatments 1, 2 and 4 slightly increased inflorescence numbers at peak flowering, but treatment 3 reduced the number of inflorescences (Figure 6.3). At the Aorangi site treatment 3 also reduced the number of inflorescences present in sward plants during late flowering (Figure 6.4). Treatment 2 increased the number
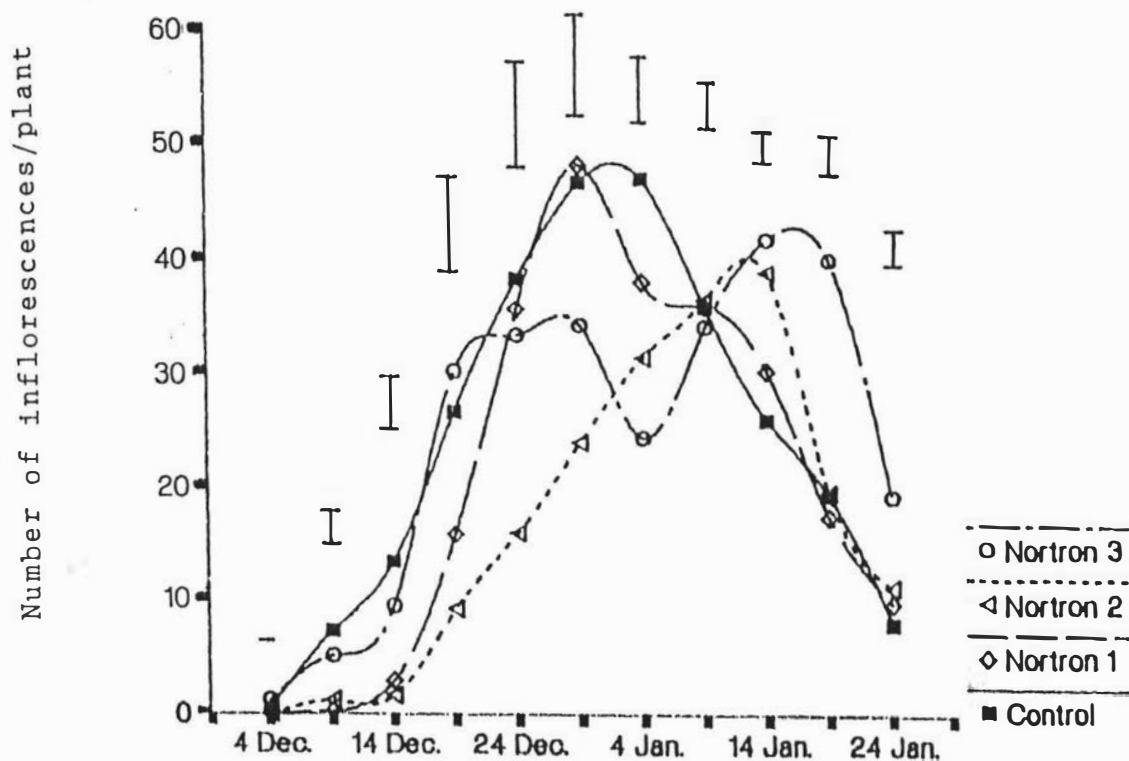
Figure 6.1. Flowering pattern in Nortron treatments
(STC site, spaced plants, 1987/88)
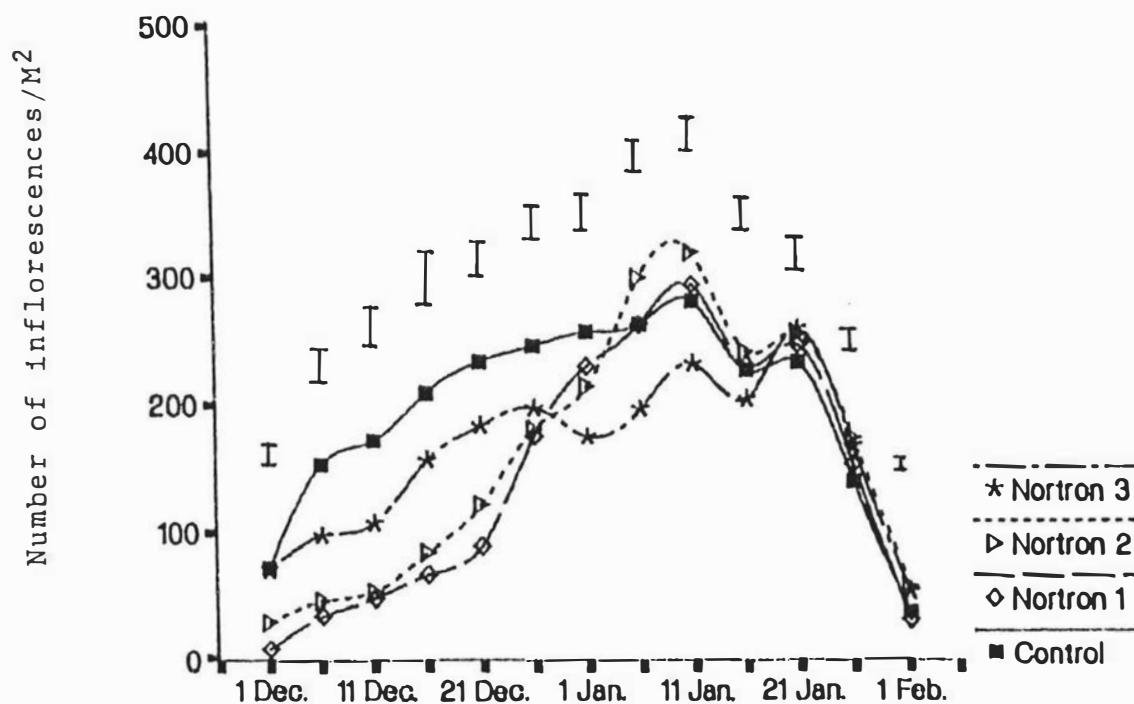
Figure 6.2. Flowering pattern in Nortron treatments
(Aorangi site, sward plants, 1987/88)
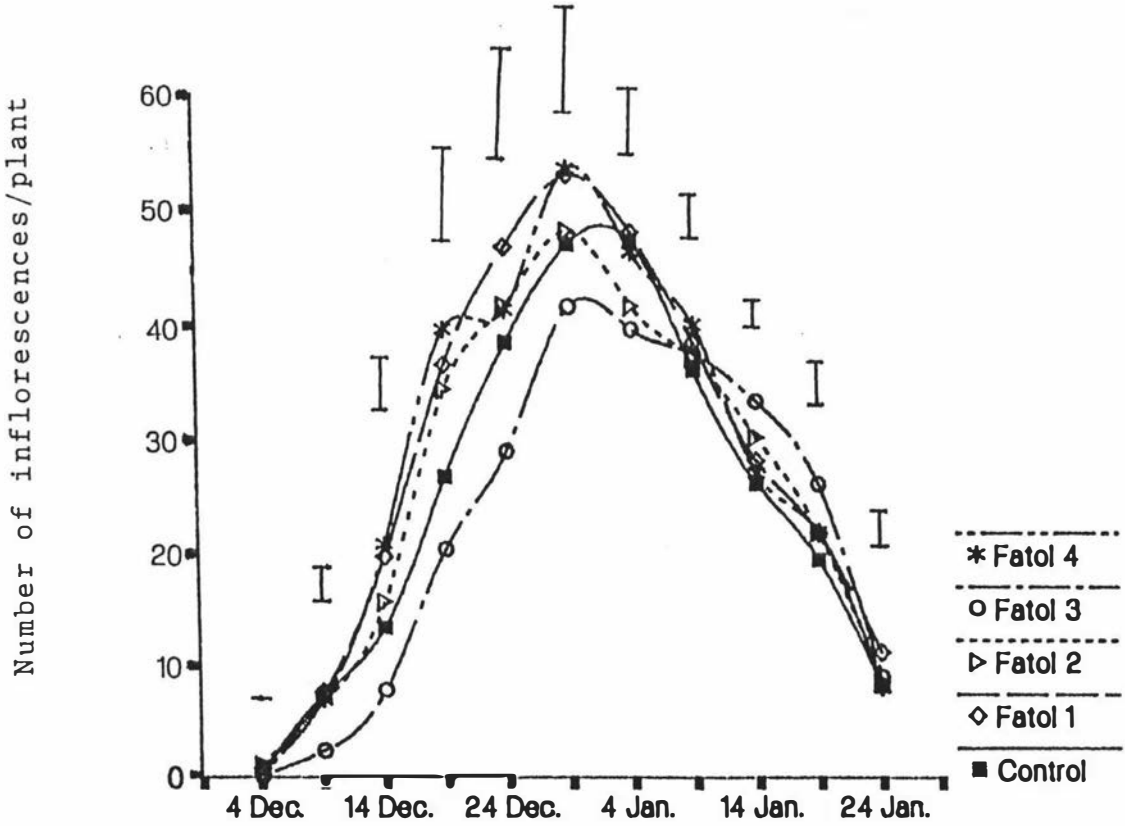
Figure 6.3. Flowering pattern in Fatol Super treatments
(STC site, spaced plants, 1987/88)
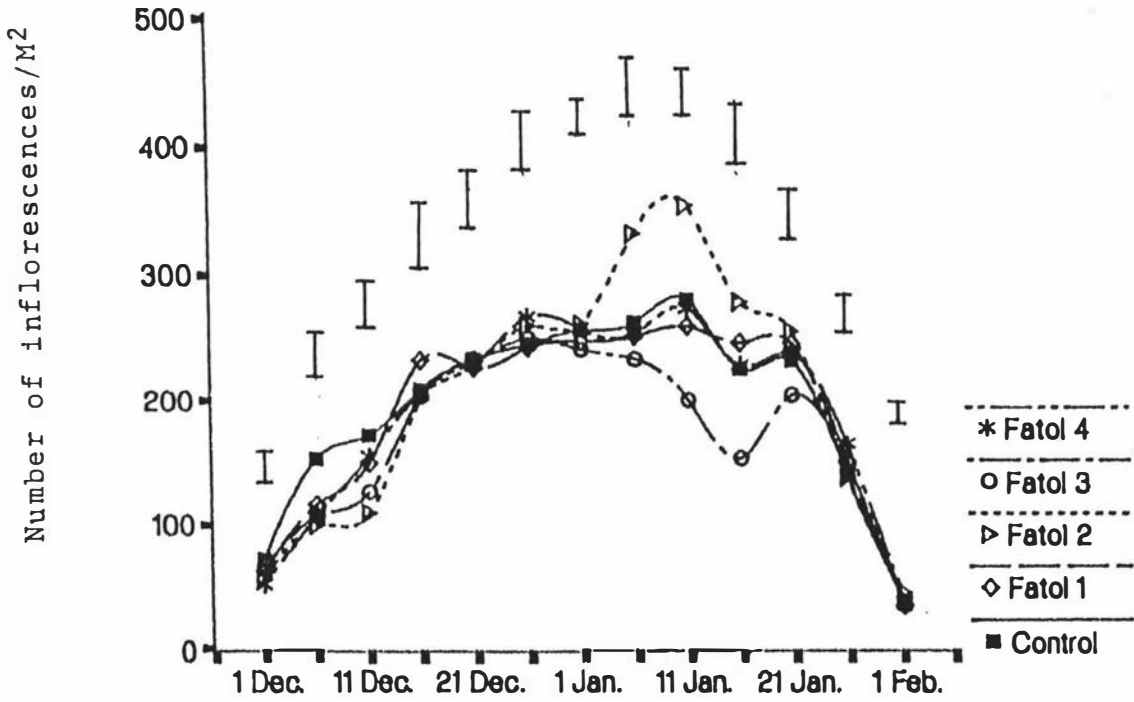
Figure 6.4. Flowering pattern in Fatol Super treatments
(Aorangi site, sward plants, 1987/88)

of inflorescences, the remaining two treatments showing no obvious effects on flowering pattern.

6.3.3. Effects on seed yield components

At the STC site, the number of florets and number of pods per inflorescence were assessed in four treatments (Nortron 2, 3 and Fatol 2, 3) during the period of most intense flowering from 15 December 1987 to 25 January 1988. While Nortron treatments obviously reduced the ability of inflorescences to bear florets (Figure 6.5), Fatol Super treatments generally increased the number of florets per inflorescence (Figure 6.6). In order to calculate potential seed yield and to compare the effects of different treatments, seed yield components (using floret numbers per inflorescence present at peak flowering on 5 January) are presented in Table 6.2. Number of pods per inflorescence were recorded once on 22 February and were found to show similar treatment responses to floret patterns. However, pod numbers were approximately 20-30% lower than the number of florets per inflorescence. The other two yield components (seed numbers per pod and thousand seed weight) showed no significant differences between the chemical treatments and the untreated control (Table 6.2).

At the Aorangi site, the number of florets per inflorescence was assessed only on 15 December 1987, there was no significant difference between the untreated control and any of the chemical treatments.
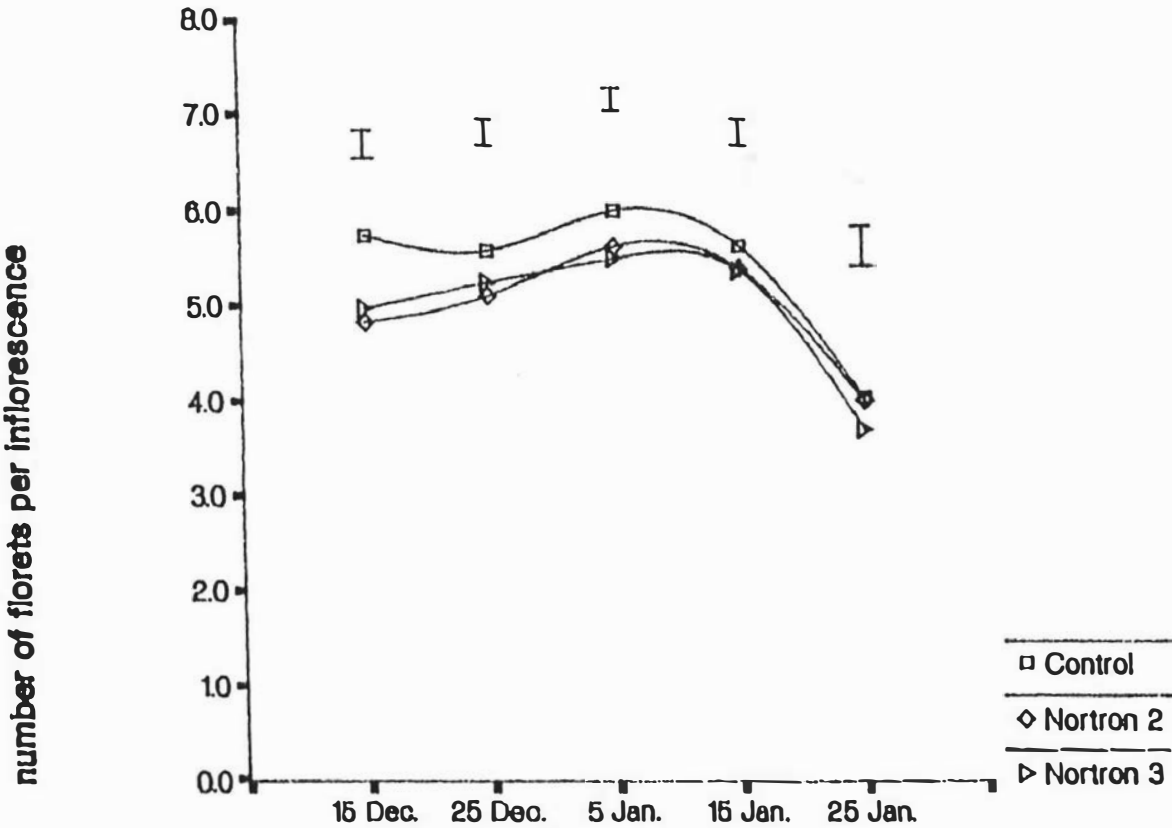
Figure 6.5. Changes in floret numbers in Nortron
treatments with time (STC site, 1987/88)
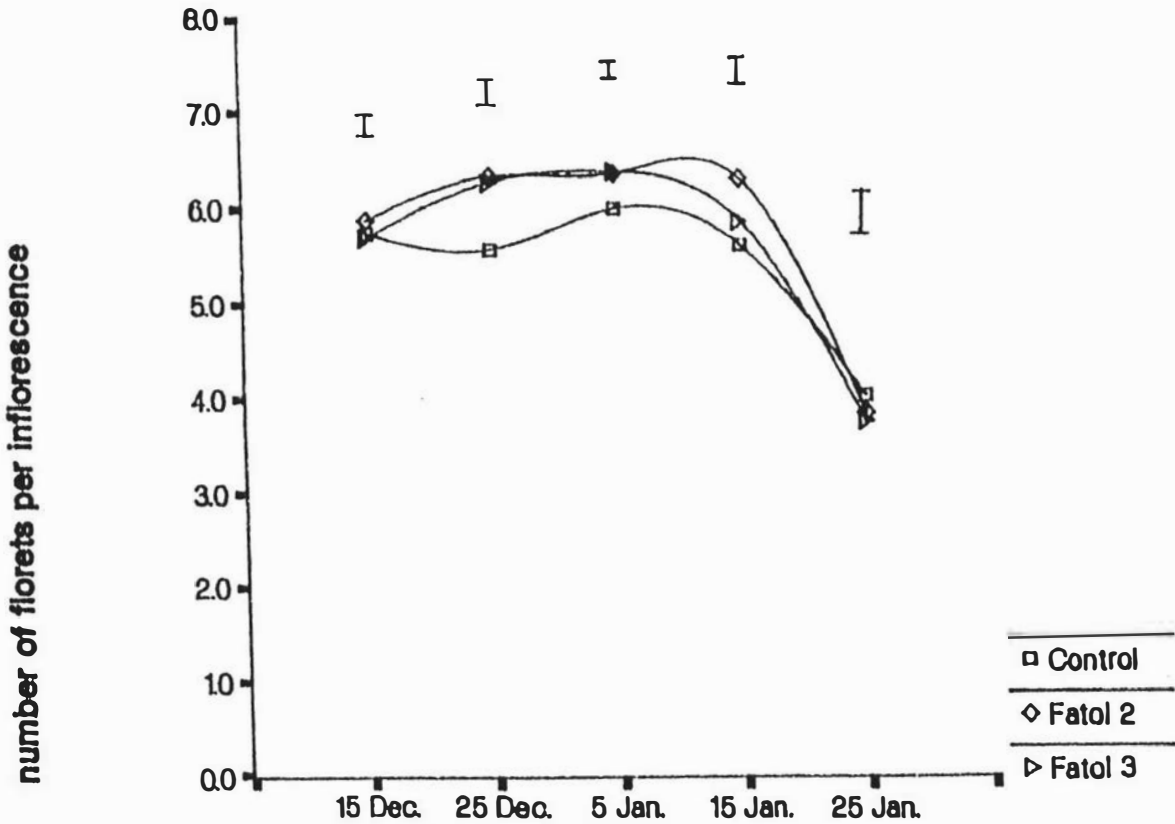
Figure 6.6. Changes in floret numbers in Fatol Super
treatments with time (STC site, 1987/88)

Table 6.2: Seed yield components (STC site)

Treatment	Inflorescence No.		Florets/	Pods/	Seeds/	1000-Seed
	Total	25 days+	Inflor.	inflor.	Pod	weight
Control	2416	1510	6.00	4.58	13.8	1.35
Nortron 1	2109	1420	na	na	13.8	1.30
Nortron 2	1706	1177	5.62*	4.12*	15.1	1.37
Nortron 3	2460	1210	5.49*	4.16*	13.3	1.54
Fatol 1	2793	1667	na	na	11.6	1.32
Fatol 2	2571	1507	6.36*	4.94*	12.9	1.36
Fatol 3	2209	1321	6.38*	4.77*	14.2	1.27
Fatol 4	2739	1622	na	na	12.3	1.37
Fatol 4*	na	na	na	na	13.1	1.25

N.B.: *; Significantly different from the Control at P=0.05.
+; Inflorescences blossoming during the 25 days of most intense flowering (peak flowering ± 12 days).
na; data not available.

6.3.4. Effects on Seed yield and seed quality

Seed yield data at the two sites are presented in Table 6.3. In spaced plants at the STC site, none of the three Nortron treatments increased seed yield. However, in the sward at the Aorangi site, early Nortron treatment (27 October) significantly increased seed yield by about 35%. The reverse effect occurred in the Fatol Super treatments. Late applications of Fatol Super to spaced plants at the STC site increased seed yield, but early applications to the spaced plants and all applications at the Aorangi sward plants resulted in no increase in seed yield.

Table 6.3: Seed yields in Nortron and Fatol super treatments

Treatment	Seed yield STC (g/plant)	Seed yield Aorangi (kg/ha)
Control	25.3	457
Nortron 1	25.4	654*
Nortron 2	25.2	500
Nortron 3	22.4	458
Fatol 1	26.0	464
Fatol 2	23.2	534
Fatol 3	29.8*	459
Fatol 4	34.6*	na
Fatol 4*	35.4*	455
L.S.D.(0.05)	3.8	107

N.B.: na; data not available.

The two chemicals used in this study had no detrimental effect on seed viability, despite the fact that Nortron had an obvious visible effect on plant growth. Seed from all treatments showed high levels of viability (>95%), although minor differences existed in the percentage of hard seeds and normal seedlings (Appendix 7).

6.4. Discussion

The injurious effects of Nortron on plant growth reduced its value as a plant regulator for manipulating shoot status in birdsfoot trefoil. Nortron is a soil conducted, slow acting chemical. Several weeks following application were needed before its effects on plant growth became visible. Early application (prior to the flower-bud stage) seemed more suitable than late application in manipulating plant structure for improved seed production. The directly injurious effect of this chemical on flowers may negate its shoot manipulating effects if it is applied at a late stage when plant reproductive growth has already commenced. Although this chemical may have other benefits, (such as weed control), it appears to offer little value for use in post-peak flowering shoot manipulation.

In the spaced plant situation at the STC site, none of the Nortron treatments improved seed yield, mainly as a result of reduced inflorescence numbers following chemical application. However, early application of this chemical resulted in yield enhancement in the sward situation. At the Aorangi site the fact that Nortron treatments 2 and 3 did not outyield the untreated control is explained by the fewer inflorescences produced in these two treatments, possibly due

to the injurious effect of the chemical. However, a higher seed yield was recorded in the Nortron 1 treatment. It is possible this increased seed yield occurred through a combined effect of this chemical on plant growth and weed control. It was observed that plants in the Nortron treatments were comparatively free of weeds (particularly white clover). Although Nortron was injurious to plant growth, the earliest (27 October) application actually did not affect plant flowering behaviour at the time of most intense flowering. Presumably this timing allowed plant recovery before full flowering of the crop (early January). This is shown by the fact that the number of inflorescences produced at peak flowering in this treatment was similar to that produced in untreated plants, despite a reduction in inflorescence numbers during early flowering.

The effect of Fatol Super was clearer. This chemical simulated the effects of hand removal treatments described earlier in chapter 4 and appears to show considerable promise in the manipulation of the post-peak flowering shoot population. However, the fact that it has only a short term effect, which visibly disappeared within ten days following application, suggests that a single application is insufficient to achieve long term advantages in controlling the continuing emergence of new shoots. Early application (pre-peak flowering) of this chemical is obviously not appropriate since it may simply reduce shoot numbers available for flowering. Post-peak flowering application, however, is likely to be beneficial in reducing competition for assimilate by late emerged shoots. The increased floret/pod numbers per inflorescence following Fatol Super application possibly indicates a competition release effect. The increased seed yields obtained following the two late applications at

the STC site, in particular, suggest Fatol Super might be of value as a plant growth regulator for reducing post- flowering vegetative shoot competition in <u>Lotus corniculatus</u>.

At the Aorangi site there were problems with spray application of this chemical in ensuring it penetrated the vegetative canopy and reached the site of activity at the crown where new shoots arise. Because of its contact-action, Fatol Super solution must drain down the stem to reach the young vegetative crown shoots. At peak flowering, sward crops of <u>Lotus corniculatus</u> have a large vegetative canopy which effectively prevents or restricts spray penetration to the crown. This tightly closed canopy at the Aorangi site perhaps accounted for the failure of this chemical to control late shoots. It was also observed at the Aorangi site that under almost entirely closed canopy conditions very few new shoots were formed naturally in the heavily shaded crown area during the post-peak flowering period.

The different responses from Fatol Super application at different times and under different cropping situations suggests that this chemical is only likely to be advantageous for post-peak flowering shoot control in crops grown in wide rows where adequate spray penetration to the crown is more readily possible. Its short term action also makes repeat application necessary. Nevertheless the improved seed yield associated with late applications at the STC site demonstrates the potential of this chemical for achieving better seed production in <u>Lotus corniculatus</u>, particularly when crops are grown at low shoot densities

Results obtained in this chapter suggest the possibility of a late shoot manipulation approach for improving seed production in Birdsfoot trefoil, although there is much more research needed to be done on this subject. The use of Fatol Super, particularly late in the season and at low plant population densities looks promising, despite the fact that there are physical problems in using this chemical at high plant populations or it may be unnecessary. The results also suggest potential in the use of Nortron applied at the early plant growth stage (prior to flower bud stage), provided it is applied early enough to allow plants to recover from its injurious effects on plant growth and flowering.

CHAPTER 7: GENERAL DISCUSSION AND CONCLUSION

The protracted flowering characteristic which results from the indeterminate growth habit of Lotus corniculatus has been a focus in discussing factors responsible for its poor seed production (MacDonald, 1946; Seaney and Henson, 1970; McGraw et al., 1986, 1986). Results in the present study comfirmed that flowering in this plant often occurs over a period of more than two months during a three year experimental period. The extended flowering period reported in this study is comparable to that described by other research reports (Joffe, 1958; McGraw and Beuselinck, 1983).

Despite the fact that the protracted flowering character of Lotus corniculatus has been consistently mentioned by previous researchers (MacDonald, 1946; Joffe, 1958; Seaney and Henson, 1970; McGraw and Beuselinck, 1983; McGraw et al., 1986; Beuselinck and McGraw, 1988), there appear to be no detailed studies which have actually investigated the cause or causes of this feature of plant development. Results in this study indicate that Lotus corniculatus has a special characteristic since a long flowering period is sustained by a continual shoot succession rather than by a continuation of flowering alone. Some other legumes such as white clover form flowers continuously along fertile stolons. Such plants exhibit a long flowering period because there can be a variable delay in the number of vegetative nodes between any two successive flower buds (Thomas, 1987). In Lotus corniculatus stems do not produce vegetative nodes between successive flowers. Also, each shoot bears only a limited numbers of flowers (usually 3-5), all of which bloom

within a comparatively short period. It is clear that in this plant,
flowering duration on individual shoots is not responsible for the
long flowering period. Protracted flowering is created by the
continuous shoot succession in that old shoots die when new shoots
are formed and become fertile rapidly. This is a continuous process,
which provides new shoots for flowering until environmental
conditions (daylength in particular) are no longer suitable for
floral initiation or floral development. Although birdsfoot trefoil
is botanically classified as an indeterminate plant because its
reproductive developmental sequence (flower bud initiation) is from
the base to the apex along a shoot, the flowering behavior of
individual shoots is more determinate since only limited numbers of
flowers are produced and a shoot ceases growth after it flowers.
However, the development of the shoot system in <u>Lotus corniculatus</u> is
indeterminate, since the production of new shoots is a continuous
process which sustains the lengthy flowering period. For this reason,
the manipulation of shoot status seems to be a most realistic
approach in attempting to improve seed production in this plant.

In this study an effort was made to investigate the relative
importance of different seed yield components in determining final
seed yield (i.e. inflorescence numbers, number of florets, pods per
inflorescence, number of seeds per pod and seed weight). A regression
technique was used to identify the relationships between seed yield
and each of these yield components.   The 1986/87 study was carried
out on intact plants (no treatments imposed). Regression results
indicate that the number of inflorescences is the only factor having
a significant correlation with final seed yield (chapter 3). In the
1987/88 study, data on seed yield and yield components were collected

from plants subjected to various treatments (including four hand removal treatments, six chemical applications and one untreated control) for multiple regression analysis (Appendix 8). Results in both 1986/87 and 1987/88 show that the number of inflorescences (per unit area or per plant) is the most important component determining final seed yield. This agrees well with similar findings by Albrechtsen, 1966; Mos, 1983; Stephenson, 1984; McGraw et al., 1986. However, by using calculated potential yield data, the regression result obtained in 1987/88 also shows that number of pods per inflorescence can have some, although less important, influence in determining final seed yield. This agrees with results by Stephenson (1984) and McGraw et al., (1986) who have shown that inflorescence number is the greatest, pod number and seed number intermediate, and seed weight the least sensitive yield component affected by cultural practices.

Since virtually no sterile shoots exist under flower induction weather conditions (a result also reported by McKee (1963) and Nitter and Kenny) (1964)), and individual shoots from different age groups have similar flower bearing ability, it is suggested that the number of shoots present at the time of most profuse flowering is an important factor influencing final seed yield in Lotus corniculatus (Chapter 2). The analysis of seed yield components also justifies the shoot manipulation strategy as a likely method for improving seed production in this species.

The two shoot manipulation approaches used in this study differed in their emphasis on shoot status and were seemingly contradictory. Pre-peak flowering shoot manipulation aimed at encouraging the production

of an increased population of sites on shoots at flowering, while efforts to manipulate the post peak-flowering shoot population aimed at depressing the production of late shoots which were incapable of flowering, but which were likely to act as a strong competitive sinks for assimilate to the detriment of assimilate available for reproductive growth.

A series of hand removal treatments (chapter 4) were designed to examine the possibility of these two approaches. Results from these hand removal treatments in spaced plants exploited the concept of shoot manipulation as a means of improving seed yield in <u>Lotus corniculatus</u> and showed that both of these approaches have potential for improving seed yields. The results have shown that shoot status in this plant is readily adjustable in terms of life span, branching behaviour and flower bearing ability by shoot manipulation.

Cutting treatments (mechanical topping), regardless of timing and severity, proved to be unsuitable as shoot manipulation methods for enhancing seed yield, since cutting was ineffective in increasing shoot branching ability or 'releasing' competition from late shoots. These findings agree well with results by previous workers (Anderson and Metcalfe, 1957; Winch, 1958; Bader and Anderson, 1962). The generally lower seed yields obtained from the cutting treatments used in this study further discourage the use of cutting as a practical method for improving seed production in this plant.

A more practical approach was to use chemicals for shoot manipulation. PP333 was selected as a pre-peak flowering manipulator. This chemical appears to be very successful in improving seed yield

in sward situations through its effectiveness in promoting lateral branching on main shoots, a result also reported by Marshall and Hides (1986, 1987) in white clover. As a result of the enhanced branching ability following PP333 application, more inflorescences were formed which eventually contributed to an increased seed yield. It appears that the most appropriate application time for this chemical should be before the majority of shoots become fertile in order to achieve maximum branch enhancement and maximum flower numbers at peak flowering. Nearly a 40% increase in final seed yield was obtained consistently in two years' experiments when PP333 was applied during the period from the pre-bud to early-flowering stage. Later application, and the application of this chemical to widely spaced plants showed little value in increasing seed yield. PP333 application was found to have no beneficial effects on plant photosynthetic activity and assimilate partitioning for seed production in Lotus corniculatus. An obvious growth retarding effect was found in PP333 treated plants. It seem likely that this effect influenced the percentage of open flowers developing into mature pods. This result is contrary to results by Hampton and Hebblethwaite (1985a, b) who found that PP333 increased the number of seeds per spikelet in perenial ryegrass. Very early application of PP333 is not recommended since its growth retardant effect may prevent plants developing to their full size at flowering.

The two chemicals chosen for post-peak flowering shoot manipulation (Nortron and Fatol Super) showed differences in their effectiveness of shoot control, effects on plant growth and duration of the shoot control. Nortron seemed to be variable in its effectiveness in controlling late shoots. The fact that increased seed yield was only

obtained following early application of this chemical suggests that Nortron may have a role in improving seed production through an effect on pre-peak flowering shoot manipulation. Its beneficial effect in manipulating structure, however, might be negated by its injurious effect on plant growth and, particularly on flowering. These effects are particularly severe if the chemical is applied too late when plants are beginning to flower or flowering. An observed weed control effect may also partially account for its success in increasing seed yield when applied early.

Fatol Super had a more desirable late shoot control effect. This chemical simulated hand removal treatments and successfully controlled late season growth of young shoots. Post-peak flowering application of Fatol Super significantly increased seed yield in spaced plants. However, in sward plants it showed only limited value at high plant populations which a tightly closed canopy restricted the penetration of the chemical to the plant crown. Since few shoots emerged naturally at the crown in plants grown in 'tight' canopies, the control of new shoots in this situation seems largely unnecessary.

This study has clearly shown that shoot manipulation is effective in improving seed yield in <u>Lotus corniculatus</u>. However, quite different effects on seed yield were achieved following the use of the three chemicals. These differences occurred mainly through their effects on plant branching behaviour, and on the differential response by plants to chemicals according to time of application and plant density. It is not surprising that different yield responses were obtained in each of the three chemical treatments and even in different timing of

application at the two experimental sites. The chemicals used have more than one effect on plant growth and development. PP333 had a beneficial branch promoting and detrimental growth retarding effects. Nortron had a growth distortion effect as well as its shoot manipulating and weed control effects. Fatol Super achieved its beneficial effect on seed production through killing young vegetative shoots during the late season. When applied during the early plant growth stage and under high plant population densities, Fatol Super is of little or no value in enhancing seed yield. However, when applied at peak flowering to spaced plant it appeared highly successful in increasing seed yield, although its short term effect may necessitate repeat applications at about 10-14 day intervals to ensure longer term control of late formed shoots.

The success or not of chemicals on enhancing seed yield was affected by both plant growth status as well as by the chemical. This study has clearly shown that improved seed yields can be obtained only in situations where the beneficial potential of the chemical is maximized and/or the detrimental effects of the chemical are minimized. Studies concerned with these aspects would appear to be useful and potentially rewarding areas of future research.

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daylength change
(sunrise-sunset)
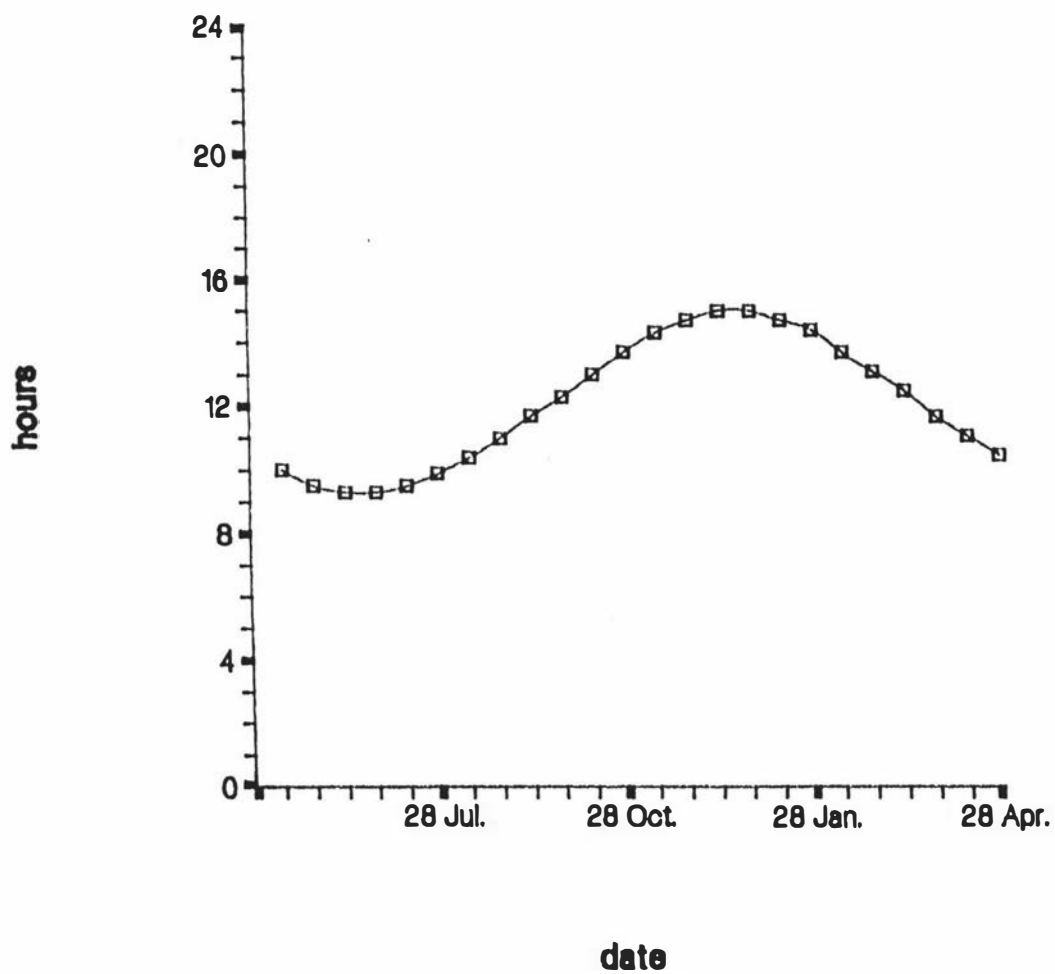
Appendix 1. Daylength change at Palmerston North, New Zealand
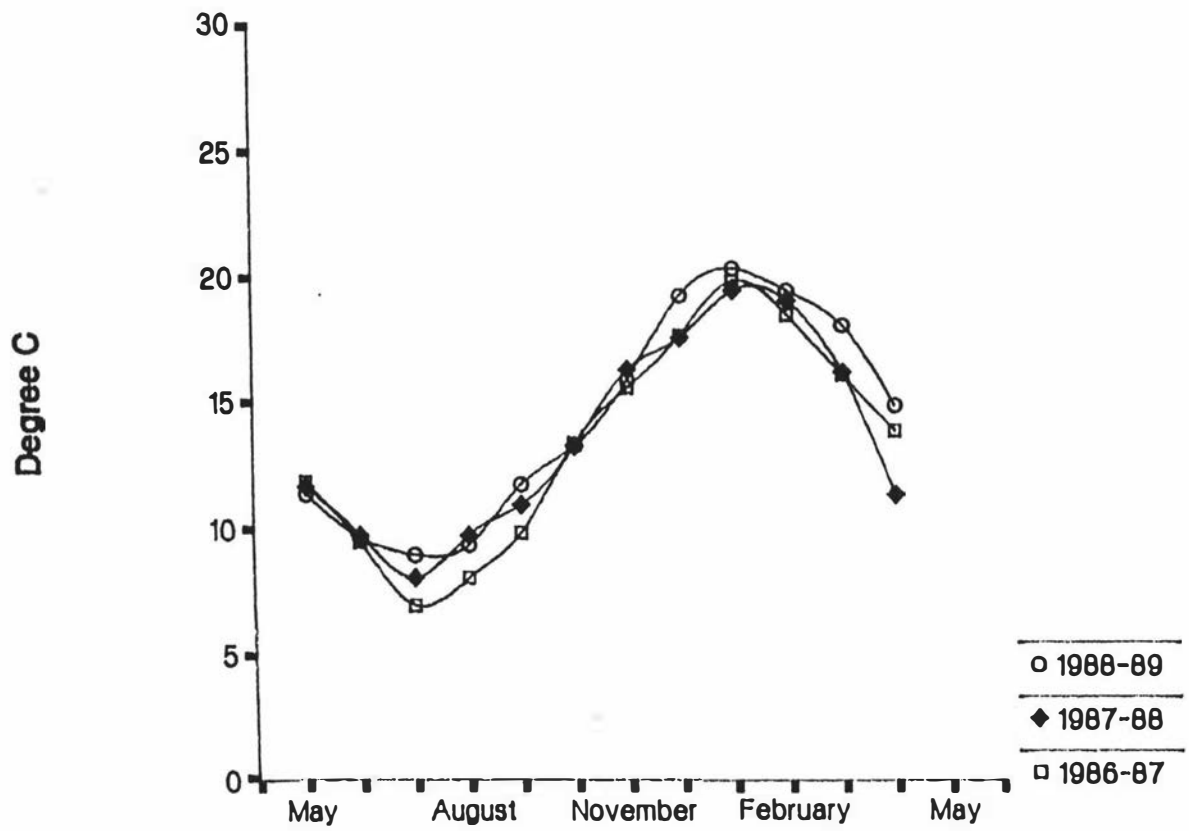
Appendix 2. Monthly temperature change at Palmerston North,
New Zealand (soil surface daily average temperature)
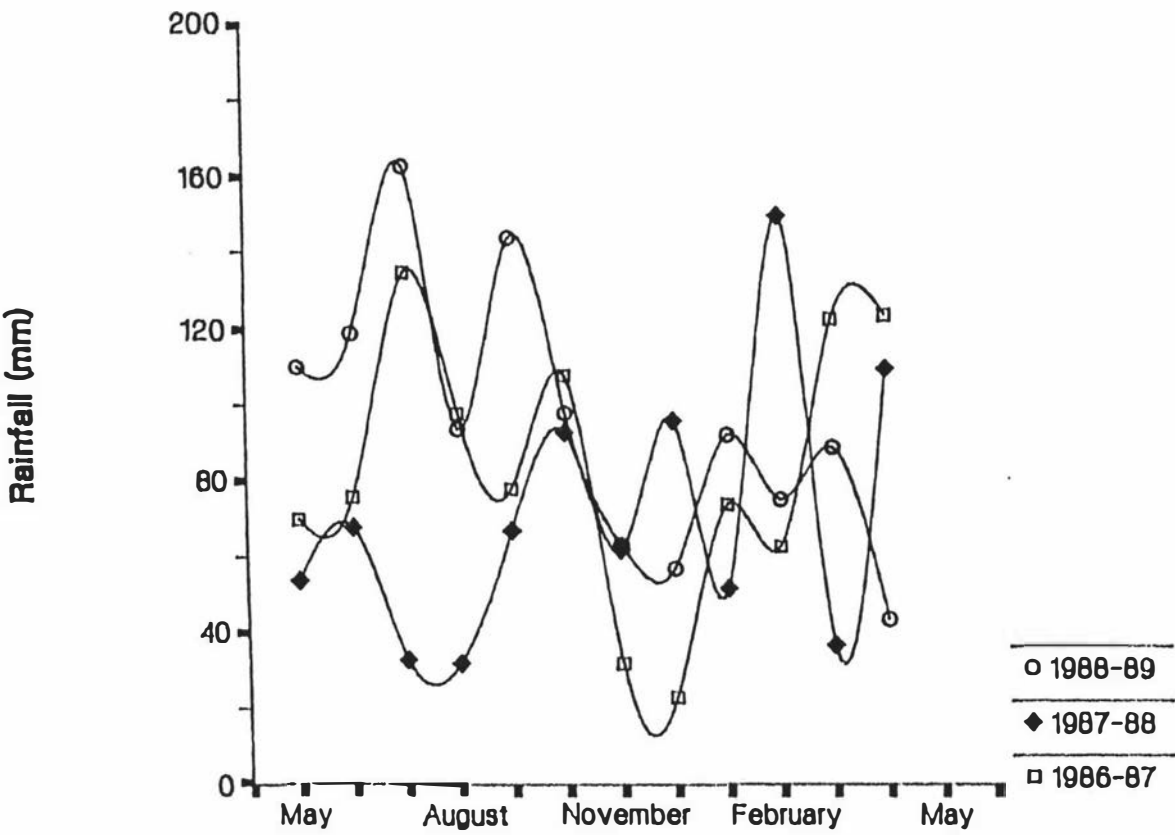
Appendix 3. Monthly rainfall at Palmerston North, New Zealand

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Appendix 4: Seed yield and yield components (STC site, 1986/87)

| Tagging date	Harvest date	Inflor. numbers/m^2	Pods/ inflor.	Seeds/ pod	1000-Seed weight	Seed yield(g)
12/12	13/1	30	4.55	13.55	1.37	7.08
19/12	28/1	84	3.98	12.61	1.32	7.79
1/1	5/2	152	4.55	14.11	1.29	12.59
11/1	20/2	34	2.97	12.24	1.33	1.64
21/1	3/3	18	2.64	14.27	1.28	0.87
31/1	12/3	35	2.32	11.54	1.34	1.26

Appendix 5: Seed yields of cutting treatents on spaced plants (g/plant) and sward plants (kg/ha)

STC site (spaced plants)	Cutting time			
	Uncut control	On 28th October	On 12th November	On 30th November
Cut (h)	35.7	36.7	38.1	26.1
Cut (L)		31.3	33.6	26.1
Aorangi site (sward plants)	Cutting time			
	Uncut control	On 27th October	On 12th November	On 27th November
	457	403	478	413

N.B.: no significant difference at p=0.05.

Appendix 6: Seed yield and yield components (STC site, 1987/88)

Treatment	Inflorescence Number	Pods per Inflor.	Seeds per Pod	1000-seed Weight(g)	Seed Yield
Control	1946	4.50	14.50	1.347	114.98
Control	2361	4.57	12.34	1.249	120.84
Control	2942	4.67	14.56	1.327	143.72
Hand 1	1861	4.57	11.97	1.416	119.93
Hand 1	3309	4.80	11.77	1.404	177.42
Hand 1	3013	5.20	15.24	1.369	123.49
Hand 2	2616	4.30	15.32	1.456	156.79
Hand 2	2743	3.77	13.87	1.322	175.74
Hand 2	3100	5.43	11.78	1.502	205.28
Hand 3	1808	4.70	13.67	1.387	127.68
Hand 3	2445	4.43	12.45	1.305	129.81
Hand 3	2298	3.90	10.68	1.381	104.21
Hand 4	1999	4.30	13.93	1.465	110.31
Hand 4	3236	4.60	13.22	1.317	142.12
Hand 4	3501	4.53	14.14	1.393	162.98
PP 2	2741	3.47	11.67	1.350	143.85
PP 2	2235	4.20	13.74	1.479	120.44
PP 2	2944	4.20	14.03	1.159	136.95
PP 3	2828	4.03	12.63	1.404	130.91
PP 3	3035	3.73	13.24	1.365	121.25
PP 3	2590	4.00	11.57	1.145	109.63
Nortron 2	1278	3.80	17.00	1.366	85.58
Nortron 2	1664	4.43	16.78	1.201	110.95
Nortron 2	2177	3.93	11.42	1.438	180.11
Nortron 3	2100	4.10	12.01	1.540	120.42
Nortron 3	2005	4.37	14.53	1.373	104.96
Nortron 3	3272	4.00	13.37	1.369	110.60
Fatol 2	2731	5.10	14.46	1.357	148.63
Fatol 2	2402	4.97	11.19	1.506	95.50
Fatol 2	2579	4.77	12.98	1.361	105.05
Fatol 3	1908	4.87	13.19	1.274	147.40
Fatol 3	2632	4.80	14.26	1.144	168.06
Fatol 3	2088	4.63	15.06	1.313	131.63

Appendix 7: Seed quality assesment (1987/88)
          (Seeds from different treatments)

Treatment	Normal seedling	Abnormal Seedling	Hard seed	Fresh ungerm.	Dead seed	Viable seed
At STC site						
Control	1	0	99	0	0	100
PP333	47	9	14	0	0	100
Nortron	27	3	70	0	0	100
Fatol	25	1	73	1	0	100
At Aorangi site						
Control	39	1	53	2	2	98
PP333	37	2	57	1	3	97
Nortron	32	0	67	1	0	100
Fatol	27	2	70	1	0	100

N.B.: (Germination test: TP, 20^0C, 50 seeds, 4 replicatess)

Appendix 8: Rregression analysis for seed yield and yield components
(raw data in Appendix 6)

A, Dependant variable Y = actual harvested seed yields

		Correlation coefficient (r) of seed yield (Y) vs.			
Variables		X1	X2	X3	X4
Single Variable		$r=0.304^{**}$	$r=0.043$	$r=0.035$	$r=0$
Two Variables	X1		$r=0.322^{**}$	$r=0.306^{**}$	$r=0.304^{**}$
	X2			$r=0.085$	$r=0.044$
	X3				$r=0.038$
Three Variable	X^1,X^2			$r=0.327^{**}$	$r=0.322^{**}$
	X^1,X^3				$r=0.306^{*}$
	X2,X3				$r=0.090$
Four Variables	X^1,X^2,X^3				$r=0.327^{**}$

B, Dependant variable Y = calculated potential yields using seed yield components.

		Correlation coefficient (r) of seed yield (Y) vs.			
Variables		X1	X2	X3	X4
Single Variable		$r=0.61^{***}$	$r=0.33^{***}$	$r=0.02$	$r=0.03$
Two Variables	X1		$r=0.83^{***}$	$r=0.74^{***}$	$r=0.65^{***}$
	X2			$r=0.34^{**}$	$r=0.35^{**}$
	X3				$r=0.07$
Three Variable	X^1,X^2			$r=0.92^{***}$	$r=0.86^{***}$
	X^1,X^3				$r=0.83^{***}$
	X2,X3				$r=0.37$
Four Variables	X^1,X^2,X^3				$r=0.99^{***}$

N.B. 1. Y = Seed yields
2. X_1 = inflorescence numbers;
X_2 = pod numbers per inflorescences;
X_3 = seed numbers per pod;
X_4 = 1,000 seed weight;
3. *, ** and *** represent 0.05, 0.01 and 0.001 significant levels, respectively

Regression procedure:
Step 1: single variable --- obtaining single variable correlation coeffecients (r) by regressing dependent variable Y against each indepentent viriable X_1, X_2, X_3 and X_4.
Step 2: two variables --- obtaining two variable correlation coeffecients (r) by regressing dependent variable Y against combination of independent variables of X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4, and X_3X_4 each time.
Step 3: three variables --- obtaining three variable correlation coeffecients (r) by regressing dependent variable Y against combination of independent variables $X_1X_2X_3$, $X_1X_2X_4$, $X_1X_3X_4$, and $X_2X_3X_4$ each time.
Step 4: four variables --- obtaining four variable correlation coeffecient (r) by regressing dependent variable Y against combination of dependent variables $X_1X_2X_3X_4$.